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FORMULATION OF TOPICAL NON-STEROIDAL
ANTI-INFLAMMATORY AGENTS

AMER NADIM ELIAS

Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

April 1987

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THE UNIVERSITY OF ASTON IN BIRMINGHAM

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AMER NADHIM ELIAS

A thesis submitted for the degree of Doctor of Philosophy, 1987

Summary

Reversed-phase high-performance liquid chromatogrpahic (HPLC) methods were developed for the assay of indomethacin, its decomposition products, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl esters. The development and application of these HPLC systems were studied. A number of physico-chemical parameters that affect percutaneous absorption were investigated. The pK_a values of indomethacin and ibuprofen were determined using the solubility method. Potentiometric titration and the Taft equation were also used for ibuprofen. The incorporation of ethanol or propylene glycol in the solvent resulted in an improvement in the aqueous solubility of these compounds. The partition coefficients were evaluated in order to establish the affinity of these drugs towards the stratum corneum. The stability of indomethacin and of ibuprofen esters were investigated and the effect of temperature and pH on the decomposition rates were studied. The effect of cetyltrimethylammonium bromide on the alkaline degradation of indomethacin was also followed. In the presence of alcohol, indomethacin alcoholysis was observed and the kinetics of decomposition were subjected to non-linear regression analysis and the rate constants for the various pathways were quantified. The non-isothermal, surfactant non-isoconcentration and non-isopH degradation of indomethacin were investigated. The analysis of the data was undertaken using NONISO, a BASIC computer program. The degradation profiles obtained from both non-iso and iso-kinetic studies show that there is close concordance in the results.

The metabolic biotransformation of ibuprofen esters was followed using esterases from hog liver and rat skin homogenates. The results showed that the esters were very labile under these conditions. The presence of propylene glycol affected the rates of enzymic hydrolysis of the esters. The hydrolysis is modelled using an equation involving the dielectric constant of the medium.

The percutaneous absorption of indomethacin and of ibuprofen and its esters was followed from solutions using an <u>in vitro</u> excised human skin model. The absorption profiles followed first order kinetics. The diffusion process was related to their solubility and to the human skin/solvent partition coefficient. The percutaneous absorption of two ibuprofen esters from suspensions in 20% propylene glycol-water were also followed through rat skin with only ibuprofen being detected in the receiver phase. The sensitivity of ibuprofen esters to enzymic hydrolysis compared to the chemical hydrolysis may prove valuable in the formulation of topical delivery systems.

Keywords

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS,
IBUPROFEN PRODRUGS,
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY,
STABILITY
PERCUTANEOUS ABSORPTION AND METABOLISM

To my mother

and sisters, Aida and Shetha.

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CHAPTER 1

INTRODUCTION

1.1 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Non-steroidal anti-inflammatory drugs are represented by variety of chemical structures which share similar effects upon acute inflammatory disorders. The use of aspirin dates from the last century and therapeutic modifications include soluble or buffered forms and enteric coated products. Other preparations include either a combination of aspirin with aluminium oxide as in aloxiprin or a sustained release from such as Levius(1). Salicylate derivatives are also available such as benorylate, salsalate and choline magnesium trisalicylate.

Indomethacin and phenylbutazone were the first alternatives to aspirin and these were followed by the propionic acid derivatives such as ibuprofen. Since then a wide variety of agents were developed. A large number of these drugs are already prescribed in the United Kingdom(1) but in spite of their similarities they differ in a number of aspects as shown in Table 1.1. Most of the drugs are available in tablets or capsules and are to be administered with food because of potential gastric irritation problems.

Some formulations are available in slow release form for example indomethacin S.R., while benorylate is often given as a suspension and some, such as naproxen and indomethacin, are available as suppositories(7).

TABLE 1.1
Non-steroidal anti-inflammatory drugs available.

Drug	Recommended clinical daily dose in arthritis (mg/day)	Frequency of administration (times daily)	Plasma half-life (hr.)	References
Aspirin[(a)]	2500-600	4	4.7-9	1,2,3
Aloxiprin[(a)]	100 mg/kg	4	-	1,4
Azapropazone	1200	4	12	1,4
Benorylate[(a)]	8000	2	1	1,4,5
Choline Salicylate[(a)]	3480-6960	3-4	-	1,4
Diclofenac	75-150	2-3	-	1,4
Fenbufen	600-900	2	10	1,4
Fenclofenac	600-1200	2	12	1,4
Fenoprofen	1200-2400	3-4	2.5	1,4
Feprazone	200-600	2-3	24	1,4
Flufenamic Acid	400-600	2-3	4	1,2,4
Flurbiprofen	150-200	3-4	3.9	1,4
Ibuprofen	600-1600	3-4	2	1,2,4
Indomethacin	75-150	2-3	2.6-11.2	1,2,3
Ketoprofen	100-200	2	1-4	1,4
Mefenamic Acid	750-1500 (max. 7 days therapy)	3	-	1,5,6
Naproxen	500-1000	2	12-15	1,4
Oxyphenbutazone	300-600	3	72	1,3,4
Phenylbutazone	300-600	3	84	1,3,4
Piroxicam	20	1-2	30-86	1,3
Salsalate[a]	975-3000	3-4	16	1,3,6
Sulindac	400	2	7-8	1,4
Tiaprofenic	300-400	3	-	1,4
Tolmetin	600-1800	3-4	1	1,4,5

(a) = Salicylate derivatives.

A comparative study of the pharmacological and clinical properties of 19 different acidic, basic and neutral anti-inflammatory agents was

published by Morsdorf and Wolf(8). The divergent physical and structural properties of these agents lead to quite different pharmacokinetic properties. Furthermore, insufficient pharmacological data made true dose-response comparisons of potency very difficult. A systematic correlation between the clinical anti-inflammatory data of 15 acidic anti-inflammatory drugs and their observed physical and pharmacological data was made by Lombardino et al.(2) (Table 1.2) towards true dose-response potency comparisons.

The role of non-steroidal anti-inflammatory drugs in the treatment of rheumatoid arthritis is shown in Fig. 1.1(1)

Stage 1	Stage 2	Stage 3	Stage 4
Mild disease → Analgesics	Moderate disease → Add non-steroidal anti-inflammatory drugs	Severe disease → Add second-line drugs (Gold, D Penicillamine)	Uncontrolled disease Add cytotoxic drugs (Methotrexate, Cyclophosphamide)

Fig. 1.1 Stages in the treatment of rheumatoid arthiritis(1).

1.2 THE PHARMACOLOGY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Non-steroidal anti-inflammatory drugs have analgesic, antipyretic and anti-inflammatory activities. The analgesia is of clinical importance and appears to be largely a peripheral rather than a central effect(7). The anti-inflammatory effects are the most important in the treatment of rheumatoid arthritis. The anti-inflammatory effect of

TABLE 1.2
Physical and clinical properties of some acidic anti-inflammatory drugs(2).

No.	Name	Formula	pK_a (a)	pK_a*	Partition Coefficient (b) in octanol/buffer		Serum half-life (h)					Carrageenan rat foot edema potency (f) ($ED_{50\%}$, mg/kg)	Clinical daily dose in arthritis (mg/day)
					buffer pH 7.4	pH 2.2	Rat	Dog	Rabbit	Monkey	Man		
1	Alclofenac	$CH_2{=}CHCH_2O-C_6H_3(Cl)-CH_2COOH$	7.2	4.6	0.61	11.4					3.5	160	1500–3000
2	Aspirin	$C_6H_4(COOH)(OCOCH_3)$	6.9	3.5	0.07	29.1					6 (g) (4.7-9)	220	2500–6000
3	Azapropazone	(structural formula: CH_3, H, $CH_2CH_2CH_3$, =O, $N(CH_3)_2$)	7.4	-	10.61	∞ (e)	2–5	2.5-6	1–2		9	200	600–1200
4	Bufexamic acid	$CH_3(CH_2)_3O-C_6H_4-CH_2CONHOH$	(c)	-	119.0	29.2					<3	620	1500–2000
5	Fenoprofen	$C_6H_5O-C_6H_4-CH(CH_3)COOH$	7.8 (d)	4.5	6.70	119	6–10	4	0.5	0.3	2.5	720	1600–2400

TABLE 1.2 continued...

No.	Name	Formula	pK_a (a)	pK_a*	Partition Coefficient(b) in octanol/ buffer		Serum half-life(h)					Carra- geenan rat foot edema potency(f) ($ED_{50\%}$, mg/kg)	Clinical daily dose in arthritis (mg/day)
					buffer pH 7.4	pH 2.2	Rat	Dog	Rab- bit	Mon- key	Man		
6	Flufenamic acid	–NH–; COOH; CF_3	6.8	3.9	119.0	2.9	3(h)	3(h)	4(4)		4(h)	95	400–600
7	Ibuprofen	$(CH_3)_2CHCH_2$–; CH_3; –CHCOOH	7.8	4.4, 5.2	∞(e)	2.9	1–2	2–3	1–2		3	45	600–1600
8	Indomethacin	CH_3O; CH_2COOH; N; CH_3; C=O; Cl	7.3	4.5	0.10	∞(e)	4	0.3		0.3	2	9	75–150
9	Ketoprofen	CH_3; –CHCOOH; C=O	7.2	–	0.97	∞(e)					1.5	33	100–300
10	Metiazinic acid	S; N; CH_3; CH_2COOH	7.3	–	9.29	50.7						40	1000–150

TABLE 1.2 continued...

No.	Name	Formula	pK_a (a)	pK_a*	Partition Coefficient(b) in octanol/ buffer		Serum half-life(h)					Carra-geenan rat foot edema potency(f) ($ED_{50\%}$, mg/kg)	Clinical daily dose in arthritis(i (mg/day)
					buffer pH 7.4	pH 2.2	Rat	Dog	Rab-bit	Mon-key	Man		
11	Naproxen	CH_3O; CH_3; CHCOOH	7.9	4.2	1.89	119	5.1	35		1.9	13.9	29	300-500
12	Niflumic acid	COOH; N; NH; CF_3	6.1	-	39.0	∞(e)	3					68	500-1000
13	Phenylbutazone	O; N; N; H; $(CH_2)_3CH_3$; O	6.1	4.4	4.80	2.7	6	6		7	72	40	300-400
14	Prenazone	O; N; N; H; $CH_2CH=C(CH_3)_2$; O	7.0	-	17.95	0.6	3.6	4.8			30	68	400-800

TABLE 1.2 continued....

No.	Name	Formula	pK_a (a)	pK_a*	Partition Coefficient(b) in octanol/buffer		Serum half-life(h)					Carra-geenan rat foot edema potency(f) ($ED_{50\%}$, mg/kg)	Clinical daily dose in arthritis(i) (mg/day)
					buffer pH 7.4	pH 2.2	Rat	Dog	Rab-bit	Mon-key	Man		
15	Sudoxicam	[structure: OH, CONH-thiazolyl, N–CH_3, SO_2 benzothiazine]	5.3	-	0.52	44.1	13	60		8	24-96	6	20

* These date from reference (4).

(a) pK_a determined at half-neutralization point in 2:1 dioxane-water using NaOH as titrant.

(b) Partition coefficient determined in n-octanol/pH 7.4 or pH 2.2 phosphate buffer.

(c) No discernible end-point was observed.

(d) The sodium salt of fenoprofen was titrated in 2:1 dioxane-H_2O using HCl as titrant.

(e) These compounds were essentially completed extracted into the n-octanol phase.

(f) Rat foot edema potencies were determined by simultaneous comparisons at three or four dose levels on the same d[ay] using phenylbutazone as a positive control.

(g) This is the half-life of salicylic acid which is derived from hydrolysis of aspirin <u>in vivo</u>.

(h) Values determined on the aluminium salt of flufenamic acid.

(i) Clinical dose, mainly for rheumatoid arthritis, is taken from literature reports; the preferred or most frequent[ly] reported dose is underlined.

aspirin was only identified in 1964(9-11). All non-steroidal anti-inflammatory drugs reduce joint inflammation pain and stiffness.

The inhibition of prostaglandin synthesis was proposed by Vane in 1971 as the possible mode of action of non-steroidal anti-inflammatory drugs(12). The therapeutic effects and gastro-intestinal adverse reactions may be explained on this basis. Prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) are mediators of inflammation and can cause increased vascular permeability, vasodilation, fever and a variety of other symptoms(13), whereas these prostanoids can exert protective actions on the gastric mucosa and small intestine(14,15). Non-steroidal anti-inflammatory drugs reduce these changes by inhibiting prostaglandin synthesis. Prostaglandins are a complex group of mediators which have many different actions and the effect of non-steroidal anti-inflammatory drugs may vary from tissue to tissue. Non-steroidal anti-inflammatory drugs inhibit the activities of several cellular or plasma enzyme systems as shown in Table 1.3 and may be considered as possible mechanisms of the anti-inflammatory action(16,17).

Other effects displayed by non-steroidal anti-inflammatory drugs are the inhibition of granulocyte and monocyte migration and phagocytosis(38); they influence lymphocyte transformation(39); they affect platelet function(40) and they stabilize lysosomal membranes(41).

Non steroidal anti-inflammatory drugs (classical) inhibit the synthesis of endogenous prostaglandins by blocking the action of the cyclo-oxygenase enzyme. A survey was presented by Cullen(42) of several new agents which act by blocking both pathways of arachidonic acid metabolism (the cyclo-oxygenase pathway leading to the prostaglandins

TABLE 1.3

Effects of non-steroidal anti-inflammatory drugs on mediators and enzyme systems(16).

1.	Uncoupling of oxidative phosphorylation(18-20)
2.	Inhibition of histidine decarboxylase and of dopadecarboxylase(21)
3.	Inhibition of proteolytic enzymes(22-24)
4.	Inhibition of mucopolysaccharide biosynthesis(25)
5.	Stabilization of plasma proteins
	- disulfide linkages(26,27)
	- protein-drug bindings(28-30)
6.	Inhibition of the Hageman factor, of Kinin systems, of fibrinolysis, etc.(31,32)
7.	Inhibition of the lymph node permeability factor(33,34)
8.	Inhibition of prostaglandin synthesis(12,35-37)

and the lipoxygenase pathway leading _inter alia_ to 5,12-dihydroxy-eicosatetraenoic acid, a potent chemotactic agent for polymorphonuclear leukocytes) together with anti-inflammatory immunomodulators and other agents currently in clinical trial.

One of these new agents, namely 3-amino-1-[(m-trifluoromethyl)-phenyl]-2-pyrazoline (BW755C), which is classified as an arachidonic acid inhibitor was found to be selective in its action on the cyclo-oxygenase enzyme and inhibits prostaglandin production in inflammatory exudate but not in the gastro-intestinal tract, thus explaining its observed nonulcerogenicity as compared with aspirin, indomethcin and ketoprofen(43).

BW755C

Non-steroidal anti-inflammatory drugs display qualitatively similar side effects. Among these is the gastrointestinal adverse reaction mediated by changes in prostaglandin metabolism. This includes dyspepsia, nausea and vomiting and can be a significant cause of gastric ulceration(41,44,45).

Healthy volunteers treated with tiaprofenic acid, aspirin or ibuprofen showed a significant blood loss in comparison to volunteers on a placebo(45). The concurrent oral administration of sodium salicylate or salicylic acid and indomethacin in rats significantly reduced the gastro-ulcerogenicity and the plasma concentrations of indomethacin(46). The simultaneous administration of sodium salicylate and indomethacin exerted similar anti-inflammatory activity as the single drugs. The ulcerogenic interaction was explained partly by the reduced indomethacin plasma concentrations and partly by a weaker inhibition by sodium salicylate of the prostaglandin system in the rat stomach. Sodium salicylate antagonised the ulcerogenic activity of ibuprofen, phenylbutazone, acetylsalicylic acid and naproxen(46).

The development of pro-drugs, which are inactive derivatives metabolised to active species during or after absorption, may help reduce the gastro-intestinal symptoms not related to prostaglandins.

Attempts to prepare anti-inflammatory prodrugs concentrated mainly on the modification of the acidic function of known anti-inflammatory drugs through esterification and/or amide formation(42).

On this basis triglyceride derivatives are able to pass through the stomach without undergoing hydrolysis and then be metabolised to provide the anti-inflammatory drug incorporated in the triglyceride structure for absorption in the intestine.

Triglycerides of aspirin (I) and indomethacin (II) were evaluated and it was observed that the therapeutic ratio of aspirin triglycerides was improved 80-fold over aspirin. However, with indomethacin only a threefold improvement was noticed. It was concluded that gastro-intestinal damage due to indomethacin is mainly produced systemically by the circulating drug where as in the case of aspirin the irritation is the result of local action on the gastric mucosa(47-50).

$COOR$, $OCOCH_3$

(I)

CH_3O, CH_2COOR, N, CH_3, C=O, Cl

(II)

$R = -CH(CH_2OCO(CH_2)_{10}CH_3)_2$

Other side effects of non-steroidal anti-inflammatory drugs include rash, headache, tinnitus, blood dyscrasias(51), hepatitis(52,53) and skin problems(54).

In order to overcome the side effects which accompanied ibuprofen therapy, a number of ibuprofen esters were prepared(55-58) and an alternative routes of administration were tested(59-63). Ibuprofen p-hydroxyphenylurea ester was tested for oral use and found to have 1.7 times the anti-inflammatory activity of aspirin and a much better taste(56).

The administration of ibuprofen and flurbiprofen topical formulations in humans and animals showed that the compounds were effective in decreasing pain and alleviate UV-erythema(64,65). In addition, it was claimed that these preparations did not have the side effects of orally or rectally administered compounds(64,65).

Dermal irritation was observed when creams or ointments containing 5% pimeprofen (2-pyridylmethyl 2-[p-(2-methylpropyl)phenyl]-propionate) were applied to rabbit's skin daily(66). The irritation was less than that introduced by a comparative drug, 5% sodium lauryl sulfate.

Unchanged ibuprofen guaiacol (ibuprofen 2-methyoxyphenyl ester) was not found in the serum of rats given the drug orally but instead, free ibuprofen was detected in concentrations similar to those obtained after the administration of ibuprofen alone(67). Ibuprofen guaiacol was apparently not hydrolysed by the gastro-intestinal mucosa but it is hydrolysed rapidly in the serum after absorption and thus preserved the pharmacological properties of ibuprofen(67).

1.3 THE SOLUBILITY AND pK_a OF INDOMETHACIN AND IBUPROFEN

Although indomethacin is practically insoluble in water it is soluble in a number of organic solvents including ethanol, ether, acetone and castor oil(68).

Krasowska and co-workers(69) determined the solubility of indomethacin in pure organic solvents (glycerol, propylene glycol, ethanol, polyethylene glycol (PEG) 200, 300, 400, 600) and in solvent-water mixtures. The data were directly correlated with the dielectric constant of the solvent-water mixture and the solubility-dielectric constant profile of indomethacin in ethanol-water systems is shown in Fig. 1.2(69) with the solubility being reciprocally related to the dielectric constant of solvent or solvent-water mixture (Table 1.4).

The influence of solvent concentration in solvent-water mixture on solubility appeared independent of the type of solvent, and lowering the polarity of the solvent mixture thus caused a general increase in the solubility of indomethacin. A solvent mixture having the dielectric constant near the optimum value (ε=5.6) is expected to give a good solubilizing effect for indomethacin(69).

The solubilization in aqueous systems by cosolvents of a number of pharmaceutical products including indomethacin was studied by Yalkowsky and Rubino(71). The solutes investigated were chosen to cover a wide range of polarities and chemical structures. The solubilization curves for the propylene glycol-water system of indomethacin and other analgesics are shown in Fig. 1.3. The solubility values of indomethacin

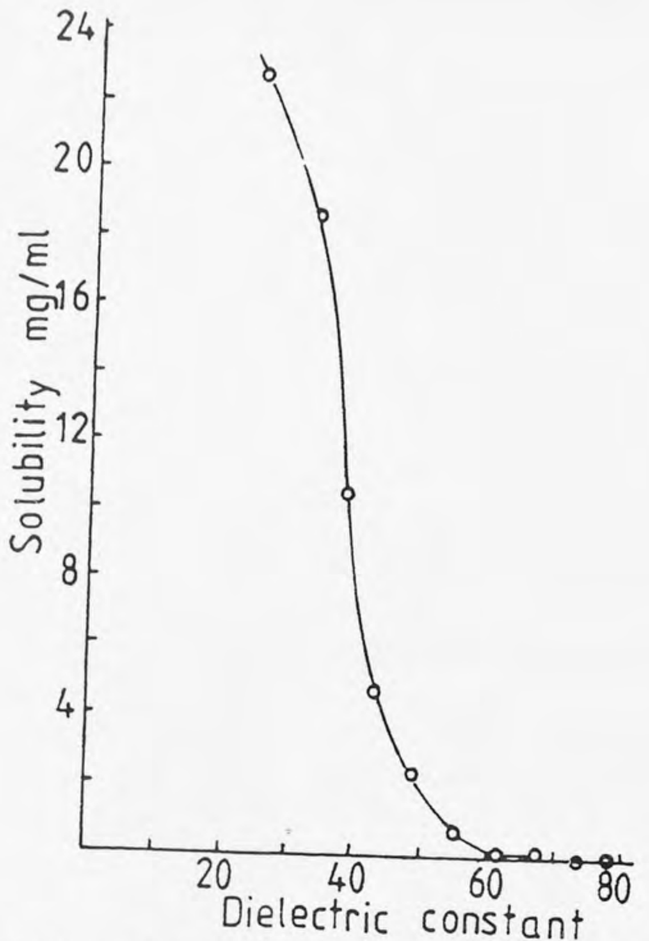


Fig. 1.2 Solubility of indomethacin at 25°C as a function of dielectric constant of ethanol-water mixtures (69).

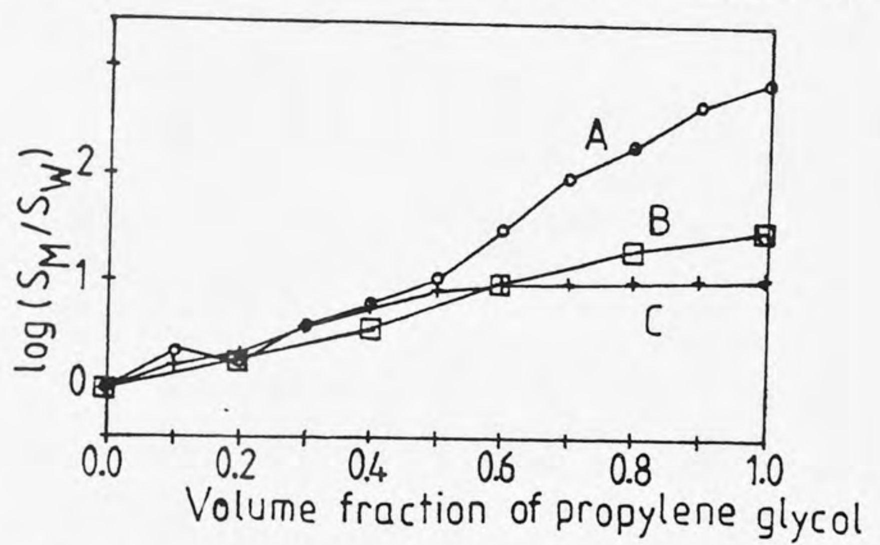


Fig. 1.3 Solubility of indomethacin, phenacetin, and p-aminoacetophenone in propylene glycol-water mixtures (data taken from refs 69 and 367) (71).

Key : (A,○) indomethacin; (B,□) phenacetin; (C,+) p-aminoacetophenone.

TABLE 1.4

The solubility of indomethacin in pure solvents and solvent-water mixtures(69).

w/w % Solvent in solvent-water mixture	Solubility mg/ml							
	Glycerol(a)	Propylene(b) glycol	Ethanol(c)	PEG 200	PEG 300	PEG 400(d)	PEG 600	Dioxane(e)
0	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
10	0.034	0.031	0.023	-	-	0.018	0.061	0.041
20	0.033	0.025	0.041	0.023	0.045	0.054	0.099	0.060
30	0.057	0.056	0.217	-	-	0.088	0.22	0.146
40	0.058	0.089	0.712	0.12	0.348	0.250	0.480	7.61
50	0.062	0.150	2.48	-	-	0.585	1.12	3.38
60	0.069	0.420	4.78	1.32	2.60	2.21	3.84	12.29
70	0.119	1.310	10.56	4.51	7.37	7.78	14.87	45.57
75	-	-	-	-	-	-	-	76.30
80	0.137	2.610	18.68	17.20	30.7	40.13	56.58	103.44
90	-	6.280	-	47.10	70.66	90.12	120.84	179.20
100	-	9.624	22.78	84.48	114.69	122.88	121.00	121.86

(a) $\varepsilon = 43.0$, (b) $\varepsilon = 32.0$, (c) $\varepsilon = 24.3$, (d) $\varepsilon = 14.31$, (e) $\varepsilon = 2.2$ dielectric constants values for pure solvents at 25°C according to Reference 70.

in this solvent system were obtained from the data published by Krasowska and co-workers(69). The curves have all been normalised by dividing the mixed-solvent solubility by the aqueous solubility. This enabled all the curves to be plotted on the same axes and facilitates comparison.

An equation was developed to describe the solubilization effect by treating a mixed solvent as a linear combination of its components. The equation correlated the solubility in the solvent system with the octanol-water partition coefficient (indomethacin = 1202.2)(71) and was used to explain both the exponential increase and the exponential decrease in aqueous solubility that are frequently observed with the addition of cosolvents. Details of the equation developed by Yalkowsky and Rubino(71) and its application to solvent systems used in this project together with its degree of success in describing the solubilization effect is discussed in Chapter 3. The authors also claimed that the equation was able to estimate the extent to which a particular drug can be solubilized and how much cosolvent would be required to accomplish a particular degree of solubilization.

Yalkowsky and others(72) correlated the octanol solubility (S_o) for a number of crystalline solutes including ibuprofen to their octanol-water partition coefficients (PC) for the estimation of their aqueous solubilities (S_w) by applying the data in the following relationship:

$$\log PC = \log SR = \log \frac{S_o}{S_w} \qquad (1.1)$$

where SR is the octanol-water solubility ratio which is defined as the octanol solubility (in moles/litre) divided by the aqueous solubility

(in moles/litre). The weak electrolytes and nonelectrolytes used conform to the regression equations and if the regression line is forced through the origin equation (1.1) became:

$$\log PC = 1.027 \log SR \tag{1.2}$$

where $r = 0.992$, $SD = 0.326$ and $n = 36$.

Fig. 1.4 shows the observed partition coefficients and solubility ratios for the solutes studied (ibuprofen log PC = 4.43; log S_w = -3.76 (observed); m.p. = 76°C and log SR (calc.) = 4.03)(72). Ibuprofen was reported to be relatively insoluble in water but readily soluble in most organic solvents(73,74).

The effect of some nonionic surfactants as well as polyethylene glycol 6000 and dextrose on the dissolution rate of ibuprofen was studied by El-Shaboury et al.(75). The surfactants produced a better drug release than polyethylene glycol and dextrose at the drug-carrier ratio studied. No interaction was observed between ibuprofen and any of the additives used. In addition, the bulkiness of the drug was not affected by the amount of surfactant added.

The relationship between the dissociation constant of a sparingly soluble acidic substance, its solubility and the pH value of the solvent stated that under controlled ionic strength and temperature conditions, the dissociation constant K_a of the acid can be determined by the solubility method(76,77). If S_o is the intrinsic solubility of the undissociated acid, $[HA]_o$, the dissociation constant can be expressed in terms of the solubilities as:

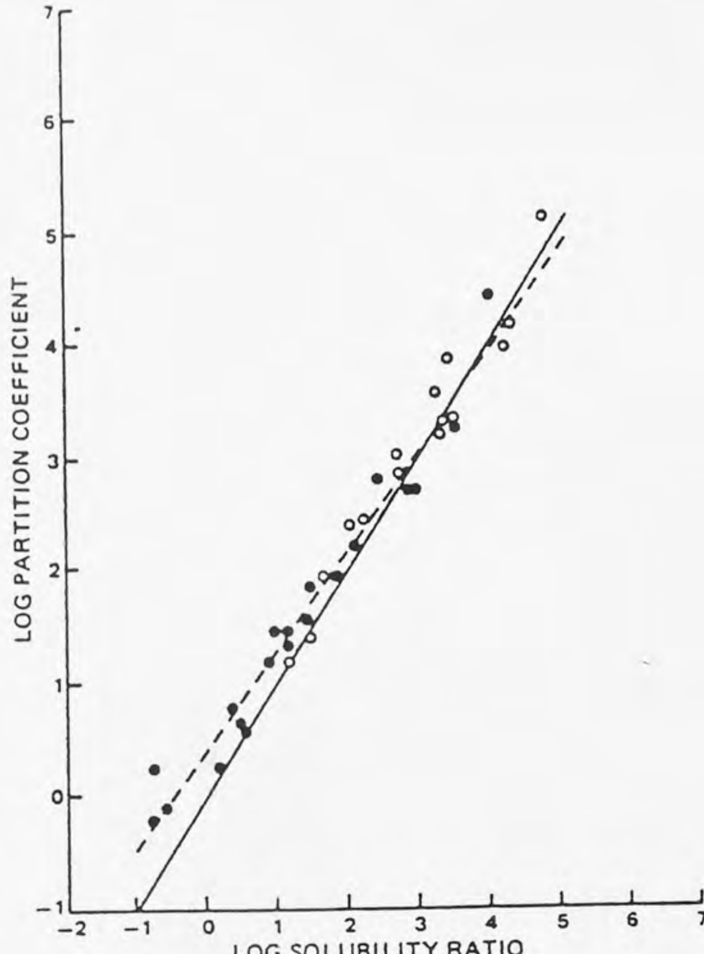


Fig. 1.4 Partition coefficients and solubility ratios of non-electrolytes (o) and weak electrolytes (●) (72).
Key: (——) theoretical line described by Eq. 1.1 ;
(----) regression line described by Eq. 1.2 .

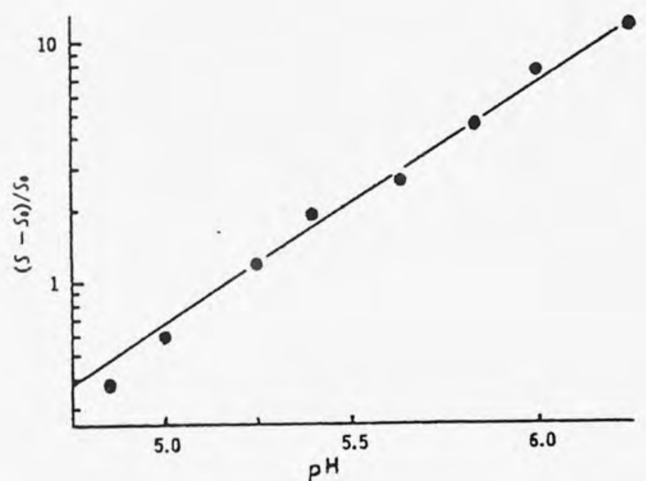


Fig. 1.5 Semilogarithmic plot of the solubility function $(S-S_0)/S_0$ against pH at 25° in accordance with $\log(S-S_0)/S_0 = pH - pK_a'$, where S is the total molar solubility of canrenoic acid and its anion at a given pH, and S_0 is the intrinsic solubility of the undissociated acid. The slope is unity, and the intercept permits the calculation of pK_a' as 5.2 (77).

$$K_a = [H^+](S-S_o)/S_o \quad (1.3)$$

$$\log[(S-S_o)/S_o] = pH - pK_a \quad (1.4)$$

where S is the total solubility of the undissociated acid in equilibrium with its anion at a given pH.

Garrett and Won(77) employed this method for the determination of the pK_a of canrenoic acid as shown in Fig. 1.5.

The effect of pH on the total solubility of indomethacin was used by Mooney et al.(78) for the determination of the dissociation constant of this weak acid at 25°C. The values obtained for the K_a and pK_a of indomethacin were 6.7×10^{-5} and 4.17 respectively(78).

Potentiometric titration is by far the most convenient method for the determination of dissociation constants and, with care, this method can give reproducible results (pK_a ± 0.03 units) for acids and bases having pK_a values between 2.5 and 11(79,80). Nonlinear plots of pH versus titrant were obtained for aqueous systems and often these plots are used to determine the dissociation constant by what is called the half-neutralization method(79). This method is based on the Henderson-Hasselbalch equation when pH = 4-10:

$$pH = pK_a + \log\left[\frac{\text{base}}{\text{acid}}\right] \quad (1.5)$$

For a large number of pharmaceutical products, the uncharged

species is so insoluble that it is very difficult to determine accurate dissociation constant in pure aqueous systems and solutions containing organic solvents were used in the potentiometric titrations(81). In order to account for the various interactions between the carboxylic acid and the solvent molecules, the concentration of water in the solvent mixture was accounted for and a plot of p_sK_a + log[H_2O] versus 1/ε should be linear where p_sK_a is the dissociation constant of the acid in the mixed solvent system and ε is the dielectric constant of the solvent mixture(79,81-83). The extrapolation of the data to the pure aqueous system (or the intercept) should give the pK_a of the acid. The derivation of the final equation used for the determination of the pK_a potentiometrically in mixed solvent systems will be discussed in detail in Chapter 3.

Goto et al.(84) determined the pK_a of indomethacin in mixed alcohol-water systems (Fig. 1.6) and the extrapolation of the data obtained by plotting p_sK_a + log [H_2O] versus 1/ε to the pure aqueous system (0% alcohol) yielded a pK_a value of 4.2 ± 0.2.

1.4 THE STABILITY OF IDOMETHACIN AND IBUPROFEN DERIVATIVES

The degradation of indomethacin has been widely investigated and it has been established that the process follows first order kinetics in solution(84-88). The effect of temperature on the first order rate of degradation of indomethacin was evaluated in alkaline medium(85) (Fig. 1.7) and the corresponding activation energies, together with other related parameters, were calculated from the Arrhenius-type plots(85) (Table 1.5). Extrapolation to room temperature allows the shelf life of the compound to be predicted.

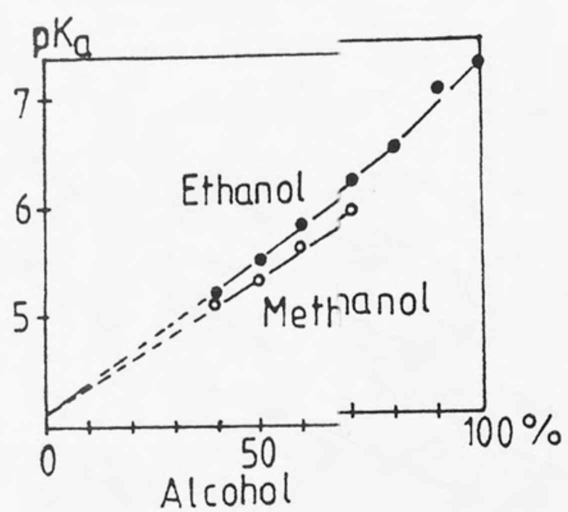


Fig. 1.6 The pK_a of indomethacin as a function of alcohol percent by volume in alcohol-water mixtures (84).

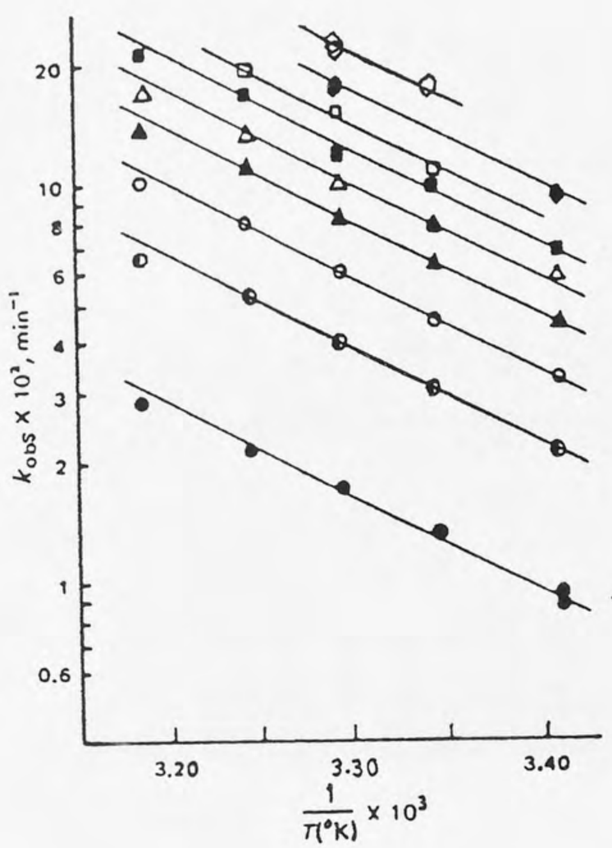


Fig. 1.7 Arrhenius plots showing temperature dependance of k_{obs}, the apparent first-order indomethacin degradation rate constant, at various hydroxide-ion concentrations (M) (85).

<u>Key</u>: ⬡, 0.01; ⬢, 0.008; □, 0.007; ■, 0.006; △, 0.005; ▲, 0.004; ○, 0.003; ◐, 0.002; and ●, 0.001.

TABLE 1.5

Activation energies and other parameters for indomethacin degradation in aqueous solution at 25°C obtained from Arrhenius-type plots(85).

Hydroxide-Ion Concentration, M	E_a kcal $mole^{-1}$	$\Delta H^{\ddagger}$ kcal $mole^{-1}$	$\Delta S^{\ddagger}$ cal $mole^{-1} deg^{-1}$	$\Delta F^{\ddagger}$ kcal $mole^{-1}$	-r	n
0.001	10.1	9.5	-35.2	20.1	0.995	6
0.002	10.0	9.4	-33.9	19.6	0.997	5
0.003	10.3	9.7	-32.3	19.3	0.998	6
0.004	10.2	9.6	-31.8	19.1	0.998	6
0.005	10.0	9.5	-32.0	19.0	0.996	8
0.006	10.3	9.7	-30.7	18.9	0.996	6
k_1	10.0	9.5	-21.4	15.8	0.997	5

Indomethacin (I) is relatively stable in neutral or slightly acidic media but it is decomposed by strong alkali(68) to p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid (II). The two products were isolated and identified by thin-layer chromatography(89). In the acidic region, further degradation of the 5-methoxy-2-methylindole-3-acetic acid product was observed. This results in the formation of 5-methoxy-2,3-dimethylindole (III) and 1-(p-chlorobenzoyl)-5-methoxy 2,3-dimethylindole which, in turn, degrade further causing discoloration of the solution(84). A proposed mechanism showing the possible routes for the hydrogen- and hydroxyl-ion catalysed reaction of indomethacin is illustrated in the Fig. 1.8(84).

The effect of varying the hydroxide-ion concentration on indomethacin degradation at various temperatures is presented in Fig. 1.9(85). The rate constant-hydroxide-ion concentration profile was linear with a positive slope suggesting a simple mechanism for the degradation reaction shown in Scheme (1.1).

$$I^{-} + OH^{-} \xrightarrow{k_1} \text{Products}$$

Scheme (1.1) The degradation of indomethacin in alkaline medium. Where I^{-} is the monodissociated species of indomethacin.

The overall first-order character of the reaction enabled the following rate law to be suggested:

Acid

Alkali

Coloured material (VI)

Coloured material (V)

(I)

(I′)

(II)

(II′)

(III)

Fig.1.8 Possible routes for hydrogen- and hydroxyl-ions catalysed reaction of indomethacin(84).

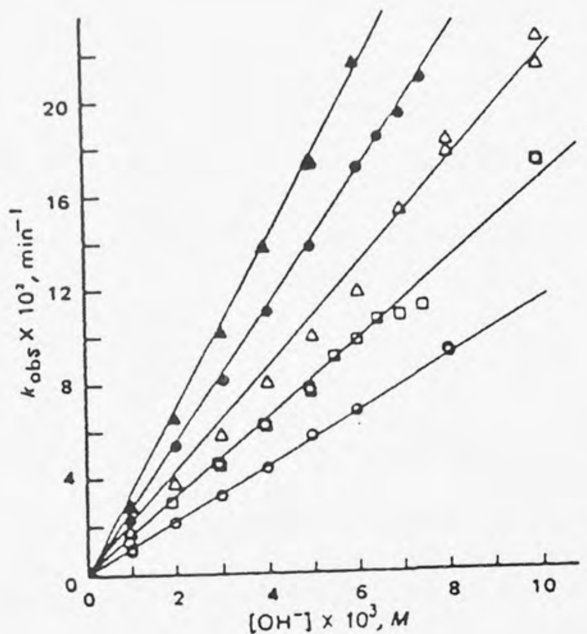


Fig. 1.9 Effect of varying concentrations of hydroxide-ion on indomethacin degradation at various temperatures (85).
Key : ○, 21.1°; □, 25.8°; △, 30.3°; ●, 35.1°; and ▲, 40.7°C .

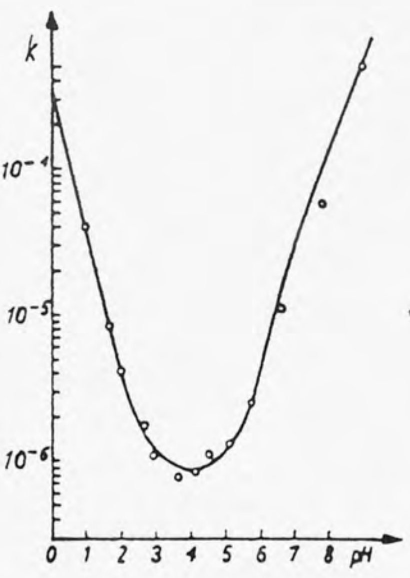


Fig. 1.10 Log k - pH profile for the degradation of indomethacin at 80°C (86) .

$$k_{obs.} = k_1 [OH^-] \quad (1.6)$$

where $k_{obs.}$ is the apparent first-order rate constant, k_1 is the second-order hydroxide-ion catalytic rate constant and the brackets signify molar concentrations.

Pawelczyk and co-workers(86) examined indomethacin degradation in both acidic and alkaline media (Fig. 1.10) and concluded that indomethacin undergoes an acid-base specific catalysis and the reaction rate was evaluated by the equation:

$$k_{obs.} = k_o + k_{H^+} [H^+] + k_{OH^-} [OH^-] \quad (1.7)$$

where k_o, k_{H^+} and k_{OH^-} are the solvent, hydrogen-ion and hydroxide-ion catalytic rate constants respectively.

It is known that indomethacin is a weak organic acid and its degree of ionization is dependent on the pH of the medium. Furthermore, in solution the hydrogen- and hydroxyl-ion-catalysed degradation rates of drugs may vary with the charge on the substrate molecule(90). Therefore, it seems that Pawelczyk and co-workers(86) have neglected considering this aspect when evaluating the degradation rate constants over the entire pH range used.

The kinetic terms that express the hydrogen- and hydroxyl-ion catalytic effect on the degradation rates have been developed by Higuchi et al.(91) and Edwards(92). The specific acid-base catalysis of degradable species was studied extensively by Garrett(90). The derived model describing the apparent first-order rate constant, k, for the loss

of a substrate at a constant pH took into account the catalytic species (H^+ or OH^- or H_2O), the specific rate constant involved and the dissociation constant of the compound. The developed equation will be discussed in Chapter 4.

Hajratwala and Dawson(85) observed a positive ionic strength effect when studying the alkaline hydrolysis of indomethacin (Fig. 1.11) which suggests that the reaction of ions of like sign plays a role in the degradation process and therefore confirms the mechanism presented earlier in Scheme (1.1).

The kinetics of the alkaline hydrolysis of indomethacin were studied in the presence of anionic(93), cationic and nonionic surfactants(93,94). The degradation followed apparent first-order kinetics. The variation of the degradation rate constants is usually treated on the assumption that the substrate S is distributed between the aqueous and micellar pseudophases and can react in each pseudophase with the rate constants k'_W and k'_M respectively. It has been postulated that the micellar Stern layer is similar to a concentrated ionic solution(95) and the concentration of hydroxide ion in the Stern layer of a cationic surfactant like cetyltrimethylammonium bromide is high. Cationic micelles therefore enhance the degradation of indomethacin(93,94) and related compounds on the basis of interaction (high binding constant) between the micellar head group and the negatively charged carboxylate moieties (Fig. 1.12a)(94).

Inhibition of degradation in the presence of anionic micelles was observed by Cipiciani et al.(94) (Fig. 1.12b). This occurred inspite of the electrostatic repulsion between the micelle head group of the

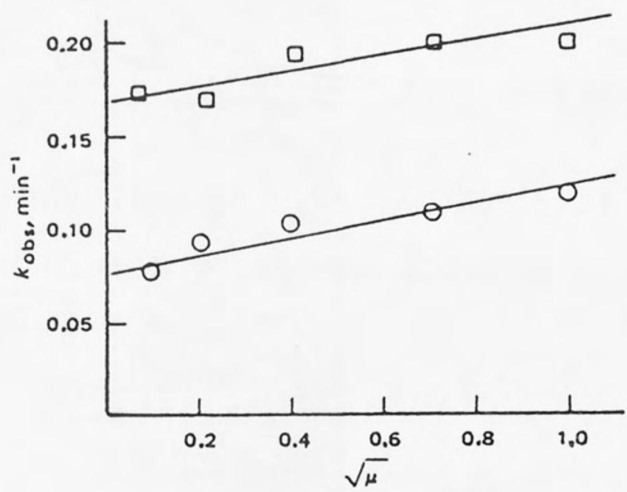


Fig. 1.11. Effect of ionic strength (μ) on the degradation of indomethacin at 25.8°C in 0.005 M (○) and 0.01 M (□) hydroxide-ion concentrations (85).

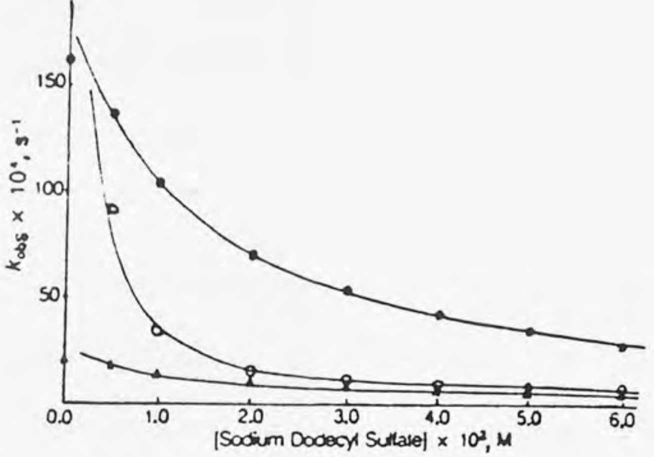


Fig. 1.12(a) Inhibition of base hydrolysis of 1, 2 and 3 in anionic micelles of sodium dodecyl sulfate in 0.05 M NaOH. The curves are simulated (94).

Key: (●) 1; (▲) 2; (o) 3 .

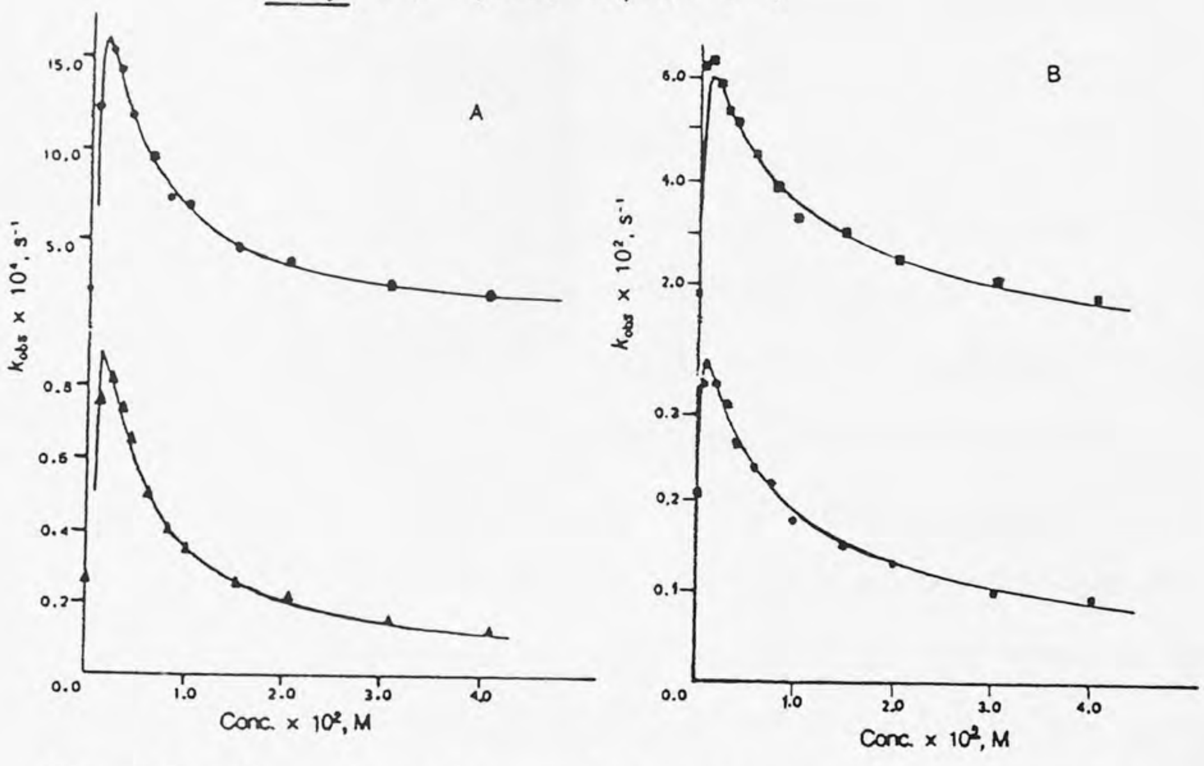


Fig. 1.12(b) Reaction of 1 and 2 in hexadecyltrimethylammonium bromide and 1×10^{-3} M (A) and 0.05 M (B) NaOH. The curve is simulated (94).

Key: A (●) 1; (▲) 2; B (■) 1; (●) 2 .

Fig. 1.12(a,b) Miceller effects on the basic hydrolysis of indomethacin and related compounds (94) .

KEY

1, R^1 = p-Cl—C_6H_4, R^2 = OCH_3

R^3 = CH_2CO_2H, R^4 = CH_3

2, R^1 = p-CH_3O—C_6H_4, R^2 = OCH_3,

R^3 = CH_2CO_2H, R^4 = CH_3

3, R^1 = p-Cl—C_6H_4, R^2 = R^3 = R^4 = H

anionic surfactant and the negatively charged carboxylate moieties which resulted in a low binding constant.

The coulombic interactions are of key importance in determining the interactions between ionice micelles and reactive species. Micellar catalysis and inhibition could be rationalised in terms of the principles which Hartley had applied to micellar effects upon equilibria(96,97). In the presence of anionic surfactants, little or no micellar effects on the observed rate constant has been described for the alkaline hydrolysis of negatively charged substrates(98,99). The anticipated effect on the alkaline hydrolysis of micelle-incorporated substrates is a strong inhibitory effect on the reaction rate. This has been interpreted in terms of a simple electrostatic exclusion of $\bar{O}H$ from the anionic micelle(99). This assumption played a key role in the formulation of the enzyme model for quantitative analysis of micellar modified reactions(99). The alkaline hydrolysis of the ester, mono-p-nitrophenyl dodecanedioate, was inhibited in the presence of the anionic surfactant lauric acid. The presence of 0.02 M laurate reduced the rate of hydrolysis of the ester to 35% of that in the absence of the surfactant(99). This inhibition effect was explained by the nature of the micelle interior, formed by the lyophobic portion of the surfactant. It was assumed that the ester substrate was incorporated into the very inner part of a micelle resulting in substantial inhibition of hydroxide ion catalysed hydrolysis(99).

Alternatively, adsorption could occur within the outer aqueous areas of the micelles where the hydroxide ion concentration might be greatly reduced because of the electrostatic factors originating at the micelle surface(99). On this basis the ionic substrate mentioned

earlier has one end of the adsorbed molecule in the aqueous region of the micelle. The ester moiety, many carbons away from the ionic end, must then be directed toward the centre of the micelle which affords protection for the ester linkage(99).

In the presence of nonionic surfactants, Krasowska(100) observed that the alkaline hydrolysis of indomethacin was inhibited. The degradation of the solubilised drug follows a first-order process in which the rate decreases with the increasing surfactant concentration. The inhibitory effect of the micelles was explained by the incorporation of indomethacin into the nonionic micelles and thus was protected from the attacking ions . The subsequent decrease in the observed rate constant when the polysorbate concentration was increased was attributed to the large miceller phase volume which lowered the dielectric constant of the medium(100). This was in agreement with an earlier report by Krasowska(101) that the rate of indomethacin hydrolysis was markedly reduced by lowering the dielectric constant of the reaction mixture.

The adsorption of non-steroidal anti-inflammatory drugs at carbon black/water surface was used as a model for the interaction of drugs at hydrophobic interfaces in the body and to give an idea on the onset of their pharmacological action(87). During the physico-chemical investigations it was found that the degradation of some of these drugs, e.g. indomethacin, phenylbutazone and azapropazone was accelerated in the presence of carbon black. This acceleration was not observed in a simple aqueous solution nor in the presence of such hydrophilic powder as silica gel.

The rate of degradation of indomethacin was observed to be greater

in the presence of carbon black than in its absence. The results obtained from solutions of different pH and temperature showed, as follows, that the R_f values of thin-layer chromatography of the degradation products were coincident to both cases of the presence of carbon black at 30°C and its absence at 60°C(87).

Degradation Products of Indomethacin Detected by TLC

Present Experimental Data			Existing Data			
Symbol	R_f values at pH 6.3[a]	R_f values at pH 8.7[a]	Symbol	Compound	λ_{max}[b] nm	R_f[c] Values
a	0.13	-	I	1-(p-chlorobenzoyl)-5-hydroxy-2-methyl-indole-3-acetic acid	265	0.15
b		0.22				
c	0.28					
d	0.36	0.38	II	5-methoxy-2-methyl-indole-3-acetic acid	280	0.37
e	0.57	0.57	III	1-(p-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid	265	0.57
f	0.64		IV	5-methoxy-2,3-dimethylindole		0.63
g	0.69					
h		0.76				
i		0.82	V	1-(p-chlorobenzoyl)-5-methoxy-2,3-dimethyl-indole		0.8
j		0.89				

(a) Degradation products at the respective pH values in the presence of carbon black at 30°C and in the absence of carbon black at 60°C
(b) from reference 102
(c) from references 84,103

In many cases, the treatment of infants and small children require that the physicians prescribe drugs to be administered in liquid dosage forms. Liquid dosage forms also may be required for adults for administration through nasogastric tubes. A number of these drugs are not available commercially in liquid dosage forms and mostly because of stability problems. Das Gupta et al.(104) studied the stability of liquid dosage forms for a number of drugs including indomethacin prepared in a suitable vehicle and required to be dispensed in quantities sufficient to last for periods of 90 days or more. Indomethacin liquid dosage form was prepared from indomethacin powder to contain 2 mg/ml in a vehicle consisting of simple syrup with 10% alcohol and has a pH of 5.2. It was noticed that the liquid dosage forms studied had limited stability and the amount remained of indomethacin after 28 and 224 days were 98.7 and 94.7% with the final pH being 5.5.

Rowe and Carless(105) prepared indomethacin microcapsules using the gelatin-acacia coacervation technique described by Nixon and Nouh(106). Furthermore, they studied the recovery of indomethacin from the prepared product. In the presence of antacid, hydrotalcite (Roussel Laboratories U.K.) the extraction of indomethacin from the microcapsules with 70% aqueous methanol resulted in a 50% recovery which was due to degradation of the indomethacin core. Whereas complete recovery of unencapsulated indomethacin in the presence of antacid was observed when subjected to the same analytical procedure. The breakdown products were identified as p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid which were identical to those following the decomposition of indomethacin in aqueous sodium hydroxide. Methyl p-chlorobenzoate was

identified as a third product in the degradation process. Further formation of this compound did not affect 5-methoxy-2-methylindole-3-acetic acid produced whereas the amount of the p-chlorobenzoic acid detected decreases as the amount of its methyl ester increases. The formation of this product was attributed to the presence of methanol and the esterification preferentially occurred on p-chlorobenzoic acid due to its more ionizable acid proton(105). It was concluded that the capsule wall had a catalytic effect in causing the decomposition of indomethacin core in the presence of moisture during the assay procedure. An alternative method was developed whereby the antacid was removed prior to analysis by adding dilute hydrochloric acid to the microcapsules. The excess acid was then decanted and the product was washed with distilled water before extraction with methanol. Subsequent esterification of p-chlorobenzoic acid during this procedure was attributed to remaining traces of the acid(105).

Hassan and Shaaban(107) devised an ultraviolet spectrometric method for the assay of indomethacin in capsules and suppositories. The method is based on the spectral changes of indomethacin due to changes in the pH of the solvent. Statistical comparison of the results obtained with those of the British Pharmacopoeia method(108) revealed that the results of the official method are 1.44 ± 2.1% higher than those of the proposed method and this was attributed to the interferences from excipients in these preparations.

High pressure liquid chromatography was used by Kwong et al.(109) to determine quantitatively indomethacin and its impurities in suppository and capsule formulations. Small quantities of p-chlorobenzoic acid (about 0.05%) were only detected in the capsule

ormulation. The degradation of indomethacin and its interaction with glycerine used in a suppository base was reported by Curran et al.(110) and is represented in Scheme (1.2). Kwong et al.(109) found that indomethacin impurities in the suppository formulations form a total of 1.4% of the amount of indomethacin included.

Scheme (1.2) The interaction of indomethacin with glycerin used in a suppository base(110).

The impurities detected in the suppository formulation were p-chlorobenzoic acid (II), 5-methoxy-2-methylindole-3-acetic acid (III), p-chlorobenzoic acid-α-monoglyceride (IV) and indomethacin-α-monoglyceride (V) (Scheme 1.2). The latter two impurities were a result of interaction of indomethacin and p-chlorobenzoic acid with glycerin used in the suppository base.

Polyethylene glycols have been found to be a suitable bases for the preparation of indomethacin suppositories(111). But recently Kahela et al.(112) reported that indomethacin is unstable in these bases and a limited shelf life was found for such a dosage form. Ekman et al.(113) studied the stability of indomethacin suppositories in a number of commercial batches. The suppository base was made of a mixture of

polyethylene glycol 300, 1500 and 6000. Analysis of the samples showed by TLC the presence of 7-8 different compounds. Data collected from mass spectrometry showed that the compounds have molecular weights differing from each other by 44 mass units corresponding to the repetitive ethylene oxide units in polyethylene glycols. In addition the mass spectra exhibited typical fragments of indomethacin which together with the molecular weights, suggested the compounds to be polyethylene glycol esters of indomethacin and they have the general structural formula:

where n= the number of ethylene oxide units.

Confirmation was done by the synthesis of the triethylene glycol ester of indomethacin and its agreement with the mass spectrum, TLC and gas chromatograms of the low-molecular weight component (n=3) separated from the sample.

The quantitative analysis of the suppositories showed that approximately 2, 3.5 and 4.5% of the original amount of indomethacin was esterified with polyethylene glycol 300 after storage times of 1, 2 and 3 years respectively. No significant amounts of indomethacin esterified with the other 2 suppository base components were observed. This was attributed to the high concentration of hydroxyl groups in polyethylene glycol 300 compared to the high-molecular weight polyethylene glycols. The effect observed by incorporating these suppository bases resulted in

decreasing the amount of indomethacin during storage, thus shortening the shelf life of the formulation(113).

The stability of gel creams containing flurbiprofen was followed. No change in the composition was observed after 90 days storage at various temperatures(64). Pimeprofen stored in an amber container was stable at room temperature for 36 months. The discolouration observed on exposure to light or over 40°C was avoided by keeping it in an amber container(114). In aqueous solutions pimeprofen was stable at pH 4-7 while it hydrolysed in alkaline or strong acidic conditions. The degradation proceeded in a pseudo first-order reaction(114).

1.5 ASSAY METHODS FOR INDOMETHACIN AND IBUPROFEN

Indomethacin and ibuprofen are well known non-steroidal anti-inflammatory, antipyretic and analgesic agents(115-119). Both agents are used separately to relieve the painful symptoms of osteoarthritis and rheumatoid arthritis (120,121). Indomethacin in combination with salicylate is frequently used in the treatment of the latter(122). The increasing use of these two compounds in medicine has resulted in the development of several pharmaceutical formulations. In addition, monitoring the stability of these formulations and the detection of any degradation products required the development of analytical methods (7,104,105,107,109,113,121,123-125).

The pharmacokinetic investigations following the administration of these compounds clinically have shown a wide range of plasma levels encountered during their therapy which indicate the need for clinical determination of plasma levels which may allow maximum theraputic effect

while minimizing adverse effects(136). This could be achieved by developing a more specific and sensitive method of analysis. Numerous approaches have been described for the determination of ibuprofen and indomethacin and among these are the following:

1.5.1 Titrimetric Methods

Indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid] and ibuprofen [(RS)-2-(4-isobutylphenyl)propionic acid] are weak organic acids. Several titrimetric methods both aqueous and non-aqueous have been developed to utilize this property. The non-aqueous ones directly titrate the acids dissolved in a suitable organic solvent with a standard solution of sodium hydroxide or sodium methoxide in the presence of a suitable indicator(137-139).

Other methods have dissolved the acids in 97% DMSO - 3% water ratio in order to extend the equivalence segment of the titration curve by several pH units when titrating the non-ionized acids with weak bases(140).

1.5.2 Spectrometric Methods

A. Colourimetry

Treatment of indomethacin with certain reagents will produce a colour reaction which permits its determination in dosage forms using a visible spectrophotometric detector. The colour complex formed by treating indomethacin with a solution of ferric chloride acidified with hydrochloric acid could be extracted with butanol and then determined

quantitatively by reading the absorbance due to the colour formed at a λ_{max} of 546 nm. The specificity of this reaction allowed the determination of indomethacin without the interference of the excipients in the capsules(123). Heating indomethacin in alkaline solution with certain reagents, including p-dimethylaminobenzaldehyde and p-toluene sulfonic acid, results in a coloured product which can be measured quantitatively in the visible region at 545 nm(124). A fast qualitative spot colour test for some drugs of abuse including indomethacin has been developed which utilizes a thin-layer chromatographic method(141).

B. Fluorimetry

Although indomethacin (I) does not exhibit native fluorescence, exposure to dilute alkali converts it to its deacylated product 5-methoxy-2-methylindole-3-acetic acid (II) (Fig. 1.13). This conversion forms the basis of a fluorimetric method for the estimation of indomethacin in biological fluids(7,125,135,142).

This includes the extraction of indomethacin from plasma or serum with a solvent consisting of isoamyl alcohol in heptane. Dilute alkali is then added to the extract to complete the conversion to the 5-methoxy-2-methylindole-3-acetic acid derivative and fluorimetry was performed (activation about 295-300 nm, fluorescence about 375-385 nm) against suitable standard concentration. The method was claimed to be accurate and precise and was not difficult to perform provided care was paid to details such as traces of alkali remaining from routine laboratory washing procedure. Additionally, indomethacin is light-sensitive, so that prolonged exposure to daylight or delay in taking readings in the spectrofluorimeter must be avoided. In contrast other

KEY	NAME
I	indomethacin
II	5-methoxy-2-methylindole-3-acetic acid (MIAA)
III	p-chlorobenzoic acid

Fig.1.13 The formation of 5-methoxy-2-methylindole-3-acetic acid from indomethacin in dilute alkali (135).

published papers have considered fluorimetric detection of indomethacin to be rather unspecific, time consuming and unsuitable for routine analysis(120,126,136). This was attributed to the fact that the method may also measure indomethacin metabolites in plasma and interference may arise if the patient is taking aspirin for salicylates are also extracted by this technique and fluoresce at a similar wavelength unless special precautions are taken.

C. Ultraviolet spectroscopy

Both ibuprofen and indomethacin absorb light in the ultraviolet region. Ultraviolet spectroscopic estimation of ibuprofen has not been developed as a separate method although its use as a sensitive method of detection in high pressure liquid chromatography is well documented. Indomethacin has three absorption bands in the UV region (Fig. 1.14). These are 230, 260 and 319 nm and the corresponding molar extinction coefficients in ethanol are 20 800, 16 200 and 6 290 cm^2/mole respectively(68).

Ultraviolet detection may be considered as rapid, sensitive and simple but its is affected by a number of factors including the presence of closely related compounds such as metabolites and degradative products. Interference by excipients from dosage forms or the change in the pH of the medium may also cause problems. These factors could negate the usage of UV detection for the analysis of indomethacin but taking into consideration the various causes of interference several authors have concluded that this method is suitable for indomethacin determination(84-88,93). p-Chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid (Fig. 1.13) are the hydrolytic degradative

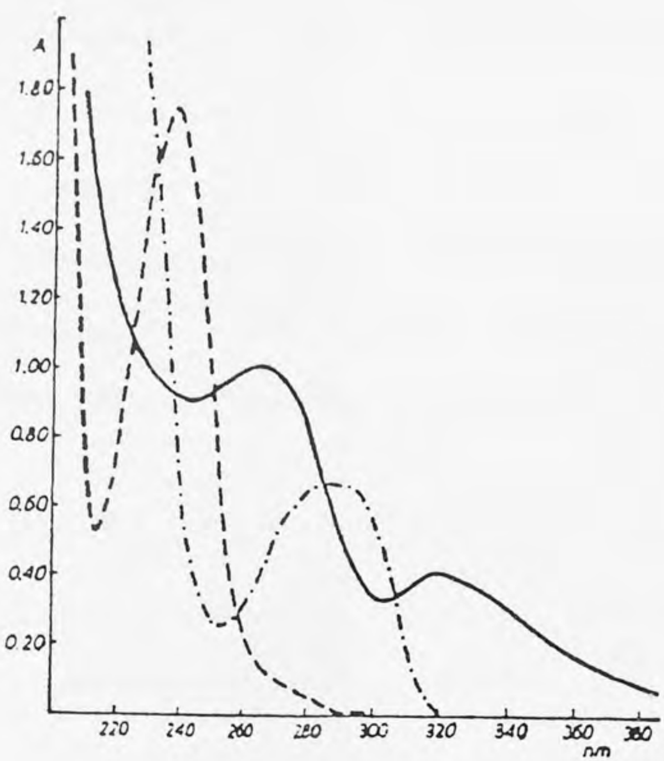


Fig.1.14. UV absorbance spectra of indomethacin and its degradation products. ——— indomethacin; —·—·— 5-methoxy-2-methylindole-3-acetic acid; - - - - p-chlorobenzoic acid. (10 % (V/v) aqueous methanolic solution, c = 20 μg/ml). (89).

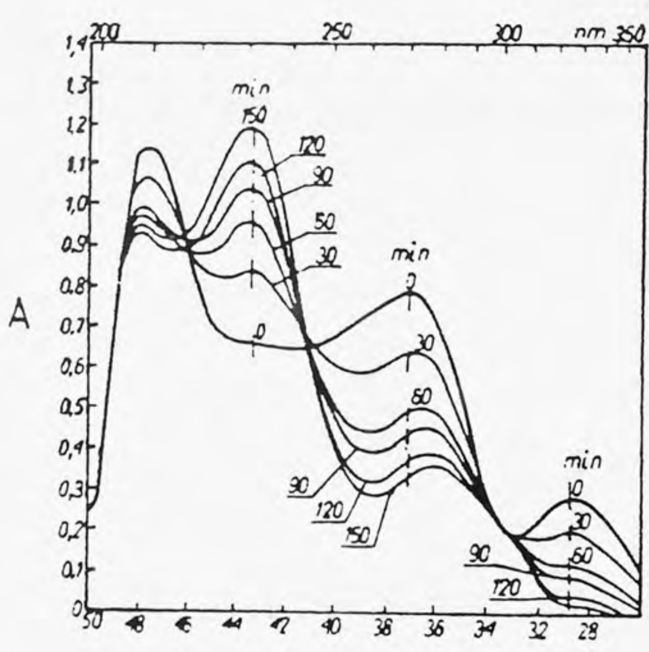


Fig. 1.15. Spectral changes of indomethacin in phosphate buffer pH 7.0 ($\mu = 0.1$) at 90°c. (86).

products of indomethacin(84,89), their ultraviolet absorption spectra are shown in Fig. 1.14 and when using direct ultraviolet spectroscopy at 320 nm these decomposition products do not interfere with the quantitation of residual indomethacin(84-88,93,143) (Fig. 1.15). Thus this method may be used to study physical properties(78) and kinetic behaviour in solutions(86,144) and in pharmaceuticals(107,145).

Others have suggested indomethacin quantitation by spectrophotometry in the presence of hydrolytic decomposition products by measuring their additive absorption at 244, 270 and 294 nm and fitting the data to mathematical equations for a three component system(146).

While on the basis of the spectral changes of indomethacin induced by changing the pH of the solvent medium (Fig. 1.16) a method for its determination in dosage forms has been developed without the interference of the excipients present. This involves absorbance measurements of both acid and alkaline solutions of the compound at 260 nm then relating differences between both values to the concentration of indomethacin(107).

1.5.3 Chromatographic methods

LIBRARY

The formulation of ibuprofen and indomethacin and their application in therapeutics has created the need for analytical methods which combine the properties of sensitivity, selectivity, reliability and specificity together with the power of resolving multicomponent mixture systems. Consideration of these demands suggests that a chromatographic technique will be most suitable. Thin-layer, paper, gas, liquid and high pressure

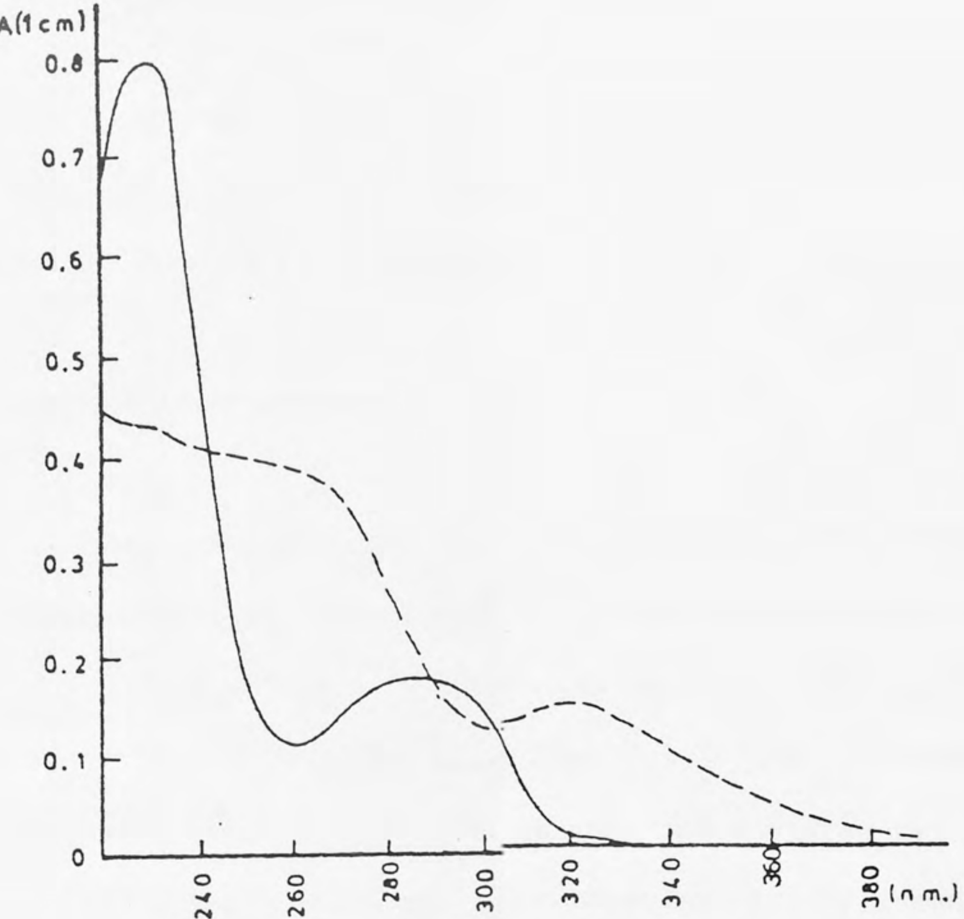


Fig. 1.16. Absorption spectra of indomethacin (0.8 mg / 100 ml) in 0.1 N hydrochloric acid in methanol-water 80 : 20 ,(– – – –) and 0.1 N sodium hydroxide , (———) (107).

liquid chromatography have been used in the assay of ibuprofen and indomethacin but although each method is capable of resolving and quantifying mixtures to a certain extent, each has its own limitation and some could only be used as complementary to others.

A. Paper and thin-layer chromatography

Several articles have reported these methods as a rapid screening procedure for the tentative identification of some non-steroidal anti-inflammatory agents(129,147-149). Both adsorption and partition phenomena may be utilized in the analytical resolution of mixtures. These methods are able to simultaneously separate the mixtures using the proper developing system, then the chromatograms are scanned for ultraviolet absorbance(147); fluorescence(148) or for radioactivity(103) to enable quantitative estimation to be made. Evaluating these methods shows their valuable resolving power but with the limitation in quanitifying the results without the resort to more complex and tedious techniques(121). Paper and thin-layer chromatography have been used for the separation and identification of the hydrolytic degradative products of indomethacin(84,86,89) and the study of indomethacin and ibuprofen plasma and urinary metabolites and conjugates(103,128), in addition they are used in the routine analysis of plasma and urine(127).

Krasowska(89) reported that p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid are the products of both the alkaline hydrolysis and the in vitro enzymatic decomposition of indomethacin(105), while the in vivo metabolic products additionally included 1-(p-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid. These metabolites are present in significant amounts in plasma but are

devoid of anti-inflammatory activity(150). Separation was by means c thin-layer chromatographic method although the actual analysis solutions was spectrophotofluorimetric using the formation of fluorescent indole derivatives of indomethacin(89). This was despite the fact that 1-(p-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid and 5-methoxy-2-methylindole-3-acetic acid may interfer due to fluorescence in the same region(143). Other workers have reported that p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid are the acid degradation products of indomethacin(86). This contradicts earlier reports that additionally other products were separated by thin-layer chromatography including 5-methoxy-2,3-dimethylindole and 1-p-chlorobenzoyl-5-methoxy-2,3-dimethylindole(84). Attempts to increase the efficiency of these methods have used:

1. radioactive compounds during the study to enable spot detection quantitatively using a radioactive counter(103).

2. a densitometer to ensure spot colour density under ultraviolet or fluorescent light when used on specially treated silica gel plates(103,127,128,148).

3. the production of colours by the introduction of specific reactions to enable quantification through the application of fluorogenic reagents(148).

Mills(128) reported an extensive study of aspects of the metabolism of ibuprofen in several animal species. Ibuprofen and its two major metabolites are shown in Fig. 1.17.

Ibuprofen

Metabolite A

Metabolite B

Fig. 1.17. Structures of ibuprofen and its major metabolites(128).

B. Gas chromatography

Gas liquid chromatography equipped with mass spectrometer or electron capture detector has been applied in the simultaneous determination of either ibuprofen or indomethacin and their metabolites or degradative products in both pharmaceuticals and in the routine analysis of biological fluids(113,128,130-132,136,151-154). Most of these procedures tried to express the sensitivity for determining minute quantities in blood or serum and being able to resolve mixtures with ease and selectivity. To establish such procedures the requirements include derivatisation, programming of temperatures, the appropriate column and the detection of the eluted materials. A pyrolysis unit attached prior to the Gas Liquid Chromatograph proved valuable in the detection of the metabolites of many anit-inflammatory drugs including ibuprofen(155,156). Although gas chromatography supplied valuable information in a study of protein binding(154), it was also able to identify the optical activity of ibuprofen metabolites from human urine(128,131). Previous informations(117) indicated that the urinary metabolites (A and B) (Fig. 1.17) from individuals receiving RS-(±)-ibuprofen to be both dextrorotatory, metabolite A, $[\alpha]_D^{20}$ +35.6 and metabolite B, $[\alpha]_D^{20}$ +38.2 when extracted from the unconjugated fraction of the urine. The low rotation of the metabolites from the (-)ibuprofen indicated that during metabolism of this isomer incomplete optical inversion occurred(128). Derivatisation of the drug under study was recommended to facilitate chromatography. Both ibuprofen and indomethacin lack an adequate vapour pressure to permit their direct gas liquid chromatography (GLC) at temperatures below 300°C so that preparation of esters by alkylation with diazoalkanes and other alkylating processes is required(127,128,132,157-161), although a

simpler GLC for the underivatised ibuprofen utilizing a 5% FFAP stationary phase on Gas-Chromatograph W HP, 80-100 mesh has been reported. The esters of both ibuprofen and indomethacin have been prepared successfully by alkylation with diazoalkanes (123,124,139, 140). Certain limitations have been brought by this derivatisation process include dealing with potentially hazardous(162) diazoalkanes and this step could introduce errors(126) into the quantitative analysis, i.e. the reaction of indomethacin with diazomethane converts a certain percentage of 1-(p-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid back to the parent compound(163). Another limitation is that most GLC methods have omitted an internal standard(163).

C. High-performance liquid chromatography (HPLC)

HPLC is also known as high pressure liquid chromatography. In 1941 Martin and Synge(164) developed the principles of this technique but only recently has it received the awaited advances in methodology and technique. This resulted in the design of a reliable system which made HPLC an ideal analytical method. Furthermore its sensitivity, accuracy, selectivity together with precision and speed of detection made it possible to determine low levels of drugs and metabolites in biological fluids and pharmaceuticals.

The advantage of HPLC over GLC is that it does not require prior derivatisation of the compounds under study and thus prevents the unnecessary exposure to hazardous derivatising compounds(126,162,163). The HPLC technique can produce good resolution of mixtures with appropriate mobile phases by simply injecting the solution mixture. A simple single step extraction may be needed to prepare the experimental

solutions prior to anlaysis(120,121,134,135,163,165-171). I technique both adsorption and partition chromatography can be use good deal of information aiding development of the mobile phase obtained from a simple thin-layer chromatographic sepa[illegible] Quantitation is ensured by using an internal standard and peak ratio to maximize method accuracy(172).

HPLC has been employed in the anlaysis and detection of ibuprofen and indomethacin for a variety of purposes using a reversed phase system. In pharmaceuticals it was able to follow the stability in dosage forms(104,105,109), detect and identify parachlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid as the alkaline degradation products of indomethacin and besides allowed conclusions that indomethacin degradation follows first order kinetics to be made(105). Furthermore it was able to detect that when analysing capsules and suppository formulation, impurities were found. It was observed that the α-monoglycerides of p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid, in addition to the free acids themselves(109), were formed.

In studying topical formulations HPLC was able to detect the influence of the vehicle composition on the penetration of indomethacin through guinea-pig skin(165). The simultaneous measurements of indomethacin and small quantities of its metabolites in rabbit plasma following percutaneous absorption of indomethacin from ointment bases in rabbits were also possible. The plasma metabolites obtained from the rabbit after indomethacin administration were 5-methoxy-2-methylindole-3-acetic acid and 1-(p-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid. In routine work for the analysis of blood, serum or biological

fluids HPLC has been used and several attempts were tried to i accuracy in detecting ibuprofen and indomethacin by deproteinizati thus lead to a more sensitive procedure with a lower sample volu 121,126,134,162,165-167,171). Detection of eluted material involved radioactive, ultraviolet, fluorimetric, potentiometric and electrochemical detectors. A dual ultraviolet detector has been used when detection is needed for eluted compounds that do not absorb at the same wavelength(104,171,173). Fluorimetric detection of ibuprofen in plasma was possible by setting the detector at an excitation wavelength of 253 nm and a band filter with an entrance transmission of 230-420 nm(166) while an ultraviolet detection(126) was able to detect indomethacin and salicylic acid together with their metabolites (Fig. 1.18) in serum without the interference observed in a direct fluorimetric method of indomethacin determination in the presence of salicylates(126). Indomethacin and its metabolites may also be detected by using fluorimetry. Biological fluids can be prepared by either treatment with alkali prior to injection(135) or the technique could make use of a postcolumn in-line alkaline hydrolysis of indomethacin to a fluorophore with the fluorimetric detector(162). This alkaline hydrolysis is needed as indomethacin is devoid of fluorescing activity, whereas its 1-(p-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid product has fluorescent properties. It was logical therefore to convert both compounds to their strongly fluorescing N-des-chlorobenzoyl derivatives(135).

1.6 PERCUTANEOUS ABSORPTION

The primary function of the skin is to separate and protect an organism from the external environment. As a barrier to chemical

Indomethacin

deschlorobenzoyl-indomethacin

4-chlorobenzoic acid

O-desmethyl-indomethacin

O-desmethyldeschlorobenzoyl indomethacin

salicylic acid

gentisic acid

salicyluric acid

gentisuric acid

Fig. 1.18. Metabolic pathways of indomethacin and salicylic acid. All compounds form conjugates with glucuronic acid at the -COOH or the -OH group (126).

transport, the skin is efficient, and the rate of diffusion c substances is much slower through the skin than through other bio membranes. Percutaneous transport must be considered whenever preparations are applied to the skin and Table 1.6 summarizes the absorption characteristics desired for various types of preparations(174). Penetration into the skin is undesirable when the activity of an agent is intended to be confined to the skin surface as in the case of various cosmetics and protective agents. Transdermal preparations intended for systemic action must traverse the skin to reach the vasculature, where absorption into the blood can occur. Whether percutaneous absorption is to be avoided or encouraged in a particular situation, it should not be overlooked since it is a requisite for the effectiveness of many topical preparations(175-180).

TABLE 1.6

Desired skin absorption characteristics of various types of preparations applied to the skin(174).

Type	Site of action	Desired absorption characteristics
Cosmetic	Skin surface	No skin absorption
Cleanser	Skin surface	No skin absorption
Dermatological	Various skin tissues	Absorption into skin but minimal uptake by blood vessels
Transdermal	Distant organ or tissue	Penetration through skin into blood at known, essentially constant rate

1.6.1 Bioavailability from Topically Applied Preprations

Percutaneous absorption occurs as a series of steps involving transport from one medium to another as well as diffusion within each medium. Active substances are almost always applied in the form of a finished preparation, such as a cream or lotion and the first step in permeation is thus diffusion of drug through the product to the skin surface. The partitioning into the outer skin layer, the stratum corneum, is followed by diffusion through this layer to the living cells of the epidermis, which lie beneath. By diffusion, the permeant is carried through the epidermis into the dermis where there is a high probability of absorption into the blood(181). The bioavailability of drug products placed on the skin and intended for systemic administration may be determined in the same way as for more traditional routes of delivery. Normally, blood or perhaps urine data could be used to compare bioavailability from a trnasdermal dosage form with that from a standard preparation. With nitroglyerin transdermal devices, delivery is quoted in terms of flux (gm/cm^2/sec.), and the dosage administered by these devices is adjusted by varying the area applied to the skin. For locally acting materials, the applicability of blood or urine analysis is limited since the amounts penetrating through the skin are normally quite small and below the sensitivity of analytical techniques. Therefore, it seems more appropriate to concentrate on drug amounts in the target tissues rather than those in the circulation(174). Techniques to measure skin tissue concentrations directly are poorly developed and the measurement of a pharmacological response that may be correlated with tissue concentrations is normal. Barry and Woodford measured vasoconstriction, which correlates with steroid anti-inflammatory activity, in studies of topical bioavailability(182-184).

Most quantitative information on percutaneous absorption has obtained from *in vitro* experiments which permits the applicati models and the calculation of parameters which can be used to compare absorption from different vehicles(185).

1.6.2 Structure of the Skin as a Permeability Barrier

The skin is one of the most heterogeneous organs of the body (Table 1.7) comprising a series of cell layers penetrated by longitudinal structures, the hair shafts and gland ducts(176,186,187) (Fig. 1.19). The barrier to diffusion resides in the stratum corneum which is the epidermal outer layer. The epidermis contains no blood vessels, and nutrients and waste products must diffuse across the dermal-epidermal

TABLE 1.7

Skin, some facts and figures(176).

An average human:	
Skin surface area	18,000 square cm.
Skin weight	9 kg
Skin weight excluding fat	3.6 kg
An average square cm of skin contains:	3 blood vessels
	10 hairs
	12 nerves
	15 sebaceous glands
	100 sweat glands
	92 cm of blood vessels
	360 cm of nerves
	3,000,000 cells.

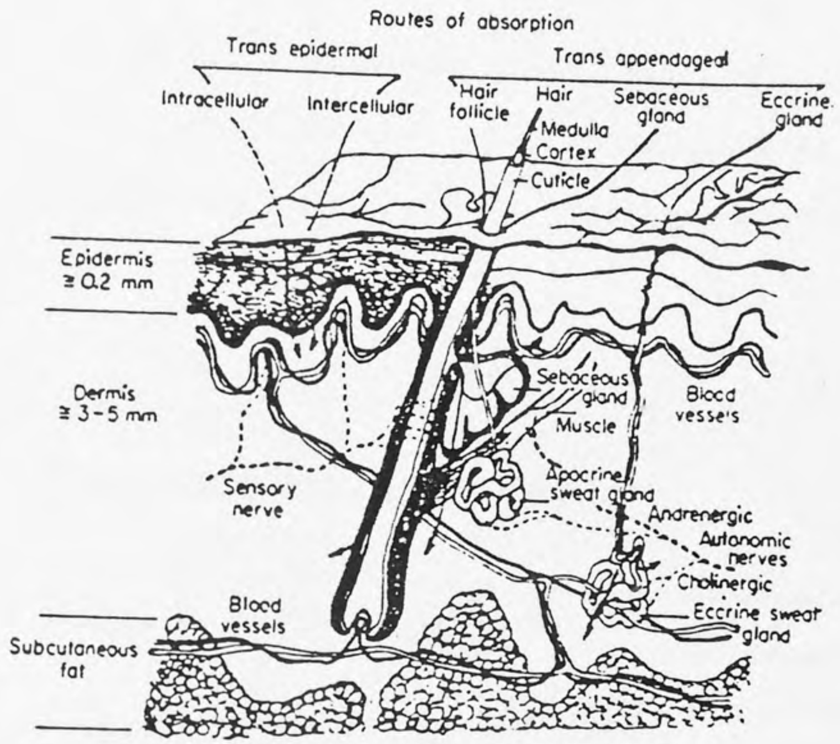


Fig. 1.19. The skin (176).

junction to maintain the viability of the epidermis. Below is dermis, which consists mostly of connective tissue containing struct fibres, blood vessels and nerve cells. This provides substance elasticity to the skin. Beneath the dermis is a layer of fatty tissue called the hypodermis or subcutaneous tissue, whose principal functions are insulation and shock absorption. None of these three layers is of uniform thickness. Some properties of the skin layers are summarized in Table 1.8(176).

TABLE 1.8

The stratified organization of human skin(176).

Layers	Epidermis	Dermis	Subcutaneous Fat
Function	Barrier	Connective tissue support	Thermal insulation, cushion
Embryonic origin	Ectoderm	Mesoderm	Mesoderm
Thickness (mm)	0.2	3-5	Variable
Thickness (ins)	1/200	1/16 - 1/8	-
pH	4.2 - 6.5	7.1 - 7.3	-
H_2O content	10 - 25%	up to 70%	Low
Cellular activity	Actively dividing cells	Mostly noncellular	Closely packed cells
Cellular contents	Keratinocytes → Keratin Melanocytes → Melanin	Fibrocytes → Collagen Fibroblasts, Histiocytes. Mastcells	Lipocytes → Lipids -
Vasculature	None	Blood vessels, Lymphatics, Sweat glands	Blood vessels

Among the various types of cells in the epidermis, those making up the stratum corneum form the actual physical barrier to most substances

that come in contact with the skin. Evidence for this is compromising of the skin barrier caused by damage to the stratum co or its removal from the skin with adhesive tape(188). Stripped s of stratum corneum have about the same resistance to permeability as the entire skin(189). This implies that transport through the dermis and through the living layers of the epidermis is much faster than through the stratum corneum. The stratum corneum consists of stratified, compressed, dead, dehydrated, keratinized cells held together by condensed protein-lipid structures. This layer is considered a two-phase system, as regions rich in protein and others rich in lipid are found within. Although relatively thin, the density and chemical makeup of this layer provide it with its barrier properties(175) (Table 1.9). In most instances, the percutaneous absorption rate depends on the transport rate through the stratum corneum.

TABLE 1.9

Composition of stratum corneum(175).

Tissue Component	Gross Chemical Characterisation	Percent	Ref
Cell membranes	lipid and nonfibrous protein	5	190,191
Intercellular material	lipid and nonfibrous protein	10	192
Cell contents	lipid, 2 α-protein, 5 β-protein, 2 nonfibrous protein, 1	85	193,194

Numbers after cellular components give a rough index of their relative proportion.

An outline of the structural and metabolic changes that accompany development of the stratum corneum is given in Table 1.10(174) and Fig. 1.20(187).

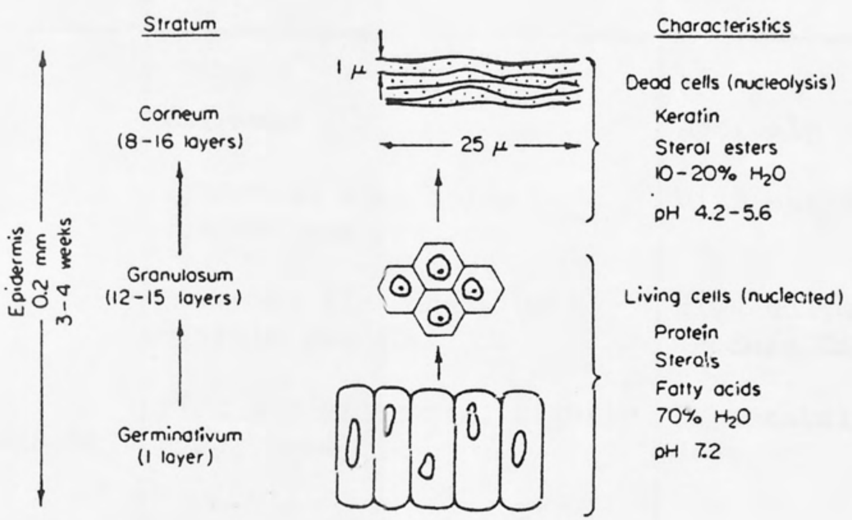


Fig. 1.20. Cell changes in the epidermis (187).

TABLE 1.10

Cells of the epidermal barrier(174).

Cell Type	Physical Description	Metabolic Description
Basal	Columnar	Actively reproducing
Prickle	Spherical with spiny projections	High enzymatic activity
Granular	Somewhat flattened, with visible granules	High enzymatic activity, produce Keratin
Horny (stratum corneum)	Flat and elongated, tightly compressed	No metabolism; cells are dead

The stratum corneum is capable of absorbing large quantities of water - upto 5 or 6 times the weight of the dry tissue(175). Small, lipophilic molecules may penetrate the stratum corneum relatively rapidly. The water sorption isotherm of stratum corneum is similar to that of other proteinaceous assemblies such as wool. If water solubility is very poor molecules may tend to remain in the stratum corneum(181). In such cases, transport across the living aqueous layers below the stratum corneum may represent the rate limiting step in the absorption process.

1.6.3 Penetration Pathways Through Skin

Several possible routes of drug transport through the skin after application can be postulated.

Fissured, broken or injured skin offers little resistance to absorption(187), but the unbroken skin, which fulfils the vital barrier

role in preventing loss of tissue fluids and components while preven the entry of external agents, presents the routes for percutar absorption as recorded in Table 1.11 and represented diagrammatical Fig. 1.21(174). The data in Table 1.11 are compiled from several literature sources(195,196). The transcellular pathway in which the drug travels through cells, is the shortest and also the most likely given the relative area presented to a diffusing molecule. The intercellular pathway avoids diffusion through cell contents, but is substantially more tortuous. The transfollicular route involves migration through the hair shaft openings, which are probably filled with sebum. This route offers substantially lower diffusional resistance to most drugs than do the other routes, but the path length is quite long and the density of hair follicles in human skin is quite low.

The stratum corneum is probably the principal rate-limiting barrier to penetration and also the principal route for the passive transepidermal diffusion and absorption of most drugs(197). The hydrated stratum corneum is a dense, effectively homogeneous phase into which small molecular weight, polar, nonelectrolytes dissolve with strong chemical interaction and through which diffusion occurs remarkably slowly. The resistance of the stratum corneum to diffusion of large molecules such as the steroids and particularly the more polar corticosteroids is so high as to make transepidermal diffusion an unlikely route for their penetration. Such compounds probably utilize the pilosebaceous apparatus as both their principal initial and continuing route for penetration. Most drugs ultimately move on through the epidermis to the dermis, where the clearance of the drug and its metabolites is quite rapid(198,199). Small amounts of some drugs,

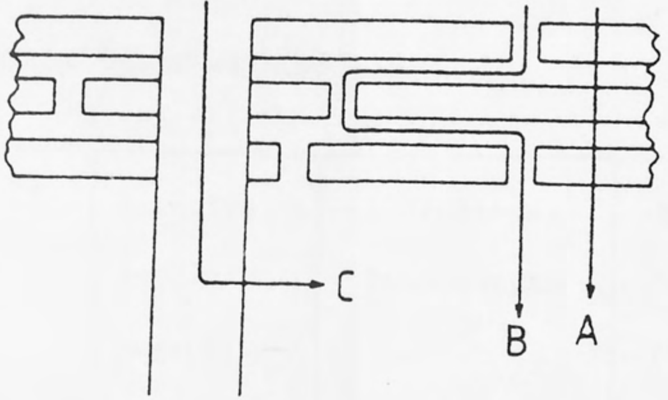


Fig. 1.21. Diagrammatic representation of pathways through stratum corneum (174):

A) transcellular

B) intercellular

C) transfollicular

however, are bound in the epidermis and form a reservoir or depot wh may provide prolonged therapy(187).

TABLE 1.11

Pathways through human skin(195,196)

Route	Relative Surface Area(%)	Diffusional path length (μm)	Relative Volume of Stratum Corneum (%)
Transcellular	99.0	25	90 - 99
Intercellular	0.7	350	1 - 10
Transfollicular	0.1	200	0.1

1.6.4 Methods and Procedures for Measuring and Analysing Skin Permeability

A. Experimental Technqiues

A variety of techniques have been used to measure skin penetration in vivo and in vitro. Many of the methods used to assess percutaneous absorption have been summarised by Stoughton(200) and also reviewed by Tregear(188) and by Ainsworth(201). These are listed in Table 1.12 (174), together with a single reference for each approach(133,182,202-205).

Human skin and skin from various animal species have been used in the in vitro experiments. Despite the differences between excised skin

and human skin in vivo, it seems reasonable to suppose that, because stratum corneum is composed of non-viable cells, and represents major barrier, in vitro and in vivo correlation might be expected in many cases. In vitro studies are convenient to perform and enable quantitative interpretation permitting insight into the mechanisms that may affect transport.

TABLE 1.12

List of techniques employed in studying percutaneous absorption(174)

Category	Type of Measurement	Sample Study (Reference)
In vivo	Blood concentration	133
	Urinary excretion	202
	Disappearance from skin surface	203
	Systemic pharmacological activity	204
	Local pharmacological activity	182
In vitro	Diffusion through excised whole skin or skin layer	195

B. Mathematical Relations

The skin barrier, as indicated in Fig. 1.8, can be regarded as a composite membrane, pierced over a small portion of its area by shunts of different but lower diffusivities(175). The stratum corneum is not simply an inert structural material but one with an affinity for the applied solute.

The steady state permeation through a rate limiting membrane under sink conditions(181) is modelled by:

$$J = DKC/h \tag{1.8}$$

In this equation, J is the steady-state flux (amount per unit area per unit time crossing the membrane), D is the diffusion coefficient within the membrane, K is the membrane/vehicle partition coefficient, h is the membrane thickness and C is the concentration of permeant dissolved in the vehicle. The term DK/h is called the permeability constant. This equation is often applied to in vitro experiments in which excised skin is utilized. If transport across the stratum corneum is rate limiting then the parameters in the equation must refer to stratum corneum and not the whole skin. The same type of relation would apply to transport from a device that contains its own rate controlling membrane.

In the case when the drug diffusion through the vehicle is the rate limiting step in percutaneous absorption, the overall penetration rate is equivalent to the rate of drug release. This situation is most likely to be observed with suspensions of relatively insoluble drugs in viscous media(181). The overall process was expressed by an equation derived by Higuchi(206):

$$\frac{dQ}{dt} = \left(\frac{C_v D_v C_s}{2t}\right)^{\frac{1}{2}} \tag{1.9}$$

where dQ/dt is the rate of drug released per unit area of surface exposure, C_s is the solubility of the drug in the vehicle, which is assumed to be small in comparison with the total drug concentration, C_v

(units/cm^3), and D_v is the diffusion coefficient for the drug in th vehicle.

1.7 PHYSIOLOGICAL FACTORS IN PERCUTANEOUS ABSORPTION

The various factors that determine skin absorption kinetics can be divided into three groups for convenience: physiological; drug and vehicle variables. Physiological factors are those that involve the properties of the barrier itself(174). It is perhaps obvious that the stratum corneum must be entire and properly formed in order to fulfil its barrier function. Agents which cause physical damage or otherwise compromise this membrane have a significant effect on percutaneous absorption. Certain environmental conditions also affect the diffusional resistance of the stratum corneum(181). Other factors include, the site and the patient's age.

1.7.1 Skin Integrity

An intact stratum corneum is required to keep foreign chemicals out of the body and needed physiological substances (including water) inside. Abrasions and cuts are short circuits for transport. Penetration rates may be increased by orders of magnitude by damage to the barrier layer(205,207). Skin diseases in which the stratum corneum is imperfectly formed also cause an increase in percutaneous absorption rate.

1.7.2 Hydration

The stratum corneum is a complex membrane that has a high affinity

or water. Although the skin surface is not easily wettable the stratum corneum is capable of taking up significant quantities of water vapour and can swell to several times its own weight when immersed in liquid water(195). In the natural condition, the water content of the stratum corneum depends on the environmental relative humidity and whether the area in question is covered or exposed. Typical water contents at 31°C are 0.2 and 0.7 g of water per gram of dry tissue at values of relative humidity of 40% and 90% respectively(175).

In general, an increase in stratum corneum hydration results in an increase in the penetration rate across skin, and the changes can be dramatic(208) (Table 1.13). A number of examples which appear in the literature have been reviewed by Idson(179). The degree of penetration enhancement by hydration varies from one permeant to another.

TABLE 1.13

Effect of hydration on percutaneous absorption of some salicylates(208)

Permeant	Absorption rate (moles/100 cm^2/hr)		
	Hydrated stratum corneum	Unhydrated stratum corneum	Hydrated/ unhydrated
Glycol salicylate	11.7	1.3	9.0
Methyl salicylate	8.6	2.7	3.2
Ethyl salicylate	2.9	1.5	2.0

1.7.3 Temperature

Raising the skin temperature results in an increase in the skin penetration rate. An activation energy can be calculated from the slope of a plot of the permeability constant against the reciprocal of absolute temperature(209). Under practical conditions, the temperature of the skin does not vary more than a few degrees. Application of a lotion or ointment with rubbing might raise the local skin temperature enough to increase penetration somewhat.

1.7.4 Anatomic Location

Feldmann and Maibach(210) reported differences in the absorption of hydrocortisone at different body sites using the same group of subjects (Table 1.14) and all of the locations on the head were more permeable than most areas on the rest of the body.

TABLE 1.14

Effect of anatomic region of application on percutaneous absorption of hydrocortisone in human(210)

Region	Ratio relative to ventral forearm
Forearm (ventral)	1
Ankle	0.14
Palm	0.83
Back	1.7
Forehead	6.0

1.8 DRUG FACTORS IN PERCUTANEOUS ABSORPTION

The vehicle may be rate controlling if the permeant is very poorly soluble or if diffusion within the vehicle is very slow. In the skin transport process, an account of the necessity for molecular transfer from one region to another and of diffusion within each region, should be made.

1.8.1 Molecular Size and Diffusivity

Although the compact structure of the stratum corneum helps to explain its effectiveness as a barrier to diffusion, its chemical heterogeneity undoubtedly plays an important role. Within the stratum corneum are hydrophilic and lipophilic regions that help to restrict movement of a drug or other foreign substances. Few reports have correlated the permeability coefficients with the size of penetrating molecules and an inverse relationship appears to exist between absorption rate and molecular weight(188,197,211-214). Small molecules penetrate more rapidly than large molecules(179,180), but within a narrow range of molecular size, there is little correlation between size and penetration rate(179).

For substances classed as small molecules, the permeability was not strongly related to molecular weight(174). A study of steroid permeation through human epidermis indicated that the chemical nature of the permeant does influence diffusivity(215). The steroids were of similar molecular size, but their diffusion coefficients, calculated from the steady-state permeability constants, varied by as much as 180-

fold. The calculated diffusion coefficients were higher for those steroids containing a relatively large number of polar groups(215). The skin penetration of high molecular weight materials such as albumin was found to be slower than that for the lower molecular weight dextran(216).

1.8.2 Partition Coefficient

Most studies relating partitioning and permeation have utilized homologous or other groups of compounds with similar chemical features. In such cases, it has generally been found that the permeability coefficient is dependent upon the partition coefficient (Table 1.15)(217).

TABLE 1.15

Parameters for penetration of some alkanols through excised human epidermis from aqueous solution(217)

Solute	$K_p \times 10^3$ (cm hr.$^{-1}$)	$D \times 10^9$ (cm^{2}sec.$^{-1}$)	K_m
Ethanol	0.8	0.66	0.9
Butanol	2.5	0.74	2.5
Hexanol	13.0	0.96	10.0
Octanol	52.0	0.77	50.0

For a homologous series of short chain alcohols applied to hydrated

skin in aqueous solution, both partitioning and permeability increased as the hydrocarbon chain was lengthened (Fig. 1.22)(175). With octanol, a relatively lipophilic material, the resistance to penetration of the stratum corneum decreased to the point that the aqueous living tissues constituted a barrier of about equal magnitude to permeation of this compound. Diffusion coefficients were estimated from permeation and sorption measurements. Values for the alcohols did not differ greatly from each other. However, this was not the case for a series of steroids where it was found that the more polar compounds had smaller diffusion coefficients(175). This was attributed to greater interaction of compounds with multiple polar groups within the stratum corneum.

1.8.3 Skin Binding

It is possible for drug molecules to bind to sites within the different regions of the skin. The term 'binding' refers to uptake of free drug molecules by skin macromolecules or surfaces. Although binding may be reversible, bound molecules are not free to diffuse. Partitioning represents a process analogous to solution in a skin tissue, and dissolved molecules can be transported. Uptake by skin tissues can be due to binding, to partitioning, or to a combination of both. Early sorption studies(175) suggested that the uptake of solutes by skin was a linear function of solute concentration. Other reports have indicated that the uptake by human epidermis follows a more complex relation in which the sorption process involved the coexistence of dissolved and mobile sorbed molecules in equilibrium with site bound and immobile molecules within the membrane. This dual sorption model was used successfully to correlate and predict the nonlinear sorption isotherm of human stratum corneum for scopolamine base from aqueous

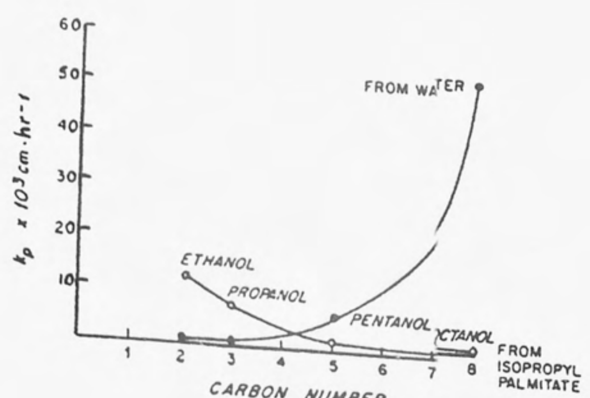


Fig. 1.22. Effect of solvent (vehicle) on permeability constant of the alcohols (175).

solutions. The model had been extensively utilized to explain the equilibrium sorption data for gases in polymers(218-221). The model postulates that sorption occurs by two mechanisms, the first mechanism being a simple dissolution producing mobile and freely diffusible molecules and the second being an adsorption process producing nonmobile molecules which do not participate in the diffusion process(222).

The total concentration of drug in the skin is assumed to be composed of two parts:

$$C_T = C_D + C_I \tag{1.10}$$

The mobile solute concentration C_D can be adequately expressed as:

$$C_D = K\ C \tag{1.11}$$

where K is the partition coefficient and C is the concentration. On the otherhand, the concentration of immobilized solute C_I was represented by an adsorption isotherm of the Langmuir form:

$$C_I = \frac{C_I^o\ bC}{1 + bC} \tag{1.12}$$

where C_I^o and b are Langmuir's isotherm constants.

Assuming that exchange between mobile and immobile species is rapid compared with the diffusion process and thereafter equilibrium exist between the two species. The steady state diffusion coefficient, D_{ss} can be written as(222):

$$D_{ss} = D \ / \ [1 + \frac{C_I^o \ b/K}{(1 + C_D \ b/K)^2}] \qquad (1.13)$$

where D is the diffusion coefficient of the solute.

A practical consequence of binding is the lag period occurring after application of the drug to the skin before theraputic amounts of the drug are absorbed(181).

1.8.4 Metabolism

Although the stratum corneum is dead, the underlying cell layers are metabolically active. Drugs that cross the stratum corneum may be metabolised before reaching target areas. This could result in some loss of drug activity. Alternatively, a prodrug might be changed into an active molecule by this process. Theoretical descriptions of metabolism combined with skin permeation have been published(223,224).

1.8.5 Thermodynamic Activity of Drug in Donor

Equation (1.8) states that permeation rate is proportional to permeant concentration. This is true for situations in which the permeant is in solution in the vehicle. Once the concentration exceeds the saturation level, there should be no further change in permeation. Excess permeant functions as a reservoir, maintaining a constant concentration over a prolonged period of time. Figure 1.23 shows the flux of benzocaine through hairless mouse skin as a function of its concentration in propylene glycol. Concentration is expressed in relation to solubility, 146 mg/ml(181). Solute association marked nonideality or alteration of skin barrier properties by the permeant

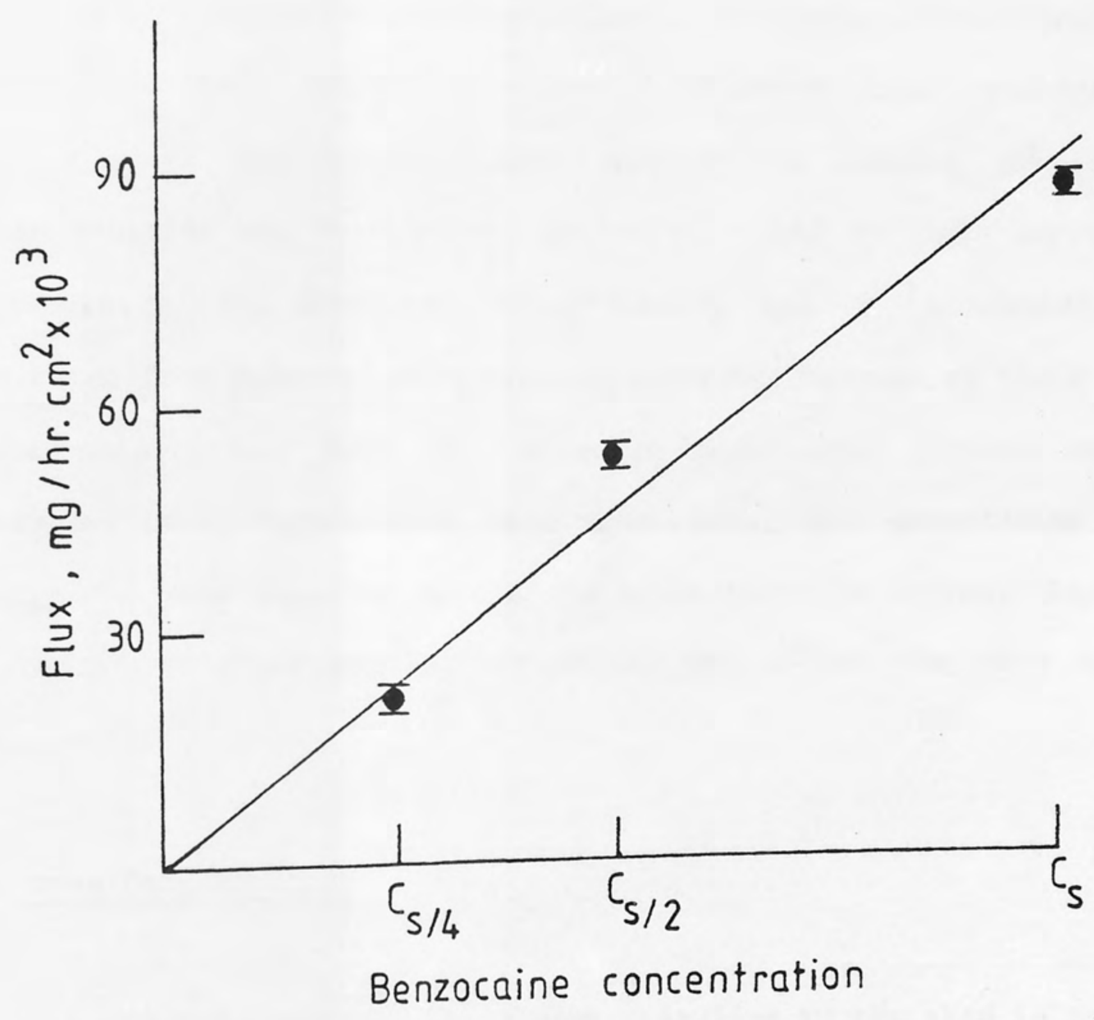


Fig. 1.23. Effect of benzocaine concentration on mean values of penetration flux through hairless mouse skin from propylene glycol solutions (181).

Bars indicate standard deviations.

would lead to nonlinearity.

1.9 FORMULATION FACTORS IN PERCUTANEOUS ABSORPTION

1.9.1 Occlusivity

Occlusive coverings increase the skin penetration rate by increasing both hydration and temperature. The increase in hydration is probably the more important effect. Plastic film coverings are regularly used in corticosteroid therapy to enhance penetration. Certain vehicles may also affect hydration. Some ointment bases, such as petrolatum, are known to be occlusive, and it is possible that penetration from bases of this type is increased because of their effect on the skin(181). Some of the newer transdermal devices such as Transderm-V (ALZA Corporation, Pólo Alto, C.A., USA) scopolamine system in which the rate limiting barrier is built into the device, changes in skin hydration after application should not affect the rate of drug absorption(174).

1.9.2 Drug Concentration

Active substances are nearly always applied to the skin in the form of a solution or dispersion. Many studies have confirmed that changes in the nature of the vehicle can profoundly influence percutaneous absorption. If the permeant is in solution, transport through the stratum corneum is likely to be rate limiting since under steady-state conditions, penetration flux is expected to be proportional to drug concentration according to equation (1.8).

Undissolved drug does not contribute to the transmembrane gradient and flux should therefore increase linearly with concentration and remain constant above the saturation concentration(174,181).

1.9.3 pH

Although the stratum corneum is a tough, chemically resistant material, it can be degraded chemically by solutions with extreme pH values. However, aqueous solutions with a pH range of 4-9(181) do not cause any measurable damage. Within this range, variation in pH can affect percutaneous absorption largely by changing the degree of ionization of a permeating compound.

In general, the unionized form of a drug will penetrate the skin more rapidly from aqueous solution than the ionized form. This is due, in part, to the difference in solubility and partition of the two species. But when saturated solutions were compared at different pH values, the flux of unionized scopolamine was still about four times that of the ionized form(225). This observation together with the lack of direct relationship between the flux and partition coefficient suggests a difference in the diffusional transfer between the two forms(225).

The relative permeability of ionized to unionized species of several drugs were reviewed recently(174). Values depended on the particular drug entity, but were less than unity in all cases. Nevertheless, some of the ionized compounds exhibited significant permeability. An example is indomethacin, reported by Inagi et al.(165). At low values of pH, at which the drug was substantially in

the unionized form, the flux value was maximal. Increasing the pH increased the fraction of ionized indomethacin and resulted in a decrease in flux. However, at the higher pH values (about pH 6.0), the flux of indomethacin was higher than expected based on permeation of unionized drug only. The flux of the unionized drug was less than twice that of the ionized species(165).

1.9.4 Solvent

There are a variety of mechanisms by which solvents can affect percutaneous absorption. A change in solvent is accompanied by an alteration of the degree of affinity of the solvent for the permeant. If affinity for the solvent is high, the permeant will tend to remain in the vehicle rather than partitioning into the stratum corneum. Alternatively, a vehicle with a smaller affinity will tend to move a permeant out of the vehicle, into the skin. This is a general solvent effect which must be taken into consideration whenever any comparison of permeation from different solvents is made.

In addition, solvents can exert more specific interactive effects, certain solvents are themselves efficient penetrants of skin. They can perhaps affect the stratum corneum and hereby influence the partitioning behaviour of drugs or cosmetic ingredients. Some solvent materials may alter the hydration of the stratum corneum. It has been suggested that osmotic gradients are responsible for the penetration enhancing effect of dimethyl sulfoxide(226). Other vehicles may cause partial dehydration to the stratum corneum, thereby reducing permeation rate. Finally, it is known that particular solvent materials (chloroform-methanol is an example) dissolve lipid materials from the stratum

corneum forming microspaces through which permeation can occur very rapidly. Interpretation of solvent effects on penetration is complicated because of the possibility of simultaneous solvent noninteractive and interative influences and it is not possible to measure solvent interaction with the skin by simply comparing the results of permeation experiments involving different solvents(174). Experiments are needed to characterise the many solvent materials that are used in drug, cosmetic and toiletry preparations. It would be very helpful to be able to assign a "solvent index" to each solvent that could be used to estimate its effect on percutaneous absorption, independent of the permeant involved(181).

1.10 OBJECTIVES OF THESIS

There is currently much interest in the topical formulation of drugs. Recently, topical administration of nitroglycerine ointment was shown to be effective for angina pectoris(227,228) and the slower absorption rate after topical administration resulted in a longer duration of action than the sublingual administration. Oral administration of non-steroidal anti-inflammatory drugs has been the principal route for the treatment of rheumatoid arthritis(229). Although the oral therapy is very effective, however the clinical efficacy is not long lasting(136) and side effects are easily provoked(41,44,45). Therefore, cutaneous application of non-steroidal anti-inflammatory drugs for local action against rheumatic and cutaneous inflammatory disorders could be advantageous. Topical application of Difflam a non-steroidal anti-inflammatory drug (benzydamine hydrochloride) as a 3% cream was more effective than placebo in the relief of symptoms associated with traumatic lesions(4,230). These

considerations are addressed in this thesis.

The task of undertaking the topical formulation of indomethacin and ibuprofen requires an understanding of various physicochemical parameters such as solubility, pH, pK_a and partition coefficient that affect the absorption process. These properties of ibuprofen and indomethacin will be studied in detail. Ester prodrugs may enhance the topical bioavailability of medication. The study of such derivatives will be undertaken. In particular, stability studies of indomethacin and ibuprofen esters and the factors which affect their rate of degradation will supply valuable information towards predicting the stability in the proposed formulation. Additionally, factors endogeneous to the skin such as its ability to act as an organ of drug metabolism is of interest since ibuprofen esters may be activated to the parent drug in the presence of skin esterases. An in vitro excised skin model will be used to study the percutaneous absorption process. This model, together with a chromatographic method for drug analysis, will enable the percutaneous absorption of drug mixtures to be monitored.

DEVELOPMENT OF ASSAY PROCEDURES FOR INDOMETHACIN, IBUPROFEN AND RELATED COMPOUNDS

The wide use of ibuprofen and indomethacin in clinical practice has increased the search for a reliable method for their analysis either in pharmaceuticals or in routine sample analysis of patients receiving therapy. Direct ultraviolet spectroscopy at 320 nm is used in this study for indomethacin quantitation in the presence of its degradative products. HPLC with ultraviolet detection is used for the analysis of complex mixtures of ibuprofen or indomethacin and in the presence of related compounds. The reliability and sensitivity of these assay methods are required to measure accurately a number of physical parameters such as solubility, pK_a and partition coefficient and in the study of their kinetic of degradation.

In order to investigate the structural changes introduced by using a number of ibuprofen esters on these parameters, and on the rates of degradation, HPLC proved to be versatile in separating and quantifying multicomponent mixtures.

Recently, topical formulations of indomethacin, ibuprofen and a number of their derivatives were tested for their local activity in animal models and human volunteers and the degree of their success raised much hope in overcoming the side effects displayed by the oral therapy of non-steroidal anti-inflammatory drug(59-66,133,165,231-237). The percutaneous absorption of indomethacin, ibuprofen and three of its esters are to be tested in this project using an excised skin

model and here HPLC may prove effective in following the absorption process.

2.1 INSTRUMENTATION

(a) The ultraviolet absorption readings were measured using a Pye Unicam SP6-400 Ultraviolet spectro-photometer, while the ultraviolet-absorption spectra were recorded using a Pye Unicam SP8000 Ultraviolet recording spectrophotometer.

(b) The pH of solutions was measured using a Radiometer PHM 64 Research pH meter with 3 decimal place display of pH.

(c) Ibuprofen, indomethacin and their related products were assayed or separated by reversed-phase HPLC using a Hypersil-ODS (5 μm) 10 cm × 4.6 mm I.D. column. The mobile phases used were 70-81% v/v methanol-water mixtures acidified with 0.2-0.3% v/v orthophosphoric acid (pH 2.5) delivered at a rate of 1 ml/min with an Altex 110A constant flow pump. 20 μl samples of assay or standard solutions were injected using a Rheodyne 7120 valve injector. Peak detection was by means of a Pye Unicam LC3 variable wavelength ultraviolet monitor operated at a wavelength of 220 and 235 nm for the detection of ibuprofen and indomethacin, respectively, together with their related compounds at an attenuation range of 0.01-0.64 A.U.F.S. with the sample flowing through an 8 μl flow cell, while the signals detected were recorded on a Pye Unicam AR 45 Linear/Log 1.0 Decade chart recorder.

(d) The infra-red spectra were recorded either in KBr discs or in nujol mulls on a Perkin-Elmer 1310 Infra-red spectrophotometer.

(e) ^{1}H-NMR spectra were recorded at 60 MHz on a Varian EM 360A spectrometer using $CDCl_3$ (Chloroform 99.8 atom % D) (Gold label) containing 1% v/v TMS and obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin, USA.

(f) The mass spectra were recorded on a Micromass spectrometer by the direct insertion technique operated with an accelerating voltage of 4 KV, a trap current of 100 μA and a source temperature of 250°C.

2.2 MATERIALS

Indomethacin, pharmaceutical grade, obtained from Merck Sharp and Dohme Limited, England.

p-Chlorobenzoic acid, thymol (B.P.), 3-hydroxy-2-naphthoic acid (GPR) (recrystallized from methanol), concentrated hydrochloric acid and boric acid (GPR) were supplied by BDH, Poole, England.

Methanol (Analar grade), orthophosphoric acid (88%, GPR), sodium hydroxide pellets (Analar grade), citric acid monohydrate (Analar grade), potassium chloride (Analar grade) and sodium dihydrogen orthophosphate dodecahydrate (SLR) were supplied by Fisons Scientific Company, England.

5-Methoxy-2-methylindole-3-acetic acid was obtained from the

Aldrich Company, Gillingham, Dorset, England.

Ibuprofen was a gift from the Boots Company, Nottingham, England.

Ethyl-, (tetrahydro-2-furanyl)methyl-,(tetrahydro-2-(2H)pyranyl)-methyl- and cyclohexylmethyl esters of ibuprofen were synthesised by the usual methods of esterification from ibuprofen and the corresponding alcohol in the Department of Pharmaceutical Sciences and kindly supplied by Dr. A. Z. Britten.

2.3 METHODS

2.3.1 Ultraviolet Spectroscopic Methods

The indomethacin contents of solutions were determined by direct ultraviolet spectroscopy at λ_{max} 320 nm (ε = 6290 cm^2/mole). The UV absorbance spectra were recorded by scanning solutions between 190-450 nm in 1 cm quartz cells against an appropriate blank.

A. Identification of indomethacin in the presence of its degradative products. Solution preparation and the recording of the UV absorption spectra.

McIlvaine buffer(238) pH 2.0 with an ionic strength of 1 M was prepared according to the table in Appendix 1, and was used in the preparation of solutions. An accurately weighed 35.7 mg ±0.2 of indomethacin was dissolved in 100 ml of ethanol to give a 1 mM stock solution. Another stock solution was prepared to contain 1 mM each of p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid by

dissolving 21.9 ±0.1 and 15.6 ±0.2 mg respectively in 100 ml of ethanol. A substock solution with a 0.03 mM strength in 10% v/v ethanol-buffer pH 2.0 was made from each stock solution. A series of solutions were then made by mixing aliquots from each substock with 10% ethanol-buffer pH 2.0 to give a final concentration of 0.003, 0.006, 0.012, 0.018, 0.024 and 0.027 mM of indomethacin and 0.027, 0.024, 0.018, 0.012, 0.006 and 0.003 mM of each of p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid. The UV absorption spectra of these mixtures together with the two substocks were recorded against a 10% ethanol-buffer pH 2.0 blank.

B. Quantitation of residual indomethacin in routine analysis and in kinetic runs. Test and standard solutions.

The decomposition of indomethacin was followed in McIlvaine's buffer pH 7.61 at 69°C. 450 ml of buffer was introduced into a 0.5 l flask and preheated to 69°C in a waterbath. When this temperature was reached, 50 mls of freshly prepared indomethacin solution in buffer (1 mM, 0.357 mg/ml) was added with constant vigorous stirring. About 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice. 5 ml portions were then acidified with 1 ml of 0.08 M hydrochloric acid to produce a stable solution at about pH 6.0 for the duration of the assay. Standard solutions were made from a stock solution in buffer pH 7.61 to contain (0.0014-0.112 mM) of indomethacin. The standards were identically treated as the test solutions. The samples and standards were analysed in the UV region against an appropriate blank.

2.3.2 High Performance Liquid Chromatographic Methods

A. Estimation of the molar extinction coefficient of flufenamic acid, ibuprofen and three of its esters. Preparation of solutions and recording of the UV absorption spectra.

Dilute solutions of compounds shown in Table 2.1 were prepared in ethanol from their stocks. The concentrations were chosen to give an ultraviolet absorbance between 0-1 absorbance units at the maximum in the 190-450 nm region.

Table 2.1

Concentration of solutions (M) used for measurement of molar extinction coefficients.

Compound	Concentration $M \times 10^6$
Ibuprofen	58.7
Flufenamic acid	18
Ethyl ester of Ibuprofen	12.6
(Tetrahydro-2-Furanyl)methyl ester of Ibuprofen	35.2
(Tetrahydro-2-(2H)pyranyl)methyl ester of Ibuprofen	25.3
Cyclohexylmethyl ester of Ibuprofen	26

B. Development of the HPLC analytical procedures for the assay and identification of ibuprofen, indomethacin and their related compounds in routine analysis, kinetic runs and permeation studies

1. HPLC Systems:

System I (Reversed-phase):

Column: Hypersil-ODS (5 μm) 10cm × 4.6 mm I.D.

Mobile phase: 65-76%v/v Methanol in water acidified with 0.2%v/v orthophosphoric acid (pH 2.5)

Flow rate: 1 ml/min.

System II (Reversed-phase):

Column: Hypersil-ODS (5 μm) 10 cm × 4.6 mm I.D.

Mobile phase: 55%v/v Acetonitrile in water acidified with 0.1%v/v orthophosphoric acid (pH 2.5)

Flow rate: 1 ml/min.

System III (Reversed-phase):

Column: Hypersil-ODS (5 μm) 10 cm × 4.6 mm I.D.

Mobile phase: 76-81%v/v Methanol in water acidified with 0.3%v/v orthophosphoric acid (pH 2.5)

Flow rate: 1 ml/min.

2. Separation of indomethacin and its degradation products in solutions and kinetic runs. Preparation of test and standard solutions

(a) Separation of indomethacin and its degradative products in routine analysis. Preparation of solutions.

Teorell-Stenhagen's buffer(239) was made according to the table in Appendix 2. A stock solution of indomethacin, p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid (MIAA) was made by dissolving accurately weighed materials in buffer pH 7.6, from which a 10-fold dilution in buffer was made to give a solution containing indomethacin, p-chlorobenzoic acid and MIAA in a concentration of 45, 13 and 20 μg/ml respectively. In establishing a quantitative estimation of the materials resolved by this technique several compounds (1 mM in 30% methanol-water) were tested for their retention times in order to select a suitable internal standard. The various materials investigated are listed in Table 2.2

(b) Separation and assay of indomethacin in kinetic runs. Preparation of sample and standard solutions.

The decomposition of indomethacin was followed in Teorell-Stenhagen's buffer pH 7.61 at 70°C. 450 ml of buffer were introduced into a 0.5 l flask and then preheated to 70°C in a waterbath. When this temperature was reached, 50 mls of freshly prepared indomethacin solution in buffer (1.68 mM, 0.6mg/ml) were then added with constant vigorous stirring. About 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature

Table 2.2

A list of the materials tested in the search for an internal standard used in the assay of the indomethacin mixture.

Mobile Phase	Compound	Retention Time (min.)
55%v/v Acetonitrile in water acidified with 0.1%v/v ortho-phosphoric acid (pH 2.5)	Aspirin[1]	1.2
	Ibuprofen[2]	5.2
	Phenacetin[3]	1.3
	Paracetamol[3]	1.4
	Chloroxylenol[3]	3.2
	Thymol[3]	4.4
70%v/v Methanol in water acidified with 02.%v/v ortho-phosphoric acid (pH 2.5)	Salsalate[1]	2.2
	Chloroxylenol[3]	3
	Thymol[3]	3.6
	Anthracine-9-Carboxylic acid[4]	3
	m-Chlorobenzoic acid[3]	2.25
	m-Bromobenzoic acid[3]	2.25
	m-Anisic acid[3]	1.75
	5-Chloro-2-nitrobenzoic acid[3]	1.6
	Indole[3]	2
	Indolyl-3-carboxylic acid[3]	1.5
	Indolyl-3-aldehyde[3]	1.6
	3,5-Dinitroso salicylic acid[3]	1.25
	3-Hydroxynaphthoic acid[3]	3.5

Compounds were supplied by:

1. Sigma Chemicals, England
2. Boots, England
3. British Drug Houses, England
4. Aldrich Company, England.

in ice. Five ml portions were then acidified with 1 ml of 0.08 M hydrochloric acid to produce a stable solution at about pH 6.0 for the duration of the assay, then the solutions were dipped in ice. Thymol (0.5 mg/ml) in 50% methanol buffer was used as an internal standard.

Two mls of each sample were mixed with 2 ml of methanol and 2 mls of the internal standard solution before analysis by HPLC.

Standard solutions were prepared in buffer from an appropriate stock solution to contain about 0.04-0.2 mM of indomethacin, MIAA and p-chlorobenzoic acid. The standards were similarly stored in ice and warmed to room temperature before acidification than they were treated as with the test solutions. No decomposition was observed in the standard solutions during this procedure as proven by the absence of MIAA peak in the HPLC chromatograms of standard solutions containing only indomethacin and p-chlorobenzoic acid.

(c) Effect of the alcohol content of the mobile phase and sample solvent on the peak ratio and the quantitative estimation of indomethacin mixture

This aspect was followed in aqueous alcoholic buffered solutions over the range of 10-100%v/v of methanol. pH 6.1 Teorell-Stenhagen's buffer was used throughout. Into each of a series of 100 ml volumetric flasks, volumes of methanol each 10 ml short of those necessary to produce the predetermined final methanol concentrations were added to the flasks together with the necessary volumes of buffer to produce 90 ml except for 10% methanol mixtures where only the 90 ml of buffer was placed. 10 mls of a methanolic solution containing indomethacin mixture

was added to each of the 100 ml containers with rapid mixing. All solutions were freshly prepared and analysed by HPLC using several mobile phases. The methanolic mixture added to the preparation contained indomethacin, p-chlorobenzoic acid and MIAA together with either thymol or 3-hydroxynaphthoic acid (3-OH.N.A.) as internal standards in concentrations of 0.45, 0.13, 0.2, 5 and 0.06 mg/ml respectively.

3. Separation and identification of ibuprofen and its esters in solutions and kinetic runs. Preparation of test and standard solutions

(a) Separation and identification of ibuprofen and its esters in routine analysis. Preparation of solutions

An HPLC separation method has been developed for the simultaneous identification of a mixture containing ibuprofen and its esters using an UV detection method. Substock solutions in ethanol of ibuprofen and its (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl- esters or ibuprofen and its ethyl and cyclohexylmethyl esters were prepared from the ethanolic stock solutions. A 10-fold dilution in Teorell-Stenhagen's buffer was made to prepare sample solutions from these two substocks. The first contained ibuprofen and its (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters in concentrations of 15, 12 and 12 μg/ml respectively in 10% v/v ethanol buffer pH 7.0. The second contained ibuprofen and its ethyl- and cyclohexylmethyl esters in concentrations of 15, 12 and 9 μg/ml respectively.

Table 2.3

A list of the materials tested in the search for an internal standard used in the assay of the ibuprofen mixture.

Material	Retention Time (min)
t-Butylphenylsalicylate[1]	6.2
Butyl p-hydroxybenzoate[2]	1.6
Alprenolol[3]	7.0
Phenylbutazone[4]	1.8
Propylgallate[4]	1.6
Vanillin[4]	1.4
Antipyrine[4]	2.0
Betamethasone-17,21-dipropionate[2]	2.2
Phenobarbitone[5]	1.4
Acebutolol[3]	3.4
Phenyl Salicylate[1]	2.4
Satolol hydrochloride[3]	2.2
Atenolol[3]	2.2
Practolol[3]	2.0
Pindolol[3]	2.8
Propranolol[2]	7.8
Flufenamic acid[2]	2.6
Ketoprofen[2]	1.6
β-estradiol[4]	1.6
Testosterone[4]	2.0
Progesterone[2]	2.6
L-Tryptophane[5]	2.8
Oxprenolol[3]	5.4
Trans-stilbene[4]	3.8
Phenanthrene[4]	3.8
Naphthalene[5]	2.2
Propyl Salicylate[1]	2.8
Anthrone[4]	2.0
Anthraquinone[4]	2.8
Anthracine[4]	4.6
Hexamethyl Benzene[4]	7.8
Hexachlorophane[6]	6.2

Compounds were separated using a mobile phase consisting of 81%v/v methanol in water acidified with 0.3%v/v orthophosphoric acid.

The Compounds were supplied by:

1. Grasser Salicylate Ltd., England
2. Sigma Chemicals, England
3. Imperial Chemical Industries, England
4. Aldrich Company, England
5. British Drug Houses, England
6. MaCarthys, England.

Due to the low solubility of the cyclohexylmethyl ester this latter solution was made in 50%v/v ethanol-buffer pH 7.0. 20 µl was then chromatographed. A number of compounds (1 mM in 30% methanol-water) (Table 2.3) were used in order to select a suitable internal standard.

(b) Separation and assay of ibuprofen and its esters in kinetic runs. Preparation of test and standard solutions

The developed HPLC system for the separation of a multicomponent mixtures constitute of ibuprofen and its esters was used to detect and follow the degradation of ibuprofen esters in Teorell-Stenhagen's buffers. To follow the decomposition of the (tetrahydro-2-(2H)pyranyl)methyl ester in buffer pH 7.0 at 70°C, 10% ethanol in the final solution was necessary due to the low solubility of the ester in aqueous solutions. 40 mls of ethanol (10 ml short of that necessary to produce the final ethanol concentration) were pipetted into a 0.5 l flask together with the necessary volume of buffer to produce 490 mls. The solution was then heated in a waterbath set at 70°C. When this temperature was reached, 10 mls of a 1.973 mM ethanolic solution of the ester was then added to the 0.5 l container with rapid stirring. About 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice. Five ml portions were then acidified with 1 ml of 0.08 M hydrochloric acid to produce a stable solution at about pH 6.0 for the duration of storage and assay. Afterwards the solutions were dipped in dry ice and then kept in the freezer (-15°C) until assayed.

Standard solutions were prepared in 10%v/v ethanol buffer pH 7.0 from their substock solutions to contain ibuprofen and its (tetrahydro-

2-(2H)pyranyl)methyl ester in a concentration of 7.74-38.7 μM and 7.94-39.2 μM respectively.

During the analysis the samples were thawed then 1 ml of hexachlorophane (7 μg/ml) in ethanol was added as an internal standard. 20 μl of these solutions were then chromatographed. The standard solutions were similarly stored in ice and warmed to room temperature before acidification they were subsequently treated as with the test solutions. No decomposition was observed in the standard solution during this procedure as proven by the absence of an ibuprofen peak in the HPLC chromatogram of a standard solution containing the (tetrahydro-2-(2H)pyranyl)methyl ester alone.

4. Separation of compounds used in the skin permeation study. Preparation of solutions

Indomethacin, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl esters were incorporated in skin permeation studies. In each test one of these compounds was used together with flufenamic acid as a standard and solutions of both materials in each case were made in 50% propylene glycol-water except when testing the cyclohexylmethyl ester with flufenamic acid when a solution in 80% propylene glycol-water was prepared. The experiment was initiated by introducing 7 ml of the solution prepared into the donor phase of a diffusion cell where a piece of skin serves as a membrane between the donor and the receiver phases, this latter comprising 13 ml of a 50% propylene glycol-water mixture. The diffusion was followed by monitoring the appearance of each of the two compounds in the receiver phase.

This was achieved by pipetting 1 ml from the receiver phase and replacing it with a 1 ml blank 50% propylene glycol-water. The samples were stored in the refrigerator until required for anlaysis.

A stock solution of each of the compounds studied was made separately by dissolving accurately weighed material in propylene glycol. For every individual compound a mixture with flufenamic acid was made in 50%v/v propylene glycol-water to give a substock solution which was used thereafter for introducing the materials into the donor phase of the diffusion cells. The concentration of the materials used in both the stock and the substock solutions are listed in Table 2.4.

The HPLC technique was utilized in the separation of these mixtures and for estimating the initial concentration of their constituents as well as determining the concentration in the samples collected to provide the pattern of diffusion for the skin permeation study of the compounds.

Three materials were used as internal standards to validate the quantitative estimation. These were hexachlorophane together with the ethyl- and the (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen. The system developed was tested for the determination of the initial concentration of all the five mixtures before proceeding to the diffusion experiment assay.

Table 2.4

The concentration of flufenamic acid and the non-steroidal anti-inflammatory agents used in the diffusion studies.

Compound	Concentration in		Flufenamic acid Concentration	
	Stock Solution mM	Substock Solution mM	Stock Solution mM	Substock Solution mM
Ibuprofen	12.2	2.44	1.87	0.374
Indomethacin	6.974	1.394	1.852	0.37
(Tetrahydro-2-furanyl)-methyl ester of ibuprofen	7.9	1.58	1.849	0.369
(Tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	2.473	0.4947	1.849	0.369
Cyclohexylmethyl ester of ibuprofen	0.6423	0.1284	1.852	0.370

As an example the determination of ibuprofen and flufenamic acid mixture will be discussed. Six samples of 1 ml each from the mixture were pipetted and mixed with (4 mls) of 50%v/v ethanol-water and 2 mls of ethyl ester (30 mg/100ml). Five standard solutions were made to contain ibuprofen and flufenamic acid in the range of 0.49-2.43 mM and 0.07-0.36 mM respectively from their stock solutions. In the assay of the initial concentrations, the samples were collected and the standards for all mixtures were treated in the same way where 1 ml was always diluted with (4 mls) of 50%v/v ethanol-water before mixing with 2 ml of the internal standard solution.

5. HPLC conditions

Column: Hypersil-ODS (5 μm) 10 cm × 4.6 mm I.D.

Detection wavelength and mobile phase:

(a) 70%v/v methanol in water acidified wtih 0.2%v/v orthophosphoric acid (pH 2.5) at 235 nm (for the separation and assay of indomethacin in the kinetic runs).

(b) 81%v/v methanol in water acidified with 0.3%v/v orthophosphoric acid (pH 2.5) at 220 nm (for the separation and assay of ibuprofen and its esters in kinetic runs).

(c) 76%v/v methanol in water acidified with 0.3%v/v orthophosphoric acid (pH 2.5) at 220 nm (for the separation and assay of indomethacin, flufenamic acid, ibuprofen and its (tetrahydro-2-furanyl)methyl- and the (tetrahydro-2-(2H)pyranyl)methyl esters in the skin permeation studies).

(d) 81%v/v methanol in water acidified with 0.3%v/v orthophosphoric acid (pH 2.5) at 220 nm (for the separation of flufenamic acid, ibuprofen and its cyclohexylmethyl ester in the skin permeation studies).

(e) 65-76%v/v methanol in water acidified with 0.2%v/v orthophosphoric acid (pH 2.5) at 235 nm (to study the effect of the alcohol content in the mobile phase on the peak ratio of indomethacin mixture).

Flowrate: 1 ml/min.

Sensitivity (A.U.F.S.):

(a) 0.16 AUFS for the assay of indomethacin in the kinetic run.

(b) 0.02 AUFS for the assay of ibuprofen's (tetrahydro-2-(2H)pyranyl)methyl ester in the kinetic run.

(c) 0.32 AUFS for the assay of the initial concentration of the mixture containing ibuprofen and flufenamic acid used in the skin permeation study (substock).

(d) 0.16 AUFS for the study of the effect of alcohol content in the mobile phase and sample solvent on the estimation of peak ratio of indomethacin mixtures.

2.4 RESULTS AND DISCUSSION

2.4.1 Identification of Ibuprofen Esters

The ethyl-,(I); (tetrahydro-2-furanyl)methyl-,(II); (tetrahydro-2-(2H)pyranyl)methyl-,(III) and the cyclohexyl methyl-,(IV) esters of ibuprofen were identified by UV, infra-red, NMR and mass spectrometric methods. Their structural formulae are shown in Fig. 2.1.

A. UV-spectrophotometric identification

Ibuprofen absorbs light in the UV-region and its esterification retained the chromophore so that all esters showed an absorption maximum at 220 nm (ethanol) (Fig. 2.2).

$$(CH_3)_2CH-CH_2-C_6H_4-CH(CH_3)-CO_2-R$$

Iburofen esters

Key	R	name of ester
I	$-CH_2CH_3$	ethyl ester
II	$-CH_2$-(tetrahydrofuran-2-yl)	(tetrahydro-2-furanyl)-methyl ester
III	$-CH_2$-(tetrahydropyran-2-yl)	(tetrahydro-2-(2H)pyranyl)-methyl ester
IV	$-CH_2$-(cyclohexyl)	cyclohexylmethyl ester

Fig. 2.1. Structures of ibuprofen esters.

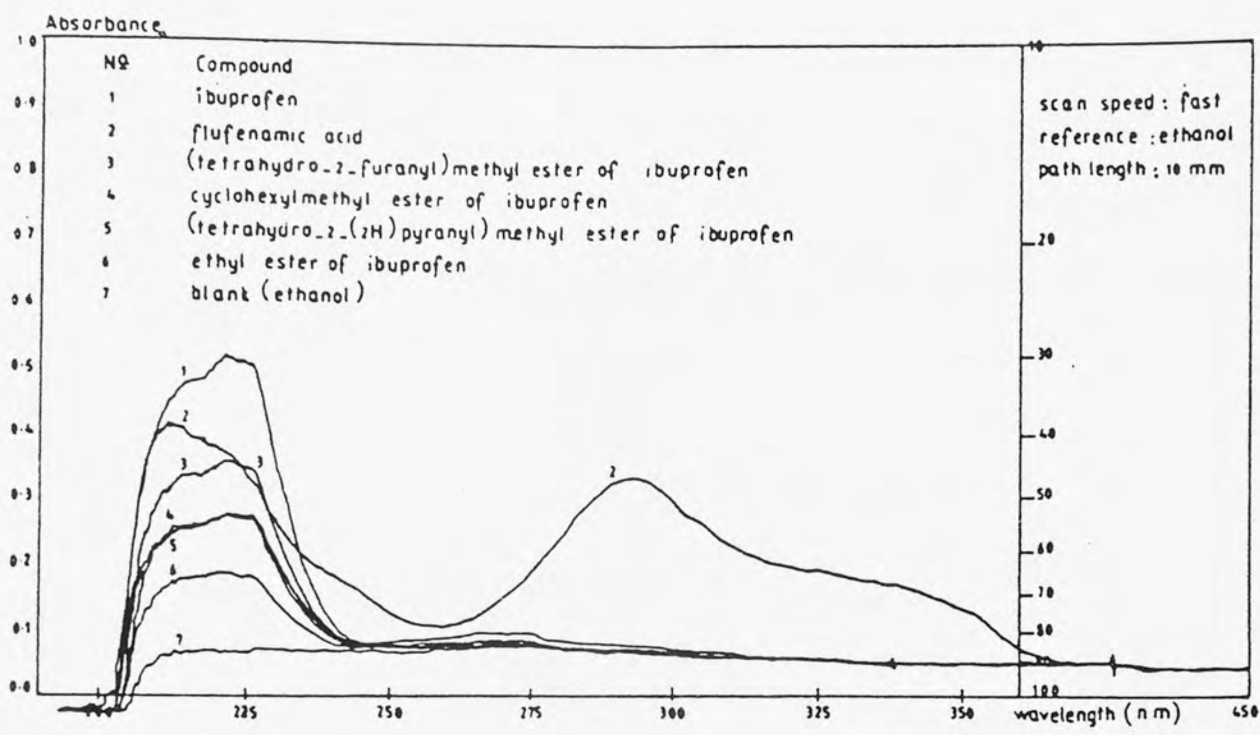


Fig. 2.2. UV-spectral recordings of flufenamic acid, ibuprofen and its esters.

B. Infra-red spectrometric identification

The infra-red spectra of ibuprofen esters showed the absence of the broad O-H stretching band between 3 300 - 2 500 cm^{-1} characteristic of a carboxylic acid functional group. In addition several common absorption peaks in the fingerprint region were present (Figs. 2.3-2.6)

ν_{max}(Neat) 2 940, 2 850 (saturated C-H stretch), 1 730 (C=O stretch), 1 510 (Aromatic-H stretch), 1 460, 1 370 (saturated C-H bending), 1 190 (C-O stretch) and 850 cm^{-1} (p-disubstituted benzene ring).

A strong band at 1 100 cm^{-1} for C-O stretch was present in the ir spectra of the (tetrahydro-2-furanyl)methyl- and the (tetrahydro-2-(2H)pyranyl)methyl esters.

C. ^{1}H-NMR spectrometric identification:

Ibuprofen:

δ_H (60 MHz, $CDCl_3$) 0.9(6H,d,$CH(\underline{CH_3})_2$), 1.45(3H,d, $\underline{CH_3}$-CH), 1.9(1H,m, $\underline{CH}(CH_3)_2$), 2.45(2H,d, $\underline{CH_2}$CH), 3.7(1H,q, $\underline{CH}$-COOH) and 7.15(4H,d,phenyl)ppm. (Fig. 2.7)

Offsetting the sweep by 2 ppm to start from 12, a singlet at δ11.3 is recorded representing the proton of the carboxylic (-COOH) group. A chemical exchange is brought about in the presence of a large excess of deuterium oxide and results in the replacement of this active proton with deuterons and the disappearance of the singlet recorded earlier at δ11.3.

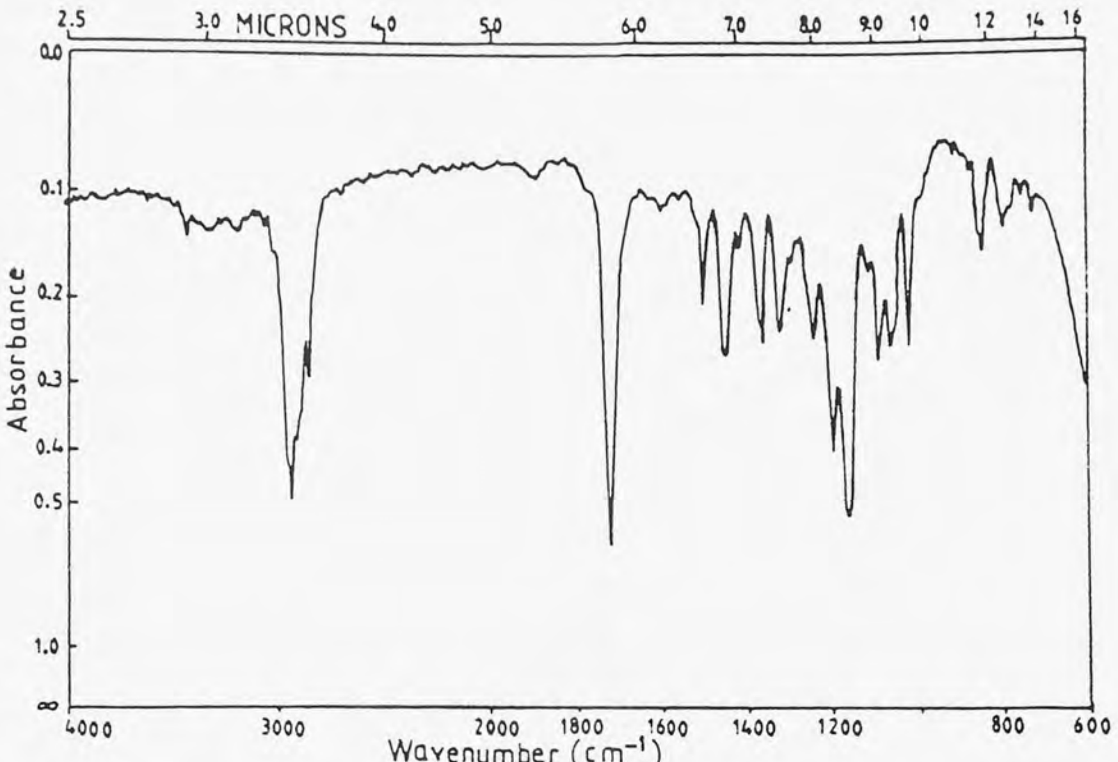


Fig. 2.3. IR spectrum of ethyl ester of ibuprofen (neat).

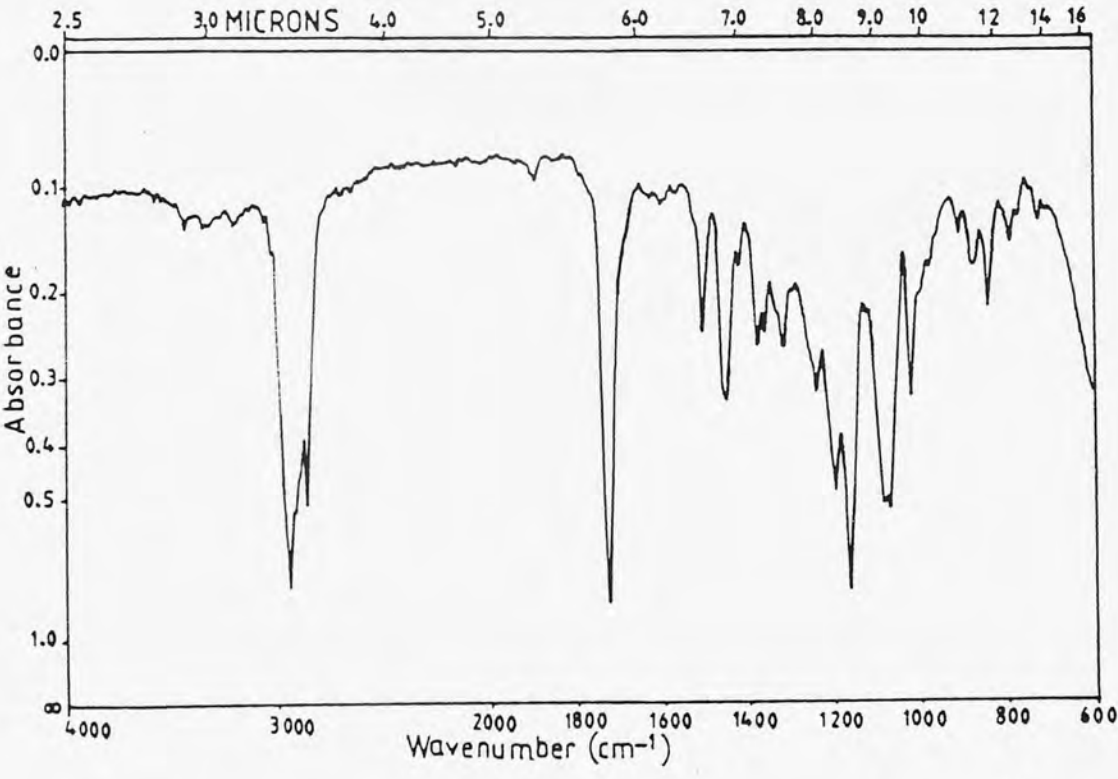


Fig. 2.4. IR spectrum of (tetrahydro-2-furanyl)-methyl ester of ibuprofen (neat).

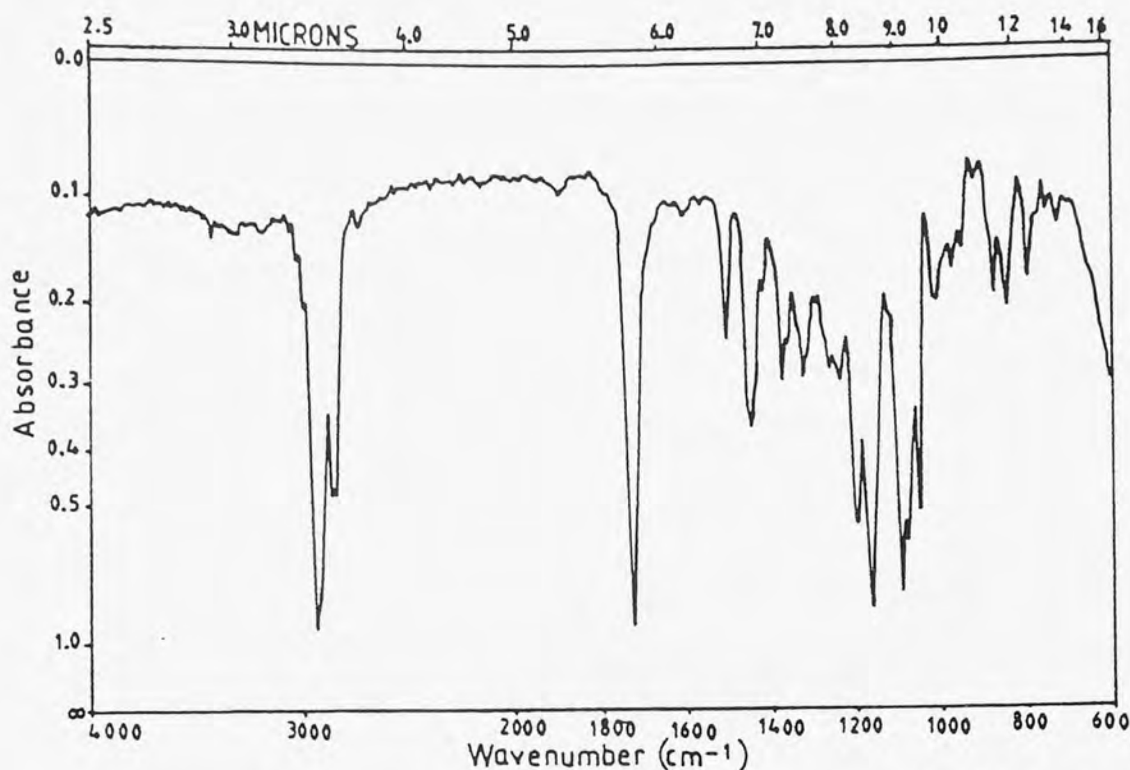


Fig. 2.5. IR spectrum of (tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen (neat).

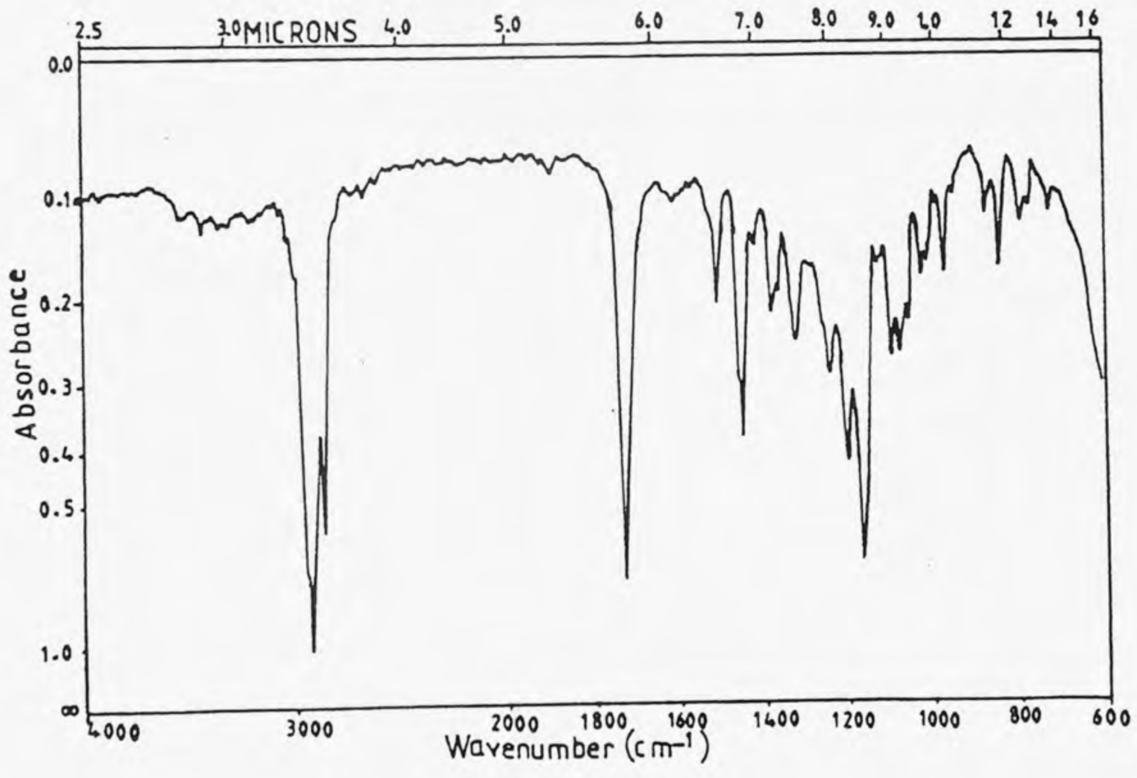


Fig. 2.6. IR spectrum of cyclohexylmethyl ester of ibuprofen (neat).

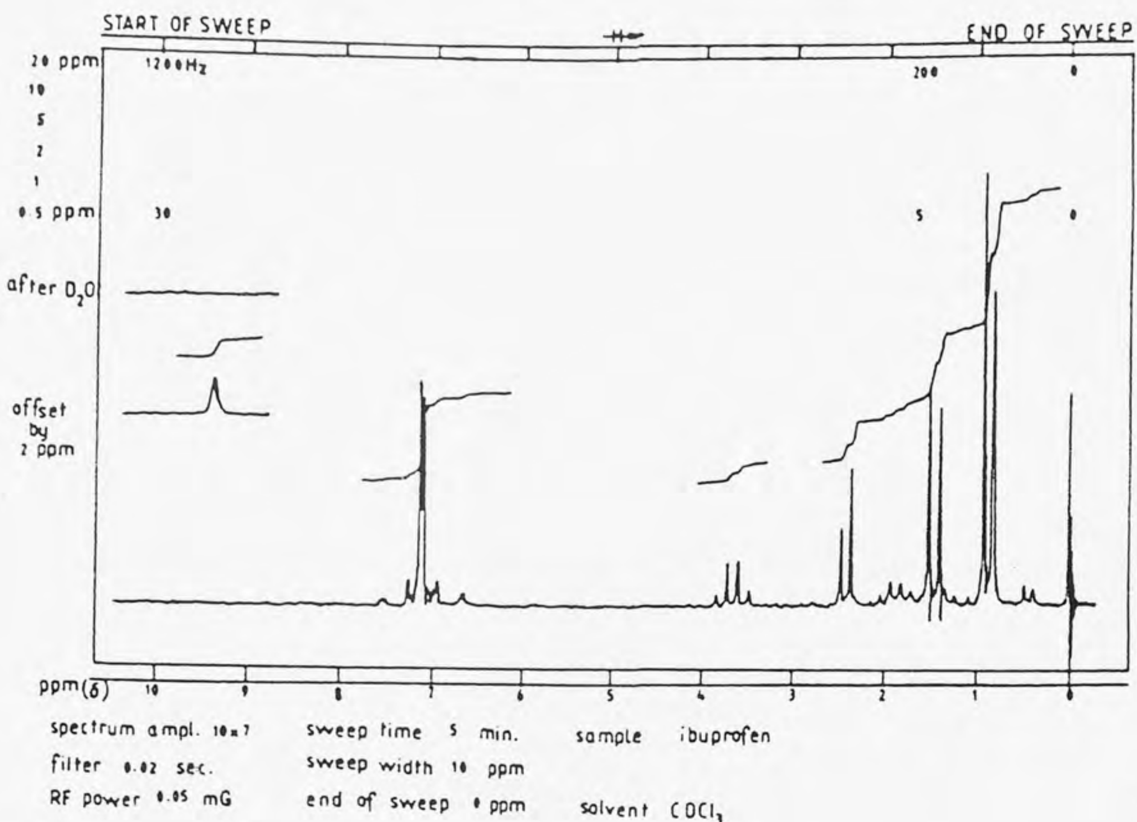


Fig. 2.7. ^1H_NMR spectrum of ibuprofen.

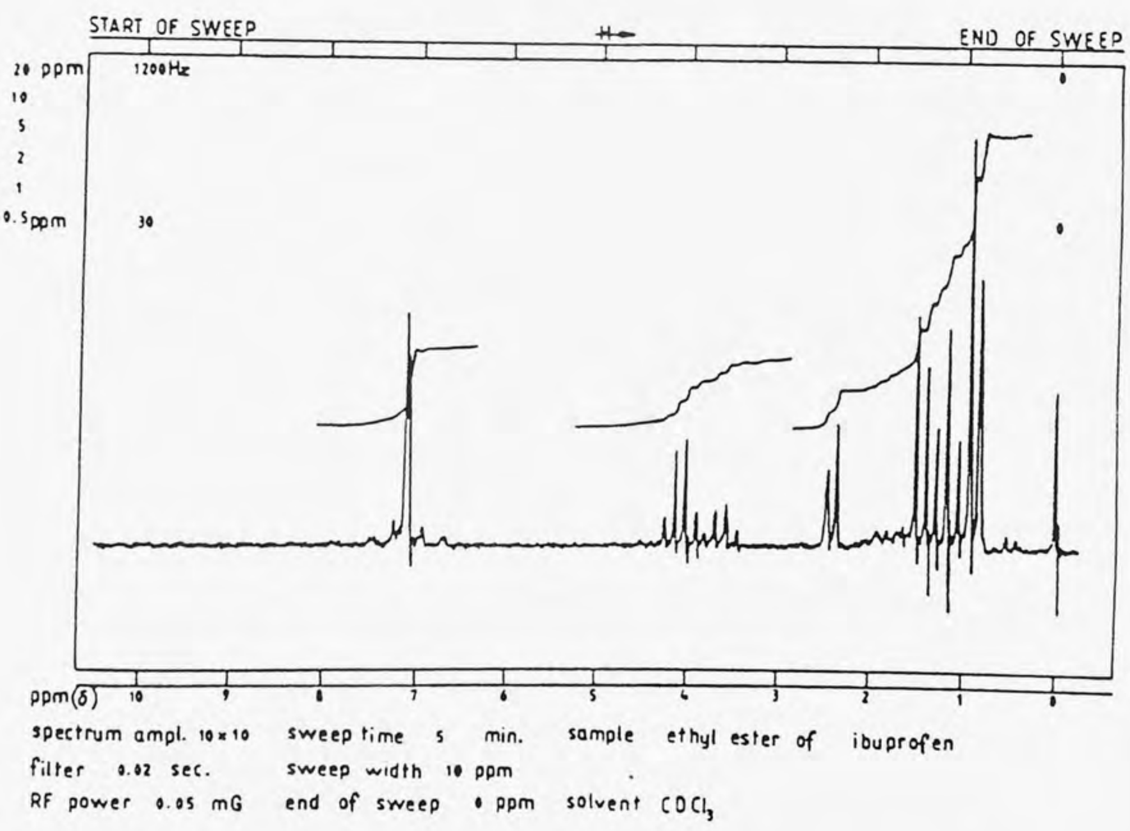


Fig. 2.8. ^1H_NMR spectrum of ethyl ester of ibuprofen.

Ethyl ester:

δ_H (60 MHz, $CDCl_3$) 0.9(6H,d, $CH(\underline{CH_3})_2$), 1.2(3H, t, $CH_2\ \underline{CH_3}$), 1.5(3H,d, $\underline{CH_3}$-CH), 1.85(1H,m, $\underline{CH}(CH_3)_2$), 2.45(2H,d, $\underline{CH_2}$-isopr.), 3.65(1H,q, $\underline{CH}$ COOH), 4.1(2H,q, $\underline{CH_2}\ CH_3$) and 7.15(4H,d,phenyl)ppm. (Fig. 2.8).

(Tetrahydro-2-furanyl)methyl ester:

δ_H (60 MHz,$CDCl_3$) 0.9(6H,d, $CH(\underline{CH_3})_2$), 1.5(3H,d, $\underline{CH_3}CH$), 1.8(4H,q,3 and 4-CH_2), 1.85(1H,m, $\underline{CH}(CH_3)_2$), 2.45(2H,d, $\underline{CH_2}$-isopr.), 3.7(1H,q, $\underline{CH}$ COOR), 3.7 (2H,m,5-CH_2), 3.7(1H,m,2-CH), 4.07(2H,d,O-$\underline{CH_2}$-R) and 7.15(4H,d,phenyl) ppm. (Fig. 2.9)

(Tetrahydro-2-(2H)pyranyl)methyl ester:

δ_H (60 MHz,$CDCl_3$) 0.9(6H,d,$CH(\underline{CH_3})_2$), 1.5(3H,d,$\underline{CH_3}$-CH), 1.5(6H,q,3, 4 and 5-CH_2), 1.85(1H,m, $\underline{CH}(CH_3)_2$) , 2.45(2H,d, $\underline{CH_2}$-isopr.), 3.5(2H,m,6-CH_2), 3.75(1H,q, $\underline{CH}$-COOR), 3.9(1H,m,2-CH), 4.05(2H,d, O-$\underline{CH_2}$-R) and 7.15(4H,d,phenyl)ppm. (Fig. 2.10).

Cyclohexylmethyl ester:

δ_H (60 MHz, $CDCl_3$) 0.9(6H,d, $CH(\underline{CH_3})_2$), 1.0(4H,m,2 and 6-CH_2), 1.5(3H,d, $\underline{CH_3}$-CH), 1.6(6H,m,3,4 and 5-CH_2), 1.8(1H,m,1-CH), 2.0(1H,m, $\underline{CH}(CH_3)_2$), 2.5(2H,d, $\underline{CH_2}$ - isopr.), 3.7(1H,q, $\underline{CH}$-COOR), 3.9(2H,d, O-$\underline{CH_2}$-R) and 7.15(4H,d,phenyl)ppm. (Fig. 2.11).

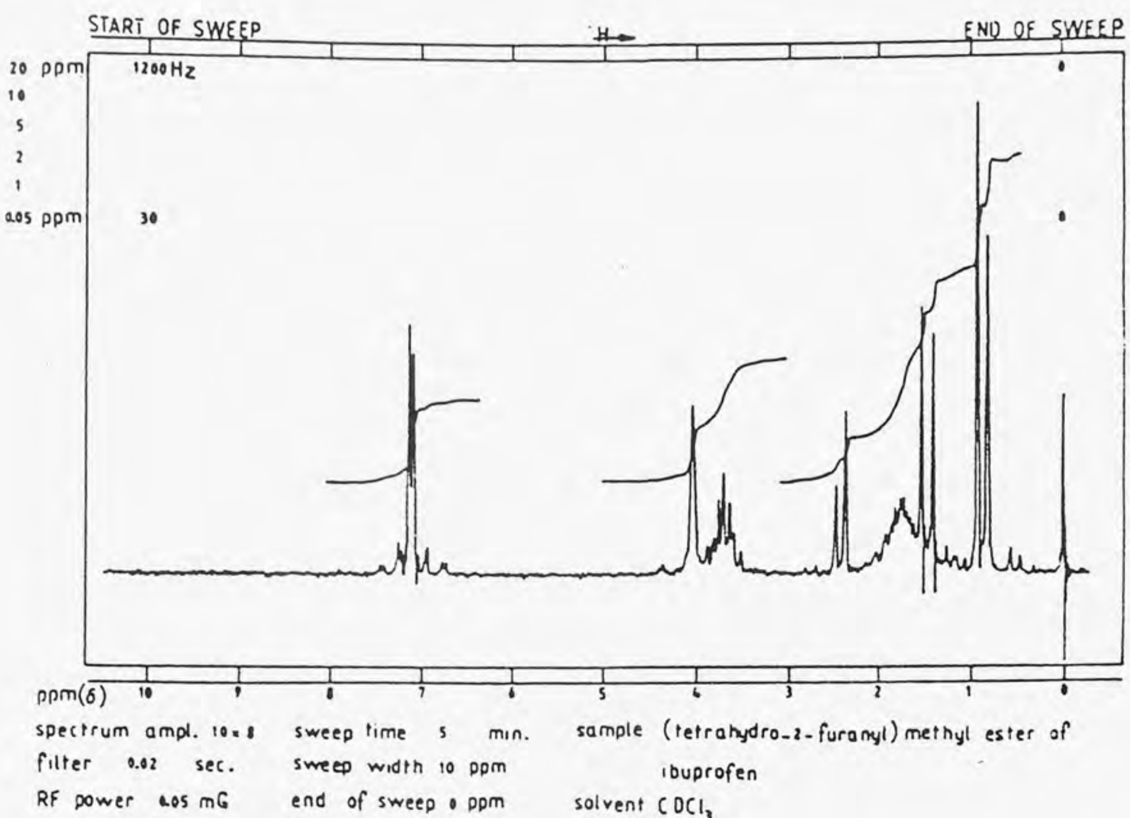


Fig. 2.9. ^{1}H_NMR spectrum of (tetahydro-2-furanyl)-methyl ester of ibuprofen.

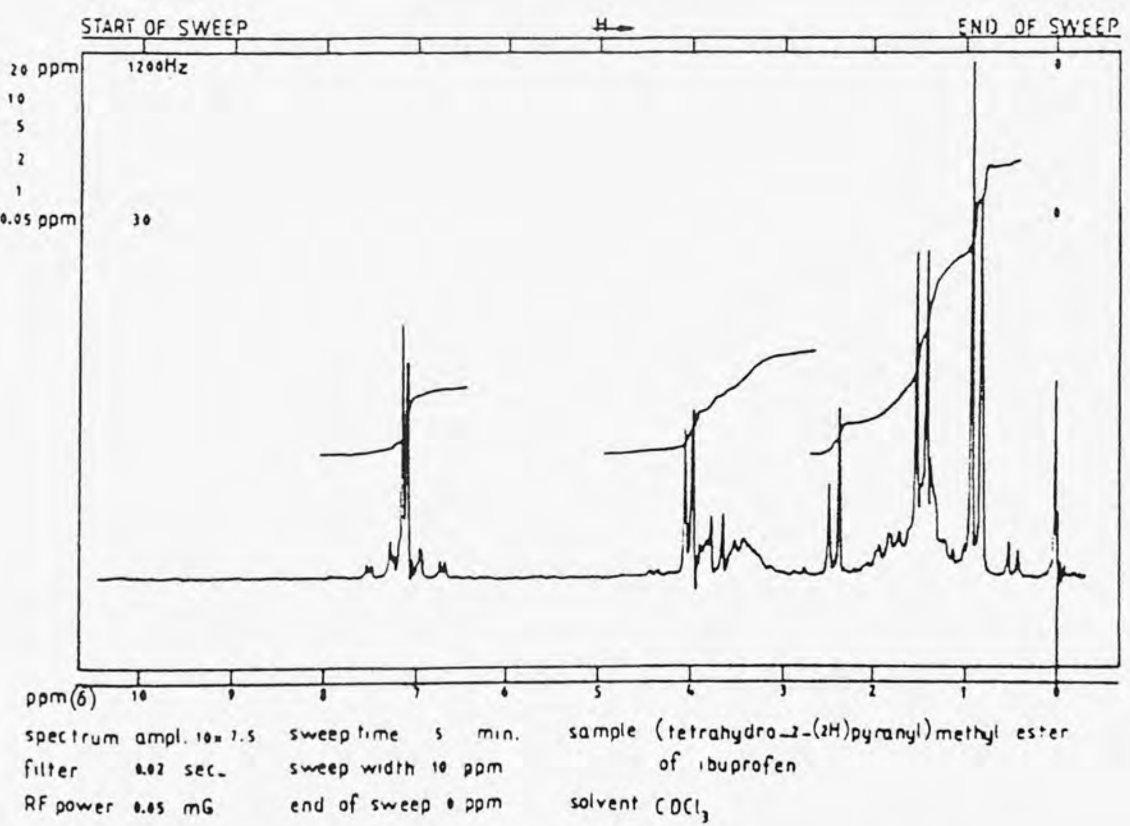


Fig. 2.10. ^{1}H_NMR spectrum of (tetahydro-2-(2H)-pyranyl)methyl ester of ibuprofen.

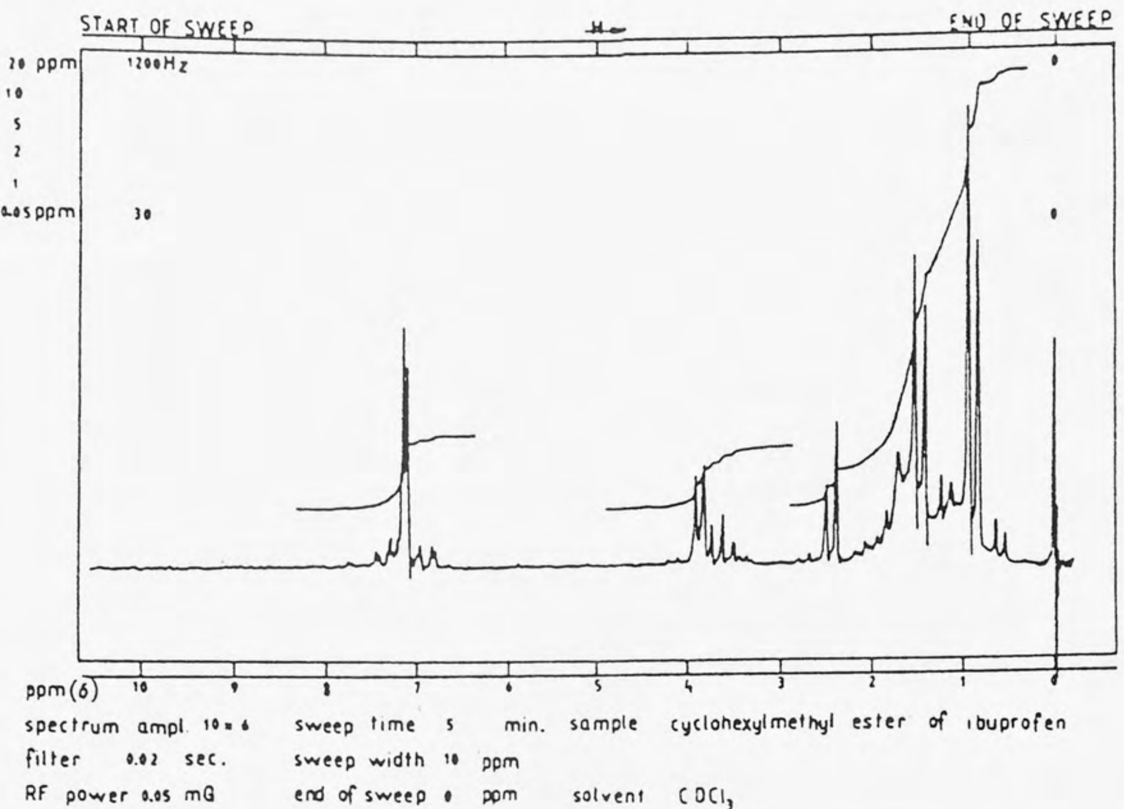


Fig. 2.11. ^{1}H_NMR spectrum of cyclohexylmethyl_ ester of ibuprofen.

D. **Mass spectral data:**

Ethyl ester:

m/z 234(M^+,18%), 191(13), 161(100), 145(4), 119(24), 117(17), 105(13) and 91(15). (Fig. 2.12).

(Tetrahydro-2-furanyl)methyl ester:

m/z 290 (M^+,1%), 206(4), 188(12), 161(61), 145(13), 119(26), 117(28), 105(7), 91(14), 84(74) and 71(100). (Fig. 2.13).

(Tetrahydro-2-(2H)pyranyl)methyl ester:

m/z 304(M^+,1%), 206(2), 188(11), 161(36), 145(9), 119(17), 117(18), 98(70), 91(10) and 85(100). (Fig. 2.14).

Cyclohexylmethyl ester:

m/z 302(M^+,12%), 259(1), 206(63), 188(2), 161(100), 145(6), 119(35), 117(30), 105(12), 96(46), 91(18) and 83(95). (Fig. 2.15).

The mass spectra for ibuprofen esters show the molecular ($M^{+\cdot}$) and fragment ions produced together with their percentage relative abundance. The molecular ions produced were in agreement with the calculated molecular weight for each of the corresponding esters. Most of these fragment ions can be explained according to the following sequences which devise the fragmentation patterns and rationalise the formation of most of the diagnostic fragment ions.

The fragmentation proceeds by the loss of an isopropyl radical to give

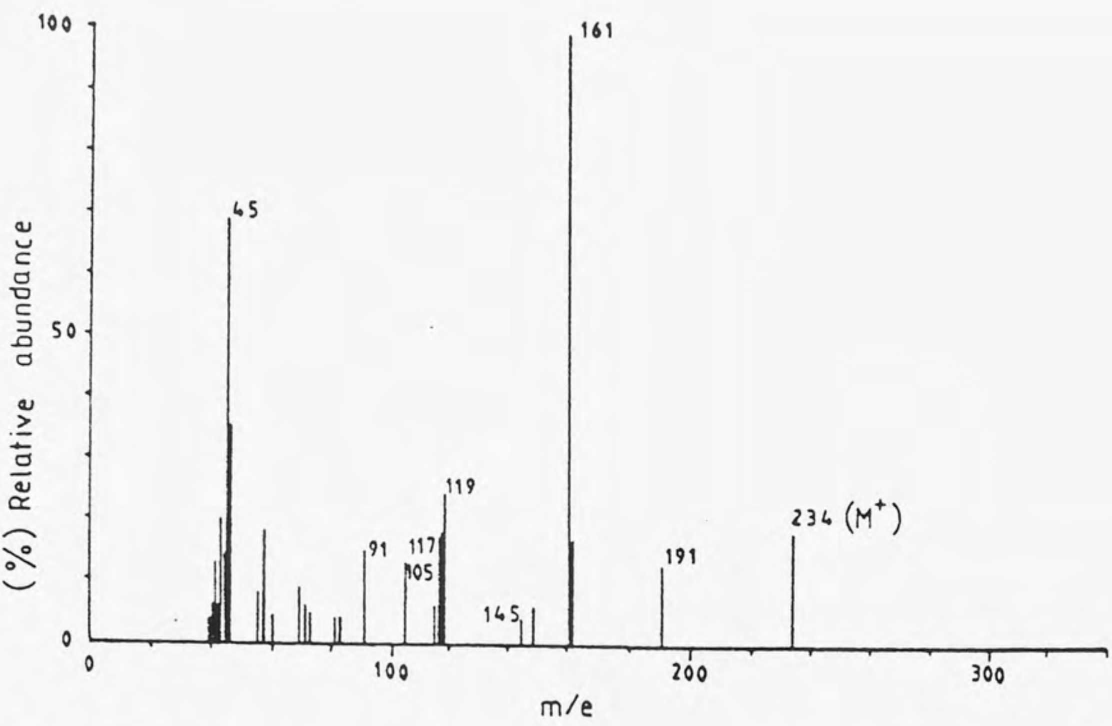


Fig. 2.12. Mass spectrum of ethyl ester of ibuprofen.

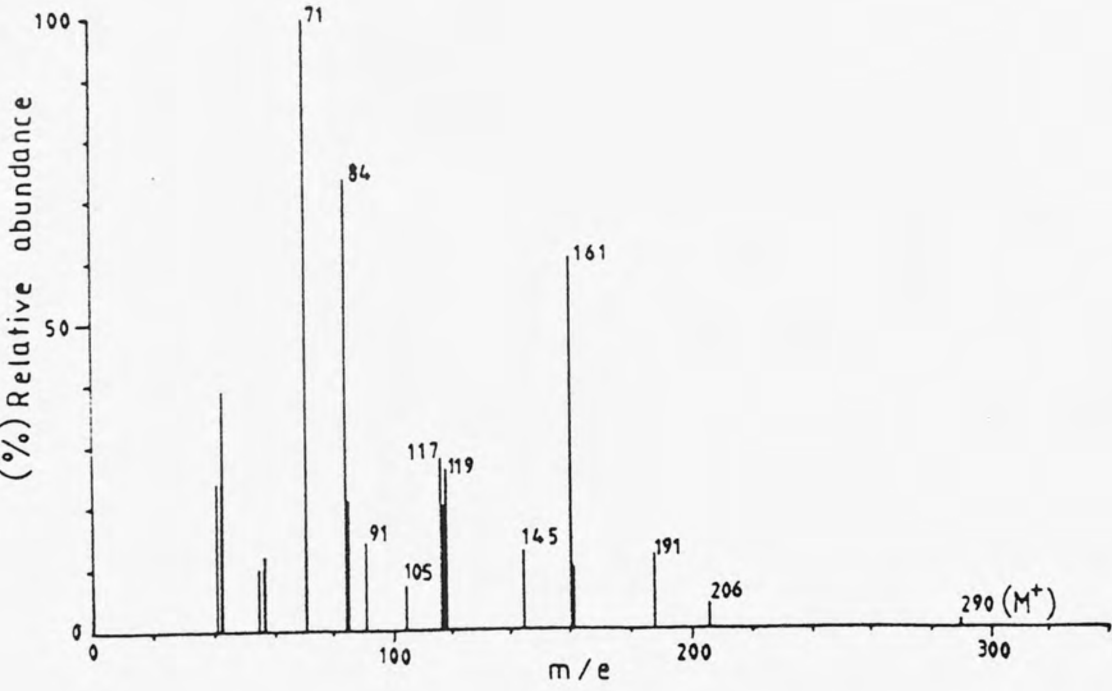


Fig. 2.13. Mass spectrum of (tetrahydro-2-furanyl)-methyl ester of ibuprofen.

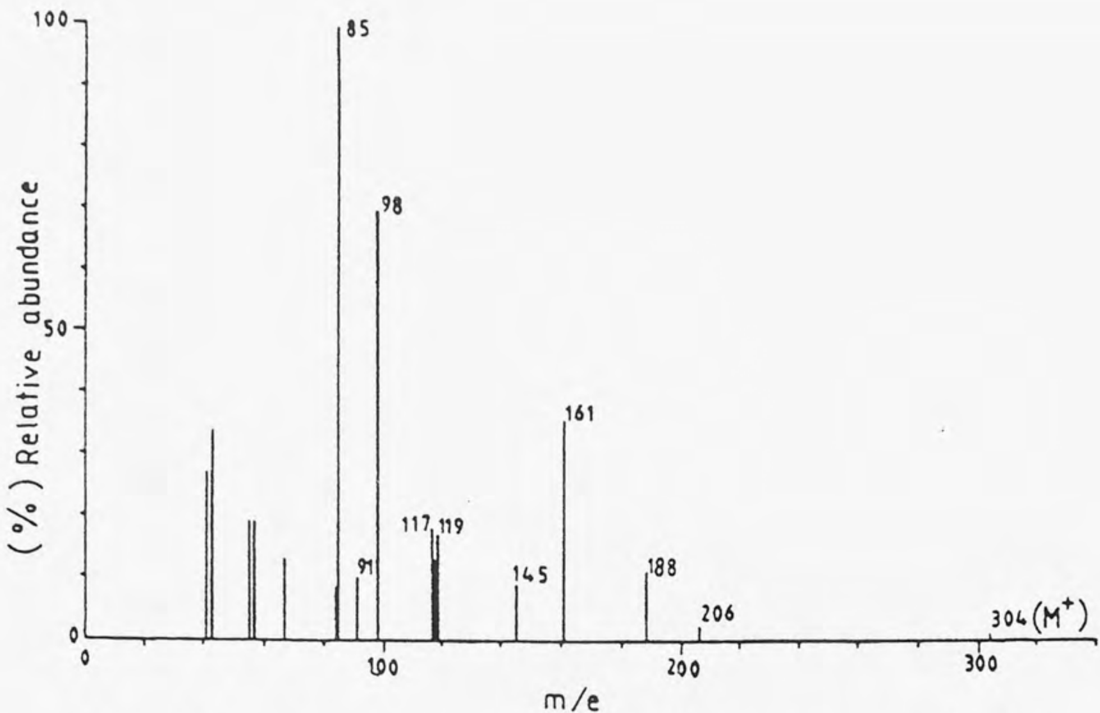


Fig. 2.14. Mass spectrum of (tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen.

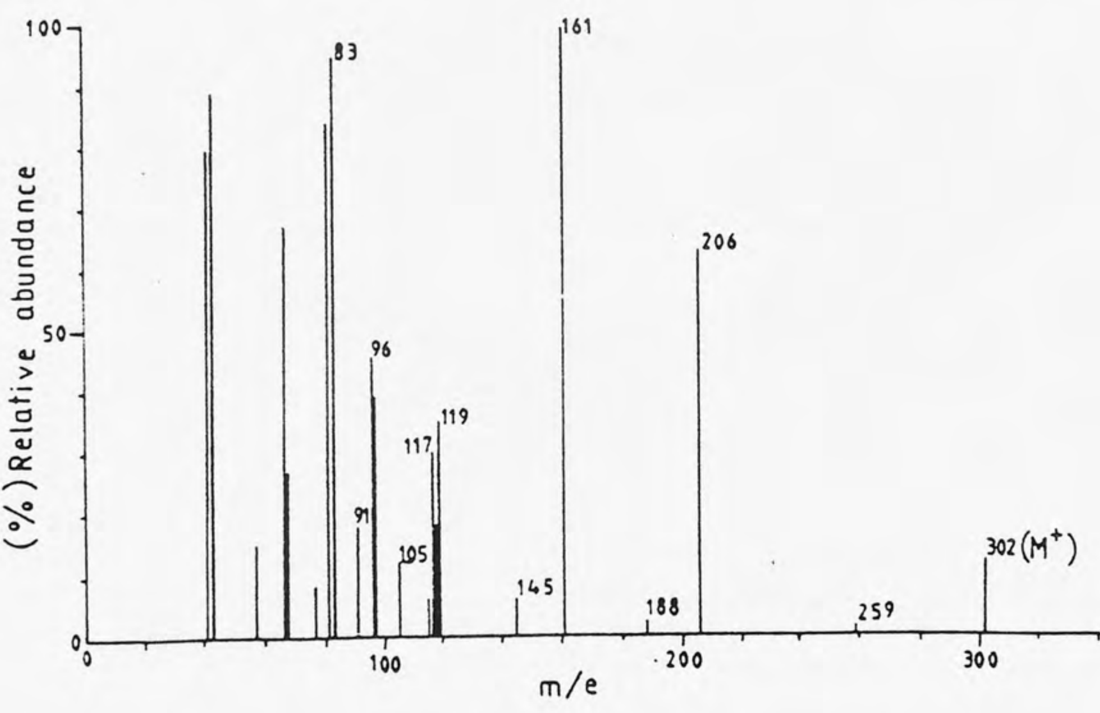


Fig. 2.15. Mass spectrum of cyclohexylmethyl ester of ibuprofen.

CH_3 CH_3 CH—CH_2—⟨benzene⟩—CH(CH_3)—C(=O)—O—R $]^{+\cdot}$ —[HĊ(CH_3)CH_3] 43 mass units

M^+

Key	R	m/e
I	$—CH_2CH_3$	234
II	$—CH_2$–(tetrahydrofuran-2-yl)	290
III	$—CH_2$–(tetrahydropyran-2-yl)	304
IV	$—CH_2$–(cyclohexyl)	302

$^+CH_2$—⟨benzene⟩—CH(CH_3)—C(=O)—OR

m/e
I = 191
II = 247
III = 261
IV = 259

(tropylium)—CH(CH_2—H)—C(=O)—OR

—[H—C(=O)—OR]

(tropylium)—CH=CH—H

m/e 117

—[CH≡CH]

26 mass units

(tropylium)

m/e 91

While the fragmentation may proceed as follows:

$M^{+\cdot}$ $\xrightarrow{-[\overset{O}{\overset{\|}{C}}-OR]}$ m/e 161

R	m/e
I	234
II	290
III	304
IV	302

m/e 161 → m/e 105

m/e 161 → $-[CH_4]$ → m/e 145

m/e 161 → → O → $-[H_2]$ → m/e 117

The rearrangment of two hydrogen atoms in the molecular ion of ibuprofen esters (McLafferty + 1 rearrangement)(240) is a characteristic decomposition reaction of esters. This will rationalise both the formation of the original acid (ibuprofen) molecular ion at m/e 206 and an alkene ion formation which predominates for propyl and higher ester(241).

m/e 290

or

m/e 304

or

m/e 302

R = $(CH_3)_2CHCH_2$–C_6H_4–$CH(CH_3)$–

rH

∝

m/e 206

+

m/e 84

or

m/e 98

or

m/e 96

rH

+

m/e 84

or

m/e 98

or

m/e 96

+

m/e 207

The rearrangement of a hydrogen atom will result in the elimination of an alcohol molecule and the formation of the ion at m/e 188.

$$\left[(CH_3)_2CHCH_2-C_6H_4-\underset{CH_3}{\overset{H}{C}}-\overset{\overset{\cdot+}{O}}{\overset{\|}{C}}-O-CH_2R\right] \xrightarrow{-[HO-CH_2R]} \left[(CH_3)_2CHCH_2-C_6H_4-\underset{CH_3}{C}=C=O^{+\cdot}\right]$$

R = tetrahydrofuranyl or tetrahydropyranyl or cyclohexyl

m/e 188

or it is possible to eliminate the acyl group as a stable neutral molecule:

$$\left[(CH_3)_2CHCH_2-C_6H_4-\underset{CH_3}{\overset{H}{C}}-\underset{\underset{O}{\|}}{C}-O-CH_2R\right]^{+\cdot} \longrightarrow [R-CH_2OH]^{+\cdot} + \left[(CH_3)_2CHCH_2-C_6H_4-\underset{CH_3}{C}=C=O\right]$$

R = tetrahydrofuranyl or tetrahydropyranyl or cyclohexyl

The alicyclic alcohol produced will undergo further fragmentation where they show an α- cleavage to loss a [CH_2-OH] radical.

$$\left[\text{tetrahydrofuran-2-yl-}CH_2OH \right]^{+\cdot} \text{ or } \left[\text{tetrahydropyran-2-yl-}CH_2OH \right]^{+\cdot} \xrightarrow[\cdot CH_2OH]{\alpha} \text{oxolanyl}^{+} \ (m/e\ 71) \text{ or } \text{oxanyl}^{+} \ (m/e\ 85)$$

while cyclohexylmethanol gives:

$$\left[C_6H_{11}CH_2OH \right]^{+\cdot} \xrightarrow[\cdot CH_2OH]{\alpha} C_6H_{11}^{+} \ (m/e\ 83)$$

2.4.2 Ultraviolet Spectroscopic Methods:

The UV absorption spectrum of indomethacin (Fig. 1.14) shows maximum absorption bands at 230, 267 and 319 nm. p-Chlorobenzoic acid and MIAA (Fig. 1.14) the degradation products of indomethacin (84,89), show clearly the absence of the band at 320 nm. This made possible the detection of indomethacin without interference by using direct ultraviolet spectroscopy at 320 nm (84-88,93,143). Consideration of this aspect when choosing the experimental wavelength for indomethacin quantitation by an UV method, a series of solutions were made containing indomethacin (0-100%, 0-0.03 mM) together with its degradative products i.e. p-chlorobenzoic acid and MIAA (100-0%, 0.03-0 mM) in 10% ethanol buffer pH 2.0. The UV absorption spectra of those solutions were recorded (Fig. 2.16). Examination of these spectra shows clearly the absence of interference on the UV absorption band of indomethacin at 320 nm.

In addition, the UV absorption of indomethacin at 320 nm as a function of its concentration gave a straight line with a correlation coefficient of 0.9998 and passing through the origin (Fig. 2.17), this means that this system obeys Beer-Lambert's law and makes possible the detection of indomethacin in solutions under these conditions.

In order to show the specificity of this method and its effectiveness in the determination of indomethacin in the presence of its degradative products, this procedure was employed in the quantitation of residual indomethacin where its decomposition was followed in McIlvaine's buffer pH 7.61 at 69°C and the UV absorption readings in a 1 cm cell of the samples taken at certain intervals were

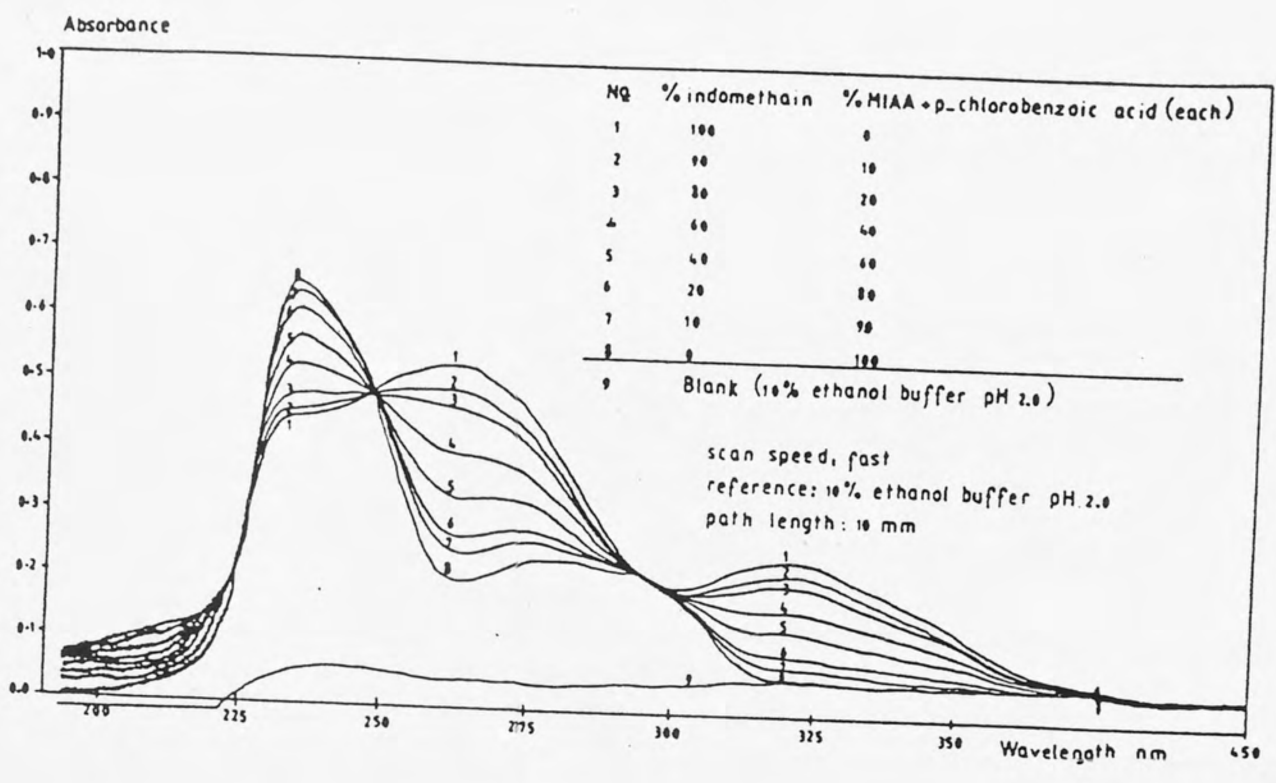


Fig. 2.16. UV_absorption spectra of indomethacin in the presence of its degradative productes

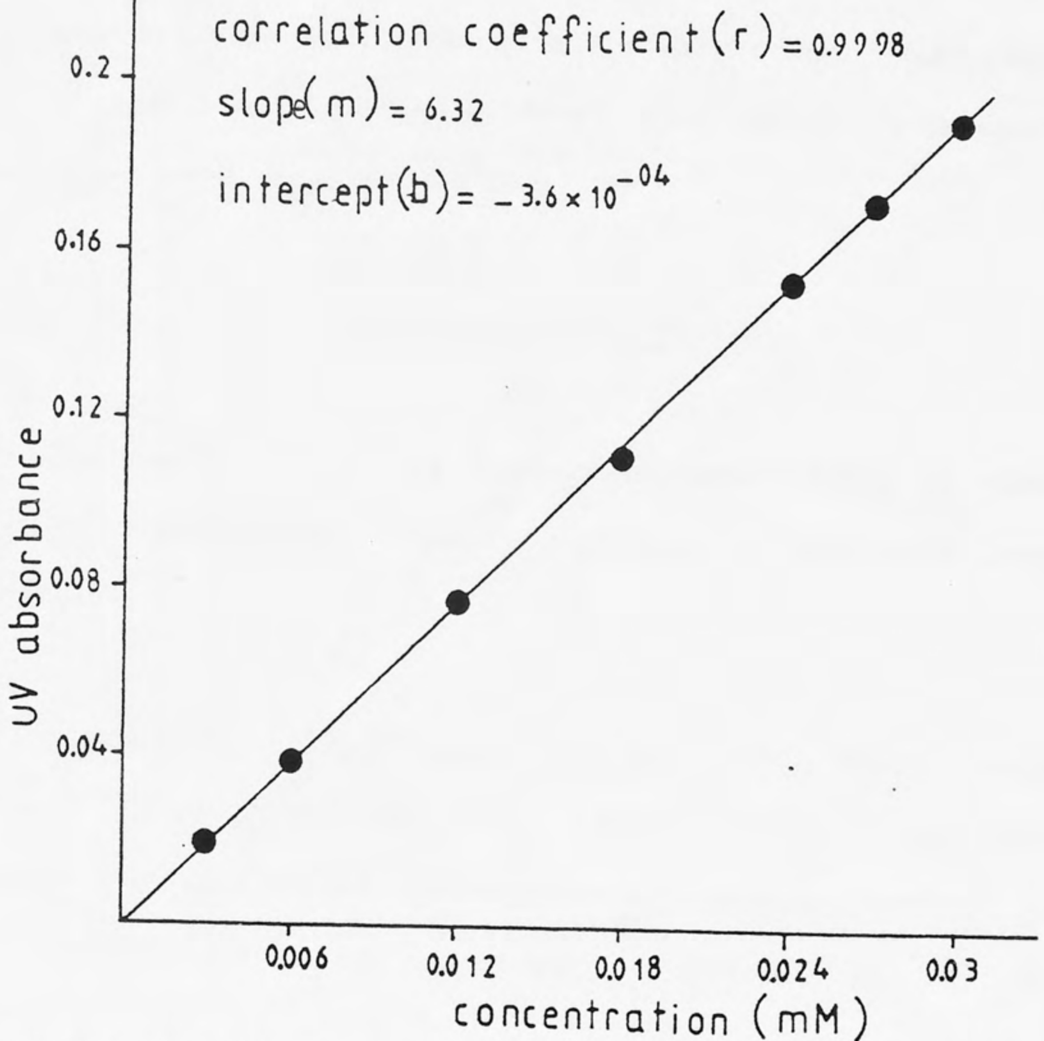


Fig. 2.17. Indomethacin UV-absorbance at 320 nm as a function of its concentration.

recorded with time (Fig. 2.18). A calibration curve was established from the concentrations of standard solutions of indomethacin vs. their UV absorptions. This curve (Fig. 2.19) has a correlation coefficient of 0.9999, slope of 6952 and an intercept of 3.23×10^{-3}. With the aid of this curve indomethacin concentrations in the samples taken were determined.

A plot of the natural logarithm of the remaining concentration of indomethacin vs. time in minutes gave a straight line (Fig. 2.20) which proves as has been reported earlier, that indomethacin degradation follows first order kinetics (84-86).

2.4.3 High Pressure liquid Chromatographic Methods:

A. Estimation of the molar extinction coefficients of flufenamic acid, ibuprofen and its esters used in the skin permeation studies:

The extinction coefficient $E^{1\%}_{1\ cm}$ and the molar extinction coefficient (ε) of flufenamic acid, ibuprofen and the ethyl- , (tetrahydro-2-furanyl)methyl-,(tetrahydro-2-(2H)pyranyl)methyl- and the cyclohexylmethyl esters of ibuprofen in ethanol at 220 nm were calculated.

The UV absorption spectra of solutions containing accurate concentrations of the above mentioned compounds (Table 2.1) in ethanol were recorded between 190-450 nm in a 1 cm cell and from which the optical density of the UV absorption band at 220 nm was measured for each compound. By using the relationship:

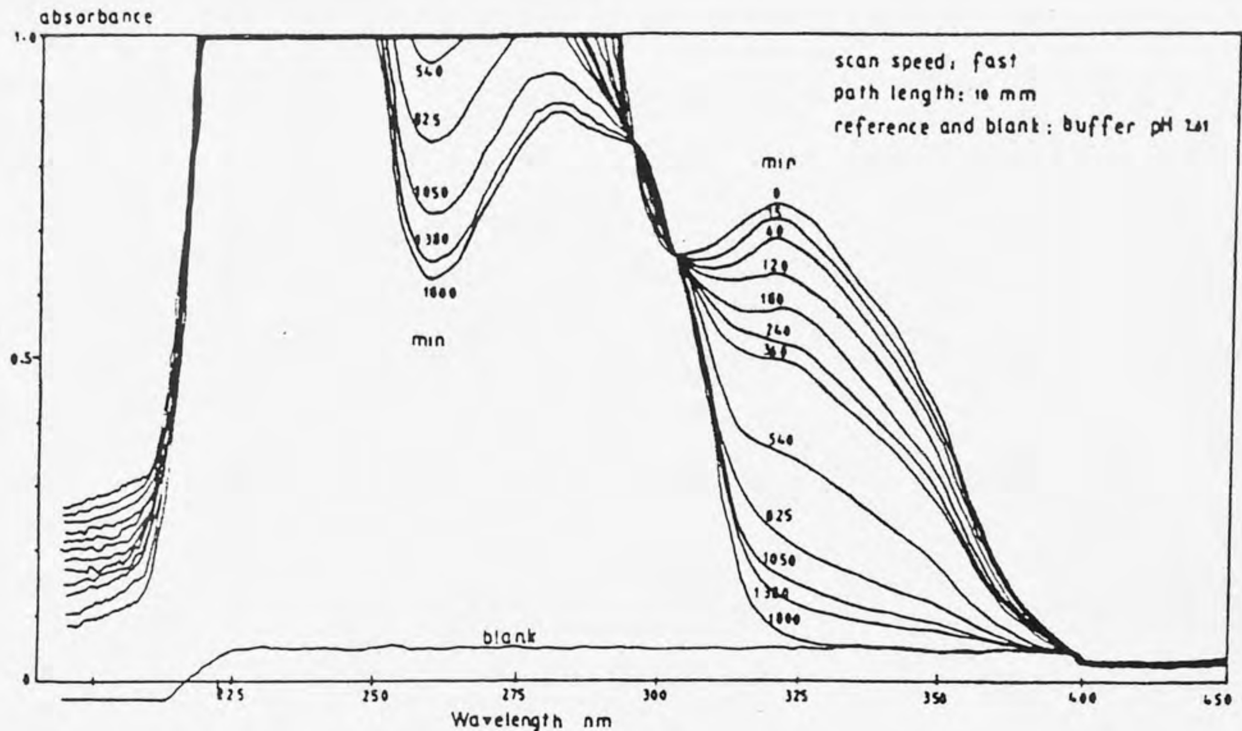


Fig. 2.18. spectral changes for the hydrolysis of 0.1 mM indomethacin in pH 7.61 buffer solution at 69°C.

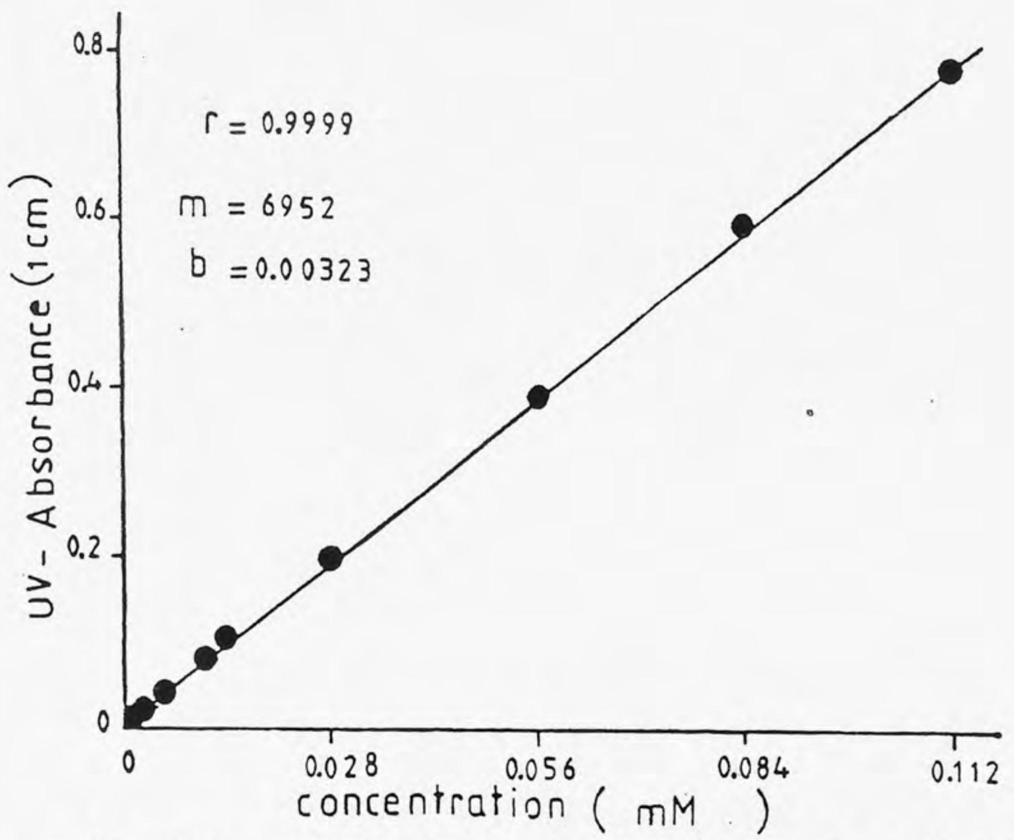


Fig. 2.19. Calibration curve for indomethacin at 320 nm in buffer pH 7.61.

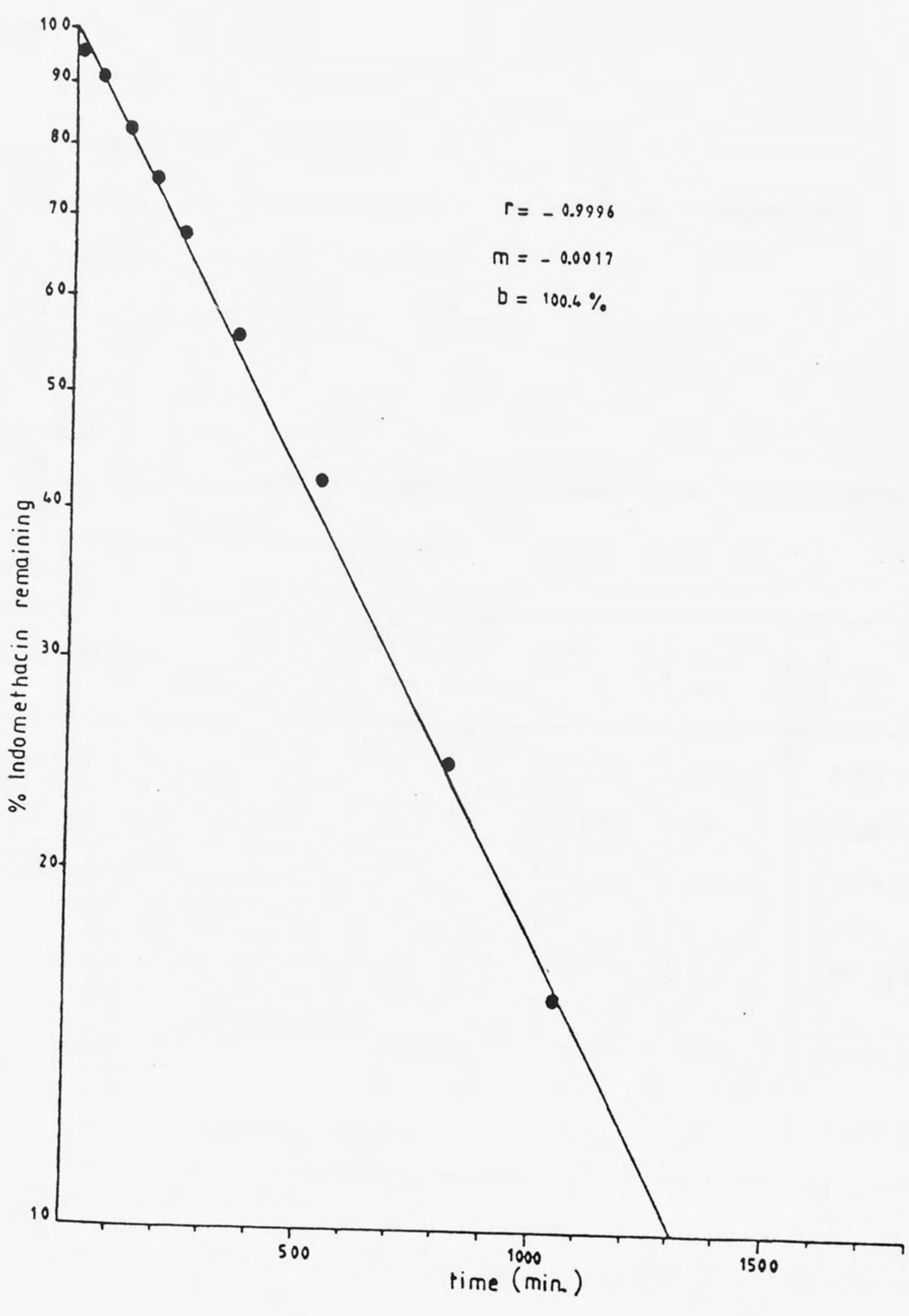


Fig. 2.20. The first order decomposition of indomethacin in buffer pH 7.61 at 69°C.

$$E = A = k.c.l.$$

where

E = Extinction

A = Absorbance or optical density

k = A constant characteristic of the solute or absorptivity

c = Concentration of solute

l = Path length through the sample.

Table 2.5

A list of the measured molar extinction coefficient for the compounds used in the skin permeation studies.

Compound	UV absorbance	$E^{1\%}_{1\ cm}$	Mol. wt.	ε
Ibuprofen	0.44	363.3	206	7484.7
Flufenamic acid	0.31	237.1	281	17215.4
(Tetrahydro-2-furanyl)-methyl ester of ibuprofen	0.285	278.5	290	8079.1
(Tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	0.205	565.5	304	8072.5
Cyclohexylmethyl ester of ibuprofen	0.205	260.1	302	7856.5
Ethyl ester of ibuprofen	0.12	404.0	234	9454.5

The absorbivity (k) can then be calculated as $E^{1\%}_{1\ cm}$ or as the molar extinction coefficient (ε) when (c) is expressed either in grams per 100 ml or in moles per litre respectively. Table 2.5 lists the absorbances measured at 220 nm for each compound together with their calculated extinction coefficients.

B. Development of the HPLC analytical procedures

The aim of this section is the development of a chromatographic analytical method which makes use of the high performance liquid chromatographic technique. The method developed introduces 20 µl of the mixtures investigated and discussed later with the aid of an injector to the top of a reversed-phase column packed with silica coated with a chemically bonded octadecylsilyl moiety. The mobile phases developed were composed of aqueous organic mixtures and were delivered at a rate of 1 ml/min and are capable of simultaneously resolving the samples concerned.

Each constituent was detected in the order of their elution from the column using an UV-spectrophotometric detector set at a pre-chosen wavelength and output was recorded on a chart recorder.

The development of such an analytical procedure included variables such as the mobile phase and the analytical wavelength, while optimisation of the analysis requires choosing a system to give a satisfactory resolution.

Standardisation of the method includes the usage of internal standards and using standard methods for calculating peak heights and ratios.

Additionally, standard or calibration curves with acceptable statistical values are required. The HPLC procedures developed were tested on a sample experiment before use in routine work. Details of

the mixtures studied and the HPLC systems developed are discussed in detail to show the various steps used during the development:

1. <u>Separation of indomethacin and its degradative products in solutions and kinetic runs:</u>

The materials which are to be separated in this system are indomethacin, p-chlorobenzoic acid, MIAA, and the chosen internal standard.

(a) Development of the mobile phase

In the process of developing the HPLC anlaytical system to resolve indomethacin and its degradative products several mobile phases were used and these were of aqueous organic mixtures in which either acetonitrile or methanol were mixed with water and acidified with orthophosphoric acid. In the process of developing the mobile phase the effect of changing the concentration of methanol or acetonitrile on the various mathematical parameters of the HPLC separation were studied.

These parameters are:

1. The retention time of each component in the mixture, t_A.

2. The capacity ratio $K' = \frac{t_A - t_o}{t_o}$

3. The number of theoretical plates $N = 16(\frac{t_A}{w_A})^2$

4. The height equivalent to a theoretical plate $H = L/N$

5. The resolution $R_s = \frac{2(t_B - t_A)}{(w_A + w_B)}$

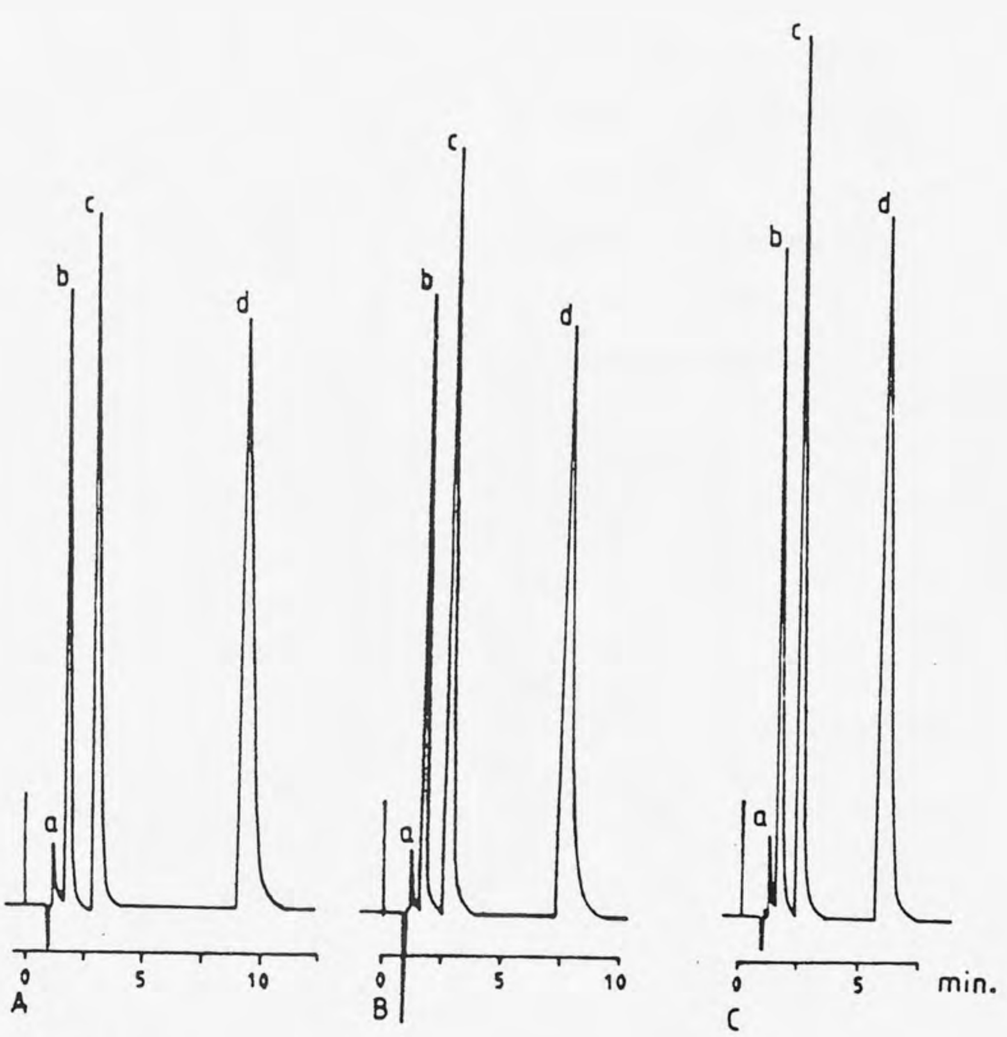
where

t_o	= retention time of unretained solute.
t_A, t_B	= retention times of compounds A and B respectively in time units (second or minute)
w_A, w_B	= peak width of compound A and B respectively, in time units
L	= Column length.

Various compositions of either methanol or acetonitrile in the mobile phases were used in order to find the optimum system to resolve the constituents and to optimize their separation.

Figure 2.21 shows the effect of changing methanol concentration in the mobile phase on the elution characteristics of the indomethacin mixture. While figures 2.22 and 2.23 display the effect of changing the proportion of methanol in the mobile phase on the retention times and the column capacity ratios respectively for the various constituents of the mixture. The column efficiency (N) together with the height equivalent to a theoretical plate (H) are displayed along with the other parameters calculated from the chromatograms in Table 2.6. Indomethacin and its degradative products are weak organic acids with pK_as around 4.

The pH of the mobile phases were thus acidified to near pH 2.5 in order that these compounds would be present in their non-ionized forms.



HPLC conditions:

Column: ODS-Hypersil (5 μm) 10 cm x 4.6 mm i.d.; Flow rate: 1 ml/min.;

Sensitivity: 0.16 A.U.F.S.; Detection wavelength: 235 nm;

Mobile phase	%Methanol in water acidified with 0.2% orthophosphoric acid.
A	65
B	67
C	70

<u>key</u>   <u>concentration (μg/ml)</u>

		concentration (μg/ml)	
a	solvent front.		
b	MIAA.	20	sample solvent: buffer pH 7.61
c	p-chlorobenzoic acid.	13	
d	indomethacin.	45	

Fig. 2.21. The effect of the methanol concentration in the mobile phase on the elution characteristics of indomethacin and its degradative products.

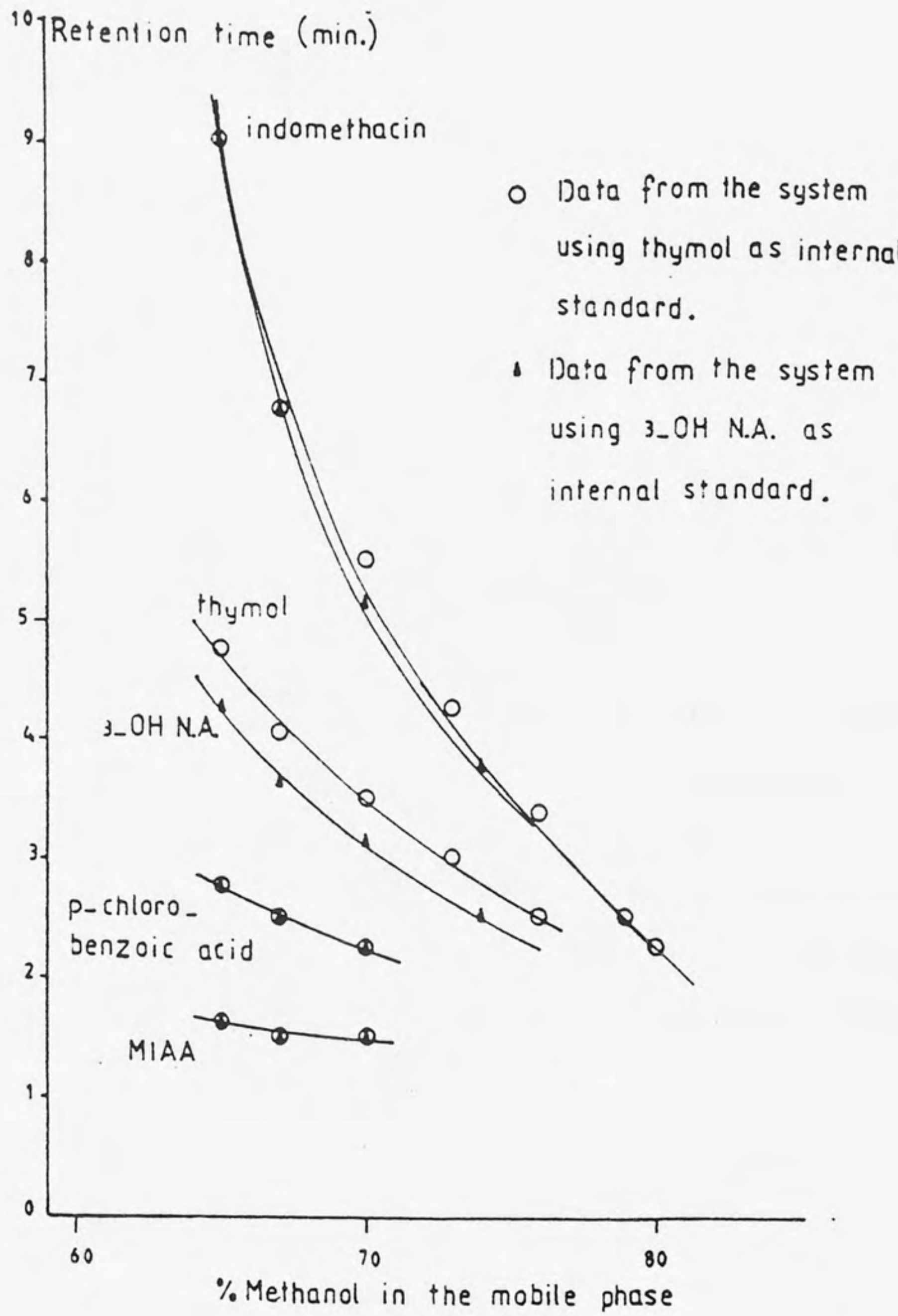


Fig. 2.22. The effect of the methanol concentration in the mobile phase on the retention times for indomethacin and its degradative products.

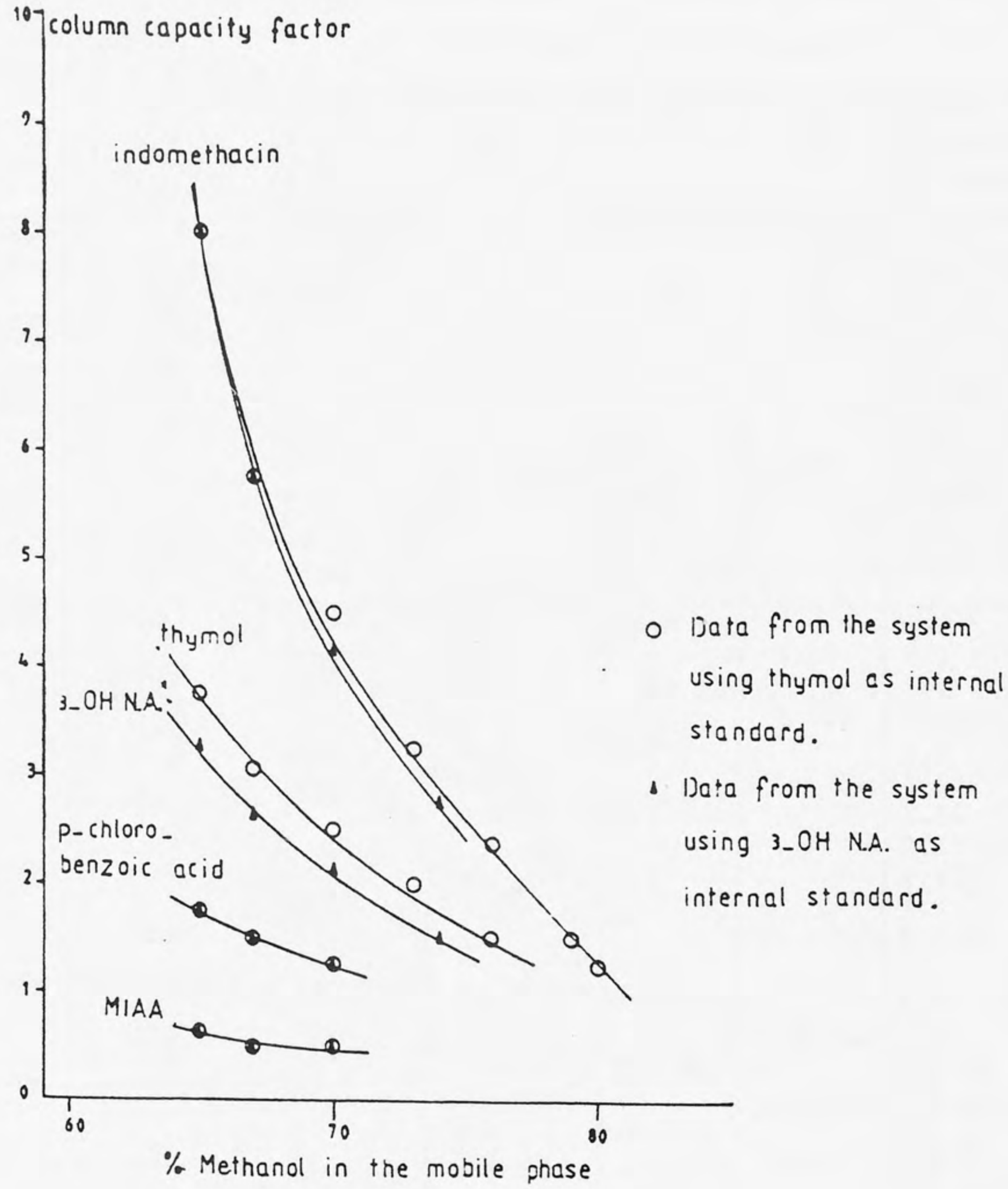


Fig. 2.23. The effect of the methanol concentration in the mobile phase on the column capacity factor for indomethacin and its degradative products.

Table 2.6

Effects of the concentration of methanol in the mobile phase on the separation of indomethacin mixture on the HPLC

Mathematical Parameter	%v/v Methanol	Materials in the Mixture				
		MIAA	p-chloro-benzoic acid	Thymol	3-OH* N.A.	Indo-methacin
Retention time (min.) t_r	65	1.625	2.75	4.75		9.0
	65	1.625	2.75		4.25	9.0
	67	1.5	2.5	4.05		6.75
	67	1.5	2.5		3.625	6.75
	70	1.5	2.25	3.5		5.5
	70	1.5	2.25		3.125	5.125
	73			3.0		4.25
	74				2.5	3.75
	76			2.5		3.375
	79					2.5
	80					2.25
Column Capacity Factor K'	65	0.625	1.75	3.75		8.0
	65	0.625	1.75		3.25	8.0
	67	0.5	1.5	3.05		5.75
	67	0.5	1.5		2.625	5.75
	70	0.5	1.25	2.5		4.5
	70	0.5	1.25		2.125	4.125
	73			2.0		3.25
	74				1.5	2.75
	76			1.5		2.375
	79					1.5
	80					1.25
N	65	75.11	158.04	231.04		324
	65	75.11	158.04		109.44	256
	67	64	130.612	149.48		182.25
	67	64	130.612		134.56	182.25
	70	92	144	196.0		215.11
	70	92	144		156.2	268.96
	73			144		184.96
	74				100	144.0
	76			177.7		201.93
	79					100
	80					81
H	65	1331.38	632.75	432.82		308.64
	65	1331.38	632.75		913.74	390.625
	67	1562.5	765.62	668.98		548.69
	67	1562.5	765.62		1070.2	548.69
	70	1086.95	694.44	510.2		464.87
	70	1086.95	694.44		1000	371.8
	73			694.44		540.65
	74				1000	694.44
	76			562.52		548.69
	79					1000
	80					1234.56

* 3-OH N.A. = 3-hydroxynaphthoic acid.

Table 2.6 continued

Mathematical Parameter	%v/v Methanol	Materials in the Mixture				
		MIAA + p-chlorobenzoic acid	p-chlorobenzoic +Thymol	p-chlorobenzoic acid + 3-OH N.A.	Thymol+ indomethacin	3-OH N.A.+ indomethacin
Resolution R_s	65	1.38	1.88		2.61	
	65	1.38		1.2		2.45
	67	1.23	1.40		1.62	
	67	1.23		1.05		1.92
	70	1.09	1.42		1.6	
	70	1.09		1.0		1.777
	73				1.111	
	74					1.111
	76				1.02	

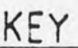
* 3-OH N.A. = 3-hydroxynaphthoic acid.

Two systems were able to combine the ability to resolve the constituents of the mixture concerned along with having favourable mathematical parameters, they are:

(i) 55%v/v Acetonitrile in water acidified with 0.1%v/v orthophosphoric acid (pH 2.5). This system resulted in the mixture constituents having column capacity ratios of: MIAA = 0.4, p-chlorobenzoic acid = 1 and indomethacin = 4.25.

(ii) 70%v/v Methanol in water acidified with 0.2%v/v orthophosphoric acid (pH 2.5).

Chromatograms representing the resolution obtained for the indomethacin mixture using these systems are shown in Fig. 2.24. Studying the chromatograms and the mathematical parameters displayed in



KEY

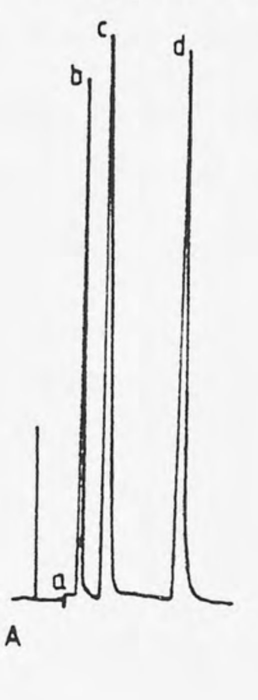
a solvent front.

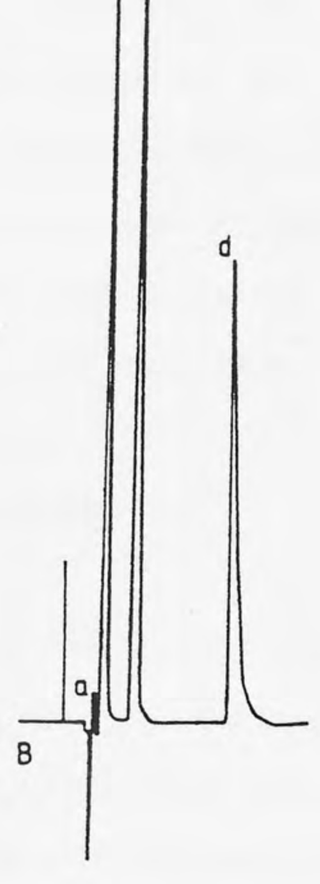
b MIAA.

c p_chlorobenzoic acid.

d indomethacin.

sample solvent: buffer pH 7.61





HPLC conditions:

Column : ODS_Hypersil (5 μm) 10 cm × 4.6 mm i.d.;
Flow rate: 1 ml/min. ; Sensitivity: 0.16 A.U.F.S.;
Detection wavelength: 235 nm; Chart speed: 1cm/2 min.;
Mobile phases: A _ 55% Acetonitrile in water acidified with 0.1% orthophosphoric acid. B _ 70% Methanol in water acidified with 0.2% orthophosphoric acid.

name	concentration(μg/ml)
indomethacin	45
p_chlorobenzoic acid	13
MIAA	20

Fig. 2.24. HPLC separation of indomethacin and its degradative products.

Table 2.6 for systems using different proportions of methanol or acetonitrile than those above show a poor resolution for the various constituents of indomethacin mixture, where for lower concentrations of methanol or acetonitrile all components tend to have higher retention times and column capacity ratios displaying a wider peak base and lead to a longer analysis time. Higher proportions of organic solvent lead to lower retention times and column capacity ratios with narrow peak base resulted in peak overlapping and poor resolution.

(b) Choosing the experimental wavelength:

Indomethacin has three maximum UV absorption bands at 230, 267 and 319 nm while p-chlorobenzoic acid has only one at 235 nm and MIAA at 235 and 290 nm. In addition, high absorption intensities are shown by the three compounds at 235 nm which makes this wavelength usable for their HPLC-UV detection.

(c) Internal standard selection and quantitative anlaysis of samples

The search for a suitable internal standard to be used in either of the proposed separation systems of mobile phases have been completed and include a number of compounds. These are:

(i) The acetonitrile system (using 55% acetonitrile in water acidified with 0.1%v/v orthophospheric acid (pH 2.5)). Several compounds were assessed and these are listed together with their retention times in Table 2.2. It is clear that thymol would overlap indomethacin if used where as chloroxylenol appears ideal and was selected as an internal standard.

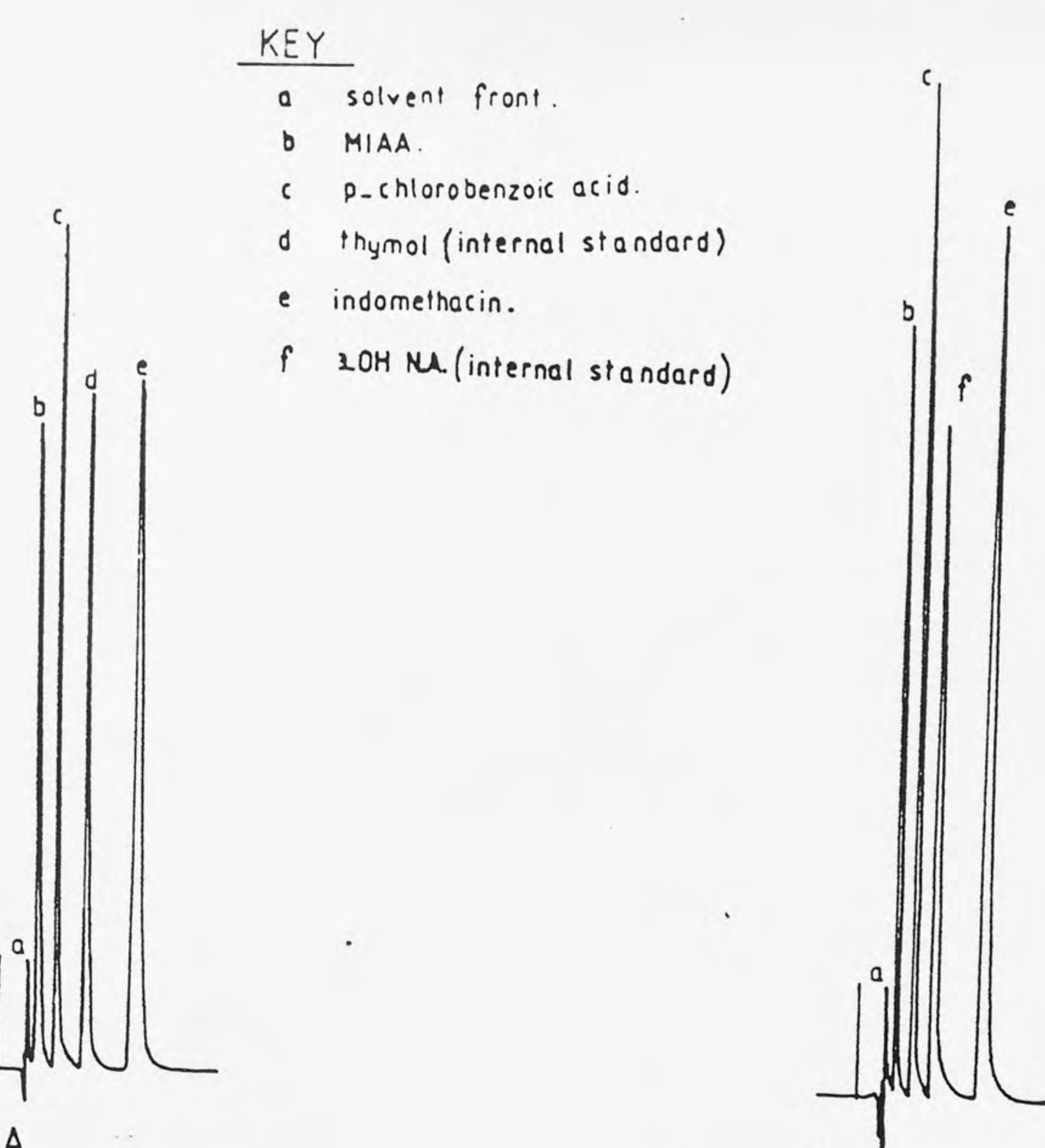
(ii) The methanol system (using 70% methanol in water acidified with 0.2% orthophosphoric acid). Several compounds were assessed and these are listed together with their retention times in Table 2.2. Thymol and 3-hydroxynaphthoic acid (3-OH N.A.) were found to be suitable for use as internal standards (Fig. 2.25).

Although both methanol- and acetonitrile-based mobile phases were adequate, the one containing methanol was preferred for economic and safety reasons. Fig. 2.26 represents calibration curves of peak ratios as a function of indomethacin, p-chlorobenzoic acid and MIAA concentrations respectively with their statistical parameters.

Following the decomposition of indomethacin in Teorell-Stenhagen's buffer pH 7.61 at 69°C was used to show the effectiveness of this analytical system and Fig. 2.27(a and b) show the first order rate of disappearance of indomethacin with time (min.) and the production of the degradation products p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid (MIAA).

(d) Effect of the alcohol content of the mobile phase and sample solvent on the peak ratio and the quantitative estimation in the indomethacin mixture:

A set of experiments carried out to study the peak ratio profiles exhibited by the constituents of a series of solutions containing a constant concentrtion each of indomethacin, p-chlorobenzoic acid and MIAA with either thymol or 3-hydroxynaphthoic acid as internal standards.



HPLC conditions:

Column : ODS-Hypersil (5 μm) 10 cm × 4.6 mm i.d.;

Flow rate: 1 ml/min. ; Sensitivity: 0.16 AUFS.;

Detection wavelength: 235 nm; Chart speed: 1 cm/2 min.;

Mobile phase : 70% Methanol in water acidified with 0.2% orthophosphoric acid.

name	concentration (μg/ml)
indomethacin	45
p-chlorobenzoic acid	13
MIAA	20
thymol	500
2OH NA.	6

sample solvent: methanol in buffer pH 6.1

Fig. 2.25. HPLC separation of indomethacin and its degradative products with the selected internal standards.

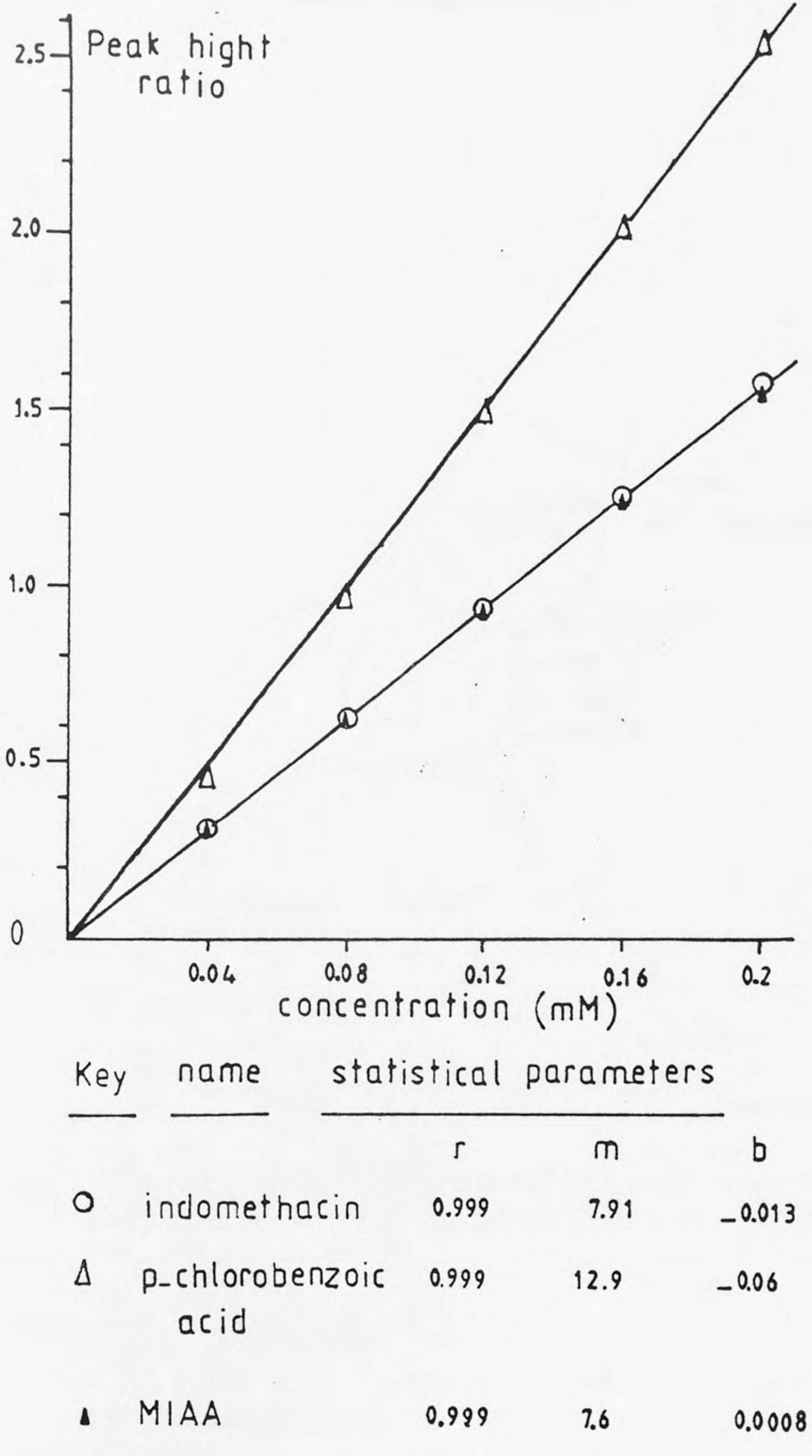


Fig. 2.26. Calibration curves for indomethacin and its degradative productes in buffer pH 7.61 .

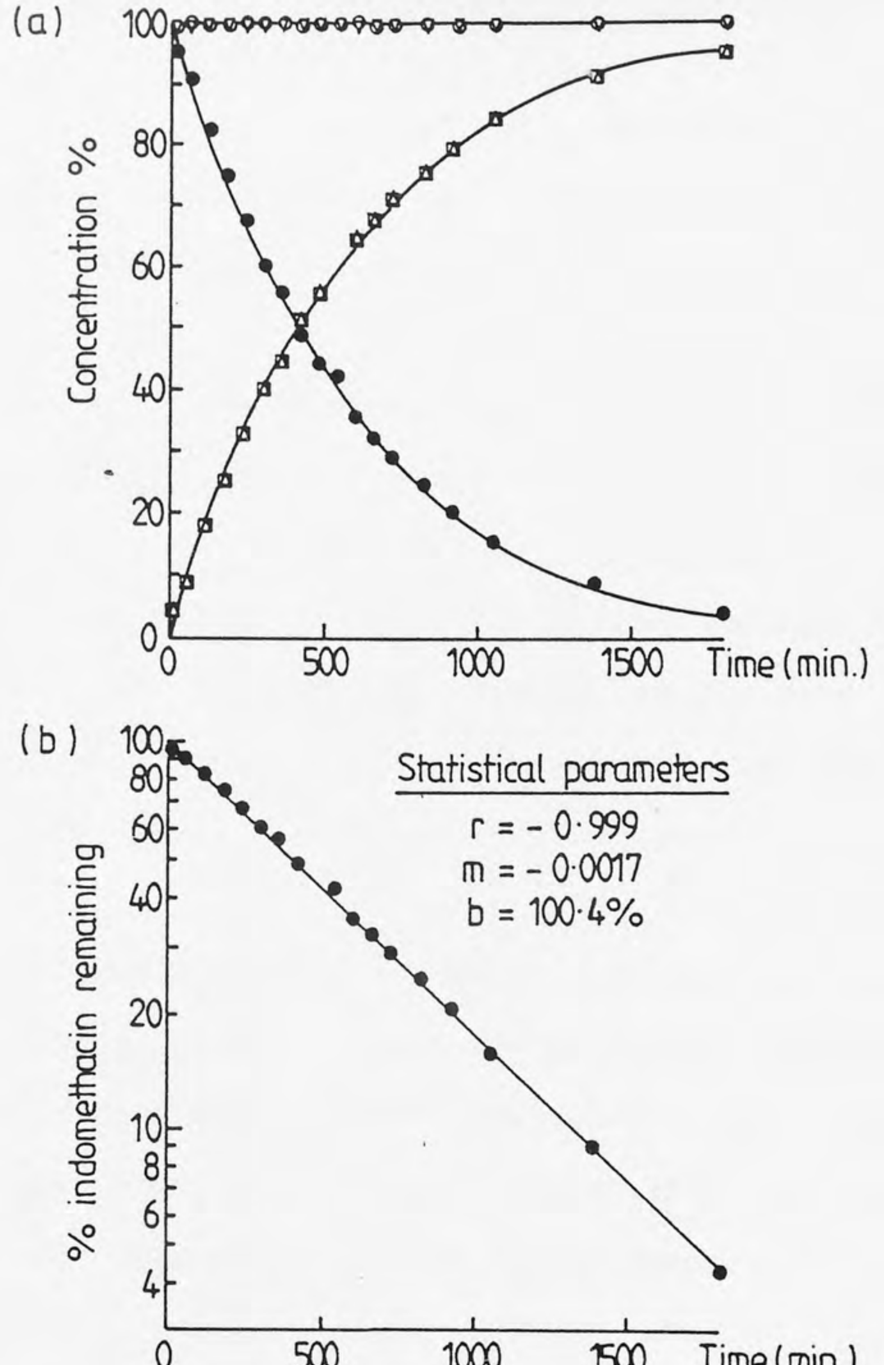


Fig. 2.27 (a) Concentration - time profiles and (b) first order plot for the degradation of indomethacin in Teorell-Stenhagen's buffer pH 7·61 at 69°C.

KEY
- ● indomethacin remaining (I)
- □ p-chlorobenzoic acid formed (II)
- △ 5-methoxy-2-methylindole-3-acetic acid formed (III)
- ○ mass balance of I + II
- ▽ mass balance of I + III

Sample solvent composition varying between 10-100% methanol by volume and mobile phase compositions in the range 65-76% methanol in water acidified with 0.2% orthophosphoric acid were investigated.

When resolving a set of sample solutions using one mobile phase, the sample constituents were subjected to a change in the sample solvent composition within the solutions of the set and displayed a considerable difference in peak heights and peak ratio profiles for each constituent although their peak areas remains constant as has been previously reported(242). This effect is due to the change in the sample solvent polarity which ultimately affects the column efficiency.

Two internal standards were selected, thymol and 3-hydroxynaphthoic acid, to eliminate possible interaction of the mixture constituents with thymol. The degradative products were studied with both internal standards using mobile phases upto 70% methanol and beyond this concentration they were eluted with the solvent front.

With indomethacin mobile phases up to 74% in methanol was used with 3-OH N.A. and up to 76% for thymol after which poor resolution and peak overlapping appeared. Figure 2.28 represents the peak height profiles of the mixture of constituents, together with thymol, as a function of the alcohol proportion in the sample solvent obtained using a mobile phase composed from 70% methanol in water acidified with orthophosphoric acid 0.2%v/v. Examination of these profiles showed that the sample constituent peak heights increase with increasing polarity of the sample solvent(243) and have a maxima when identical alcohol proportions are present in both the sample solvent and the mobile phase. This does not

sample solvent: methanol in buffer pH 6.1

key	name	concentration(μg/ml)
▲	indomethacin	45
●	p-chlorobenzoic acid	13
▲	MIAA	20
○	thymol (internal standard)	500

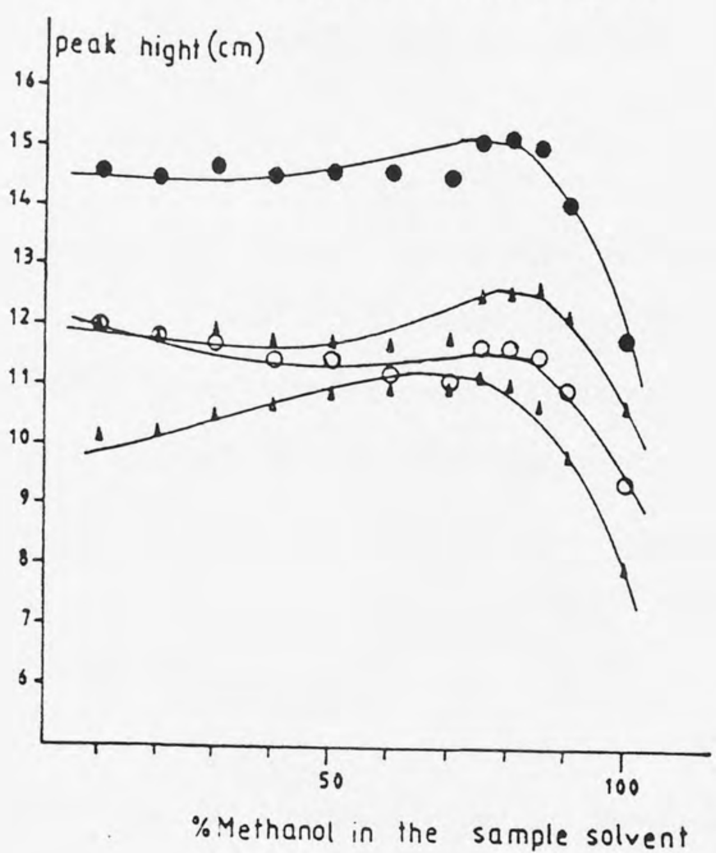


Fig. 2.28. Profiles for the effect of the methanol concentration in the sample solvent on the peak hights for indomethacin and its degradative products using 70% Methanol in water acidified with 0.2% orthophosphoric acid mobile phase .

necessarily maximise the column efficiency but gives a good separation of the sample constituents. Alternatively, as the sample solvent alcohol concentration increases over that of the mobile phase the sample peak heights decreases and drastic degradation in column efficiency is observed resulting in poor resolution and peak base broadening(242).

In a set of test solutions the effects observed on the peak heights of each constituent brought about by changes in the polarity of the sample solvent or that of the mobile phase should not affect in any way their peak areas(243) when they represent the amount of material present. The separation of one set of solutions was carried out using a mobile phase containing 67% of methanol with the signals recorded on a chart recorder at a speed of 1 mm/sec to allow sufficient space on the chart for each peak when separated.

An assessment of the peak areas for each constituent obtained from chromatograms of the test solutions was done by the weighing method assuming a uniform thickness throughout the chart paper and is displayed in Table 2.9 together with the corresponding standard deviation which are in close agreement with the literature(242).

It is interesting to observe that increasing the polarity of the mobile phase also led to a decrease in the samples peak heights which is in agreement with observations by Giddings(244). This can be explained on the basis that the sample will occupy more theoretical plates and hence reduce separation efficiency. This effect is more pronounced when the sample solvent polarity is less than that of the mobile phase.

Table 2.7

Lack of effect of change in sample solvent methanol content on peak height areas of indomethacin, its decomposition products and the internal standard, 3-hydroxynaphthoic acid.

Sample solvent methanol %v/v	Weight of peaks (gm)			
	MIAA	p-chloro-benzoic acid	3-OH N.A.	Indomethacin
100	0.04801	0.07304	0.11506	0.13004
90	0.04795	0.07305	0.11504	0.13
85	0.04804	0.07305	0.11501	0.13
80	0.04799	0.07301	0.11505	0.12995
75	0.04805	0.07296	0.115	0.12996
70	0.04796	0.07295	0.11406	0.13
60	0.04805	0.07299	0.1151	0.13
50	0.04802	0.07302	0.11495	0.13005
40	0.04798	0.07295	0.11499	0.13
30	0.04801	0.07298	0.115	0.13
20	0.04795	0.073	0.11494	0.13
10	0.04800	0.073	0.1149	0.13

Standard deviations were:

MIAA = 3.604×10^{-5}

p-chlorobenzoic acid = 3.59×10^{-5}

3-OH N.A. = 5.6×10^{-5}

Indomethacin = 2.73×10^{-5} .

The examination of the peak ratio profiles of the sample constituents revealed that those of MIAA and p-chlorobenzoic acid obtained for each mobile phase used show a maximum near the same proportion of alcohol in the mobile phase. Decreasing the polarity of the mobile phase i.e. increasing its alcohol concentration resulted in higher peak ratios and with the maximum always placed near the

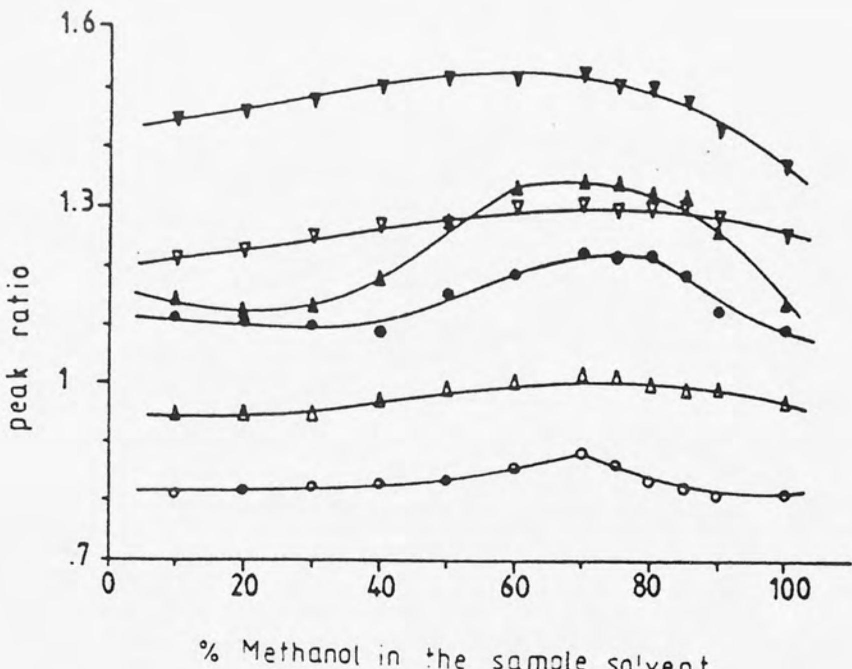
concentration of the alcohol in the mobile phase.

5-Methoxy-2-methylindole-3-acetic acid and p-chlorobenzoic acid were studied with mobile phases with up to 70% methanol content and Figs. 2.29 and 2.30 show the peak ratio profiles. The profiles obtained from indomethacin separation (Fig. 2.31) show basically an increase in the peak ratio when decreasing the polarity of the sample solvent with high mobile phase polarity. This could be attributed to poor column capacity ratio and resolution. Furthermore, increasing the alcohol content in the mobile phase produced higher peak ratio profiles as expected up to 70% methanol concentration after which lower profiles were exhibited.

In contrast to the ones resolved by lower mobile phase alcohol concentration these displayed a maximum near the alcohol concentration of the mobile phase while the previous ones show a drastic increase in peak ratio around that value. Such results indicate that optimisation of the sample solvent after choice of the appropriate mobile phase is essential.

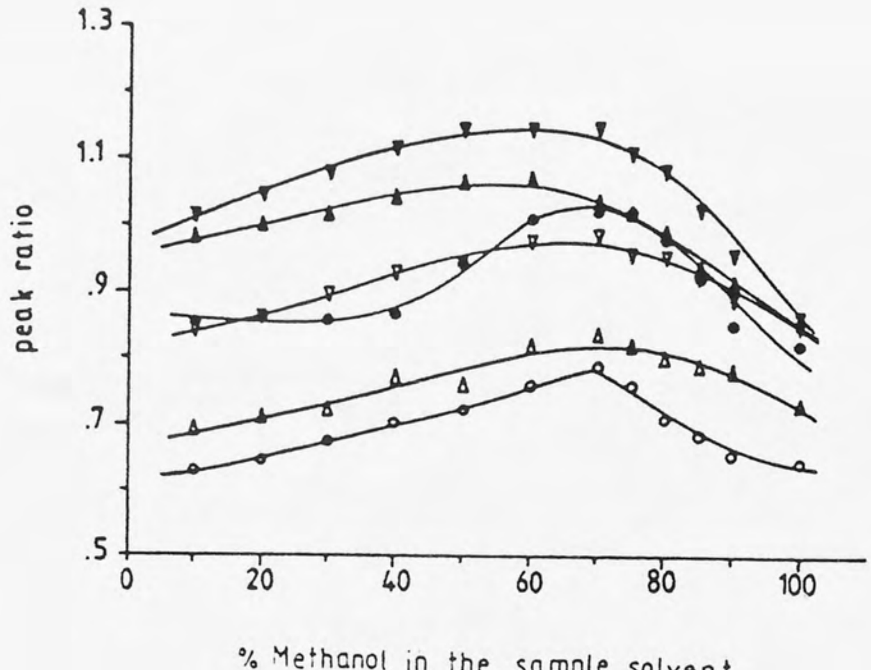
2. The development of the HPLC analytical system for the identification and assay of ibuprofen and its esters

The development of the HPLC analytical procedure was carefully planned to include the steps discussed previously for the identification of indomethacin. The development of a mobile phase for the quantitative estimation of ibuprofen and its esters included trials of a number of systems using methanol in water acidified with orthophosphoric acid.



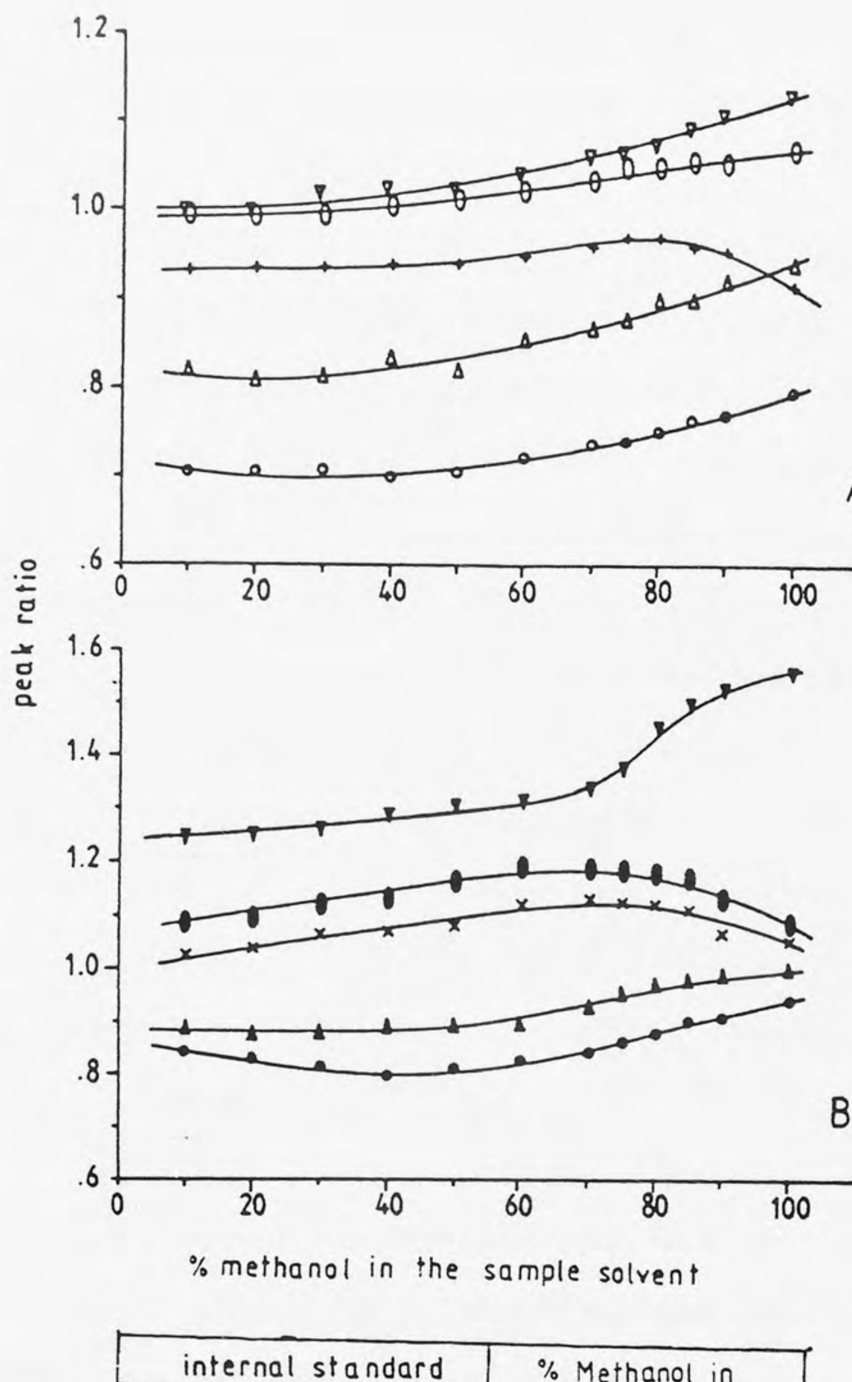
	internal standard		% methanol in the mobile phase
	thymol	3-OH N.A.	
key	▼	▼	70
	▲	▲	67
	○	●	65

Fig. 2.29. Profiles for the effect of the methanol concentration in the sample solvent on the peak ratio for MIAA (20 μg/ml) using several mobile phases.



key	internal standard		% methanol in the mobile phase.
	thymol	3-OH N.A.	
	▼	▼	70
	▲	▲	67
	○	●	65

Fig. 2.30. Profiles for the effect of the methanol concentration in the sample solvent on the peak ratio for p-chlorobenzoic acid (13 μg/ml) using several mobile phases.



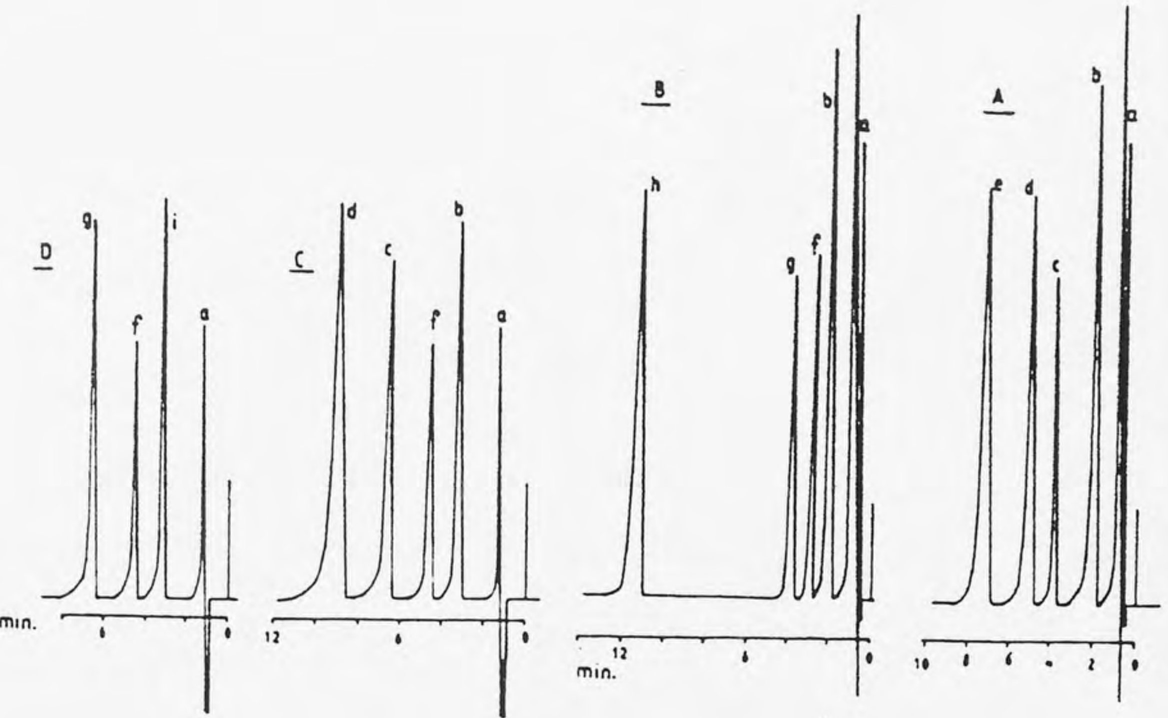
	internal standard		% Methanol in the mobile phase
	thymol	3OH N.A.	
	A	B	
key	+		76
		×	74
	o	⬮	73
	▼	▼	70
	▲	▲	67
	○	●	65

Fig. 2.31. Profiles for the effect of the methanol concentration in the sample solvent on the peak ratio for indomethacin (45 µg/ml) using several mobile phases.

The system was composed of 31%v/v methanol in water and acidified with 0.3%v/v orthophosphoric acid. This was selected over others for it gave a good separation of ibuprofen and its esters. Due to the fact that both ethyl- and the (tetrahydro-2-furanyl)methyl esters of ibuprofen have the same retention time, Fig. 2.32(a) shows the separation of ibuprofen, its (tetrahydro-2-furanyl)methyl ester and the (tetrahydro-2-(2H)pyranyl)methyl ester, with retention times of R_t = ibuprofen = 1.8 (min.), (tetrahydro-2-furanyl)methyl ester = 3.7 (min.) and the (tetrahydro-2-(2H)pyranyl)methyl ester = 4.8 (min.). Fig. 2.32(b) shows the separation of the ethyl- and cyclohexylmethyl esters with retention times of ethyl = 3.7 (min.) and cyclohexylmethyl ester = 11 (min.). Ibuprofen is a weak organic acid with a pKa reported to be 4.5(245) so the pH of the mobile phase was adjusted to pH = 2.5 with orthophosphoric acid 0.3%v/v to ensure that ibuprofen was present in its non-ionized form. The pH has no effect upon the esters as they are chemically neutral.

The analytical wavelength chosen was 220 nm (Fig. 2.2). The quantitative analysis of ibuprofen and its esters involved choosing a suitable internal standard and the use of calibration curve. Several materials were assessed and these are listed together with their retention times in Table 2.3.

In addition, the quantitative analysis made use of standard or calibration curves which showed acceptable statistical factors, when peak ratios as a function of concentration for each material were used. Fig. 2.33 represents calibration curves for ibuprofen and its (tetrahydro-2-(2H)pyranyl)methyl ester used for the quantitation of the samples using peak ratios obtained from the experiment following the



HPLC conditions:

Column : ODS-Hypersil (5 μm) 10 cm × 4.6 mm i.d.;

Flow rate: 1 ml/min. ; Sensitivity: 0.02 A.U.F.S. ;

Detection wavelength: 220 nm; Chart speed: 1cm/2 min.;

Mobile phases:	% Methanol in water acidified with 0.3 % orthophosphoric acid.
A, B	81
C, D	76

a = solvent front.

Fig. 2.32. HPLC separation of ibuprofen (b) and its (tetrahydro-2-furanyl) methyl ester (c), (tetrahydro-2-(2H)pyranyl) methyl ester (d), ethyl ester (g) and cyclohexylmethyl ester (h) together with flufenamic acid (f), indomethacin (i) and hexachlorophane (e).

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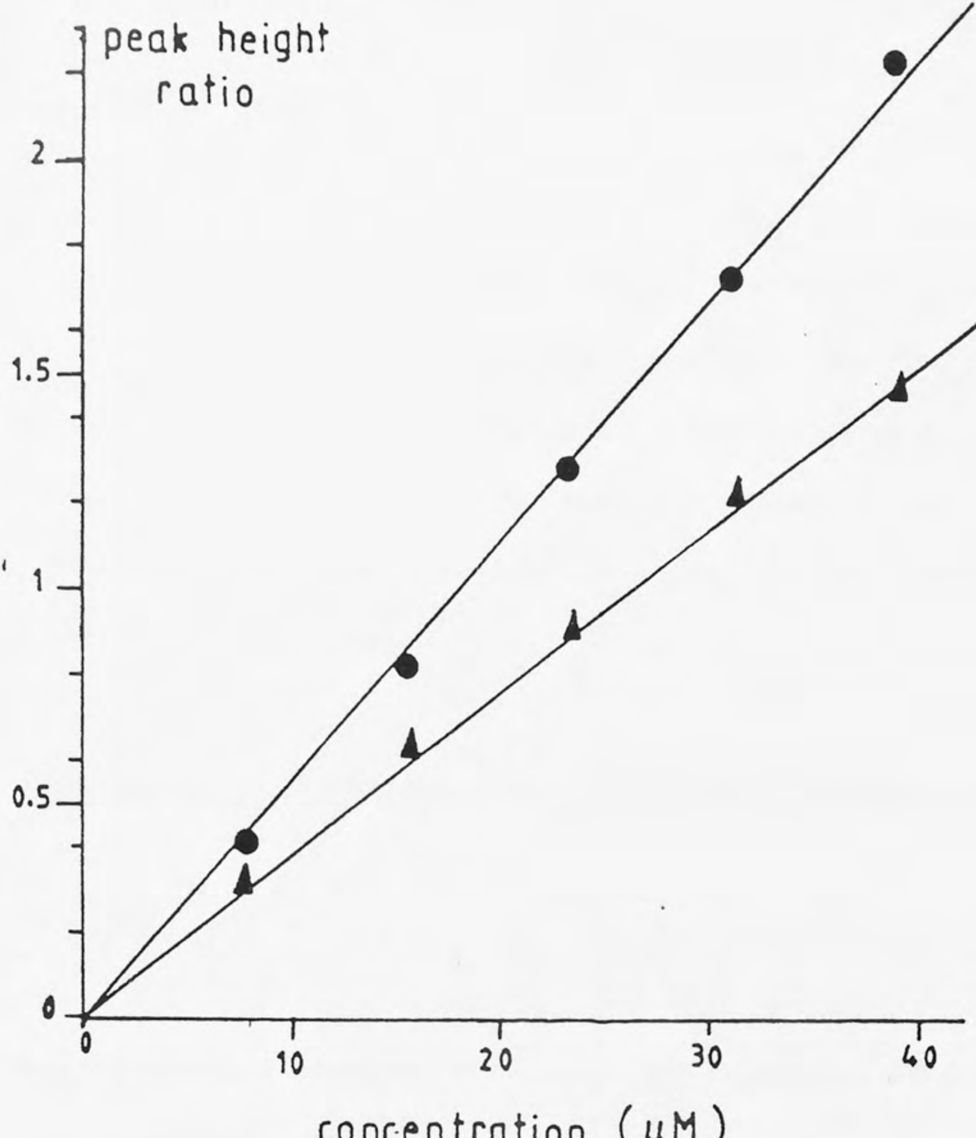

Key	name	statistical parameters		
		r	m	b
●	ibuprofen	0.999	58814	-0.069
▲	(tetrahydro-2-(2H)-pyranyl)methyl ester	0.999	37158	0.041

Fig. 2.33. Calibration curves for iburofen and its (tetrahydro-2-(2H)pyranyl) methyl ester in buffer pH 7.0 .

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ester degradation in Teorell-Stenhagen's buffer pH 7.0 at 70°C. Figure 2.34 shows the first order rate of degradation of the (tetrahydro-2-(2H)pyranyl)methyl ester with time under these conditions together with the production of ibuprofen. Both samples and standards were carefully made to contain the same composition of solvent in their solutions to prevent any discrepancy in peak ratio of both samples and standards produced due to differences in solvent composition. All the solutions were then mixed with a suitable solution of the selected internal standard, hexachlorophane.

3. Separation of compounds used in the skin permeation studies:

The HPLC analytical system for the analysis of indomethacin, ibuprofen and its esters during the skin permeation studies were designed to resolve mixtures of any of the compounds used with a marker compound, flufenamic acid.

Several mobile phases were tested composed of methanol in water acidified with 0.3%v/v orthophosphoric acid, but due to the fact that flufenamic acid has a retention time intermediate between ibuprofen and its (tetrahydro-2-furanyl)methyl ester, it was impossible to resolve all components with the system used for the kinetic studies of the esters. Poor resolution was observed due to peaks related to ibuprofen overlapping with those from flufenamic acid and the (tetrahydro-2-furanyl)methyl ester. A system containing 76%v/v methanol in the mobile phase was thus used for analysis of mixtures containing ibuprofen with either its (tetrahydro-2-furanyl)methyl ester or its (tetrahydro-2-(2H)pyranyl)methyl ester and flufenamic acid (Fig. 2.32(c)).

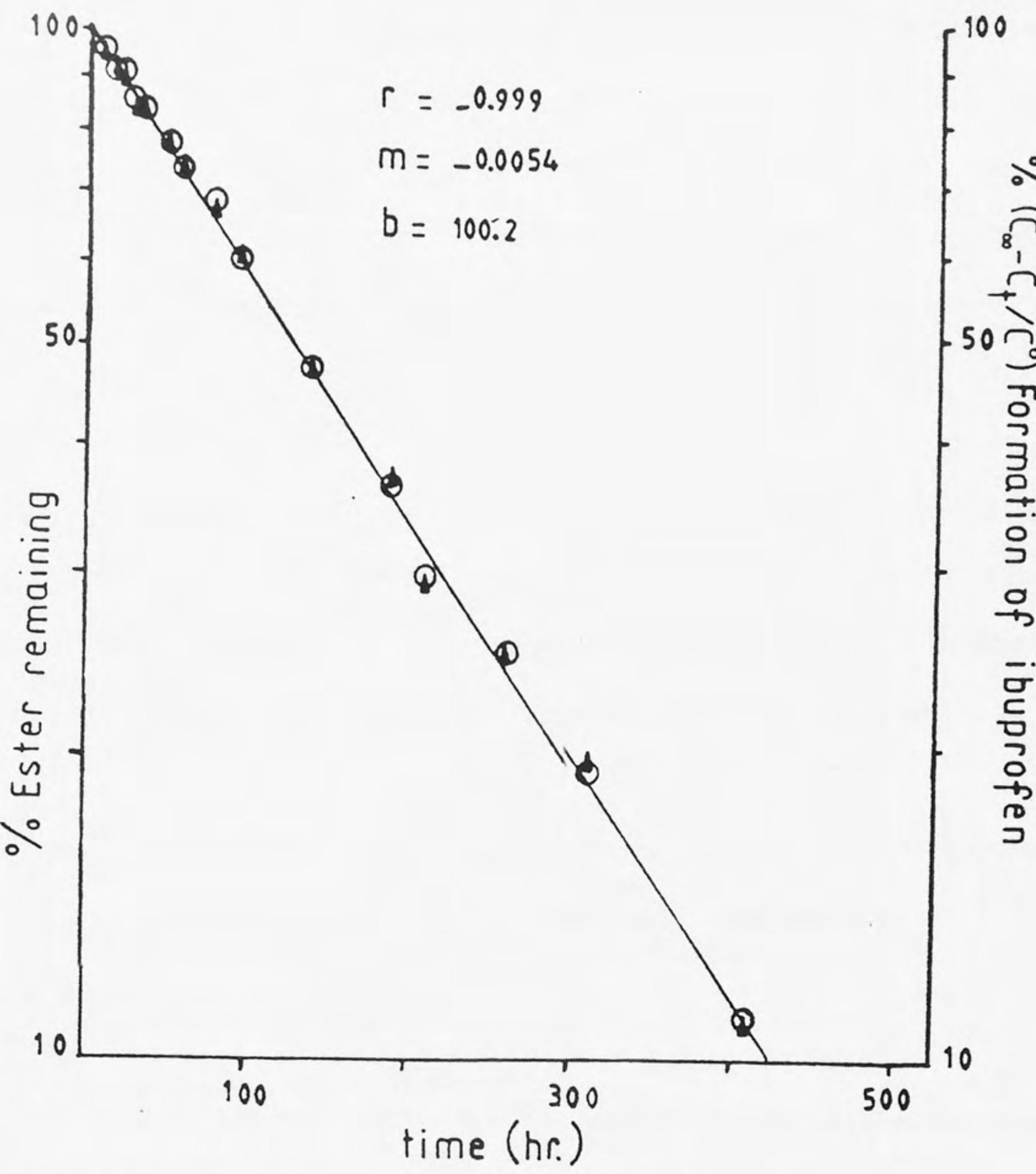

Fig. 2.34. The first order decomposition of (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen (○) and the formation of ibuprofen (▲)(normalised to ester concentration) in buffer pH 7.0 at 70°C.

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Due to the long R_t of the cyclohexylmethyl ester this mobile phase was not appropriate. A mobile phase (Fig. 2.32(b)) with 81% methanol was used for the separation of mixtures containing this ester with flufenamic acid and ibuprofen if produced as a result of degradation. The analytical wavelength (220 nm) was the same as that used in the kinetic runs.

Hexachlorophane (7 µg/ml), the ethyl ester of ibuprofen (10 µg/ml) and the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen (10 µg/ml) were selected as internal standards. Table 2.8 shows the internal standards chosen for the different mixtures analysed.

Figure 2.35 shows typical calibration curves for ibuprofen and flufenamic acid together with their statistical parameters.

Table 2.8

The choice of the internal standard for analysing the different mixtures used in the skin permeation studies.

Mixture	Internal Standard
Ibuprofen + flufenamic acid	Ethyl ester of ibuprofen
(Tetrahydro-2-furanyl)methyl ester of ibuprofen + flufenamic acid	(Tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen
(Tetrahydro-2-(2H)pyranyl)methyl-ester of ibuprofen + flufenamic acid	Ethyl ester of ibuprofen
Cyclohexylmethyl ester of ibuprofen + flufenamic acid	Hexachlorophane
Indomethacin + flufenamic acid	Ethyl ester of ibuprofen

r = 0.999

m = 547.4

b = 0.009

peak ratio

1.0
0.5

1
2

r = 0.999

m = 1111.3

b = 0.008

0.4
0.3
0.2
0.1

0.1
0.2
0.3
0.4

concentration (mM)

Fig. 2.35. Calibration curves for ibuprofen (●) and flufenamic acid(▲) in 50% propylene glycol water mixture.

Table 2.9 shows the concordance found between theoretical and experimentally determined concentrations of ibuprofen and flufenamic acid in the diffusion mixture.

Table 2.9

Estimation of the initial conentrations of ibuprofen and flufenamic acid in diffusion mixtures and correlation with the theoretical values.

Material in the Mixture	Correlation of the Initial Concentration with that expected from Weight Data* and the Observed Standard Deviation
Ibuprofen	99.93 ± 0.0727
Flufenamic acid	99.98 ± 0.194

* mean of six replicates.

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CHAPTER 3

THE DETERMINATION OF SOLUBILITY AND pK_a

3.1 INTRODUCTION

Several parameters play a key role in controlling drug efficacy. Aqueous solubility, pK_a and partition coefficient are important physicochemical properties which may be used to model drug availability. A knowledge of the molecular weight, chemical structure, ionization constant and the solubility in both aqueous and in mixed solvents, gives a better understanding of drug behaviour and therefore enable optimisation of its formulations. An orally administered drug must dissolve in the gastrointestinal fluid before it can be bioavailable and both the dissolution rate and the maximum amount of the drug that can be dissolved may be controlled by the solubility of the drug in the medium(246).

Insufficient aqueous solubility may result in a drug showing poor activity whereas structural modifications designed to improve solubility may enhance the observed effect. Quantitative study of the relationship between aqueous solubility and chemical structure is under intensive study by Martin and co-workers(247) to predict and model these effects.

The developed solubility relationships(72,246,248-250) deal with ideal and regular solutions and are based mostly on the concept of solubility parameters as proposed by Hildebrand(251,252). These are not well suited to explaining the aqueous solubility of non-polar substances and weak electrolytes. This is due to limitations such as the lack of

readily available thermodynamic data and the difficulty in determining such data.

A need is thus apparent for an understanding of the factors which govern the solubility in polar solvents for which regular solution theory is not applicable. To this end investigations were undertaken in which the solubility relationships with respect to dielectric constant of mixtures(248,253,254), partition coefficient(71,72,247,249,255), volume fraction and the chain length in a series of compounds(250) were studied. Hansch and co-workers(256) have correlated the molecular structure of a series of chemically related compounds with biological activity using multiple regression models based upon the partition coefficient. Additionally, the solubility of weak electrolytes (weak acids or weak bases) at a given pH is governed by the ionization constants. These determine the concentration of the ionized and non-ionized species of the drug and therefore the pH can control the dissolution of these drugs in biological fluids(79).

3.2 THEORY

3.2.1 The Effect of pH on the Solubility of Weak Acids

The observed solubility (S) of a weak acid (HA) in aqueous solution at a given pH is equal to the sum of two terms, the solubility of the neutral molecule, $[HA]_0$, which equals the intrinsic solubility (a saturated solution) and the solubility of the ionized form $[A^-]$ (far from saturated). Thus

$$S = [HA]_o + [A^-] \qquad (3.1)$$

combining equation (3.1) with the K_a expression of the acid yields:

$$S = [HA]_o + \frac{K_a [HA]_o}{[H^+]} \qquad (3.2)$$

substituting the intrinsic solubility by the term (S_o) we may write:

$$S = S_o \left(1 + \frac{K_a}{[H^+]}\right) \qquad (3.3)$$

where $[H^+]$ is the hydrogen ion concentration at equilibrium.

The logarithmic transformation of equation (3.3) yields the pK_a of the weak acid as:

$$pK_a = pH - \log \left(\frac{S-S_o}{S_o}\right) \qquad (3.4)$$

The total solubility of the weak acid as a function of pH under controlled temperature can be used to determine its pK_a, but because solubilities are highly sensitive to the presence of foreign ions and in order for the values obtained to be thermodynamically accurate, it is necessary to do the determination at a constant ionic strength. So from equation (3.2) a plot of (S) versus $1/[H^+]$ will yield an intercept of $[HA]_o$ or the intrinsic solubility and a slope of $K_a[HA]_o$, from which K_a is obtained by dividing the slope by the intercept.

Alternatively, the method described by Krebs and Speakman(76) allows the intrinsic solubility (S_o) to be determined at a pH where the acid exists totally as its neutral species. This can be achieved by determining the solubility of the weak acid in a solution of 0.1 M HCl. A further two readings can be determined at similar pH values in order to confirm the true (S_o). The solubility is then determined at the pH suspected to be near the acid's pK_a and equation (3.4) is used to calculate the approximate pK_a. This is followed by solubility measurements at a series of pH values at a constant ionic strength well distributed within the range $pK_a \pm 1$.

3.2.2 Potentiometric Titrations for the Determination of pK_a

Potentiometric titration of a weak acid (HA) by alkali can be used to determine pK_a. A problem arises when the compound under study is poorly soluble in water but is highly soluble in aqueous organic mixtures. The pK_a of the acid may be determined in a mixed solvent system such as aqueous ethanolic mixtures.

However, the alcohol will generally weaken the acid strength and thus the pK_a will be raised with increasing alcohol concentration.

Extrapolation of the pK_a data of the acid to the pure aqueous system will enable an estimation of the aqueous pK_a.

Non-linear relationships are often encountered and therefore low levels of the organic component are recommended to obtain a reliable result(79,82-84,257,258). The logarithmic titration method described by

Li Wan Po and Irwin (257) can be used to obtain the pK_a of a weak acid when potentiometrically titrated by alkali provided that no precipitation takes place and the method was applied to determine the pK_a of some tricyclic anti-depressants in which aqueous alcoholic solutions were used to provide high solubility condition for these bases(257). The equation used to describe this model is:

$$pK_a = pH + \log \frac{a - [H_3O^+] + [OH^-]}{b + [H_3^+O] - [OH^-]} \qquad (3.5)$$

where a is the initial molar concentration of an acid HA in a mixture with its salt M^+A^- of molar concentration b.

Benet and Goyan(79) faced a problem in converting what they call the P_sK_a value (those obtained in partly aqueous solutions) to pK_a values although their P_sK_a values were obtained with high accuracy but they couldnot extend that to the aqueous dissociation constants. In addition, they recommended that aqueous methanolic mixtures among other solvent systems be used because it provides less error than others.

Furthermore, in order to avoid the extrapolation of the non-linear portion of the curves it was recommended that extrapolation from the linear plot of $P_sK_a + \log[H_2O]$ against $1/\varepsilon$ (where ε is the dielectric constant of the mixed solvent)(79,83) be undertaken. The expression of the P_sK_a for a weak acid obtained in a mixed solvent system in the form of $P_sK_a + \log[H_2O]$ was modelled by Shedlovsky(83) and Benet and Goyan(79) so that extrapolation will result in the pK_a value of the pure aqueous system. The dissociation constant of a weak acid in mixed solvents at equilibrium is

$$HA + S \rightleftarrows SH^+ + A^- \qquad (3.6)$$

$$HA + H_2O \rightleftarrows H_3^+O + A^- \qquad (3.7)$$

where (S) is one of the components of the mixed solvent. But since water has a high proton affinity, equation (3.6) can be written as (3.7). If the mixed solvent contains sufficient quantity of water and low concentration of the acid then the dissociation constant in a mixed solvent should be written as

$$K = \frac{[A^-]\ [H_3^+O]}{[HA]\ [H_2O]} \qquad (3.8)$$

where square brackets indicate concentration.

The strength of the acid in water is expressed in the form of K_a which includes the constant term $[H_2O]$.

$$K_a = \frac{[A^-]\ [H_3O^+]}{[HA]} \qquad (3.9)$$

In mixed solvents this term is not equivalent or constant. As a result K should be used for the estimation of the dissociation constant of an acid in mixed solventes in place of K_a(82). Due to the fact that the acitivity of water in mixed solvents containing electrolytes is not known activities are replaced by the concentration of water.

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An extensive view of this problem was presented by Shedlovsky(83). He proposed that on dissolving the acid in water or alcohol a series of events occur. These include the force linkage of the acid with the solvent molecules followed by the transfer or proton shift from the acid carboxylic group to the bound solvent molecules. This ion pair re-arrangement then dissociates into its ionic components. These events are represented in the following equations with their mass action constants proposed by Shedlovsky, where equations (3.10), (3.11), (3.12) and (3.13) (3.14) (3.15) are for those using water or alcohol as solvents respectively together with their mass action constants K_1 to K_6.

The overall constants for each solvent are K_H and K_R:

$$HA + H_2O \rightleftarrows HA.H_2O \quad (3.10) \qquad HA + ROH \rightleftarrows HA.ROH \quad (3.13)$$

$$HA.H_2O \rightleftarrows H_3^+O.A^- \quad (3.11) \qquad HA.ROH \rightleftarrows RH_2^+O.A^- \quad (3.14)$$

$$H_3^+O.A^- \rightleftarrows H_3^+O + A^- \quad (3.12) \qquad RH_2O^+.A^- \rightleftarrows RH_2^+O + A^- \quad (3.15)$$

$$K_1K_2K_3 = \frac{[H_3O^+][A^-]\ f^2}{[HA][H_2O]} = K_H \qquad (3.16)$$

$$K_4K_5K_6 = \frac{[RH_2^+O][A^-]\ f^2}{[HA][ROH]} = K_R \qquad (3.17)$$

where f represents the activity coefficient. And therefore if both water and alcohol are used in the mixed solvent then he proposed that two types of hydrogen ion will be present, H_3^+O and H_2O^+R and their sum should be equal to the carboxylate ion for electroneutrality.

Furthermore, the equilibrium constant (K) obtained from the potentiometric titration experiments should contain both kinds of hydrogen ions in its expression:

$$K = \frac{[A^-][H_3^+O + H_2O^+R]\ f^2}{(HA)} \qquad (3.18)$$

where brackets refer to concentration, the paranthesis to activity and (f) to activity coefficient.

But the experimental dissociation constant is:

$$K = K_H[H_2O] + K_R[HOR] \qquad (3.19)$$

As expressed by Shedlovsky and on making the assumption that

(a) the volume concentrations for all the electrically neutral species can be taken as their activities, and

(b) that coulombic forces control the ionic dissociation expression in equations (3.12) and (3.15),

then by employing the Bjerrum's theory of ionic dissociation(259,260), Shedlovksy obtained:

$$K_H = B_H \exp(-b) \quad \text{and} \quad K_R = B_R \exp(-b) \qquad (3.20)$$

where $b = e^2/\varepsilon Tka$ in which, e is the electronic charge, ε is the dielectric constant of the mixed solvent, k is the Boltzmann constant, T is the absolute temperature and a is the average ionic diameter(79,259,261-265), then:

$$Ke^b = B_H[H_2O] + B_R[HOR] \qquad (3.21)$$

dividing this equation by the total number of moles of solvent/l (S) where:

$$S = [H_2O] + [HOR] \qquad (3.22)$$

then

$$Ke^b/S = B_H X + B_R(1-X) = (B_H - B_R)X + B_R \qquad (3.23)$$

where X is the mole fraction of water. While considering the model proposed earlier it is clear that K_1 and K_4 which represent dipole-dipole interactions and that K_3 and K_6 which represent ion pair dissociation are not very different in magnitude due to the fact that the alkyl group plays a minor role in these processes when its position is distal from the molecular site of interest. In addition, the proton

shift represented by equations (3.11) and (3.14) occurs more readily in water. Therefore K_2 is greater than K_5 and B_R is smaller than B_H. On the assumption that $B_H \gg B_R$ then

$$Ke^b/S \approx B_H X \quad \text{or} \quad Ke^b/[H_2O] \approx B_H \qquad (3.24)$$

and

$$\ln K - \ln[H_2O] + (\frac{e^2}{kTa})(\frac{1}{\varepsilon}) = \ln B_H \qquad (3.25)$$

or

$$P_sK_a + \log[H_2O] = (\frac{e^2}{(2.303)Tka})(\frac{1}{\varepsilon}) - \log B_H \qquad (3.26)$$

then a plot of ($P_sK_a + \log[H_2O]$) against ($1/\varepsilon$) should be linear and (a) can be obtained from the slope(79,83).

3.2.3 Prediction of pK_a Values of Weak Acids using the Taft Equation:

The Taft equation models the ionization constants of aliphatic and alicyclic acids with special attention to the free energy changes affecting these constants:

$$pK = pK^\circ - \rho^* \Sigma (\sigma^*) \qquad (3.27)$$

where pK° is the ionization constant of the parent compound, (ρ^*) is a constant for the particular reaction and (σ^*) is a constant that is a characteristic of a given substituent. This equation defines $\sigma^* = 0$ for

the methyl group giving $\sigma^* = 0.49$ for a hydrogen atom. If the value of σ^* for a substituent (-R) is known then σ^* for $-CH_2R$ can be estimated from the relation:

$$\sigma^*(-CH_2R) \simeq 0.4 \times \sigma^* (-R) \qquad (3.28)$$

while σ^* for the group

```
  X
  |
-C-Y
  |
  Z
```

can be estimated from the σ^* values of $-CH_2X$, $-CH_2Y$ and $-CH_2Z$, so

$$\sigma^*(-CXYZ) \simeq \sigma^*(-CH_2X) + \sigma^*(-CH_2Y) + \sigma^*(-CH_2Z) \qquad (3.29)$$

The Taft equation for aliphatic carboxylic acid having the formula R'R"CHCOOH is:

$$pK_a = 4.8 - 0.66\, \Sigma\, \sigma^* \qquad (3.30)$$

by calculating the value of $\Sigma\sigma^*$ from that of (σ^* of R' + σ^* of R") the pK_a of the acid can be estimated. A collection of published Taft equations and σ^* values are listed by Perrin et al.(266).

3.2.4 The Solubility in Mixed Solvent Systems

The solubility of weak or nonelectrolytes in aqueous or mixed solvent systems might deviate from the well established solubility relationships that deals with regular and ideal solutions. Therefore other relationships have been proposed in the literature which are concerned with correlating the solubility data for nonpolar compounds with other physical parameters such as the partition coefficient of the solute, melting point of the crystalline solid(71,72,249) and the dielectric constant(267-275) of the solvent system. These relationships were used to account thermodynamically for the deviation observed when applying the regular solution theory and may be used to obtain the solubility parameters for the compounds studied.

An equation relating the solubility data with the octanol-water partition coefficient has been proposed by Yalkowsky and Rubino(71) and is given by:

$$\log(S_f/S_w) = (A \log P_{o/w} + D)\, f \qquad (3.31)$$

where S_f is the solubility of the solute in a mixed solvent of volume fraction (f) of cosolvent and S_w is the solubility of the solute in water, $P_{o/w}$ is the octanol-water partition coefficient of the solute and (A and D) are constants which depend only on the partitioning systems.

Equation (3.31) was derived originally from an earlier equation used by the authors where the aqueous solubility of a solute is:

$$\log S_w = \frac{-\Delta S_f(mp - 25)}{1364} - \log P_{o/w} + C_w \qquad (3.32)$$

where ΔS_f and mp are the entropy of fusion and the melting point (°C) of the solute while C_w is a constant which is dependent upon the units chosen for solubility.

The solubililty of the solute in a mixed solvent system (S_c) is:

$$\log S_c = \frac{-\Delta S_f(mp - 25)}{1364} - \log P_{o/c} + C_c \qquad (3.33)$$

where the subscript (c) represents cosolvent. But the solubility of the solute in a mixed solvent system can also be obtained by the relationship:

$$\log S_f = f \log S_c + (1-f) \log S_w \qquad (3.34)$$

which can be rewritten as:

$$\log S_f = \log S_w + f(\log S_c - \log S_w) \qquad (3.35)$$

Then substituting equations (3.32) and (3.33) into equation (3.35) gives an equation which expresses the solubility of the solute in terms of its

aqueous solubility and the octanol-water partition coefficient, so:

$$\log S_f = \log S_w + f(\log P_{o/w} - \log P_{o/c} - C_w + C_c) \qquad (3.36)$$

and $(\log P_{o/w} - \log P_{o/c} - C_w + C_c)$ can be assumed to be equivalent to (S) which is defined as the solubilizing power of the cosolvent and therefore

$$\log S_f/S_w = sf \qquad (3.37)$$

According to Hansch the partition coefficient of the solute between any solvent and water can be related to the octanol water partition coefficent is given by the solvent regression equation:

$$\log P_{c/w} = a \log P_{o/w} + b \qquad (3.38)$$

where (a) and (b) are constants depending on the partitioning system. By analogy, the cosolvent-octanol partition coefficient can be related to the octanol-water partition coefficient is:

$$\log P_{o/c} = A \log P_{o/w} + B \qquad (3.39)$$

and therefore the value of (S) can be expressed as

$$S = A \log P_{o/w} + B + C_c - C_w \tag{3.40}$$

and assuming that:

$$B + C_c - C_w = D \quad \text{then } S = A \log P_{o/w} + D$$

and substituting this value for (S) into equation (3.37) will result in equation (3.31). Applying this equation with no intercept to a number of compounds, Yalkowsky and Rubino obtained the values of (A) and (D) so

$$\log S_f/S_w = (0.714 \log P_{o/w} + 0.174)\, f \tag{3.41}$$

where $r = 0.981$.

Additionally, Paruta(267,270,274), correlated the effect of the dielectric constant of sucrose-water solvent systems on the solubility of a number of compounds and by studying this effect he was able to optimise the solubility of pharmaceuticals in this mixture.

3.3 MATERIALS AND METHODS

3.3.1 Solubility Determinations:

A. Solubility and pK_a Determinations of Ibuprofen and Indomethacin

Excess solid of either ibuprofen or indomethacin was shaken in suitable iodine flasks (100 or 250 ml) which McIlvaine buffer adjusted to constant ionic strength of 1M with potassium chloride(238) in a

thermostatic water bath (Mickel Laboratory Engineering Company, England) until a constant concentration of solute was found in the supernatant solution.

One hundred millilitres of McIlvaine buffer were shaken with ibuprofen or indomethacin in a 250 ml flask and for pHs higher than pH 7.0 a 100 ml flask containing 50 mls of the buffer was shaken with indomethacin. The solubility of ibuprofen was determined at 25°C over a pH range of 3.9 to 5.6 while that of indomethacin was determined at 37°C over a pH range of 4.38 to 7.98. In all cases equilibrium was reached within 40 hours.

After equilibrium the excess undissolved solid was separated by filtration using an 8 μm membrane filters (Millipore Corp., Mass., U.S.A.) and the filtrate was diluted as necessary prior to analysis. All glassware and filter holders were kept at the corresponding temperature for the determination of solubility to prevent any precipitation due to changes in temperature. The first few ml of the filtrate were discarded in order to prevent the error obtained from the loss of the material on the filtration beds and the filtrate was kept at the appropriate temperature for sampling, dilution and pH reading. The pH of the filtrate was measured using a Radiometer pHM64 three decimal place pH meter (Radiometer-Copenhagen) calibrated at the corresponding temperature. Indomethacin solubility determination at pH values higher than 6.5 required that the filtrates were diluted prior to analysis with the respective buffer containing 10% of 0.1M hydrochloric acid in order to produce a stable solution (at about pH 6.0) for the duration of the assay. The solubilities of ibuprofen and indomethacin were measured at pH 2.0 and in 0.1M hydrochloric acid following the same procedure at the

corresponding temperatures.

The solubilities were calculated from the concentrations of the samples anlaysed taking into consideration the dilution factor. Sample dilution was as follows:

Material		pH	Dilution Factor
Indomethacin		7.986	500
		7.823	500
		7.35	100
		7.085	100
		6.692	10
		6.085	10
		5.875	5
		5.407	2.5
		5.274	-
		5.161	-
		4.88	-
		4.704	-
		4.581	-
		4.388	-
		2.0	-
	in	0.1 M hydrochloric acid	-
ibuprofen		5.6	10
		5.21	10
		4.7	10
		4.46	10
		4.25	10
		4.01	10
		3.9	10
		2.0	5
	in	0.1 M hydrochloric acid	5

Indomethacin samples were analysed by direct ultraviolet spectroscopy at 320 nm. Standard solutions were made from a stock solution (0.56mM) to contain 1.4 - 111.8 μM of indomethacin. The standards were treated in exactly the same way as the test solutions. The samples and standards were analysed against an appropriate blank solution in the ultraviolet region. HPLC analysis was used for the determination of the solubility of ibuprofen in buffer, for the measurement of the intrinsic solubilities of both compounds in 0.1 M

hydrochloric acid and pH 2.0 buffer, and for the estimation of indomethacin solubility at pH 7.98 and 7.35. Prior to analysis by HPLC 2 ml of each sample were mixed with 2 mls of ethanol and 1 ml of the internal standard solution. All samples were run in triplicate.

B. Solubility Determination in Ethanol-water Mixtures

The solubility of ibuprofen and its (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters were determined in a series of solutions containing 5, 10, 15, 20, 25, 30, 35 and 40% of ethanol in water and that of the cyclohexylmethyl ester of ibuprofen was determined over the range 20-40% of ethanol. Excess of each material was shaken in a thermostatic water bath at 25°C with the corresponding aqueous ethanolic solution and in all cases equilibrium was reached within 40 hours. Excess material was separated by centrifugation at 4200 rpm and 2 ml of solutions containing 95, 90, 85, 80, 75, 70, 65 and 60% ethanol-water were mixed with 2 ml of the supernatents prepared in 5, 10, 15, 20, 25, 30, 35 and 40% ethanol-water respectively to give mixtures containing 50% ethanol in water. Further dilution was made with a solution of 50% ethanol in water and the solubilities were calculated from the concentrations of the samples analysed taking into consideration the dilution factor. Sample dilution was as follows:

Material studied	% ethanol in Water	Dilution Factor
ibuprofen	5	5
	10	10
	15	10
	20	20
	25	50
	30	100
	35	250
	40	500

continued...

Material studied	% ethanol in Water	Dilution Factor
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	5	2
	10	2
	15	2
	20	5
	25	5
	30	10
	35	10
	40	20
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	5	2
	10	2
	15	2
	20	5
	25	5
	30	10
	35	20
	40	80
Cyclohyexylmethyl ester of ibuprofen	20	2
	25	2
	30	2
	35	2
	40	4

Four mls of each sample were mixed with 1 ml of the internal standard solution prior to analysis by HPLC.

C. Solubility Determination in Propylene Glycol-Water Mixtures at 25°C

The solubility of flufenamic acid, indomethacin, ibuprofen and its (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl-esters were determined in a series of solutions containing 20, 40, 50, 60 and 80% propylene glycol in water.

The solubility of the cyclohexylmethyl ester of ibuprofen was determined in 40, 50, 60, 70 and 80% propylene glycol water mixtures. The procedure followed the same method described in part (B) and 2ml of

the supernatents made in 20, 40, 50, 60, 70 and 80% propylene glycol water were mixed with 2 ml of solutions containing 80, 60, 50, 40, 30 and 20% ethanol-water respectively to give mixtures containing 50% alcohol in water. Further dilution was made with a solution of 50% ethanol-water and the solubilities were calculated from the concentrations of the samples anlaysed taking into consideration the dilution factor. Sample dilution was as follows:

Material studied	% Propylene glycol in water	Dilution Factor
indomethacin	20	10
	40	20
	50	20
	60	50
	80	200
flufenamic acid	20	10
	40	20
	50	20
	60	80
	80	1000
ibuprofen	20	20
	40	40
	50	80
	60	400
	80	2000
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	20	5
	40	40
	50	40
	60	250
	80	500
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	20	5
	40	20
	50	20
	60	100
	80	250
cyclohexylmethyl ester of ibuprofen	40	2
	50	2
	60	5
	70	20
	80	125

Four ml of each sample were mixed with 1 ml of the internal standard solution prior to anlaysis by HPLC.

D. Aqueous Solubility Determination at 25°C

The aqueous solubility of flufenamic acid, indomethacin, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl esters were determined by the same method as described in section (B) except that the excess solid ibuprofen, flufenamic acid and indomethacin was filtered using an 8 μm Millipore membrane filter and the filtrate was collected in the same manner described in section (A). Two ml of each sample solution were diluted with 2 ml of ethanol to give mixtures containing 50% ethanol in water. Ibuprofen mixture was further diluted with a solution of 50% ethanol-water and the dilution factor was equal to a value of 5. The aqueous solubility of the cyclohexylmethyl ester of ibuprofen was determined in the following manner due to the low water solubility of this ester causing analytical problems.

Four portions of the saturated suspension of the ester in water (≈ 40 mls each) were centrifuged at 4200 rpm for 1 hour and 30 ml samples of the supernatent from each tube were extracted with 12 mls of ether. The ethereal extract was concentrated in a test tube and the ether was evaporated to dryness under Nitrogen (using Tecam Dri Block DB-3). The residue was dissolved in ethanol and water added to 15 ml to give 50% ethanol water solutions.

Four ml of each sample solution were mixed with 1 ml of the internal standard solution prior to anlaysis by HPLC. In sections (B),

(C) and (D) samples were run at least in triplicates.

E. HPLC Analysis

1. HPLC Conditions

Column: Hypersil-ODS (5 μm) 10 cm × 4.6 mm I.D.

Mobile Phase:

(a) 70% Methanol in water acidified with 0.2% orthophosphoric acid (pH = 2.5) for indomethacin solutions.

(b) 76% Methanol in water acidified with 0.3% orthophosphoric acid (pH = 2.5) for solutions of flufenamic acid, ibuprofen and its (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)-methyl esters.

(c) 81% Methanol in water acidified with 0.3% orthophosphoric acid (pH = 2.5) for solutions of cyclohexylmethyl ester of ibuprofen.

Flow Rate: 1 ml/min.

Detection wavelength:

235 nm for indomethacin

220 nm for all the remaining compounds.

Sensitivity: 0.01 - 0.04 A.U.F.S.

2. Details of test and standard solutions together with the internal standards used in each case:

The test solutions to be analysed were prepared and diluted as described earlier in sections (A), (B), (C) and (D) to contain an ethanol concentration of 50% v/v.

Standard solutions were prepared from a suitable stock solution and treated in the same way as the sample solutions. The concentration range for each standard solution used is listed in Table 3.1.

TABLE 3.1

Composition of the standard solutions used in the solubility study

Material studied	Compound contained in standard solution	Concentration range µg/ml	Internal Standard and concentration µg/ml
indomethacin	indomethacin	1.2 - 6	Thymol, 500
flufenamic acid	flufenamic acid	0.5 - 2.5	ethyl ester of ibuprofen, 12
ibuprofen	ibuprofen	2 - 10	ethyl ester of ibuprofen, 12
(tetrahydro-2-furanyl)methyl ester of ibuprofen	ibuprofen (tetrahydro-2-furanyl)methyl-ester of ibuprofen	4 - 20 6 - 30	(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen,12
(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen	ibuprofen (tetrahydro-2-(2H)-pyranyl)methyl-ester of ibuprofen	2 - 10 3 - 15	ethyl ester of ibuprofen, 12
cyclohexylmethyl-ester of ibuprofen	ibuprofen cyclohexylmethyl-ester of ibuprofen	2 - 10 1.5 - 1.75	Hexachlorophane 0.7

Four mls of samples or standards were mixed with 1 ml of internal standard and 20 µl was chromatographed in the HPLC.

3.3.2 Measurement of Partition Coefficient

The octanol-water partition coefficient of flufenamic acid (pH 3.9), indomethacin (pH 4.4), ibuprofen (pH 4.5) and its (tetrahydro-2-

furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl-esters were determined. In addition, the octanol - 0.1 M hydrochloric acid partition coefficient for the three acids was also determined.

Octanol (SLR grade, Fisons Chemical Co., England) was saturated with water or 0.1 M hydrochloric acid in separate flasks by shaking overnight in a thermostatic waterbath at 25°C. From a stock solution in water-saturated octanol or 0.1 M hydrochloric acid-saturated octanol a 50 ml substock solution was prepared for each of the compounds studied and a list of the compounds used with their concentration is given as follows:

Compound	Amount in mg per 50 ml of the Octanol substock solution
flufenamic acid	50[a]
indomethacin	50[a]
ibuprofen	120[a]
(tetrahydro-2-furanyl)methyl ester of ibuprofen	100[b]
(tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen	100[b]
Cyclohexylmethyl ester of ibuprofen	80[b]

(a) prepared in water-saturated octanol and also in 0.1 M hydrochloric acid-saturated octanol.
(b) prepared in water-saturated octanol.

Ten ml of the octanol was shaken with 200 ml of water or 0.1 M hydrochloric acid as specified earlier for about 3 hrs and allowed to equilibrate at 25°C for 48 hrs in a thermostatic waterbath. The phases were separated by centrifugation at 3600 rpm for 1 hr and the

concentration in each phase was determined by HPLC where the two phases separated by centrifugation were treated in the following manner. Three aliquots of 5 ml from the aqueous or acidified aqueous layer of each determination in the set were diluted with ethanol (5 ml) to give 50% ethanol-water mixtures. While three other aliquots of 1 ml each from the octanol layer were diluted to give a mixture of 50% alcohol water (ethanol + octanol). This dilution was made prior to the HPLC analysis (Section 3.3.1, part E).

For the cyclohexylmethyl ester of ibuprofen due to its low aqueous solubility, the amount in the aqueous layer was determined in a similar way to that described in the determination of the aqueous solubility but the residue obtained was dissolved in 10 ml of 50% ethanol water. The HPLC conditions were similar to those described for the solubility determinations. All determinations were run in triplicates and sets of solutions for each of the aqueous and the octanol layers were prepared in 50% alcohol water as described earlier. The standard solutions were prepared from the octanol stocks and diluted to give a final solution in 50% alcohol water (ethanol + octanol) having the range of concentrations described in Table 3.1. The internal standards were made according to Table 3.1 and mixed with either the test or standard solutions as in the solubility section.

3.3.3 Potentiometric Titration of Ibuprofen

Freshly prepared solutions of ibuprofen (4.854×10^{-4}M) containing 20, 30, 40, 50 and 60%v/v ethanol in cooled boiled double distilled water were made by dilution from an ethanolic stock solution of ibuprofen (9.708×10^{-3}M) and the required amount of ethanol. Twenty

five mls from each ibuprofen solution were placed into a Radiometer TTA60 titration cell fitted with a jacketed wall attached to a Churchill re-circulating thermostatic water bath to keep the temperature constant in the cell at 25°C. The cell was equipped with a combined glass electrode with a silver-silver chloride reference system attached to a Radiometer PHM64-3 decimal pH meter, a paddle stirrer, a flushing source of nitrogen gas, a thermometer, and a port for the delivery of the titrant. The ibuprofen solution in the cell was titrated at 25°C by the addition of 0.01 M sodium hydroxide (usually in 0.3 μl aliquots) delivered from 1 ml burette graduated to 1/100 of a ml. Five replicates from each solution of ibuprofen in ethanol were used and the values obtained were averaged.

Data and statistics were calculated on an ICL 1904S computer using a Fortran IV program given the title PKA written by Dr. W. J. Irwin (Department of Pharmaceutical Sciences).

3.4 RESULTS AND DISCUSSION

3.4.1 The Determination of the pK_a Values for Ibuprofen and Indomethacin

A. **Solubility data and pK_a determination:**

The solubility values were determined in McIlvaine's buffer adjusted to a constant ionic strength of 1M with potassium chloride. The solubility of indomethacin was determined in this buffer at 37°C over a pH range of 4.38 to 8 and the values obtained from direct UV analysis of the solubility data at 320 nm are listed in Table 3.2, the values representing an average of three determinations. Earlier reports

indicated that indomethacin is subjected to decomposition by hydrolysis in aqueous solutions(84,85,276). According to these reports it was

TABLE 3.2

The solubility of indomethacin in McIlvaine's buffer (μ = 1M)

pH	Solubility mg/100 ml	
	UV analysis data	HPLC analysis data
7.986	705.223	704.9
7.823	546.134	
7.35	169.26	169.43
7.085	84.689	
6.692	35.415	
6.085	11.176	
5.875	5.809	
5.407	2.111	
5.274	1.565	
5.161	1.184	
4.88	0.705	
4.704	0.498	
4.581	0.423	
4.388	0.302	
2.0		0.1242
in 0.1 M hydrochloric acid		0.1248

clear that below pH = 5 the half life for hydrolysis was longer than the time required for equilibrium during the solubility studies. In the alkaline and slightly acidic regions indomethacin will degrade but the amount in solution will remain constant after equilibration as replacement of degraded material from the solid reservoir will occur. Filtrates were diluted immediately with the respective buffer containing 10% of 0.1M hydrochloric acid in order to produce a stable solution at about pH 6.0 for the duration of the assay. HPLC analysis of solubility

samples at pH 7.98 and 7.35 gave values which matched those obtained from the UV analysis of the samples. These values are also given in Table 3.2.

Figure 3.1 shows the effect of pH on the solubility of indomethacin in McIlvaine's buffer (μ = 1M) at 37°C (r = 0.9992, slope = 0.955). It was reported earlier that the pK_a of a monoprotic weak acid can be determined by measuring the total solubility of the acid as a function of pH under controlled ionic strength and temperature conditions(77,78,277). This method can be applied to acids with poor aqueous solubility such as indomethacin. According to equation (3.3) a plot of the total solubility of indomethacin (S) versus $1/[H^+]$ yields a slope of $K_a[HA]_o$ and an intercept of $[HA]_o$ or the intrinsic solubility. Dividing the slope by the intercept gives K_a, the dissociation constant. Figure 3.2 shows such a plot for indomethacin with a correlation coefficient of 0.9999. The intrinsic solubility, K_a and pK_a for indomethacin obtained from this figure were $3.5 M \times 10^6$ (0.1255 mg/100ml), 6.048×10^{-5} and 4.21 respectively.

The intrinsic solubility of indomethacin determined in 0.1 M hydrochloric acid and pH 2.0 buffer obtained by HPLC was $3.49 M \times 10^6$ (0.1248 mg/100ml). While the intrinsic solubility of ibuprofen determined in 0.1M hydrochloric acid and pH 2.0 (Teorell-Stenhagen's buffer) was equivalent to 2.75 mg/100ml. Following the method of Krebs and Speakman and using equation (3.4) the solubility data obtained for ibuprofen can be used to determine its pK_a. Table 3.3 records the determination of ibuprofen pK_a by the solubility method.

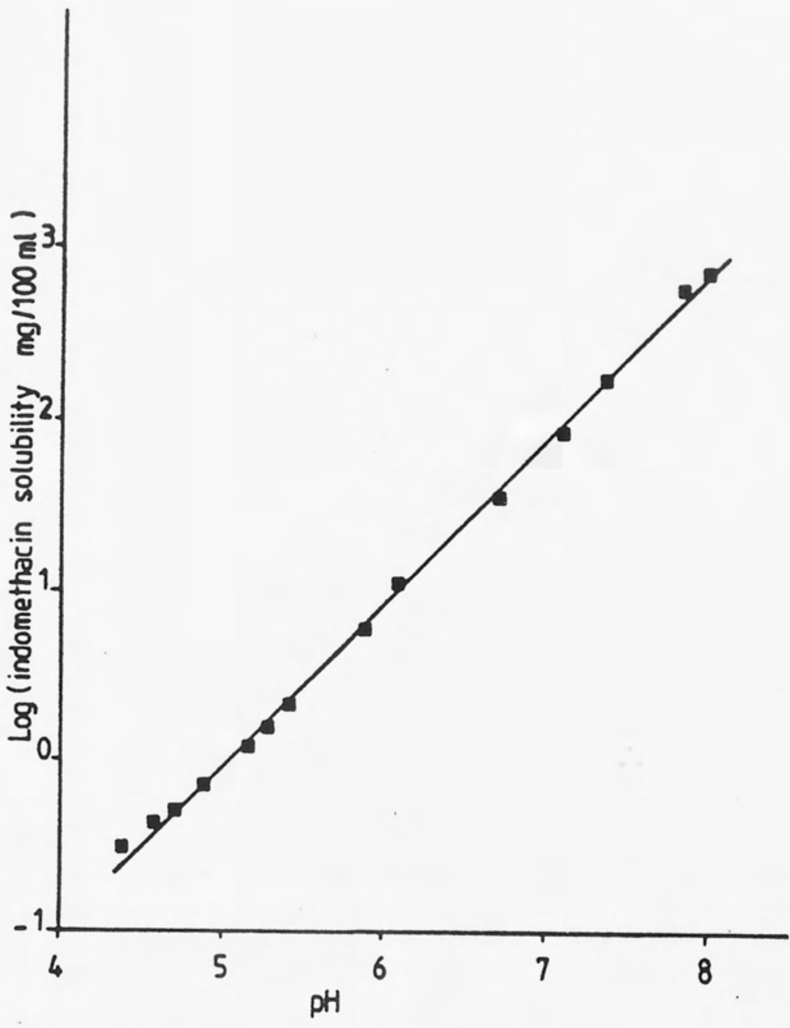

Fig. 3.1 Effect of pH on the solubility of indomethacin in McIlvaine buffer (μ = 1 M) at 37° C .

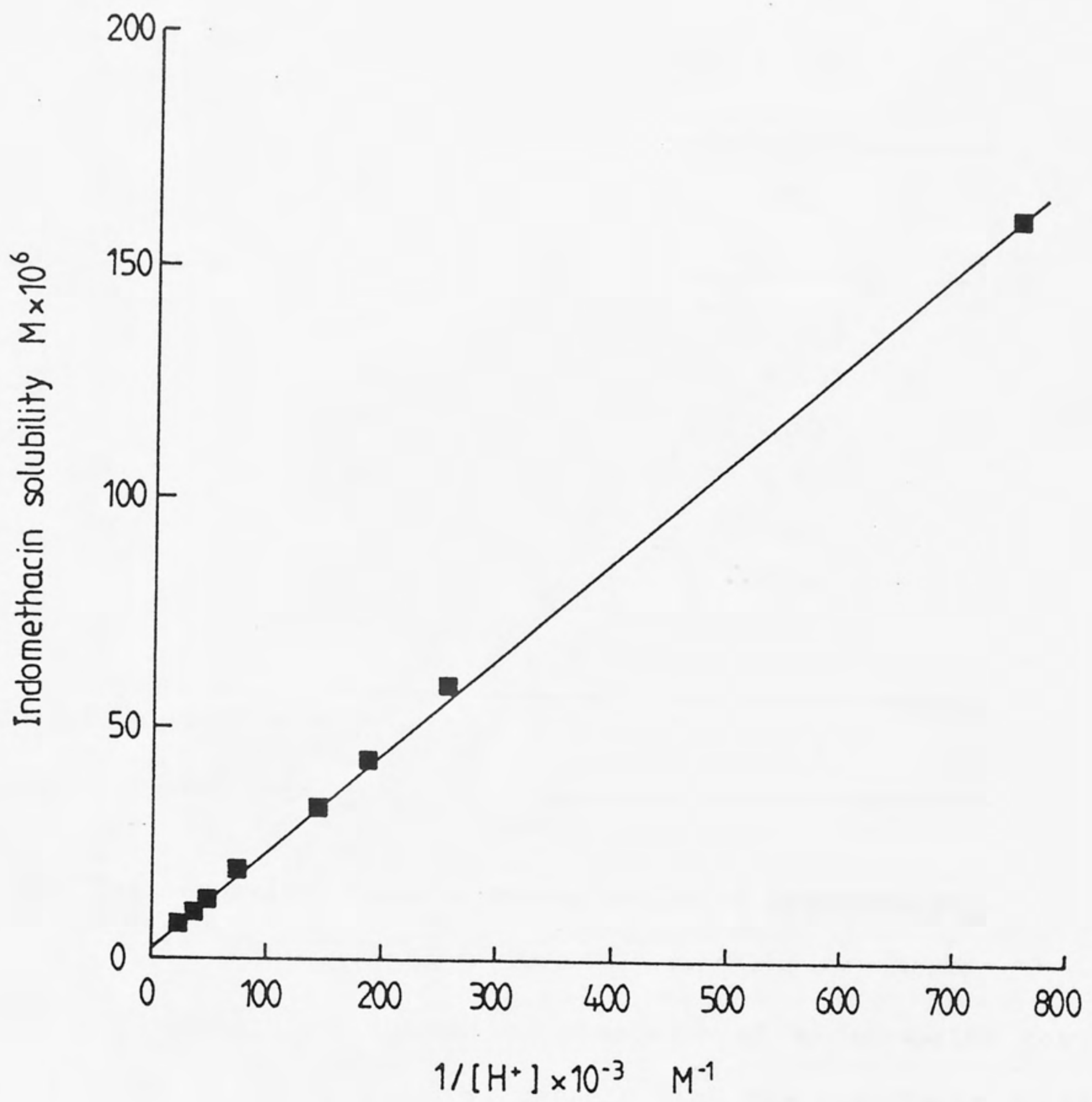

Fig. 3.2 Plot of total solubility of indomethacin at 37°C versus $1/[H^+]$ for equilibrated McIlvaine buffers ($\mu = 1M$)

TABLE 3.3

The determination of the dissociation constant of ibuprofen by the solubility method.

Temperature 25°C,

S_o = 2.7588 mg/100 ml in 0.1 M hydrochloric acid = 1.339×10^{-4}M

pH	S (Total Solubility (mg/100 ml)	pK_a
3.9	3.031	4.465
4.01	3.737	4.465
4.25	4.383	4.48
4.46	4.985	4.553
4.7	7.39	4.475
5.21	17.57	4.48
5.6	39.97	4.47

Result = pK_a = 4.471 ± 0.008 at μ = 1M and 25°C

B. The "Taft equation" and the Determination of Ibuprofen pK_a.

The determination of ionization constants of a derivative for a weak acid using the Taft equation depends upon the additivity of the free energy changes affecting the ionization constant of the parent compound (pK°) expressed by equation (3.27)

$$pK = pK^\circ - \rho^* \Sigma (\sigma^*) \qquad (3.27)$$

The Taft equation describes the ionization constant of an acid with the general formula R'R"CHCOOH expressed by equation (3.30) was:

$$pK_a = 4.8 - 0.66 \Sigma \sigma^*$$

and when ibuprofen has the structural formula

$$\begin{array}{c} CH_3 \\ \diagdown \\ CH - CH_2 - C_6H_4 - CH(CH_3) - COOH \\ \diagup \\ CH_3 \end{array}$$

CH_3 can be considered as R' and $-C_6H_4-4-CH_2CH(CH_3)_2$ as R" and the ionization constant for ibuprofen can be predicted by calculating the sum of (σ^*) for the two groups and applying it to equation (3.30). A list given by Perrin et al.(266) for the values of σ^* of certain groups are given as follows:

Group	σ^*
$-CH_3$	0.00
$-C_6H_5$	0.75
$-C_6H_4-4-CH_3$	0.59
$-C_6H_4-4-C_2H_5$	0.59
$-C_6H_4-4-CH(CH_3)_2$	0.56
$-C_6H_4-4-C(CH_3)_3$	0.52
$-CH_2-CH(CH_3)_2$	-0.19

Equation (3.29) may be employed in the calculation of σ^* for a group, from the values of σ^* for its constituents when available. In the case when values are unknown the $\Sigma\sigma^*$ can be estimated using the assumption that the constituted parts of the group are additive. Thus the calculation of the σ^* for R" is:

$-C_6H_4-4-CH_2CH(CH_3)_2$

1. $\Sigma\sigma*$:

$\sigma*$ for $-C_6H_5$	=	0.75
$\sigma*$ for $-CH_2-CH(CH_3)_2$	=	-0.19
$\Sigma*$ for R"	=	+0.56

or

2.

$\sigma*$ for $-C_6H_4-4-CH(CH_3)_2$	=	0.56
$\sigma*$ for $-CH_3$	=	0.00
Then $\Sigma\sigma*$ for R"	=	+0.56

applying this value in equation (3.30) gives:

$$pK_a = 4.8 - 0.66\ \Sigma\sigma*$$

$$\Sigma\sigma* \text{ for } R'R'' = 0 + 0.56 = 0.56$$

and

$$pK_a = 4.8 - 0.66\ (0.56)$$
$$= 4.4304$$

The pK_a value of ibuprofen obtained by both the solubility and by applying the Taft equation were in close agreement with the value given by Albert and Serjeant(278) which is 4.4.

C. The Determination of Ibuprofen Dissociation Constant by Potentiometric Titration:

The P_sK_a or more properly pK values of ibuprofen obtained in aqueous ethanolic systems using the logarithmic potentiometric titration are listed in Table 3.4 with their statistical parameters.

TABLE 3.4

The P_sK_a values of ibuprofen in aqueous ethanolic solutions.

% Ethanol	The dissociation constant K			P_sK_a	
	Value	Standard deviation	95% error limits	Value	95% error limits
20	0.1348×10^{-4}	0.165×10^{-5}	0.123×10^{-4}–0.146×10^{-4}	4.9	4.885–4.9
30	0.5×10^{-5}	0.273×10^{-6}	0.481×10^{-5}–0.52×10^{-5}	5.30	5.283–5.31
40	0.195×10^{-5}	0.103×10^{-6}	0.186×10^{-5}–0.203×10^{-5}	5.70	5.691–5.72
50	0.834×10^{-6}	0.571×10^{-7}	0.79×10^{-5}–0.87×10^{-6}	6.07	6.056–6.1
60	0.365×10^{-6}	0.232×10^{-7}	0.347×10^{-6}–0.383×10^{-6}	6.44	6.416–6.45

A plot showing the effect of ethanol in the solvent system on the P_sK_a(pK) of ibuprofen is illustrated in Fig. 3.3. The line has a correlation coefficient of 0.999 and extrapolation of the data to the pure aqueous system represents the pK_a of ibuprofen. This y intercept is equal to 4.114 which gives a value for the K_a of 7.69×10^{-5} . Figure 3.4 plots the dielectric constants of aqueous ethanolic mixtures as a function of their ethanol concentration according to Sorby et al.(279). The following are the values of the dielectric constants of aqueous ethanolic mxitures used in the potentiometric titrations.

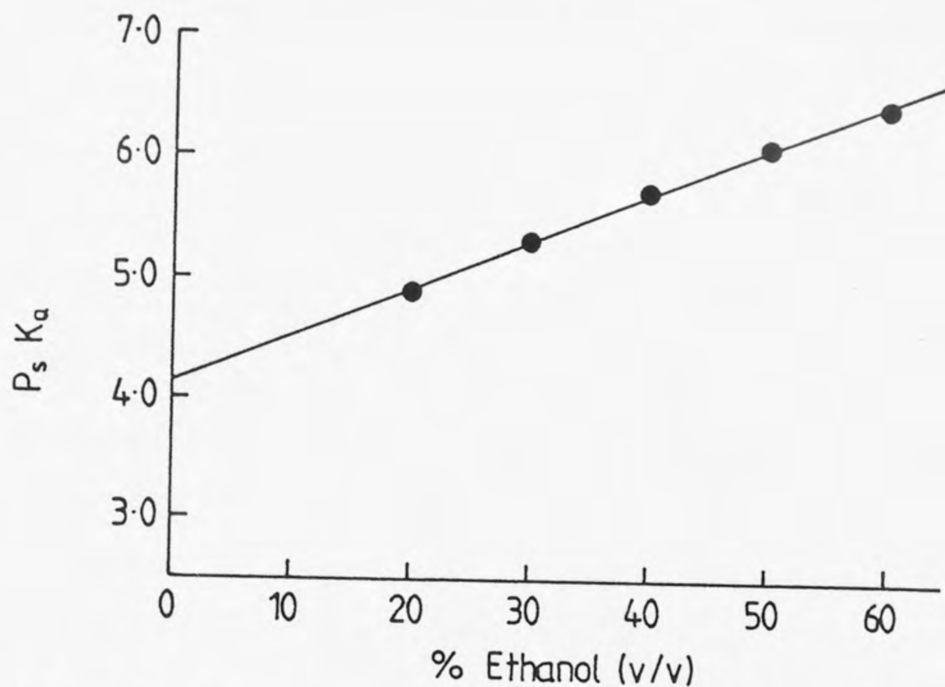

Fig. 3.3 The effect of ethanol in the solvent system on the $P_s K_a$ (pK) of ibuprofen at 25°C

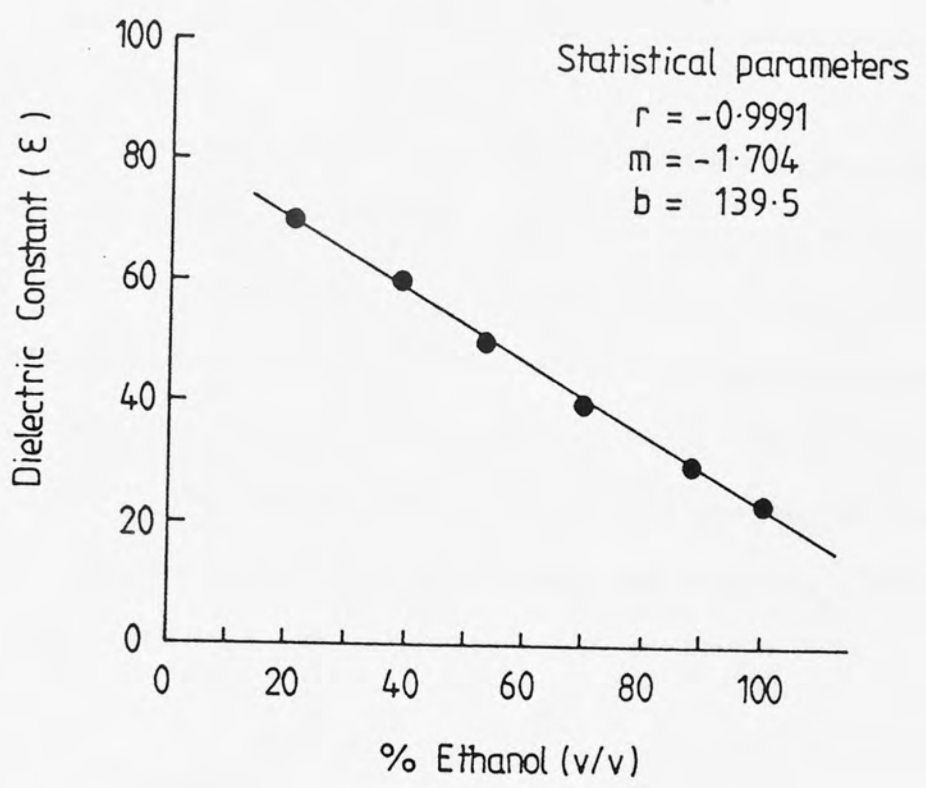

Fig. 3.4 The dielectric constants of aqueous ethanolic mixtures as a function of their ethanol concentration (279). (From. J. Pharm. Sci., 1963, 52, 1149-1153)

Additionally, the values of $[H_2O]$ and of $P_sK_a + \log[H_2O]$ at 25°C are included:

% Ethanol	Dielectric Constant (ε)	1/ε	$[H_2O]$	$P_sK_a + \log[H_2O]$
20	70.09	0.0142	44.31	6.6
30	64.00	0.0156	38.77	6.88
40	58.00	0.0173	33.23	7.2
50	52.00	0.0192	27.69	7.5
60	47.00	0.0212	22.15	7.84

Following the findings by Shedlovsky and Yasuda concerning the inclusion of $[H_2O]$ when relating the effect of the solvent system dielectric constant on the P_sK_a of the acid a plot of ($P_sK_a + \log[H_2O]$) versus 1/ε of the aqueous ethanolic system resulted in a linear relationship with a correlation coefficient of 0.999, and a slope of 175.86. The intercept which represents the pK_a in the pure aqueous system is equal to 4.12 (Fig. 3.5). In accordance with Shedlovsky and equation (3.26) the slope of Fig. 3.5 is equal to the value of

$$\left(\frac{e^2}{(2.303)\ Tak} \right)$$

The value of a, which is the average ionic diameter, will be equal to

$$\text{slope} = \frac{243.3}{a(\text{in A°})}$$

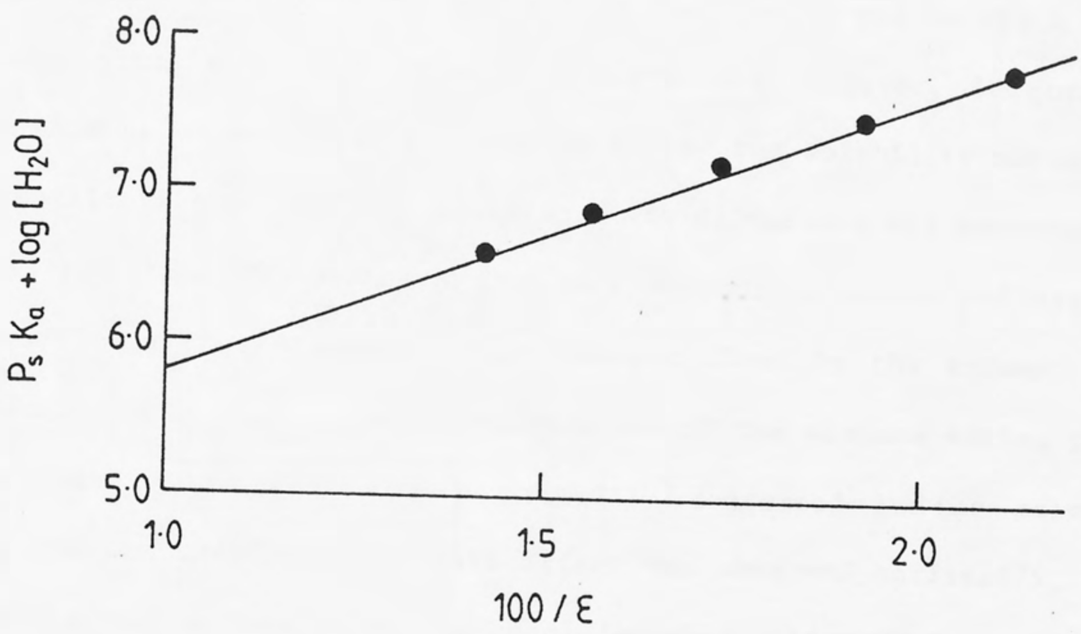

Fig. 3.5 Variation of $P_s K_a$ values of ibuprofen with the dielectric constant of the ethanol water solutions.

and

$$a = \frac{243.3}{175.86} = 1.383\ A°$$

The intercept is equivalent to ($-\log B_H$) and B_H will thus be equal to 7.585×10^{-5}.

Although the data obtained from the potentiometric titration of ibuprofen in mixed solvent system were treated in two different ways in order to obtain the pK_a of the acid, this resulted in values which were quite consistant with each other. A difference, however, is noticed between this value and those obtained by either the solubility method or by the application of the Taft equation. The difference was encountered when extrapolating the data to the pure aqueous solution and can be attributed to a thermodynamic effect brought about by the solvent and through changes in the alcohol concentration of the mixture during this process when the alcohol content steadily decreased in the solvent system. Due to the fact that this effect was observed earlier(79,257) in sets of accurate data it can be concluded that this method may give a poorer guide to the pK_a of the acid.

3.4.2 Solubilization by Cosolvents

Increasing the aqueous solubility of a poorly water soluble drug is an objective in pharmaceutical formulation especially if the drug is to be administered parentrally or orally in liquid form. The solubility of such compounds can be increased by careful study of their physical parameters together with their chemical structures. The aim is to establish an aqueous system which matches the polarity of the drug in order to increase its solubility. Careful control of the vehicle pH is

a means of increasing the solubility of weak electrolytes. This is not possible for nonelectrolytes and other means of solubilization are to be used. These methods include the use of cosolvents, surfactants and complexing agents. The use of cosolvents is evident in a number of pharmaceutical formulations as a means of increasing aqueous solubility and a relationship proposed by Yalkowsky and Rubino(71) describes solubilization in aqueous system by cosolvents. Among the cosolvents which can be used are ethanol and propylene glycol. Propylene glycol is commonly used as cosolvent as it is nontoxic, inexpensive and stable in addition to being a good solvent for many drugs. The solubility of ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl esters was determined in aqueous ethanolic mixtures (0-40% ethanol) at 25°C (Fig. 3.6) and in propylene glycol-water mixtures (0-80% propylene glycol). Indomethacin and flufenamic acid solubilities at 25°C were also determined and Fig. 3.7 and Table 3.5 record the solubility of these compounds in the two solvents.

Examination of the solubility Figures 3.6 and 3.7 shows the deviation of the solubility data form the already established solubility relationships which deal with regular solutions since these relationships were concerned with aqueous and semiaqueous solutions of relatively nonpolar substances. The solubility data show the lack of relation between the solute solubility in a mixed binary aqueous solvent S_f and the volume fraction of the cosolvent f, reported to exist for many drug systems:

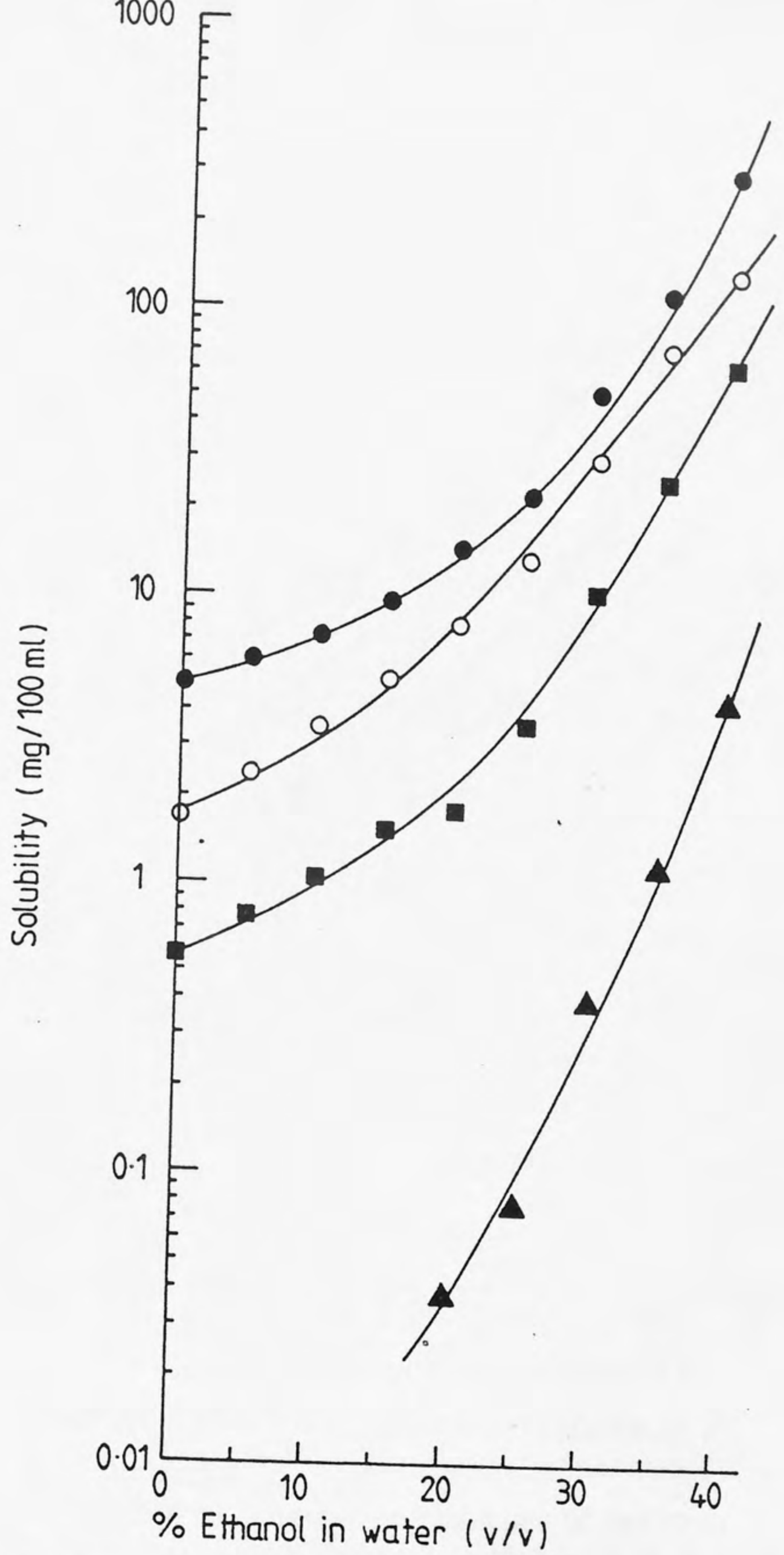

Fig. 3.6 The solubility of ibuprofen and its esters in ethanol water mixtures at 25° C.

KEY
- ● Ibuprofen
- ○ (tetrahydro - 2 - furanyl) methyl ester
- ■ (tetrahydro - 2 - (2H) pyranyl) methyl ester
- ▲ Cyclohexylmethyl ester

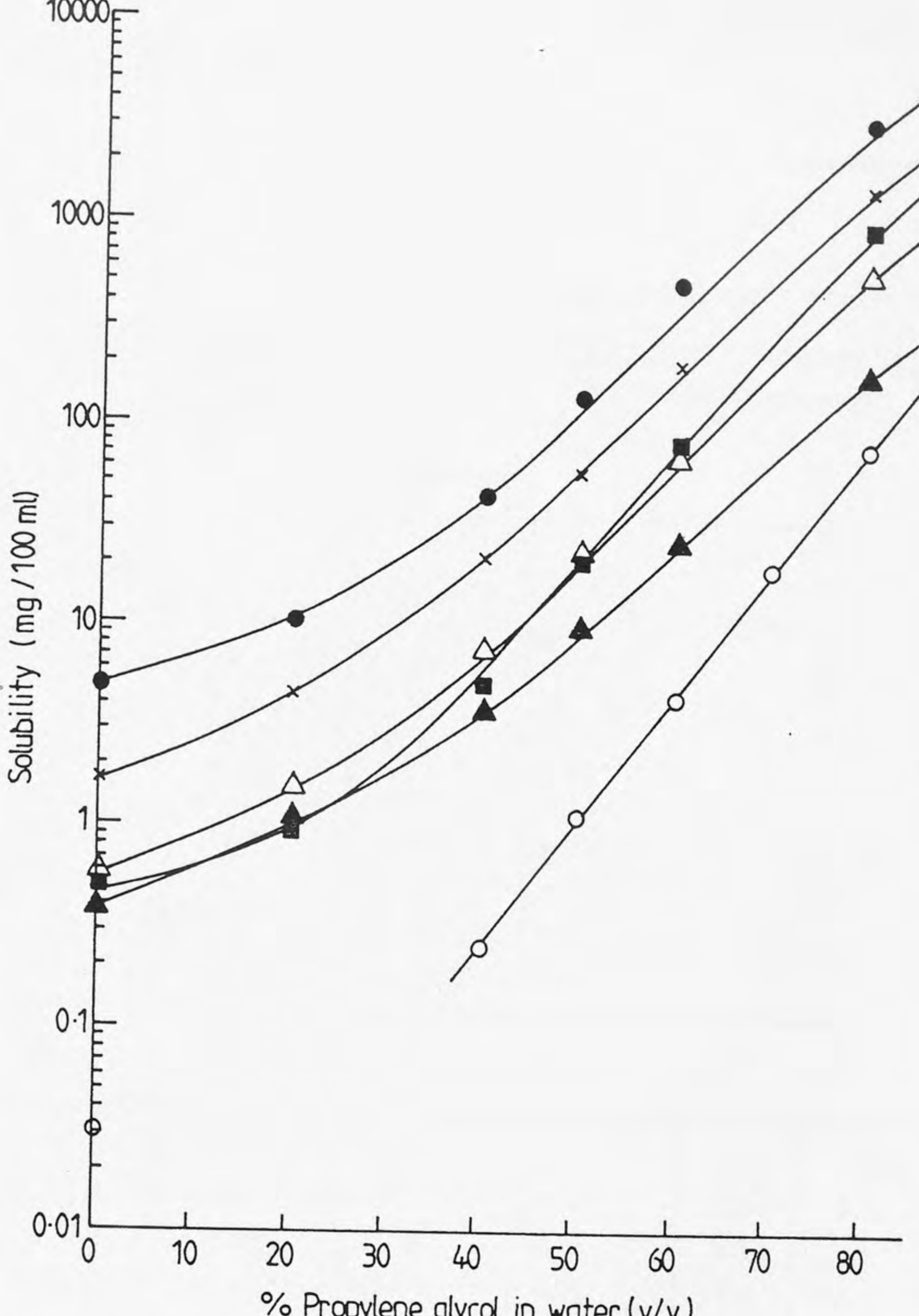

Fig. 3.7 Solubility in propylene glycol water mixtures at 25°C

KEY
- ● Ibuprofen
- × (tetrahydro-2-furanyl)methyl ester of ibuprofen
- △ (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen
- ○ Cyclohexylmethyl ester of ibuprofen
- ■ Flufenamic acid.
- ▲ Indomethacin

TABLE 3.5

The solubility data in mixed solvent systems for the compounds under study.

% Cosolvent v/v in water	Compound Solubility mg/100 ml					
	ibuprofen	(tetrahydro-2-furanyl)-methyl ester of ibuprofen	(tetrahydro-2-(2H)-pyranyl)-methyl ester of ibuprofen	cyclohexyl-methyl ester of ibuprofen	Indo-methacin	flufenamic acid
ETHANOL						
0	4.99	1.71	0.585	0.03	0.378	0.49
5	6.2	2.4	0.76			
10	7.3	3.6	1.02			
15	9.55	5.3	1.5			
20	14.6	7.9	2.07	0.037		
25	22.6	13.8	4.03	0.075		
30	51.5	31.5	11.97	0.3		
35	116.5	73.6	22.76	1.12		
40	305.0	135.0	43.92	4.4		
PROPYLENE GLYCOL						
20	10.0	4.53	1.5		1.08	0.946
40	45.87	21.32	7.0	0.265	3.7	4.92
50	134.9	57.25	23.68	1.14	9.24	19.85
60	493.0	196.6	65.4	4.33	25.5	76.65
70				19.6		
80	3080.3	1444.2	549.6	77.0	172.75	924.5

$$\log S_f = \log S_{f=0} + af \quad (3.42)$$

where $S_{f=0}$ is the solubility in water and the value of a is characteristic of the system. It was (254,267-270,274,275) indicated that the best correlation between solubility parameters and dielectric

constants was obtained with those solvents which associate primarily through hydrogen bonding where as Hildebrand(252) has carefully noted that justification for the theory of solubility parameters rests upon the London dispersion forces and that its use for polar substances is somewhat questionable. In order to describe the solubilization effect by the cosolvents the solubility data in propylene glycol-water mixtures will be used. Application of equation (3.31) proposed by Yalkowsky and Rubino(71) may model the amount of the drug solubilized by the cosolvent through the octanol water partition coefficient. The solubilization curves for the propylene glycol water system are given in Fig. 3.8 which represents $\log(\frac{S_M}{S_w})$ as a function of the volume fraction of propylene glycol (f). The degree to which equation (3.31) can be considered successful in describing the solubilization effect can be estimated from the "Residual values" calculated from the least squares model.

Table 3.6 shows the extent to which equation (3.31) was able in predicting the aqueous solubility by comparing the calculated value of the slope from the octanol-water partition coefficient ($m = A \log P_{o/w} + D$) for each compound and that obtained from the slopes in Fig. 3.8.

The residual values recorded in Table 3.6 show that the equation was nearly successful with two of the six compounds where the values were less than unity. This deviation in the slope accounts for the curvature in the graphs but it is not accounted for in the equation. This effect is due to solvent interactions and nonideality involving the solute(71).

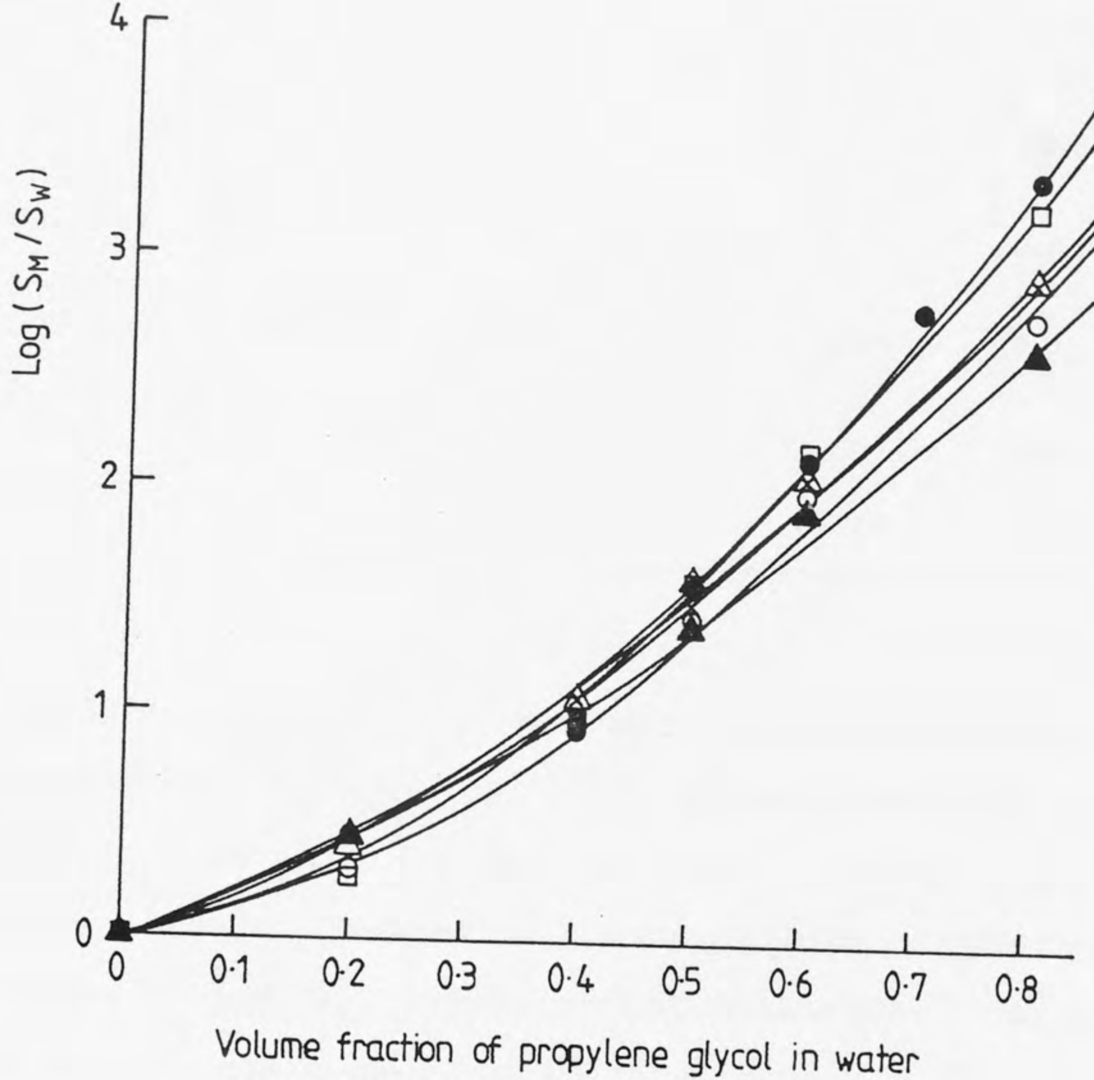

Fig. 3.8 Plot of log (S_M/S_W) versus volume fraction (f) of propylene glycol water at 25°C

KEY
- ○ Ibuprofen
- □ Flufenamic acid
- × (tetrahydro-2-furanyl) methyl ester of ibuprofen
- △ (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen
- ▲ Indomethacin
- ● Cyclohexylmethyl ester of ibuprofen

TABLE 3.6

Slopes and octanol-water partition coefficient data for various compounds in propylene glycol-water mixtures

Compound	log P	Slope		
		observed	predicted	residual
Ibuprofen	3.24	3.60	2.48	1.12
Flufenamic acid	3.45	4.19	2.63	1.56
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	3.75	3.71	2.85	0.86
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	3.80	3.77	2.89	0.88
Cyclohexylmethyl ester of ibuprofen	4.05	4.26	3.07	1.19
Indomethacin	2.738	3.31	2.12	1.19

The interactions displayed by water-propylene glycol systems make this mixture nonideal in that it is not a linear combination of its components. Other parameters may also play a role including the dielectric constant discussed by Paruta (267-275). The solute interactions may include interaction between the molecules of the solute as well as those between the solute and the solvent.

Yalkowsky and Rubino(71) introduced another form of equation (3.31) where a nonideality component of the solubilization curves (S_n) was quantified by the equation

$$\log S_n = Ef + Ef^2 \qquad (3.43)$$

where E is constant. Using this value in equation (3.31) then

$$\log(S_f/S_w) = (A \log P_{o/w} + G)f - Ef^2 \tag{3.44}$$

where G = D + E and using this equation for the data originally used for equation (3.31) Yalkowsky and Rubino obtained:

$$\log(S_f/S_w) = (0.714 \log P_{o/w} + 0.794)f - 0.103f^2 \tag{3.45}$$

considering the value for ($0.103f^2$) to be negligible the residual value may be presented as follows:

Compound	Slope		
	Observed	Predicted	Residual
Ibuprofen	3.6	3.1	0.5
Flufenamic acid	4.19	3.26	0.93
Indomethacin	3.31	2.75	0.56
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	3.71	3.47	0.24
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	3.77	3.83	-0.06
Cyclohexylmethyl ester of ibuprofen	4.26	3.68	0.58

This result provides a better model of the solubility data with all residual values being less than unity.

Applying the principle proposed by Paruta the solubility data were plotted againast the 1/ε (inverse of the dielectric constant) of the propylene glycol-water mixture (Fig. 3.9). The dielectric constants of propylene glycol-water mixtures are presented in Fig. 3.10 according to Sorby et al.(279).

The solubility curves in Figs. 3.8 and 3.9 show that Fig. 3.9 presents a better description of the solubilization effect.

The correlation coefficients of the solubility plots for each compound are as follows and show that the dielectric constant may in certain cases account for the deviation in the solubility data and the curvature in the graphs obtained.

Compound	Correlation Coefficients	
	Fig. 3.8	Fig. 3.9
Ibuprofen	0.982	0.991
Flufenamic acid	0.975	0.996
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	0.988	0.994
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	0.987	0.994
Cyclohexylmethyl ester of ibuprofen	0.968	0.995
Indomethacin	0.989	0.996

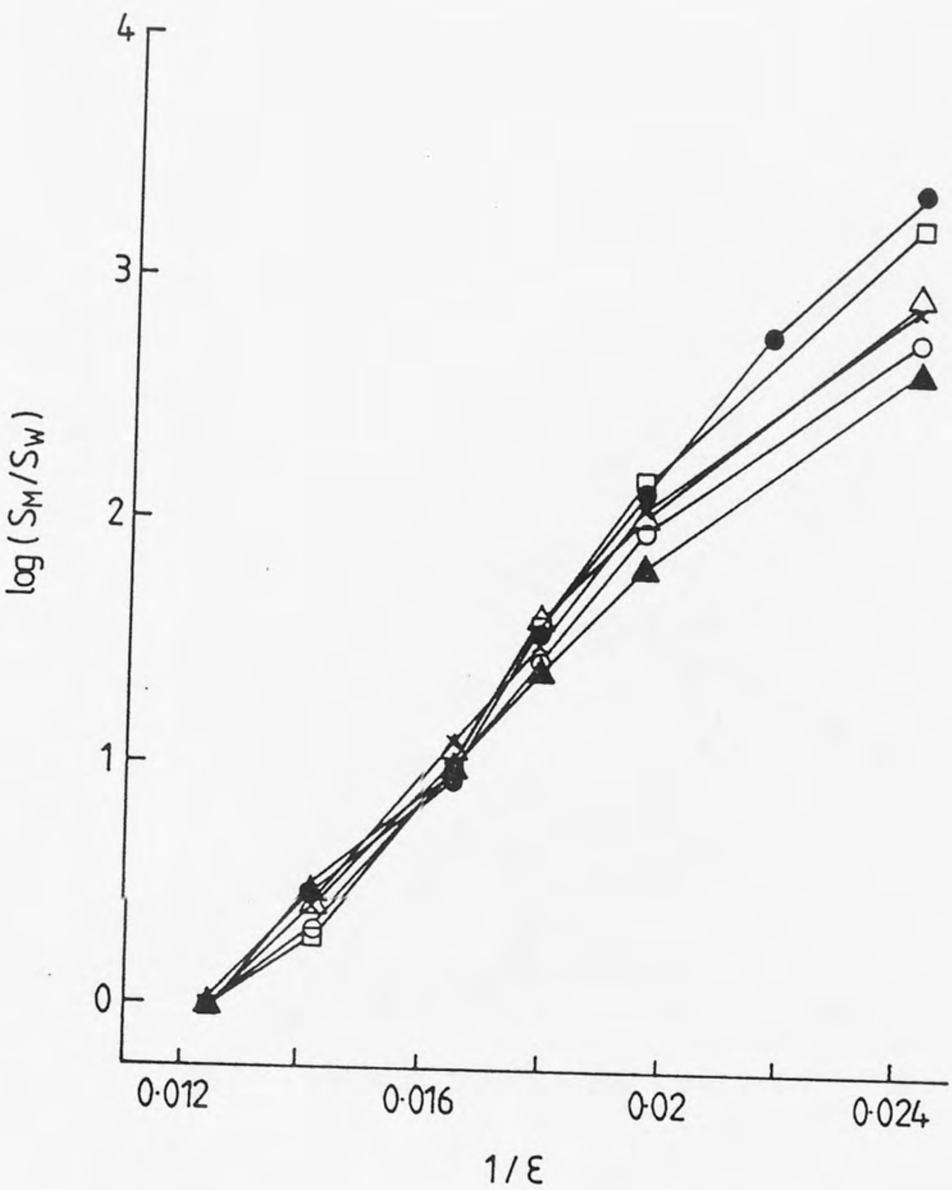

Fig. 3.9 Plot of $\log(S_M/S_W)$ versus $1/\varepsilon$ of propylene glycol water at 25°C.

KEY
- ○ Ibuprofen
- □ Flufenamic acid
- × (tetrahydro-2-furanyl) methyl ester of ibuprofen
- △ (tetrahydro-2-(2H) puranyl) methyl ester of ibuprofen
- ▲ Indomethacin
- ● Cyclohexylmethyl ester of ibuprofen

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CHAPTER 4

DRUG DECOMPOSITION KINETICS: THE KINETICS OF HYDROLYSIS OF INDOMETHACIN AND IBUPROFEN ESTERS

4.1 INTRODUCTION

Initial studies with ibuprofen, its derivatives and indomethacin indicated that cosolvents were likely to be required because of the drugs' limited solubilities in purely aqueous systems. During the screening for suitable solvents for the formulations and for assay by high-performance liquid chromatography it was observed that p-chlorobenzoic acid esters were formed in some of the formulations containing indomethacin. Since glycols and alcohols were the most likely cosolvents for use in the proposed topical formulations, the decomposition was studied in greater detail.

4.2 THEORY

The hydrolysis of indomethacin has been widely investigated and an emphasis was given to show that it follows first order kinetics(84-88,93) and this is confirmed in the present studies.

Under constant temperature and pH conditions the concentration change with time can be represented as:

$$\ln C_t = \ln C_o - kt \qquad (4.1)$$

where

C_t = concentration at time t.

C_o = initial concentration.

The temperature dependence of indomethacin degradation has been investigated(85,86) and the effect of temperature on the first order rate of degradation is given by the Arrhenius equation(90):

$$\log k = \log A - \frac{E_a}{2.303\ RT} \qquad (4.2)$$

where

k = the specific rate constant at temperature T

A = the frequency factor

E_a = the energy of activation

R = the universal gas constant, and

T = the absolute temperature in Kelvin

from which the Arrhenius parameters can be determined.

Indomethacin has been found to undergo specific acid-base catalysed hydrolysis(84-87). The observed rate constant, $k_{obs.}$, for the hydrolysis of a weak acid such as indomethacin (IH), under these conditions can be represented by the following equation:

$$k_{obs.} = (k_1+k_2[H^+] + k_3[OH^-])/(1 + K_a/[H^+]) + (k_4 + k_5[H^+] + k_6[OH^-])/(1 + [H^+]/K_a) \qquad (4.3)$$

where K_a is the dissociation constant of indomethacin and [ ] are concentration terms for the enclosed ions. By appropriate choice of pH

values over which the reaction is followed, the drug species being studied can be selected. k_1, k_2 and k_3 refer to rate constants for the non-dissociated acid species. k_1 represents the rate constant for the non-catalysed reaction, k_2 the hydrogen ion catalytic coefficient and k_3 the hydroxyl ion catalytic coefficient. k_4, k_5 and k_6 are the corresponding constants for the ionized acid.

Considering the effect of pH on the degradation of indomethacin in aqueous solutions, Schemes 4.1a to 4.1f are possible:

$$IH \xrightarrow{k_1} \text{Products} \qquad \text{Scheme 4.1a}$$

$$IH + H^+ \xrightarrow{k_2} \text{Products} \qquad \text{Scheme 4.1b}$$

$$IH + OH^- \xrightarrow{k_3} \text{Products} \qquad \text{Scheme 4.1c}$$

$$I^- \xrightarrow{k_4} \text{Products} \qquad \text{Scheme 4.1d}$$

$$I^- + H^+ \xrightarrow{k_5} \text{Products} \qquad \text{Scheme 4.1e}$$

$$I^- + OH^- \xrightarrow{k_6} \text{Products} \qquad \text{Scheme 4.1f}$$

Scheme 4.1 Possible routes of degradation of indomethacin in aqueous solutions.

where I^- is the monodissociated species of indomethacin and Schemes 4.1a and 4.1d indicate the solvent catalytic effect, that is, unionized water may be considered as the reacting species in a pH independent reaction.

Schemes 4.1b and 4.1e represent the specific hydrogen ion catalysed decomposition of indomethacin in acidic solutions while Schemes 4.1c and 4.1f represent the specific hydroxide ion catalysed decomposition of indomethacin in alkaline solutions. The overall velocities for the reactions represented in Schemes 4.1a to 4.1f are given by:

$$\frac{d[IH]_T}{dt} = k_1[IH] \qquad (4.4)$$

$$\frac{d[IH]_T}{dt} = k_2[IH][H^+] \qquad (4.5)$$

$$\frac{d[IH]_T}{dt} = k_3[IH][OH^-] \qquad (4.6)$$

$$\frac{d[IH]_T}{dt} = k_4[I^-] \qquad (4.7)$$

$$\frac{d[IH]_T}{dt} = k_5[I^-][H^+] \qquad (4.8)$$

$$\frac{d[IH]_T}{dt} = k_6[I^-][OH^-] \qquad (4.9)$$

$[IH]_T$ represent the total amount of the acid which is equal to:

$$[IH]_T = [IH] + [I^-] \qquad (4.10)$$

and due to the overall first order character of the reactions:

$$-\frac{d[IH]_T}{dt} = k_{obs.}\,[IH]_T \tag{4.11}$$

and when:

$$K_a = \frac{[I^-][H^+]}{[IH]} \tag{4.12}$$

so equation (4.10) can be rewritten as

$$[IH]_T = [IH](1 + K_a/[H^+]) \tag{4.13}$$

or

$$[IH]_T = [I^-](1 + [H^+]/K_a) \tag{4.14}$$

Combining these equations and substituting in the corresponding rate laws for the reactions given in Schemes 4.1a to 4.1f will clearly show that the pH dependence of the specific acid-base catalysed hydrolysis of indomethacin can be summarized in terms of the general rate law:

$$\frac{d[IH]_T}{dt} = ((k_1 + k_2[H^+] + k_3[OH^-])/(1 + K_a/[H^+]))[HI] +$$

$$((k_4 + k_5[H^+] + k_6[OH^-])/(1 + [H^+]/K_a))[I^-] \tag{4.15}$$

and for which the observed rate constant was given in equation (4.3).

The pH-dependence for the hydrolysis of esters (S) is another example of this type of catalysis(280) where the general rate law is

given by

$$\frac{dP}{dt} = (k_1 + k_2[H^+] + k_3[OH^-])S \qquad (4.16)$$

where P represents the Product and:

$$k_{obs.} = k_1 + k_2[H^+] + k_3[OH^-] \qquad (4.17)$$

which summarize the pH dependence of the ester hydrolysis.

The dielectric constants of the solvent compositions were calculated from:

$$\varepsilon_{solvent} = x\,\varepsilon_x + (1 - x)\varepsilon_y \qquad (4.18)$$

where

x = mole fraction of the solvent component

ε_x and ε_y = dielectric constant of the pure solvents(279).

For example, the dielectric constant of aqueous methanol (50%v/v) was calculated as follows:

$$x = \frac{(50 \times 0.8435)/32}{(50 \times 0.8435)/32 + (50 \times 1.01345)/18} = 0.3187$$

$$\varepsilon_{\substack{\text{methanol-water}\\(50\%v/v)}} = (0.3187 \times 33.7) + (1 - 0.3187) \times 80 = 65.24$$

4.3 MATERIALS AND METHODS:

4.3.1 The Effect of Temperature on the Alkaline Hydrolysis of Indomethacin and (tetrahydro-2-(2H)pyranyl)methyl ester of Ibuprofen

A. The effect of temperature on the alkaline hydrolysis of indomethacin

The decomposition of indomethacin in Teorell-Stenhagen's buffer pH 9.7 was conducted at various temperatures (30, 40, 46, 52.5 and 58.5°C). During the reaction runs the indomethacin content of the solutions was determined by direct ultraviolet spectroscopy at 320 nm. In each case, 450 ml of buffer, pH 9.7, was introduced into a 0.5 litre flask and preheated to the required temperature in a water bath. When this temperature was reached 50 mls of freshly prepared indomethacin solution in buffer (1 mM, 0.357 μg/ml) was added with constant, vigorous stirring. About 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice. Five ml portions were then acidified with 1 ml of 0.08 M hydrochloric acid to produce a solution at about pH 6.0 which was stable for the duration of the assay. Standard solutions were made from freshly prepared stock solutions in buffer, pH 9.7, to contain 0.0014 - 0.112 mM of indomethacin. The standards were treated in every respect as with the test solutions. The samples and standards were analysed in the ultraviolet region at 320 nm against an appropriate blank using a 1 cm cell using a Pye Unicam SP6-400 Ultraviolet Spectrophotometer.

B. The effect of temperature on the alkaline hydrolysis of (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen

The hydrolysis of the ibuprofen ester in 10% ethanol-Teorell-Stenhagen's buffer mixtures, pH 7.0, was conducted under various isothermal conditions (57°, 67°, 70°, 75° and 96°C).

The remaining ester and the ibuprofen formed were determined using HPLC. Preparation of the reaction mixtures, sample and standard solutions followed the method described earlier in 2.2.3 for the hydrolysis of (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen.

4.3.2 The Effect of pH on the Degradation of Indomethacin and Ibuprofen Esters

A. The effect of pH on the degradation of indomethacin at 69°C

The decomposition of indomethacin at a temperature of 69°C was conducted in Teorell-Stenhagen's buffer with the following pH values: 9.526, 9.3, 9.016, 8.88, 8.74, 8.42, 8.03, 7.61, 7.15, 6.55, 6.05, 5.45, 5.16, 4.34, 4.05, 3.63, 3.0, 2.5, 2.0 and 1.4. The indomethacin content of the solution during the reaction was determined by direct ultraviolet spectroscopy at 320 nm.

For pH values higher than pH 5.0, the reaction mixture at the required pH was prepared and 450 mls of Teorell-Stenhagen's buffer, preheated to 69°C was introduced into a 0.5 l flask. When the temperature had equilibrated 50 mls of freshly prepared indomethacin solution in buffer (1 mM, 0.357 µg/ml) was added with constant

stirring. At pH values lower than 5.0, 10% ethanol in the final solution was necessary to increase the low solubility of indomethacin.

Forty ml of ethanol (10 ml short of that necessary to produce the final ethanol concentration) were pipetted into a 0.5 l flask together with the necessary volume of buffer of the required pH to produce 490 mls. The solution was then heated in a water bath to 69°C.

Ten mls of a 1.5 mM ethanolic indomethacin solution was then added with rapid stirring. In all cases about 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ince. For pH values higher than 7.0, 5 ml portions were then quenched by acidification with 1 ml of 0.08 M hydrochloric acid. For pH values higher than pH 5.0 the standard solutions were made to contain 0.0014 - 0.112 mM from a freshly prepared stock solution in buffer at the specified pH value. Lower than pH 5.0 the standard solutions were made from freshly prepared stock solution in 10% ethanol-buffer at the required pH value to contain 0.003 - 0.03 mM of indomethacin. The samples and standards were analysed in the ultraviolet region against an appropriate blank using 1 cm cell.

B. The effect of pH on the hydrolysis of (tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen at 70°C

The hydrolysis of the ester at 70°C was conducted in Teorell-Stenhagen's buffer over a pH range between 2 and pH 9.6. The value of these pH points are as follows: 9.6, 9.0, 8.7, 8.46, 8.14, 7.496, 7.0, 6.58, 6.19, 5.0, 3.97, 3.5, 2.97, 2.5 and 1.9. At each pH the preparation and analysis of the reaction mixture, sample and standard

solutions in 10% ethanol-buffer mixtures were conducted using the method described earlier in 2.2.3 for the degradation of the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen.

One ml of 0.08 M hydrochloric acid was added to the samples collected of pH values higher than pH 7.0 in order to quench the reaction before freezing the solution in dry ice prior to storage at -15°C. Standard solutions were made in 10% ethanol buffer mixtures of the specific pH value.

Standard solution made for pH 2.0 included 7.74 - 38.7 μM of the ethylester of ibuprofen in addition to ibuprofen and its (tetrahydro-2-(2H)pyranol)methyl ester.

C. The hydrolysis of the (tetrahydro-2-furanyl)methyl- and cyclohexylmethyl esters of ibuprofen in Teorell-Stenhagen's buffer pH 7.0 at 70°C.

The hydrolysis of each of the two esters was followed in Teorell-Stenhagen's buffer pH 7.0 at 70°C. The reaction mixtures were prepared containing 10% ethanol for the (tetrahydro-2-furanyl)methyl ester and 30% ethanol for the cyclohexylmethyl ester of ibuprofen in the final solution because of the low solubilities of the esters in aqueous solutions. To follow the decomposition of the (tetrahydro-2-furanyl)-methyl ester of ibuprofen 40 mls of ethanol (10 ml short of that necessary to produce the final ethanol concentration) were pipetted into a 0.5 l flask together with the necessary volume of buffer to produce 490 mls. For the decomposition of the cylcohexylmethyl ester of ibuprofen 140 ml of ethanol (10 ml short of that necessary to produce

the final ethanol concentration) were pipetted into a 0.5 l flask together with 50 mls of buffer made to contain a salt concentration ten times that required in the final solution in order to maintain constant ionic strength and then the necessary volume of water was added to produce 490 ml. This approach also helped to overcome problems of zero time decomposition in the samples used. Final adjustment to pH 7.0 were carried out with 1 M hydrochloric acid where necessary. Volume changes induced by this treatment were negligible.

These solutions were heated in a water bath at 70°C. When this temperature was reached, 10 ml of 1.973 mM ethanolic (tetrahydro-2-furanyl)methyl ester solution or 10 ml of 1.654 mM ethanolic cyclohexylmethyl ester solution was added with rapid stirring.

The experiments were conducted using the method described earlier in 2.2.3 for the hydrolysis of the (tetrahydro-2-(2H)pyranyl)methyl-ester of ibuprofen. Standard solutions for the (tetrahydro-2-furanyl)-methyl ester were made in 10% ethanol-buffer pH 7.0 from stock solutions to contain ibuprofen and its (tetrahydro-2-furanyl)methyl ester in a concentration of 7.74 - 38.7 μM and 7.84 - 3.92 μM respectively. Standard solutions for the cyclohexylmethyl ester were made in 30% ethanol buffer pH 7.0 from substock solutions to contain ibuprofen and its cyclohexylmethyl ester in a concentration of 7.74 - 38.7 μM and 6.61 - 33.08 μM respectively. Solutions were analysed by HPLC.

4.3.3 Solvent effects on the Alkaline Hydrolysis of Indomethacin

A. Synthesis of methyl and ethyl p-chlorobenzoates

These were synthesised as described in the literature(281,282) by esterification of the free acid with methanol or ethanol in the presence of concentrated sulphuric acid. Product identification was by melting point determination (44°C) for methyl p-chlorobenzoate (Lit. 43 - 43.5°C (281)) and by boiling point determination ($b.p._{10}$ 107°C, $b.p._{760}$ 237-239°C) for ethyl p-chlorobenzoate (Lit. $b.p._{760}$ 238°C (282)). Infra-red, nuclear magnetic resonance and mass spectra were consistent with the required esters and reported data(283-287).

B. The effect of methanol on the alkaline hydrolysis of indomethacin

The decomposition of indomethacin in aqueous alcoholic buffered solutions at 40°C over the range of 0 to 70%v/v of methanol- pH 9.7 Teorell-Stenhagen's buffer was monitored.

To maintain constant ionic strength, a buffer with a salt concentration ten times that required in the final solution was made. Fifty millilitres were pipetted into each of a series of 500 ml flasks and volumes of methanol each 10 ml short of those necessary to produce the predetermined final methanol concentrations were added to the flasks together with the necessary volumes of water to produce 490 ml. The solutions obtained were heated in water baths to 40°C.

Ten ml of a 3 mg/ml methanolic indomethacin solution was added to each of the 500 ml containers with rapid stirring. Final adjustments to

pH 9.7 were carried out with 1M hydrochloric acid where necessary. Volume changes were negligible.

For decomposition in the purely aqueous buffered solution of indomethacin 50 ml of buffer solution and 400 ml of water were prewarmed to 40°C. Fifty ml of a freshly prepared indomethacin solution (0.6 mg/ml) was then added with constant vigorous stirring. In all cases about 10 ml volumes were sampled at appropriate intervals and the reaction quenched by cooling to room temperature in ice. Five mls portions were then quenched with 1 ml of 0.08 M hydrochloric acid.

Standard solutions were prepared from appropriate stock solutions to contain 0.04 - 0.2 mM of each of indomethacin, p-chlorobenzoic acid, 5-methoxy-2-methylindole-3-acetic acid and methyl p-chlorobenzoate in either buffer pH 9.7 or aqueous alcoholic solutions containing the required concentration of methanol. Similarly a solution was monitored at 60°C containing 1 μM of indomethacin in Teorell-Stenhagen's buffer pH 9.7.

Standard solutions were similarly stored in ice and warmed to room temperature before acidifying as with test solutions. No decomposition was observed in the standard solutions during this procedure as proven by the absence of a 5-methoxy-2-methylindole-3-acetic acid peak in the HPLC chromatogram of a standard solution containing indomethacin, p-chlorobenzoic acid and methyl p-chlorobenzoate.

C. Effect of ethanol on the alkaline degradation of indomethacin

The decomposition of indomethacin was followed in 40% ethanol-

Teorell-Stenhagen's buffer pH 9.7 at 40°C using the method described earlier for the degradation of indomethacin in aqueous methanolic solutions in section 4.3.3.B.

D. The degradation of 5-methoxy-2-methylindole-3-acetic acid in buffer pH 2.0

The degradation of 5-methoxy-2-methylindole-3-acetic acid was followed in Teorell-Stenhagen's buffer pH 2.0. 10% ethanol in the final solution was necessary due to the low solubility of the acid in aqueous solutions. Forty mls of ethanol (10 ml short of that necessary to produce the final ethanol concentration) were pipetted into a 0.5 l flask together with the necessary volume of buffer pH 2.0 to produce 490 mls. The solution was then heated in a water bath to 60°C.

Ten mls of a 1.5 mM ethanolic 5-methoxy-2-methylindole-3-acetic acid solution was added with rapid stirring. About 5 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice. p-Chlorobenzoic acid (0.06 μM) in 50% ethanol buffer was used as an internal standard. Two mls of each sample were mixed with 2 mls of the internal standard solution before anlaysis by HPLC.

Standard solutions were prepared in 10% ethanol buffer pH 2.0 from an appropriate stock solution to contain 6 - 30 μM of the acid.

E. The degradation of indomethacin in buffer pH 2.0

The degradation of indomethacin in 10% ethanol-Teorell-Stenhagen's

buffer pH 2.0 was followed at 60°C using the method described for the degradation of indomethacin in 4.3.2.A.

Samples were quenched by cooling to room temperature with ice. The analysis was performed by means of HPLC in addition to the ultraviolet method described in 4.3.2.A. For HPLC anlaysis, 0.1 mg/ml thymol in 50% ethanol-buffer was used as an internal standard and 2 mls of sample was mixed with 2 ml of the internal standard solution before analysis. Standard solutions were prepared in buffer from an appropriate stock solution to contain 0.003 - 0.03 mM of each indomethacin and p-chlorobenzoic acid.

Similarly, solutions of indomethacin (1 μM) were followed at 60°C in buffer pH 2.0, 10% ethanol buffer pH 2.0 and in 10% methanol buffer pH 2.0.

F. The hydrolysis of methyl p-chlorobenzoate in 40% methanol-Teorell-Stenhagen's buffer pH 9.7 at 40°C

The decomposition of methyl p-chlorobenzoate was followed in aqueous Teorell-Stenhagen buffer solutions, pH 9.7, at 40°C in the presence of 40%v/v of methanol.

To maintain constant ionic strength a buffer with a salt concentration ten times that required in the final solution was made. Fifty millilitres were pipetted into a 0.5 l flask and 190 mls of methanol (10 ml short of that necessary to produce the predetermined final methanol concentration was added to the flask together with the necessary volume of water to produce 490 ml. The solutions obtained

were then heated to 40°C. Ten ml of a 1.764 mM methanolic ester solution was added with rapid stirring.

Final adjustment to pH 9.7 was carried out with 1 M hydrochloric acid with negligible volume changes. About 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice and acidifying 5 ml portions with 1 ml of 0.08 M hydrochloric acid.

5-Methoxy-2-methylindole-3-acetic acid (0.5 mM) in 50% methanol buffer was used as an internal standard. Four mls of each sample were mixed with 1 ml of the internal standard solution before analysis by HPLC. Standard solutions were made from an appropriate stock solution to contain 7.056 - 35.2 μM of each p-chlorobenzoic acid and its methyl ester.

The standards were stored in ice and warmed to room temperature before acidifying as with the test solutions. No decomposition was observed in the standard solutions during this procedure as proven by the absence of p-chlorobenzoic acid peak in the HPLC chromatogram of a standard solution containing methyl p-chlorobenzoate alone.

G. The effect of hydrotalcite pretreatment on the degradation of indomethacin

Approximately 550 mls of either methanol (Analar) or 50% methanol-water mixture were placed into two separate 1 l flasks and stirred vigorously overnight in the presence of 0.5 g hydrotalcite (Russel). Each batch was filtered and 490 mls from each were placed in a separate

0.5 l flask.

Ten mls of a 0.6 mg/ml methanolic indomethacin solution was added to each flask with rapid stirring and each flask was equipped with a condenser. The solution was brought to the boil gently in a water bath and monitored for indomethacin and its degradative products using HPLC over a period of 2.0 hours.

H. Solutions containing either p-chlorobenzoic acid alone or a mixture of both 5-methoxy-2-methylindole-3-acetic acid and p-chlorobenzoic acid (0.03 mM each) in 40% methanol-Teorell-Stenhagen's buffer pH 9.7

Solutions containing either p-chlorobenzoic acid or a mixture of both 5-methoxy-2-methylindole-3-acetic acid and p-chlorobenzoic acid (0.03 mM each) in 40% methanol-Teorell-Stenhagen's buffer pH 9.7 were monitored at 40°C using HPLC for the appearance of any new peaks.

4.3.4 The Effect of Cetyltrimethylammonium Bromide on the Degradation of Indomethacin

A. Surface tension measurements

Surface tensions were measured using the Wilhelmy plate method(288). The surface tension instrument was constructed mainly from a microbalance which consist of three parts (C_i Electronics, England) and attached to a chart recorder (W+W). The microbalance unit measures any changes in weight exerted by a balanced hanging glass plate (made from a 0.5 cm wide glass slide cover glued to a piece of wire).

Ten to fifteen mls of the test or standard solutions were placed in a shallow water jacketed cell attached to a Churchill constant temperature controller set at 20°C and the cell was placed on a moving plane which during the surface tension measurements can elevate the cell and its contents sufficiently to allow contact with the glass plate. The plane may then be lowered gently to break the adhesion force exerted by the molecules of the liquid surface on the glass plate due to its surface tension and this work is in the form of an extra weight on the side of the glass plate which is recorded as the machine response. This increases in magnitude until the tension reaches its maximum at which point the attachment between the glass and the surface of the fluid breaks. The machine response is used in a calibration curve constructed from pure compounds of known surface tension ($mN.m^{-1}$) at 20°C as follows: n-hexane (HPLC grade, Fisons), 18.43; acetone (SLR, BDH), 23.7; benzene (Analar, Fisons), 28.85; benzyl alcohol (SLR,BDH), 39; ethylene glycol (Analar, Fisons), 47.7; glycerol (B.P. Grade, MaCarthys), 63.4 and double distilled water, 69.9(289).

1. From a stock solution of indomethacin (3.4×10^{-4}M) in Teorell-Stenhagen's buffer pH 9.7 a series of solutions were made in buffer and their surface tension measurements were conducted at 20°C. These solutions contained the following concentrations of indomethacin $M \times 10^5$: 0.8, 1.7, 3.4, 6.81, 10.2, 12.65, 15.3, 17.025, 20.3 and 25.53.

2. The determination of the critical micelle concentration of cetyltrimethylammonium bromide (CTMB) in the presence and absence of indomethacin.

(a) The CMC of CTMB was determined in Teorell-Stenhagen's buffer pH 9.7 at 20°C by measuring the surface tension of a series of solutions of the surfactant in buffer made from an appropriate stock solution (2.5×10^{-4}M) and containing a range of CTMB concentrations between 0 and 1.7×10^{-4}M.

(b) The effect of the presence of indomethacin on the CMC of CTMB was studied by measuring the surface tension of a series of surfactant solutions in buffer pH 9.7 at 20°C in the presence of each of the following concentrations of indomethacin $M \times 10^5$: 3.4, 6.8, 10.2, 13.6, 17.0 and 20.4. The surfactant solutions were prepared from a stock solution in buffer (2.5×10^{-4}M) and contained a range of CTMB concentrations between 0 and 2×10^{-4}M.

B. The degradation of indomethacin in the presence of CTMB

1. The effect of CTMB on the alkaline hydrolysis of indomethacin at 60°C.

(a) Preparation of CTMB solutions

A stock solution of CTMB (0.0274 M) was prepared in Teorell-Stenhagen's buffer pH 9.7 and containing 3%v/v ethanol as a cosolvent. From this solution a substock was prepared containing 1.371 mM of CTMB in 3%v/v ethanol-Teorell-Stenhagen's buffer pH 9.7.

(b) The alkaline degradation of indomethacin in the presence of CTMB

The decomposition of indomethacin was followed in Teorell-Stenhagen's buffer pH 9.7 at 60°C in the presence of CTMB. The concentrations of CTMB used were as follows in $M \times 10^4$: 0, 1.371, 2.743, 5.48, 13.71, 27.43, 41.15, 54.8, 68.5, 82.3, 109.7, 137, 164.6, 219.4 and 274.3.

CTMB concentrations in the range between 1.371×10^{-4} and 13.71×10^{-4}M were made from the substock solution and the rest of the surfactant concentrations were made from the stock solution.

The reaction mixtures were made by introducing the required concentration of the surfactant into a series of 0.5 l flasks and volumes of ethanol necessary to produce a final ethanol concentration of 3%v/v were added to the flasks together with the necessary volumes of buffer pH 9.7 to produce 450 mls. The solutions obtained were then heated to 60°C.

Fifty ml of freshly prepared indomethacin solution (0.6 mg/ml) were added to each with rapid constant stirring. Approximately 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice and 5 ml portions were acidified with 1 ml of 0.08 M hydrochloric acid. Samples were analysed using both direct ultraviolet spectroscopy and HPLC.

Standard solutions for the ultraviolet assay of indomethacin were made from freshly prepared stock solution in buffer pH 9.7 to contain 0.014 - 0.112 mM of indomethacin and the required concentration of CTMB

where necessary. The samples and standard solutions in the ultraviolet assay were analysed for indomethacin at 320 nm using a 1 cm cell against an appropriate blank.

The HPLC assay used thymol (0.5 mg/ml) in 50% ethanol-water as an internal standard. Two mls of the sample solutions were mixed with 2 mls of the internal standard solution before analysis by HPLC. Standard solutions were prepared in buffer containing the required concentration of CTMB where necessary from an appropriate stock to contain 0.04 - 0.2 mM of each indomethacin, p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid.

2. <u>The degradation of indomethacin in 10% ethanol-Teorell-Stenhagen's buffer pH 2.0 at 60°C in the presence of 0.0137 M of CTMB</u>

The reaction mixture was prepared following the method described for the degradation of indomethacin in 10% ethanol-Teorell-Stenhagen's buffer pH 2.0 at 60°C together with the required concentration of CTMB to produce 0.0137 M from its stock solution. Procedures described earlier in 4.3.3.E were followed.

The standard solution incorporated the required concentration of CTMB to produce 0.0137 M. The samples and standards were analysed by HPLC.

C. The determination of indomethacin solubility in 10% ethanol-buffer pH 2.0 in the presence and absence of 0.0137 M CTMB at 40°C

The solubility of indomethacin in 10% ethanol-Teorell-Stenhagen's

buffer pH 2.0 at 40°C in the presence and absence of 0.0137 M CTMB was determined following the method described in 3.3.1.A for the solubility determination of indomethacin with the thermostatic water bath set at 40°C instead of 25°C. After equilibration, care was taken to filter the suspension using equipment prewarmed at 40°C.

The indomethacin concentration was determined by direct ultraviolet spectroscopy at 320 nm. Standard solutions were made to include 0.0137 M CTMB for the solubility determination of indomethacin in the presence of the surfactant.

D. Recording the ultraviolet spectra of indomethacin, CTMB and mixture of the two compounds in Teorell-Stenhagen's buffer pH 9.7

The ultraviolet spectra of solutions containing either indomethacin (3.4×10^{-5}M) or CTMB (2.74×10^{-3}M) and that of a mixture of both indomethacin (3.4×10^{-5}M) and CTMB (2.05×10^{-3}M) were recorded between 190 - 450 nm using a 1 cm cell against an appropriate blank using a Pye Unicam SP 800 Ultraviolet Recording Spectrophotometer.

E. The effect of CTMB on the ultraviolet absorption readings of indomethacin at 320 nm in Teorell-Stenhagen's buffer pH 9.7

1. The ultraviolet absorbance of indomethacin in the absence of CTMB at 20°C

From an appropriate freshly prepared stock solution of indomethacin in Teorell-Stenhagen's buffer pH 9.7, solutions were made containing 3.4, 6.8, 10.2, 13.6, 17.0 and 20.4 $M \times 10^5$ of indomethacin. The

ultraviolet absorbances of these solutions were measured at 320 nm using 1 cm cell against an appropriate blank.

2. The ultraviolet absorbance of indomethacin in the presence of CTMB at 20°C

The ultraviolet absorbance of a series of solutions containing a fixed concentration of indomethacin in the presence of a range of CTMB concentrations were measured by direct ultraviolet spectroscopy at 320 nm against an appropriate blank using a 1 cm cell. Each series contained CTMB in a concentration range between 0 - 0.00125 M together with one of the following indomethacin concentrations ($M \times 10^5$): 3.4, 6.8, 10.2, 13.6, 17.0 and 20.4.

4.3.5 HPLC Conditions

Column: Hypersil - ODS(5 μm) 10 cm × 4.6 mm I.D.

Detection wavelength and mobile phase:

(a) 70%v/v Methanol in water acidified with 0.2% orthophosphoric acid (pH=2.5) at 235 nm (for the separation of indomethacin and its degradative products).

(b) 65%v/v Methanol in water acidified with 0.02% orthophosphoric acid (pH=2.5) at:

(1) 235 nm was used for the separation of the hydrolytic solution of indomethacin in buffer pH 2.0 at 0.01 sensitivity (AUFS).

(2) 235 nm was used for the separation of the hydrolytic solution of indomethacin when ethyl p-chlorobenzoate was one of the decomposition products.

(3) 235 nm was used for the separation of the hydrolytic solution of indomethacin in buffer pH 9.7 at 0.01 sensitivity (AUFS).

(4) 267 nm was used for following the disappearance of 5-methoxy-2-methylindole-3-acetic acid in buffer pH 2.0 at 0.02 sensitivity (AUFS).

4.4 RESULTS AND DISCUSSION

4.4.1 The Influence of Temperature on the Alkaline Hydrolysis of Indomethacin and (tetrahydro-2-(2H)pyranyl)methyl Ester of Ibuprofen

The hydrolysis of indomethacin in Teorell-Stenhagen's buffer pH 9.7 was conducted at various temperatures. The first order decomposition of indomethacin reported by several other workers(84-88,93) is confirmed as shown in Fig. 4.1 and the respective rate constants equivalent to the absolute slopes of lines in Fig. 4.1 were determined by linear regression analysis and are recorded in Table 4.1.

Concentration-time profiles for the degradation of (tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen in Teorell-Stenhagen's buffer pH 7.0 conducted at various isothermal conditions are shown in Fig. 4.2 and a chromatogram illustrating the separation of the ester from its degradative product ibuprofen is shown in Fig. 4.3.

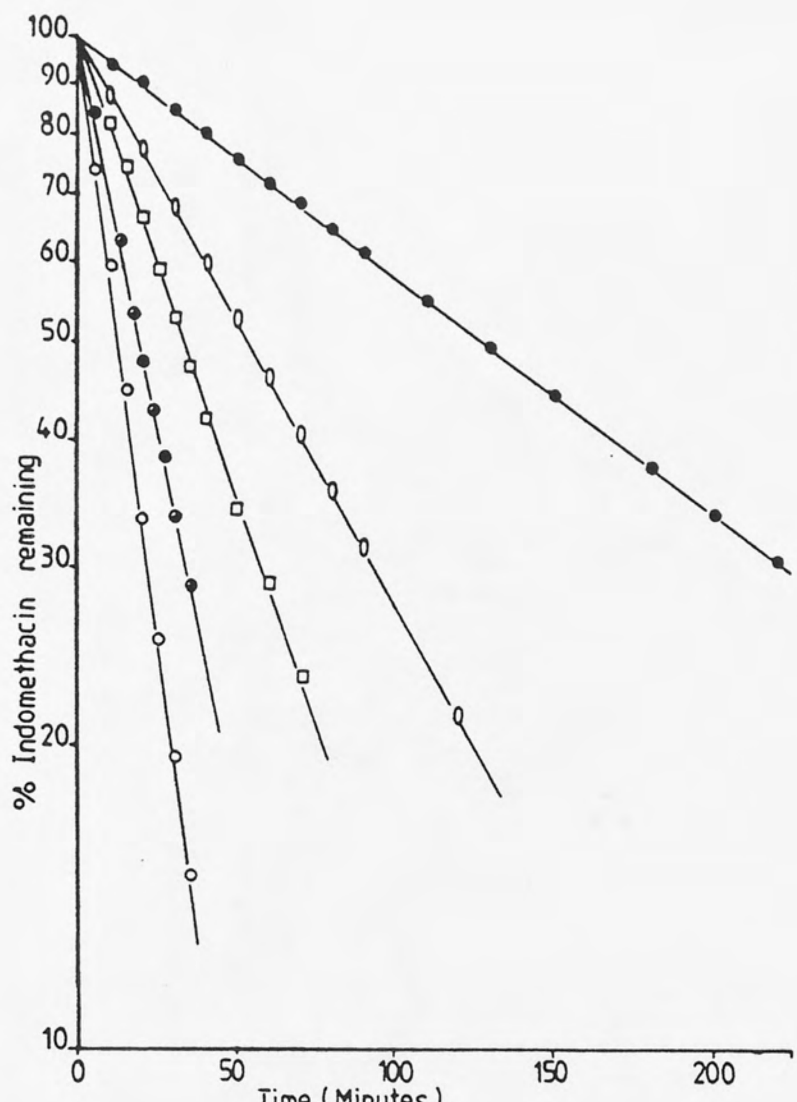


Fig. 4.1 Effect of temperature on the first order rate decomposition of indomethacin in pH 9.7 buffer. Temperature : (°C) ●: 30 , 0 : 40 , □ : 46 , ◑ : 52.5 , ○ : 58.5.

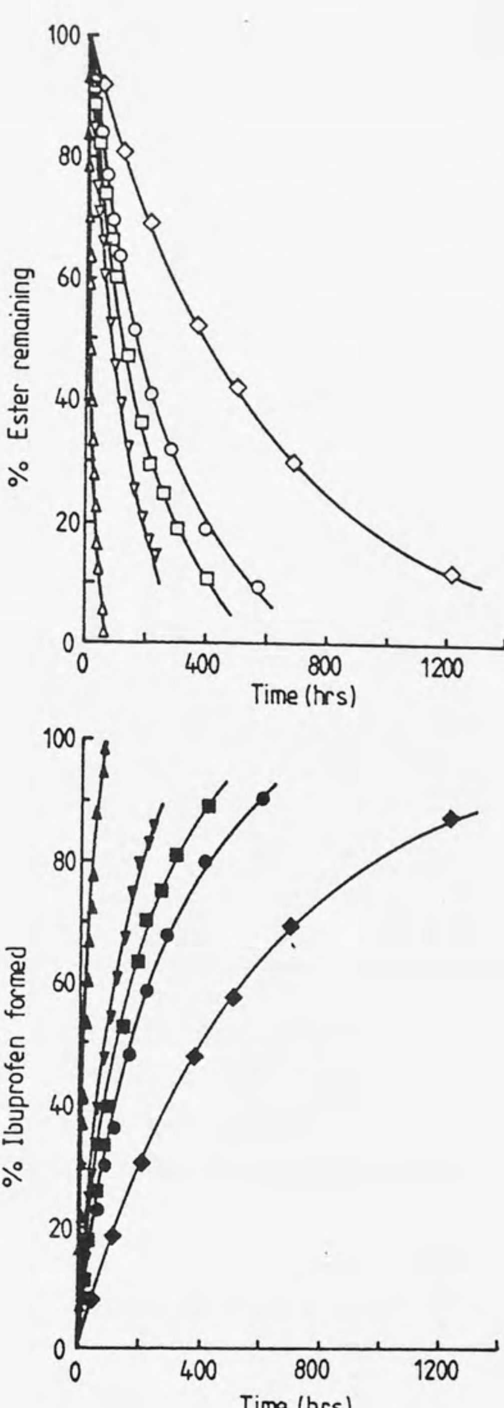
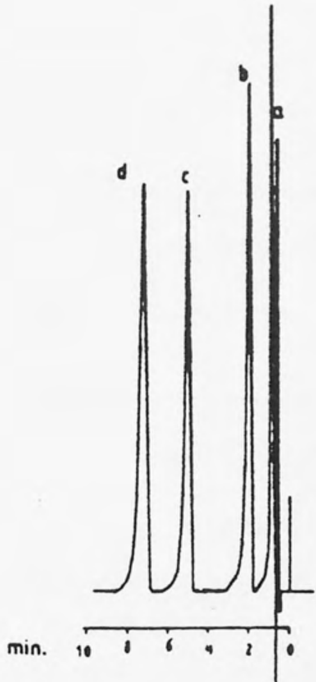


Fig. 4.2 Concentration - time profiles for the effect of temperature on the degradation of the (tetrahydro-2-(2H)pyranyl)methyl-ester of ibuprofen in Teorell-Stenhagen buffer pH 7·0.

KEY

(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen remaining.	ibuprofen formed	temperature °C
△	▲	96
▽	▼	75
□	■	70
○	●	67
◇	◆	57



HPLC conditions:

Column : ODS_Hypersil (5 μm) 10 cm x 4.6 mm i.d.;
Flow rate: 1 ml/min. ; Sensitivity: 0.02A.U.F.S.;
Detection wavelength: 220 nm; Chart speed: 1cm/2 min.;

Mobile phases: 81 % Methanol in water acidified with 0.3 % orthophosphoric acid.

a = solvent front.

Fig. 4.3 HPLC separation of ibuprofen(b), its (tetrahydro_2-(2H)pyranyl) methyl ester (c) and hexachlorophane (d).

TABLE 4.1

Effect of temperature on the first order hydrolysis of indomethacin in Teorell-Stenhagen's buffer pH 9.7.

Temperature °C	Rate Constant of Hydrolysis k $min.^{-1} \times 10^2$ (r = regression coefficent)
30	0.537 (r = 0.9991)
40	1.281 (r = 0.9992)
46	2.087 (r = 0.9994)
52.5	3.620 (r = 0.9994)
58.5	5.443 (r = 0.9993)

TABLE 4.2

Effect of temperature on the first order decomposition of (tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen in Teorell-Stenhagen's buffer pH 7.0.

Temperature °C	Rate constant of ester hydrolysis k $hr.^{-1} \times 10^3$ (r = regression coefficent)	Rate constant of ibuprofen formation k $hr.^{-1} \times 10^3$
57	1.725 (r = 0.9993)	1.722
67	4.046 (r = 0.9992)	4.05
70	5.37 (r = 0.9998)	5.365
75	8.207 (r = 0.999)	8.21
96	43.61 (r = 0.9997)	43.64

Semilogarithmic plots of these profiles gave straight lines which indicate that the ester degradation follows first order kinetics (Fig. 4.4) with the respective rate constants being listed in Table 4.2. The rate constants for the formation of ibuprofen were also measured and are similarly recorded.

The effect of temperature on the decomposition rate constant for indomethacin at pH 9.7 is shown in Fig. 4.5 (r=0.9997). While the effect on that for the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen at pH 7.0 is shown in Fig. 4.6 (r=0.9998). Both sets of data showed good adherence to the Arrhenius relationship and the activation energies and other related thermodynamic parameters are shown in Table 4.3.

TABLE 4.3

Activation energies and other related parameters for the degradation of indomethacin and (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen at 25°C in aqueous buffer solution at pH 9.7 and pH 7.0 respectively.

Compound	E_a (kJ/Mole)	A	ΔH* (kJ/Mole)	ΔS* (J/Mole/deg.)	ΔF* (kJ/Mole)
Indomethacin	68.325	3.23×10^{9} $min.^{-1}$	68.325	-28.818	76.91
Tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	84.203	3.59×10^{10} $hr.^{-1}$	84.203	25.24	76.68

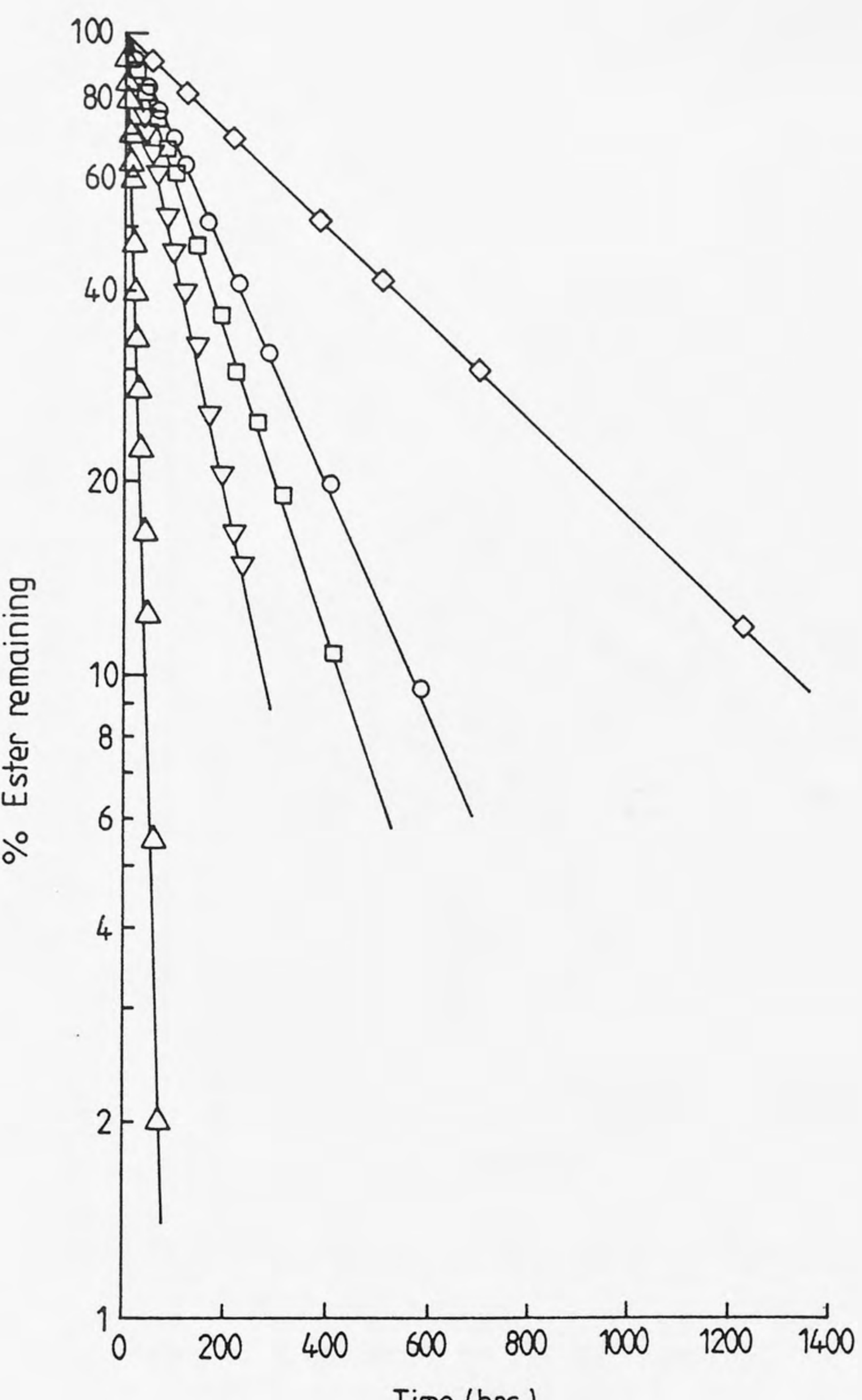


Fig. 4.4 Effect of temperature on the first order rate decomposition of (tetrahydro - 2 - (2H) pyranyl) methyl ester of ibuprofen in pH 7·02 buffer.

<u>KEY</u>

Temperature (°C)	
△	96
▽	75
□	70
○	67
◇	57

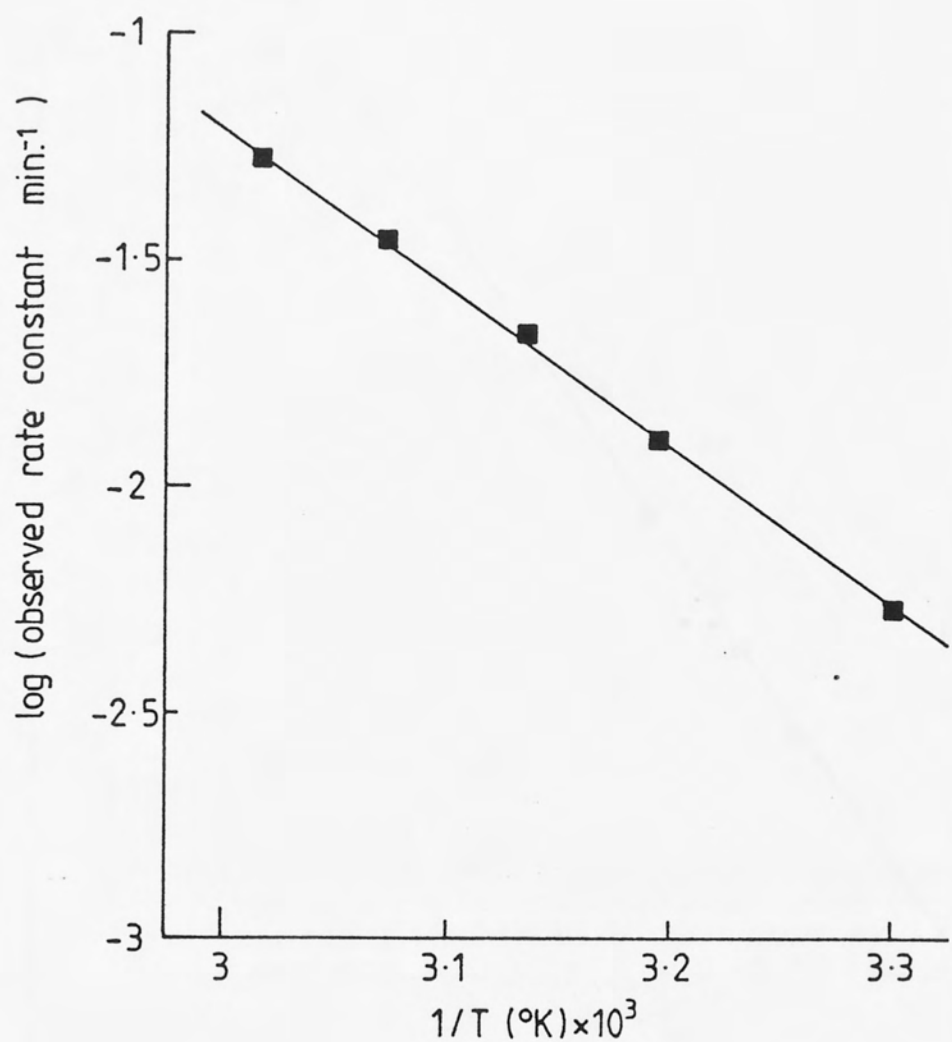


Fig. 4.5 Effect of temperature on the decomposition rate constant for indomethacin at pH 9·7

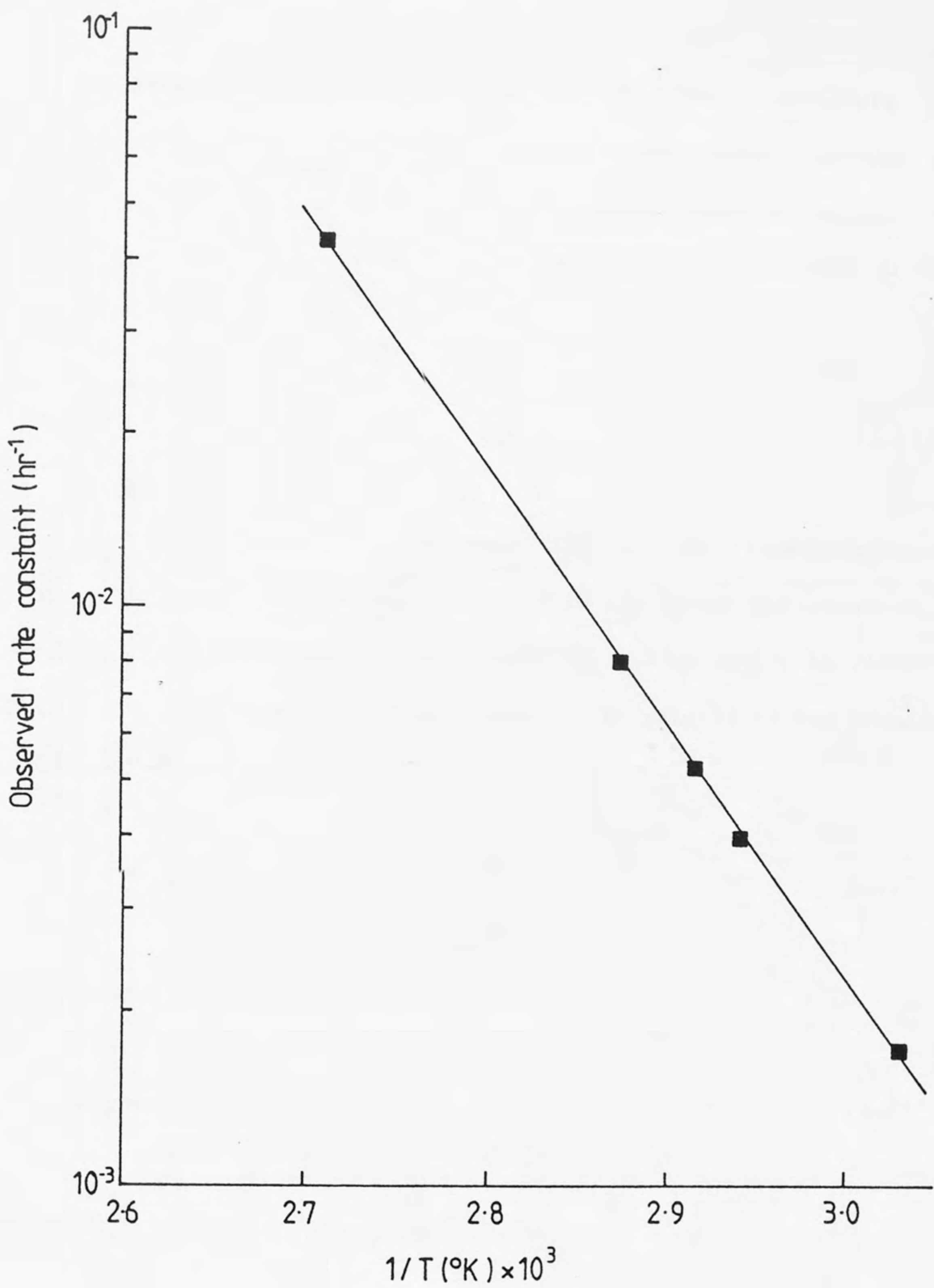


Fig. 4.6 Effect of temperature on the decomposition rate constant for (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen at pH 7·0

These parameters include ΔF^*, ΔS^* and ΔH^* (290) which are the respective differences between the standard free energy, entropy and enthalpy in the transition state and in the normal reactant state. The Arrhenius A factor is related to the entropy of activation of the transition state:

$$A = v.e^{\Delta S^*/R} \tag{4.19}$$

where v is the frequency of decomposition of the transition state complex and is equivalent to $(\frac{RT}{Nh})$ where R is the molar gas constant, T is the absolute temperature, N is Avogadro's number and h is Planck's constant. The Arrhenius activation energy E_a is related to the enthalpy of activation of the transition state:

$$E_a = \Delta H^* = \Delta E^* + P\,\Delta V^* \tag{4.20}$$

For most practical purposes $\Delta V^* = 0$, hence

$$E_a = \Delta E^* \tag{4.21}$$

where as

$$\Delta F^* = \Delta H^* - T\,\Delta S^* \tag{4.22}$$

4.4.2 The Effect of pH on the Degradation of Indomethacin and Ibuprofen Esters:

A. The hydrolysis of indomethacin in Teorell-Stenhagen's buffer was conducted under various pH conditions at 69°C. The data in Fig. 4.7

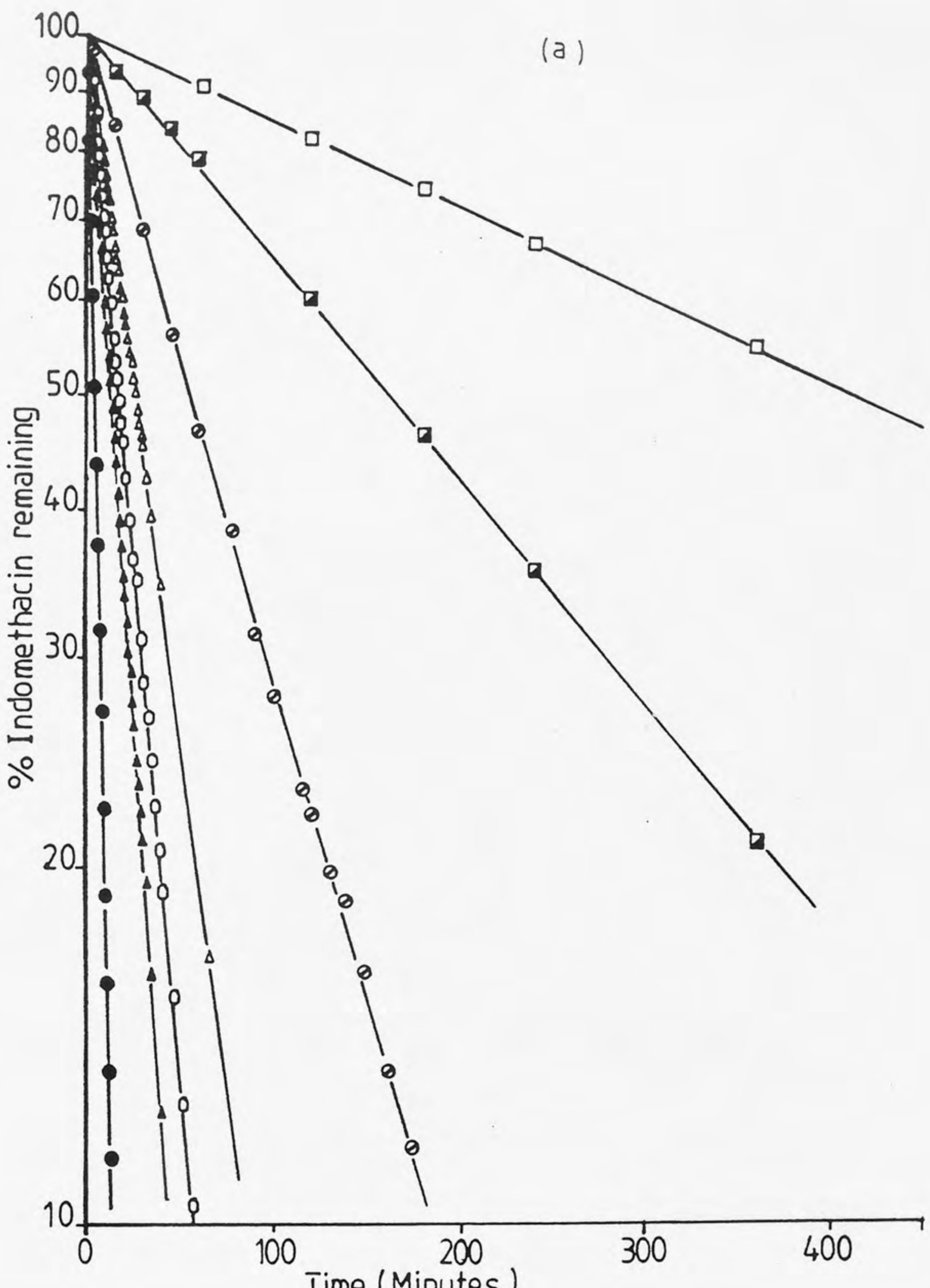


Fig. 4.7(a,b) Effect of pH on the first order decomposition of indomethacin at 69 °C. pH : □ : 7.61, ◪ : 8.03, ⌀ : 8.42, △ : 8.75, ○ : 8.88, ▲ : 9.02, ● : 9.53.

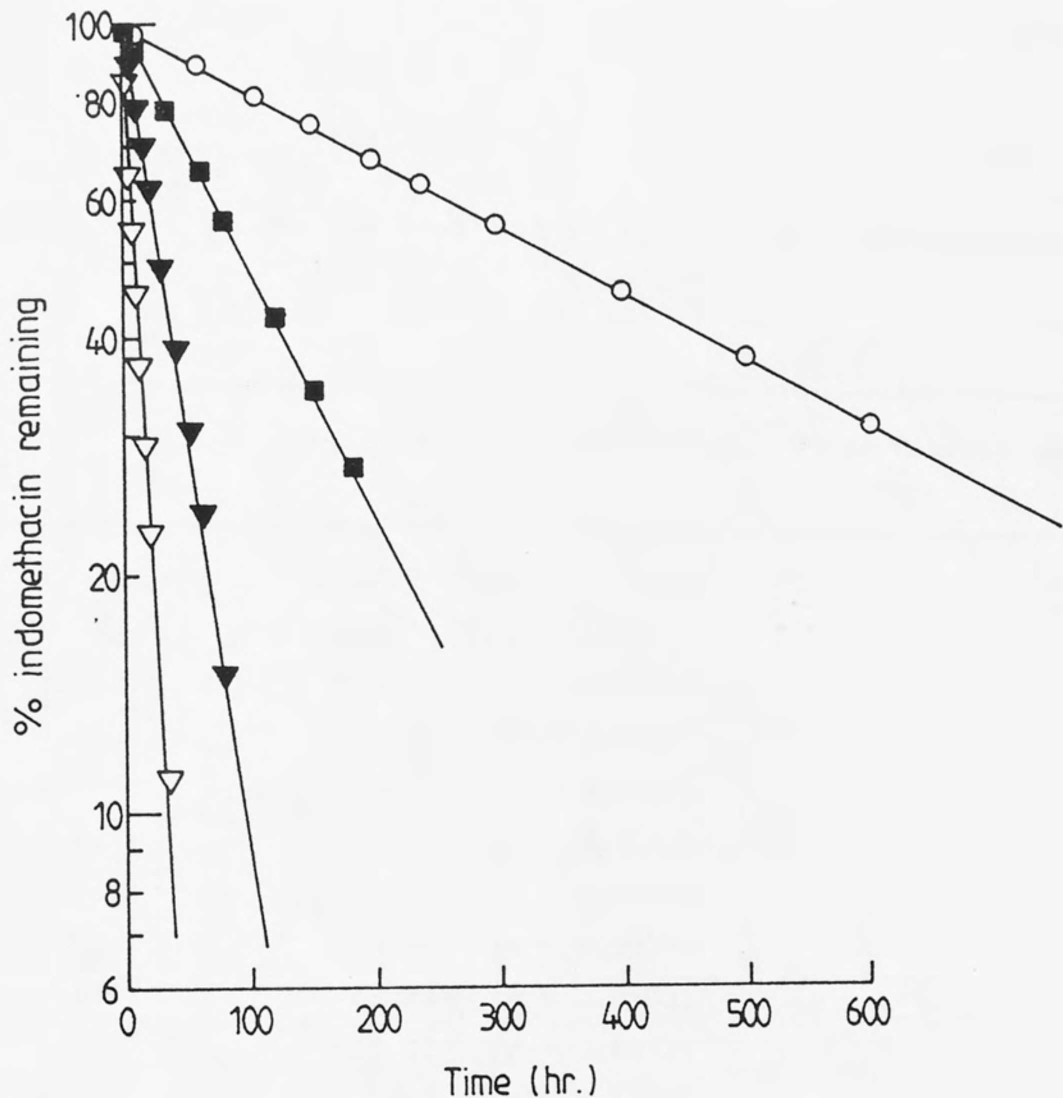


Fig. 4.7 (b)

KEY	pH
▽	1·4
■	2·5
○	3·1

show that in the buffered systems used in the present study, the decomposition was first order throughout the pH range employed and the rate constants equivalent to the absolute slopes of lines were determined by linear regression analyses and are recorded in Table 4.4.

TABLE 4.4

The effect of pH on the first order decomposition of indomethacin at 69°C.

pH	$k_{obs.}$ $min.^{-1}$ × 10^5 (r = regression coefficient)	
9.526	16754.0	(r = 0.9999)
9.3	9683.0	(r = 0.9991)
9.016	5175.0	(r = 0.9994)
8.88	4012.0	(r = 0.999)
8.748	2678.9	(r = 0.9998)
8.421	1254.0	(r = 0.9992)
8.030	423.5	(r = 0.9997)
7.61	166.4	(r = 0.9999)
7.156	63.52	(r = 0.9993)
6.555	25.02	(r = 0.9996)
6.056	9.17	(r = 0.9991)
5.456	4.57	(r = 0.9999)
5.16	4.06	(r = 0.999)
4.342	1.92	(r = 0.9992)
4.055	3.5	(r = 0.9997)
3.639	3.42	(r = 0.9995)
3.1	3.21	(r = 0.9998)
2.5	12.0	(r = 0.9993)
2.05	39.49	(r = 0.9991)
1.4	200.0	(r = 0.9992)

The rate-pH profile for the hydrolysis of indomethacin in aqueous buffer solutions at 69°C is shown in Fig. 4.8. This is in agreement with earlier reports that indomethacin was found to undergo the acid-base specific catalysed hydrolysis(86). With a pK_a of 4.22, over the alkaline pH range, indomethacin is essentially fully ionized, furthermore, the hydrogen ion concentration is negligible relative to the hydroxyl ions. Under such conditions, if the reaction is base catalysed and is not subject to other catalytic reactions, equation (4.3) reduces to

$$k_{obs.} = k_6[OH^-]$$

and $\log k_{obs.} = \log k_6 K_w + pH.$

A plot of $\log k_{obs.}$ against pH should therefore give a straight line of slope equal to unity. Analysis of the data shows that the effect of pH on the alkaline decomposition rate constant for indomethacin at 69°C (Fig. 4.9) gave a straight line ($r = 0.9993$) with a positive slope of 1.056 which is in close agreement with earlier reports that in the alkaline medium indomethacin hydrolysis is base catalysed(85).

The hydroxide ion catalytic rate constant k_6 at 69°C obtained from the intercept was equal to 1.518×10^3 L/mol./min.. At low pH indomethacin is present in the undissociated form. Futhermore, the hydrogen ions will be present in predominant concentration over the hydroxyl ions. Under such conditions specific hydrogen ion catalysis will be observed if the reaction is not subject to other catalytic reactions and equation (4.3) will be reduced to

$$k_{obs.} = k_2 [H^+].$$

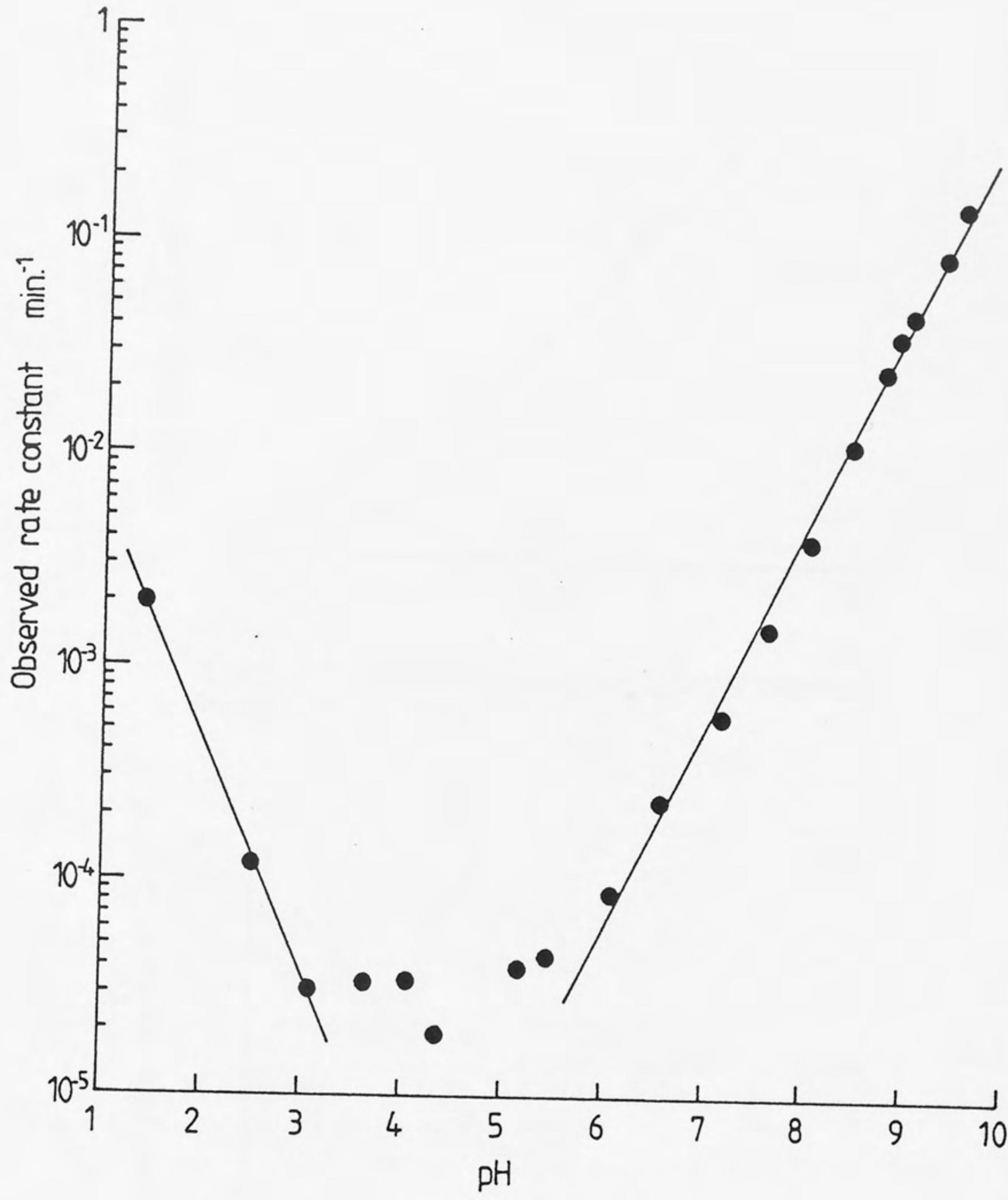


Fig. 4.8 Rate - pH profile for the specific acid-base catalysed hydrolysis of indomethacin at 69°C.

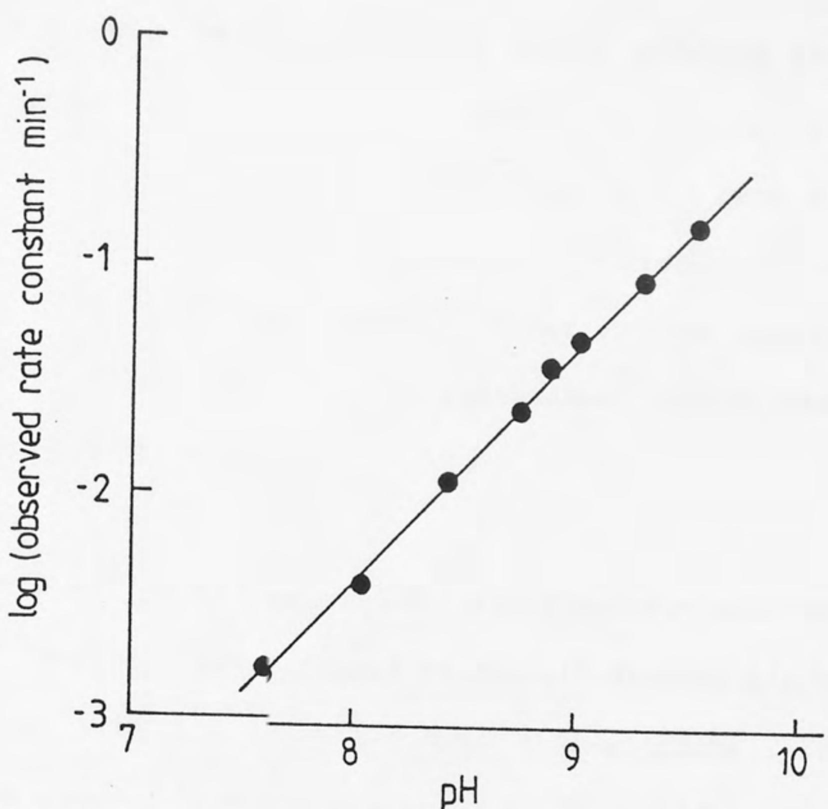


Fig. 4.9 Effect of pH on the alkaline decomposition rate constant for indomethacin at 69°C.

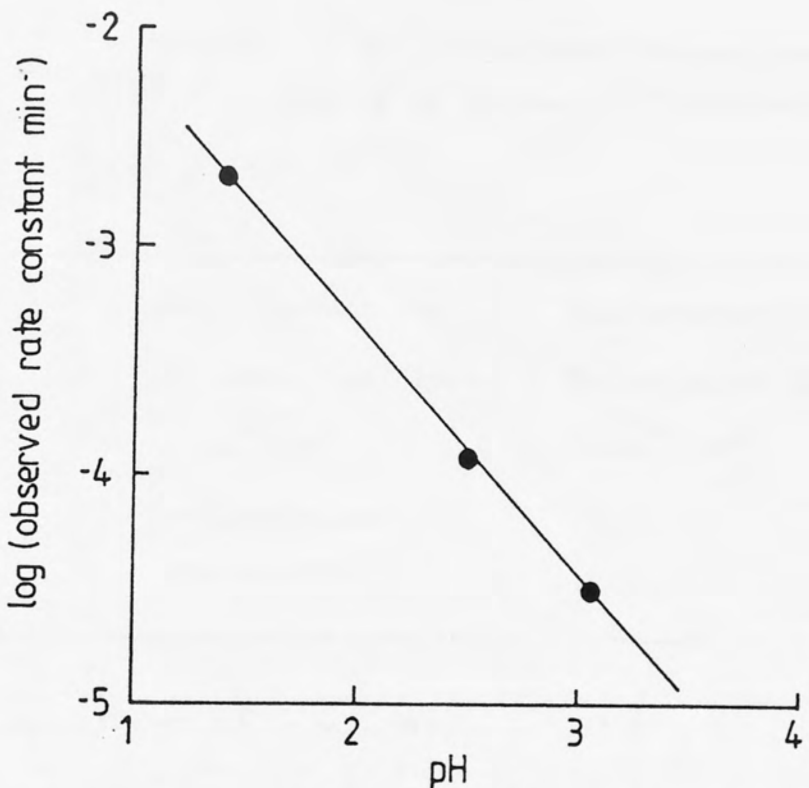


Fig. 4.10 Effect of pH on the acidic decomposition rate constant for indomethacin at 69°C.

A plot of logk against pH should therefore give a straight line with a negative slope equal to unity. The effect of pH on the acidic decomposition rate constant of indomethacin (Fig. 4.10) gave a straight line (r = 0.9992) with a slope of -1.06. The hydrogen ion catalytic rate constant k_2 at 69°C obtained from the intercept was equal to 0.059 L/mol./min.. Above pH 3, there is a pH independent region, the plateau extending over some 3 pH units.

B. The hydrolysis of the (tetrahydro-2-furanyl)methyl- and cyclohexylmethyl esters of ibuprofen was followed in Teorell-Stenhagen's buffer pH 7.0 at 70°C (Figs. 4.11 and 4.12) and HPLC chromatograms illustrating the separation of ibuprofen esters are shown in Fig. 4.13.

TABLE 4.5

Rate constants for the degradation of (tetrahydro-2-furanyl)methyl- and cyclohexylmethyl esters of ibuprofen in ethanol - Teorell-Stenhagen's buffer pH 7.0 at 70°C.

Compound	Rate constant for the ester hydrolysis $k\ hr.^{-1} \times 10^4$ (r=regression coefficient)	Rate constant for the formation of ibuprofen $k\ hr.^{-1} \times 10^4$
(tetrahydro-2-furanyl)-methyl ester of ibuprofen	57.32 (r = 0.9999)	57.31
cyclohexylmethyl ester of ibuprofen	4.428 (r = 0.9999)	4.429

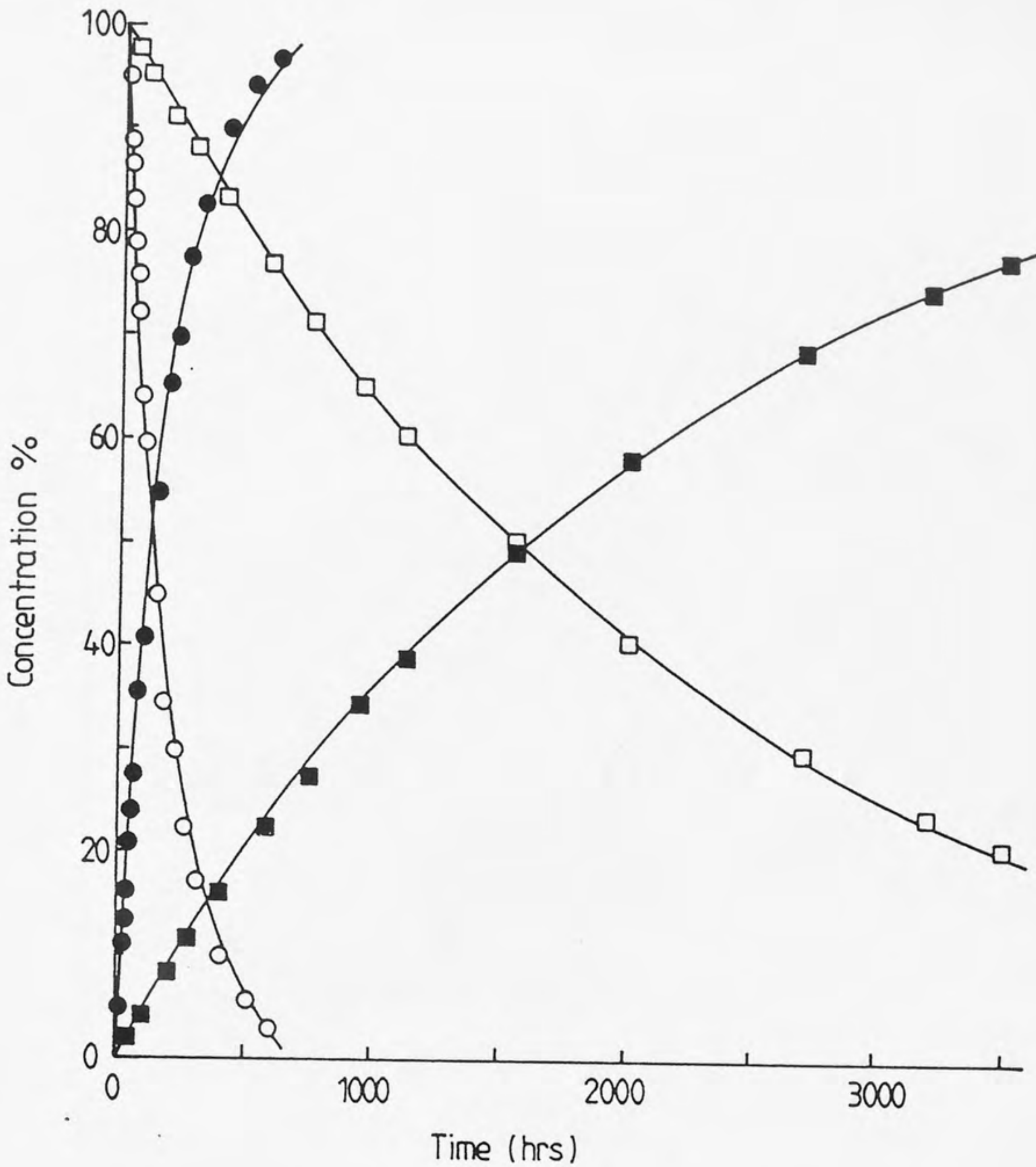


Fig. 4.11 Concentration - time profiles for the hydrolysis of (tetrahydro-2-furanyl) methyl and cyclohexylmethyl esters of ibuprofen in Teorell - Stenhagen's buffer pH 7·0 at 70°C.

KEY ○ (tetrahydro - 2 - furanyl) methyl ester remaining
● ibuprofen formed
□ Cyclohexylmethylester remaining
■ ibuprofen formed

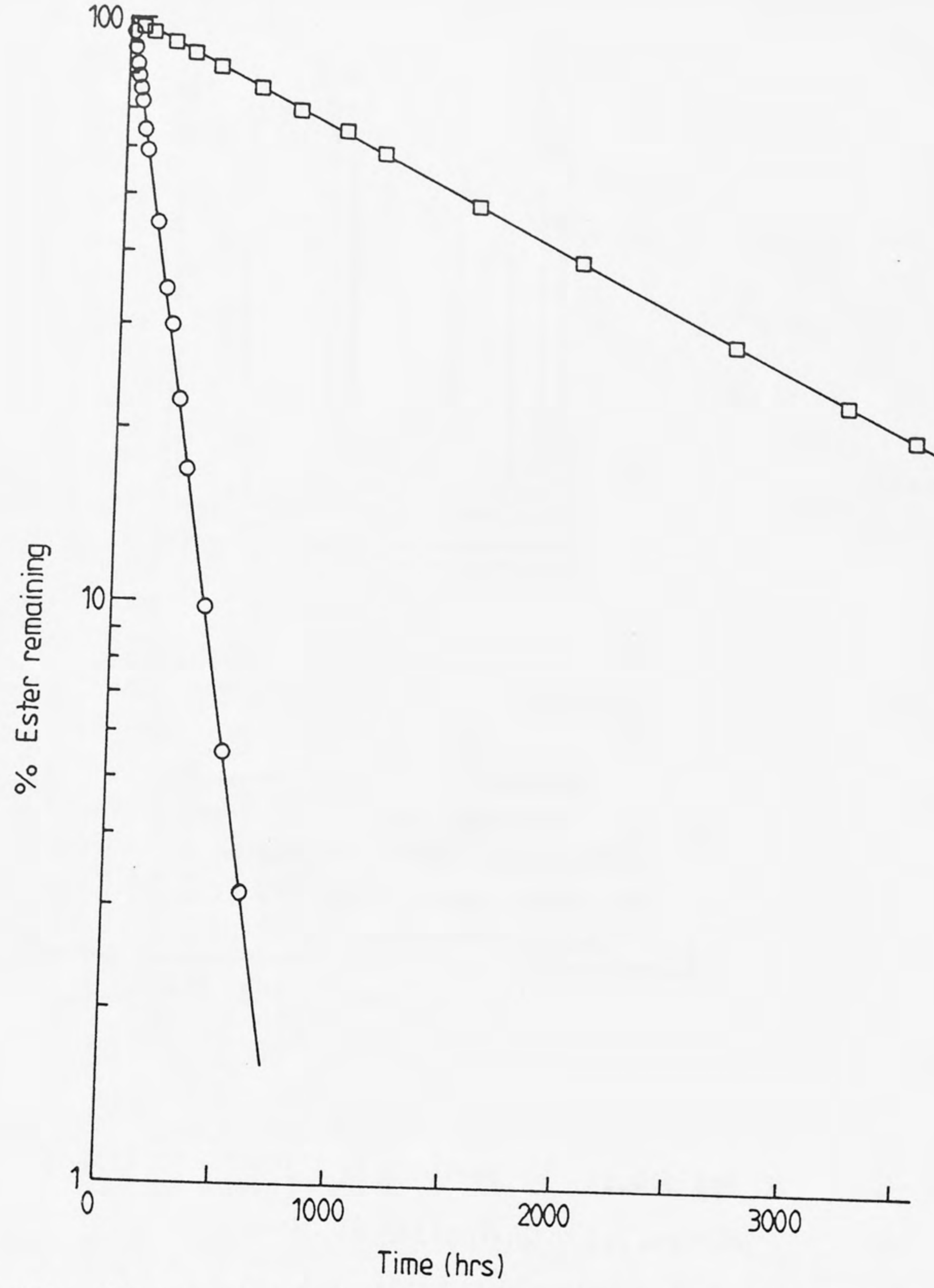
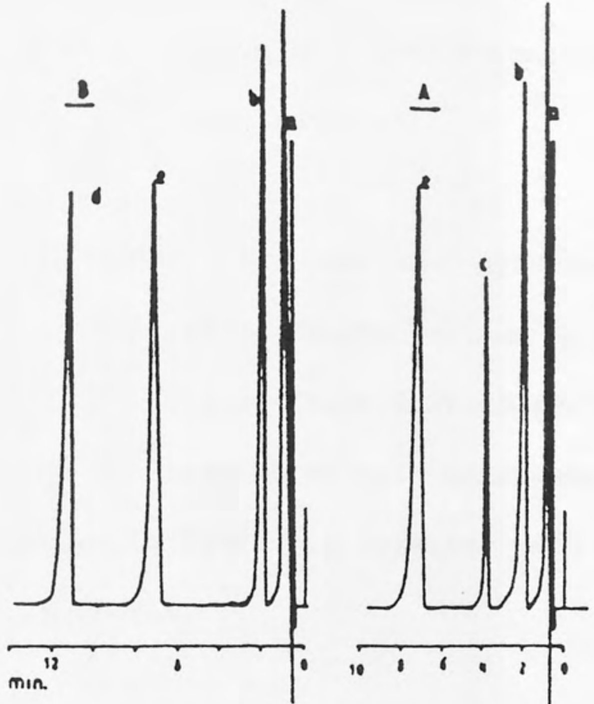


Fig. 4.12 First order plots for the hydrolysis of (tetrahydro-2-furanyl)-methyl and cyclohexyl methyl esters of ibuprofen in Teorell-Stenhagen's buffer pH 7·0 at 70°C.

KEY
○ (tetrahydro-2-furanyl) methyl ester
□ Cyclohexylmethyl ester



HPLC conditions:
Column : ODS-Hypersil (5 μm) 10 cm x 4.6 mm i.d.;
Flow rate: 1 ml/min. ; Sensitivity: 0.02 A.U.F.S.;
Detection wavelength: 220 nm; Chart speed: 1 cm/2 min.;
Mobile phases : 81% Methanol in water acidified with 0.3% orthophosphoric acid.

a = solvent front.

Fig. 4.13 HPLC separation of ibuprofen (b) and its (tetrahydro-2-furanyl)-methyl ester (c), cyclohexylmethyl-ester (d) and hexachlorophane (e).

The respective first order rate constants are recorded in Table 4.5. The rate constants for the formation of ibuprofen were also measured and are similarly recorded.

C. The first order plots for the hydrolysis of the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen over a range of pH values between 2 and pH 9.6 in 10% ethanol-Teorell-Stenhagen's buffer at 70°C are shown in Fig. 4.14 and the respective rate constants for the hydrolysis of the ester are recorded in Table 4.6 together with the rate constants for the formation of ibuprofen.

The effect of pH on the decomposition rate constant of the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen at 70°C is shown in Fig. 4.15. Examination of this rate-pH profile indicates that the ester undergoes specific acid-base catalysis.

In the alkaline region a plot of log k versus pH for the hydrolysis at 70°C again gives a straight line (Fig. 4.16) ($r = 0.9998$) with a positive slope of 1.003. The hydroxide ion catalytic rate constant k_3 at 70°C was calculated from the intercept and is equal to 4.94×10^4 L/mol./hr.. At low pH, specific hydrogen ion catalysis was again observed and a straight line (Fig. 4.17) ($r = 0.9999$) with a negative slope of a value equal to -1.01 was obtained from a plot of log k versus pH. The value of k_2 which represent the hydrogen ion catalytic rate constant at 70°C was calculated from the intercept to be equal to 1.4501 L/mol./hr..

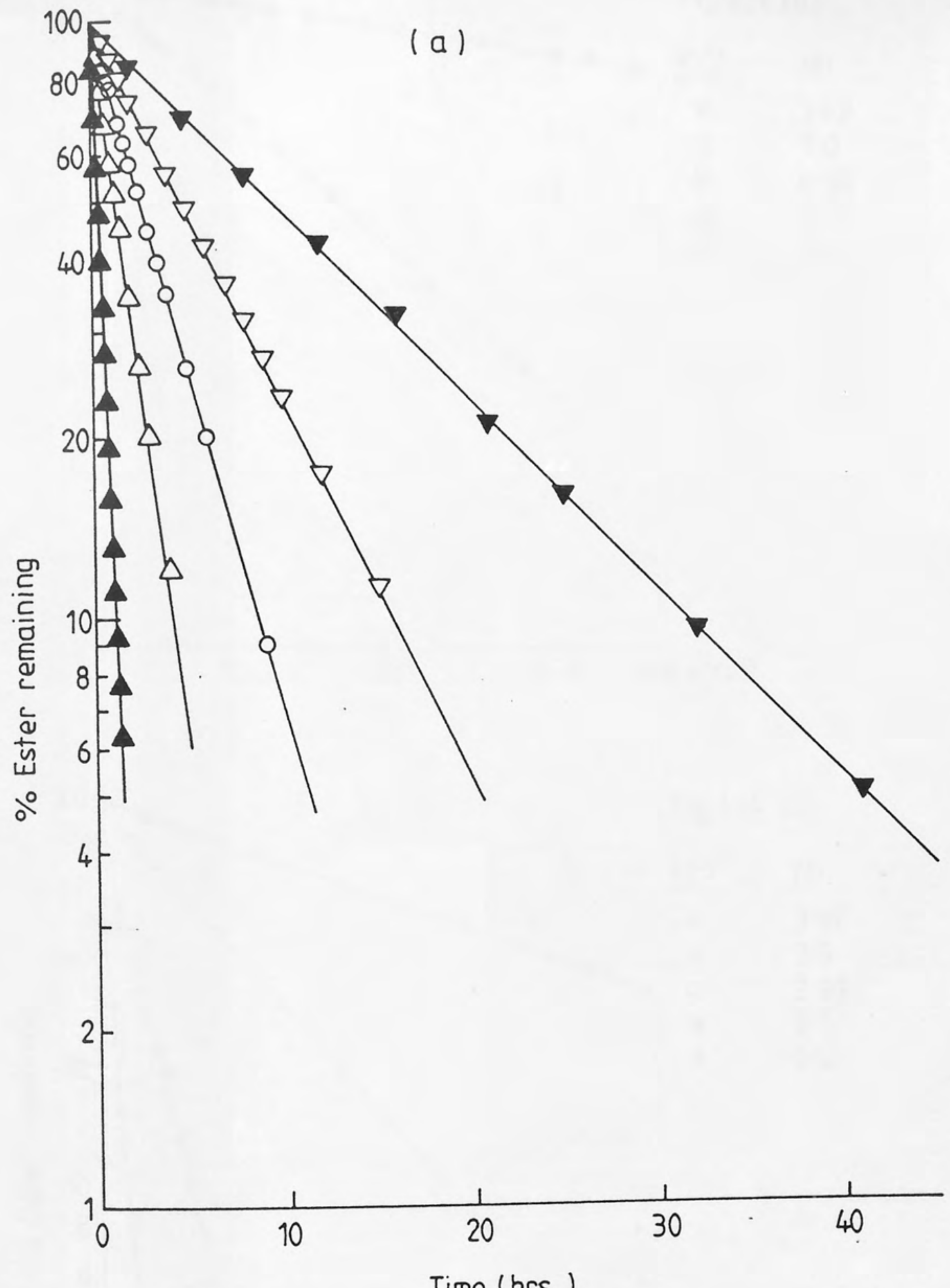


Fig. 4.14 (a,b,c) Effect of pH on the first order rate decomposition of (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen at 70° C

KEY
▲ 9·6 pH
△ 9·0 pH
○ 8·7 pH
▽ 8·46 pH
▼ 8·14 pH

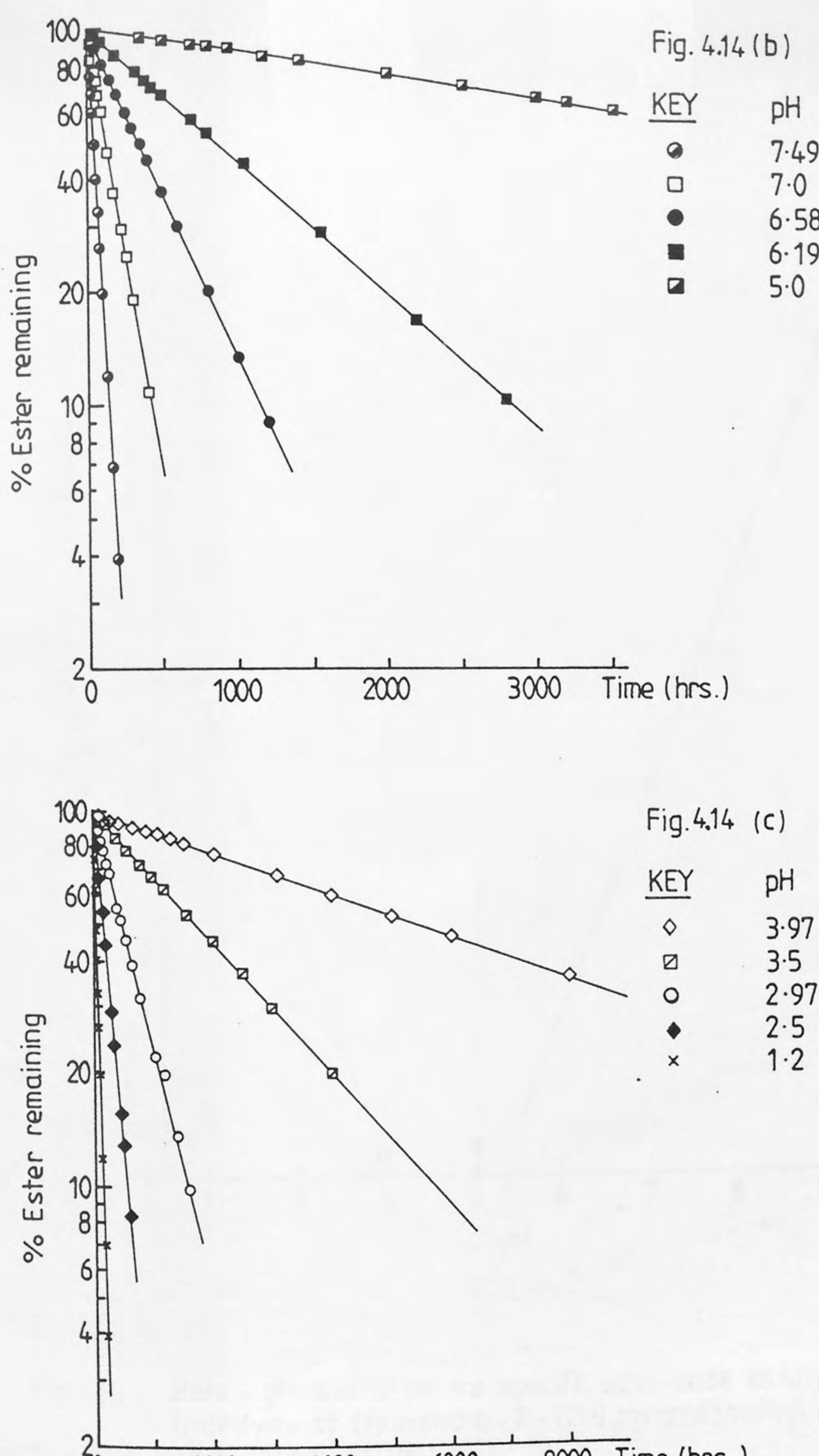


Fig. 4.14 (b)
KEY
pH
7·49
7·0
6·58
6·19
5·0
% Ester remaining
100
80
60
40
20
10
8
6
4
2
0
1000
2000
3000
Time (hrs.)
Fig. 4.14 (c)
KEY
pH
3·97
3·5
2·97
2·5
1·2
% Ester remaining
100
80
60
40
20
10
8
6
4
2
0
2000
4000
6000
8000
Time (hrs.)

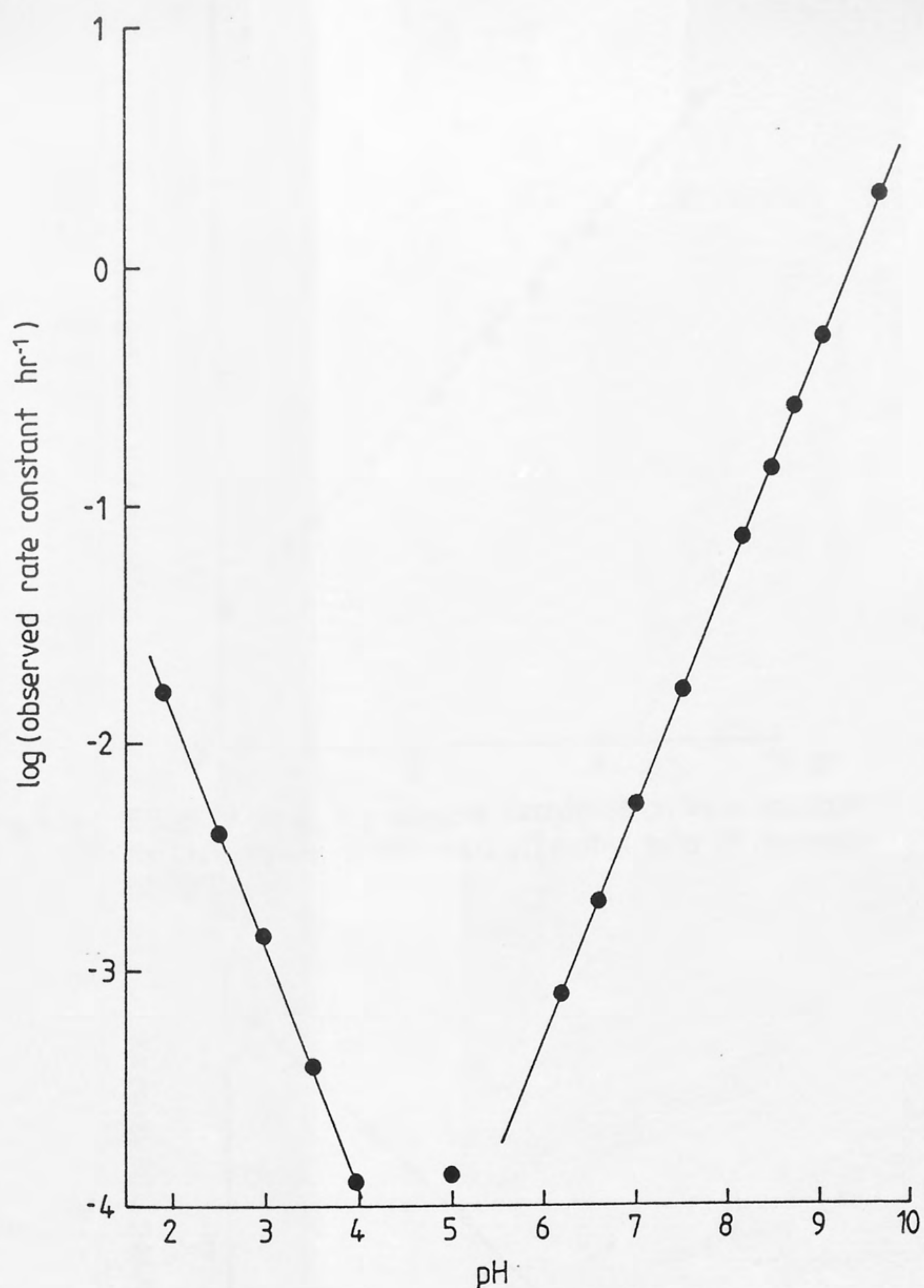


Fig. 4.15 Rate - pH profile for the specific acid-base catalysed hydrolysis of (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen at 70° C

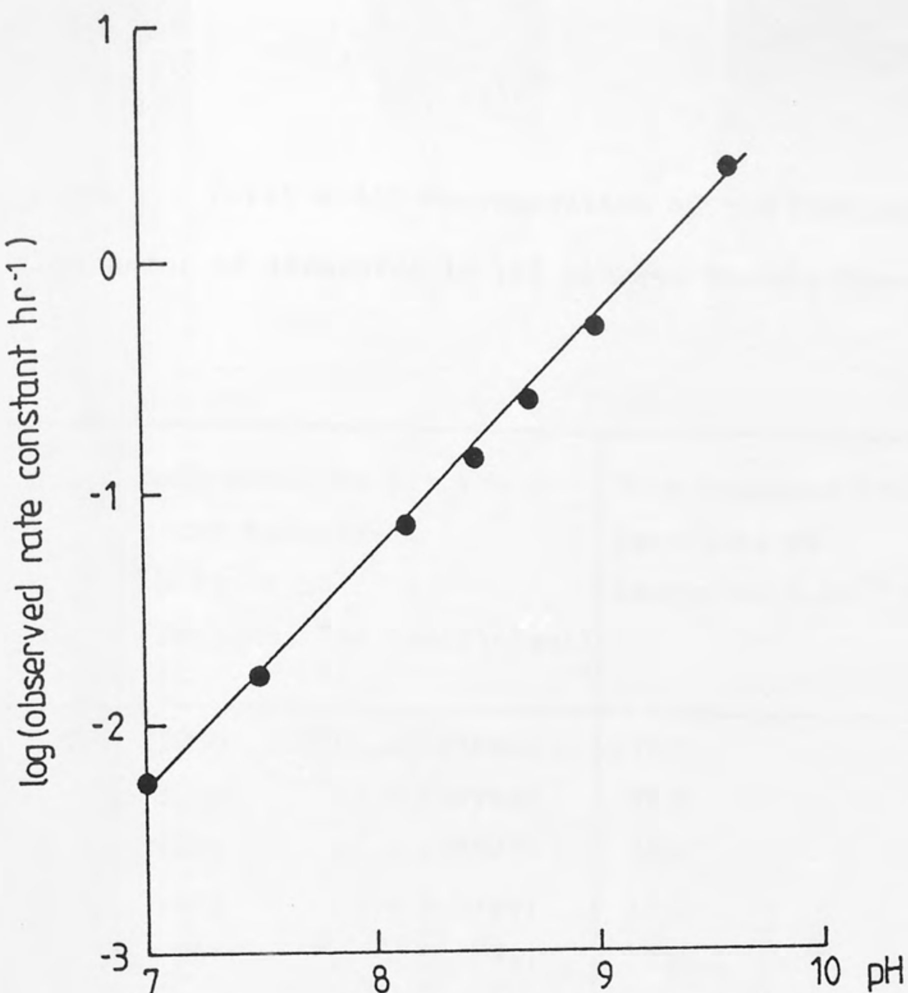


Fig. 4.16 Effect of pH on the alkaline decomposition rate constant for (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen at 70°C

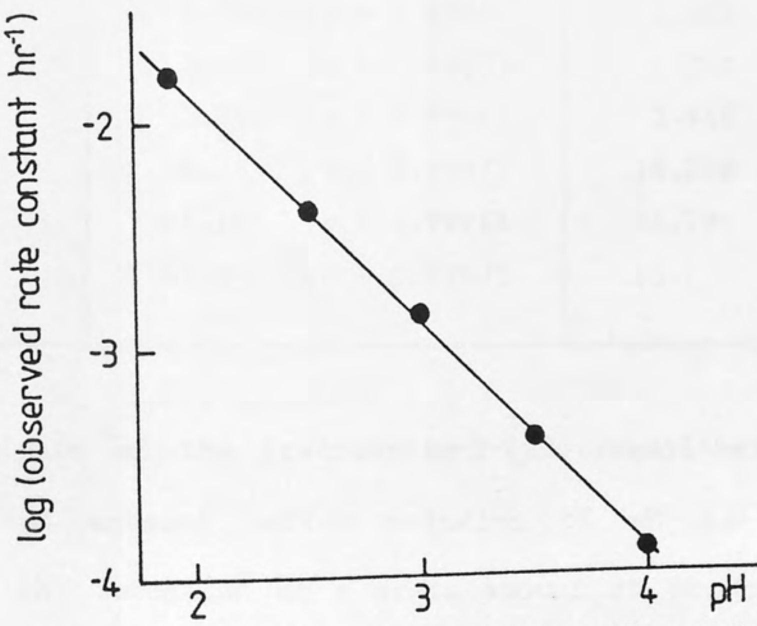


Fig. 4.17 Effect of pH on the acidic decomposition rate constant for (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen at 70°C

TABLE 4.6

The effect of pH on the first order decomposition of the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen in 10% ethanol-Teorell-Stenhagen's buffer at 70°C.

pH	Rate constant for the ester hydrolysis $k\ hr.^{-1} \times 10^4$ (r=regression coefficient)		Rate constant for the formation of ibuprofen $k\ hr.^{-1} \times 10^4$
9.6	22000	(r = 0.9991)	22005
9.0	5330	(r = 0.9990)	5332
8.7	2661	(r = 0.9992)	2661
8.46	1450	(r = 0.9999)	1451
8.14	731.0	(r = 0.9994)	730.9
7.49	165.0	(r = 0.9996)	164.8
7.0	57.3	(r = 0.9998)	57.3
6.58	20.0	(r = 0.9993)	20.1
6.19	8.139	(r = 0.9991)	8.138
5.0	1.361	(r = 0.999)	1.362
3.97	1.283	(r = 0.9997)	1.282
3.5	3.946	(r = 0.9998)	3.946
2.97	14.249	(r = 0.9992)	14.249
2.5	41.195	(r = 0.9991)	41.194
1.9	165.0	(r = 0.9997)	165.1

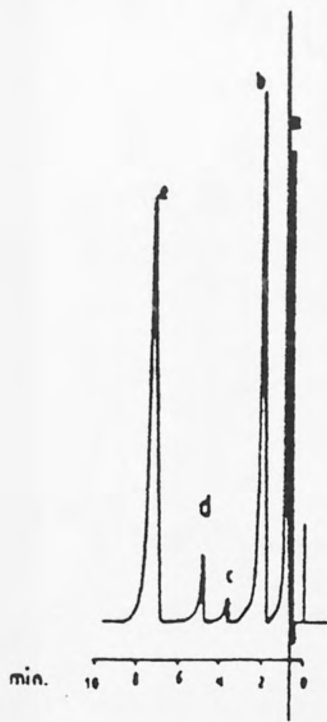
The hydrolysis of the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen in 10% ethanol buffer solution of pH 2.0 at 70°C is accompanied by the formation of a trace amount of the ethyl ester of ibuprofen.

Under acidic condition, the presence of ethanol led to the esterification of ibuprofen. The amount of ibuprofen transformed into

its ethyl ester after the completion of the hydrolysis of its (tetrahydro-2-(2H)pyranyl)methyl ester was less than 6% over a period of 500 hours (Fig. 4.18). A control solution containing ibuprofen in 10% ethanol-buffer pH 2.0 resulted in the formation of a trace amount of its ethyl ester.

4.4.3 Solvent Effect on the Alkaline Hydrolysis of Indomethacin:

The products of decomposition of indomethacin (I) under alkaline condition are known to be 5-methoxy-2-methylindole-3-acetic acid (II) and p-chlorobenzoic acid (III) (Fig. 4.19)(84,89,105). Both products are also formed under neutral and acidic conditions with the overall rate of disappearance of indomethacin showing a minimum at pH 4 (Fig. 4.8). The data were obtained using isopH analysis and are in close agreement with those of Pawelczyk, Knitter and Alejska(86). Earlier data reported by Goto and others(84) however showed that in the acid region products other than (II) and (III) were formed and these authors postulated that the unidentified products were themselves products of the intermediates. In the absence of analytical data to elucidate the structures of these products, it is not possible to confirm the mechanism proposed. Since these products may interfere with the ultraviolet spectroscopic assay used in the kinetic studies, it was felt essential to exclude this possibility by a more discriminative method of analysis. High pressure liquid chromatography was therefore resorted to and Fig. 4.20(a) illustrates the separation of indomethacin from its two major products. In decomposed alkaline solutions of indomethacin (1 μM in buffer pH 9.7) there was no hint of additional products in the form of peak shoulders or altered ultraviolet spectra using the peak ratioing technique(172) (Fig. 4.21).



HPLC conditions:

Column : ODS-Hypersil (5 μm) 10 cm x 4.6 mm i.d. ;

Flow rate: 1 ml/min. ; Sensitivity: 0.02 A.U.F.S. ;

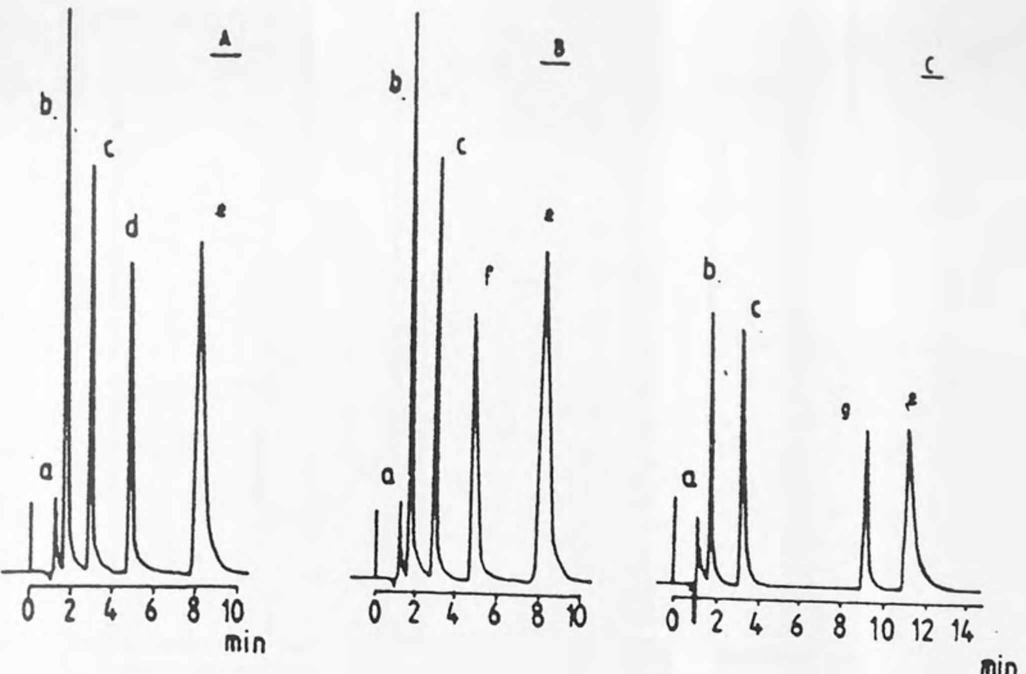
Detection wavelength: 220 nm; Chart speed: 1cm/2 min.;

Mobile phases : 81% Methanol in water acidified with 0.3% orthophosphoric acid.

a = solvent front.

Fig. 4.18 HPLC separation of ibuprofen(b) and its ethyl ester (c), (tetrahydro-2-(2H)pyranyl) methyl ester(d) and hexachlorophane (e) [in aqueous alcoholic buffered solution pH 2].

Fig. 4.19 Indomethacin (I) and its decomposition products, 5-Methoxy-2-methylindole-3-acetic acid (II), p-chlorobenzoic acid (III), methyl p-chlorobenzoate (IV) and ethyl p-chlorobenzoate (V).



HPLC conditions:

Column : ODS-Hypersil (5 μm) 10 cm x 4.6 mm i.d. ; Flow rate: 1 ml/min. ;

Sensitivity: 0.16 AUFS. ; Detection wavelength: 235 nm;

Mobile phase	% Methanol in water acidified with 0.2% orthophosphoric acid.
A, B	70
C	65

sample solvent: buffer pH 9.7

KEY

a solvent front.

b 5-methoxy-2-methylindole-3-acetic acid

c p-chlorobenzoic acid

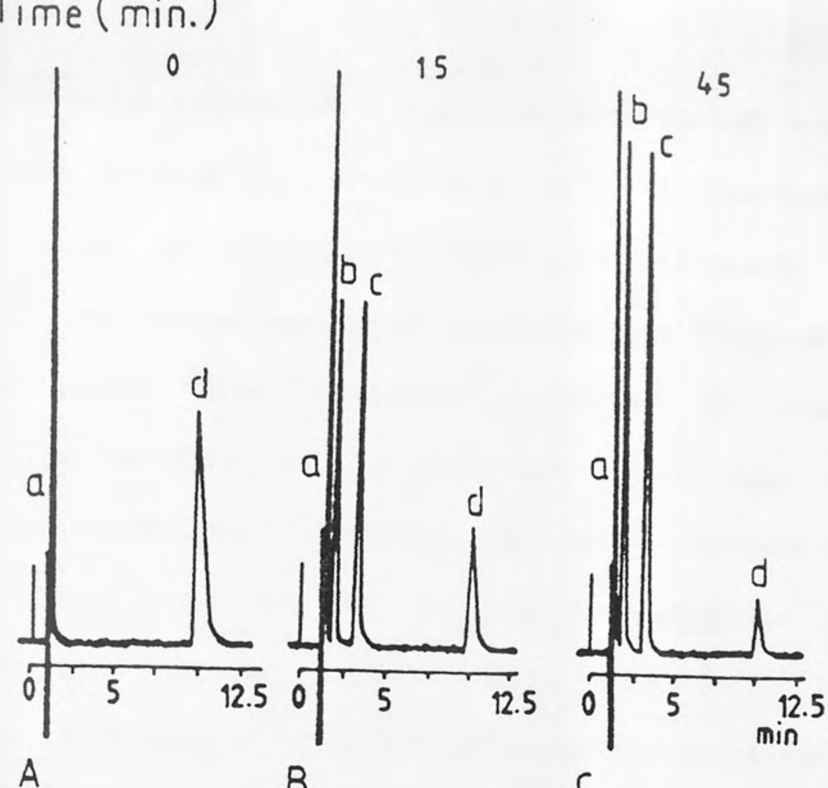
d thymol

e indomethacin

f methyl p-chloro benzoate.

g ethyl p-chloro benzoate.

Fig. 4.20 HPLC separation of indomethacin and its degradative products.



HPLC conditions:

Column : ODS_Hypersil (5 μm): 10 cm × 4.6 mm i.d.;

Flow rate: 1 ml/min. ; Sensitivity: 0.01 A.U.F.S.;

Detection wavelength: 235 nm; Chart speed: 1 cm/2 min.;

Mobile phase : 65 % Methanol in water acidified with
0.2 % orthophosphoric acid.

KEY

a solvent front.

b MIAA.

c p-chlorobenzoic acid.

d indomethacin.

sample solvent: buffer pH 9.7

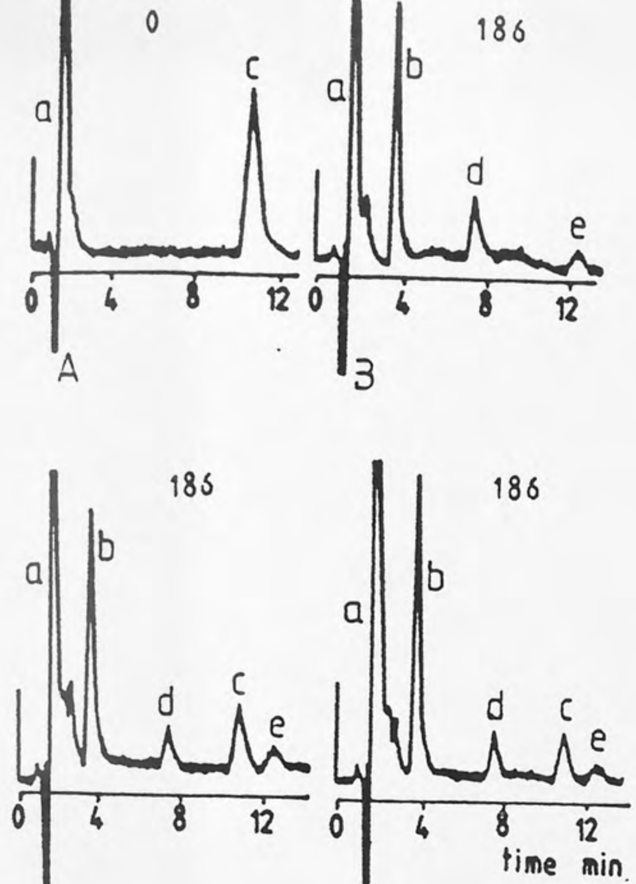
Fig. 4.21 HPLC chromatograms of indomethacin (1 μM) decomposition in Teorell-Stenhagen's buffer pH 9.7 at 60°C.

In the acid region pH 2 however, several new compounds could be detected upon storing the solutions at 60°C. It was interesting to note that no trace of 5-methoxy-2-methylindole-3-acetic acid could be detected in the chromatograms of the solutions (Fig. 4.22a). When the acidic solutions also contained methanol or ethanol the HPLC decomposition profile was surprisingly much less complex. Two unidentified peaks plus indomethacin and p-chlorobenzoic acid were observed (Fig. 4.22c and d) in the chromatograms of aqueous and hydroalcoholic solutions of indomethacin (1 μM) in pH 2.0 buffer. The hydrolysis of indomethacin in 10% ethanol-Teorell-Stenhagen's buffer pH 2.0 at 60°C was followed using both ultraviolet spectroscopy and HPLC. At any time, according to the HPLC data, the mass balance of the residual indomethacin and the generated p-chlorobenzoic acid corresponds to the initial indomethacin concentration as shown in Fig. 4.23. The first order plot of this profile is shown in Fig. 4.24 and the respective rate constants obtained from both analytical techniques were in good agreement with each other and recorded in Table 4.7. The rate constant for the formation of p-chlorobenzoic acid was also measured from the HPLC data and similarly recorded.

TABLE 4.7

The rate constants for the degradation of indomethacin in 10% ethanol-Teorell-Stenhagen's buffer pH 2.0 at 60°C obtained by ultraviolet and HPLC analytical techniques.

Rate Constant for the hydrolysis of indomethacin k $hr.^{-1} \times 10^3$ (r = regression coefficient)		Rate constant for the formation of p-chlorobenzoic acid k $hr.^{-1} \times 10^3$
Ultraviolet Spectroscopy	HPLC	HPLC
4.167 (r=0.9990)	4.169 (r=0.9996)	4.171



HPLC conditions:

Column : ODS-Hypersil (5 µm) 10 cm × 4.6 mm i.d. ;

Flow rate: 1 ml/min. ; Sensitivity: 0.01 A.U.F.S. ;

Detection wavelength: 235 nm; Chart speed: 1 cm/2 min.;

Mobile phase : 65 % Methanol in water acidified with 0.2 % orthophosphoric acid.

KEY

a	solvent front
b	p-chlorobenzoic acid
c	indomethacin
d,e	two unidentified peaks

Fig. 4.22 High performance liquid chromatograms of indomethacin (1 µM) decomposition in Teorell-Stenhagen's buffer pH 2 (B) and in the presence of 10 % methanol (C) or 10 % ethanol (D) at 60°C.

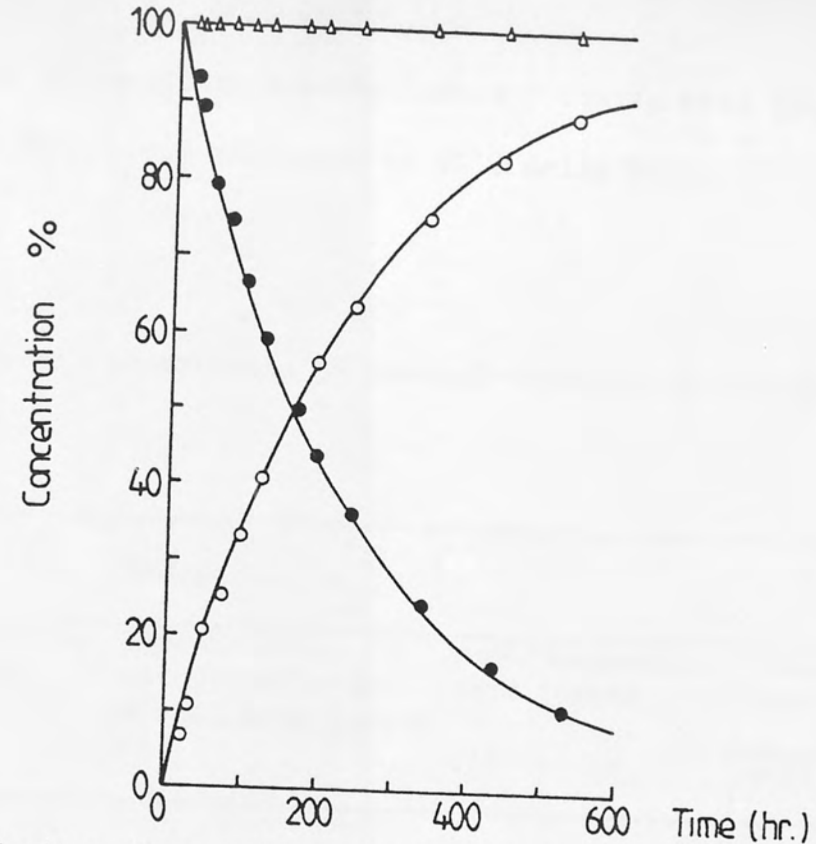


Fig. 4.23 Concentration - time profiles showing the degradation of indomethacin in Teorell - Stenhagen's buffer pH 2·0 at 60°C.

KEY ● indomethacin , ○ p-chlorobenzoic acid, △ mass balance.

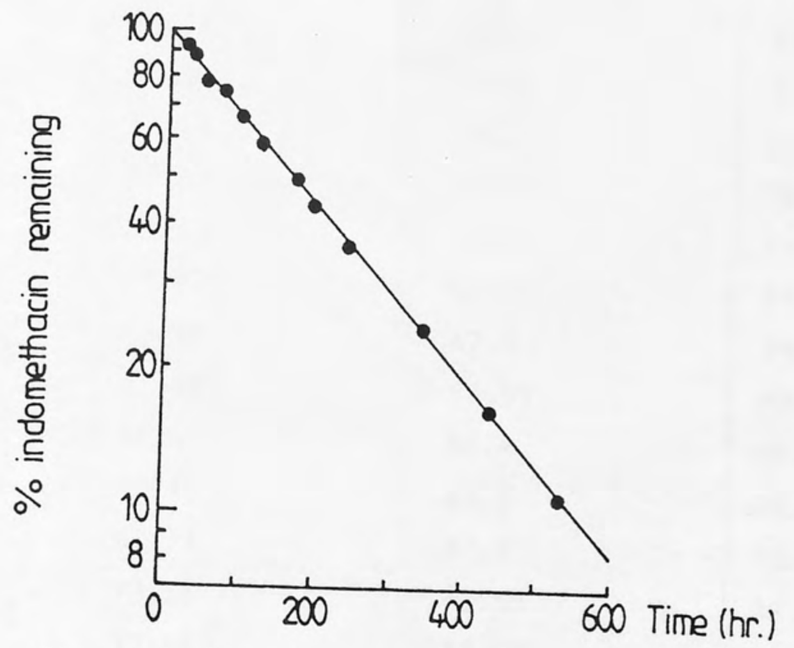


Fig. 4.24 First order plot for the degradation of indomethacin in 10% ethanol - Teorell Stenhagen's buffer pH 2·0 at 60°C.

A solution of 5-methoxy-2-methylindole-3-acetic acid (30 μM) in 10% ethanol buffer pH 2.0 was monitored at 60°C using HPLC.

TABLE 4.8

The degradation of indomethacin in Teorell-Stenhagen's buffer pH 9.7 at 40°C.

Time (min.)	Concentration %			Total (%) mass balance	
	Indomethacin remaining I	5-methyl-2-methylindole-3-acetic acid formed II	p-chlorobenzoic acid formed III	I+II	I+III
5	95.8	4.2	4.15	100	99.95
10	92.95	7.15	7.05	100.1	100
15	88.8	11.13	11.15	99.93	99.95
20	87.1	12.85	12.9	99.95	100
25	84.4	15.58	15.5	99.98	99.9
30	82.1	17.89	17.9	99.99	100
40	76.8	23.16	23.1	99.96	99.9
50	72.7	27.25	27.3	99.95	100
60	67.1	32.82	32.87	99.92	99.97
80	59.0	40.97	40.95	99.97	99.95
100	53.0	46.98	47.0	99.98	100
120	46.4	53.58	53.55	99.98	99.95
140	41.5	58.43	58.4	99.93	99.9
160	36.5	63.48	63.5	99.98	100
180	32.2	67.74	67.7	99.94	99.9
210	26.4	73.56	73.55	99.96	99.95
240	22.5	77.44	77.47	99.94	99.97
300	15.7	84.0	84.23	99.7	99.93
330	13.0	86.95	86.95	99.95	99.95
360	10.9	89.09	89.0	99.99	99.9
422	7.4	92.58	92.5	99.98	99.9
480	4.9	95.1	95.1	100	100
540	3.1	96.84	96.5	99.94	99.6

The first order rate of disappearance of 5-methoxy-2-methylindole-3-acetic acid is shown in Fig. 4.25 (r = 0.9999) with a rate constant of 0.153 $hr.^{-1}$. It is interesting to note that the two unidentified peaks detected earlier in the chromatograms of indomethacin solutions in pH 2.0 buffer stored at 60°C were recorded too under these conditions (Fig. 4.26).

The hydrolysis of indomethacin in Teorell-Stenhagen's buffer pH 9.7 was followed at 40°C using HPLC.

Concentration-time profiles for the degradation of indomethacin and the formation of both 5-methoxy-2-methylindole-3-acetic acid and p-chlorobenzoic acid are shown in Fig. 4.27. The sum of the concentrations of either p-chlorobenzoic acid or 5-methoxy-2-methylindole-3-acetic acid with that of residual indomethacin corresponds to the initial indomethacin concentration throughout the period of the study as shown in Table 4.8. First order plot for the degradation of indomethacin in buffer pH 9.7 at 40°C is shown in Fig. 4.28 and the corresponding rate constant is recorded in Table 4.9 together with that for the formation of both degradative products. Hydroalcoholic solutions of indomethacin in buffer pH 9.7 containing either methanol or ethanol led to the rapid formation of a peak which would be identified as either methyl or ethyl p-chlorobenzoate by comparing with an authentic specimens.

The alcoholysis or esterification of amides has been reported to proceed as easily as the corresponding acid(291-301) where the neutral alcoholysis of amides may be formally written as

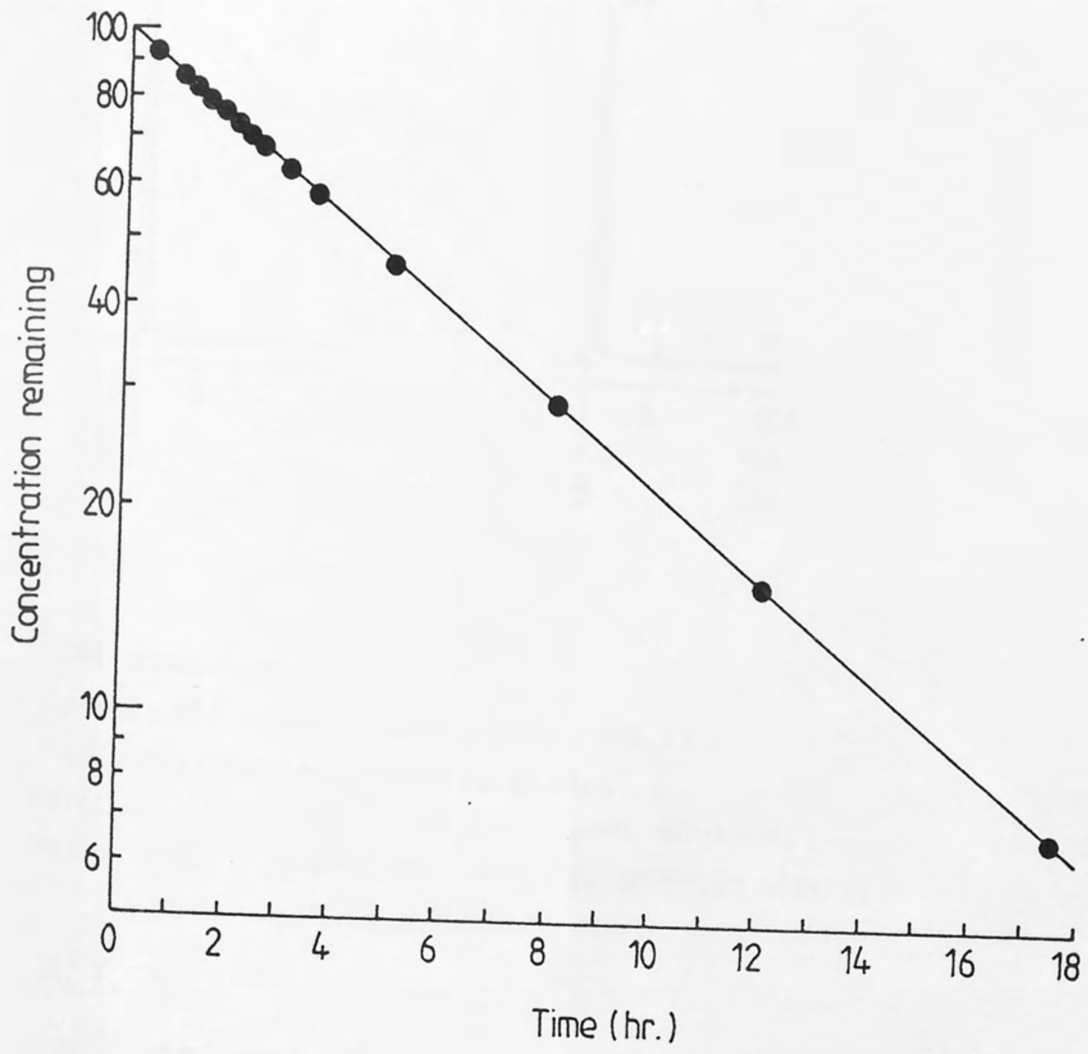
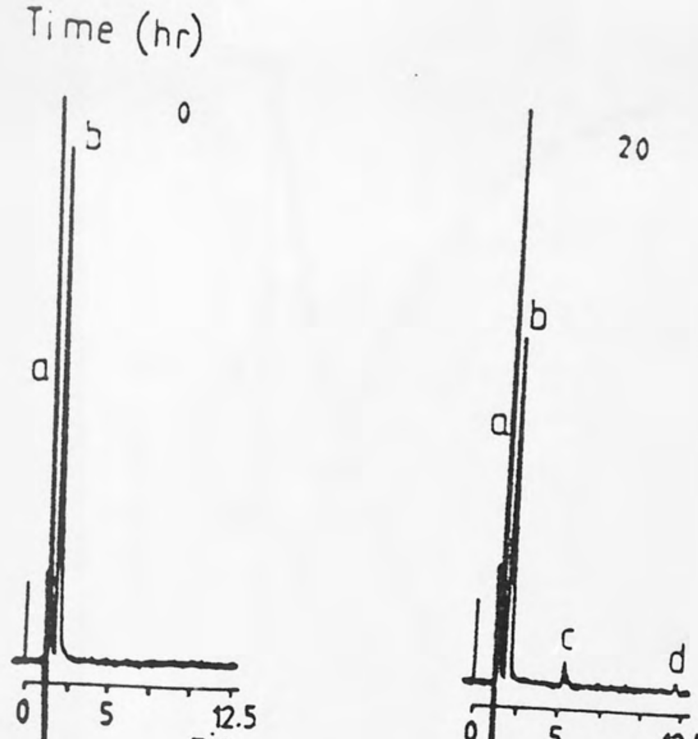


Fig. 4.25 First order plot for the degradation of 5-methoxy-2-methylindole-3-acetic acid in Teorell-Stenhagen's buffer pH 2·0 at 60°C.



HPLC conditions:

Column: ODS-Hypersil (5 μm): 10 cm × 4.6 mm i.d.;
Flow rate: 1 ml/min.; Sensitivity: 0.02 A.U.F.S.;
Detection wavelength: 267 nm; Chart speed: 1 cm/2 min.;
Mobile phase: 65 % Methanol in water acidified with 0.2 % orthophosphoric acid.

KEY

a	solvent front.
b	5-methoxy-2-methylindole-3-acetic acid
c,d	unidentified peaks

Fig. 4.26 High performance liquid chromatograms of 5-methoxy-2-methylindole-3-acetic acid decomposition in 10% aqueous ethanolic buffered solutions pH 2 at 60°C.

-286-

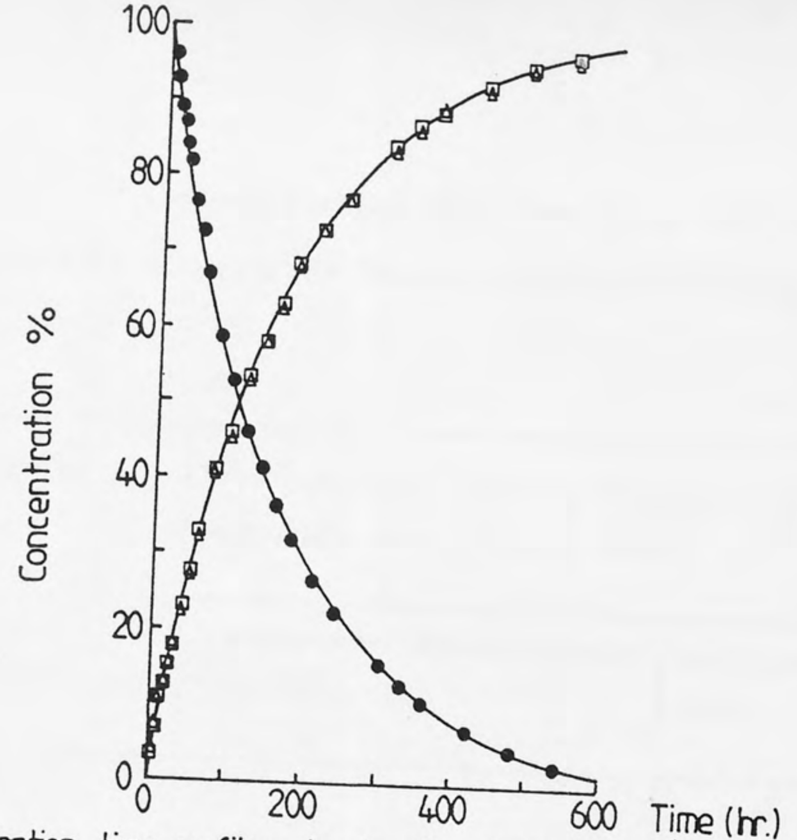


Fig. 4.27 Concentration-time profiles showing the degradation of indomethacin in Teorell-Stenhagen's buffer pH 9·7 at 40°C.

KEY ● indomethacin remaining; □ 5-methoxy-2-methylindole-3-acetic acid formed, △ p-chlorobenzoic acid formed

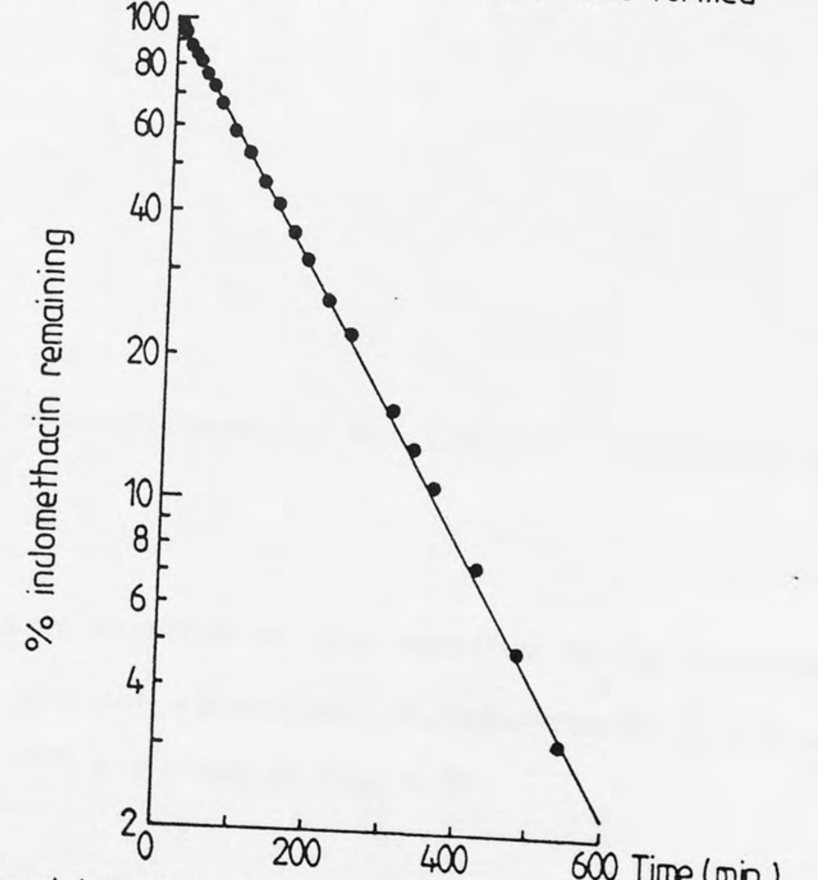


Fig. 4.28 First order plot for the degradation of indomethacin in Teorell-Stenhagen's buffer pH 9·7 at 40°C.

TABLE 4.9

The rate constants for the degradation of indomethacin and the formation of its two degradative products in Teorell-Stenhagen's buffer pH 9.7 at 40°C.

Rate constant for the degradation of indomethacin $k\ min^{-1} \times 10^2$ (r=regression coefficient)	Rate constants for the formation of the degradation products $k\ min^{-1} \times 10^2$	
	5-methyoxy-2-methylindole-3-acetic acid	p-chlorobenzoic acid
0.6196 (r = 1.000)	0.6194	0.6197

$$ROH + \rangle N - C(=O)R' \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ROC(=O)R' + \rangle NH \qquad (4.23)$$

Mechanistically, however, there may exist several kinetically equivalent schemes.

Little has been reported on this reaction in the alkaline region. Typical profiles for the alcoholysis of indomethacin in 30% methanol-buffer pH 9.7 at 40°C are shown in Fig. 4.29.

Rowe and Carless(105) isolated methyl p-chlorobenzoate when extracting indomethacin from microcapsules with methanol and ascribed this as a catalytic effect by the components of their gelatin-acacia-

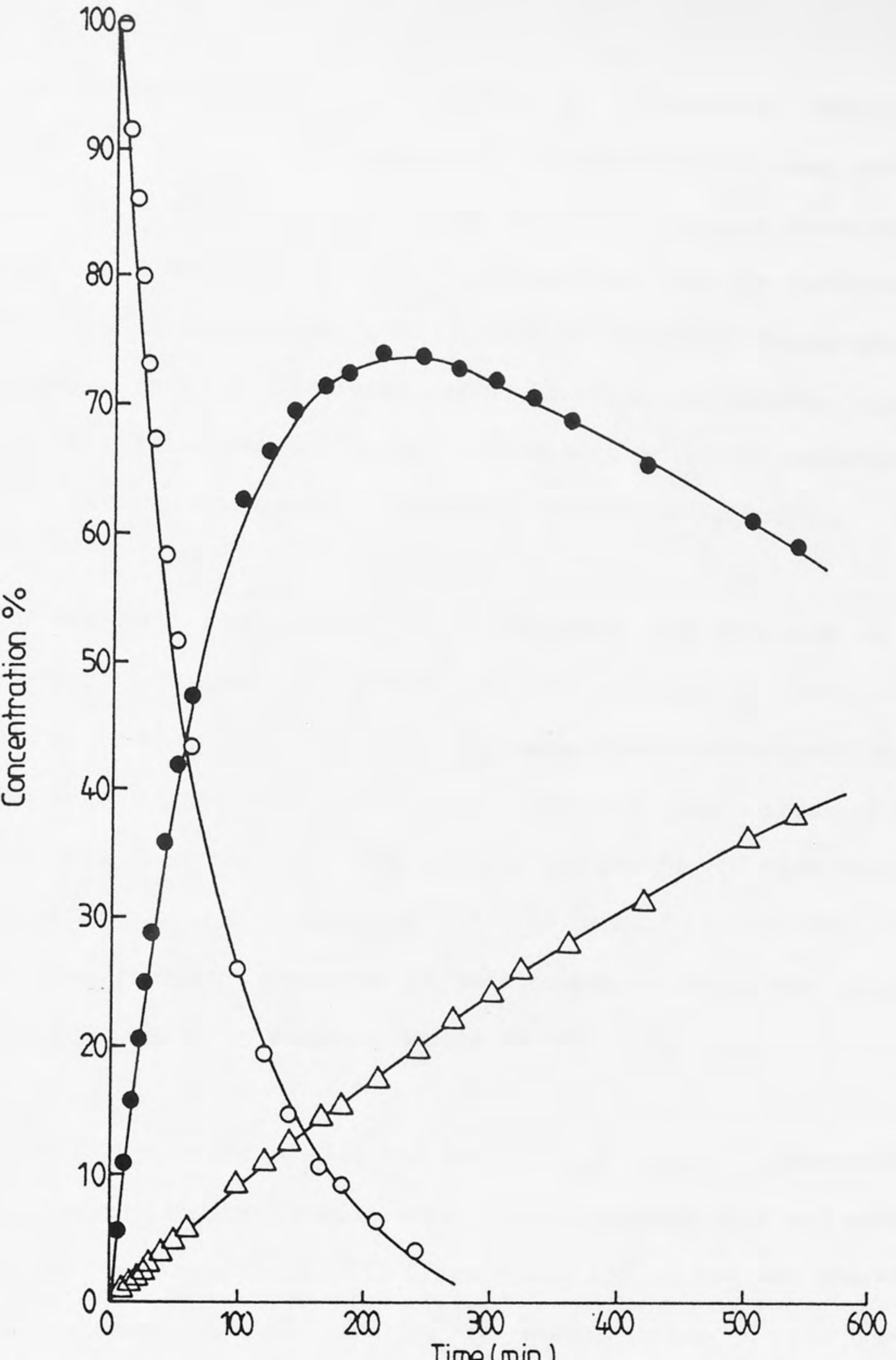


Fig. 4.29 The time-course for the degradation of indomethacin in 30% methanol-Teorell-Stenhagen's buffer pH 9·7 at 40° C.

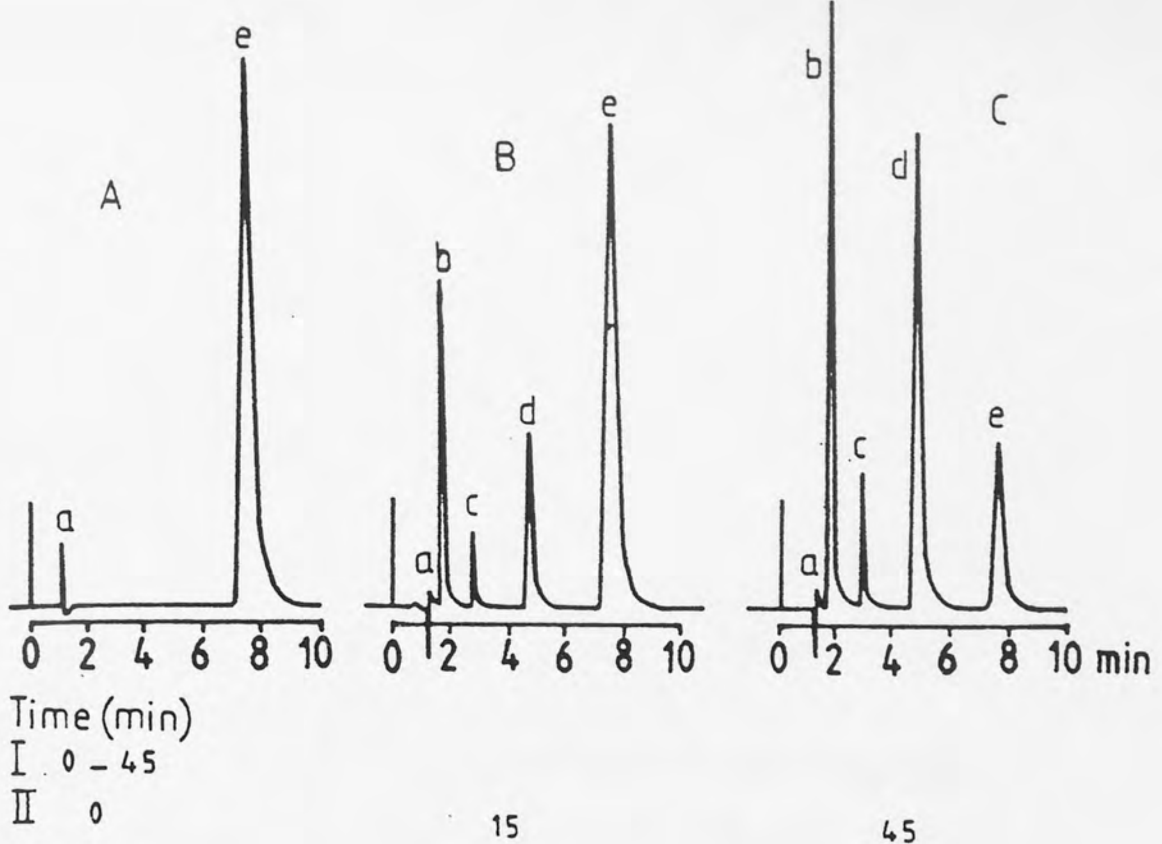
KEY ○ indomethacin remaining
● methyl p-chlorobenzoate formed
△ p-chlorobenzoic acid formed

based microcapsules and to the presence of indomethacin degradative products as impurities. Our experiments indicate that the same reaction takes place without the need to reflux alkaline alcoholic solutions of indomethacin. However, water seems to be essential for the formation of the methyl p-chlorobenzoate. Additionally, no ester was formed when p-chlorobenzoic acid is refluxed with alkaline methanolic aqueous solutions nor following refluxing indomethacin with dry hydrotalcite (Magnesium Carbonate hydroxide) containing methanol (Fig. 4.30).

The degradation of methyl p-chlorobenzoate was followed in 40% methanol-Teorell-Stenhagen's buffer pH 9.7 mixture at 40°C (Fig. 4.31). The semilogarithmic plot of the concentration-time profile for the degradation of the ester under these conditions gave a straight line which indicate that the ester degradation follows first order kinetics (Fig. 4.32) with a rate constant of $7.9 \times 10^{-4} \text{min.}^{-1}$ ($r = 0.9999$) while that obtained from the formation of p-chlorobenzoic acid was equal to 7.87×10^{-4} min.^{-1}. The proposed mechanism is in Fig. 4.33.

The HPLC separation enabled the simultaneous assay of indomethacin, 5-methoxy-2-methylindole-3-acetic acid, p-chlorobenzoic acid and methyl (IV) or ethyl p-chlorobenzoate(V) (Fig. 4.20b and c) and was therefore ideal for elucidating the kinetics of decomposition of the parent nonsteroidal anti-inflammatory agent.

In earlier reports it has been shown that in the HPLC anlaysis of acids and esters(242) the presence of different amounts of cosolvents exerted significant effects on the heights of the HPLC peaks and could lead to highly erroneous results. The extent to which this occurs with indomethacin was assessed earlier in 2.2.3.



HPLC conditions:

Column : ODS-Hypersil (5 μm) 10 cm × 4.6 mm i.d.;
Flow rate: 1 ml/min.; Sensitivity: 0.16 A.U.F.S.;
Detection wavelength: 235 nm; Chart speed: 1 cm/2 min.;
Mobile phase : 70% Methanol in water acidified with
0.2% orthophosphoric acid.

KEY

a solvent front.
b 5-methoxy-2-methyl-indol-3- acetic acid
c p-chlorobenzoic acid
d methyl p-chlorobenzoate
e indomethacin

Fig. 4.30 High performance liquid chromatograms of indomethacin decomposition in dry- (I) and 50 % water hydrotalcite-containing methanol (II).

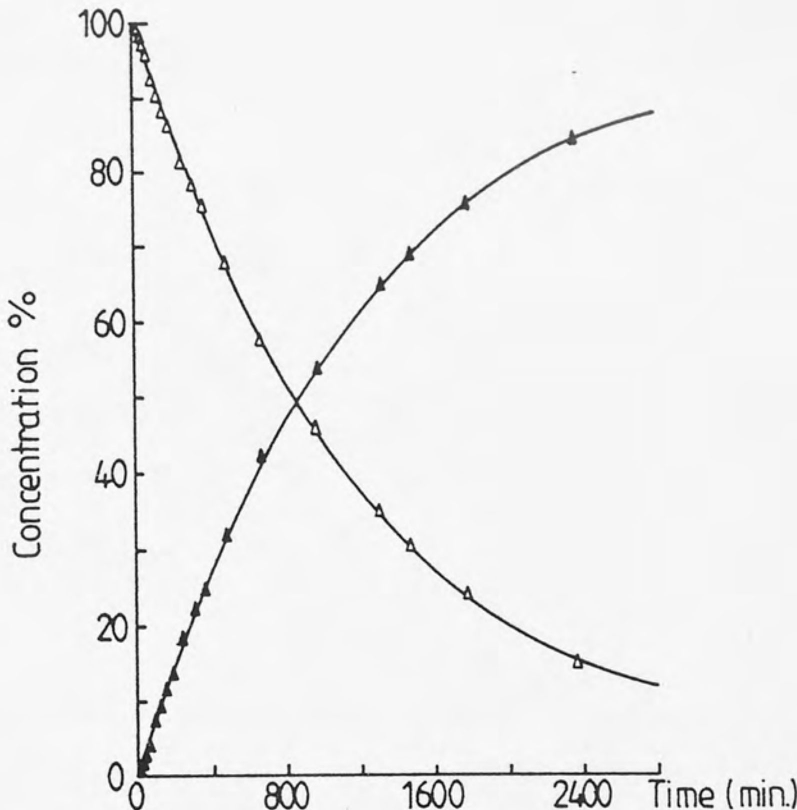


Fig. 4.31 Concentration - time profile for the hydrolysis of methyl p-chlorobenzoate in 40% methanol-Teorell-Stenhagen buffer pH 9·7 at 40°C.

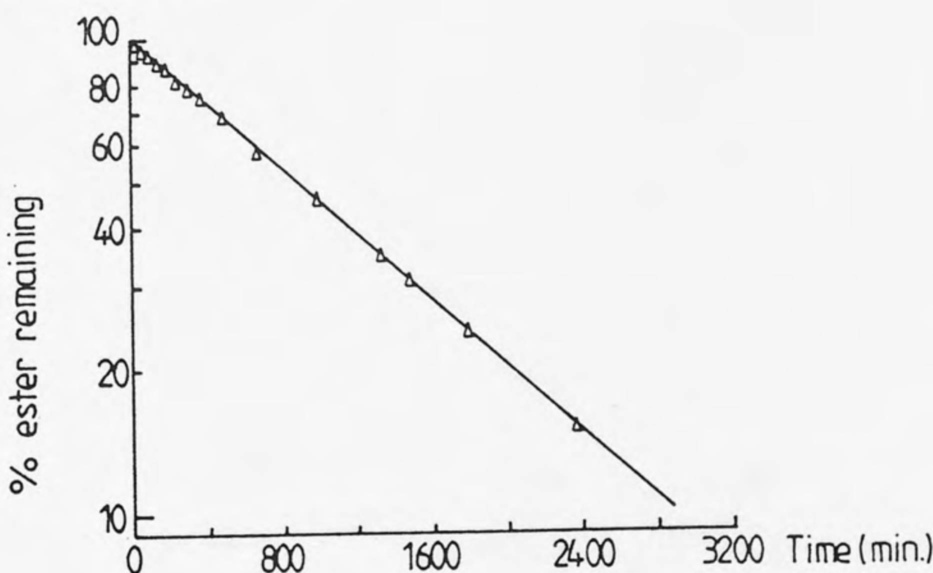
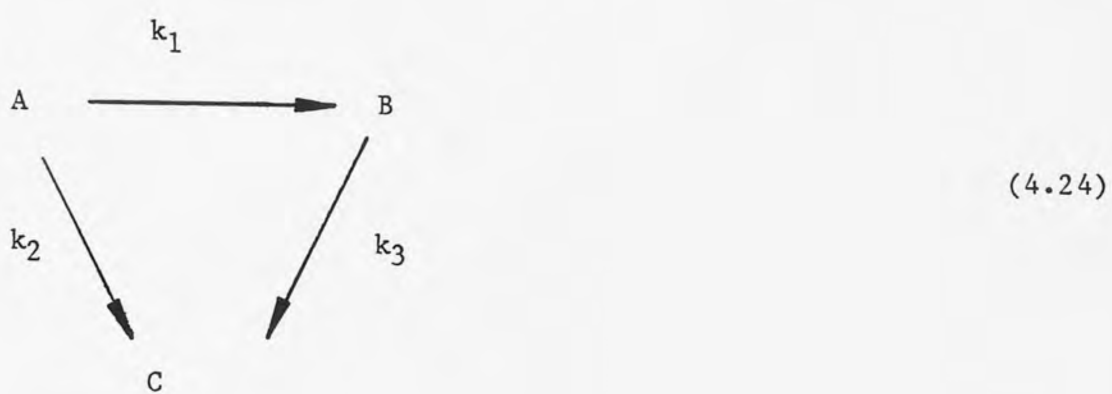


Fig. 4.32 First order plot for the hydrolysis of methyl p-chlorobenzoate in 40% methanol-Teorell-Stenhagen buffer pH 9·7 at 40°C.

KEY △ methyl p-chlorobenzoate
▲ p-chlorobenzoic acid

Fig. 4.33 The proposed mechanism of indomethacin degradation in alkaline aqueous - alcoholic systems.

The mechanism of decomposition of indomethacin in alkaline methanolic or ethanolic aqueous systems appear to be consistent with schemes 4.2(a and b), and the kinetic model may be presented by equation (4.24)



where A = indomethacin

B = the intermediate ester of p-chlorobenzoic acid

C = p-chlorobenzoic acid

k_1 = rate constant for the alcoholysis of indomethacin

k_2 = rate constant for the hydrolysis of indomethacin

k_3 = rate constant for the hydrolysis of the formed ester of p-chlorobenzoic acid.

Reversibility was excluded by monitoring for p-chlorobenzoic acid methyl ester and indomethacin in a system initially consisting solely of equimolar concentrations of p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid but maintained at the same pH and solvent composition. No p-chlorobenzoic acid methyl ester or indomethacin could be detected during storage under the same conditions as the test samples. The indomethacin, p-chlorobenzoic acid and methyl p-chlorobenzoate form a closed system with their molar concentrations

CH_3O CH_2COOH N CH_3 C=O Cl

k_1 CH_3OH, OH^-

$COOCH_3$ Cl

OH^- k_2

OH^- k_3

COOH Cl

Scheme 4·2(a)

CH_3O CH_2COOH N CH_3 C=O Cl

k_1 C_2H_5OH, OH^-

$COOC_2H_5$ Cl

OH^- k_2

OH^- k_3

COOH Cl

Scheme 4·2(b)

Scheme 4·2(a,b) Models showing possible routes of alcoholysis and hydrolysis of indomethacin in alkaline aqueous alcoholic systems.

TABLE 4.10

The degradation of indomethacin in 10% methanolic-Teorell-Stenhagen's buffer pH 9.7 at 40°C.

Time (min.)	Concentration %						
	indomethacin remaining (I)	5-methoxy-2-methyl-indole-3-acetic acid formed (II)	p-chloro-benzoic acid formed (III)	methyl p-chloro-benzoate formed (IV)	Total (%) mass-balance		
					III+IV	I+II	I+III+IV
2	94.44	5.64	0.37	6.06	6.43	100.08	100.87
5	90.08	9.3	0.73	9.86	10.59	99.38	100.67
10	83.79	15.75	0.88	15.92	16.8	99.54	100.49
15	76.57	23.45	1.1	22.66	23.76	99.82	100.13
20	70.82	28.32	1.39	27.42	28.81	99.14	99.63
25	66.68	33.14	1.47	31.67	33.74	99.82	99.82
30	59.94	38.01	1.61	36.6	38.21	97.95	98.15
40	52.35	47.36	2.2	44.76	46.96	97.11	99.31
50	44.07	55.24	2.5	52.01	54.51	99.31	98.58
60	37.9	61.47	2.86	57.62	60.48	99.37	98.38
80	26.85	71.04	3.67	66.65	70.32	97.89	97.17
100	20.33	78.07	4.03	72.59	76.62	98.4	96.95
120	14.44	83.96	4.91	77.28	82.19	98.4	96.63
145	10.55	86.62	5.43	80.88	86.31	97.17	96.86
165	7.16	91.5	6.09	84.03	90.12	98.66	97.28
180	5.41	93.0	6.58	85.12	91.7	98.41	97.11
215	3.02	93.93	7.48	85.75	93.23	96.95	96.25
265	1.76	96.77	8.14	87.59	95.73	98.53	97.49

summing to the initial concentration, throughout the period of study (Table 4.10). The postulated mechanism of decomposition suggests that for every mole of (III) or (IV) formed, a mole of 5-methoxy-2-methylindole-3-acetic acid (II) is formed (Fig. 4.34). Although the absence of unidentified peaks in the chromatograms suggests that (II)

Fig. 4.34 The mechanism of decomposition of indomethacin in aqueous-methanolic systems.

does not decompose further, it does not provide conclusive proof as the analytical technique could well be unsuitable for resolving the possible products. To test that (II) is indeed an end product and not an intermediate at least within the time scale of the study, concordance between the sum of the molar concentrations of p-chlorobenzoic acid (III) and its methyl ester (IV) and the molar concentration of (II) and was checked by linear least squares analysis. Although this may appear to be an excessively cautious approach in preformulation work, it was deemed necessary because of the well known problems associated with skin allergies induced by topical applications and more notably in the present context, by non-steroidal anti-inflammatory drugs. The data shown in Fig. 4.35 demonstrate that no unidentified product was formed in any significant concentration during the decomposition of indomethacin in the system studied. The linear relationship could be described by equation (4.25):

$$y = 0.9747\ x + 0.001818\ (r = 0.9998) \qquad (4.25)$$

where (y) is the molar concentration of (II) and (x) is the sum of the molar concentration of (III) and (IV). The slope of (0.9747) relative to a predicted value of unity and the insignificant intercept confirms the validity of the proposed model.

In the buffered systems investigated each of the decomposition pathways would be expected to be a first order or pseudo first order process with respect to the decompositing entity. The integrated rate equations for the decomposition are therefore the same as reported earlier(302) for the transesterification of salicylates and they are

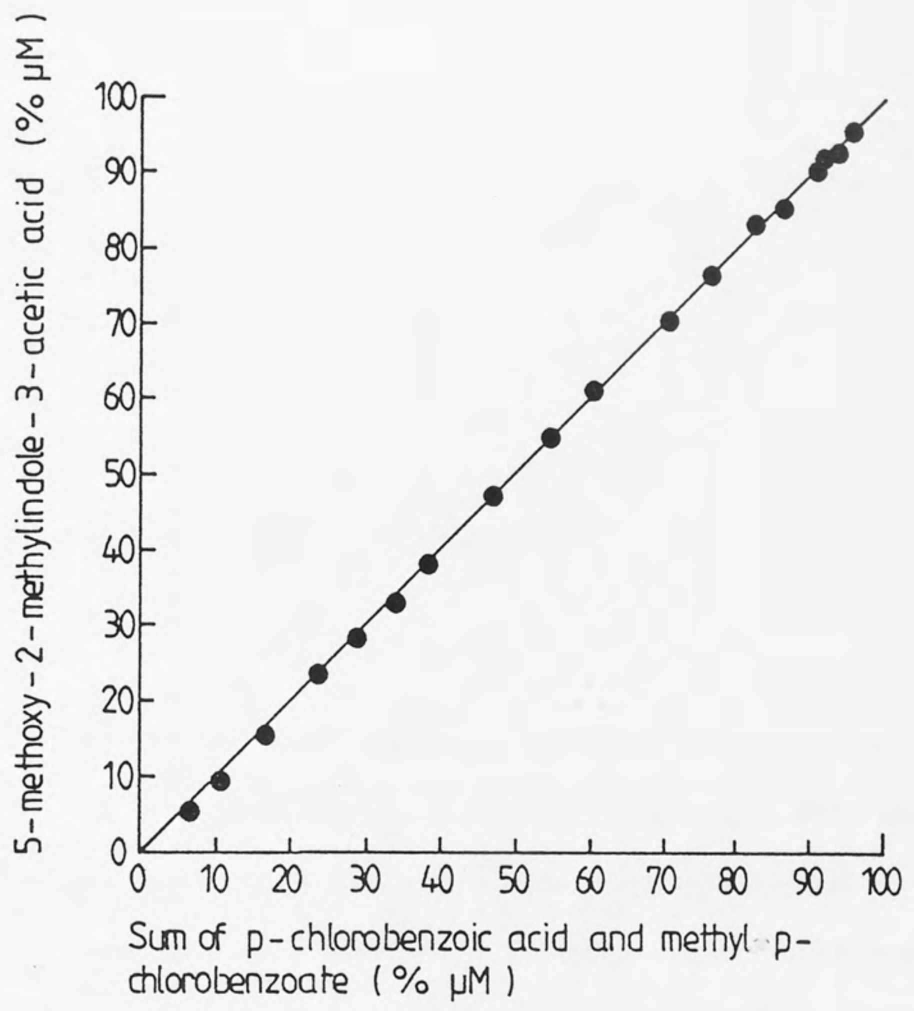


Fig. 4.35 The relationship between the concentration of 5-methoxy-2-methylindole-3-acetic acid and the sum of concentration of p-chlorobenzoic acid and methyl p-chlorobenzoate formed during the degradation of indomethacin in 10% methanol Teorell-Stenhagen's buffer pH 9·7 at 40°C.

given by:

$$A_t = A_o \cdot e^{-(k_1+k_2).t} \tag{4.26}$$

$$B_t = A_o \cdot k_1 \cdot \frac{e^{-k_3 t} - e^{-(k_1+k_2).t}}{(k_1 + k_2 - k_3)} \tag{4.27}$$

$$C_t = A_o \cdot [1 - \frac{k_1 e^{-k_3 t} + (k_2 - k_3) \cdot e^{-(k_1+k_2).t}}{(k_1 + k_2 - k_3)}] \tag{4.28}$$

The overall disappearance of indomethacin in alkaline methanolic aqueous systems at 40°C is shown in Fig. 4.36 and the respective rate constants equivalent to the absolute slope of lines were determined by linear regression analyses and are recorded in Table 4.11. The excellent linearity confirms the overall first order degradation expected of equation (4.26), the logarithmic form of which is

$$\ln A_t = \ln A_o - (k_1 + k_2).t \tag{4.29}$$

The measured slopes thus yield as a sum of the alcoholysis and hydrolysis rate constants (k_1 and k_2) for the disappearance of indomethacin.

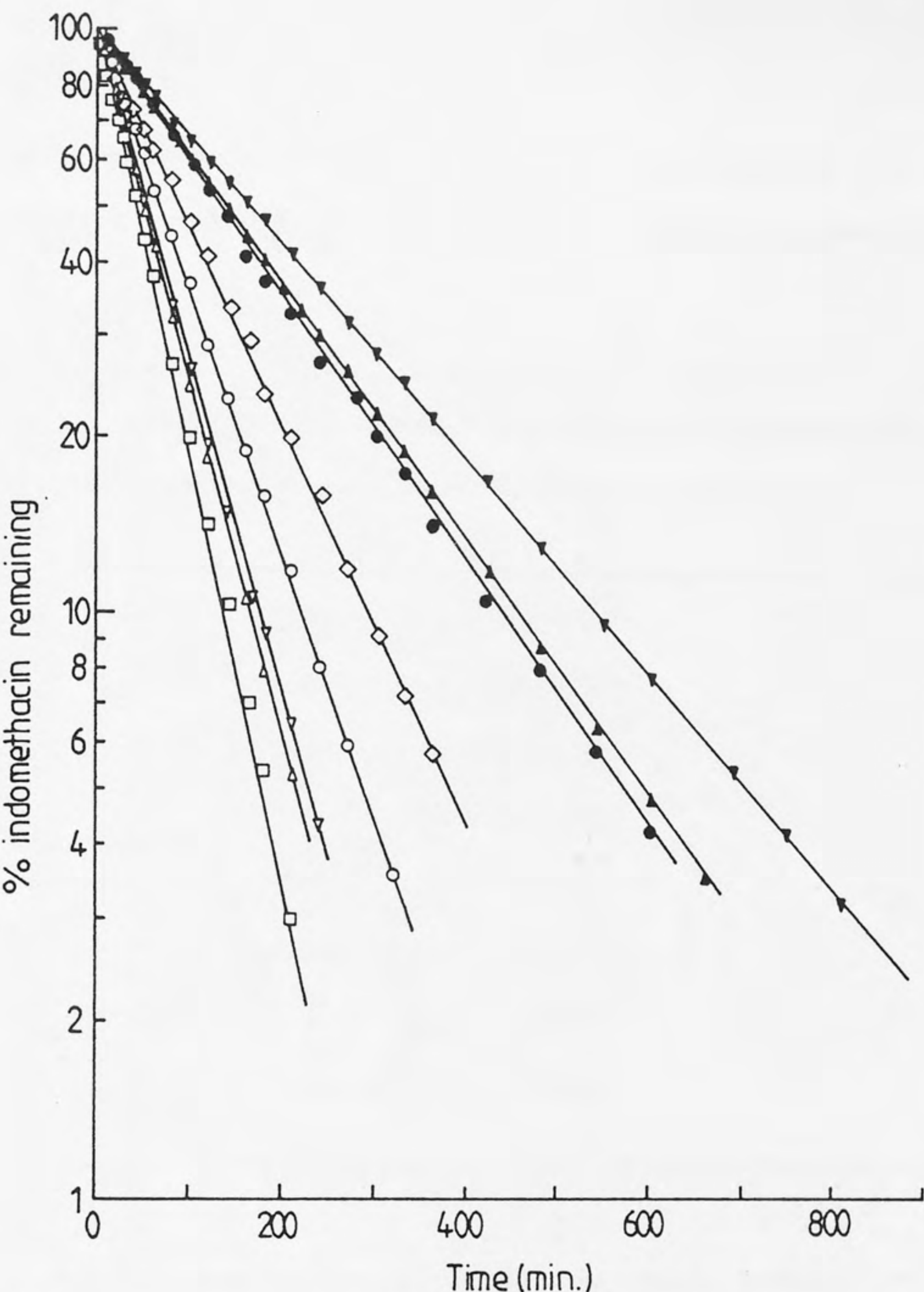


Fig. 4.36 First order plots showing the overall degradation of indomethacin in various concentrations of aqueous methanolic alkaline solutions pH 9·7 at 40°C.

KEY

	methanol % (v/v)
□	10
△	20
▽	30
○	40
◇	50
●	60
▲	65
▼	70

TABLE 4.11

The overall degradation rate constants of indomethacin in various concentrations of methanol-Teorell-Stenhagen's buffer mixtures, pH 9.7 at 40°C.

% Methanol	(k_1+k_2): The overall degradation of indomethacin $k\ min.^{-1} \times 10^3$	(r = regression coefficient)
10	13.94	(r = 0.999)
20	14.0	(r = 0.9995)
30	13.0	(r = 0.9996)
40	10.0	(r = 0.9992)
50	7.8	(r = 0.9993)
60	5.2	(r = 0.9991)
65	5.0	(r = 0.9999)
70	4.2	(r = 0.9994)

Non-linear regression analysis of the data yielded the rate constants shown in Table 4.12 by fitting the time courses of the reactants and products to equations (4.26), (4.27) and (4.28) using the FORTRAN version of NONLIN(NONREG). The relationship between the overall rate of disappearance of indomethacin obtained from the slopes of lines in Fig. 4.36 and the sum of the alcoholysis and hydrolysis rate constants ($k_1 + k_2$) for the disappearance of indomethacin was checked by linear least square analysis. The linear relationship obtained (Fig. 4.37) has a slope of 1.02 relative to a predicted value of unity and the insignificant intercept confirms the validity of the proposed kinetic model.

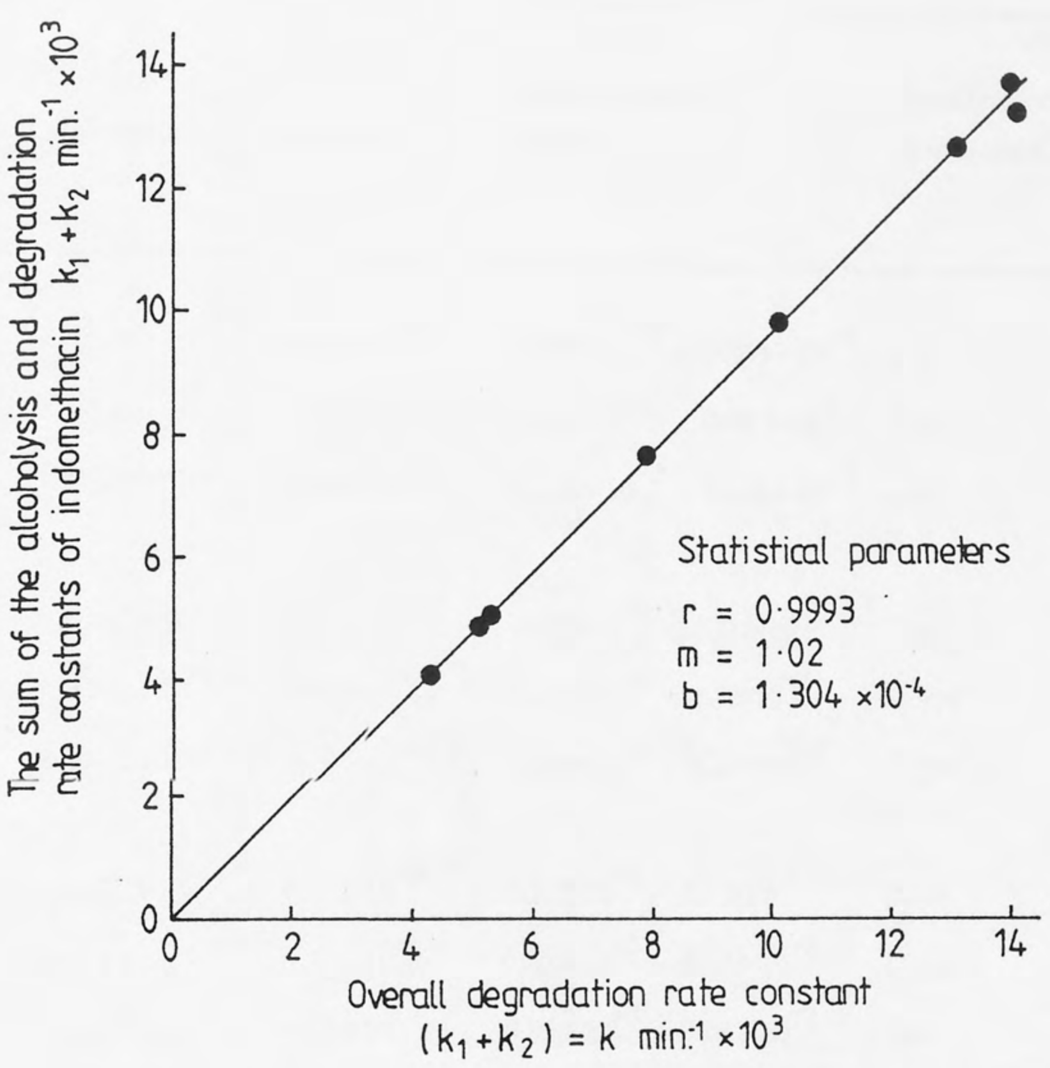


Fig. 4.37 The relationship between the overall rate of degradation and the sum of the alcoholysis and degradation rate constants of indomethacin in various aqueous-methanolic solutions, pH 9·7 at 40°C.

TABLE 4.12

Rate constants for the degradation of indomethacin in aqueous-alcoholic alkaline solutions, pH 9.7 at 40°C.

% methanol	Calculated rate constant $min.^{-1}$	Standard deviation	95% Confidence limits	Correlation Coefficient
10	$k_1=0.999\times10^{2}$	0.12×10^{-5}	$0.9992\times10^{-2} - 0.9997\times10^{-2}$	1.0
	$k_2=0.395\times10^{2}$	0.988×10^{-6}	$0.395\times10^{-2} - 0.395\times10^{-2}$	1.0
	$k_3=0.1666\times10^{2}$	0.445×10^{-6}	$0.1665\times10^{-2} - 0.1667\times10^{-2}$	1.0
20	$k_1=0.113\times10^{-1}$	0.112×10^{-3}	$0.111\times10^{-1} - 0.116\times10^{-1}$	1.0
	$k_2=0.213\times10^{-2}$	0.847×10^{-4}	$0.196\times10^{-2} - 0.23\times10^{-2}$	0.999
	$k_3=0.116\times10^{-2}$	0.319×10^{-4}	$0.109\times10^{-2} - 0.122\times10^{-2}$	0.999
30	$k_1=0.117\times10^{-1}$	0.122×10^{-3}	$0.115\times10^{-1} - 0.12\times10^{-1}$	0.999
	$k_2=0.11\times10^{-2}$	0.866×10^{-4}	$0.934\times10^{-3} - 0.128\times10^{-2}$	1.0
	$k_3=0.892\times10^{-3}$	0.299×10^{-4}	$0.832\times10^{-3} - 0.952\times10^{-3}$	1.0
40	$k_1=0.958\times10^{-2}$	0.721×10^{-4}	$0.943\times10^{-2} - 0.972\times10^{-2}$	1.0
	$k_2=0.43\times10^{-3}$	0.561×10^{-4}	$0.318\times10^{-3} - 0.542\times10^{-3}$	0.999
	$k_3=0.649\times10^{-3}$	0.237×10^{-4}	$0.602\times10^{-3} - 0.697\times10^{-3}$	0.999
50	$k_1=0.761\times10^{-2}$	0.786×10^{-4}	$0.746\times10^{-2} - 0.777\times10^{-2}$	0.999
	$k_2=0.203\times10^{-3}$	0.583×10^{-4}	$0.872\times10^{-4} - 0.319\times10^{-3}$	0.999
	$k_3=0.172\times10^{-3}$	0.214×10^{-4}	$0.129\times10^{-3} - 0.215\times10^{-3}$	0.999

continued....

TABLE 4.12 continued

% methanol	Calculated rate constant $min.^{-1}$	Standard deviation		95% Confidence limits	Correlation Coefficient
60	$k_1=0.506\times10^{-2}$	0.321×10^{-4}		0.5×10^{-2} - 0.513×10^{-2}	1.0
	$k_2=0.134\times10^{-3}$	0.272×10^{-4}		0.801×10^{-4} - 0.188×10^{-3}	1.0
	$k_3=0.187\times10^{-3}$	0.141×10^{-4}		0.159×10^{-3} - 0.215×10^{-3}	0.999
65	$k_1=0.482\times10^{-2}$	0.267×10^{-5}		0.418×10^{-2} - 0.483×10^{-2}	1.0
	$k_2=0.218\times10^{-3}$	0.137×10^{-5}		0.215×10^{-3} - 0.221×10^{-3}	1.0
	$k_3=0.319\times10^{-2}$	0.359×10^{-7}		0.248×10^{-6} - 0.391×10^{-6}	1.0
70	$k_1=0.391\times10^{-2}$	0.103×10^{-4}		0.389×10^{-2} - 0.393×10^{-2}	1.0
	$k_2=0.303\times10^{-3}$	0.93×10^{-5}		0.284×10^{-3} - 0.321×10^{-3}	1.0
	$k_3=0.289\times10^{-10}$	0.577×10^{-5}	(-)	0.115×10^{-4} - 0.115×10^{-4}	1.0
% Ethanol					
40	$k_1=0.1828\times10^{-2}$	0.394×10^{-4}		0.174×10^{-2} - 0.19×10^{-2}	0.999
	$k_2=0.236\times10^{-2}$	0.398×10^{-4}		0.228×10^{-2} - 0.244×10^{-2}	1.0
	$k_3=0.1357\times10^{-8}$	0.614×10^{-4}	(-)	0.1229×10^{-3} - 0.1229×10^{-3}	0.999

The close agreement between the predicted and observed concentrations of indomethacin and its decomposition products can be seen in Figs 4.38 and 4.39.

The effect of solvent on the rate of decomposition of drugs was studied extensively by Amis(303). He suggested that the solvent may alter the rate without influencing the mechanism by changing the forces between the reacting particles and hence altering the stability of the transition state. The influence of the dielectric constant of the mixed solvent on the electrostatic forces among reacting particles was used as a basis for explaining this phenomenon. The dielectric constant of various aqueous-methanolic systems is shown in Table 4.13.

TABLE 4.13

The dielectric constant of various aqueous-methanolic systems at 25°C.

Methanol in Water (%v/v)	Dielectric Constant
0	80
10	77.71
20	75.15
30	72.26
40	68.99
50	65.24
60	60.9
65	58.2
70	55.83
80	49.83
90	42.58
100	33.7

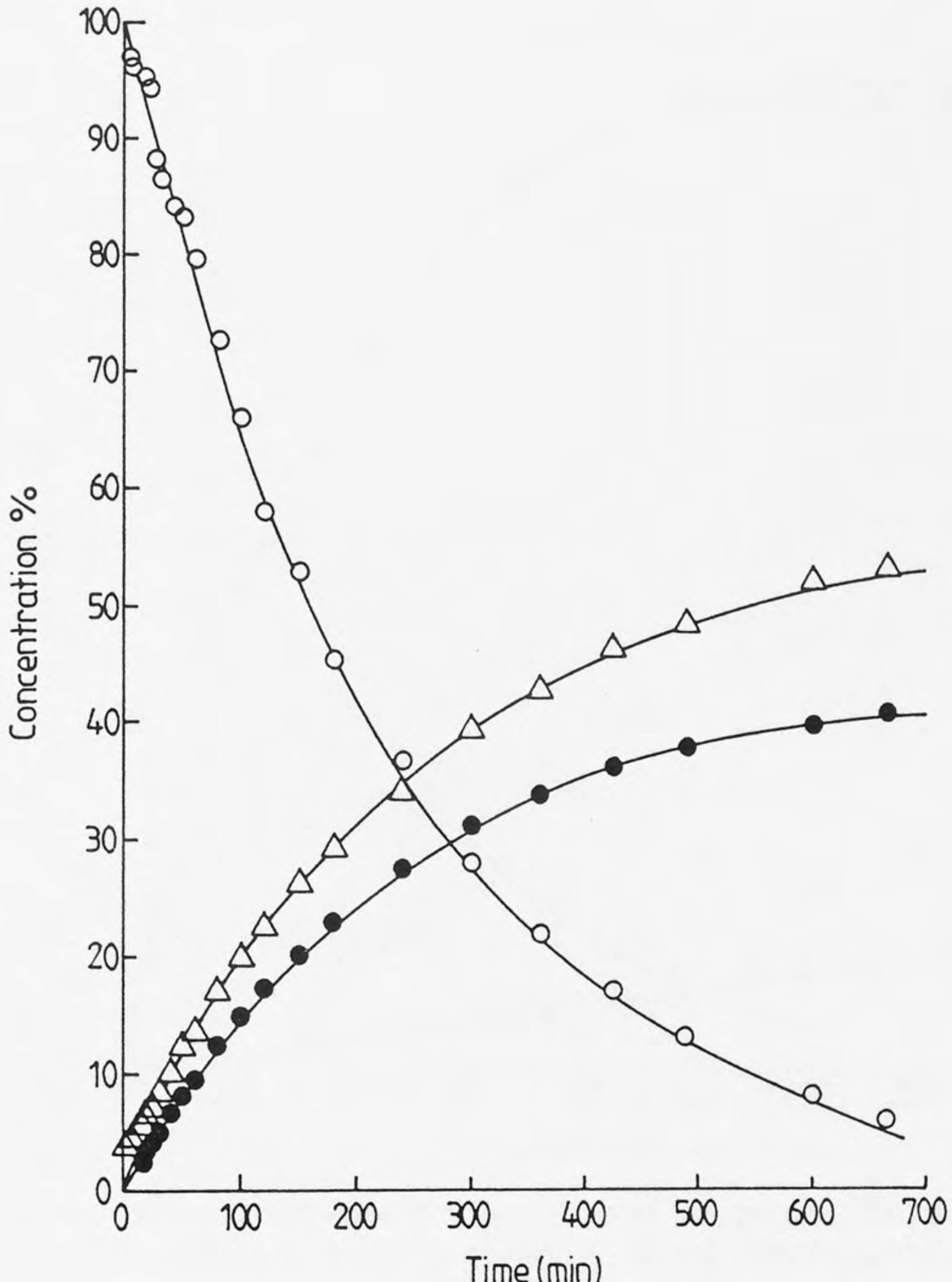


Fig. 4.38 Concentration - time profiles showing the degradation of indomethacin in 40% ethanol Teorell - Stenhagen's buffer pH 9·7 at 40° C.

KEY
- ○ indomethacin remaining
- ● ethyl p - chlorobenzoate formed
- △ p - chlorobenzoic acid formed

} Observed data

The continuous lines represent the calculated data obtained from the kinetic model.

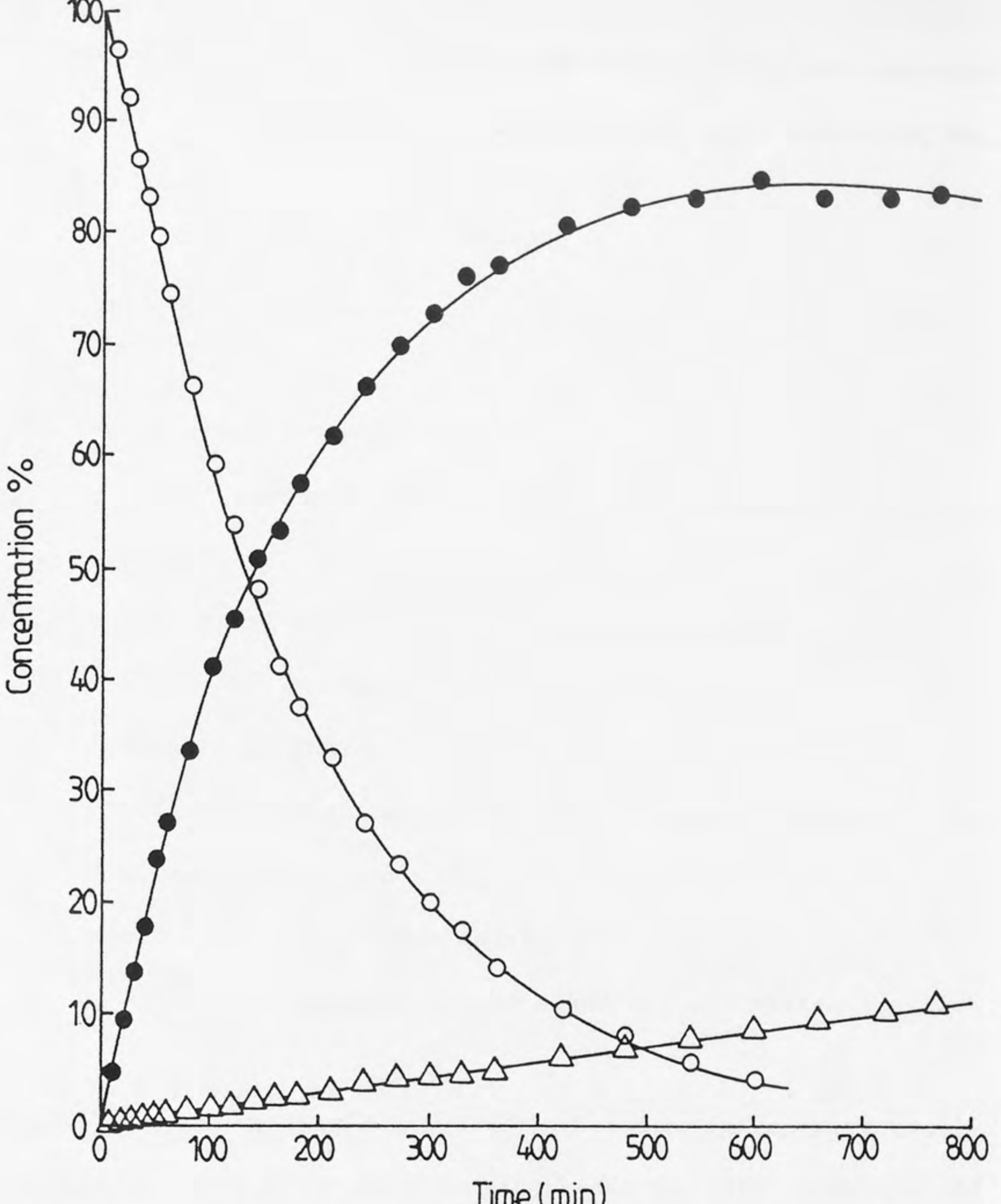


Fig. 4.39 Concentration - time profiles showing the degradation of indomethacin in 60% methanol Teorell - Stenhagen's buffer pH 9·7 at 40°C.

KEY
- ○ indomethacin remaining
- ● methyl p-chlorobenzoate formed
- △ p-chlorobenzoic acid formed

} observed data

The continuous lines represent the calculated data obtained from the kinetic model

The effect of the dielectric constant on the specific rate constant for a reaction at constant temperature is expressed quantitatively(304, 305) by the equation:

$$\ln k = \ln k_{\varepsilon=\infty} - \frac{z_A z_B N e^2}{RTr\varepsilon} \quad (4.30)$$

where k = the specific rate constant

$k_{\varepsilon=\infty}$ = rate constant in a medium of infinite dielectric constant

z_A, z_B = electrical charges on the reacting species

N = Avogadro's number

e = electronic charge

r = interionic distance within the activated complex

R = universal gas constant

T = temperature in degree Kelvin (°K), and

ε = dielectric constant of the mixed solvent system.

The effect of methanol on the degradation of indomethacin in Teorell-Stenhagen's buffer pH 9.7 at 40°C is displayed in Fig. 4.40(a,b and c). The alcoholysis rate constant of indomethcin (k_1) followed the relationship in equation (4.30) closely and a plot of $\ln k_1$, versus $1/\varepsilon$ is shown in Fig. 4.41(a). Least squares analysis of the data gives:

$$\ln k_1 = -1.934 - \frac{198.74}{\varepsilon} \quad (r = -0.9992,\ n=8)$$

the value of $\ln k_{\varepsilon=\infty}$ which is the rate constant in a medium of infinite dielectric constant can be calculated from the intercept and it is equal to 0.14456 $min.^{-1}$. The negative slope indicates that the ions in the

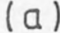


Fig. 4.40 Concentration - time profiles showing the effect of methanol concentration on the degradation of indomethacin in Teorell - Stenhagen's buffer pH 9·7 at 40°C.

(a) The disappearance of indomethacin in alkaline methanolic aqueous solutions

KEY methanol % (v/v)

▲	20
△	40
●	50
○	70

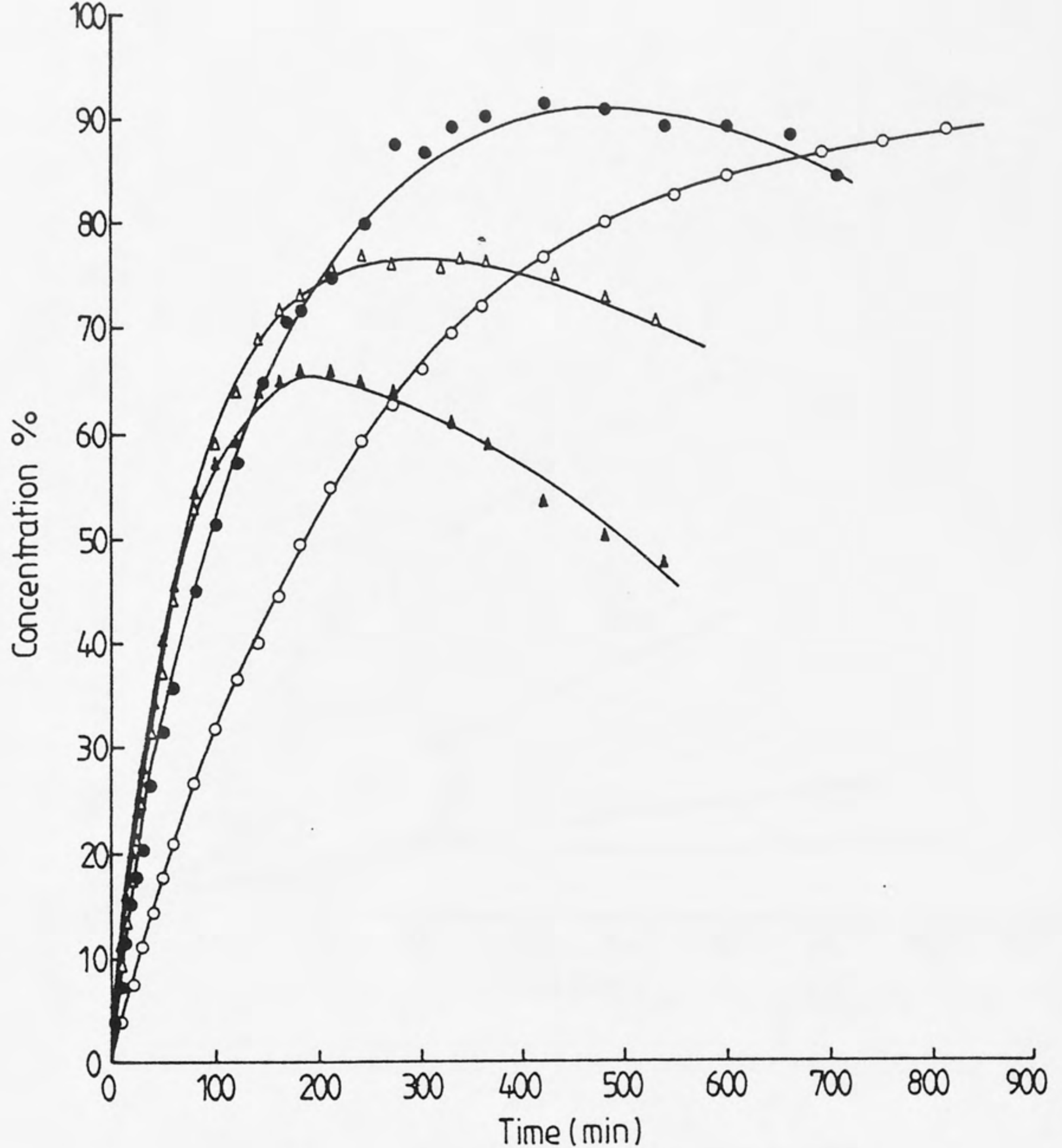


Fig. 4.40b. Formation and disappearance of methyl p-chlorobenzoate in alkaline methanolic aqueous solutions.

KEY methanol % (v/v)

▲	20
△	40
●	50
○	70

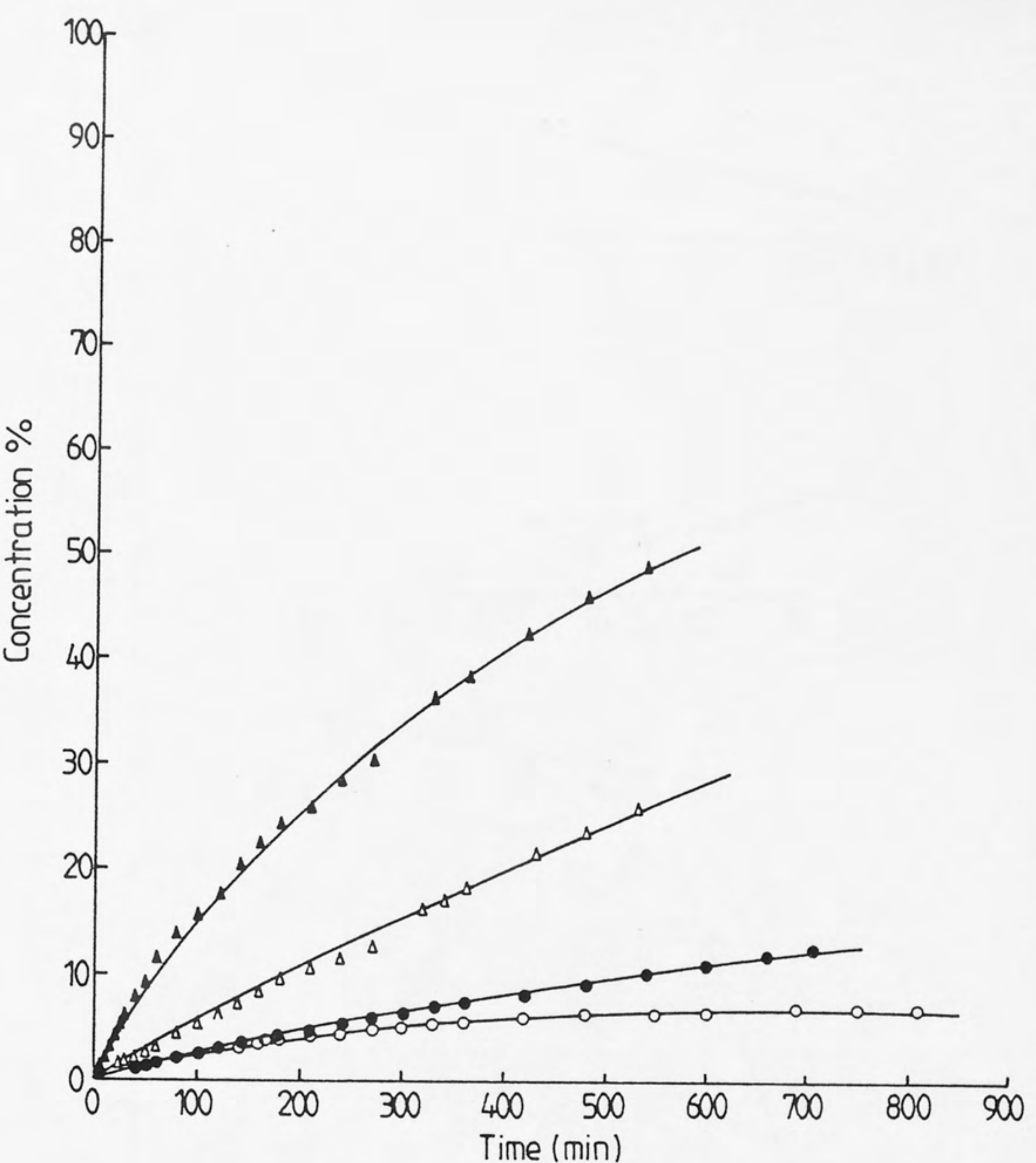


Fig. 4.40 c. Formation of p-chlorobenzoic acid in alkaline methanolic aqueous solutions

KEY methanol % (v/v)

▲ 20

△ 40

● 50

○ 70

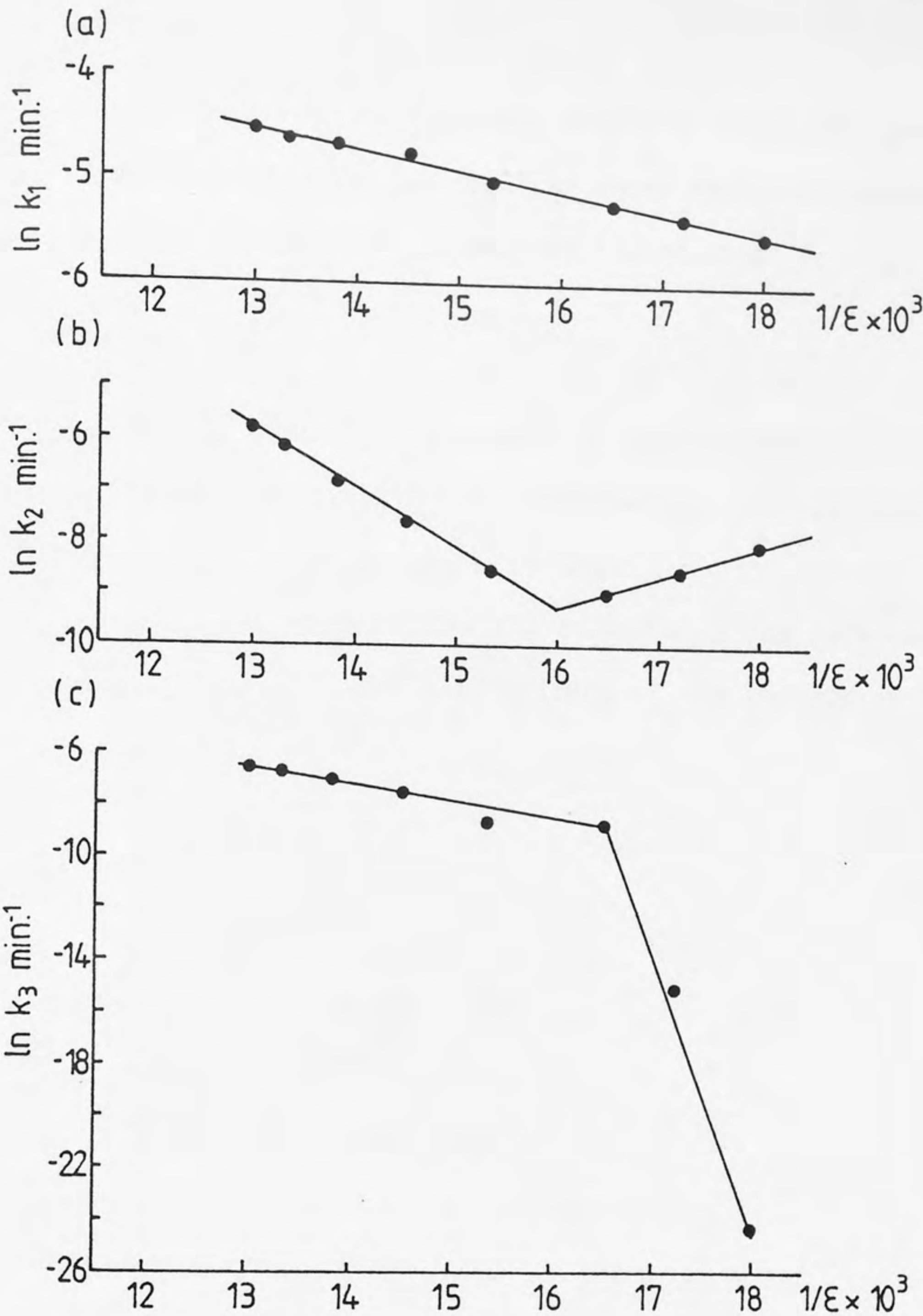


Fig. 4.41 Effect of the solvent dielectric constant on the degradation of indomethacin in Teorell-Stenhagen's buffer pH 9·7 at 40°C

(a) The effect on the alcoholysis rate constant (k_1) of indomethacin
(b) The effect on the degradation rate constant (k_2) of indomethacin
(c) The effect on the degradation rate constant (k_3) of methyl p-chlorobenzoate

transition state have like charges and suggests that the reaction involves an attack of hydroxide ion upon the monodissociated species of indomethacin which confirms the mechanism illustrated in Fig. 4.33 proposed earlier.

The effect of the dielectric constant on the degradation constant of indomethacin (k_2) and that of the degradation rate constant of methyl p-chlorobenzoate (k_3) is shown in Fig. 4.41 (b and c). The relationship obtained was biphasic and the linear sections intersect at about 50%v/v methanol level. The coefficients of the regression lines are:

Low alcohol level:

$$\ln k_2 = 9.318 - \frac{1164.9}{\varepsilon} \quad (r = -0.9992;\ n=5)$$

$$\ln k_3 = 2.025 - \frac{658.4}{\varepsilon} \quad (r = -0.965;\ n=5)$$

High alcohol level:

$$\ln k_2 = -19.027 + \frac{612.15}{\varepsilon} \quad (r = 0.9993;\ n=3)$$

$$\ln k_3 = 164.54 - \frac{10472.0}{\varepsilon} \quad (r = -0.9975;\ n=3)$$

The deviation of the ln k versus $1/\varepsilon$ plots from linearity in lower dielectric constant values of the mixed solvent have been explained in several studies(306-310) in terms of preferential adsorption of water on the reactant ions. In systems upto 30 or 40% by weight of the organic component in the mixed solvent systems the reactant ions cling rather

exclusively to the more polar component. This relative affinity for water exists until the latter is replaced by the larger molar volume of the organic component.

In studying the influence of the dielectric constant on the alcoholysis rate constant of indomethacin (k_1), the electrostatic contribution to the free energy increase ($\Delta G^*_{e.s.}$) (311) in forming the activated complex is given by

$$\Delta G^*_{e.s.} = \frac{z_A z_B e^2 N}{\varepsilon r^*} \tag{4.31}$$

in a system containing 30% methanol by volume and having a value of 72.26 for the dielectric constant, the calculated value of $\Delta G^*_{e.s.}$ at 40°C was equal to 7.157 kJ mol.$^{-1}$.

Equation (4.30) can be rewritten as:

$$\ln k = \ln k_{\varepsilon=\infty} + \frac{A}{T\,\varepsilon} \tag{4.32}$$

where A is equal to $-z_A z_B e^2 N/Rr^*$.

From the relationship between entropy and equilibrium constant(311) it is found that the electrostatic contribution to the entropy of activation is given by

$$(\Delta S^*_o)_{e.s.} = R\left(\frac{\partial(T \ln K^*)}{T}\right) \tag{4.33}$$

$$= \frac{z_A z_B e^2 N}{r^* \varepsilon^2} \left(\frac{d\varepsilon}{dT}\right) \tag{4.34}$$

$$= - \frac{RA}{\varepsilon^2} \left(\frac{d\varepsilon}{dT}\right) \tag{4.35}$$

In aqueous solutions ε is about 80 and $\left(\frac{d\ln\varepsilon}{dT}\right)$ is about -0.0046; hence

$$(\Delta S_o^*)_{e.s.} = 1.13 \times 10^{-4} A \tag{4.36}$$

the value of A can be calculated from the slope of ln k versus $1/\varepsilon$ and it is equal to (-62205.62) and $(\Delta S_o^*)_{e.s.}$ = -7.029 J/mol./deg. for the alcholysis of indomethacin at 40°C.

4.4.4 Effect of Cetyltrimethylammonium Bromide on the Hydrolysis of Indomethacin in Aqueous Buffer Solutions

The variation of rate constants with surfactant concentrations is usually treated on the assumption that the substrate S, is distributed between the aqueous and micellar pseudophases and can react in each pseudophase with the rate constants k'_W and k'_M respectively(93,94).

$$\begin{array}{ccc} S_W + D_n & \overset{K_S}{\rightleftharpoons} & S_M \\ k'_W \searrow & & \swarrow k'_M \\ & \text{Products} & \end{array} \tag{4.37}$$

where D_n represents the micellar surfactant and its concentration is that of the analytical concentration less that of the monomeric surfactant:

$$[D_n] = [D_T] - CMC \tag{4.38}$$

where CMC is the critical micelle concentration and K_S is the equilibrium constant for substrate binding:

$$K_S = \frac{[S_M]}{[S_W][D_n]} \tag{4.39}$$

The basic hydrolysis of an activated amide involves the initial attack of the hydroxide ion on the amide, followed by base catalysed decomposition of the tetrahedral intermediate to products as shown below:

$$R-\overset{O}{\overset{\|}{C}}-N\langle \underset{k_2}{\overset{k_1[OH^-]}{\rightleftharpoons}} R-\underset{OH}{\overset{O^-}{C}}-N\langle \xrightarrow{k_3[OH^-]} RCO_2^- + H-N\langle \tag{4.40}$$

The first order rate constant $k_{obs.}$(94) is given by:

$$k_{obs.} = \frac{k_1 k_3 [OH^-]^2}{k_2 + k_3[OH^-]} \tag{4.41}$$

Surface tension measurements for cetyltrimethylammonium bromide and indomethacin solutions were conducted using the Wilhelmy plate method(288). A calibration curve was constructed from several pure compounds with known surface tensions at 20°C (Fig. 4.42). Variation of indomethacin concentration exerted no effect on the surface tension of solutions made in Teorell-Stenhagen's buffer pH 9.7 at 20°C (Fig. 4.43). The cmc of cetyltrimethylammonium bromide (CTMB) was measured in the absence of indomethacin in Teorell-Stenhagen's buffer pH 9.7 at 20°C, and was found to be 4.5×10^{-5} M (Fig. 4.44). In the presence of

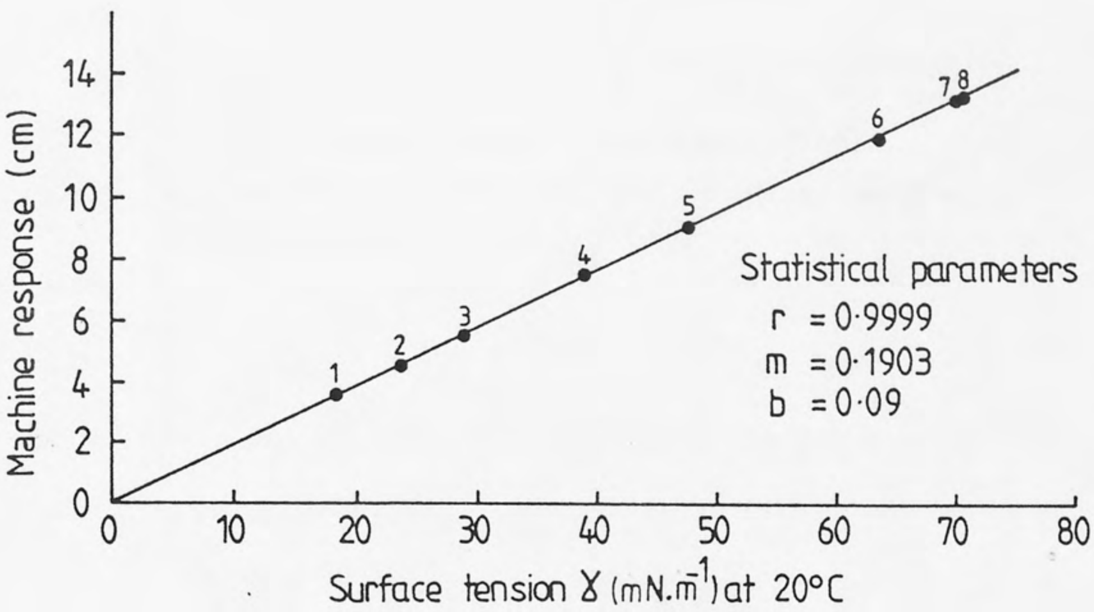


Fig. 4.42 Calibration curve for the measurement of surface tension of cetyltrimethylammonium bromide solutions at 20°C

KEY	Materials
1	n - Hexane
2	Acetone
3	Benzene
4	Benzyl alcohol
5	Ethylene glycol
6	Glycerol
7	Distilled water
8	Teorell - Stenhagen buffer pH 9·7

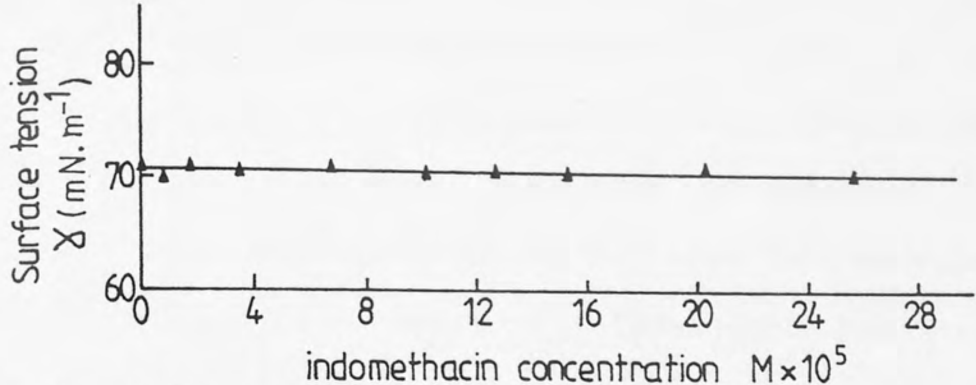


Fig. 4.43 Surface tension of indomethacin solutions in Teorell-Stenhagens buffer pH 9·7 at 20°C.

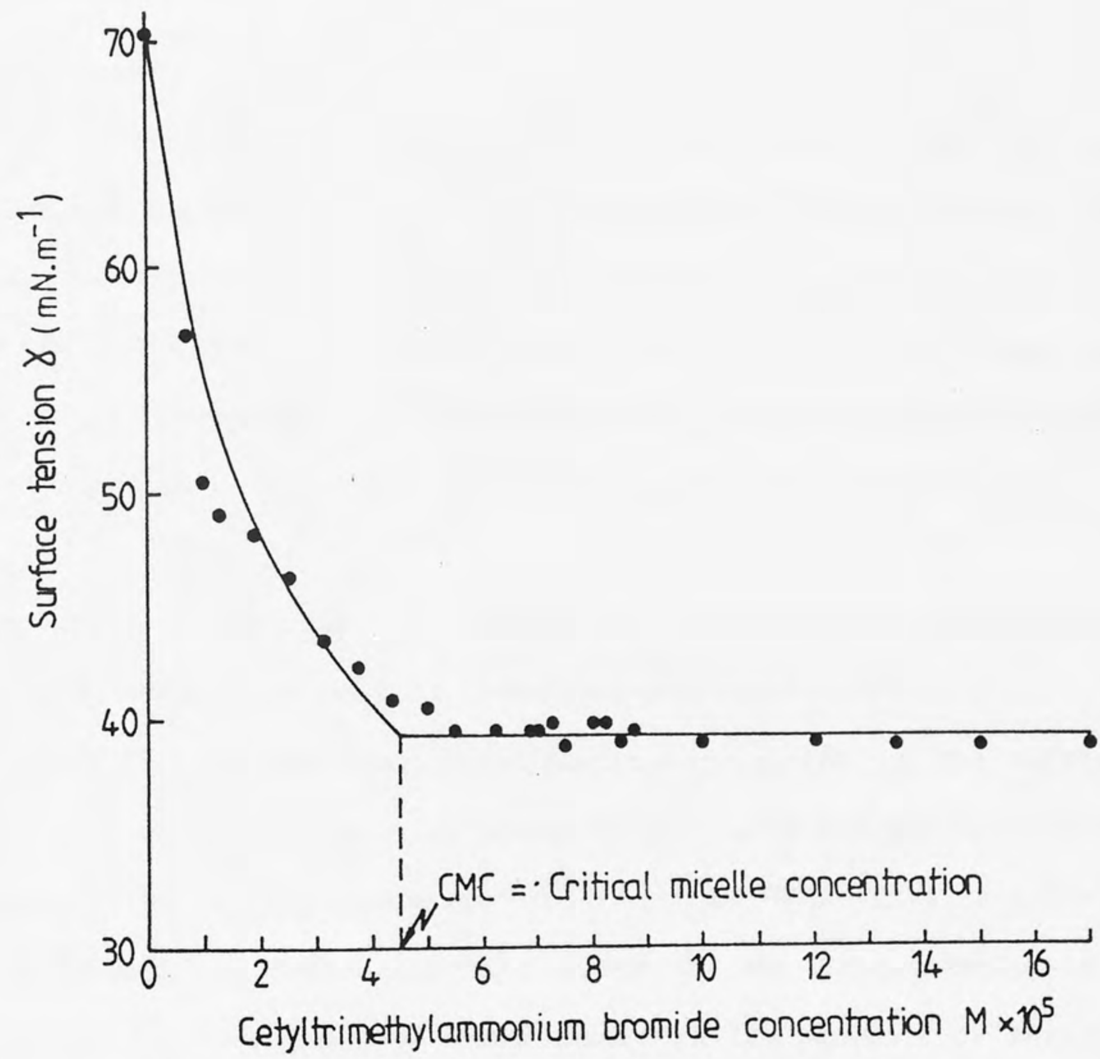


Fig. 4.44 The effect of the concentration of cetyltrimethylammonium bromide on the surface tension of the surfactant solutions in Teorell-Stenhagen's buffer pH 9·7 at 20°C.

indomethacin, surface tension measurements show that the cmc of CTMB in Teorell-Stenhagen's buffer at 20°C was affected as shown in Fig. 4.45. From Fig. 4.45 the cmc value of CTMB in solutions containing 17×10^{-5}M of indomethacin is equal to 1.25×10^{-5}M. The value of the cmc of CTMB obtained in the presence of indomethacin indicates clearly that even the lowest concentration of the surfactant used to study its effect on indomethacin degradation CTMB was present in the micellar form.

The hydrolysis of indomethacin (60.8 μg/ml, 17×10^{-5}M) in Teorell-Stenhagen's buffer pH 9.7 was conducted in the presence of various concentrations of cetyltrimethylammonium bromide - a cationic surfactant - at 60°C. The hydrolytic process was followed using both ultraviolet spectroscopy at 320 nm and HPLC and both p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid were detected.

At any time, throughout the study, the sum of the concentration of either p-chlorobenzoic acid or 5-methoxy-2-methylindole-3-acetic acid formed with that of residual indomethacin corresponds to the initial concentration of indomethacin as shown in Fig. 4.46 for the hydrolysis of indomethacin in the presence of 5.48×10^{-4}M of cetyltrimethylammonium bromide. Semilogarithmic plots of the concentration-time profiles for the hydrolysis of indomethacin in the presence of various surfactant concentration gave straight lines which indicate that indomethacin degradation followed first order kinetics (Fig. 4.47a and b), and these findings are in close agreement with an earlier report by Cipiciani and others(94) which indicate that the rate of indomethacin degradation in the presence of micelles of CTMB is first order in alkaline solutions.

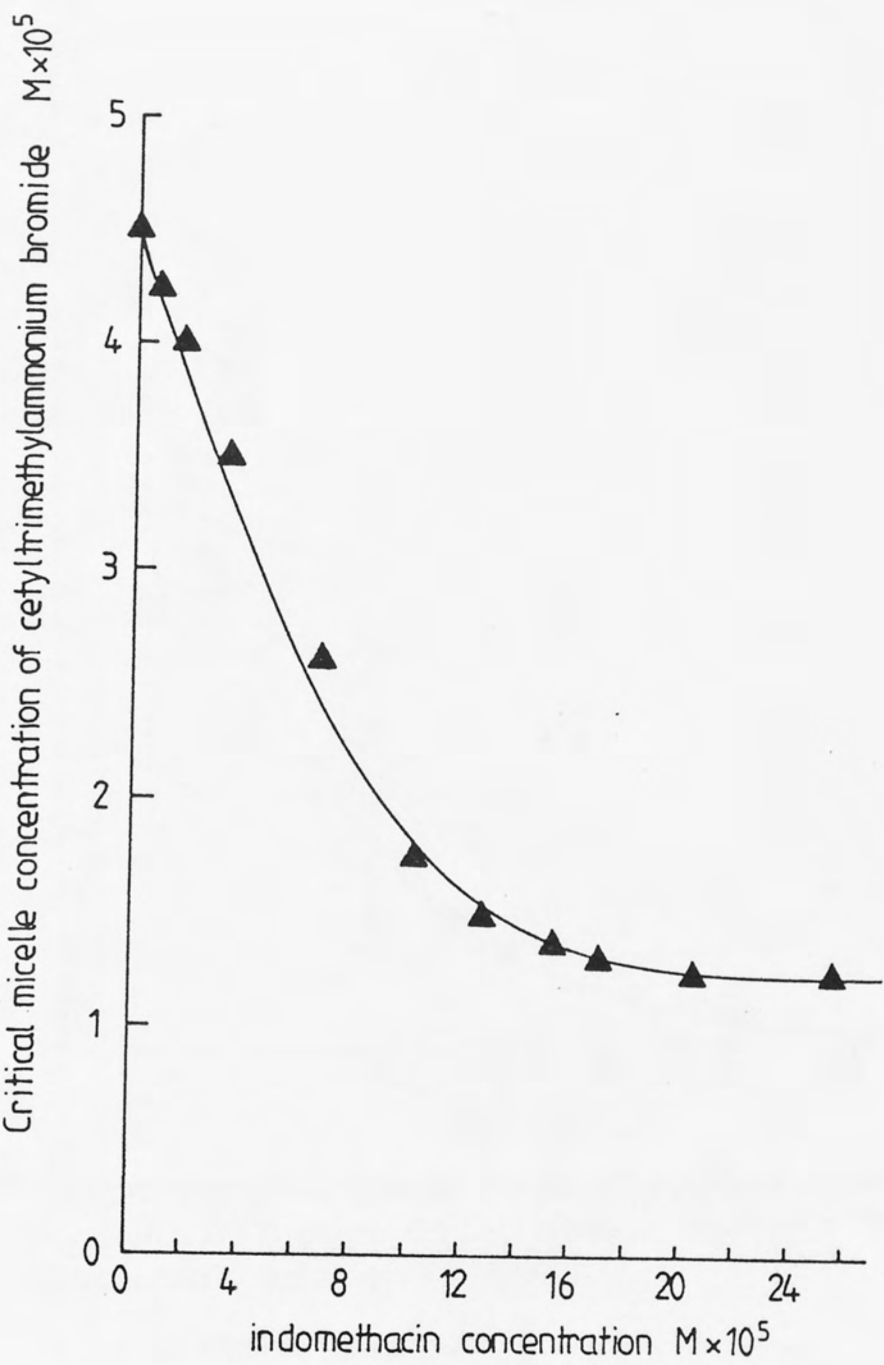


Fig. 4.45 The effect of indomethacin concentration on the critical micelle concentration of cetyltrimethylammonium bromide at 20°C.

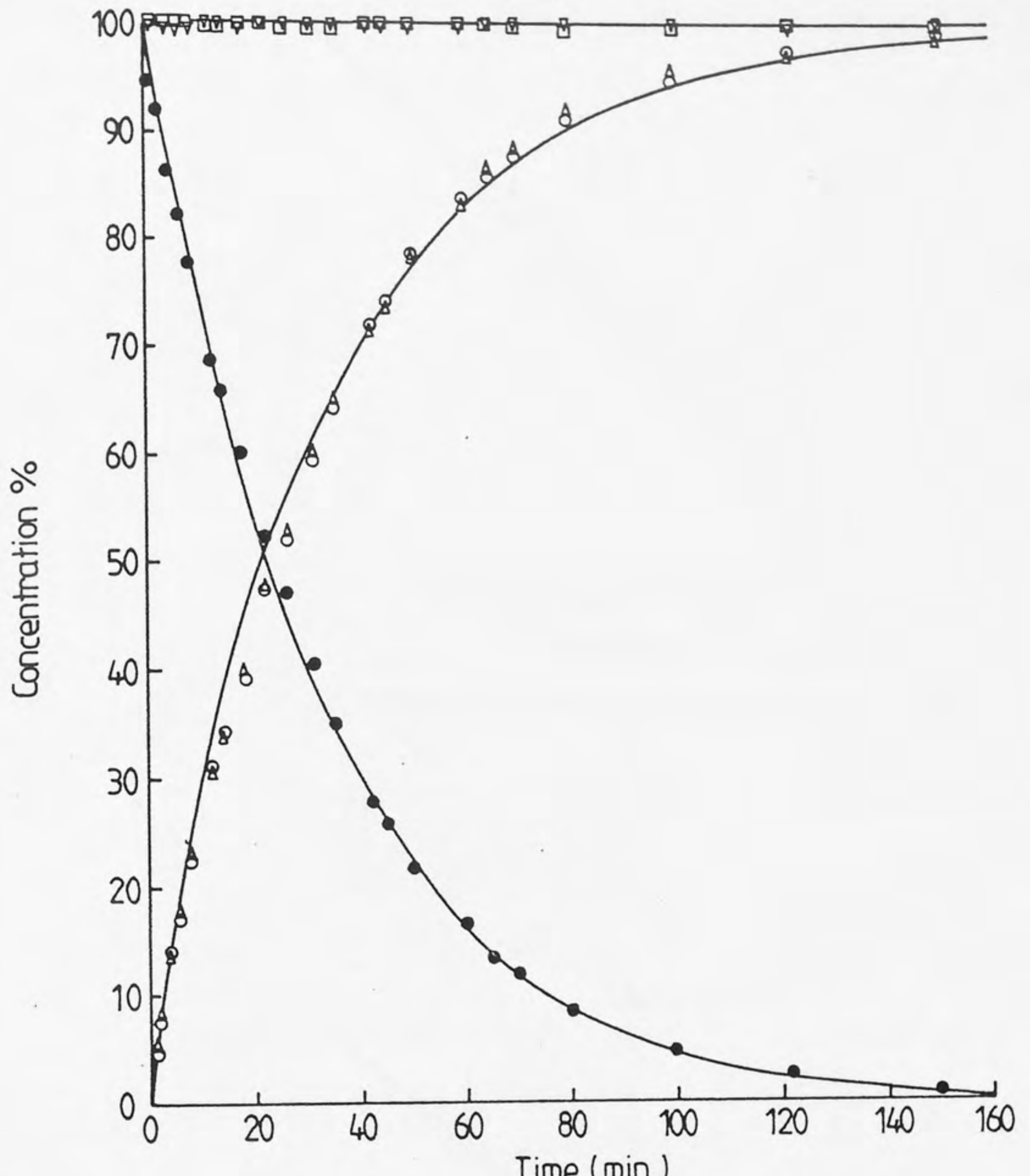


Fig. 4.46 Concentration time profiles for the degradation of indomethacin by $5 \cdot 48 \times 10^{-4}$ M cetyltrimethylammonium bromide in Teorell-Stenhagen's buffer pH 9·7 at 60°C.

KEY

- ● indomethacin remaining (I)
- ○ p-chlorobenzoic acid formed (II)
- △ 5-methoxy-2-methylindole-3-acetic acid formed (III)
- □ mass balance of I + II
- ▽ mass balance of I + III

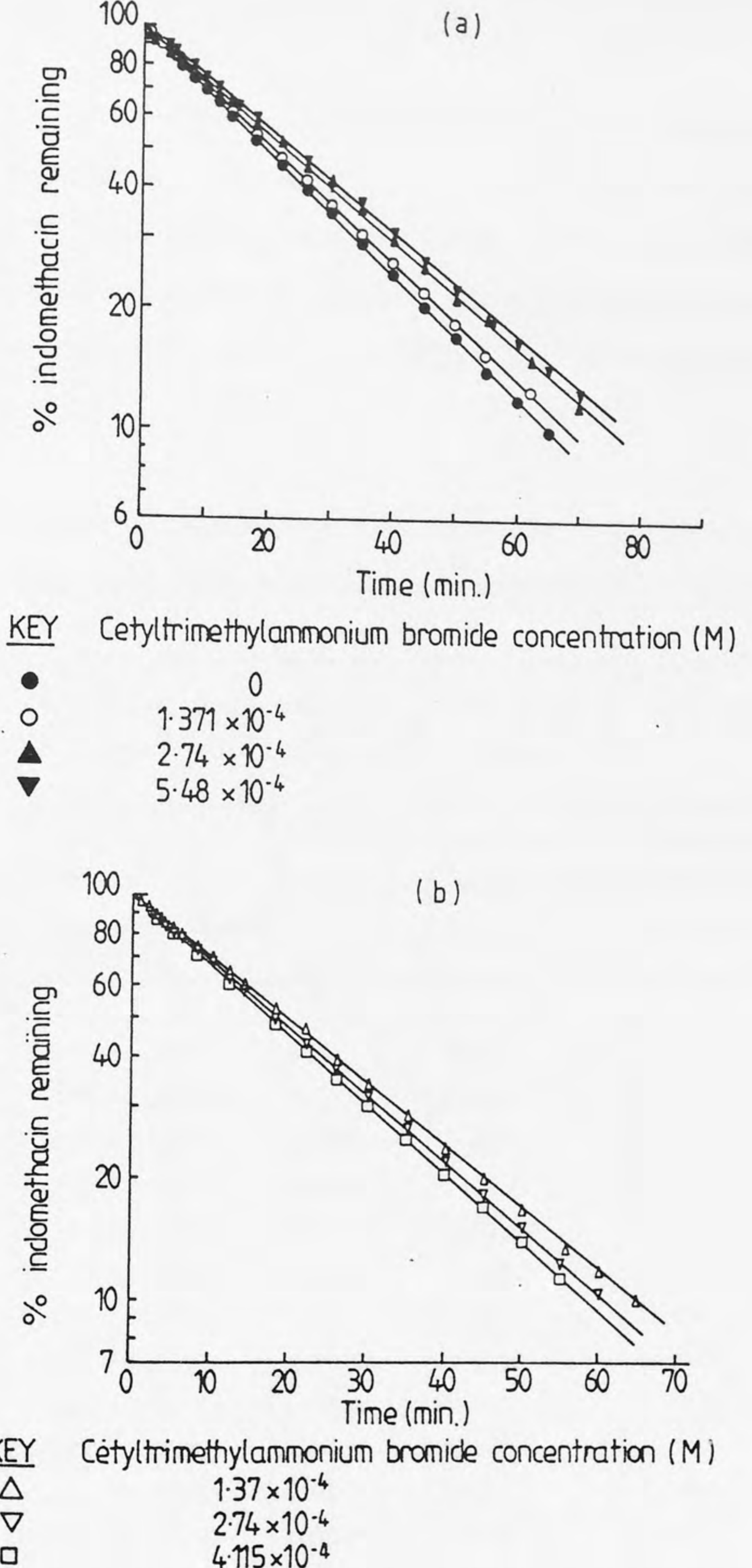


Fig. 4.47 Effect of cetyltrimethylammonium bromide concentration on the first order rate of degradation of indomethacin in Teorell-Stenhagen's buffer pH 9·7 at 60°C

The respective rate constants for the degradation process obtained from both ultraviolet spectroscopy and HPLC are recorded in Table 4.14 with both sets of data showing good agreement. The rate constant for the formation of p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid are similarly recorded. The effect of cetyltrimethyl-

TABLE 4.14

The effect of cetyltrimethylammonium bromide on the first order rate of indomethacin degradation in Teorell-Stenhagen's buffer pH 9.7 at 60°C.

Concentration of cetyltrimethyl ammonium bromide $M \times 10^4$	Degradation rate constant of indomethacin k $min.^{-1} \times 10^2$		Rate constant of formation k $min.^{-1} \times 10^2$	
	Ultraviolet (r = regression coefficient)	HPLC	p-chloro-benzoic acid	5-methoxy-2-methylindole-3-acetic acid
0	3.498 (0.9999)	3.5	3.497	3.499
1.371	3.336 (0.9998)	3.311	3.336	3.337
2.743	3.035 (0.9996)	3.971	3.035	3.034
5.48	2.944 (0.9997)	3.059	2.945	2.944
13.71	3.527 (0.9997)	3.4	3.527	3.526
27.43	3.757 (0.9999)	3.745	3.756	3.757
41.15	3.92 (0.9999)	3.95	3.91	3.93
54.8	4.003 (0.9991)	4.0	4.001	4.004
68.5	4.046 (0.9994)	4.04	4.045	4.044
82.3	4.062 (0.9993)	4.06	4.06	4.063
109.7	4.04 (0.9994)	4.04	4.04	4.039
137	3.994 (0.9993)	4.001	3.992	3.991
164.6	3.962 (0.9993)	3.96	3.961	3.961
219.4	3.962 (0.9996)	3.965	3.961	3.962
274.3	4.002 (0.9991)	4.0	4.001	4.002

ammonium bromide concentration on the degradation rate constant of indomethacin in buffer pH 9.7 at 60°C is shown in Fig. 4.48. Examining the data in Fig. 4.48 for the alkaline hydrolysis of indomethacin in the presence of the cationic surfactant indicates that the cationic micelles enhance the decomposition of indomethacin when the concentration of cetyltrimethylammonium bromide is higher than 5.48×10^{-4}M. The findings can be explained taking into consideration the different electrostatic interactions between the substrates and the head group charge of the surfactants. Cipiciani(94) accounted for the value of the indomethacin binding constants (K_S) obtained in the presence of sodium dodecyl sulfate and hexadecyltrimethylammonium bromide which were of values equal to 80 and 2500 M^{-1} respectively, where the electrostatic repulsion between the head groups of sodium dodecyl sulfate and the negatively charged carboxylate moieties of indomethacin attributed to the low value of K_S obtained while the interaction in the presence of hexadecyltrimethylammonium bromide resulted in the relatively high binding constant value.

On the other hand it has been postulated that the micellar stern layer is similar to a concentrated ionic solution(95), and the concentration of the hydroxide ion in the Stern layer of a cationic surfactant is so high that the tetrahedral intermediate will always proceed to products, even though the analytical concentration of the hydroxide ion in solution may be low(312). Furthermore, the slight increase in the rate of indomethacin degradation in the presence of hexadecyltrimethylammonium bromide was due to a small increase in K_S which was caused by a salting-out effect of sodium hydroxide which drives the substrate into the micelles(313).

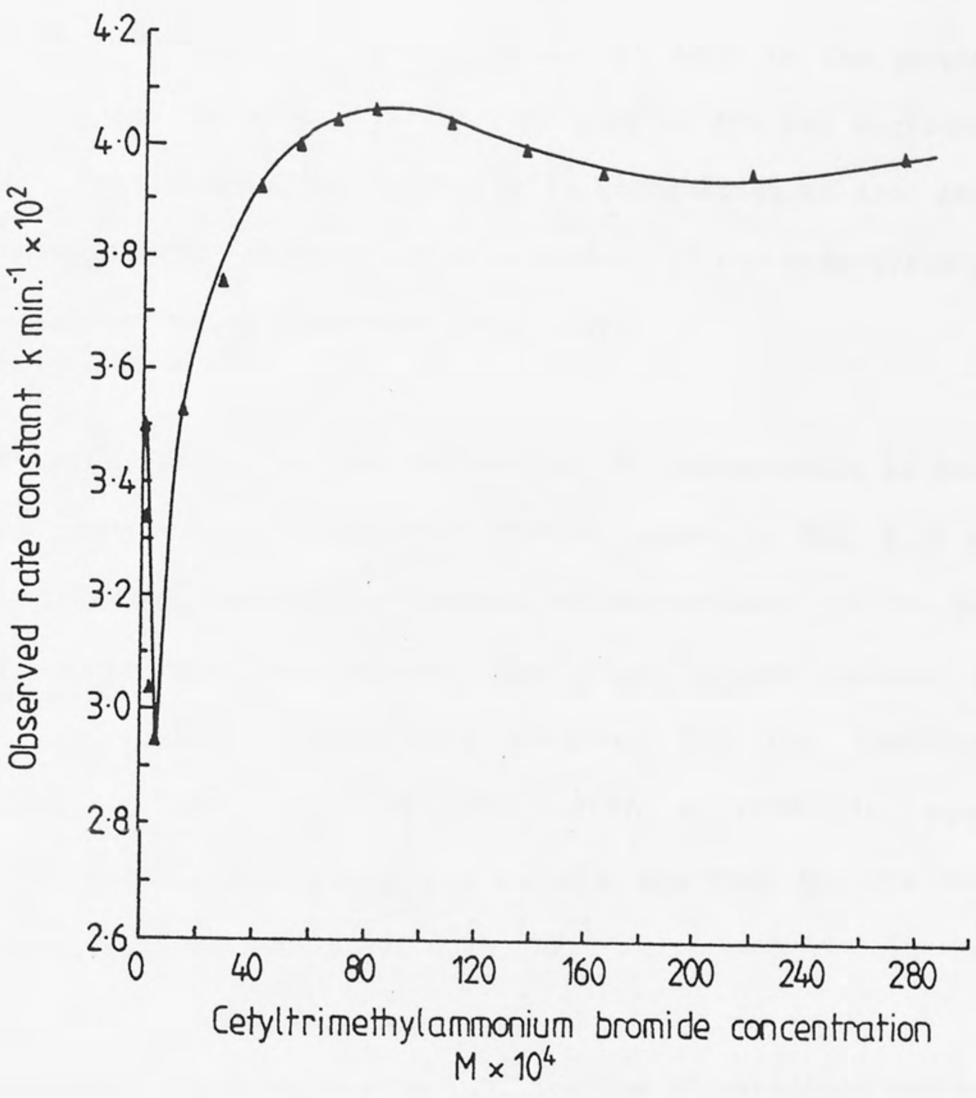


Fig. 4.48 Effect of cetyltrimethylammonium bromide concentration on the decomposition rate constant of indomethacin in Teorell - Stenhagen's buffer pH 9·7 at 60°C.

In the acidic region indomethacin hydrolysis was followed in 10% ethanol-Teorell-Stenhagen's buffer pH 2.0 at 60°C in the presence of 0.0137 M CTMB and the concentration-time profile for the degradation of indomethacin is shown in Fig. 4.49. It is interesting to note that only p-chlorobenzoic acid was detected as a product of the hydrolytic process with mass-balance being preserved (Fig. 4.49).

First order plots for the degradation of indomethacin in buffer pH 2.0 in the presence and absence of CTMB are shown in Fig. 4.50 and the data show that the degradation process of indomethacin in the presence of 0.0137 M of CTMB was slower than that in the absence of the surfactant. The respective rate constant for the hydrolysis of indomethacin in the presence of 0.0137 M CTMB is equal to 6.996×10^{-4} $hr.^{-1}$ (0.999) in pH 2.0 at 60°C and that for the formation of p-chlorobenzoic acid is $6.991 \times 10^{-4} hr.^{-1}$.

This effect indicates that solubilization of unionized indomethacin into the micellar phase has taken place to a certain degree and thus limits the effect of the hydrogen ions on indomethacin molecules. The hydrogen ions are concentrated in the aqueous phase due to the possible repulsion between these ions and the head group charge of the cationic surfactant.

The solubility determination of indomethacin in 10% ethanol buffer pH 2.0 in the absence and presence of 0.0137 M CTMB confirm the positive effect induced by the presence of the surfactant which resulted in a (52)-fold increase in the solubility of indomethacin at 40°C. The solubility of indomethacin obtained in 10% ethanol-buffer pH 2.0 was

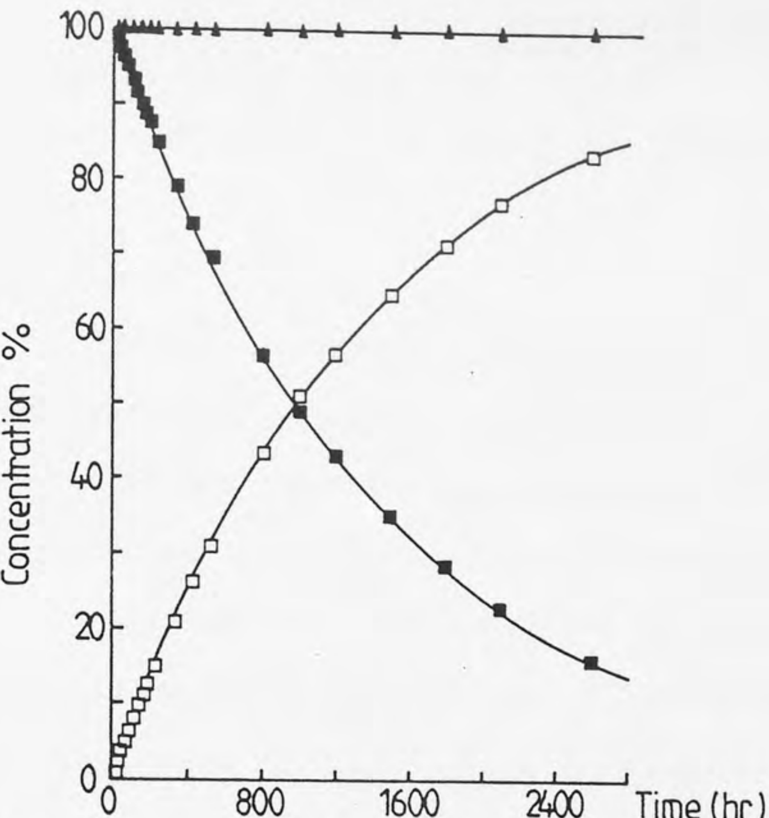


Fig. 4.49 Concentration-time profiles for the degradation of indomethacin by 0·0137 M Cetyltrimethylammonium bromide in 10% ethanol - Teorell Stenhagen buffer pH 2·0 at 60° C.

KEY ■ indomethacin remaining
□ p-chlorobenzoic acid formed
▲ mass balance

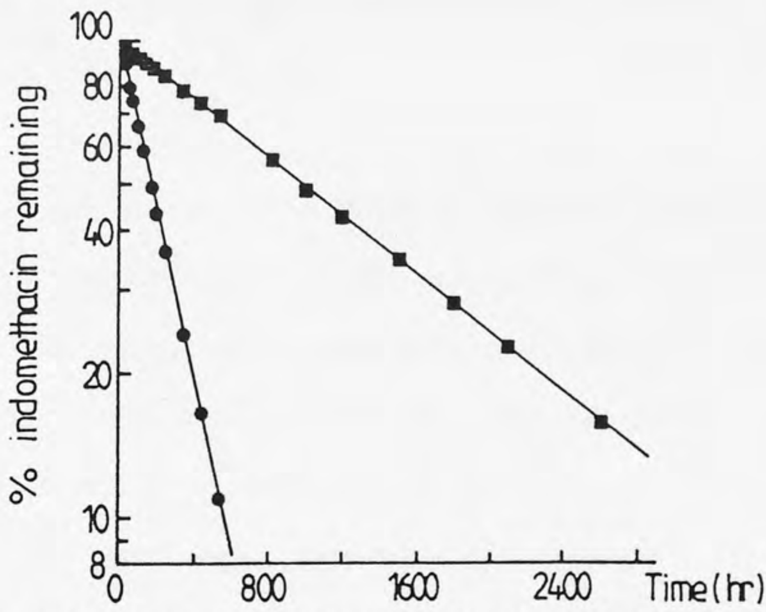


Fig. 4.50 The effect of presence and absence of cetyltrimethylammonium bromide on the first order rate of degradation of indomethacin in 10% ethanol - Teorell Stenhagen buffer pH 2·0 at 60°C.

KEY ● absence of cetyltrimethylammonium bromide
■ 0·0137 M of cetyltrimethylammonium bromide

equal to 1.98 mg/100ml while that in the presence of 0.0137 M CTMB was equal to 102.7 mg/100ml at 40°C.

The effect of CTMB on the ultraviolet absorption of indomethacin was studied and typical absorption spectra of indomethacin (3.4×10^{-5}M) and CTMB (2.74×10^{-3}M) in Teorell-Stenhagen's buffer pH 9.7 are shown in Figs. 4.51 and 4.52. At pH 9.7 indomethacin λ_{max} are 225, 267 and 320 nm while that for CTMB is 218 nm. In the case of an indomethacin solution (3.4×10^{-5}M) containing (2.05×10^{-3}M) of CTMB in buffer pH 9.7, it was noticed that the λ_{max} of indomethacin at 225 nm was affected by the presence of the surfactant while no spectral effect was observed upon the λ_{max} of indomethacin at 320 nm (Fig. 4.53). In the absence of CTMB a plot of the ultraviolet absorption at λ_{max} 320 nm for a series of solutions containing different concentrations of indomethacin versus indomethacin concentrations is shown in Fig. 4.54 ($r = 0.9998$, $m = 5941.1$ and $b = 0.019$).

In solutions containing a mixture of indomethacin and CTMAB in Teorell-Stenhagen's buffer pH 9.7 it was noticed that varying the concentration of the surfactant affected the ultraviolet absorption of indomethacin at λ_{max} 320 nm. Plots of the absorbance changes of indomethacin at 320 nm as a function of the CTMB concentrations show that increasing the surfactant concentration reduced the absorbance value of indomethacin until a maximum effect was obtained at a given surfactant concentration and beyond which the absorbance changes are more nearly constant. Figure 4.55 represents the typical absorbance variations observed in this study. These findings are in close agreement with the data obtained by Clarke and others(314) for the effect of CTMB and sodium lauryl sulfate on the ultraviolet absorption

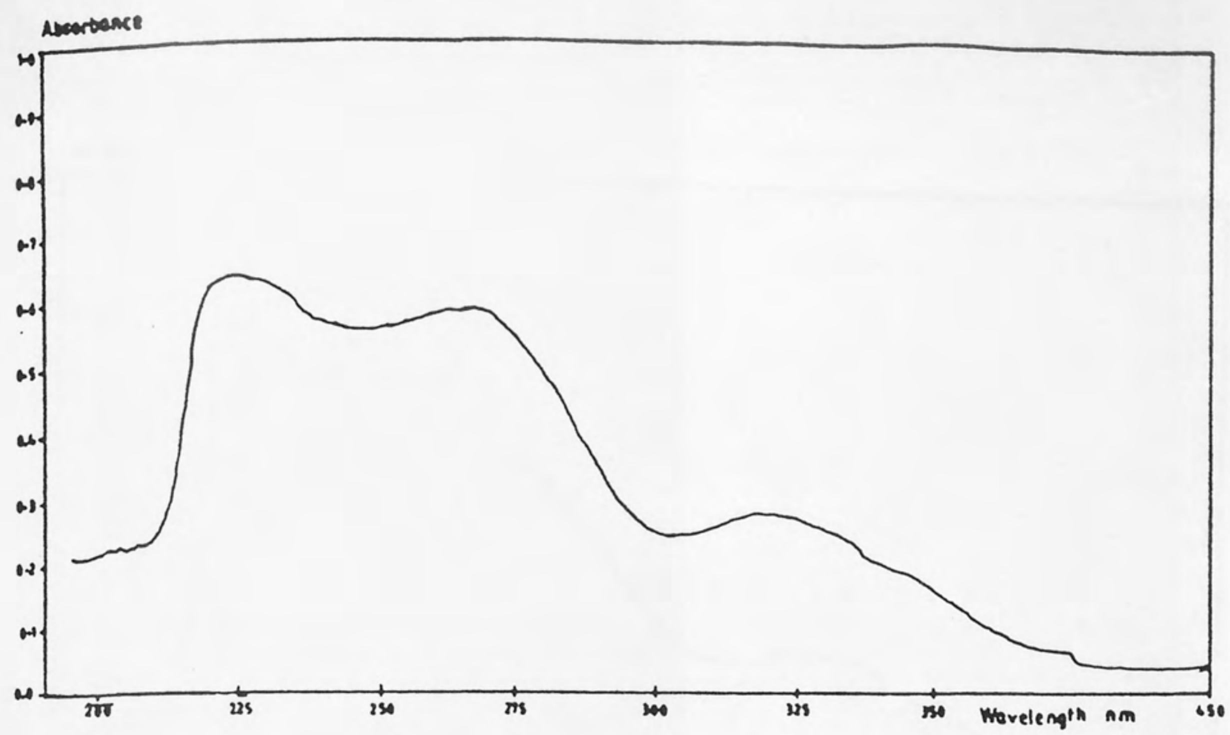


Fig.4.51 UV_absorption spectrum of indomethacin(3.4×10^{-5} M) in buffer pH 9.7 .

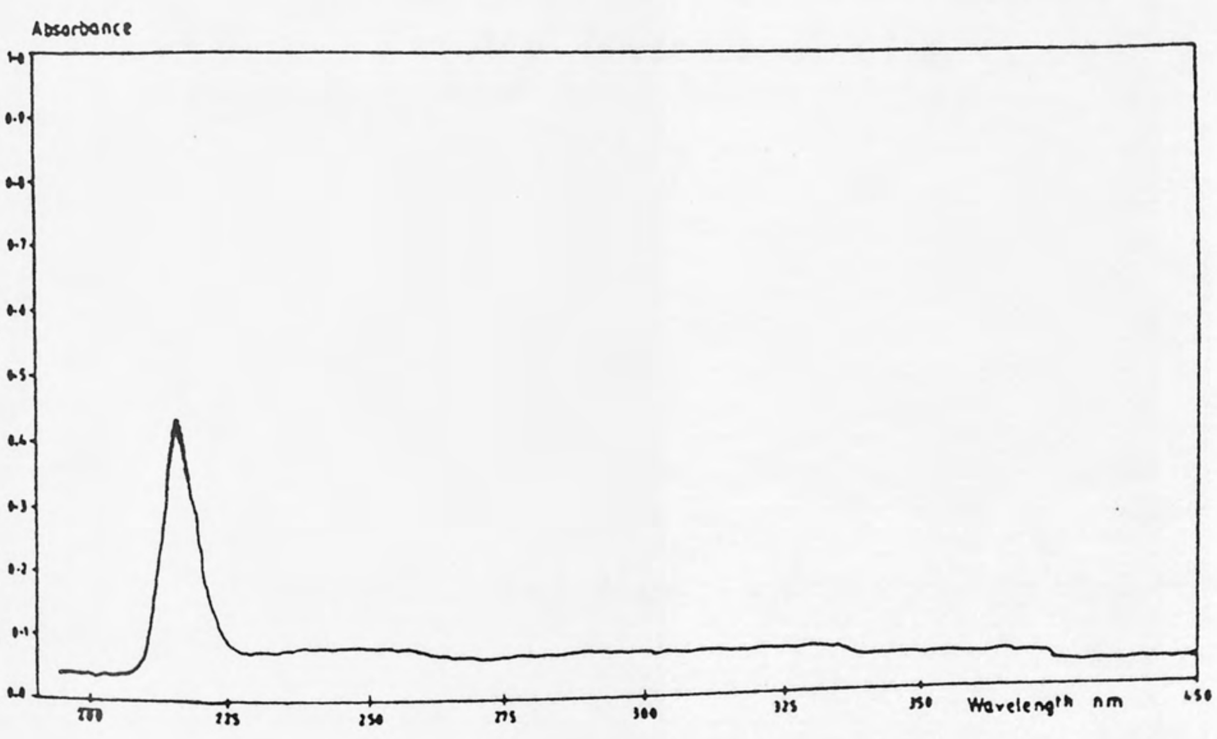


Fig.4.52 UV_absorption spectrum of CTMB(2.74×10^{-3} M) in buffer pH 9.7 .

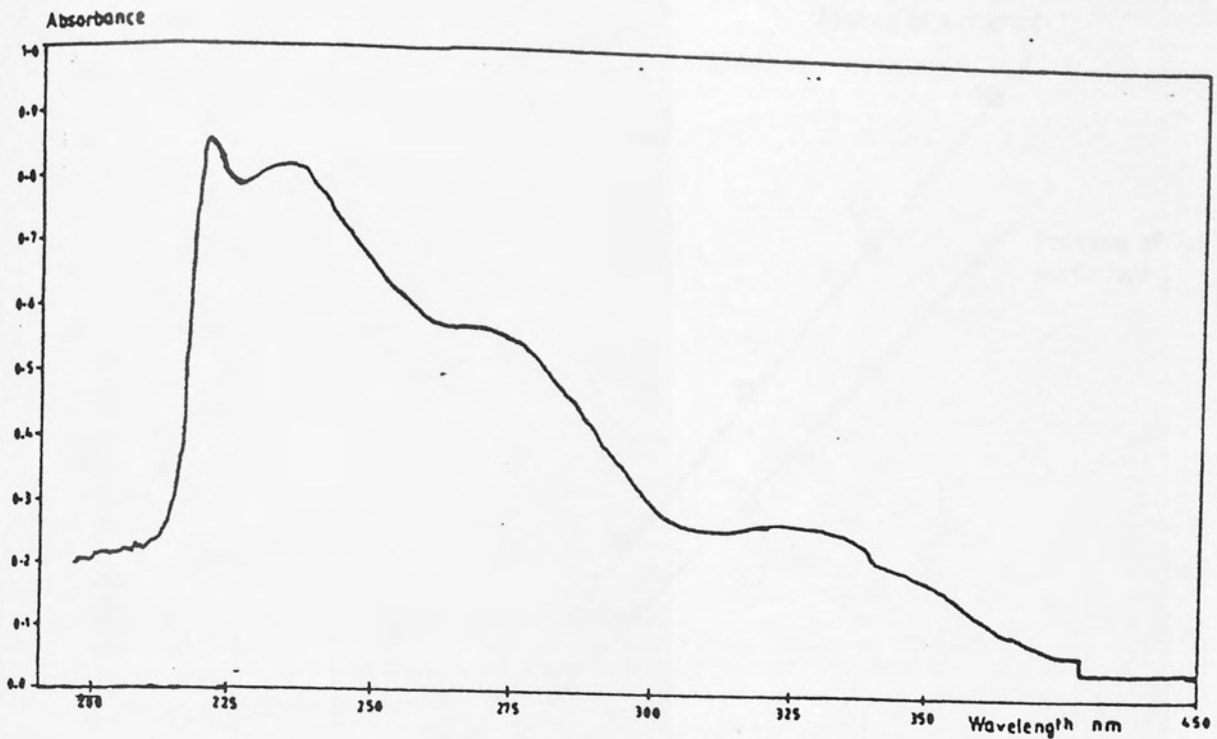


Fig. 4.53 UV_absorption spectrum of indomethacin (3.4×10^{-5}M) in the presence of CTMB (2.05×10^{-3}M).

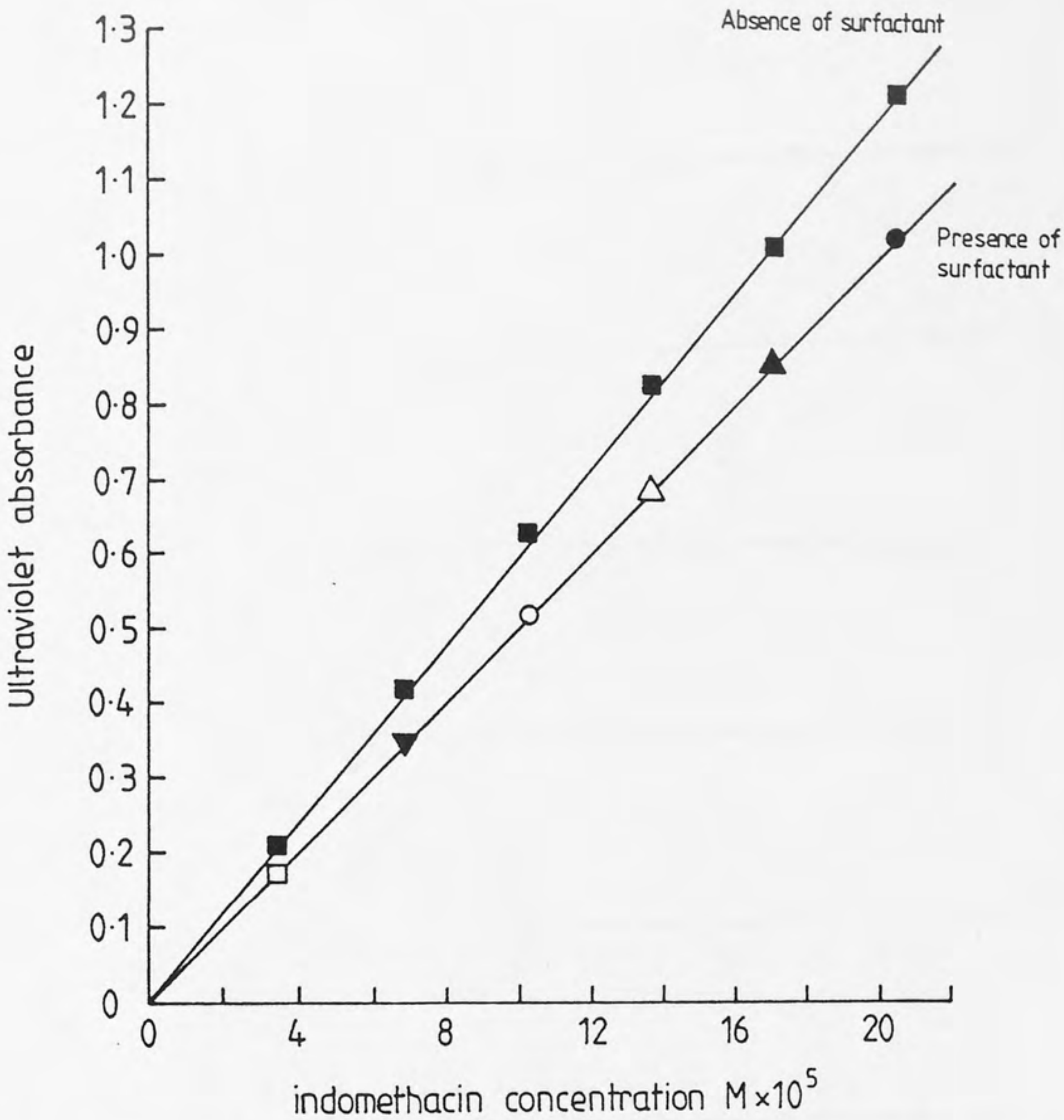


Fig. 4.54 Variation of the ultraviolet absorbance of indomethacin at 320 nm versus the concentration of indomethacin in the presence and absence of cetyltrimethylammonium bromide at 20°C.

KEY Concentration of cetyltrimethylammonium bromide $M \times 10^4$ in indomethacin solutions.

- ■ absence of surfactant
- □ 1·25
- ▼ 1·70
- ○ 2·125
- △ 2·5
- ▲ 3·0
- ● 3·75

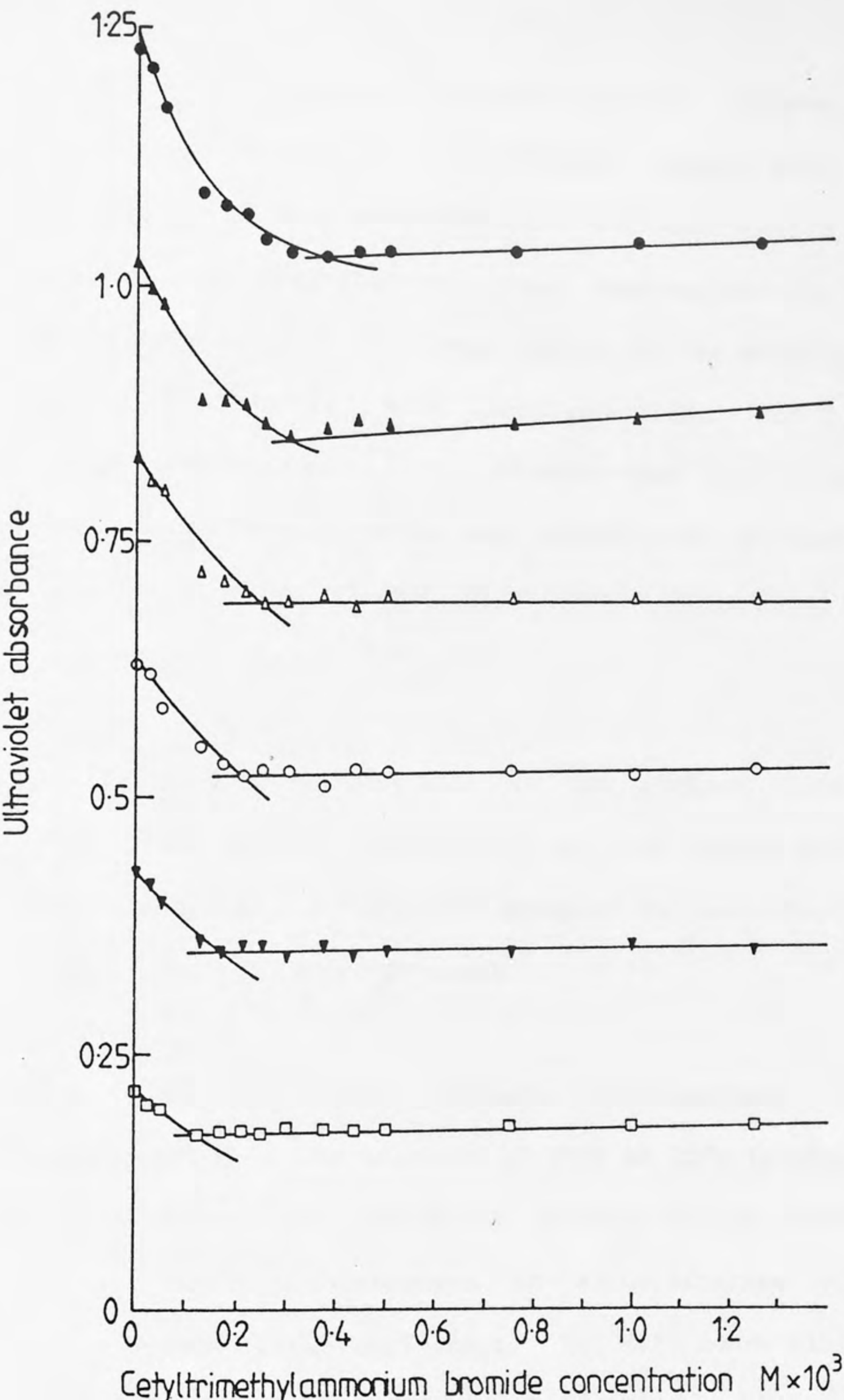


Fig. 4.55 Ultraviolet absorbance variation at 320nm with cetyltrimethyl ammonium bromide concentration for indomethacin solutions in Teorell - Stenhagen's buffer pH 9·7 at 20°C.

KEY	indomethacin initial concentration $M \times 10^5$
●	20·4
▲	17·0
△	13·6
○	10·2
▼	6·8
□	3·4

of benzoic acid and other aromatic carboxylic acids. However, the manner in which the absorbance of indomethacin varies with CTMB concentrations does suggest that solubilization has occurred(315,316). Clarke and others(314) also suggested that these observations are found to be essentially independent of the formal charge of the micelle, the anion and neutral carboxylic acid configurations, and added electrolytes. Furthermore, their data indicate that the anion and neutral forms of the carboxylic acids are solubilized in both the cationic and anionic micelles without significant changes in their energetics.

It appears likely that indomethacin is not surface bound but instead is located at the micelle surface with its ring moiety directed towards the aliphatic chain and the polar group at or near the Stern layer, with access to the aqueous environment.

The variation of the ultraviolet absorbance of indomethacin at 320 nm versus its concentration in the presence of CTMB at 20°C is shown in Fig. 4.54. The data in Fig. 4.54 present the ultraviolet absorption of these solutions at maximum interaction or solubilization effect introduced by the presence of the surfactant. The data concerning the interaction of indomethacin with CTMB or the solubilization data obtained from the ultraviolet absorption study indicate that the maximum amount of indomethacin interacting or solubilized is directly related to the concentration of CTMB causing the effect as shown in Fig. 4.56. The linear relationship between the amount of indomethacin solubilised and the concentration of CTMB presented in Fig. 4.56 ($r = 0.9985$) has a (y) intercept value equal to 8×10^{-5} M which represent the concentration of a solution containing CTMB at the cmc. The value for the cmc of CTMB is

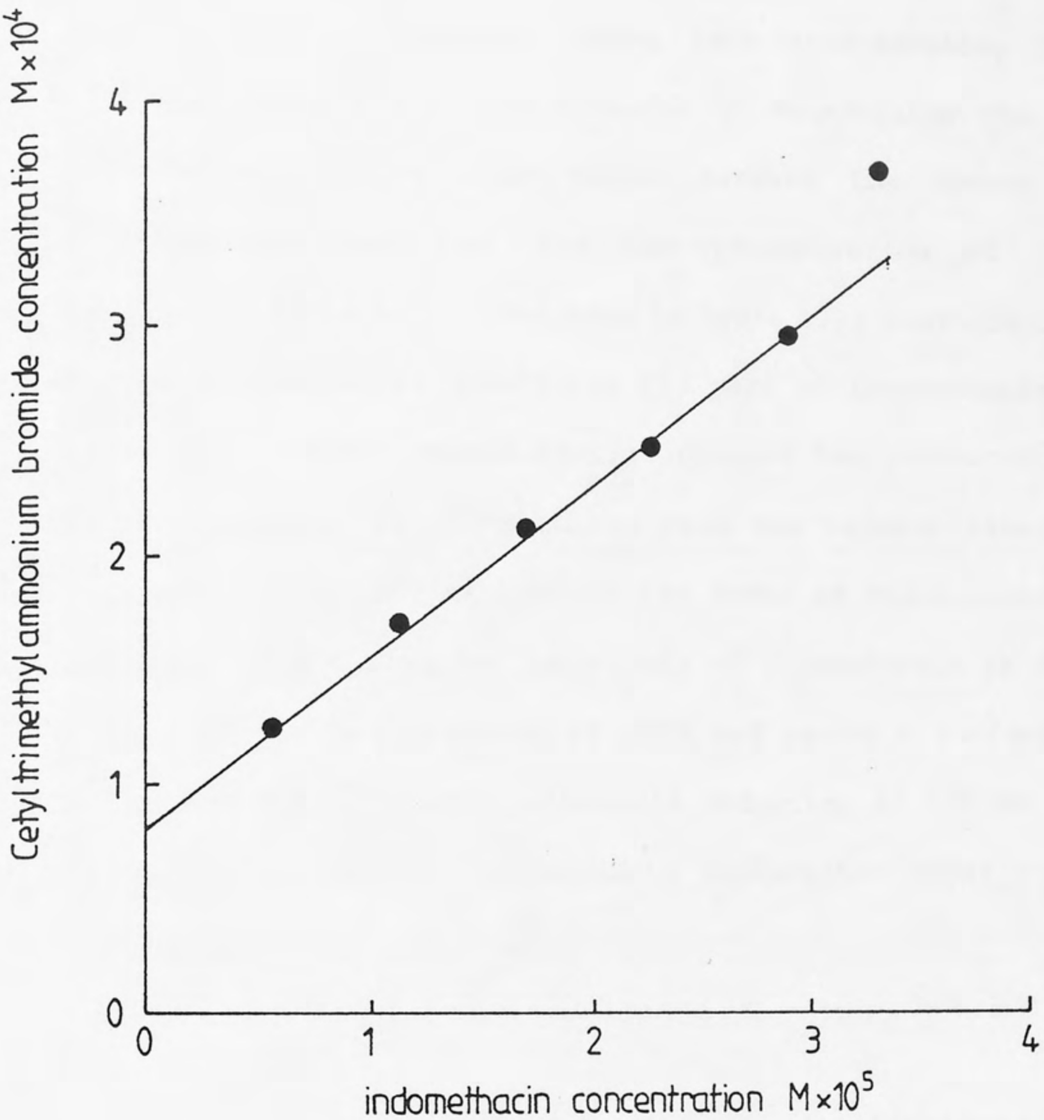


Fig. 4.56 The relationship between the amount of indomethacin interacted and the concentration of cetyltrimethylammonium bromide in Teorell-Stenhagen's buffer solutions pH 9·7 at 20°C.

closely in agreement with the value of 4.5×10^{-5} M for the cmc obtained by the surface tension measurements taking into consideration that surface tension measurements are more specific in determining the cmc value of a surfactant. The relationship between the amount of indomethacin solubilized and that for the concentration of CTMB interacted is shown in Table 4.15. The data in Table 4.15 indicate that (8) parts of CTMB are needed to solubilize (1) part of indomethacin in buffer pH 9.7 at 20°C and the solubilization process has protected 16% of the total concentration of indomethacin from the effects alkaline conditions. These findings may explain on the basis of solubilization the early inhibition of the alkaline hydrolysis of indomethacin at 60°C observed in Fig. 4.48 in the presence of CTMB and up to a surfactant concentration of 5.48×10^{-4} M with an overall reduction of 16% in the value of the rate constant for indomethacin degradation under these conditions.

TABLE 4.15

The relation between the amount of indomethacin solubilized and the presence of cetyltrimethylammonium bromide in the medium.

Initial concentration of indomethacin $M \times 10^5$	Indomethacin solubilized $M \times 10^5$	CTMB interacted ($CTMB_x$) $M \times 10^4$	($CTMB$ interacted)-CMC $M \times 10^4$	$\frac{CTMB_x - CMC}{\text{indomethacin solubilized}}$	indomethacin solubilized % w/v
3.4	0.56	1.25	0.45	8.03	16.44
6.8	1.12	1.7	0.9	8.03	16.4
10.2	1.68	2.125	1.325	7.88	16.4
13.6	2.24	2.5	1.7	7.58	16.4
17.0	2.88	3.0	2.2	7.638	16.9
20.4	3.28	3.75	2.95	8.99	16.0

CHAPTER 5

NON-ISOTHERMAL, SURFACTANT, NON-ISOCONCENTRATION AND NON-ISOpH KINETICS IN FORMULATION STUDIES

5.1 INTRODUCTION

The effect of continuous change in temperature, surfactant concentration and pH on indomethacin was studied in three independent experiments. The advantages are that compared to experiments run with constant temperature, surfactant concentration or pH significant time saving may be achieved. In contrast to experiments involving the simultaneous variation of two or more parameters, which affect decomposition, data handling is much simplified and microcomputer programs used for the analysis of non-isothermal data(317) can be used for anlaysis without modification.

5.2 THEORY

The hydrolysis of indomethacin was confirmed earlier in Section 4.4 to follow first order kinetics under isothermal and isopH conditions. The concentration change with time was presented in equation (4.1). When the test solutions are subjected to a change in temperature or surfactant concentration or pH, deviations from linearity are observed. During continuous changes the tangent at any point on the ln C_t versus time curve gives the decomposition constant (k) at that particular temperature, surfactant concentration or pH. In the differential form

$$k = \frac{-d(\ln C_t)}{dt} \qquad (5.1)$$

This applies to iso-pH with increasing temperature, isopH isothermal with changing surfactant concentration and isothermal with changing pH conditions. As shown previously(317), the ln C_t data can be fitted to a polynomial function of time:

$$\ln C_t = a_o + a_1 t + a_2 t^2 + a_3 t^3 + \ldots.. + a_n t^n \qquad (5.2)$$

This can then be differentiated at the sampling points to yield the corresponding rate constants.

$$\frac{d(\ln C_t)}{dt} = -k = a_1 + 2a_2 t + 3a_3 t^2 + \ldots + na_n t^{n-1} \qquad (5.3)$$

where a_1, a_2, a_3 .....a_{n-1} are the polynomial coefficients, t is time and n is the order of the polynomial.

5.3 MATERIALS AND METHODS

5.3.1 The Non-Isothermal Study:

The experimental set up is illustrated in Fig. 5.1. The temperature control dial was rotated at a constant speed using the drive

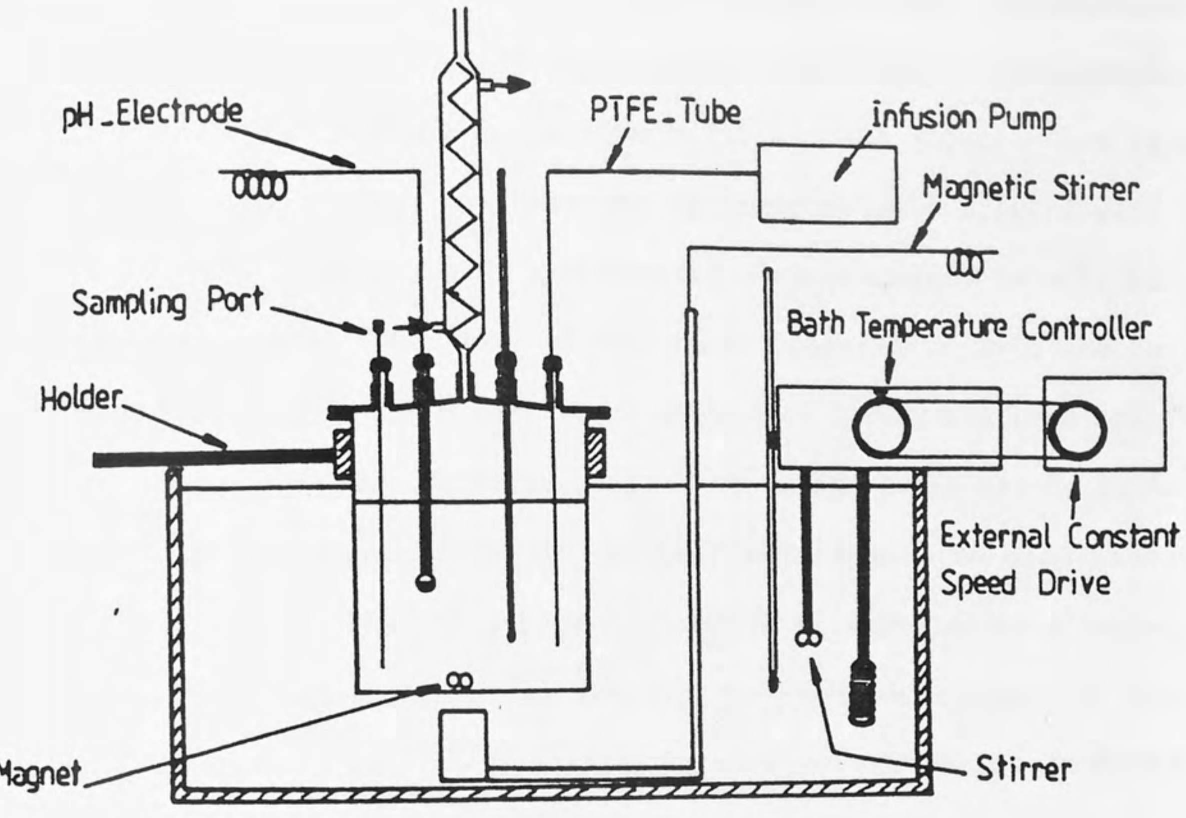


Fig. 5.1 Diagramatic representation of the equipment used for the non-isothermal and non-isopH studies.

mechanism of an infusion pump (Braun-Melsungen). Speeds of rotation were altered to suit the desired extent of decomposition. Indomethacin solution was prepared in Teorell-Stenhagen's buffer pH 9.7 according to the method described earlier in Section 4.3.1 and the solution was kept at 25°C in a water bath. The solution was continuously stirred with a magnetic stirrer. Losses due to evaporation were minimized by attaching a condenser to one of the necks of the glass container. Both the test solution and the bath temperatures were monitored during the runs and it was found that a constant difference of about 1 degree was set up during the run. Only the temperature of the test solutions were used in the calculations. About 10 ml volumes were sampled at appropriate intervals and the reaction was quenched by cooling to room temperature in ice. Time and temperature of the reaction mixture were recorded instantly with each sample. Treatment of samples, preparation of standard solutions and analysis followed the method described earlier in Section 4.3.1.

5.3.2 The Non-IsopH Study:

Indomethacin solution was prepared in Teorell-Stenhagen's buffer pH 7.25 according to the method described earlier in Section 4.3.2 and kept in a water bath at 69°C. For the non-isopH runs, the set up was as described for the non-isothermal work except that instead of driving the temperature control dial, the infusion pump was set up to drive a glass syringe containing one molar sodium hydroxide solution. The rate of delivery was fixed at about 2.11 ml hr^{-1}. Under the conditions of the experiment the total volume change brought about by the base addition did not exceed 1% of the initial volume of test solution and volume

correction was therefore not necessary in the calculation. All pH measurements were done using a Radiometer pHM64 3 decimal place pH meter. About 10 ml volumes were sampled at appropriate intervals and time together with pH of the reaction mixture were recorded instantly. Treatment of samples, preparation of standard solution and analysis followed the method described earlier in 4.3.2.

5.3.3 The Surfactant Non-Isoconcentration Study

Indomethacin solution was prepared in Teorell-Stenhagen's buffer pH 9.7 according to the method described earlier in Section 4.3.4.B and the solution was kept in a water bath at 60°C. For the surfactant non-isoconcentration runs, a Gilson peristaltic pump-minipuls II (Model HP-8, Gilson Co., France) was set up to deliver a 20%w/v of cetyltrimethylammonium bromide in 50%v/v ethanol-buffer pH 9.7 from a 25 ml burette. The rate of delivery was fixed as about 3.95 ml/10 minutes through a 1.5 mm i.d. silescol silicone rubber tube (Esco Rubber Co., England). Five ml volumes were sampled as appropriate intervals and the reaction was quenched by acidification with 1 ml of 0.08 M hydrochloric acid and cooled to room temperature in ice. Time and volume of the surfactant solution added were recorded instantly with each sample. Under the conditions of the experiment, volume corrections were made. Sample treatment and preparation of standard solutions followed the method described earlier in 4.3.4.B. Standards were treated in every respect as with the test solutions. During the reaction runs, the indomethacin content of the solutions were determined by direct ultraviolet spectrophotometry at 320 nm.

5.4 RESULTS AND DISCUSSION

The computer program (NONISO) used for data anlaysis does not require linear increases in temperature or pH with time(317). Typical temperature, pH and surfactant concentration-time profiles used in the present experiments are shown in figures 5.2, 5.3 and 5.4 respectively. The first order hydrolysis of indomethacin was reported by several other workers(84-88) and the effect of temperature on the first order decomposition of indomethacin was shown in Fig. 4.1. The rate constants at various experimental temperatures were determined fitting the time-temperature-concentration data from the non-isothermal set to the program NONISO and are recorded in Table 5.1.

The validity of the non-isothermal approach used in obtaining the hydrolysis rate constant is shown by plotting the isothermal data on the same graph (Fig. 5.5). Both sets of data showed good adherence to the Arrhenius relationship. The Arrhenius parameters were as expected in good agreement with each other and are recorded in Table 5.2.

As previously reported(85) the hydrolysis of indomethacin is base catalysed. The data in Fig. 4.7(a) show that in the alkaline systems used the decomposition was first order throughout the pH range employed. The rate constants at various experimental pHs were determined by analysing the time-pH-concentration data from the non-isopH set with the computer program and are recorded in Table 5.3.

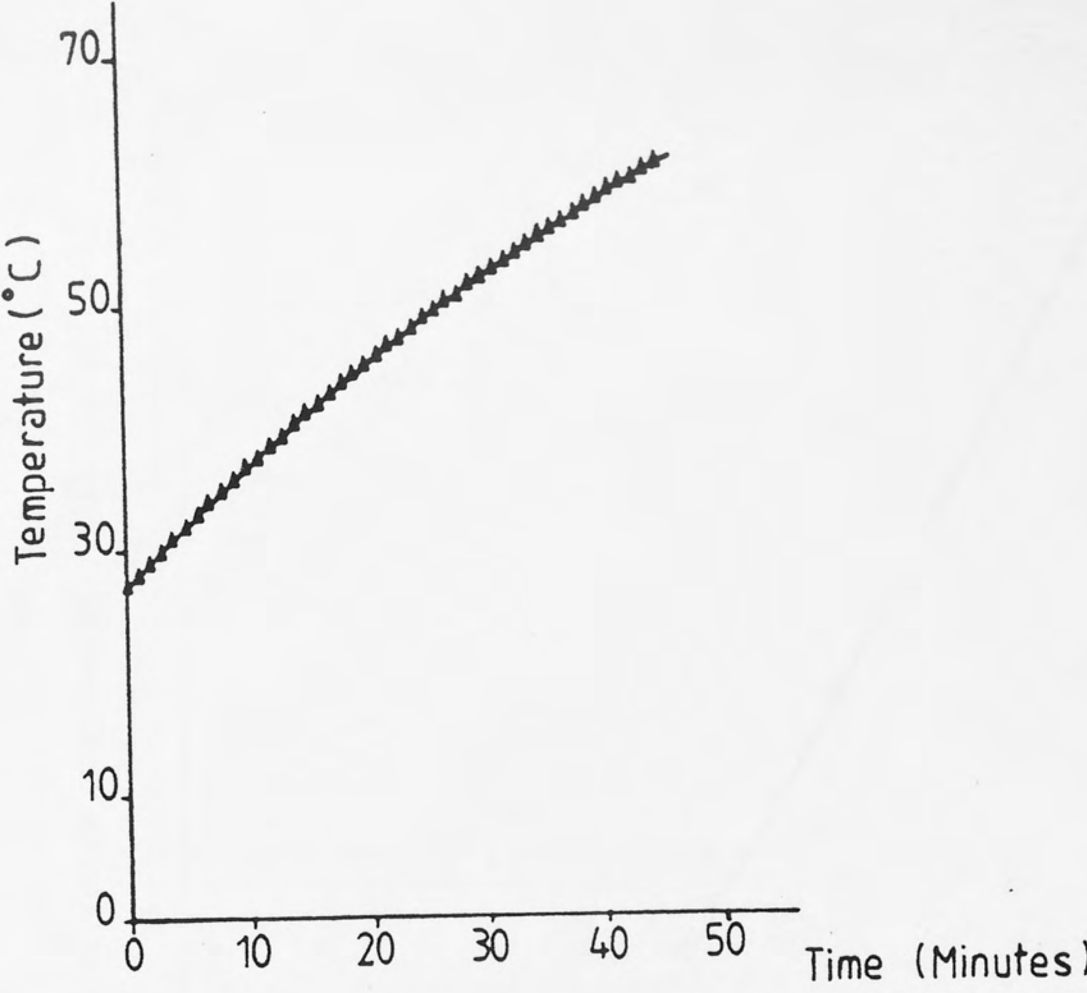


Fig. 5.2 Temperature - time profile for the non-isothermal study.

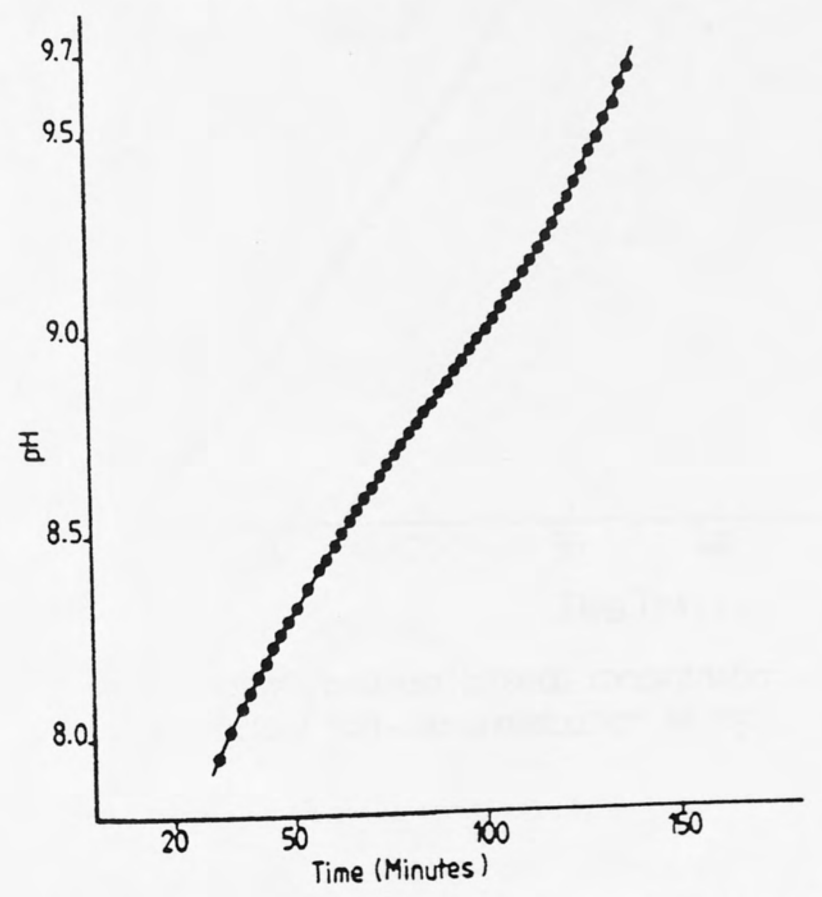


Fig. 5.3 pH - time profile for the non-isopH study.

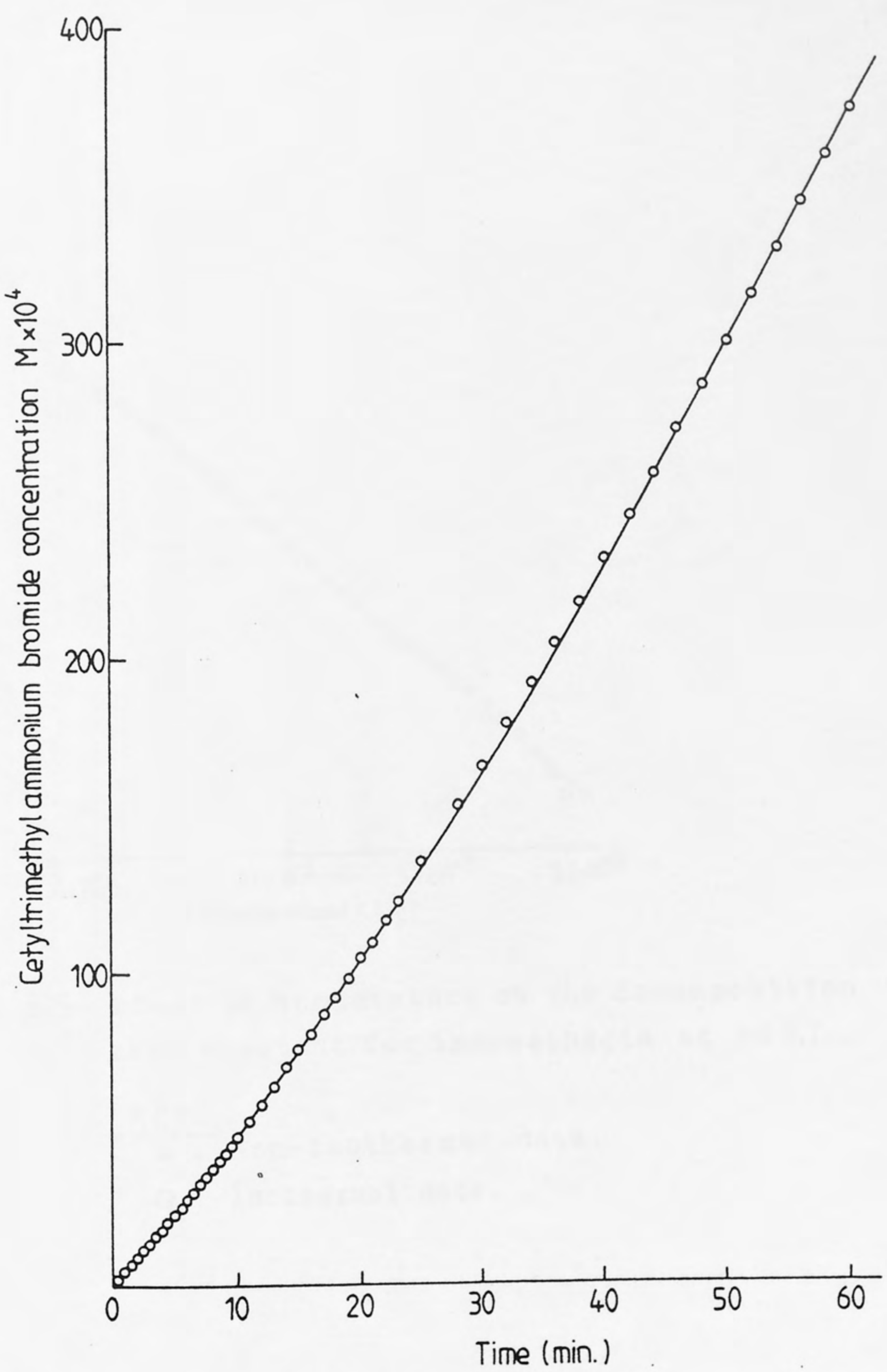


Fig. 5·4 Cetyltrimethylammonium bromide concentration - time profile for the surfactant non-isoconcentration study.

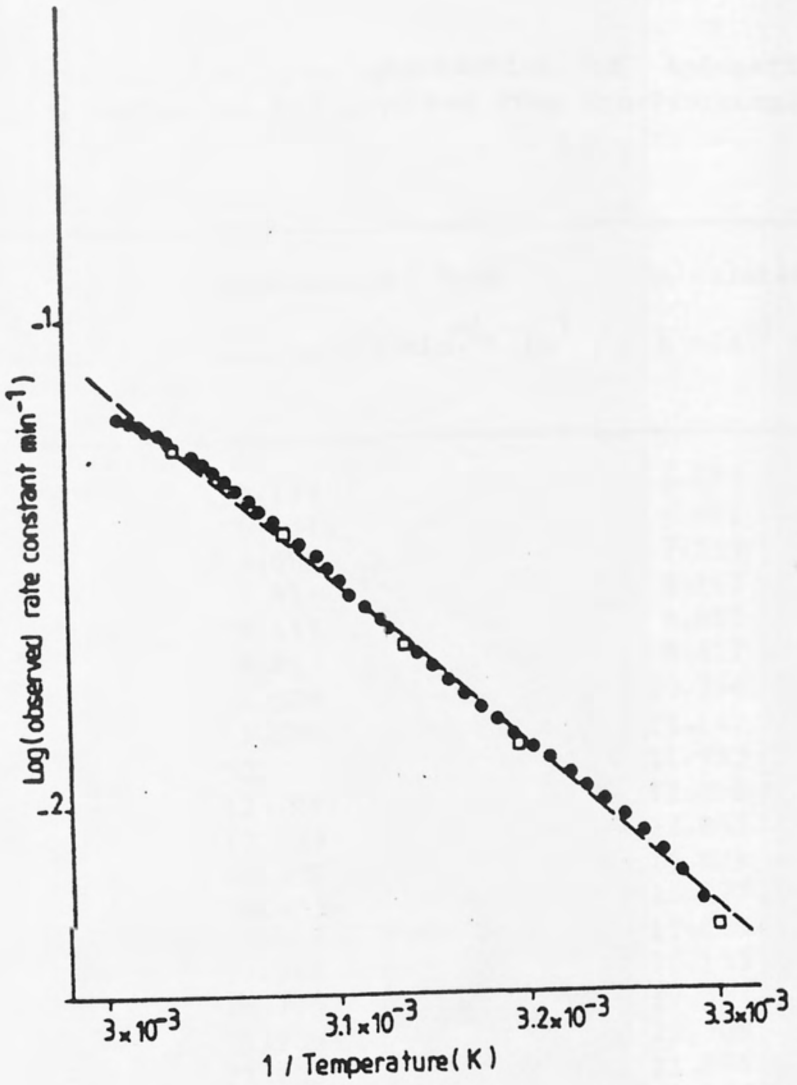


Fig. 5.5 Efect of temperature on the decomposition rate constant for indomethacin at pH 9.7 .

KEY

● : Non-isothermal data.

□ : Isothermal data.

TABLE 5.1

Rate constants for the degradation of indomethacin in Teorell-Stenhagen's buffer pH 9.7 obtained from non-isothermal experiment using NONISO.

Temperature °C	Experimental Rate constant k $min.^{-1} \times 10^3$	Calculated rate constant k $min.^{-1} \times 10^3$
30.8	6.132	6.377
31.8	6.931	6.942
32.8	7.686	7.553
33.7	8.41	8.145
34.7	9.116	8.853
35.7	9.817	9.617
36.6	10.524	10.356
37.5	11.249	11.147
38.4	12	11.993
39.3	12.789	12.898
40.2	13.623	13.865
41.1	14.509	14.899
41.9	15.455	15.877
42.8	16.466	17.048
43.6	17.548	18.155
44.4	18.705	19.327
45.2	19.939	20.569
46	21.253	21.884
46.7	22.649	23.097
47.4	24.125	24.372
48.2	25.682	25.908
49	27.317	27.537
49.6	29.028	28.811
50.2	30.81	30.145
50.9	32.659	31.772
51.7	34.567	33.729
52.3	36.527	35.27
53	38.531	37.148
53.7	40.568	39.118
54.3	42.627	40.882
55	44.697	43.033
55.6	46.763	44.958
56.2	48.811	46.962
56.8	50.823	49.047
57.4	52.783	51.217
58.2	54.671	54.246
58.7	56.467	56.223
59.3	58.15	58.681
59.9	59.695	61.237
60.4	61.079	63.445
61	62.275	66.19
61.6	63.256	69.043

TABLE 5.2

Arrhenius parameters measured from isothermal and non-isothermal degradation of indomethacin in Teorell-Stenhagen's buffer pH 9.7.

Parameters	Values	
	Isothermal	Non-isothermal
E_a ($kJ.mol.^{-1}$)	68.3	65.4
A ($min.^{-1}$)	3.231×10^9	1.115×10^9
r (regression coefficient)	-0.9997	-0.9989

TABLE 5.3

Rate constants for the degradation of indomethacin in Teorell-Stenhagen's buffer at 69°C obtained from non-isopH experiment using NONISO.

pH	Experimental Rate Constant k $min.^{-1} \times 10^3$	Calculated Rate Constant k $min.^{-1} \times 10^3$
7.507	1.184	1.346
7.607	1.798	1.710
7.709	2.358	2.181
7.749	2.594	2.400
7.836	3.118	2.955
7.901	3.575	3.451
7.964	4.098	4.011
8.024	4.696	4.629
8.085	5.373	5.355
8.125	5.870	5.892
8.162	6.405	6.437
8.199	6.977	7.032
8.235	7.588	7.663
8.27	8.236	8.331
8.305	8.924	9.057
8.338	9.651	9.800
8.37	10.417	10.579
8.4	11.224	11.364
8.432	12.072	12.267

TABLE 5.3 continued....

pH	Experimental Rate Constant k $min.^{-1} \times 10^3$	Calculated Rate Constant k $min.^{-1} \times 10^3$
8.461	12.963	13.147
8.492	13.9	14.157
8.521	14.885	15.172
8.548	15.92	16.183
8.577	17.011	17.344
8.605	18.161	18.543
8.631	19.376	19.731
8.657	20.663	20.995
8.636	22.03	22.501
8.715	23.483	24.115
8.741	25.035	25.66
8.769	26.694	27.434
8.793	28.475	29.053
8.82	30.389	30.988
8.847	32.453	33.052
8.872	34.682	35.086
8.898	37.095	37.333
8.925	39.712	39.82
8.952	42.555	42.472
8.977	45.646	45.086
9.009	49.011	48.666
9.037	52.678	52.032
9.065	56.675	55.631
9.099	61.035	60.337
9.125	65.79	64.202
9.157	70.976	69.301
9.188	76.633	74.627
9.222	82.8	80.94
9.252	89.521	86.952
9.288	96.841	94.759
9.324	104.809	103.266

Comparison of the isopH with the non-isopH data (Fig. 5.6) shows that here again, there was close concordance in the results. With its pka of 4.22, over the alkaline pH-range chosen for the non-isopH study, indomethacin is essentially fully ionised. Under such conditions the reaction is base catalysed and is not subject to other catalytic reactions and therefore equation (4.3) reduces to $k_{obs.} = k_6[OH^-]$.

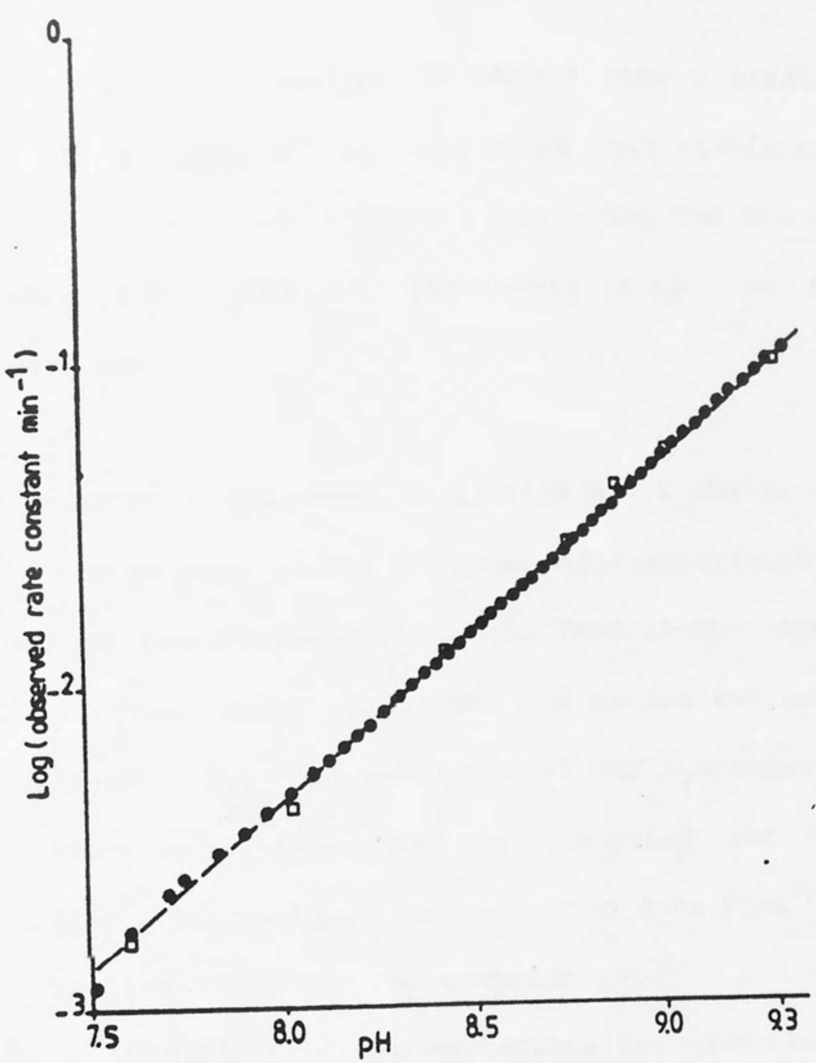


Fig. 5.6 Effect of pH on the decomposition rate constant for indomethacin at 69 °C .

KEY

● : Non-isopH data.

□ : IsopH data.

A plot of log k against pH should give a straight line of slope equal to 1. Analysis of the data shows that within experimental error, this was so. The value of this slope using the non-isopH approach was 1.04 while that obtained previously with the traditional isopH experiments was 1.07.

As previously discussed in Section 4.4.4 the data in Fig. 4.47 and Table 4.14 show that in the presence of cetyltrimethylammonium bromide the decomposition of indomethacin in Teorell-Stenhagen's buffer pH 9.7 at 60°C was first order throughout the entire surfactant concentration range employed. The rate constants at various experimental surfactant concentrations were determined by analysing the time - surfactant concentration - indomethacin concentration data from the surfactant non-isoconcentration set with the computer program and values recorded in Table 5.4. Comparison of the surfactant isoconcentration with the non-isoconcentration data (Fig. 5.7) shows that both sets are in close agreement.

Examining the surfactant non-isoconcentration data in Fig. 5.7 for the alkaline hydrolysis of indomethacin indicates that here again the cationic micelles of cetyltrimethylammonium bromide (CTMB) enhanced the decomposition of indomethacin.

The addition of CTMB to indomethacin solution in the surfactant non-isoconcentration set at 60°C affected the ultraviolet absorption of indomethacin at λ_{max} 320 nm. The absorbance changes of indomethacin at 320 nm as a function of the CTMB concentrations was similar to the

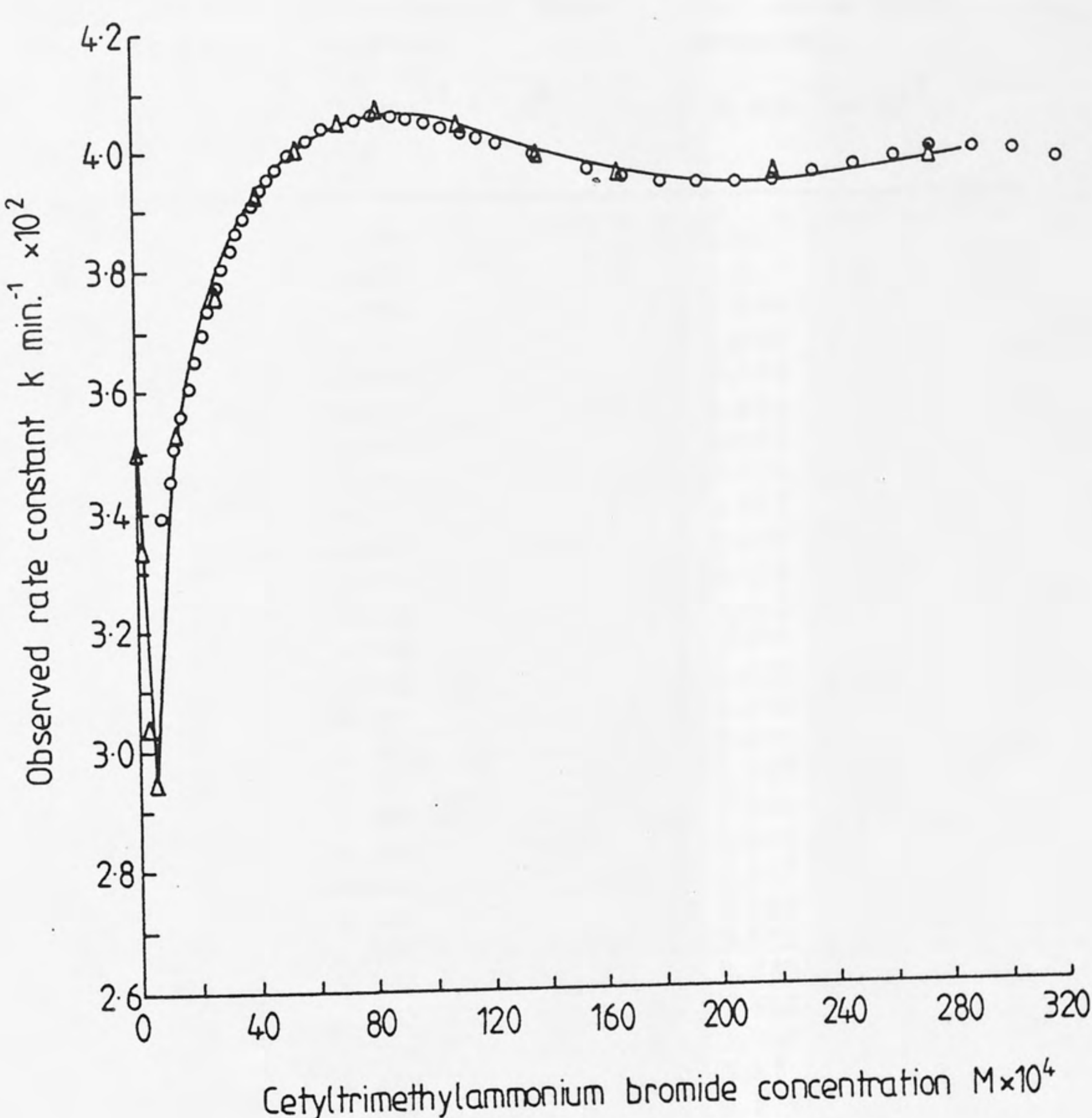


Fig. 5.7 Effect of cetyltrimethylammonium bromide concentration on the decomposition rate constant of indomethacin in Teorell - Stenhagen's buffer pH 9·7 at 60°C.

KEY △ Surfactant isoconcentration data
○ Surfactant non - isoconcentration data

TABLE 5.4

Rate constants for the degradation of indomethacin in Teorell-Stenhagen's buffer pH 9.7 at 60°C obtained from surfactant non-isoconcentration experiment using NONISO.

Cetyltrimethylammonium bromide concentration $M \times 10^3$	Experimental Rate constant $k\ min.^{-1} \times 10^2$	Calculated Rate constant $k\ min.^{-1} \times 10^2$
0.924	3.395	3.88
1.185	3.453	3.867
1.358	3.508	3.858
1.593	3.56	3.844
1.818	3.608	3.824
2.057	3.653	3.835
2.299	3.695	3.805
2.526	3.734	3.808
2.79	3.77	3.807
2.99	3.803	3.803
3.275	3.834	3.826
3.497	3.862	3.823
3.755	3.888	3.825
4.002	3.912	3.825
4.279	3.933	3.826
4.532	3.952	3.825
4.781	3.97	3.827
5.292	3.999	3.831
5.876	4.022	3.832
6.414	4.039	3.837
7.045	4.05	3.843
7.599	4.058	3.851
8.139	4.061	3.853
8.685	4.061	3.862
9.259	4.058	3.867
9.868	4.053	3.87
10.471	4.046	3.876
11.064	4.038	3.88
11.681	4.028	3.887
12.32	4.019	3.89
13.582	3.999	3.89
15.454	3.973	3.909
16.661	3.960	3.916
18	3.953	3.921
19.294	3.95	3.926
20.622	3.953	3.933
21.939	3.961	3.941
23.319	3.972	3.948
24.691	3.985	3.957
26.046	3.998	3.97
27.478	4.009	3.98
28.897	4.014	3.993
30.331	4.009	4.009
31.802	3.992	3.992

effect observed earlier in Fig. 4.55. Increasing the surfactant concentration reduced the absorbance value of indomethacin until a maximum effect was obtained at a surfactant concentration of 4.88×10^{-4} M. This occurred after a period of some 1.5 minutes. Absorbance changes after this time were due solely to the degradation of indomethacin. The early time points ($t<1.5$ minutes) were not used in the calculation of rate constants. The initial effect of the surfactant on ultraviolet absorption prevented the observation of the early inhibition of the alkaline hydrolysis of indomethacin at 60°C shown earlier in Fig. 4.48 in the presence of CTMB. The value of 4.88×10^{-4} M obtained for the cmc of CTMB in the surfactant non-isoconcentration set is closely in agreement with that of 5.48×10^{-4}M obtained in the surfactant isoconcentration set. Goddard and Benson reported earlier that the cmc value of cationic surfactants increases with temperature(318) and this may explain the value obtained for the cmc of CTMB at 60°C. With an initial indomethacin concentration of 20.4×10^{-5}M, the cmc of CTMB at 4.88×10^{-4}M resulted in solubilizing 15.5% of indomethacin.

The indomethacin data reported here reaffirms(317,319-327) the usefulness of the non-isothermal approach to obtaining stability data for hydrolabile drugs. More importantly, the data show that the same approach can be used for monitoring the effect of pH or surfactant concentration changes on the decomposition rates. In using such an approach, however, several theoretical and/or practical limitations have to be borne in mind. The most important is that changes in temperature, pH or surfactant concentration can alter the decomposition pathways and can lead to erroneous results in estimating product shelf-lives. Using

the isothermal, surfactant isoconcentration and isopH approaches, deviations can be rapidly detected from plots such as those shown in Fig. 4.1, 4.7a and 4.47. With non-isopH, surfactant non-isoconcentration and non-isothermal work, the absence of such primary curves makes this system more fallible in this respect and it is therefore essential that as a pre-requisite to using such systems, the decomposition pathways of the compound under study should be fully understood.

Practically, several limitations became obvious during the work. Some of these limitations are specific to the compound under study. Indomethacin is poorly soluble in the unionised form. Figure 3.1 gives its pH-solubility profile. During the pH programming in the non-isopH work it is important to ensure that solubility is not exceeded at any point. Ideally, at low pH values the experiment should be initially with a solution whose pH is then adjusted upwards. At low pH values limitations in solubility lead to problems in assay and separate runs are required if wider pH ranges are required. The wide variation in rate constants with pH also means that in practice a pH change programme such as that shown in Fig. 5.3 is unsuitable, when it is necessary to follow the changes over a wide pH range. Assay precision becomes a problem in the region where the compound is most stable. At the extremes of pH values, volume corrections are necessary as the buffer capacities of the systems increase. Despite these limitations, it is clear that in the stability screening of hydrolabile drugs such as indomethacin, the non-isopH and the surfactant non-isoconcentration approaches offer a useful addition to the techniques available to the product formulator.

CHAPTER 6

METABOLIC TRANSFORMATION OF IBUPROFEN ESTERS

6.1 INTRODUCTION

The principal aim of using a prodrug is to improve the theraputic effects of the parent compound by conversion of the prodrug into the required medicinal species at either the site of administration or elsewhere within the body.

The liver is well known as a major site for drug metabolism. But the lung and the skin also possess a range of metabolic activities(223,328-332). The skin also serves as a reservoir for steroids and an important site for their metabolism and biosynthesis(333-335).

The skin contains a number of enzymes and can carry out most of the metabolic drug biotransformation of the liver including oxidation, hydroxylation, reduction, hydrolysis, deamination, dealkylation and conjugation to sulphate and glucuronide(336,337). Reports have shown that most of the enzymic activity of the skin is localised in the epidermal layer(338) and the skin contains large amount of esterases which promote the hydrolysis of a number of esters(338-340). The differences in the topical potency of the parent drug and its derivatives may be explained on the basis of the penetration properties of each compound and on the degree of susceptibility to metabolic inactivation in the skin. The design of topically applied drugs and their delivery systems can be assisted by a good understanding of how

such enzymes affect the activity of these drugs. Esterification has been shown to be efficient in modulating the topical activity and transport properties for a number of medicinal compounds(341,342). Therefore, the susceptibility of ibuprofen esters to esterase was investigated in this study by producing full kinetic profiles in order to rationalise any differences in their topical availability.

6.2 MATERIALS AND METHODS:

6.2.1 Materials

A. Hog Liver esterase:

The hog liver esterase (Sigma Chemicals) used in the metabolic studies was a carboxylic ester hydrolase obtained from hog liver and suspended in 3.2 M ammonium sulphate solution adjusted to pH 8. The claimed activity was that 1 unit hydrolyzed 1 μl of ethyl butyrate to butyric acid and ethanol per minute at pH 8 and 25°C. Each mg of protein was equivalent to 160 units and each ml of solution contained 10.9 mg of protein. The term esterase is used throughout the text for this preparation.

B. Diluted enzyme solution:

The original solution (0.92 ml/vial or 1600 units) was diluted to 20 ml to give 80 units/ml and from this solution a series of solutions were prepared to contain 64, 48, 32 and 8 units/ml by the addition of appropriate amounts of 3.2 M ammonium sulphate solution.

C. Tris buffer solution:

Each litre of pH 8.09 buffer solution contained 6.05 g of tris(hydroxymethyl)aminomethane and 279 ml of 0.1 M hydrochloric acid solution in water.

D. Tris buffer solution, concentrated:

To maintain constant ionic strength a buffer solution with a salt concentration ten times that required in the final solution was made. Each litre of this buffer contained 60.575 g of tri(hydroxymethyl)-aminomethane and 27.9 ml of 1 M hydrochloric acid solution in water, pH 8.094.

E. Acidified acetonitrile solution:

This was prepared to contian 50%v/v acetonitrile in 0.024 M aqueous hydrochloric acid solution.

F. Cutaneous esterase:

Cutaneous esterase was obtained from rat skin. The abdominal areas of freshly killed rats were mechanically depilated and skinned. The skin pieces were weighed (about 15-16 g), cut into tiny pieces and suspended in 20 ml of iced Tris buffer solution of pH 8.094. The mixture was twice homogenised by passing through a french press (Aminco) pre-cooled to -4°C before use in order to minimize heat induced denaturation of the cutaneous enzymes during processing. The specimens were kept in an iced bath whenever possible to minimize loss of enzyme

activity. The homogenates were diluted with cooled buffer at about 4°C and centrifuged at 1200 rpm at 4°C for 1 hour. The supernatant was filtered through 1.2 μm Millipore-MF membrane filter (Millipore Corp., USA) and diluted to 200 ml with buffer and stored at -15°C until used.

G. Internal standard solutions:

Hexachlorophane dissolved in acidified acetonitrile solution was used as internal standard for assaying ibuprofen and its esters. The internal standard concentrations used were as follows:

Ibuprofen ester	Initial Concentration of Ibuprofen ester μM	The Concentration of the internal standard solution μg/ml
(tetrahydro-2-furanyl)-methyl ester	76.2	5.0
	62.95	5.0
	47.93	2.5
	34.6	2.5
	17.5	2.5
(tetrahydro-2-(2H)pyranyl)-methyl ester	48	5.0
cyclohexylmethyl ester	52.2	2.5

6.2.2 Test Solutions and Procedure:

A. Enzymic hydrolysis by esterase:

1. The enzymic hydrolyses of the (tetrahydro-2-furanyl)methyl- and

the (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen were carried out at 37°C in solutions containing 20%v/v propylene glycol and 80% pH 8.09 Tris buffer solution. The final aqueous glycol buffer had a pH Of 8.14. The propylene glycol was necessary because of the poor aqueous solubility of the esters. At this relatively high pH, the enzymic activity is optimum but non-enzymic hydrolysis can take place too. To overcome this problem during the preparation of the ester solutions, the esters were predissolved in propylene glycol of appropriate concentration such that the final concentration after mixing with Tris buffer solutions were as required. 5.1 millilitres of concentrated Tris buffer solution were introduced into a 100 ml flask together with the necessary volumes of water to produce 40.8 ml. The solution obtained was then heated in a water bath at 37°C. When this temperature was reached, 10.2 ml of the ester-propylene glycol solution were added with rapid stirring. Two 1 ml aliquots were taken immediately as the initial samples in all cases, to leave 49 ml of solution. One ml of the enzyme solution was introduced and the resulting solution thoroughly mixed with the timer being started simultaneously. Adjustment was made for the 1 ml volume addition to the test solution. One ml aliquots were taken at appropriate time intervals. One ml of the internal standard solution of appropriate concentration (see section under title internal standard solution), and 1 ml of acidified acetonitrile solution was added to quench the enzymic reaction. From preliminary studies it was found that addition of these solutions stopped the enzymic hydrolysis and the mixed solutions were stable for at least 1 week at 4°C. Controls were carried out without the enzymes. Since the enzyme solution contained ammonium sulphate,

experiments with 1 ml of 3.2 M ammonium sulphate added instead of 1 ml of enzyme solution were undertaken for comparison. The concentration of ibuprofen esters, the reaction conditions and the composition of the test solutions were as follows:

Ibuprofen ester	Initial Concentration (μM)	Incubation Medium	Enzyme Concentration	
			Units %	Strength[a] × Volume[b] (unit/ml) (ml)
(tetrahydro-2-(2H)-pyranyl)methyl ester	48	20 % propylene glycol-Tris buffer mixture	160	80 × 1
	48		128	64 × 1
	48		96	48 × 1
	48		64	32 × 1
	48		16	8 × 1
(tetrahydro-2-furanyl)methyl ester	76.2		160	80 × 1
	76.2		128	64 × 1
	76.2		96	48 × 1
	76.2		64	32 × 1
	76.2		16	8 × 1
	62.95		16	8 × 1
	47.93		16	8 × 1
	34.6		16	8 × 1
	17.5		16	8 × 1

(a) Strength: Is the concentration of the diluted enzyme solution (units/ml)

(b) Volume: The volume used is for each 50 ml sample solution.

2. The effect of propylene glycol concentration on the enzymic hydrolysis of the (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl esters of ibuprofen by 96 units %v/v of esterase was investigated in propylene glycol-Tris buffer mixtures, pH 8.1 at 37°C. The enzymic hydrolysis of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)-methyl esters were followed in the presence of 20, 30, 40, 50 and 70%v/v propylene glycol-Tris buffer mixtures while the hydrolysis of the cyclohexylmethyl ester was followed in the presence of 50, 55, 60 and 70%v/v propylene glycol-Tris buffer mixtures. To overcome the problem of non-enzymic hydrolysis, ibuprofen esters were predissolved in propylene glycol. The reaction mixtures were prepared by introducing 5.1 ml of concentrated Tris buffer solution into each of a series of 100 ml flasks. Volumes of propylene glycol, each 10.2 ml short of those necessary to produce the predetermined final propylene glycol concentration, were added to the flasks together with the necessary volume of water to produce 40.8 ml. The solution thus obtained was warmed in a water bath at 37°C. When this temperature was reached, 10.2 ml of the ester solution in propylene glycol was added to the containers with rapid stirring. Two 1 ml aliquots were taken immediately as the initial samples in all cases to leave 49 ml of solution. One ml of 48 units/ml of the diluted enzyme solution was introduced into each of the 100 ml containers and the reaction was followed using the method described earlier. The initial concentration of ibuprofen esters in the test solution used for studying the effect of propylene glycol on the enzymic hydrolysis by 96 units %v/v of esterase were as follows:

Ibuprofen ester	% propylene glycol in the reaction mixture	Initial concentrations μM
(tetrahydro-2-furanyl)methyl-ester	20, 30, 40, 50 and 70	76.2
(tetrahydro-2-(2H)pyranyl)-methyl ester	20, 30, 40, 50 and 70	48
cyclohexylmethyl ester	50, 55, 60 and 70	52.2

A control experiment for the hydrolysis of the cyclohexylmethyl ester in 50%v/v propylene glycol-Tris buffer at 37°C was carried out without the enzyme.

B. The Enzymic Degradation of Ibuprofen Esters by Cutaneous Esterases

The enzymic degradation of the (tetrahydro-2-furanyl)methyl- and the (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen by rat Skin homogenate was followed in 20 and 50%v/v propylene glycol-Tris mixture pH 8.1 at 37°C while the hydrolysis of the cyclohexylmethyl ester was followed in 50% propylene glycol-Tris buffer mixture due to the lower aqueous solubility of this ester. The reaction mixture was prepared by introducing 10 ml of the rat skin homogenate into a series of 100 ml flasks. Four ml of concentrated Tris buffer solution was added and

volumes of propylene glycol, each 2 ml short of those necessary to produce the predetermined final propylene glycol concentration, were added to the flasks together with the necessary volumes of water to produce 48 ml. The solutions obtained were then warmed in a water bath at 37°C. When this temperature was reached, the metabolic reactions were initiated by the introduction of 2 ml ester solution in propylene glycol to each mixture with rapid stirring. One ml aliquots were taken at appropriate time intervals. One ml of the internal standard solution of the appropriate concentration (see section under internal standard solutions) and 1 ml of acidified acetonitrile solution was added to quench the enzymic reaction before HPLC anlaysis.

The concentration of ibuprofen esters in the stock solutions and initial concentration in the test solutions were as follows:

Ibuprofen ester	Ester Concentration	
	Stock solution mM	Initial concentration in the test solution μM
(tetrahydro-2-furanyl)methyl-ester	1.905	76.2
(tetrahydro-2-(2H)pyranyl)-methyl ester	1.200	48
cyclohexylmethyl ester	1.305	52.2

6.2.3 Standard solutions

It is important that the solvent of each standard solution was the same as that of each test solution in respect to the propylene glycol and Tris buffer composition. The standard solutions were treated in every respect as with the test solutions. The concentration range and the compounds contained in the standard solutions were as follows:

Ibuprofen ester and initial concentration (μM)	Compounds contained in standard solutions and concentration range(μM)	Hexachlorophane (internal standard) (μg/ml)
(tetrahydro-2-furanyl)-methyl ester	(tetrahydro-2-furanyl)-methyl ester and ibuprofen	
76.2	16 - 80	5
62.95	13 - 65	5
47.93	10 - 50	2.5
34.6	72 - 36	2.5
17.5	4 - 20	2.5
(tetrahydro-2-(2H)-pyranyl)methyl ester	(tetrahydro-2-(2H)-pyranyl)methyl ester and ibuprofen	
48	9.6 - 48	5
cyclohexylmethyl ester	cyclohexylmethyl ester and ibuprofen	
52.2	10.44 - 52.2	2.5

6.2.4 Assay of ibuprofen and its esters:

A reversed-phase high-performance liquid chromatography (HPLC) system was used for assaying ibuprofen and its esters. The equipment

used was as previously described in section 2.1(c). The mobile phase consisted of 81%v/v methanol in water acidified with 0.3%v/v orthophospheric acid and was delivered at 1.0 ml/min. Separations were achieved using a 10 cm × 4.6 mm i.d. Hypersil-ODS 5 μm (Shandon, U.K.) Column. Peak detection was at 220 nm and at sensitivity setting ranging from 0.01 - 0.04 AUFS.

6.3 RESULTS AND DISCUSSION

The enzymic biotransformation profiles of the (tetrahydro-2(2H)-pyranyl)methyl-, (tetrahydro-2-furanyl)methyl- and cyclohexylmethyl-esters of ibuprofen were investigated using a pure carboxylic ester hydrolase extracted from hog liver as a model enzyme since a purified cutaneous esterase was not available. This enzyme was chosen because of its low specificity and because it is commonly found in animal tissues. The medium used in this study was buffered to pH 8 with Tris buffer solution as this corresponds to the optimal value for this enzyme.

Propylene glycol was incorporated to increase the solubility of ibuprofen esters. Propylene glycol was chosen as cosolvent because it is commonly used in topical formulations.

The enzymic and base-catalysed reactions were quenched by the addition of an acidic solvent such that the final pH was 2-4. Under these conditions the test solutions could be kept for a week at 4°C, but the samples were analysed within three days. The esterase initiates hydrolysis of the ester bond and liberates ibuprofen and both the disappearance of the parent ester and the formation of ibuprofen were

monitored.

Figures 6.1 and 6.2 show the concentration-time profiles for the hydrolysis of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)-pyranyl)methyl- esters of ibuprofen respectively when incubated in 20% propylene glycol-Tris buffer pH 8.14 at 37°C in the absence of esterase. Semilogarithmic plots of these profiles showed that the rates of disappearance of both ibuprofen esters were first order (Figs. 6.3 and 6.4) and the respective rate constants were determined and recorded in Table 6.1. The rate constants for the formation of ibuprofen were also measured and are similarly recorded.

TABLE 6.1

The degradation of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen in 20% propylene glycol-Tris buffer pH 8.14 at 37°C.

Ibuprofen ester	rate constant of ester hydrolysis k $hr.^{-1} \times 10^4$ (r=regression anlaysis)	rate constant of ibuprofen formation k $hr.^{-1} \times 10^4$
(tetrahydro-2-furanyl)-methyl ester	4.07 (r = 0.9998)	4.1
(tetrahydro-2-(2H)-pyranyl)methyl ester	4.56 (r = 0.9995)	4.5

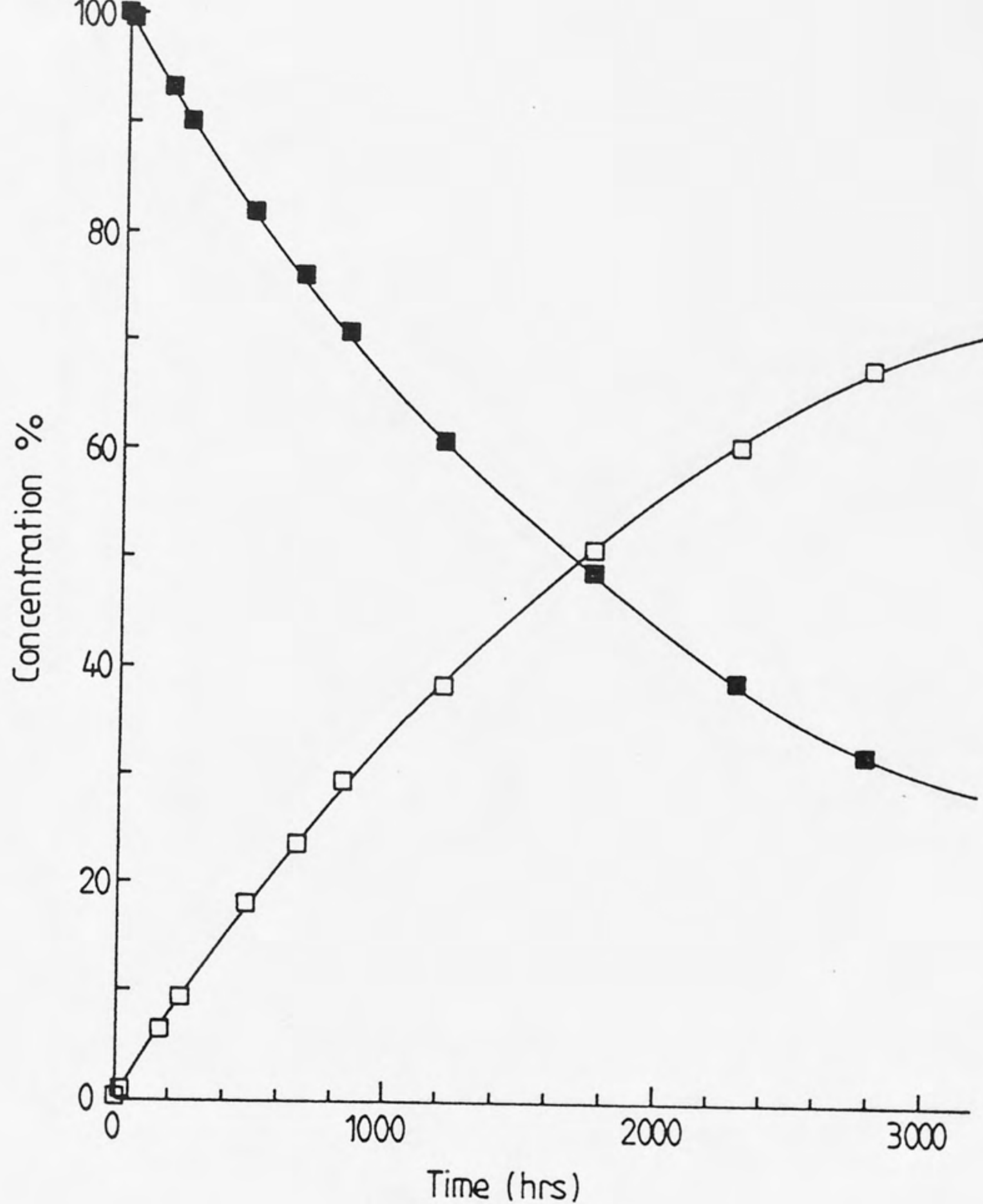


Fig. 6.1 Concentration - time profile for the hydrolysis of (tetrahydro - 2 - furanyl) methyl ester of ibuprofen in 20% propylene glycol - Tris buffer pH 8·14 at 37°C.

KEY ■ (tetrahydro - 2 - furanyl) methyl ester of ibuprofen remaining
□ ibuprofen formed

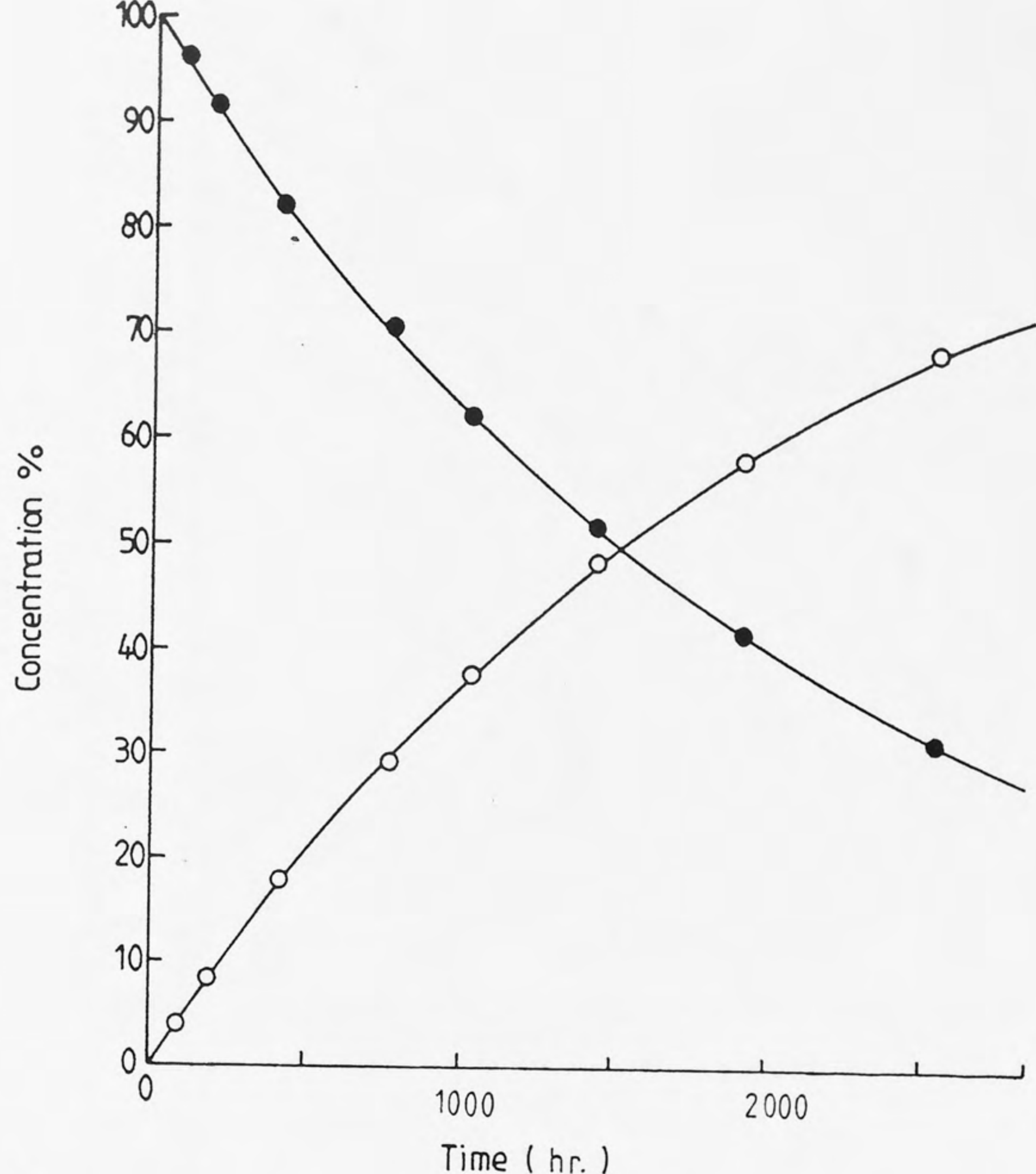


Fig. 6.2 Concentration - time profile for the hydrolysis of (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen in 20% propylene glycol - Tris buffer pH 8·14 at 37°C

KEY

● (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen remaining.

○ ibuprofen formed

Fig. 6.3 First order plot for the hydrolysis of (tetrahydro-2-furanyl) methyl ester of ibuprofen in 20% propylene glycol -Tris buffer pH 8·14 at 37°C.

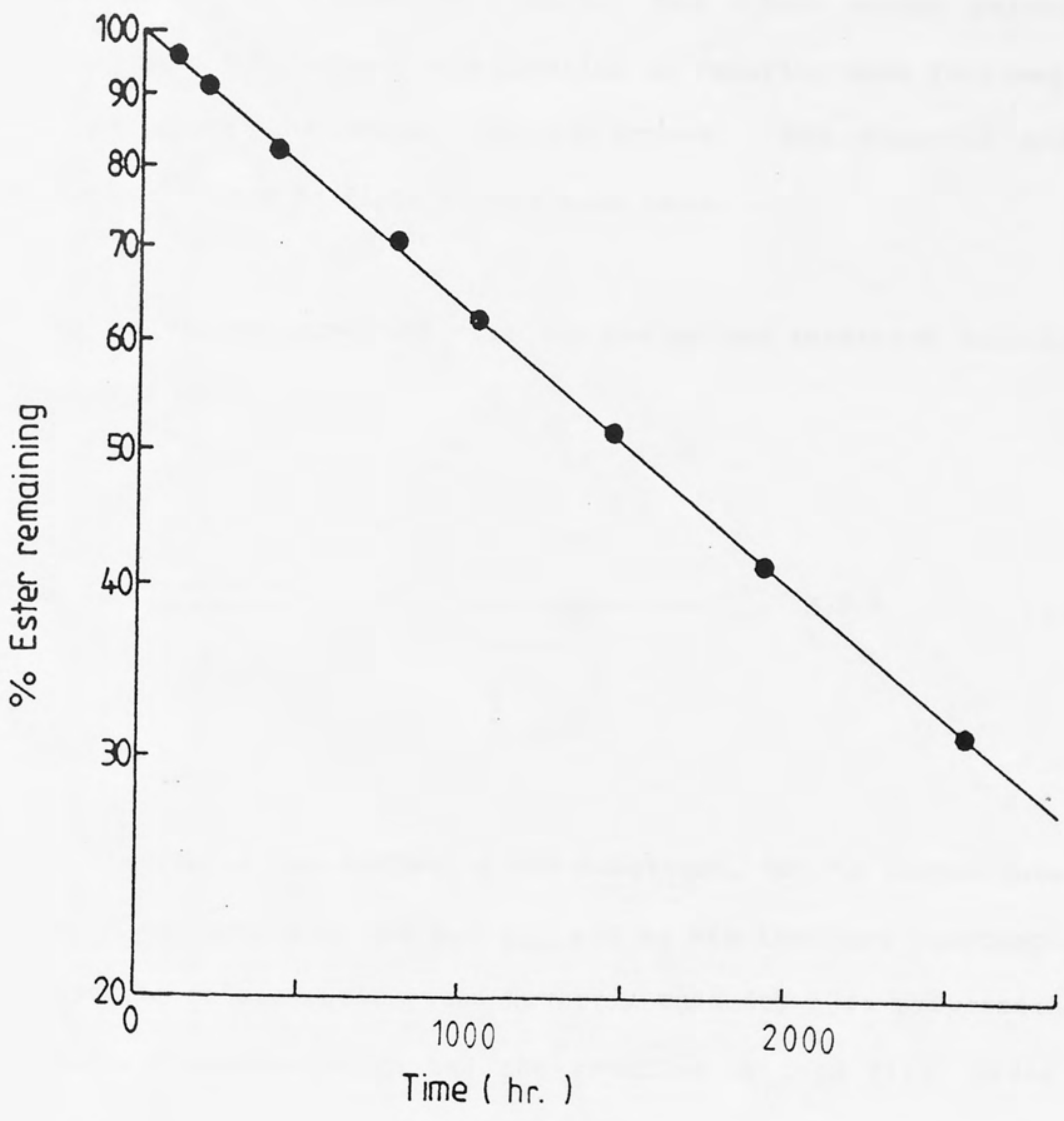


Fig. 6.4 First order plot for the hydrolysis of (tetrahydro - 2 - (2H) pyranyl) methyl ester of ibuprofen in 20% propylene glycol -Tris buffer pH 8·14 at 37°C

In the study of enzyme kinetics, the substrate concentration is usually chosen to be in excess such that the enzyme is saturated and the order of reaction is zero. In this study, the objective was to observe the intrinsic enzymic hydrolysis rates of the drugs rather than the characteristics of the enzyme itself. The enzyme merely served as a model esterase. Therefore, the kinetics of reaction were followed under conditions where the enzyme was in excess. The observed order of reaction would then be first rather than zero.

For an enzyme-catalysed reaction the enzyme substrate relation can be expressed by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (6.1)$$

where E represents the enzyme, S the substrate, ES the enzyme-substrate complex, P the products and k_1, k_{-1} and k_2 are the rate constants. At low substrate concentration, the reaction velocity v is proportional to the substrate concentration and the reaction is thus first order with respect to the substrate. But at high substrate concentration, the rate becomes constant and independent of the substrate concentration. The enzyme is saturated with its substrate and the reaction becomes zero order with respect to the substrate. Therefore, a distinctive feature of enzyme catalysed reaction is the phenomenon of saturation with substrate. The constant steady-state velocity v is given by the Michaelis-Menten equation(343,344):

$$v = \frac{V}{1 + \frac{K_m}{[S]}} \qquad (6.2)$$

where V is the maximum velocity obtained at high substrate concentration or it is the velocity constant for the break down of the enzyme-substrate complex, [S] is the concentration of the free substrate and K_m a quantity termed the 'Michaelis constant' which is the equilibrium dissociation constant for the reaction. Although K_m is independent of [S] and [E] it usually changes with pH, temperature and substrate concentrations. Equation (6.2) can be rewritten as

$$\frac{1}{v} = \frac{1}{V} + \frac{K_m}{V} \cdot \frac{1}{[S]} \qquad (6.3)$$

The initial velocities for the enzymic hydrolysis of a series of solutions containing different initial concentrations of the (tetrahydro-2-furanyl)methyl ester of ibuprofen in 20% propylene glycol-Tris buffer pH 8.13 at 37°C were obtained in the presence of 16 units %v/v of esterase. Examination of the data showed that increasing the initial concentration of ibuprofen ester for the given concentration of esterase resulted in increasing the initial velocity of degradation (Table 6.2). The data were linearized using the lineweaver-Burke method(345-347) by plotting $\frac{1}{v}$ against $\frac{1}{A}$ as shown in Fig. 6.5. The linear relationship (r = 0.9998) presented in Fig. 6.5 has an intercept equivalent to $\frac{1}{V}$ and a positive slope ($\frac{K_m}{V}$) of value equal to 31.242 min. This indicates that the substrate concentration was well below the enzyme capacity and the degradation process should therefore approximate

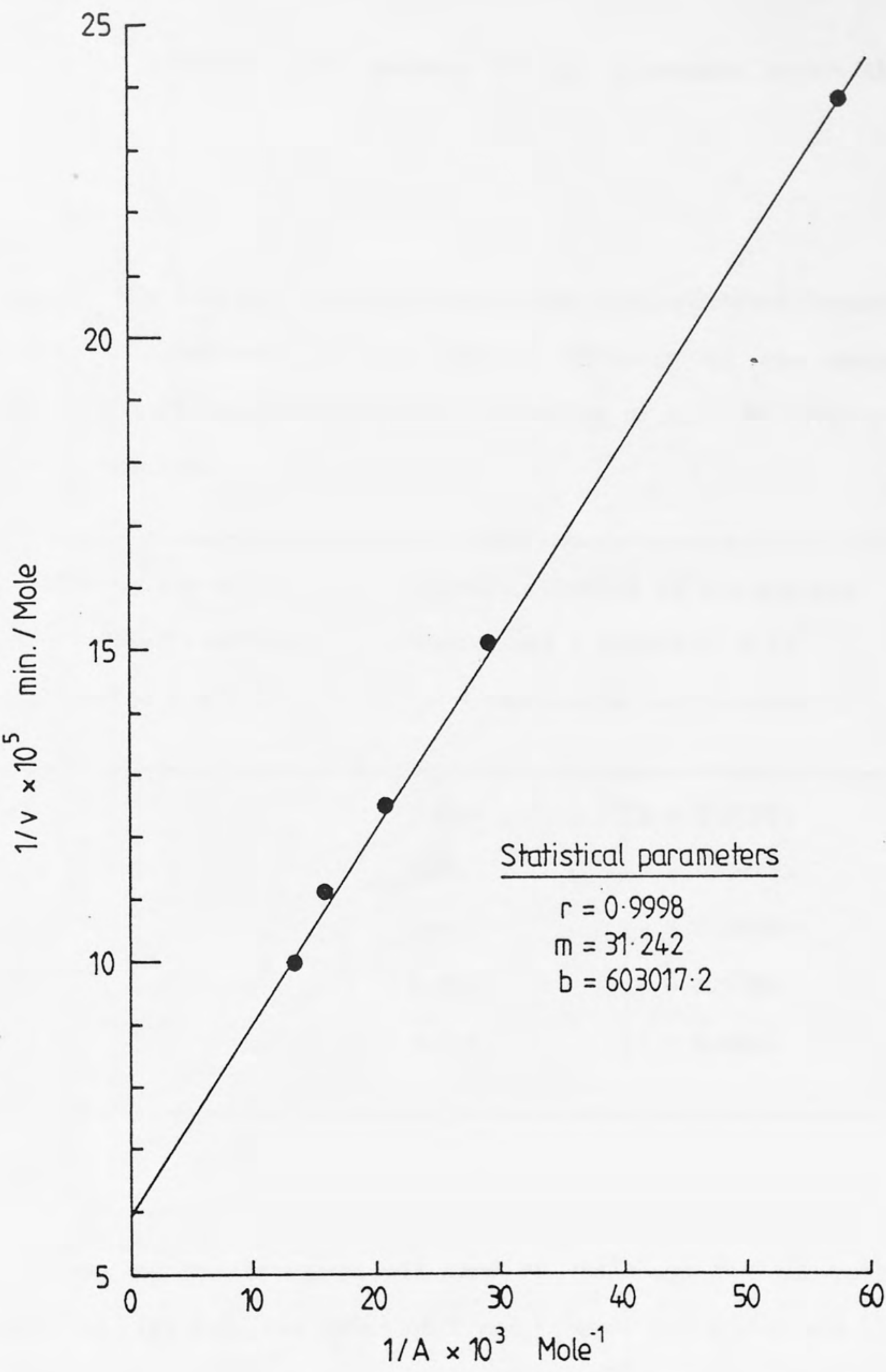


Fig. 6.5 Plot of 1/v versus 1/A for the enzymic hydrolysis of the (tetrahydro-2-furanyl) methyl ester of ibuprofen by 16 Units% esterase in 20% propylene glycol-Tris buffer pH 8·14 at 37°C.

to first order kinetics with respect to the substrate under these conditions.

TABLE 6.2

The effect of the initial concentration of the (tetrahydro-2-furanyl)-methyl ester of ibuprofen on the initial velocity of the enzymic hydrolysis in 20%v/v propylene glycol-Tris buffer pH 8.14 at 37°C by 16 units %v/v of esterase.

Initial concentration of the (tetrahydro-2-furanyl)methyl-ester of ibuprofen A M $\times 10^5$	Initial velocity of the enzymic hydrolysis v mole/min. $\times 10^6$ (r = regression coefficient)	
7.62	1.025	(r = 0.9999)
6.295	0.9	(r = 0.9995)
4.79	0.8	(r = 0.9998)
3.46	0.66	(r = 0.999)
1.75	0.419	(r = 0.999)

From the values of the intercept and that of the slope for the linear relationship in Fig. 6.5, the value of V and K_m were calculated and they are equal to 1.65×10^{-6} mole/min. and 5.154×10^{-5} mole respectively.

The enzymic hydrolysis of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl- esters of ibuprofen was monitored in the presence of different concentrations of esterase in 20%v/v propylene glycol-Tris buffer pH 8.14 at 37°C as shown in Figs. 6.6 and 6.7

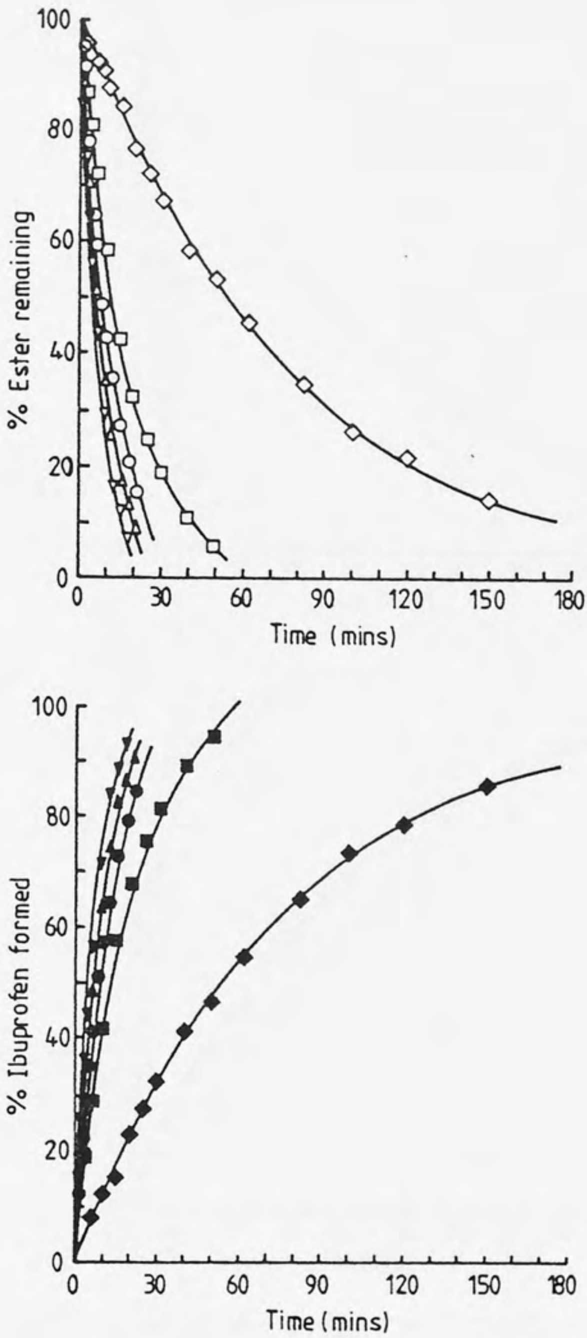


Fig. 6.6 Concentration–time profiles for the enzymic hydrolysis of (tetrahydro-2-furanyl) methyl ester of ibuprofen by esterase in 20% propylene glycol-Tris buffer pH 8·14 at 37° C

KEY

(tetrahydro-2-furanyl) methyl ester of ibuprofen remaining	Ibuprofen formed	esterase concentration (units %)
▽	▼	160
△	▲	128
○	●	96
□	■	64
◇	◆	16

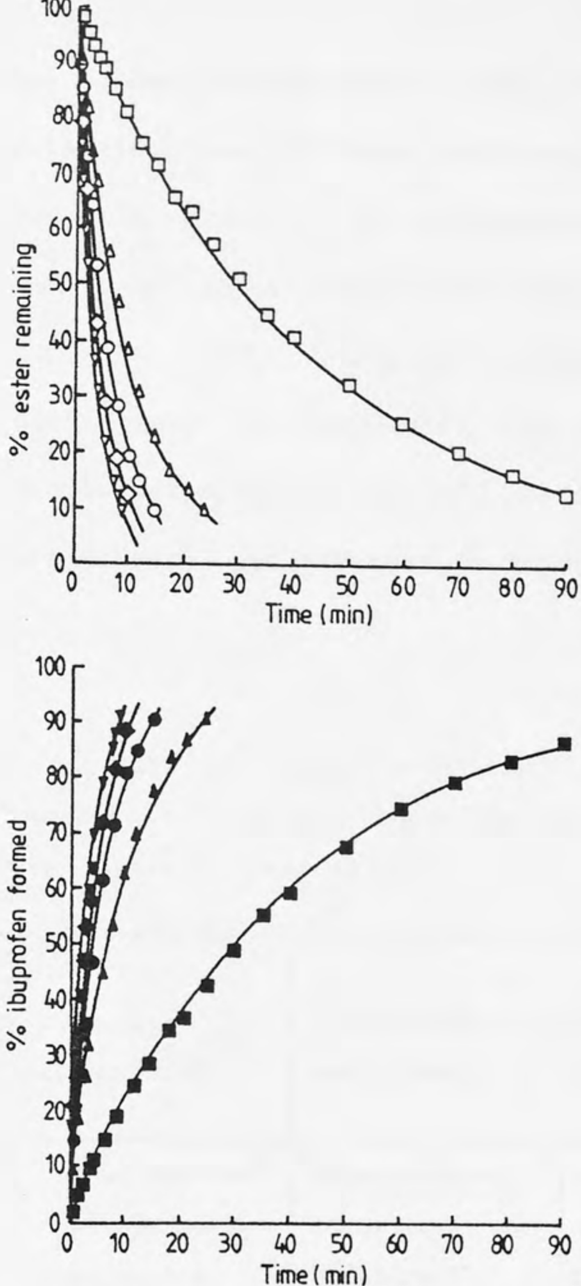


Fig. 6.7 Concentration-time profiles for the enzymic hydrolysis of (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen by esterase in 20% propylene glycol-Tris buffer pH 8·14 at 37°C

KEY (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen remaining	ibuprofen formed	esterase concentration (units %)
▽	▼	164
◇	◆	128
○	●	96
△	▲	64
□	■	16

respectively. The esterase concentrations were 16, 64, 96 and 128 and 160 units %v/v. Semilogarithmic plots of these profiles gave straight lines which indicate that the kinetics of disappearance of both ibuprofen esters were first order under these conditions as shown in Figs. 6.8 and 6.9 respectively. This would again suggest that the enzyme capacity was not saturated. The respective rate constants for the disappearance of both ibuprofen esters as well as those for the appearance of ibuprofen were measured and recorded in Table 6.3.

TABLE 6.3

The enzymic hydrolysis of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen by esterase in 20%v/v propylene glycol-Tris buffer pH 8.14 at 37°C.

Estrase concentration units %v/v	(tetrahydro-2-furanyl)-methyl ester of ibuprofen		(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	
	rate constant of ester hydrolysis $k\ min.^{-1} \times 10^2$ (r=regression coefficent)	rate constant of ibuprofen formation $k\ min.^{-1} \times 10^2$	rate constant of ester hydrolysis $k\ min.^{-1} \times 10^2$ (r=regression coefficient)	rate constant of ibuprofen formation $k\ min.^{-1} \times 10^2$
16	1.28(r=0.9996)	1.27	2.25(r=0.9999)	2.2
64	5.6 (r=0.9997)	5.5	9.79(r=0.9996)	9.8
96	8.73(r=0.9995)	8.7	15.66(r=0.9998)	15.7
128	11.33(r=0.9996)	11.2	21.03(r=0.9997)	21.08
160	14.42(r=0.999)	14.4	26.04(r=0.9993)	26.05

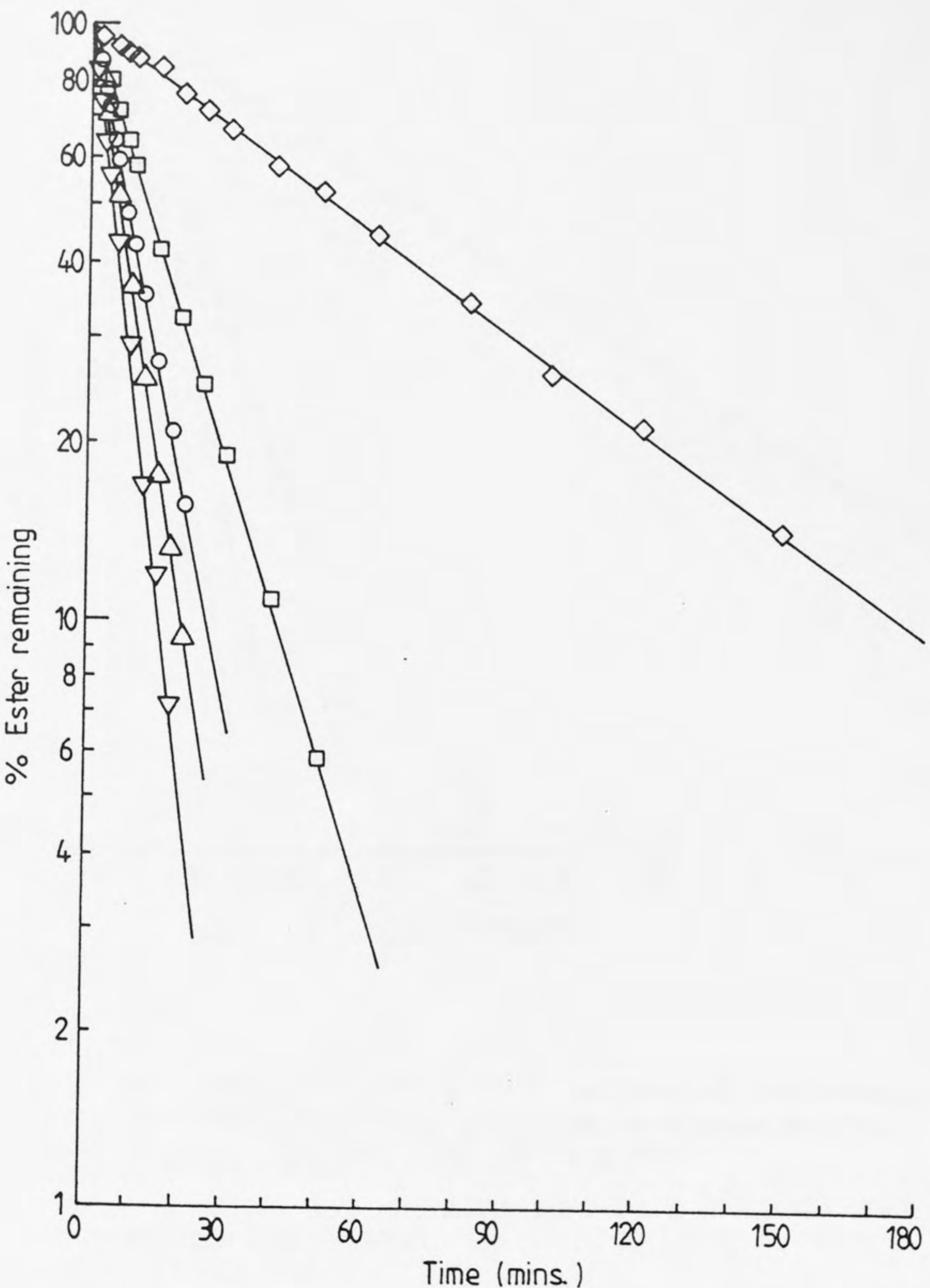


Fig. 6.8 First order plots for the enzymic hydrolysis of (tetrahydro-2-furanyl) methyl ester of ibuprofen by esterase in 20% propylene glycol - Tris buffer pH 8·14 at 37°C.

KEY

	esterase concentration (Units %)
▽	164
△	128
○	96
□	64
◇	16

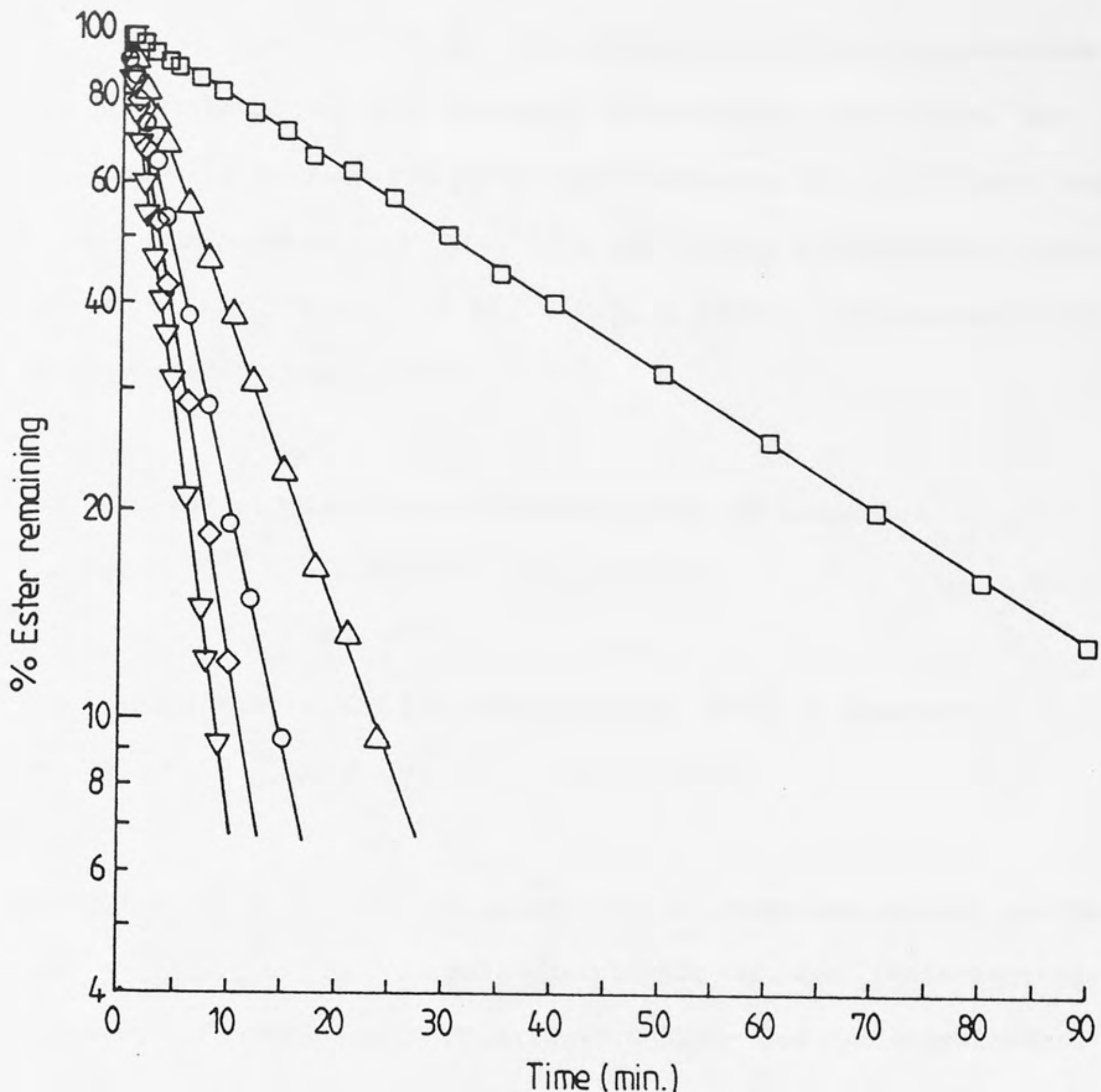


Fig. 6.9 First order plots for the enzymic hydrolysis of (tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen by esterase in 20% propylene glycol-Tris buffer pH 8·14 at 37°C

KEY esterase concentration (units %)

▽	164
◇	128
○	96
△	64
□	16

Linear regression analysis of the data showed that the rate constants were highly dependant on the esterase concentration and there was a linear relationship between the enzyme concentration (C, units %v/v) and the observed rate constant(k, $min.^{-1}$) in the enzyme concentration range of 16-60 units %v/v (Figs. 6.10 and 6.11); a linear relationship which could be expressed by the equations:

1. For the (tetrahydro-2-furanyl)methyl ester of ibuprofen
   $k = 9.1 \times 10^{-4} C - 1.7 \times 10^{-3}$ $(r = 0.9997)$

2. For the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen
   $k = 1.7 \times 10^{-3} C - 5.3 \times 10^{-3}$ $(r = 0.9996)$

The effect of different concentrations of propylene glycol in the substrate solution on the enzymic hydrolysis of the (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl- esters of ibuprofen by 96 units %v/v esterase were investigated. The enzymic hydrolysis of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl- esters of ibuprofen by esterase was followed in the presence of 20-50 %v/v propylene glycol-Tris buffer mixtures pH 8.1 at 37°C (Figs. 6.12 and 6.13 respectively). Semi-Logarithmic plots of these profiles showed that the rate of disappearance was first order (Figs. 6.14 and 6.15). The respective rate constants were determined and are recorded in Table 6.4. The rate constants for the formation of ibuprofen were also measured and are similarly recorded.

The enzymic hydrolysis of the cyclohexylmethyl ester of ibuprofen was followed in the presence of 50, 55 and 60%v/v propylene glycol-Tris buffer mixtures pH 8.1 at 37°C. It was observed that the

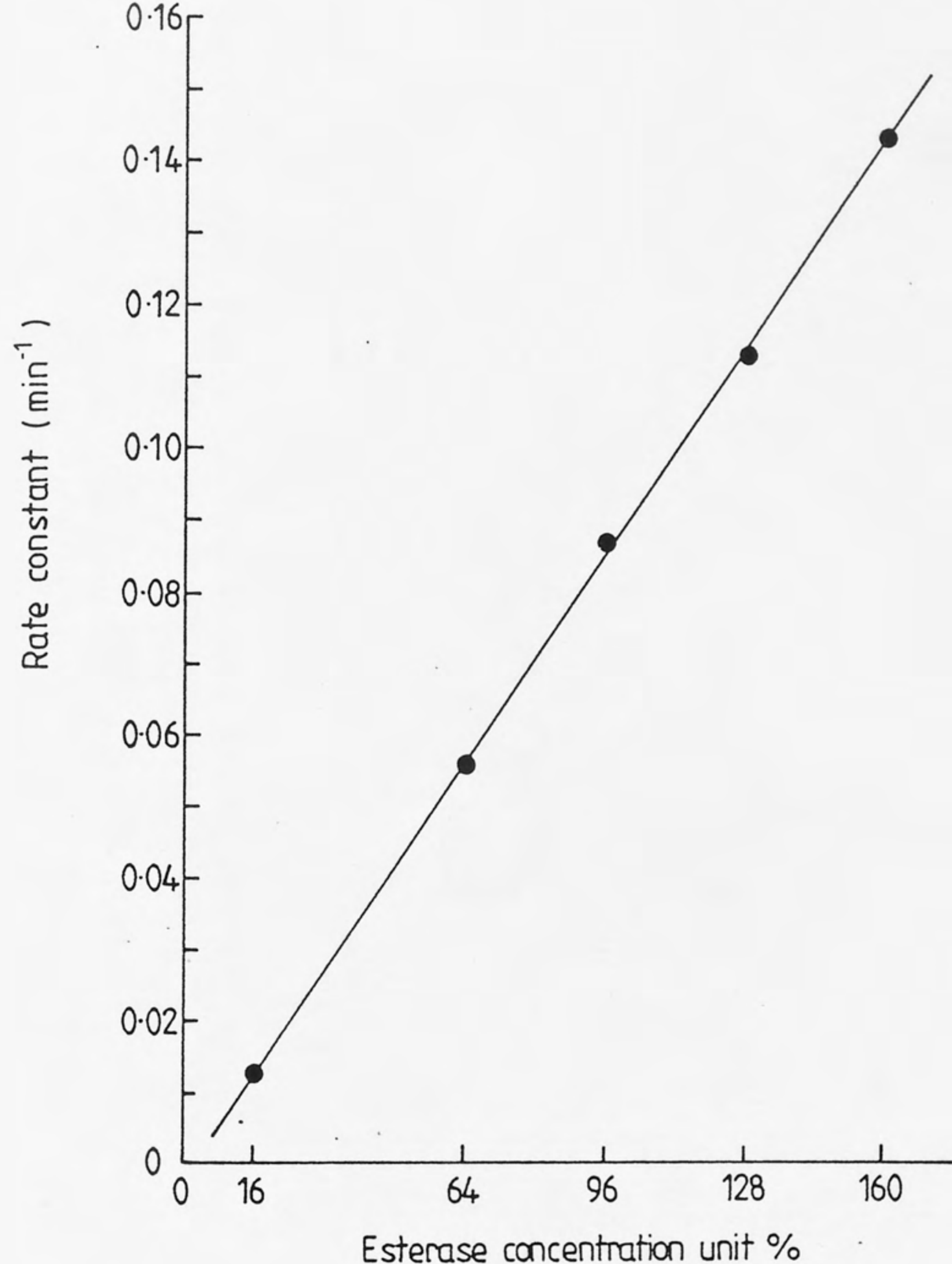


Fig. 6.10 Effect of enzyme concentration on the hydrolysis rate of (tetrahydro-2-furanyl) methyl ester of ibuprofen by esterase in 20% propylene glycol-Tris buffer pH 8·14 at 37°C

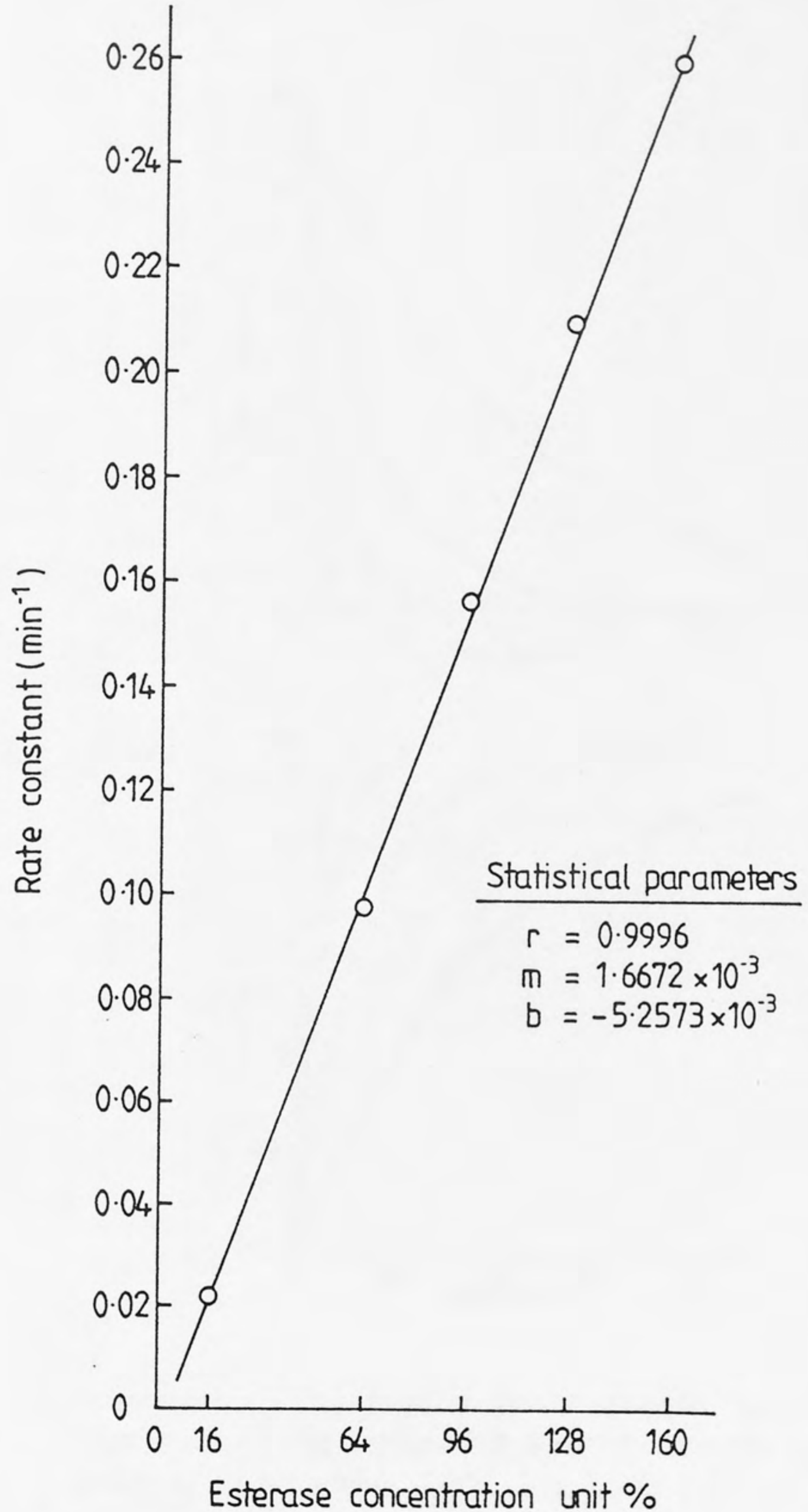


Fig. 6.11 Effect of enzyme concentration on the hydrolysis rate of (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen by esterase in 20% propylene glycol -Tris buffer pH 8·14 at 37°C

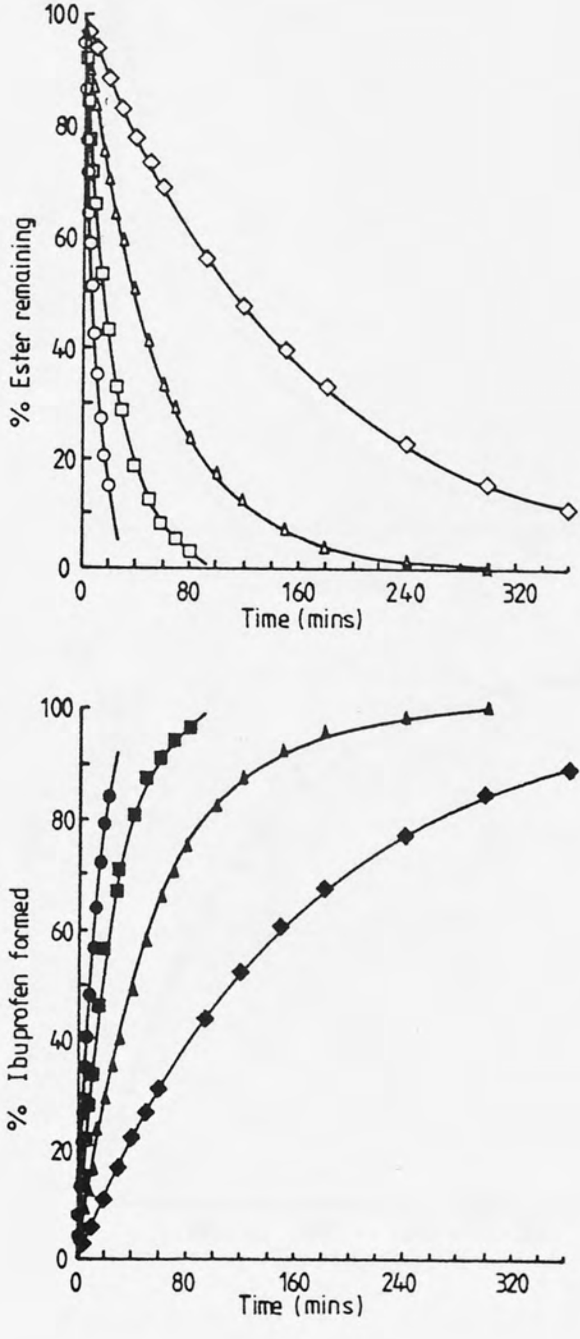


Fig. 6.12 Concentration - time profiles for the enzymic hydrolysis of (tetrahydro-2-furanyl) methyl ester of ibuprofen by (96 unit %) esterase in propylene glycol - Tris buffer mixtures pH 8·1 at 37°C.

KEY	(tetrahydro-2-furanyl) methyl ester remaining	ibuprofen formed	propylene glycol % (v/v)
	○	●	20
	□	■	30
	△	▲	40
	◇	◆	50

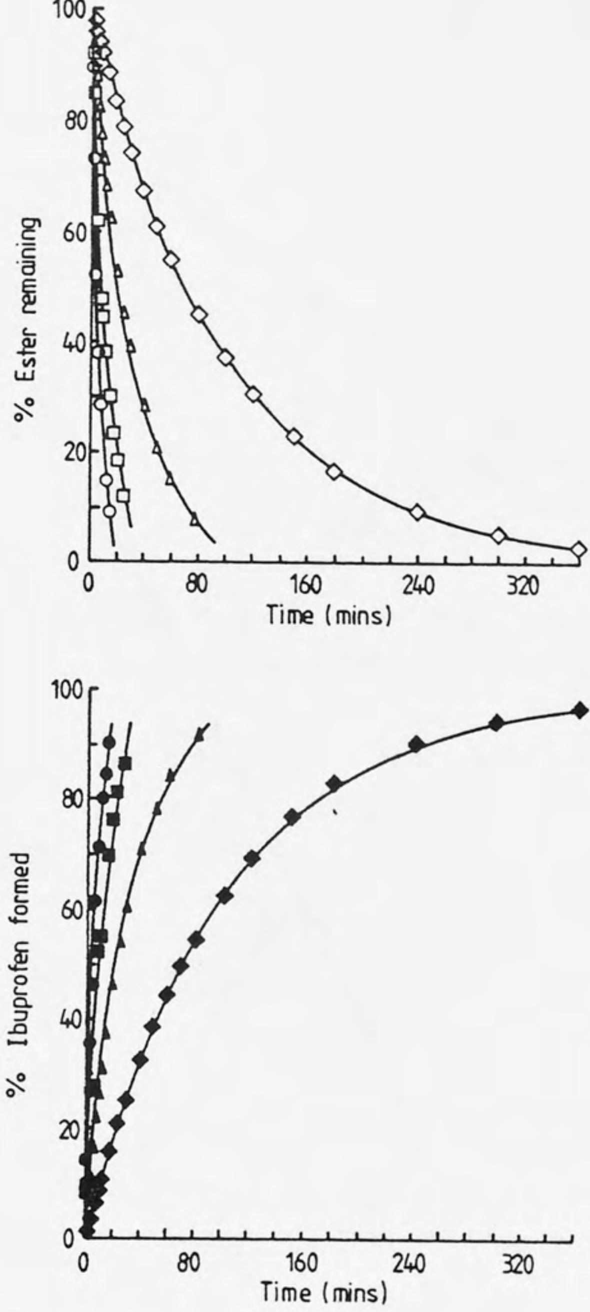


Fig. 6.13 Concentration - time profiles for the enzymic hydrolysis of (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen by (96 unit %) esterase in propylene glycol-Tris buffer mixtures pH 8·1 at 37°C.

KEY	(tetrahydro-2-(2H)pyranyl) methyl ester remaining	ibuprofen formed	propylene glycol % (v/v)
	○	●	20
	□	■	30
	△	▲	40
	◇	◆	50

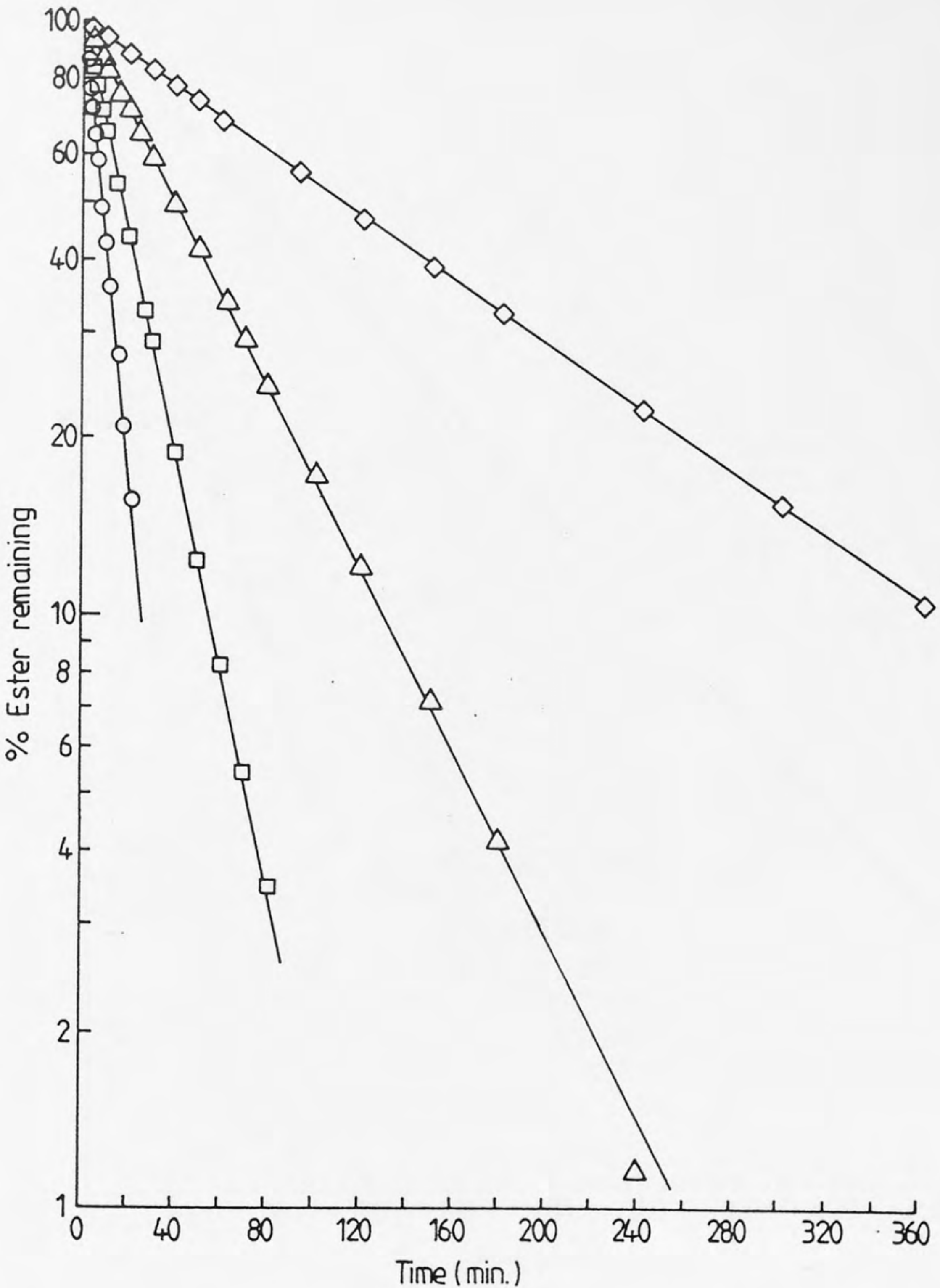


Fig. 6.14 First order plots for the enzymic hydrolysis of (tetrahydro-2-furanyl)methyl ester of ibuprofen by (96 units %) esterase in propylene glycol - Tris buffer mixtures pH 8·1 at 37°C.

KEY	(tetrahydro-2-furanyl) methyl ester	propylene glycol % (v/v)
	○	20
	□	30
	△	40
	◇	50

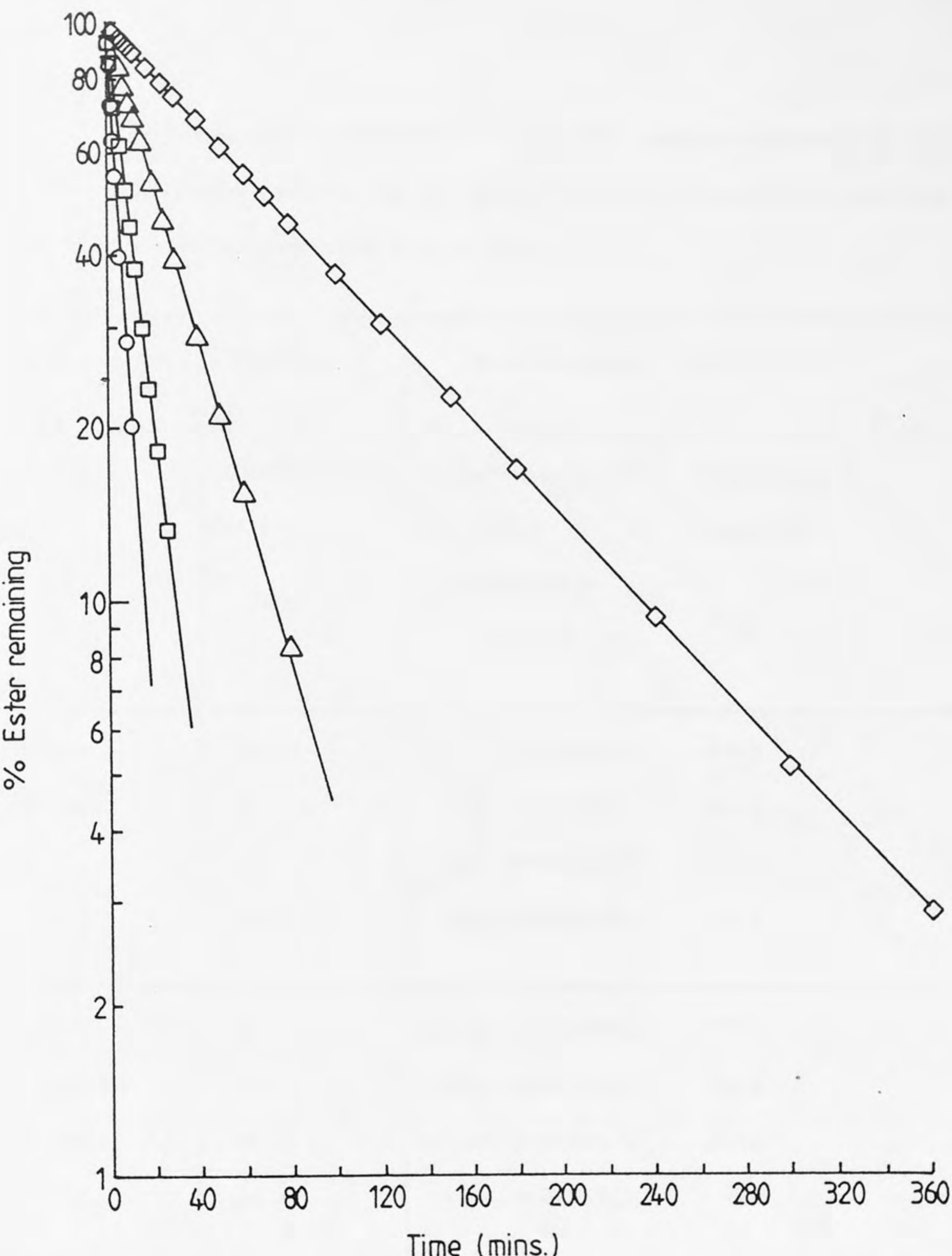


Fig. 6.15 First order plots for the enzymic hydrolysis of (tetrahydro - 2 - (2H) pyranyl) methyl ester of ibuprofen by (96 units %) esterase in propylene glycol - Tris buffer mixtures pH 8·1 at 37° C

KEY	(tetrahydro - 2 - (2H)pyranyl) methyl ester	propylene glycol % (v/v)
	○	20
	□	30
	△	40
	◇	50

TABLE 6.4

Effect of propylene glycol concentration on the enzymic hydrolysis rate constant of ibuprofen esters by 96 units %v/v of esterase in propylene glycol-Tris buffer mixtures pH 8.1 at 37°C.

Ibuprofen ester	Propylene glycol concentration %v/v	Rate constant k $min.^{-1} \times 10^3$	
		Disappearance of the ester (r=regression coefficient)	Formation of ibuprofen
(tetrahydro-2-furanyl)methyl-ester	20	87.3 (r=0.9995)	87.0
	30	41.4 (r=0.999)	41.1
	40	17.5 (r=0.9995)	17.4
	50	6.2 (r=0.9999)	6.3
(tetrahydro-2-(2H)pyranyl)-methyl ester	20	156.6 (r=0.9998)	157.0
	30	80.2 (r=0.9999)	80.8
	40	31.1 (r=0.9992)	31.0
	50	9.8 (r=0.9991)	9.6
		Rate constant k $hr.^{-1}$	
cyclohexylmethyl-ester	50	0.3355	0.3350
	55	0.0907	0.0910
	60	0.0593	0.0590

cyclohexylmethyl ester of ibuprofen became more resistant to esterase activity as the concentration of propylene glycol increased in the substrate mixture. This can be attributed to denaturation of the enzyme protein due to the presence of high concentrations of the glycol but, in spite of this the reaction followed first order kinetics as shown in Fig. 6.16. The respective rate constants for the hydrolysis of the cyclohexylmethyl ester of ibuprofen were calculated by fitting the concentration-time data for the disappearance of the ester to the following equation:

$$\ln(C_t - C_\infty) = \ln(C_o - C_\infty) - kt \qquad (6.4)$$

where C_o represents the initial concentration, C_t the concentration at time t, C_∞ the concentration at infinite time and k is the reaction rate. The measured rate constants are listed in Table 6.4. The rate constants for the formation of ibuprofen were also measured and similarly recorded.

No significant hydrolysis was observed when ibuprofen esters were incubated with 70%v/v propylene glycol-Tris buffer mixtures pH 8.1 at 37°C. This can be attributed to complete denaturation of the enzyme.

Esters are chemically neutral molecules and requires that Z_B which represent the valence of the ester molecule in equation (4.33) is set equal to zero. Therefore in order to illustrate the effect of changing the dielectric constant, equation (4.33) is no longer applicable in this case and it can be rewritten as(348):

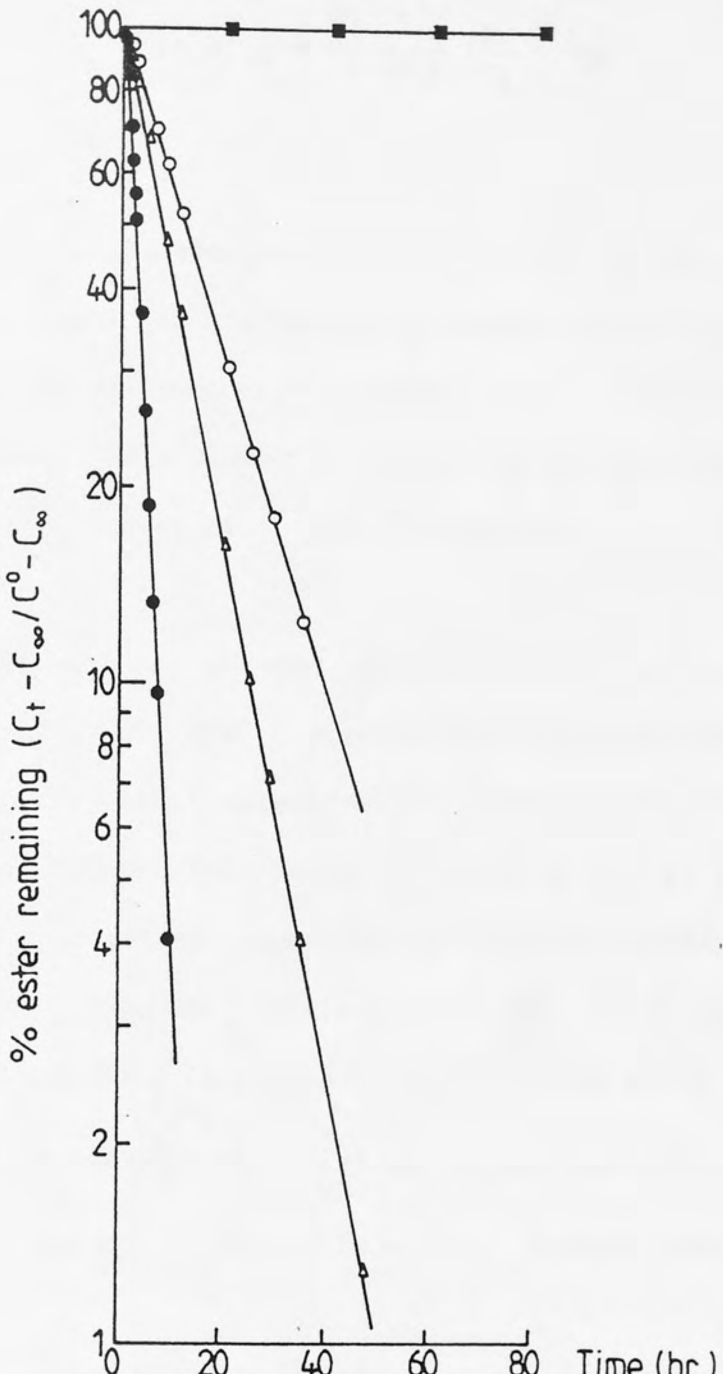


Fig. 6.16 First order plots for the enzymic hydrolysis of cyclohexylmethyl-ester of ibuprofen by 96 units % of esterase in propylene glycol - Tris buffer mixtures pH 8·1 at 37°C.

C_t = ester concentration (M) at time t.

C_∞ = ester concentration (M) at equilibrium.

C^o = initial concentration of the ester (M).

<u>KEY</u> propylene glycol % (v/v)

● 50

△ 55

○ 60

■ Control

$$\ln k_o = \ln k_{\varepsilon=\infty} + \frac{N z_A^2 e^2}{2\, RT\, \varepsilon}\left(\frac{1}{r_A} - \frac{1}{r^*}\right) \qquad (6.5)$$

where z_A is the charge on the ion A, r_A is the radius of the ion and r^* is the radius of the activated complex and a plot of ln k_o against $\frac{1}{\varepsilon}$ should therefore give a straight line. Such straight line plots have been found for a number of reactions and the slopes are consistent with reasonable values of r_A and r^*(349-351).

The effect of the solvent dielectric constant on the enzymic degradation of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen by 96 units %v/v of esterase in propylene glycol-Tris buffer mixtures pH 8.1 at 37°C show the dependence of the degradation rate constant on the solvent composition and a plot of ln $k_{obs.}$ versus $\frac{1}{\varepsilon}$ presented in Figs. 6.17 and 6.18 followed closely the relationship in equation (6.5). These plots have ln $k_{\varepsilon=\infty}$ as the intercept and $\frac{z_A^2 e^2 N}{2RT}\left(\frac{1}{r_A} - \frac{1}{r^*}\right)$ or A/T as the slope. The respective values of $k_{\varepsilon=\infty}$ and A, calculated from least squares analysis of the data gave:

1. (tetrahydro-2-furanyl)methyl ester of ibuprofen:

   $\ln k_o = 7.9339 - \frac{728.2}{\varepsilon}$ ($r = -0.9999$, $n=4$);

   $k_{\varepsilon=\infty} = 2790.3$ min.$^{-1}$ and A at 37°C = -22.6×10^4.

2. (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen:

   $\ln k_o = 9.1449 - \frac{768.5}{\varepsilon}$ ($r = -0.9992$, $n=4$);

   $k_{\varepsilon=\infty} = 9366.7$ min.$^{-1}$ and A at 37°C = -23.8×10^4.

The negative slopes obtained in Figs. 6.17 and 6.18 are in close agreement with the findings of Laidler and Eyring(352) for the effect of

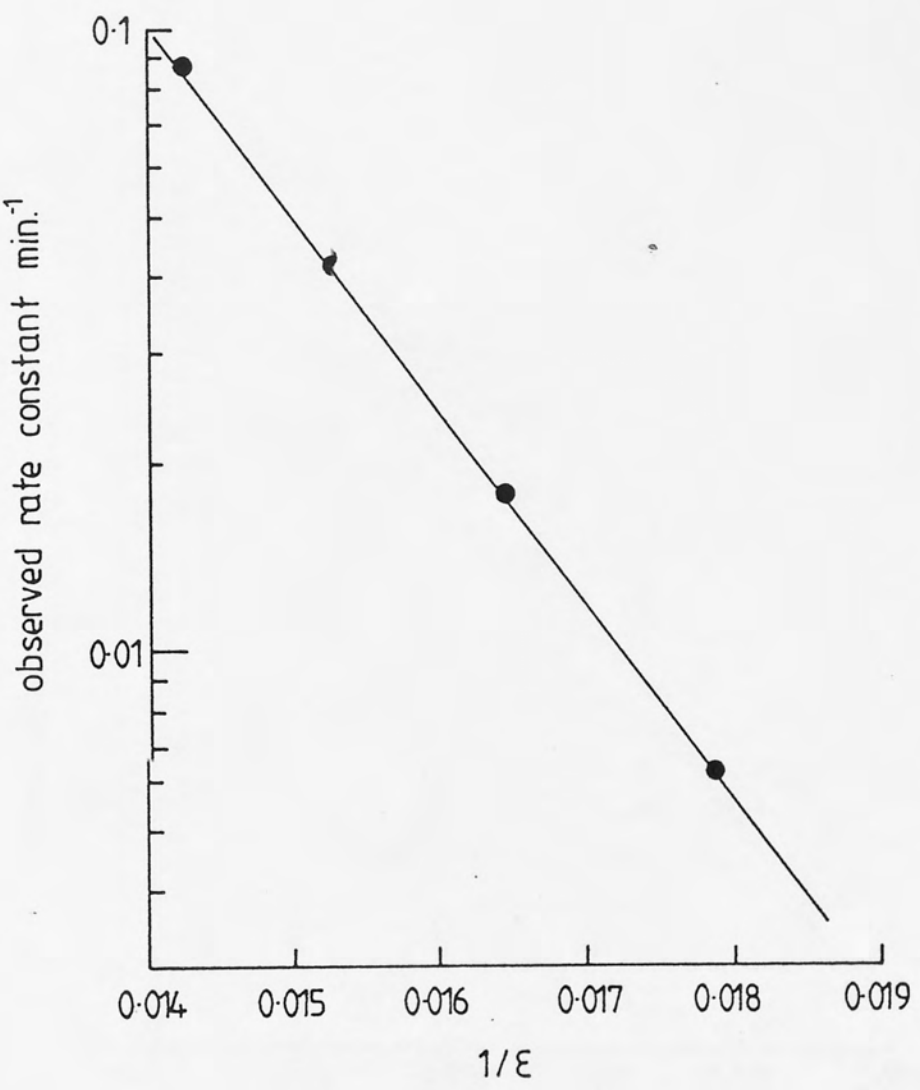


Fig. 6.17 The effect of propylene glycol concentration on the decomposition rate constant of (tetrahydro-2-furanyl)-methyl ester of ibuprofen by (96 units %) esterase in propylene glycol-Tris buffer mixtures pH 8·1 at 37°C.

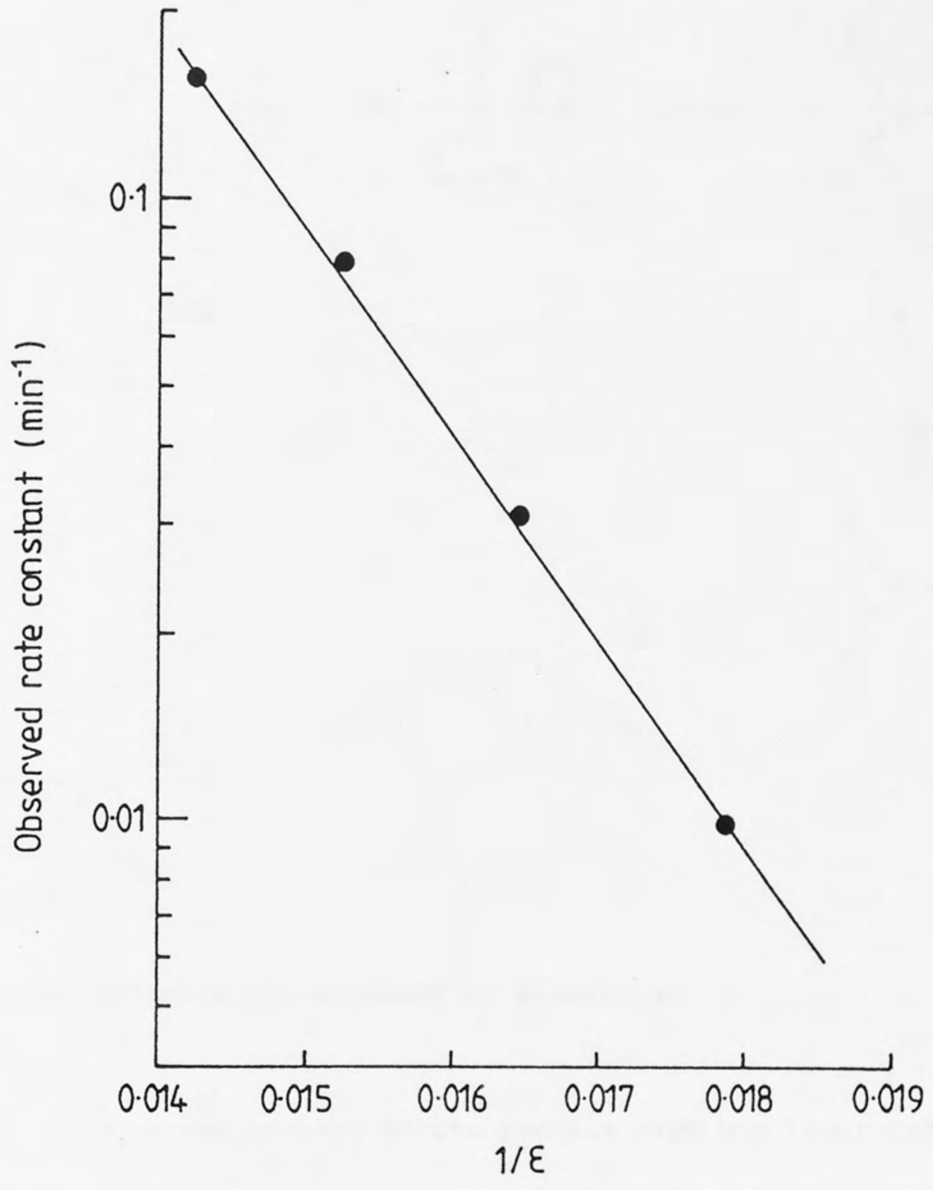


Fig. 6.18 The effect of propylene glycol concentration on the decomposition rate constant of (tetrahydro-2-(2H)-pyranyl) methyl ester of ibuprofen by (96 units %) esterase in propylene glycol - Tris buffer mixtures pH 8·1 at 37°C

solvent on the alkaline degradation of esters. The mechanism of reaction is(351,352):

$$HO^{-} + \begin{matrix} R \\ \diagdown \\ C = O \\ \diagup \\ R'O \end{matrix} \longrightarrow HO - \begin{matrix} R \\ | \\ C \\ | \\ O'R \end{matrix} - O^{-} \longrightarrow \begin{matrix} R \\ \diagdown \\ C = O \\ \diagup \\ HO \end{matrix}$$

$+ \ R'O^{-}$

$\rightleftharpoons$

$$\begin{matrix} R \\ \diagdown \\ C = O \\ \diagup \\ ^{-}O \end{matrix}$$

$+ \ R'OH$

where the arrows indicate the movement of electrons.

To study whether the results of the studies with hog liver esterase can be extrapolated to skin enzymes, the kinetics of hydrolysis of ibuprofen esters were followed using homogenates of whole skin from rats. The disappearance of the parent ester as well as the formation of ibuprofen were monitored. The biotransformation of the (tetrahydro-2-furanyl)methyl- and (tetrahydryo-2-(2H)pyranyl)methyl esters of ibuprofen by rat skin homogenate was followed in 20 and 50%v/v propylene glycol-Tris buffer mixtures pH 8.14 at 37°C and due to the low aqueous solubility of the cyclohexylmethyl ester of ibuprofen its degradation was followed in the presence of 50% by volume as shown in Figs. 6.19 and

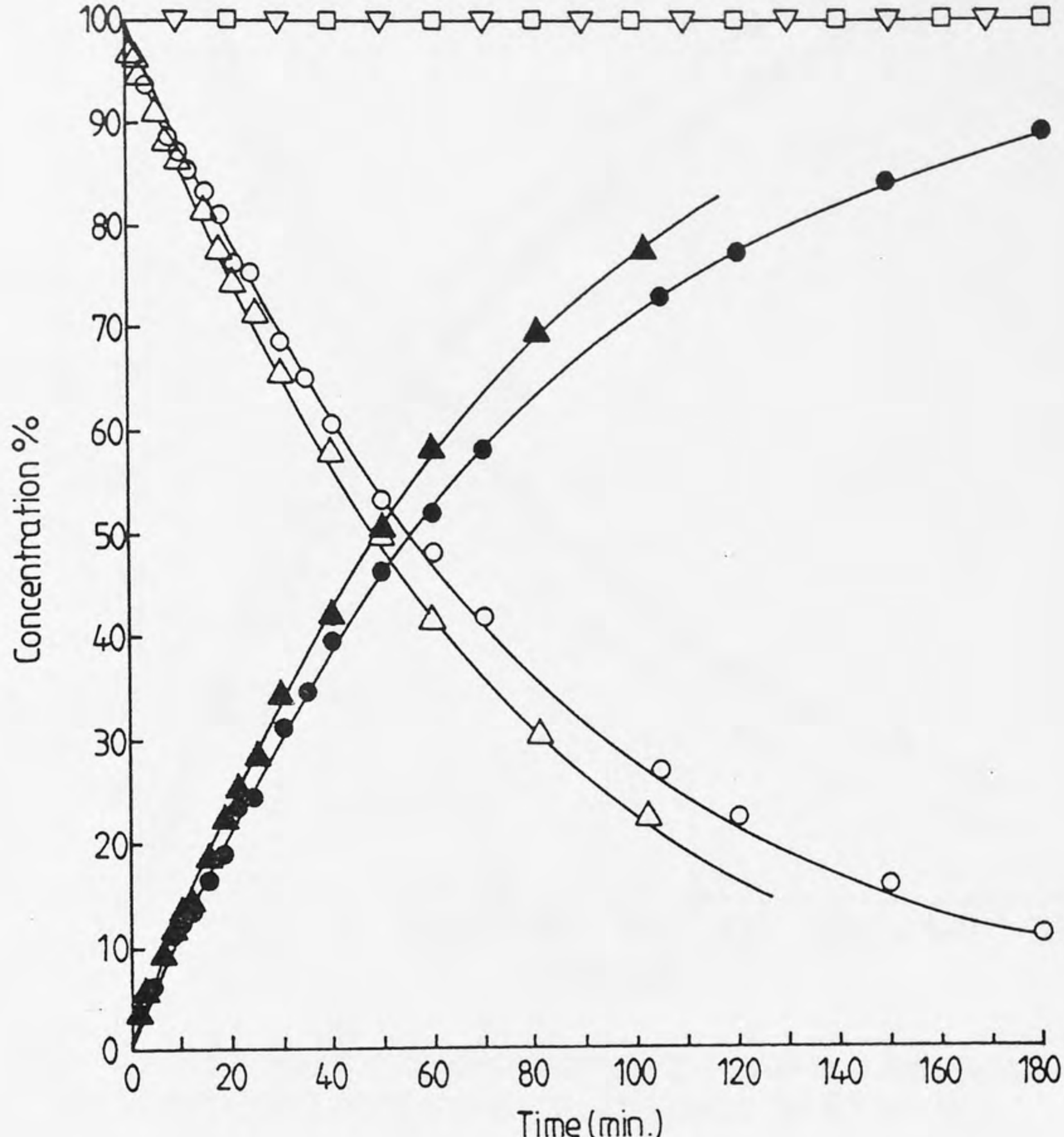


Fig. 6.19 Biotransformation of ibuprofen esters by rat skin homogenate in 20% (v/v) propylene glycol - Tris buffer mixture pH 8·14 at 37°C.

KEY

[△ (tetrahydro-2-furanyl) methyl ester remaining
▲ ibuprofen formed
□ control]

[○ (tetrahydro-2-(2H)pyranyl) methyl ester remaining
● ibuprofen formed
▽ control]

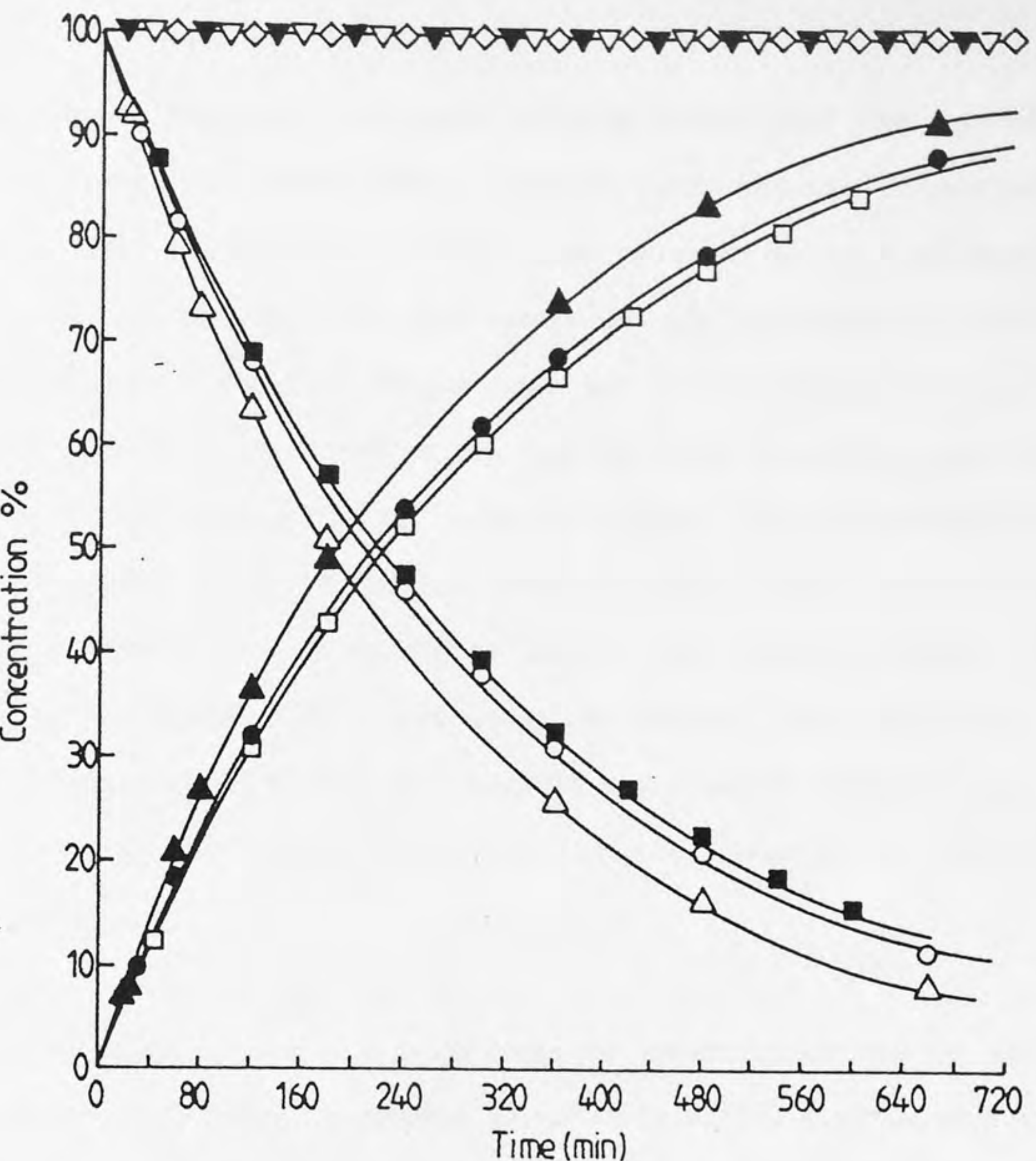


Fig. 6.20 Biotransformation of ibuprofen esters by rat skin homogenate in 50% (v/v) propylene glycol - Tris buffer pH 8·1 at 37°C.

Ester concentration $= (C_t - C_\infty)/(C^o - C_\infty)$

C_t = Concentration at time t.
C_∞ = equilibrium concentration
C^o = initial concentration

KEY

[△ (tetrahydro-2-furanyl) methyl ester remaining
▲ ibuprofen formed
▽ control]

[○ (tetrahydro-2-(2H)pyranyl) methyl ester remaining
● ibuprofen formed
▼ control]

[■ cyclohexylmethyl ester remaining
□ ibuprofen formed
◇ control]

6.20. Semi-logarithmic plots of these profiles showed that the rate of disappearance were first order (Figs. 6.21 and 6.22) and the respective rate constants for the hydrolysis of the parent esters as well as those for the formation of ibuprofen were measured and recorded in Table 6.5. Examination of the data showed that rat skin esterases were not discriminative between ibuprofen esters and the rate constants were in close agreement with each other for each set of data. The concentration of propylene glycol in the substrate mixture affected the activity of the cutaneous esterases and ibuprofen esters were more resistant to hydrolysis in the presence of 50%v/v propylene glycol. This may be due to a slight denaturation of the skin enzymes and causing the hydrolysis rate constants to be slower than those in the presence of 20%v/v propylene glycol.

The rate constants for the hydrolysis of ibuprofen esters by the cutaneous esterase in 50%v/v propylene glycol-Tris buffer mixture pH 8.1 at 37°C were calculated by fitting the concentration-time data for the disappearance of the ester to equation (6.4).

It was observed that ibuprofen esters were more resistant to hydrolysis by rat skin enzymes than by hog liver esterase and the latter was more specific in its hydrolytic effect than the cutaneous enzymes. The specificity of the hog liver esterase upon ibuprofen esters can be attributed to differences in the interaction at the active site.

A semiquantitative comparison between the rat skin enzymes and the hog liver esterase showed that each 10 ml of the homogenate is equivalent to approximately 16 units %v/v of esterase if it is used for the (tetrahydro-2-furanyl)methyl ester of ibuprofen and to 8 units %v/v

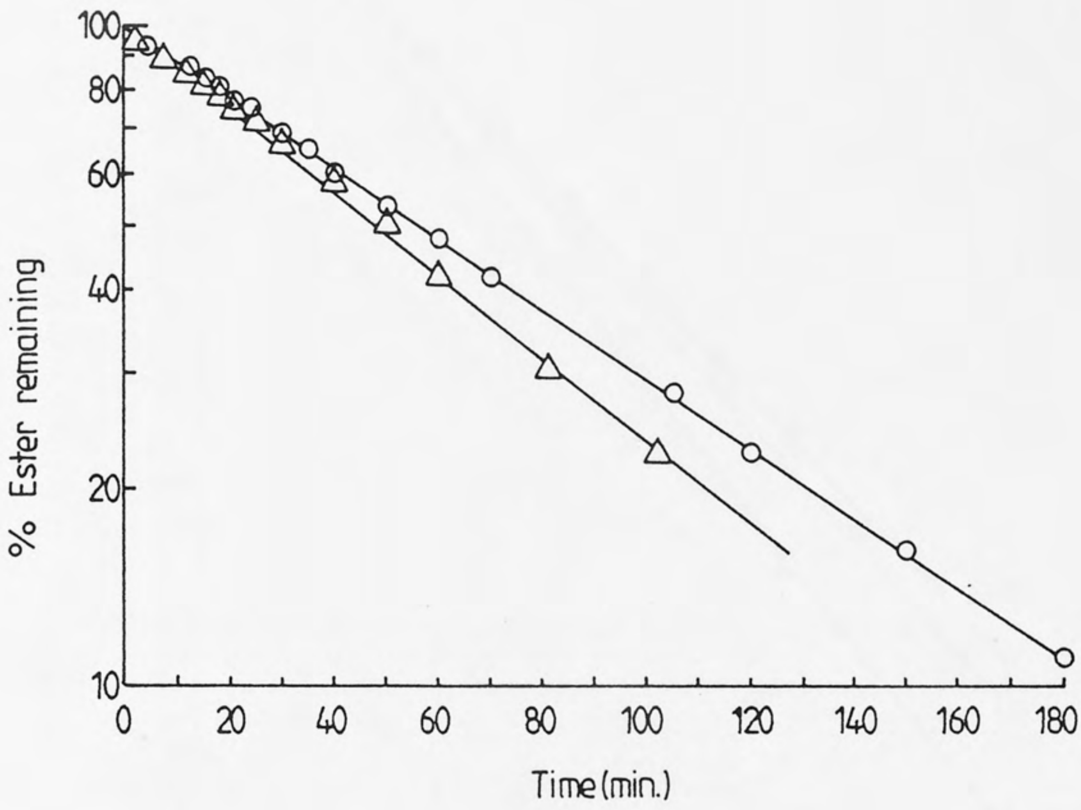


Fig. 6.21 First order plots for the biotransformation of ibuprofen esters by rat skin homogenate in 20% (v/v) propylene glycol - Tris buffer pH 8·14 at 37°C.

KEY △ (tetrahydro - 2 - furanyl) methyl ester
○ (tetrahydro - 2 - (2H) pyranyl) methyl ester

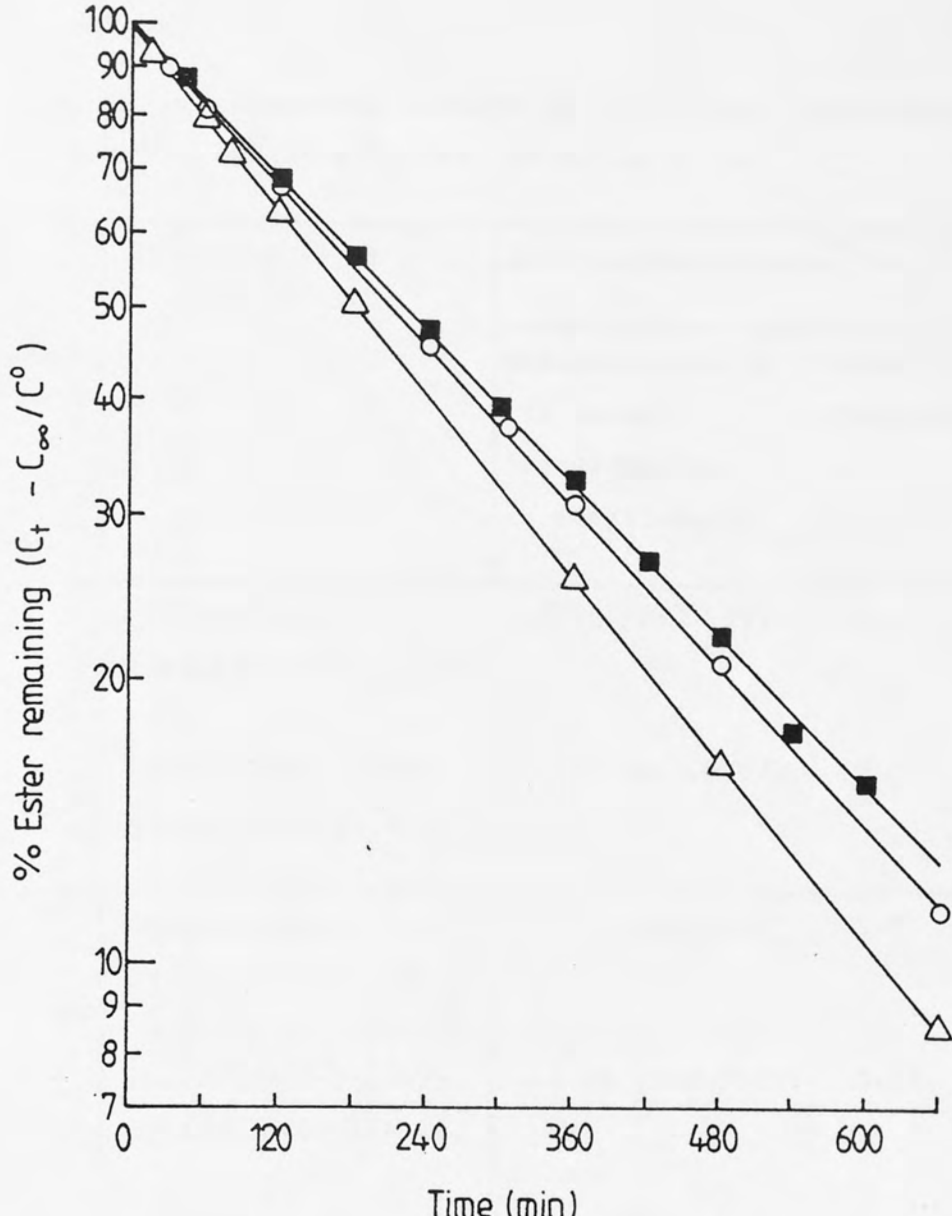


Fig. 6.22 First order plots for the biotransformation of ibuprofen esters by rat skin homogenate in 50% (v/v) propylene glycol - Tris buffer pH 8·1 at 37°C.

KEY
△ (tetrahydro-2-furanyl) methyl ester
○ (tetrahydro-2-(2H)pyranyl) methyl ester
■ Cyclohexylmethyl ester

TABLE 6.5

Biotransformation of ibuprofen esters by rat skin homogenates, in propylene glycol-Tris buffer mixtures, pH 8.1 at 37°C.

Propylene glycol concentration %v/v	Ibuprofen ester	Rate constant k $min.^{-1} \times 10^3$	
		Disappearance of the ester (r=regression coefficient)	Formation of ibuprofen
20	(tetrahydro-2-furanyl)methyl ester	14.45 (r=0.999)	14.5
	(tetrahydro-2-(2H)-pyranyl)methyl ester	12.29 (r=0.9997)	12.25
50	(tetrahydro-2-furanyl)methyl ester	3.74 (r=0.9999)	3.8
	(tetrahydro-2-(2H)-pyranyl)methyl ester	3.25 (r=0.9999)	3.27
	cyclohexylmethyl-ester	3.095(r=0.999)	3.1

of esterase if it is used with the (tetrahydro-2-(2H)pyranyl)methyl-ester of ibuprofen in a reaction mixture consisting of 20%v/v propylene glycol-Tris buffer pH 8.14 at 37°C.

The addition of the enzyme cofactor NADPH to the reaction mixtures was dismissed when a report by Cheung and others(353) showed that no significant effect was observed on the cutaneous esterase activity of skin homogenates against betamethasone-21-valerate.

CHAPTER 7

RELEASE AND PENETRATION OF TOPICAL NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

7.1 INTRODUCTION

There is currently much interest in the topical delivery of drugs for systemic or subepidermal effects(210,354-356) and the nonsteroidal anti-inflammatory agents represent a group of drugs which is particularly attractive in this respect (133,232,233,357). Topical formulations of non-steroidal anti-inflammatory drugs can be used for the treatment of sunburn(358) and alleviation of muscular rheumatism(359,360) because of their analgesic and anti-inflammatory activity and thus avoiding the risk of systemic adverse effects. The availability of the drug in adequate amounts at the required site of action will ensure the effectiveness of the drug formulation. The topical delivery of the drug depends on its release from the formulated preparation and its penetration through the skin barrier mainly manifested by the stratum corneum. Either of these two processes may be rate limiting and are governed by the nature of the drug and the composition of the vehicle in which it is formulated. On penetrating the skin barrier, the drug will proceed relatively rapidly through the remainder of the skin layers and into the circulation. In order to enhance the skin penetration properties for a medicinal agent, esterification has been employed for a number of compounds and its has proved effective in increasing their potency. This is probably due to a decrease in the polarity of the parent compound(175) which enhances absorption.

Skin penetration can be tested *in vitro* using a diffusion cell equipped with animal or human skin and this procedure can give a good indication of the penetration potential of the compound under study. However, results obtained with identical vehicle composition may lack reproducibility even using the same skin and, therefore, it is essential to include a reference material as a marker in all skin diffusion studies. This enables penetration profiles to be normalised before comparison.

7.2 THEORY

Diffusion is defined as a process of mass transfer of individual molecules of a substance, brought about by random molecular motion and associated with a concentration gradient(361). The transport or flow of molecules through the barrier is a particularly convenient way to study diffusion and at steady state the process is described by Fick's first law. The amount, M, of material flowing through a unit cross section, S, of a barrier in unit time t, is known as the flux, J.

$$J = \frac{dM}{S.dt} \qquad (7.1)$$

The flux is proportional to the concentration gradient, $\frac{dC}{dx}$:

$$J = - D \frac{dC}{dx} \qquad (7.2)$$

where J is the flux in $gm/cm^2/sec.$, D is the diffusion coefficient of the permeant in cm^2/sec, C its concentration in gm/cm^3, x the distance in cm of movement perpendicular to the surface of the barrier and dC/dx

is the gradient in concentration. The negative sign of equation (7.2) reflects the fact that the direction of flow is down the gradient in concentration. A schematic representation of the diffusant concentration profile through a membrane is presented in Fig. 7.1. In all cases the permeant on either side of the membrane is in equilibrium with the respective surface layer of membrane, and the concentration just inside the membrane surface can be related to the concentration in the adjacent solution, C, by the expressions:

$$C_1 = C_d K \text{ at the upstream surface } (x=0) \qquad (7.3)$$

$$C_2 = C_r K \text{ at the downstream surface } (x=h) \qquad (7.4)$$

where C_d is the concentration in the donor phase, C_r is the concentration in the receptor side, h is the thickness of membrane and K is the distribution coefficient where as C_1 and C_2 are the concentrations in the membrane on the left (donor) and on the right (receptor) sides respectively. It is normal for the concentration curve to increase or decrease sharply at the boundaries of the barrier layer since the affinity of many drugs is greater for the membrane than for water. In general, most drugs have low solubility in polymeric membranes and on the assumption that diffusion coefficients and distribution coefficients are constants, thus in the steady state, equation (7.2) can be integrated to give(206)

$$J = \frac{M}{s.t} = D \frac{C_1 - C_2}{h} \qquad (7.5)$$

$$= D \frac{\Delta C}{h} \qquad (7.6)$$

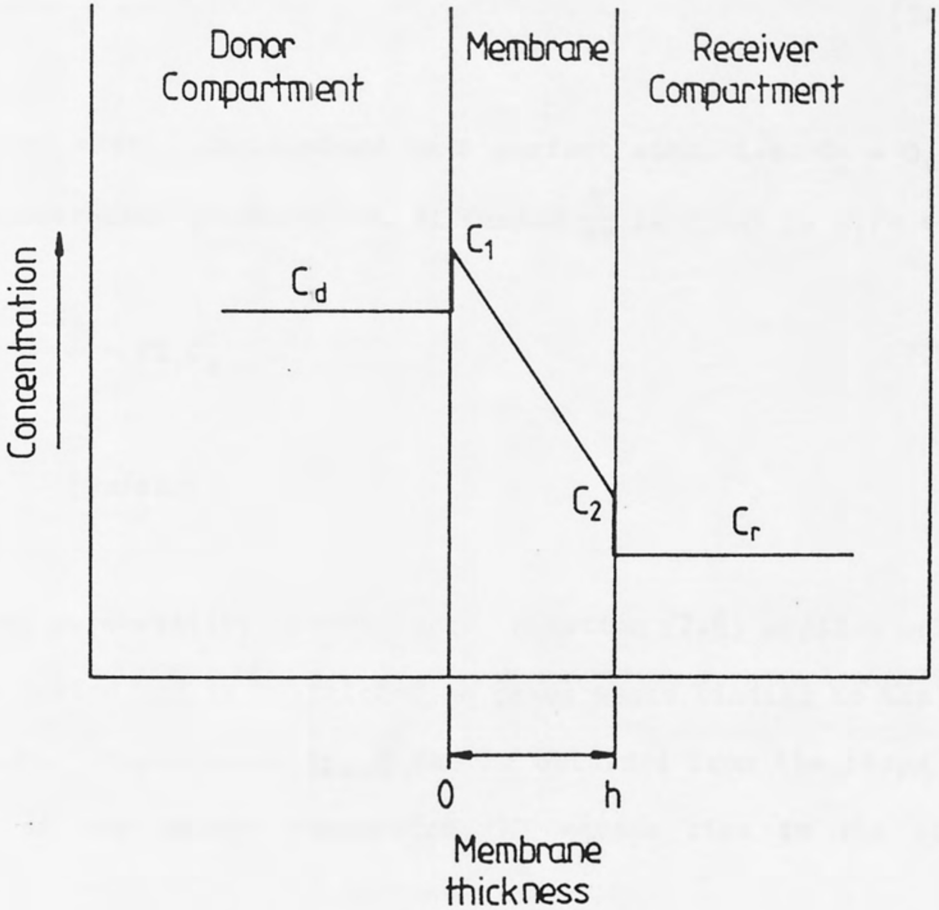


Fig. 7.1 Schematic representation of the concentration gradient across the membrane of a diffusion cell.

Since the concentration within the membrane is usually not known; equation (7.5) is frequently written as:

$$J = \frac{DSK\ (C_d - C_r)}{h} \tag{7.7}$$

if the receptor side is maintained in a perfect sink, i.e. $C_2 = 0$, such that the concentration gradient for diffusion $\frac{dC}{dx}$ is equal to C_1/h so:

$$\frac{M}{t} = \frac{DSK\ C_d}{h} = PS\ C_d \tag{7.8}$$

in which $P = \frac{DK}{h}$ (cm/sec)

where P is the permeability coefficient. Equation (7.8) applies only to steady state period and is restricted to cases where binding to the skin does not occur. Experimentally, P can be obtained from the slope of a linear plot of the amount transfered (M) versus time in the steady state(361).

$$M = PS\ C_d t \tag{7.9}$$

providing that C_d remains relatively constant throughout time. This can be achieved by using a saturated solution in the presence of excess solid which results in zero-order release being observed.

Systems using nonconstant sources of drug show an appreciable change in C_d with time and thus unit thermodynamic activity of the drug cannot be maintained within the donor phase. A donor phase of volume V_d separated by the rate controlling membrane from the receiving fluid, of

volume V_r. Let the time-dependent mass of drug in the donor phase be M_d and that in the receiver be M_r then the total mass of drug M_o is given by

$$M_o = M_d + M_r \tag{7.10}$$

and if all the drug is initially within the donor phase:

$$M_d = M_o \text{ at } t = 0 \ .$$

The concentrations and masses are related as follows

$$M_d = C_d V_d \tag{7.11}$$

$$M_r = C_r V_r \tag{7.12}$$

Then P can be obtained from the slope of ln M_d or ln C_d versus time(362) where

$$\ln M_d = \ln M_o - \frac{PSt}{V_d} \tag{7.13}$$

or

$$\ln C_d = \ln C_o - \frac{PSt}{V_d} \tag{7.14}$$

The Time Lag

Constant-activity dosage form give constant release in the steady state, but during the initial stages of drug transfer from the donor phase to the receiver phase the rate of flow and the concentration at any point within the membrane varies with time, this is called the non-

steady state period. At later times the rate of diffusion is constant and the system is at steady state. When the steady state portion of the line is extrapolated to the time axis the point of intersection is known as the lag time L(362). This is the time required for a diffusant to establish a quasi-stationary state or a uniform concentration gradient within the membrane separating the donor from the receptor phases. The lag time is given by

$$L = \frac{h^2}{6D} \tag{7.15}$$

7.3 MATERIALS AND METHODS

7.3.1 The Diffusion of Indomethacin, Flufenamic Acid, Ibuprofen and its Esters Through Human Skin from Solutions made in Propylene Gylcol-Water Mixtures at 37°C

The diffusion of indomethacin, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl-esters through human skin was evaluated at 37°C according to the method described earlier in section 2.3.2.B part 4 in the presence of flufenamic acid and using a modified form of the Franz diffusion cell(363) illustrated in Fig. 7.2a.

Excised human skin, stored at -15°C, was kindly supplied by the Birmingham Accident Hospital. The skin pieces, consisting mainly of the stratum corneum and part of the epidermis (between 0.0095 - 0.025 cm in thickness), were thawed before use in saline, washed with warm water and mounted as a membrane between the two compartments of the diffusion cell. The donor and the receiver compartments of the diffusion cell

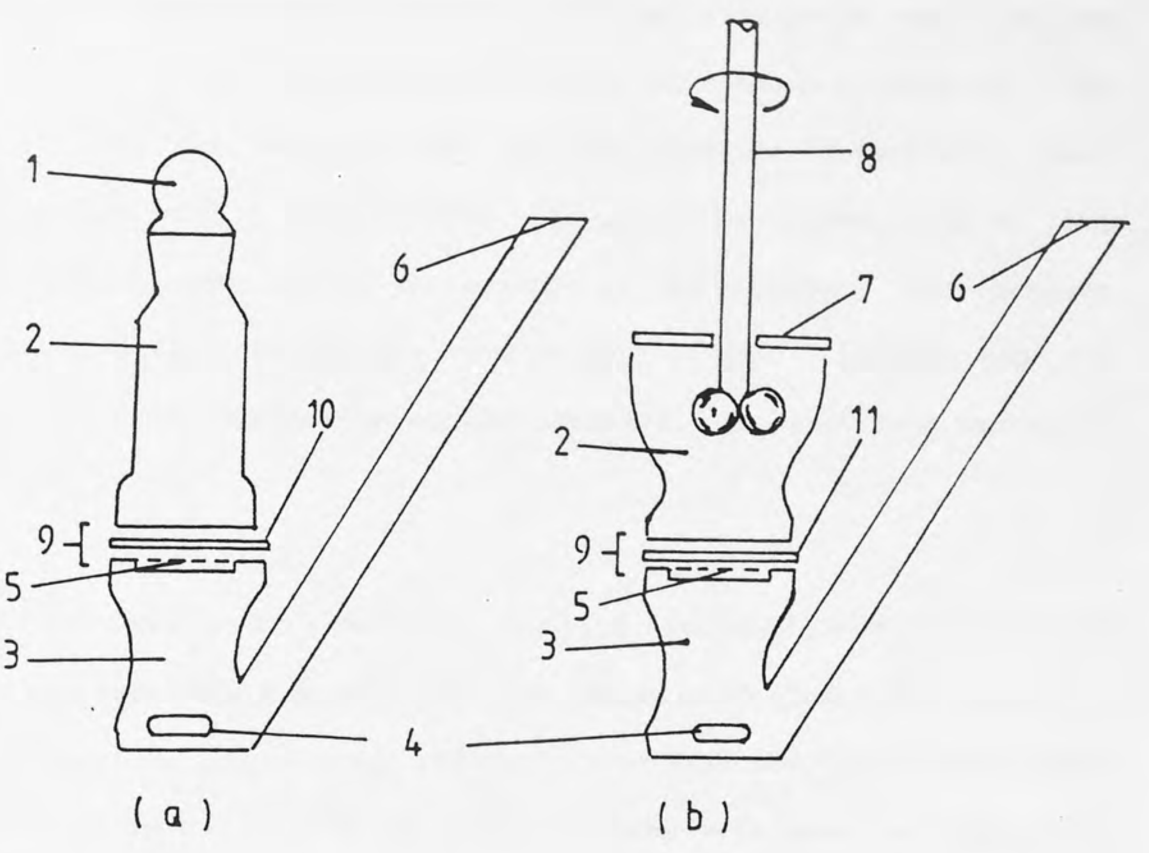


Fig. 7.2 (a,b) Schematic diagrams of the glass diffusion cells used in the permeation studies through human skin(a) and rat skin (b).

Key 1, Glass stopper ; 2, donor compartments ; 3, receiver compartments ; 4, teflon coated stirring bars ; 5, support screen ; 6, sampling ports (sealed with parafilm) ; 7, perspex cover ; 8, glass stirrer ; 9, compartments clamped across ground glass surfaces ; 10, human skin ; 11, rat skin .

were clamped together after mounting the skin membrane between them. The skin membrane with an effective diffusion diameter of 2 cm was supported by a stainless steel mesh screen positioned beneath it. The donor part and the sampling part of the receiver compartments were tightly closed with a glass stopper and parafilm respectively so that solvent loss by evaporation was reduced to the minimum. The receiver phase was stirred constantly using a bar magnetic stirrer and the diffusion cell was immersed after construction in a water bath warmed at 37°C.

In each evaluation a substock solution containing a mixture of one of the test compounds together with flufenamic acid (reference compound) made in propylene glycol-water was introduced into the donor compartment of the diffusion cell. The substock solutions were made to contain the concentrations listed earlier in Table 2.4. In the receiver phase a blank solution of propylene glycol-water was introduced. All substocks and blanks were made to contain 50% propylene glycol-water except when testing the cyclohexylmethyl ester of ibuprofen with flufenamic acid when a mixture of 80% propylene glycol-water was used. The volumes introduced into the donor and receiver compartments of the diffusion cell of each mixture and blank solution together with the thickness of the human skin membranes used are as follows:

Mixture studied	Volume of substock solution introduced (ml)	Volume of blank solution introduced (ml)	Vehicle composition (% propylene glycol in water)	Thickness of human skin membrane (cm)
indomethacin + flufenamic acid	6.5	13	20	0.02
ibuprofen + flufenamic acid	6.5	13	20	0.011
(tetrahydro-2-furanyl)methyl-ester of ibuprofen + flufenamic acid	7.5	13.5	20	0.0095
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen + flufenamic acid	6.5	13	20	0.01
Cyclohexylmethyl ester of ibuprofen + flufenamic acid	7	13	80	0.025

The samples collected from the receiver phase were diluted with 4 ml of 50% ethanol in water before mixing with 2 ml of the internal standard. The internal standards chosen for the analysis of the different mixtures evaluated are listed in Table 2.8 while their concentrations are discussed earlier in section 2.4.3.B part 3. The standard solutions were prepared to contain the same vehicle composition as well as treated in every respect as with the test solutions. The samples and standards were analysed by HPLC (Section 7.3.5). The constituents of the standard solutions together with their concentration range are as follows:

Mixture studied	Compounds contained in standard solutions	Concentration range (μM)
indomethacin + flufenamic acid	indomethacin flufenamic acid	28.4 - 142 7.11 - 35.5
ibuprofen + flufenamic acid	ibuprofen flufenamic acid	60.6 - 303.1 8.89 - 44.4
(tetrahydro-2-furanyl)-methyl ester of ibuprofen + flufenamic acid	ibuprofen (tetrahydro-2-furanyl)methyl ester flufenamic acid	30 - 150 58.14 - 290.7 8.87 - 44.3
(tetrahydro-2-(2H)-pyranyl methyl ester of ibuprofen + flufenamic acid	ibuprofen (tetrahydro-2-(2H)-pyranyl methyl ester flufenamic acid	12 - 60 12.3 - 61.5 8.87 - 44.3
Cyclohexylmethyl ester of ibuprofen + flufenamic acid	ibuprofen cyclohexylmethyl ester flufenamic acid	6 - 30 69.8 - 349.1 2.13 - 10.66

Control solutions of the same initial concentrations used in the diffusion study and having a volume of 50 mls were made in the same vehicle for each of the mixtures studied. The controls were monitored in the presence of absence of human skin sections at 37°C for the same duration as in the diffusion study.

7.3.2 The Determination of the Diffusion Coefficients

The diffusion coefficients of flufenamic acid, indomethacin, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)-pyranyl)methyl- and cyclohexylmethyl esters were determined at 37°C from

their individual diffusion data. The diffusion process was monitored through Polyvic membrane filters (Millipore Corporation, USA) (0.0115 cm in thickness) from solutions made in 50% propylene glycol-water and using a modified Wurster permeability cell(364) (Fig. 7.3).

The diffusion cell consists of a pair of half cells in mirror image which is totally closed after assembled. The sampling ports are tightly closed with glass stoppers, so that solvent loss can be minimized. Each half cell is stirred with a bar magnetic stirrer at a constant speed and the cell, after constructions was immersed in a water bath at 37°C. Two diffusion cells were used during the evaluations and they were calibrated initially with a solution of methyl nicotinate (Sigma Chemicals) (7.5 mM) in 50% propylene gylcol water. The effective diameter of the membrane filter exposed to the diffusion process was equal to 4 and 3.85 cm when employed in the diffusion cells named Cell 1 and Cell 2 respectively.

The experiment was initiated by introducing 115 ml of a solution of each compound in 50% propylene glycol-water into the donor compartment of the diffusion cell, where the Millipore filter serves as a membrane between the donor and the receiver phases, this latter comprising of 115 ml of 50% propylene glycol-water mixture. The diffusion was followed by monitoring the appearance of the compound in the receiver phase. This was achieved by pipetting 1 ml from the receiver phase and replacing it with a 1 ml blank 50% propylene glycol-water. The samples were diluted with 4 ml of 50% ethanol-water before mixing with 1 ml of the internal standard solution.

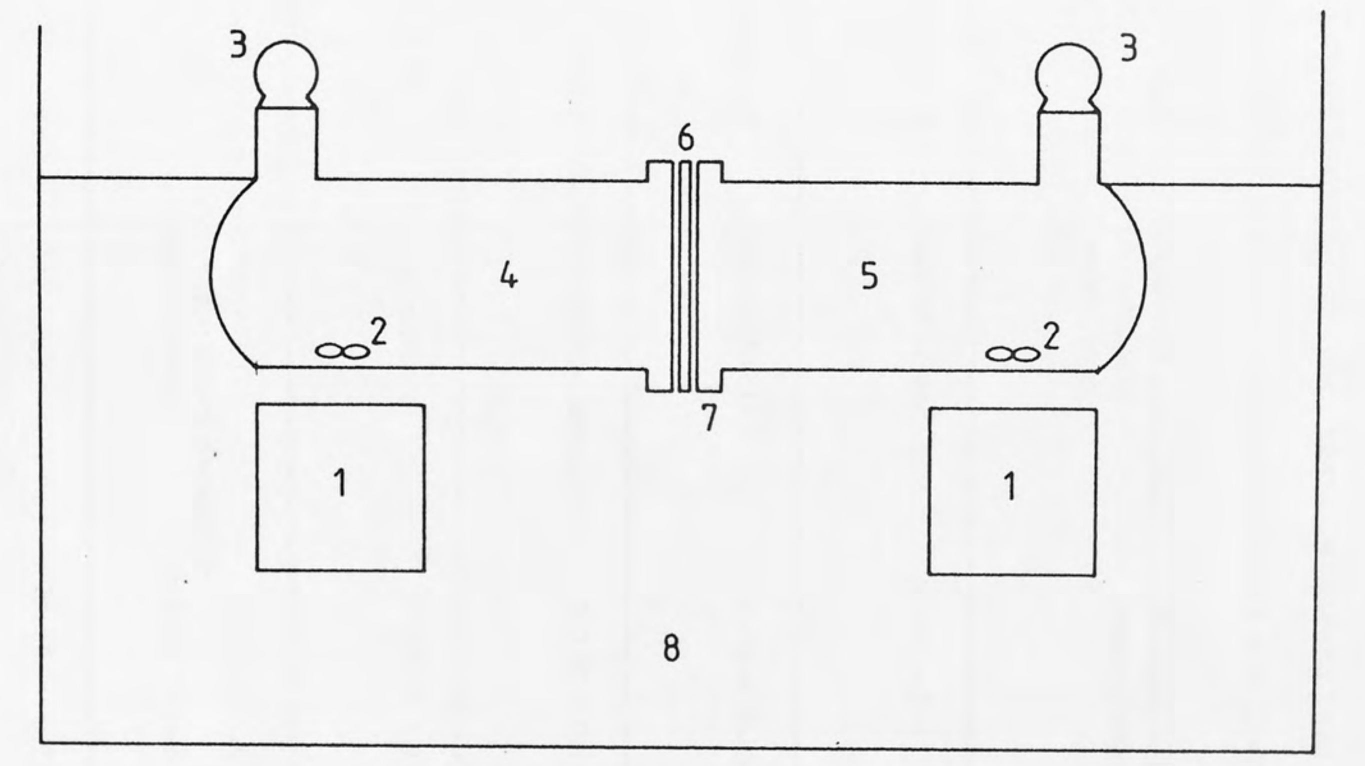


Fig. 7.3 Schematic diagram of the glass diffusion cell used to determine diffusion coefficient.

<u>KEY</u> : 1, Synchronous under-water stirrers; 2, Teflon coated stirring bars; 3, Sampling ports; 4, Donor compartment (115 ml); 5, Receiver compartment (115 ml); 6, Membrane filter (Millipore Co.); 7, Ground glass surfaces; 8, Cell compartments are placed in a water bath at 37°C.

Standard solutions were prepared in 50% propylene glycol-water and they were treated in every respect as with the test solutions. The test and standard solutions were analysed by HPLC (Section 7.3.5.).

The constituents of the test, standard and internal standard solutions together with their concentrations are listed as follows:

Compound studied and initial concentration (mM)	Compound contained in standard solutions	Concentration range (mM)	Internal Standard and concentration (mM)
methyl nicotinate, 7.5	methyl nicotinate	0.7 - 3.5	indomethacin, 0.7
indomethacin, 0.3	indomethacin	0.03 - 0.15	flufenamic acid ,0.2
flufenamic acid, 0.36	flufenamic acid	0.036 - 0.18	ethyl ester of ibuprofen, 1.28
ibuprofen, 2.25	ibuprofen	0.225 - 1.125	ethyl ester of ibuprofen, 1.28
(tetrahydro-2-furanyl)-methyl ester of ibuprofen, 1.6	ibuprofen (tetrahydro-2-furanyl)-methyl ester	0.16 - 0.8 0.16 - 0.8	flufenamic acid, 0.2
(tetrahydro-2-(2H)-pyranyl) methyl ester of ibuprofen, 0.5	ibuprofen (tetrahydro-2-(2H)-pyranyl methyl ester	0.05 - 0.25 0.05 - 0.25	flufenamic acid, 0.2
cyclohexylmethyl ester of ibpurofen, 0.4	ibuprofen cyclohexylmethl ester	0.08 - 0.4 0.08 - 0.4	flufenamic acid, 0.2

Experiments were run in triplicate.

7.3.3 The Diffusion of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl Esters of Ibuprofen through Rat Skin

The diffusion of the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen through rat skin was evaluated at 37°C from suspensions made in 20% v/v propylene glycol-water using the diffusion cell illustrated in Fig. 7.2b. The abdominal areas of freshly killed male rats were mechanically depilated and skinned. The skin pieces (approximately 0.03 cm in thickness) were mounted as a membrane between the two compartments of the diffusion cell. The diffusion cell was constructed in a similar way to the method described earlier in Section 7.3.1 except that the wide necked donor compartment was used. During the experiment this was covered with a perspex sheet and sealed with parafilm to reduce the evaporation process. In each test one of the esters was used together with flufenamic acid as a standard and suspensions of both materials in each case were made in 20% propylene glycol-water. The experiment was initiated by introducing 10 ml of the suspension prepared into the donor compartment of the diffusion cell. An inlet in the centre of the perspex cover allowed the usage of a glass stirrer which was attached to a Heidolph motor (Type RZR 50, West Germany) operating at 60 rpm in order to prevent the separation of the suspension constituents.

Twelve and 13.5 mls of 20% propylene glycol-water were introduced into the receiver compartments of the cells containing the (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen respectively in their donor compartments. The diffusion was

followed by monitoring the appearance of each of the two compounds in the well stirred receiver phase. This was achieved by pipetting 1 ml from the receiver phase and replacing it with a 1 ml blank 20% propylene glycol in water. The sample was placed into a test tube containing 1 ml of acidified acetonitrile solution (for preparation see section 6.2.1.E) and simultaneously mixed in order to quench any enzymic degradation process which may be taking place in the solution. The samples were further mixed with 1 ml of an internal standard solution before anlaysis by HPLC (section 7.3.5). Standards were made in 20% propylene glycol-water and treated in every respect as with the test solutions. The constitutents and concentrations of the standard solutions together with the chosen internal standards are as follows:

Mixture studied in the suspension	Compounds contained in standard solutions	Concentration Range (μM)	Internal standard and concentration (μM)
(tetrahydro-2-furanyl)-methyl ester of ibuprofen + flufenamic acid	ibuprofen (tetrahydro-2-furanyl)-methyl ester flufenamic acid	15 - 75 15 - 75 4 - 20	(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen, 100
(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen + flufenamic acid	ibuprofen (tetrahydro-2-(2H)-pyranyl)methyl ester flufenamic acid	15 - 75 15 - 75 4 - 20	(tetrahydro-2-furanyl)-methyl ester of ibuprofen, 100

Control solutions having initial concentrations of that used in the human skin controls and 50 mls in volume were made in 20% propylene glycol-water and monitored in the presence and absence of rat skin sections (100 - 200 mg) at 37°C for the same duration as in the diffusion study.

The suspension introduced into the donor compartment was used at the end of the diffusion study to measure the solubility of the compounds included in the mixture. The method followed the technique described earlier in section 3.3.1.C.

7.3.4 The Determination of Partition Coefficient

A. Determination of the partition coefficent of flufenamic acid, indomethacin, ibuprofen and its esters between propylene glycol-water and human skin.

The partition coefficient of indomethacin, flufenamic acid, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)-pyranyl)methyl- and cyclohexylmethyl esters between various propylene glycol-water systems and human skin were studied individually at 37°C. the binary systems consisted of 20, 40, 50, 60 and 80% propylene glycol in water except for the cyclohexylmethyl ester where the systems consisted of 40, 50, 60, 70 and 80% propylene glycol in water. The concentrations of compounds dissolved in the propylene glycol-water mixtures were as follows:

% propylene glycol in the binary system	Concentrations of compounds evaluated (mM)					
	ibuprofen	flufenamic acid	indomethacin	(tetrahydro-2-furanyl)-methyl ester of ibuprofen	(tetrahydro-2-(2H)-pyranyl)-methyl ester	Cyclohexyl-methyl-ester of ibuprofen
20	0.484	0.0711	0.167	0.155	0.0493	
40	0.969	0.142	0.335	0.344	0.098	0.1
50	1.212	0.177	0.7	0.585	0.246	0.2
60	1.212	0.177	0.7	0.585	0.246	0.22
70						0.36
80	1.212	0.177	0.7	0.585	0.246	0.4

Twenty mls of these solutions were placed into 50 ml containers and equilibrated with skin sections of known weight (100 - 200 mg each) in a shaking water bath at 37°C for the duration of up to 5 days. One ml aliquots of the propylene glycol-water phase were sampled every day and after dilution the concentration of its constituents was determined by HPLC (Section 7.3.5) until the equilibrium state was reached. One ml of 80, 60, 50, 40, 30 and 20% ethanol in water was added to the samples made in 20, 40, 50, 60, 70 and 80% propylene glycol-water respectively to give mixtures containing 50% alcohol (ethanol and propylene glycol) in water. The mixtures were further diluted with 3 ml of 50% ethanol water before mixing with 2 ml of the internal standard solution. The standard solutions were prepared to contain the same vehicle composition and were treated in every respect as the test solutions. The constituents of the standard solutions and their concentration range together with the internal standard chosen are as follows:

Compound studied	Compounds contained in standard solutions	Concentration range (mM)	Internal Standard and concentration (mM)
indomethacin	indomethacin	0.15 - 0.75	flufenamic acid, 0.2
flufenamic acid	flufenamic acid	0.04 - 0.2	ethyl ester of ibuprofen, 1.28
ibuprofen	ibuprofen	0.25 - 1.25	ethyl ester of ibuprofen, 1.28
(tetrahydro-2-furanyl)-methyl ester of ibuprofen	ibuprofen	0.12 - 0.6	flufenamic acid, 0.2
	(tetrahydro-2-(2H)-pyranyl)methyl ester	0.12 - 0.6	
(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen	ibuprofen	0.06 - 0.3	flufenamic acid, 0.2
	(tetrahydro-2-(2H)-pyranyl)methyl ester	0.06 - 0.3	
cyclohexylmethyl ester of ibuprofen	ibuprofen	0.08 - 0.4	flufenamic acid, 0.12
	cyclohexylmethyl-ester	0.08 - 0.4	

The skin/solution partition coefficient was then determined from the concentration of each compound in the skin and the equilibrium concentration in the solution using

$$K = \frac{\text{g of compound/g of skin}}{\text{g of compound/g of vehicle}}$$

The amount in the skin was determined from the initial and the equilibrium concentrations. The densities of solutions containing 20, 40, 50, 60, 70 and 80% propylene glycol in water were determined using a pycnometer at 25°C and they were equal to 1.0145, 1.0293, 1.035, 1.0384, 1.0392 and 1.0405 g/ml respectively. All samples were run in quadriplicate.

B. The determination of the partition coefficient of flufenamic acid, (tetrahydro-2-furanyl)methyl ester of ibuprofen and (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen between 20% propylene glycol-water and rat skin.

The determination of the partition coefficient followed the same method described earlier in Section 7.3.4.A except that rat skin sections were used. Test and standard solutions were prepared and treated in every respect according to this method before analysis by HPLC (Section 7.3.5).

C. Determination of the partition coefficient of flufenamic acid, indomethacin, ibuprofen and its esters between Polyvic membrane filter and 50% propylene glycol-water mixtures.

The partition coefficient of flufenamic acid, indomethacin,

ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)-pyranyl)methyl- and cyclohexylmethyl esters between Polyvic membrane filter (Millipore Corporation, USA) and 50% propylene glycol-water mixtures were determined at 37°C according to the method described earlier in Section 7.3.4.A. Sections of the membrane filter (100 - 200 mg in weight) were used instead of the human skin. Test and standard solutions were prepared and treated as described earlier in the same section.

All partition coefficient samples were run in quadriplicate.

7.3.5 HPLC Analysis

A reversed-phase high-performance liquid chromatography system was used for assaying indomethacin, flufenamic acid, ibuprofen and its esters. The equipment used was as previously described in Section 2.1C. A mobile phase consisted of 76%v/v methanol in water acidified with 0.3% orthophosphoric acid (pH = 2.5) was used for the analysis of indomethacin, flufenamic acid, methyl nicotinate, ibuprofen and its (tetrahydro-2-furanyl)methyl- and (tetrahydro-2-(2H)pyranyl)methyl-esters except that for the analysis of the cyclohexylmethyl ester of ibuprofen a mobile phase having 81%v/v methanol was used. Both mobile phases were delivered at 1.0 ml/min. Separations were achieved using a 10 cm × 4.6 mm i.d. Hypersil-ODS 5μm (Shandon, U.K.) column. Peak detection was at 220 nm and at a sensitivity setting ranging from 0.01 - 0.32 AUFS except for the anlaysis of methyl nicotinate where a wavelength of 235 nm was used.

7.4 RESULTS AND DISCUSSION

7.4.1 Human Skin Permeation Studies

Permeation studies of indomethacin, ibuprofen and its (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl esters through human skin were evaluated using a modified form of the Franz diffusion cell(363).

Earlier reports indicated possible variation in the permeability of human skin within and between specimens(365) attributable to several endogenous and external factors(174,181,366). The integrity of the stratum corneum, as well as its hydration and temperature status in addition to the presence or absence of occlusions are among the endogenous factors which affect the permeability of the skin(174,181,366). While permeant factors such as partitioning, skin binding and concentration in addition to vehicle factors such as pH, solvent and surfactants were among the external factors which may affect the permeability of the skin(174,181).

A reference compound, flufenamic acid, was therefore included into the diffusion study in order to account for the factors which may affect the permeability and to assist in presenting the data obtained with identical vehicle composition in a suitable way for comparison. The skin penetration was monitored from solutions delivered into the donor phase of the diffusion cell made in 50% propylene glycol-water mixture with pH values of 4.5-4.6. This pH range is well within the region of 4-9 recommended by Zatz(174,181) in order that little damage to the stratum corneum may be brought by the permeant and its vehicle.

The use of solutions as the donor phase did not maintain a unit thermodynamic activity due to the depletion of the source. Typical diffusion patterns are shown in Fig. 7.4 for the fractional release of ibuprofen and flufenamic acid through human skin from a solution in 50% propylene glycol-water.

The calculation of the amount released through human skin included correction for the samples pipetted from the receiver phase. Similar corrections were made in the diffusion experiments which will be discussed later for the purpose of calculating the amount released into the receiver phase. The total amount of each compound recovered at infinite time, represented by the amount left in the donor phase and that released into the receiver phase show the lack of evidence in support of skin binding and was recorded as follows:

Mixture studied	Correlation between the amount recovered at infinite time from both donor and receiver compartments with the amount introduced originally
indomethacin	99.9
flufenamic acid	99.89
ibuprofen	99.88
flufenamic acid	99.87
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	99.92
flufenamic acid	99.86
(tetrahydro-2-(2H)pyranyl)methyl-ester of ibuprofen	99.85
flufenamic acid	99.89
Cyclohexylmethyl ester of ibuprofen	99.95
flufenamic acid	99.83

This allowed the calculation of the fraction remaining at time t in the donor phase to be determined by mass balance.

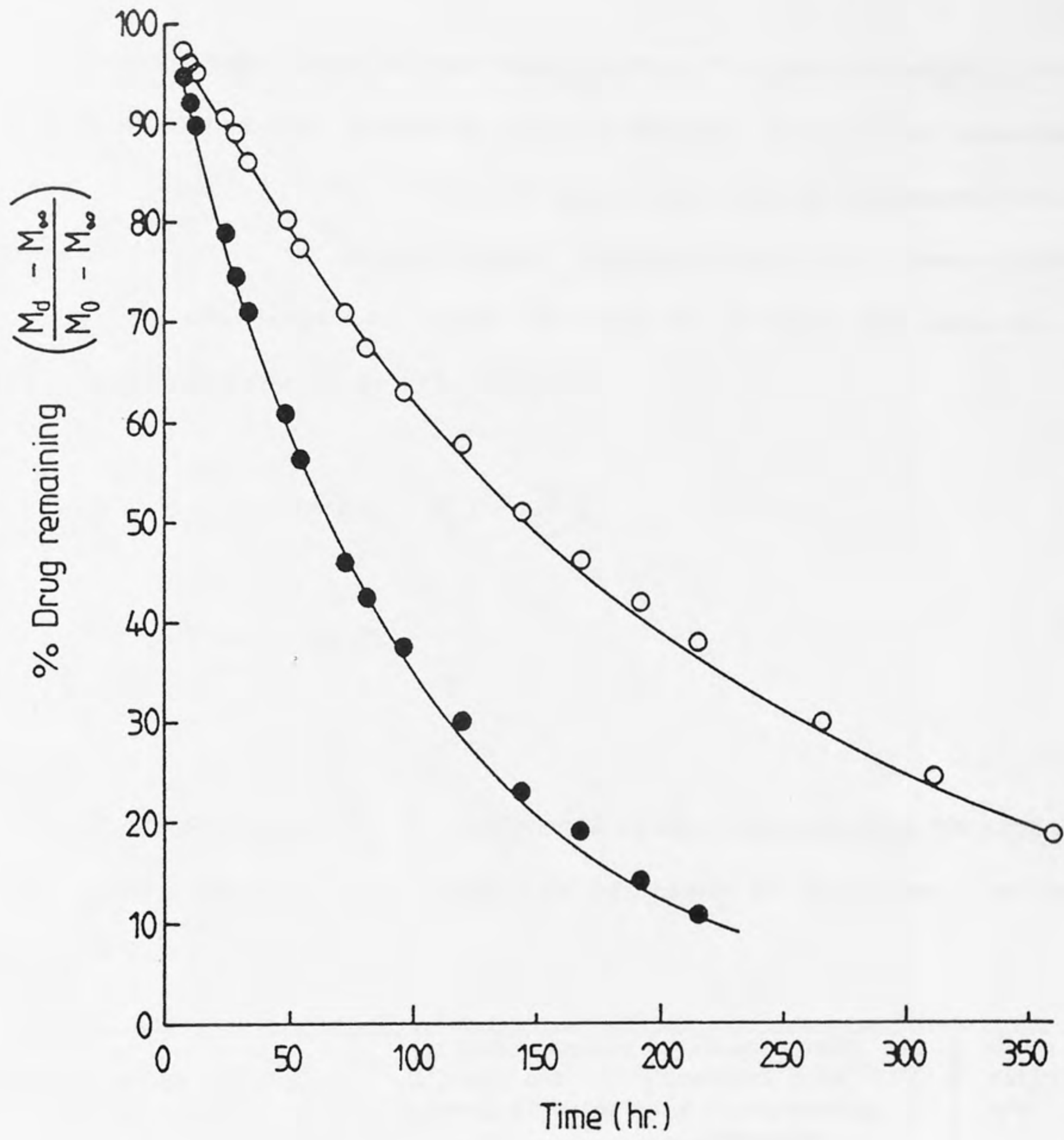


Fig. 7.4 In vitro fractional release of ibuprofen and flufenamic acid from 50% propylene glycol - water solution through human skin at 37°C.

KEY Drug remaining in the donor phase

● flufenamic acid
○ ibuprofen
M_d mass of the drug in the donor phase at time t
M_0 initial mass of the drug in the donor phase
M_∞ mass of the drug in the donor phase at infinite time

Semilogarithmic plots of the data in Fig. 7.4 gave straight lines which indicate that the diffusion process follows first order kinetics as shown in Fig. 7.5. The diffusion rate constants of flufenamic acid together with each of indomethacin, ibuprofen and its esters were equivalent to the slopes of lines obtained by fitting the data to a slightly modified form of equation (7.13):

$$\ln (M_d - M_\infty) = \ln (M_o - M_\infty) - \frac{PS}{V_d} t \qquad (7.16)$$

and are recorded in Table 7.1.

TABLE 7.1

The diffusion rate constants of flufenamic acid, indomethacin, ibuprofen and its esters through human skin from solutions in propylene glycol-water at 37°C.

Mixture evaluated	Diffusion medium % propylene glycol in water	Diffusion rate constant k $hr^{-1} \times 10^3$ (r = regression coefficient)	Slope ratio a/b
1 Indomethacin (a) + flufenamic acid (b)	50	2.62 (r= 0.9992) 19.57 (r= 0.9997)	0.133
2 Ibuprofen (a) + flufenamic acid (b)	50	4.565 (r= 0.9996) 10.18 (r= 0.9993)	0.4484
3 (tetrahydro-2-furanyl)-methyl ester of ibuprofen (a) + flufenamic acid (b)	50	4.282 (r= 0.9997) 8.78 (r= 0.999)	0.4876
4 (tetrahydro-2-(2H)-pyranyl)-methyl ester of ibuprofen (a) + flufenamic acid (b)	50	5.77 (r= 0.9991) 7.95 (r= 0.999)	0.725
5 cyclohexylmethyl ester of ibuprofen (a) + flufenamic acid (b)	80	1.1 (r= 0.9995) 1.4 (r= 0.9992)	0.7857

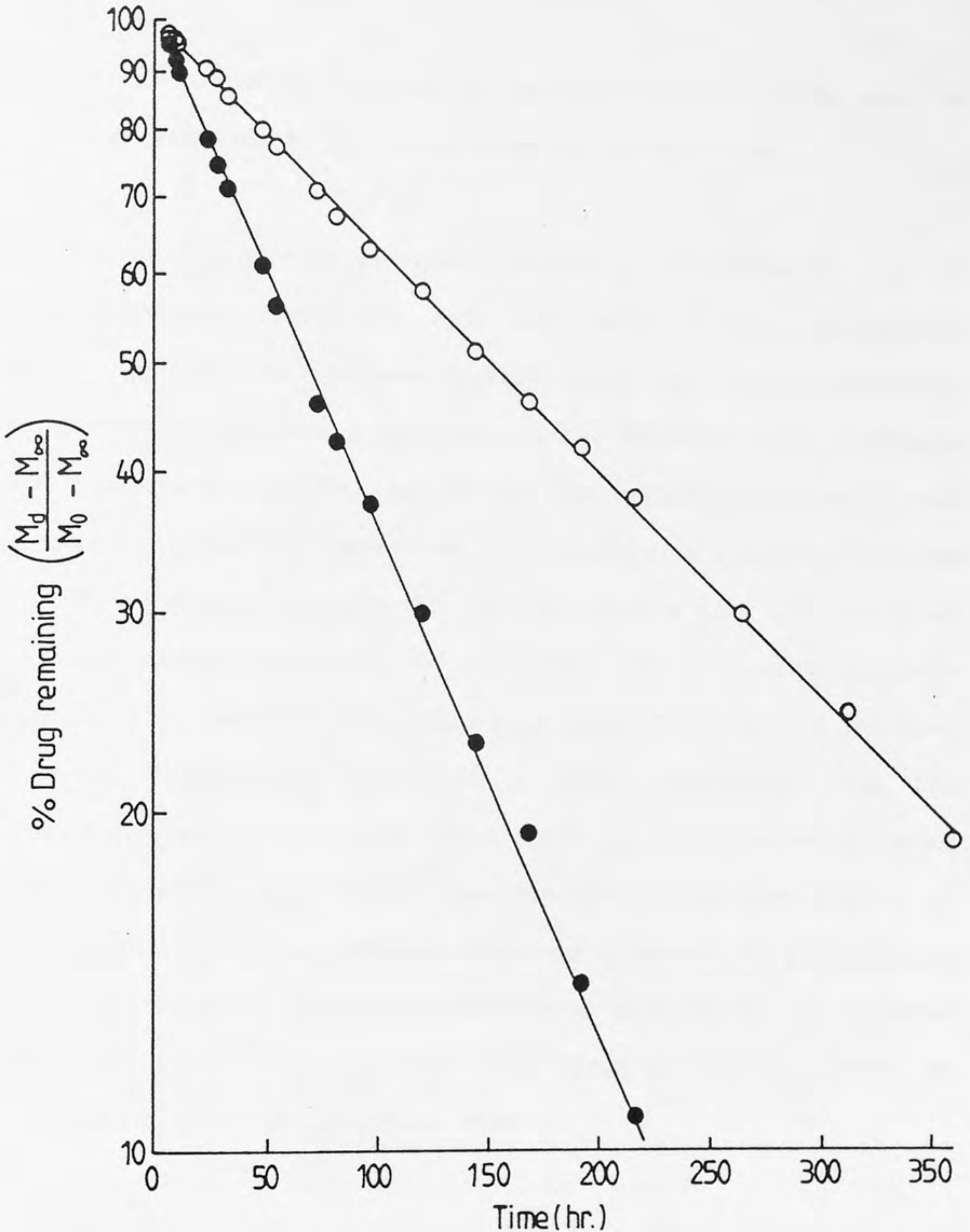


Fig. 7.5 First order plots showing the in vitro fractional release of ibuprofen and flufenamic acid from a 50% propylene glycol-water solution through human skin at 37°C.

KEY — Drug remaining in the donor phase

- ● flufenamic acid
- ○ ibuprofen
- M_d mass of the drug in the donor phase at time t
- M_0 initial mass of the drug in the donor phase
- M_∞ mass of the drug in the donor phase at infinite time

The definition of M_∞ included in equation (7.16) is the mass or amount of the drug left in the donor phase at infinite time.

No sign of degradation was observed during the diffusion runs of mixtures containing flufenamic acid and each of the susceptable compounds. In order to overcome factors which may cause variability within and between specimens, the ratio of the diffusion rate constants for each compound studied to that of its accompanying flufenamic acid was used as an index for comparison in systems with identical vehicle composition. The data in Table 7.1 suggest clearly that the ratios of the (tetrahydro-2-furanyl)methyl- and that of the (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen were higher than that of the pure acid and of indomethacin which is a clear indication that the esterification process and thereafter changes in partitioning enhanced the skin penetration properties. Controls containing each mixture of compounds evaluated in the diffusion runs and incubated at 37°C for the same period were made in propylene glycol-water mixtures in the presence and absence of human skin sections. Both types of controls showed no sign of degradation of the ibuprofen esters.

Controls made in the absence of human skin sections showed the lack of change in their concentrations and is represented as follows:

Material in the mixture	Correlation between the concentration determined for each sample* with that originally introduced and the observed standard deviation
indomethacin	99.94 ± 0.97
flufenamic acid	99.96 ± 0.98
ibuprofen	99.93 ± 0.95
flufenamic acid	99.9 ± 0.673
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	99.91 ± 0.589
flufenamic acid	99.93 ± 0.663
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	99.97 ± 0.92
flufenamic acid	99.9 ± 0.698
cyclohexylmethyl ester of ibuprofen	99.9 ± 0.95
flufenamic acid	99.93 ± 0.99

* mean of fifteen samples.

The partition coefficients of indomethacin, flufenamic acid, ibuprofen and its esters between human skin and propylene glycol-water mixtures (binary system) were determined at 37°C (Table 7.2). The data in Table 7.2 show that the partition coefficient was dependent upon the concentration of propylene glycol and increases with decreasing the amount of propylene glycol in the system. Figures 3.6 and 3.7 show that the solubility of a compound with limited aqueous solubility can be improved or increased by the addition of a cosolvent. While in the case of skin diffusion the partition coefficient between the skin and the vehicle is one of the parameters which can be modified to facilitate the drug penetration (equation (7.13)). Therefore the partition coefficient can be optimized by decreasing the solubility of the drug in the vehicle. Figures 7.6(a,b) show the effect of propylene glycol concentration in the binary system on the partition coefficient of the

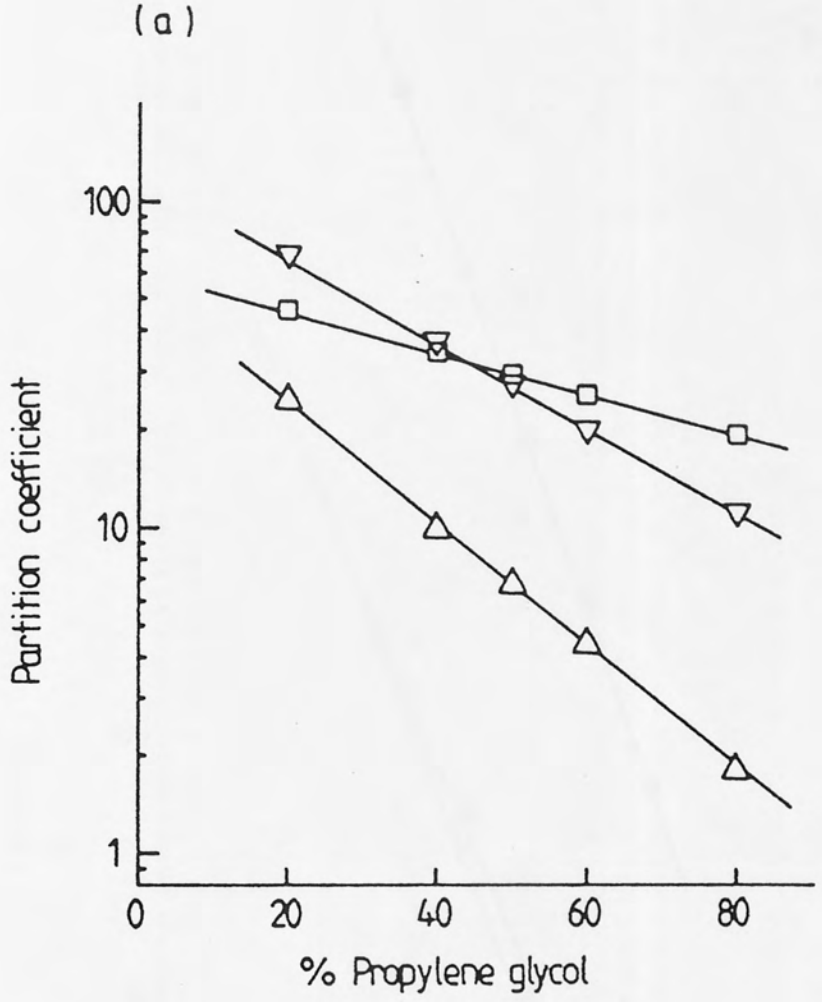


Fig. 7.6 (a,b) The effect of propylene glycol concentration on the partition coefficient between human skin and propylene glycol - water at 37° C.

KEY
▽ indomethacin
□ flufenamic acid
△ ibuprofen

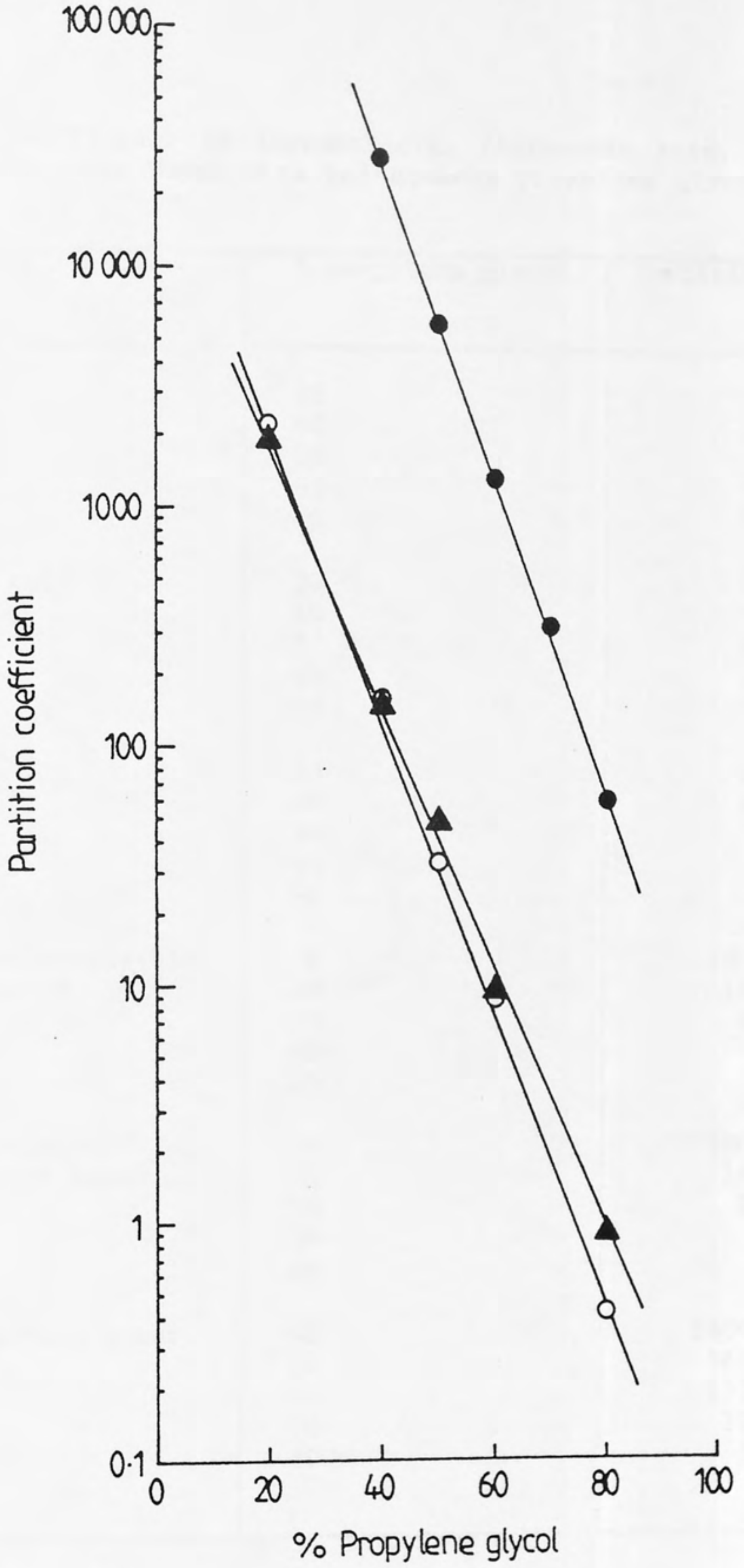


Fig. 7.6 (b)

KEY ▲ (tetrahydro-2-furanyl)methyl ester of ibuprofen
○ (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen
● cyclohexylmethyl ester of ibuprofen

TABLE 7.2

Partition coefficient of indomethacin, flufenamic acid, ibuprofen and its esters between human skin and aqueous propylene glycol mixtures at 37°C.

Compound	% propylene glycol	Partition coefficient
indomethacin	20	70.0
	40	38.0
	50	29.0
	60	21.0
	80	11.49
flufenamic acid	20	47.0
	40	35.2
	50	30.5
	60	26.4
	80	20.0
ibuprofen	20	25.0
	40	10.0
	50	6.8
	60	4.4
	80	1.8
(tetrahydro-2-furanyl)-methyl ester of ibuprofen	20	1900.0
	40	151.28
	50	49.0
	60	9.5
	80	0.88
(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen	20	2200.0
	40	160.0
	50	33.4
	60	9.0
	80	0.42
cyclohexylmethyl ester of ibuprofen	40	28000.0
	50	5673.7
	60	1312.17
	70	317.86
	80	58.72

compounds studied with the compounds produced by the esterification of ibuprofen showing improved partition coefficients over the free acid. Analysis of the data in Fig. 7.6 showed that the partition coefficients for each compound evaluated between the propylene glycol-water mixture

and the skin were highly dependent on the concentration of propylene glycol in the binary system and the relationship follows closely the expression proposed by Yalkowsky and Flynn(367):

$$\log (PC)_f = \log (PC)_w + \beta f \qquad (7.17)$$

where $(PC)_f$ is the partition coefficient of a substance between a binary system with volume fraction f of a given cosolvent (vehicle) and an immiscible solvent (or skin); $(PC)_w$ is the partition coefficient between water and the immiscible solvent and β is a constant.

The treatment of the data according to equation (7.17) gave:

1. For indomethacin

   $\log (PC)_f = \log (128.46) - 0.0131 f$ ($r = -0.9998$, $n=5$)

2. For flufenamic acid

   $\log (PC)_f = \log (61.83) - 6.136 \times 10^{-3} f$ ($r = -0.9999$, $n=5$)

3. For ibuprofen

   $\log (PC)_f = \log (59.14) - 0.01892 f$ ($r = -0.9997$, $n=5$)

4. For the (tetrahydro-2-furanyl)methyl ester of ibuprofen

   $\log (PC)_f = \log (2.6025 \times 10^4) - 0.056 f$ ($r = -0.999$, $n=5$)

5. For the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen

   $\log (PC)_f = \log (4.2815 \times 10^4) - 0.062 f$ ($r = -0.9993$, $n=5$)

6. For the cyclohexylmethyl ester of ibuprofen

$$\log (PC)_f = \log (1.21 \times 10^7) - 0.066f \quad (r= -0.9996, n=5)$$

The permeability coefficient $P(\frac{DK}{h})$ and the diffusion coefficient (D) of indomethacin, flufenamic acid, ibuprofen and its esters through human skin from solutions made in propylene glycol-water mixtures were calculated according to equation (7.13) from their corresponding diffusion rate constants $(\frac{PS}{V_d})$ recorded earlier in Table 7.1. Values are recorded in Table 7.3 together with those of flufenamic acid as internal standard.

TABLE 7.3

The permeability and diffusion coefficients of flufenamic acid, indomethacin, ibuprofen and its esters through human skin from aqueous propylene glycol mixtures at 37°C

Mixture studied	% propylene glycol in the diffusion medium	Permeability coefficient P cm/hr × 10^3	Diffusion coefficient D $cm^2/hr \times 10^6$
indomethacin	50	5.42	3.737
flufenamic acid		40.4	26.22
ibuprofen	50	9.44	15.27
flufenamic acid		21.05	7.591
(tetrahydro-2-furanyl)-methyl ester of ibuprofen	50	10.22	1.981
flufenamic acid		20.95	6.52
(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen	50	11.93	3.571
flufenamic acid		16.44	5.39
cyclohexylmethyl ester of ibuprofen	80	2.45	1.043
flufenamic acid		3.119	3.898

The diffusion coefficient is one of the key factors which affect

the rate of diffusion and can be used for the interpretation of the kinetics of interfacial transfer. A number of methods have been devised in the literature for the measurement of the diffusion coefficient and several types of diffusion cells have been constructed for this purpose(361,364,368-370).

One method for the estimation of the diffusion coefficient standardises the system with methyl or ethyl nicotinate. The method calibrates the diffusion cell by calculating the diffusion rates (k_s) of the standard (e.g. methyl nicotinate) of known diffusion coefficient D_s across the chosen membrane. The rate of diffusion k_x for the test compound, is then determined to give the diffusion coefficient D_x(368) provided that the system variables are controlled.

$$D_x = D_s k_x / k_s \tag{7.18}$$

The diffusion coefficients of indomethacin, flufenamic acid, ibuprofen and its esters from solutions in 50% propylene glycol-water were determined at 37°C using a modified form of the Wurster permeability cell(364,369) equipped at the interface with Polyvic membrane filter (Pore Size: 2 μm , Composition: Polyvinylchloride copolymer + trace amount of silica) (Millipore Corporation, USA) and calibrated with methyl nicotiante (D_s = 0.0432 cm^2/hr.)(371). Solutions made in 50% propylene glycol-water were used throughout. The release process followed first order kinetics when unit thermodynamic activity was not maintained by the solutions in the donor phase. The diffusion rate constants (k_x), equivalent to the absolute slopes of lines in Fig. 7.7(a,b), were determined by fitting the data to equation (7.16) and parameters are recorded in Table 7.4.

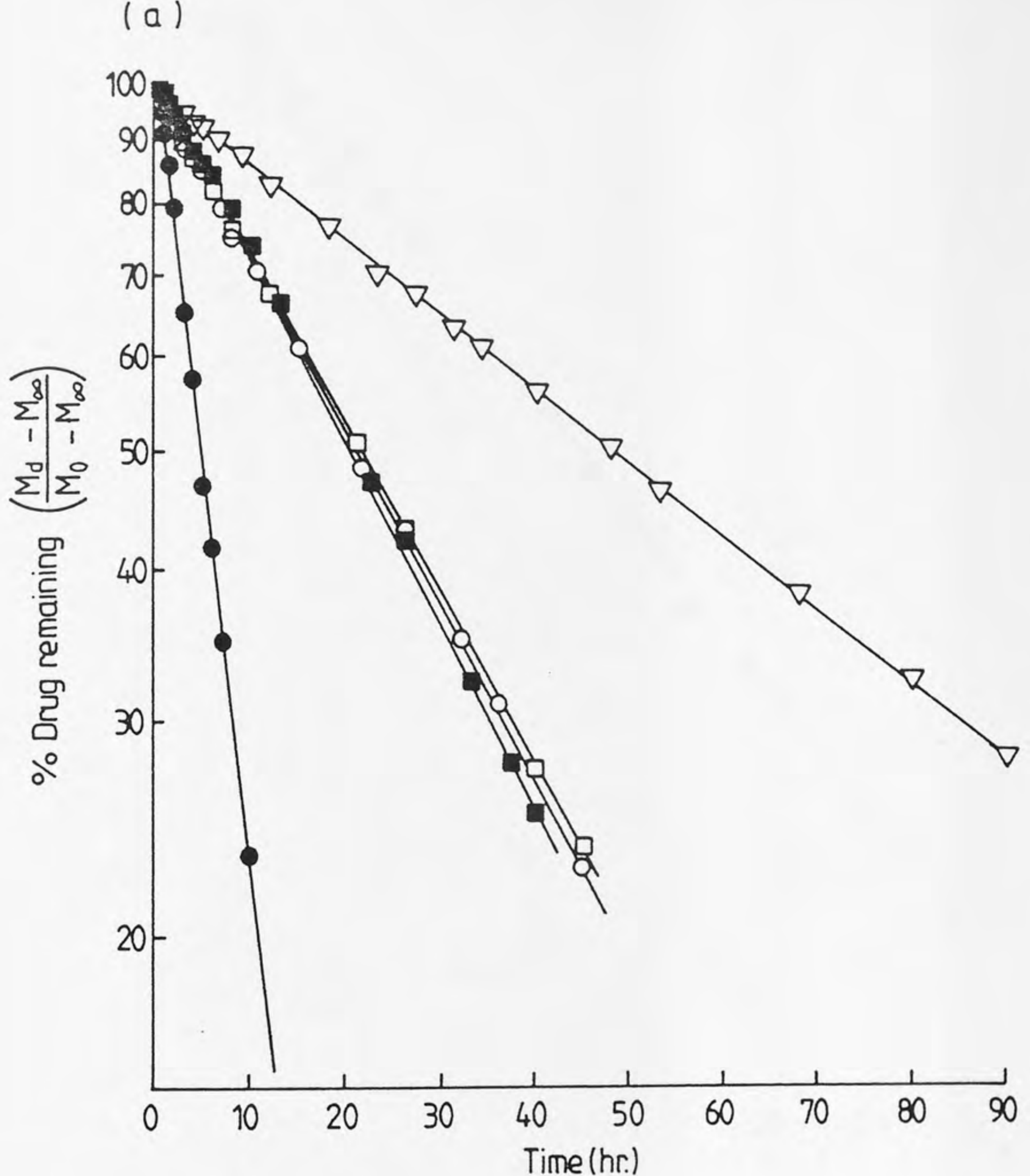


Fig. 7.7 (a,b) First order plots showing the fractional release of flufenamic acid, methyl nicotinate, indomethacin, ibuprofen and its esters from 50% propylene glycol-water solutions through a membrane filter at 37°C, used for the purpose of calculating the diffusion coefficient.

KEY

Cell	Symbol	Material
1	■	(tetrahydro-2-furanyl)methyl ester of ibuprofen
	▽	Cyclohexylmethyl ester of ibuprofen
	○	ibuprofen
	□	indomethacin
	●	methyl nicotinate

(Methyl nicotinate was used to calibrate the diffusion cell)

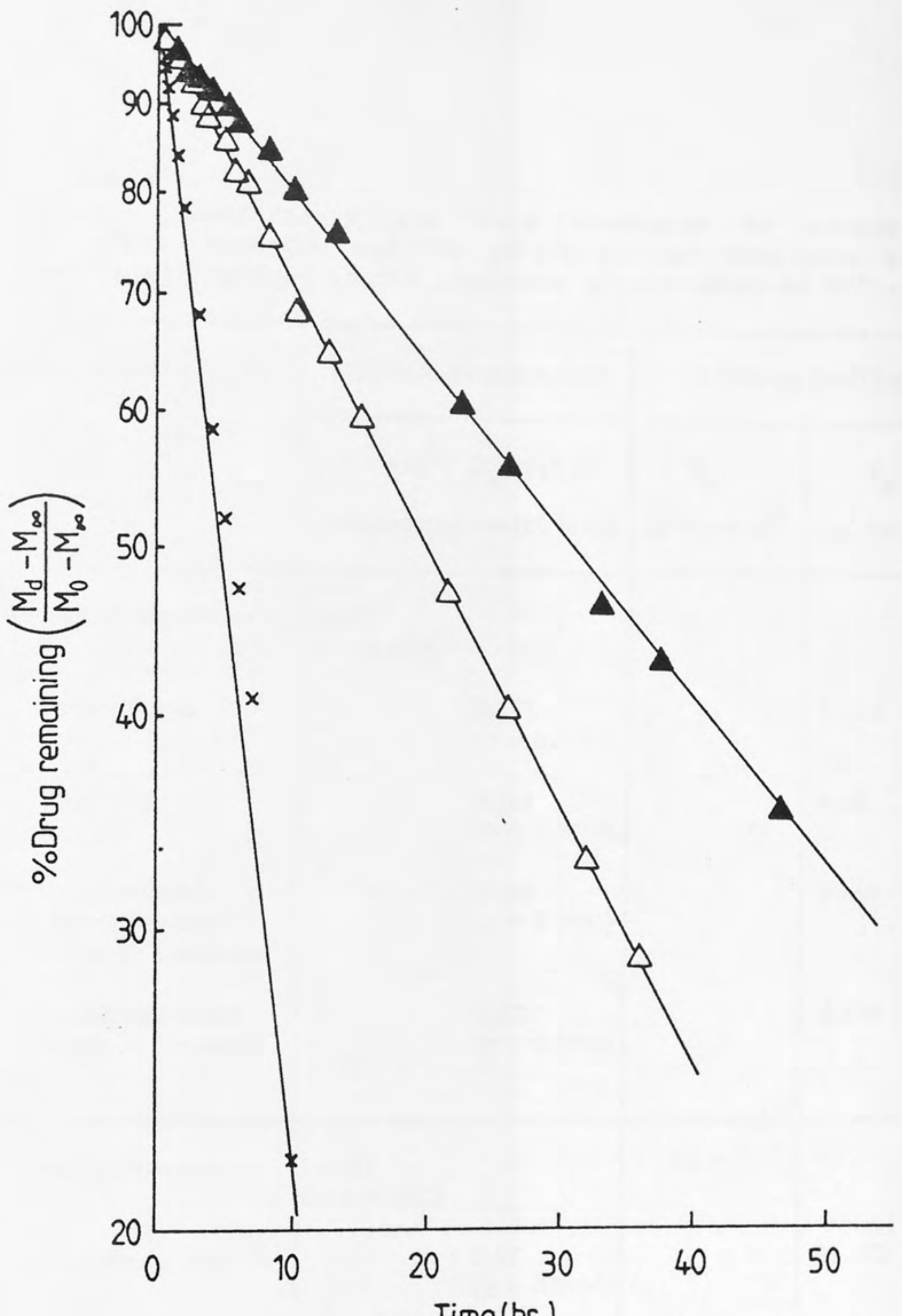


Fig. 7.7 (b)

KEY

Cell	Symbol	Material
2	▲	(tetrahydro-2-(2H)pyranyl) methyl ester of ibuprofen
	△	flufenamic acid
	×	methyl nicotinate

(Methyl nicotinate was used to calibrate the diffusion cell)

TABLE 7.4

The diffusion coefficients and rate constants of indomethacin, flufenamic acid, ibuprofen and its esters through Millipore membrane filter from solutions made in 50% propylene glycol-water at 37°C.

Diffusion Cell	Material studied	Diffusion rate Constant		Diffusion Coefficient	
		k_s $hr.^{-1} \times 10^2$	k_x $hr.^{-1} \times 10^2$	D_s	D_x
		(r=regression coefficient)		$cm^2/hr \times 10^3$	$cm^2/hr \times 10^3$
1	methyl nicotinate	16.022 (r = 0.9991)		43.2	
	indomethacin		3.173 (r = 0.9997)		8.553
	ibuprofen		3.219 (r = 0.9999)		8.68
	(tetrahydro-2-furanyl)methyl-ester of ibuprofen		3.436 (r = 0.9991)		9.266
	cyclohexylmethyl-ester of ibuprofen		1.371 (r = 0.9992)		3.696
2	methyl nicotinate	13.042 (r = 0.9992)		43.2	
	flufenamic acid		3.47 (r = 0.9994)		11.493
	(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen		2.244 (r = 0.9996)		7.434

The fraction remaining in the donor phase was calculated following the same principle discussed earlier for the diffusion process through human skin. The total amount of each compound recovered at infinite time shows the lack of binding to the membane filter and recorded as follows:

Diffusion cell	Compound evaluated	Correlation between the amount recovered* at infinite time from both donor and receiver compartments with the amount introduced originally
1	Methyl nicotinate	99.89
	indomethacin	99.9
	ibuprofen	99.91
	(tetrahydro-2-furanyl)-methyl ester of ibuprofen	99.86
	cyclohexylmethyl ester of ibuprofen	99.87
2	methyl nicotinate	99.93
	(tetrahydro-2-(2H)-pyranyl)methyl ester of ibuprofen	99.96
	flufenamic acid	99.9

* mean of three readings.

The diffusion coefficients of the above mentioned compounds were calculated by substituting their corresponding rate constants k_x in equation (7.18) and are similarly recorded in Table 7.4.

According to equation (7.16) the diffusion rate constant is equal to $\frac{KDS}{V_d h}$ and therefore the knowledge of the parameters involved can be used to calculate the value of the diffusion coefficient.

The partition coefficient (K) of indomethacin, flufenamic acid, ibuprofen and its esters were determined at 37°C between the membrane

filter and solutions made in 50% propylene glycol-water and are recorded as follows:

Material studied	Partition coefficient* between the membrane filter and propylene glycol-water
indomethacin	0.382
flufenamic acid	0.863
ibuprofen	1.249
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	0.903
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	1.204
cyclohexylmethyl ester of ibuprofen	1.711

* mean of four readings.

The diffusion coefficients calculated from substituting these parameters into the value of the slopes were in close agreement with those obtained from equation (7.18) and are recorded as follows:

Material studied	Diffusion coefficent D $cm^2/hr. \times 10^3$
indomethacin	8.728
flufenamic acid	4.561
ibuprofen	2.713
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	4.005
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	2.117
cyclohexylmethyl ester of ibuprofen	0.843

The diffusion of indomethacin, flufenamic acid, ibuprofen and its esters was monitored through human skin and Millipore membrane filter at 37°C from solutions made in 50% propylene glycol-water. Values varied between the two membranes with smaller coefficient being obtained for the diffusion through the skin.

7.4.2 Diffusion Studies Through Rat Skin

For many purposes, *in vitro* penetration experiments are advantageous. They may be prefered for evaluating a series of compounds or for comparing several formulations of a drug to see which has the most favourable skin penetration characteristics. The main reason for accepting the results of experiments using excised skin is the fact that the stratum corneum, the principle diffusional barrier in skin, is dead tissue even in the living organism and provided it is not damaged, resistance to diffusion may still be expected *in vitro*. Drugs absorbed through living skin may be changed enzymatically; but this would occur after the rate-limiting transport process has taken place.

In order to simulate the effect of living skin, pieces obtained from freshly killed rats can be used in the diffusion cells. The susceptability of ibuprofen esters to hydrolysis by the cutaneous esterases present in rat skin homogenates was discussed in Section 6.4. Due to the low aqueous solubility of the esters it was recommended that the concentration of the cosolvent to be added was minimized to reduce denaturation of the protein. From the diffusion equations (7.8) and (7.13), the partition coefficient between the skin and vehicle and the concentration of the drug in solution in the vehicle are among the parameters which can be modified to facilitate drug penetration. The

partition coefficient is a measure of the ability of the drug to escape from the vehicular phase and is defined as the equilibrium solubility of drug in the surface of the stratum corneum (barrier) relative to its solubility in the vehicle. Therefore, according to the data in Table 7.2 the value of the partition coefficient was improved with decreasing the concentration of propylene glycol in the binary phase. The concentration of the drug in the vehicle (C_d) is a measure of its thermodynamic activity. With a nonconstant source, as in the case of release from solutions, the activity decreases as the drug diffuses through the membrane and the release rate falls exponentially with time. Unit thermodynamic activity ensures constant release of drug, following zero order kinetics, by using a saturated solution in the presence of excess solid drug.

The diffusion of mixtures containing either the (tetrahydro-2-furanyl)methyl- or (tetrahydro-2-(2H)pyranyl)methyl esters of ibuprofen together with flufenamic acid through rat skin (from freshly killed animals) was followed at 37°C from suspensions made in 20% propylene glycol-water. Ibuprofen and flufenamic acid were monitored on the receiver side of both diffusion runs without a trace of the ester introduced originally in the donor phase and the amounts released with time are shown in Figs 7.8 and 7.9.

The detection of ibuprofen in the receiver side indicates that the rat skin cutaneous enzymes are still active. The enzymic effect was expected and taken into consideration when planning the experiment thus diffusion samples were treated immediately with acidified acetonitrile in order to quench any reaction. No trace of the parent esters was found in the receiver phase suggesting that hydrolysis is substantially

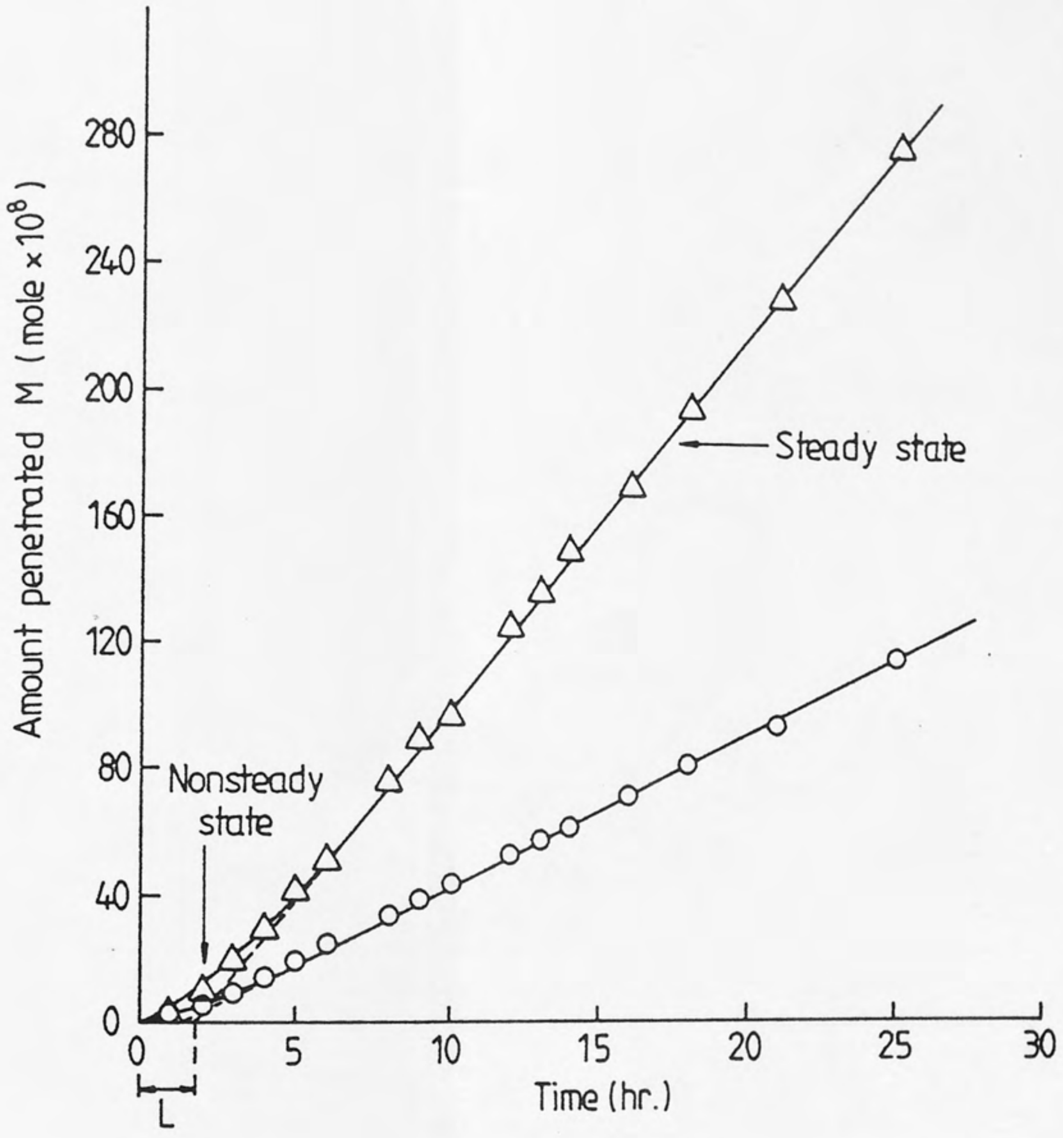


Fig. 7.8 The diffusion of-flufenamic acid and (tetrahydro-2-(2H) pyranyl) methyl ester of ibuprofen through rat skin from a suspension in 20% propylene glycol-water at 37°C.

KEY. ○ ibuprofen formed
△ flufenamic acid

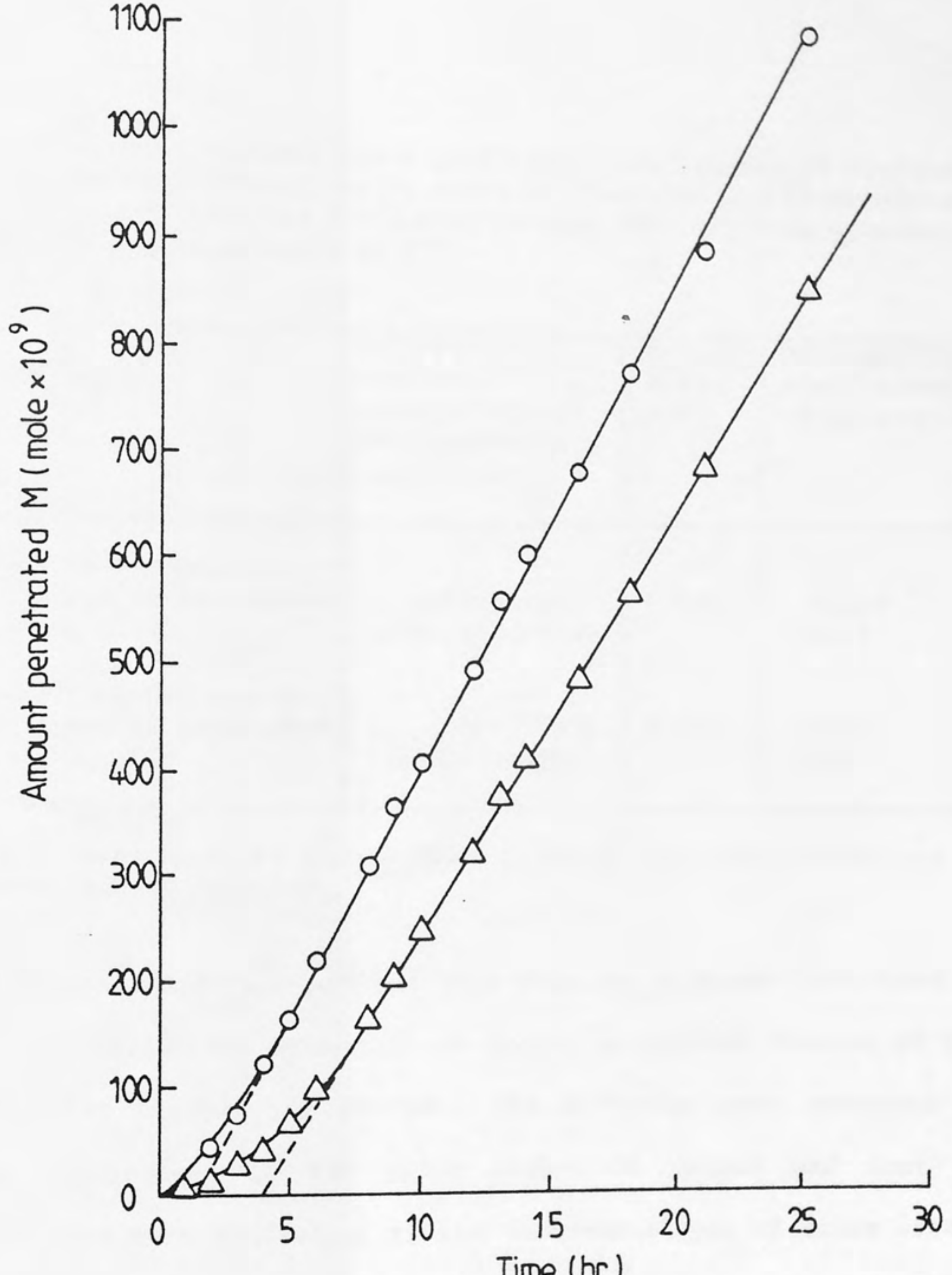


Fig. 7.9 The diffusion of flufenamic acid and (tetrahydro-2-furanyl) methyl ester of ibuprofen through rat skin from a suspension in 20% propylene glycol-water at 37°C

KEY : ○ ibuprofen formed
△ flufenamic acid

faster than diffusion for these compounds.

TABLE 7.5

The diffusion rate constants and permeability coefficients of flufenamic acid, (tetrahydro-2-furanyl)methyl ester of ibuprofen and (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen through rat skin from suspensions in 20% propylene glycol-water at 37°C

Mixture evaluated	Diffusion rate constant k mole/hr.$\times 10^8$ (r= regression coefficient)	Slope Ratio a/b	Permeability coefficients P cm/hr.$\times 10^2$
a (tetrahydro-2-furanyl)-methyl ester of ibuprofen*	4.622(r=0.999)	1.160	9.405
b flufenamic acid	3.983(r=0.9993)		37.14
a (tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen*	4.766(r=0.9997)	0.400	9.817
b flufenamic acid	11.89(r=0.9998)		110.87

* rate constants were the steady state rates of ibuprofen normalised to its ester amount in moles.

The data in Figs. 7.8 and 7.9 show that the mixtures introduced in the form of suspensions were able to ensure a constant release of the drug after the initial lag period. The diffusion rate constants of ibuprofen (normalised to its ester amount in moles) and that of flufenamic acid were equivalent to the absolute slopes of lines at the steady state according to equation (7.9) and are recorded in Table 7.5. The ratio of the diffusion rate constants of the esters (represented by ibuprofen) to that of their accompanied flufenamic acid (reference compound) (Table 7.5) show clearly that the diffusion process was improved when using a suspension in 20% propylene glycol-water compared to solutions. The permeability coefficients were calculated from the values of the diffusion rate constants (= PSC_d) and are similarly recorded in Table 7.5. The solubility of each compound in 20%

propylene glycol-water was substituted for the value of C_d in equation (7.9).

No degradation of the ibuprofen ester was detected in the donor phase.

Compound	Solubililty (mg/100 ml) in 20% propylene glycol-water mixture
flufenamic acid	0.947
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	4.531
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	1.51

Controls made in 20% propylene glycol-water in the absence of rat skin sections failed to show any sign of degradation. The lack of change in the concentration of these controls is represented as follows:

Material in the Mixture	Correlation between the concentration determined for each sample* with that originally introduced and the observed standard deviation
(tetrahydro-2-furanyl)methyl-ester of ibuprofen	99.95 ± 0.865
flufenamic acid	99.93 ± 0.993
(tetrahydro-2-(2H)pyranyl)-methyl ester of ibuprofen	99.94 ± 0.99
flufenamic acid	99.98 ± 0.91

* mean of fifteen samples.

Rat cutaneous enzymes preserved some of their activity even under the unfavourable pH condition of the vehicles made in 20% propylene glycol-water (pH = 4.5) and resulted in the hydrolysis of ibuprofen esters to a certain extent in the mixtures employed for the determination of the partition coefficients and in controls monitored in the presence of rat skin. No values were therefore calculated for the partition coefficients.

CHAPTER 8

SUMMARY AND CONCLUSION

Reversed-phase high-performance liquid chromatographic methods were developed for the assay of indomethacin and its degradation products, flufenamic acid and ibuprofen and its ethyl-, (tetrahydro-2-furanyl)methyl-, (tetrahydro-2-(2H)pyranyl)methyl- and cyclohexylmethyl-esters. The developed mobile phases consisted of aqueous methanolic mixtures acidified with orthophosphoric acid to give a final pH of 2.5. These methods were found to be efficient in the separation of these compounds during the determination of their physical properties, and for kinetic and skin permeation studies. The resolution of indomethacin from its degradation products, p-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid and the sensitivity of the HPLC method were advantageous over the ultraviolet spectrophotometric method. The sample solvent alcohol content was found to affect the chromatography of each individual constituent in the mixture and therefore, the solvent composition of samples and standards were always identical.

To aid the design of a topical drug delivery system, the physico-chemical properties of the drugs were studied. Indomethacin and ibuprofen are weak organic acids and their pK_a values were found to be 4.2 and 4.5 respectively. Unionized species penetrate skin more readily than do the conjugate ionic species and, therefore, pH of the vehicle may be an important factor in determining absorption rate.

Poor aqueous solubility was exhibited by ibuprofen, its esters and by indomethacin. This was improved by the incorporation of a cosolvent,

as ethanol or propylene glycol, into the medium. The relationship between the amount of the drug solubilized and the cosolvent concentration did not fit the established solubility relationships. This solubility enhancement is related to the lowering of the dielectric constant of the medium and this can be attributed to a reduction in the solvent polarity and the effect of the cosolvent in separating the aggregated water molecules.

Kinetic studies were used to follow the stability of idomethacin and ibuprofen esters and the effect of several parameters on their decomposition rates. Indomethacin was found to be more labile than the (tetrahydro-2-(2H)pyranyl)methyl ester of ibuprofen and their activation energies were 68.3 (in Teorell-Stenhagen's buffer pH 9.7) and 84.2 (in 10% ethanol-Teorell-Stenhagen's buffer pH 7.0) kJ/mole respectively. Both compounds underwent the specific acid-base catalysed hydrolysis.

The non-iso approach for studying the effect of temperature, pH and surfactant concentration on the stability of indomethacin offers a significant reduction in experimental effort. The analysis of the data was undertaken using NONISO, a BASIC computer program. The data obtained from both iso and non-iso kinetic studies were in close agreement with each other. The NONISO program provided a rapid and versatile method for the analysis of the non-iso data and a full kinetic profile was obtained from one single experiment. Several theoretical and/or practical limitations may affect the non-iso kinetic approach. These may include the changes in the dependence on temperature or pH during the decomposition process and thus may lead to erroneous results in estimating the decomposition parameters and profiles. Such limitations may be avoided and deviations can be rapidly detected from

plots obtained from iso-kinetic runs which make them essential as a pre-requisite to using this data analysis.

The low aqueous solubility of indomethacin may require the incorporation of cosolvent such as alcohols and glycols into formulations. Under alkaline conditions, indomethacin alcoholysis and degradation was readily observed in the presence of methanol or ethanol. The developed HPLC system was used to elucidate the decomposition pathways. The hydrolysis was modelled using equations involving the dielectric constant of the medium. This reaction may compromise stability assessments, pharmaceutical integrity and delivery profiles. It may largely be avoided by lowering the pH of the medium to pH 6.0, and suggesting that an acidic pH is a pre-requisite for the topical formulation of indomethacin using vehicles based on alcohols or glycols.

The metabolic biotransformation of ibuprofen esters using esterase from hog liver and rat skin homogenates showed that the esters were very labile under these conditions. This activity has great pharmacological and toxicological importance because the skin is one of the largest organs of the body and contributes significantly to the ability of the body to metabolise drugs. The sensitivity of ibuprofen esters to cutaneous esterases, upon application to the skin, may result in a better delivery of the parent acid through absorption of the ester and subsequent cutaneous metabolism.

The percutaneous absorption of indomethacin, and of ibuprofen and its esters was followed using an _in vitro_ excised skin model. The diffusion results were correlated with physico-chemical parameters of

the drugs discussed earlier.

The kinetics of diffusion from solutions and suspensions were also studied. The diffusion of ibuprofen esters through rat skin confirmed the earlier enzymatic observations and only ibuprofen was detected in the receiver phase in such experiments. The activation of ibuprofen esters following percutaneous absorption is of importance in their topical formulations. Future work needs to be conducted on *in vitro-in vivo* correlations and the fitting of mathematical models to *in vivo* data.

APPENDIX 1

Preparation of Constant Ionic Strength McIlvaine Buffered Solutions(238)

pH desired at 25°C	Composition G/litre Solution $Na_2HPO_4 \cdot 12 H_2O$	$H_3C_6H_5O_7 \cdot H_2O$	Buffer System Ionic Strength M	G. KCl Added Per Litre of Solution to Produce Ionic Strength of 1M
2.2	1.43	20.6	0.0108	74.5
2.4	4.44	19.7	0.0245	72.7
2.6	7.8	18.7	0.041	71.5
2.8	11.35	17.7	0.0592	70.2
3.0	14.7	16.7	0.0771	68.7
3.2	17.7	15.8	0.0934	67.6
3.4	20.4	15.0	0.112	66.2
3.6	21.5	14.2	0.128	64.9
3.8	25.4	13.6	0.142	64.0
4.0	27.6	12.9	0.157	62.8
4.2	29.7	12.3	0.173	64.7
4.4	31.6	11.7	0.19	60.4
4.6	33.4	11.2	0.21	58.9
4.8	35.3	10.7	0.232	57.2
5.0	36.9	10.2	0.256	55.5
5.2	38.4	9.75	0.278	53.8
5.4	40.0	9.29	0.302	52.1
5.6	41.5	8.72	0.321	50.6
5.8	43.3	8.32	0.336	49.5
6.0	45.2	7.74	0.344	48.9
6.2	47.5	7.12	0.358	47.9
6.4	49.6	6.47	0.371	46.9
6.6	52.1	5.72	0.385	45.8
6.8	55.4	4.79	0.392	44.5
7.0	58.9	3.7	0.427	42.7
7.2	62.3	2.74	0.457	40.4
7.4	65.0	1.91	0.488	38.2
7.6	67.2	1.35	0.516	36.0
7.8	68.6	0.893	0.540	34.3
8.0	69.6	0.589	0.559	32.9

(From Anal. Chem., 1956, 28(7), 1179-1180)

APPENDIX 2

Preparation of Teorell-Stenhagen's Buffer(239)

Stock Solution A: To citric acid and phosphoric acid solutions (Ca. 100 ml), each equivalent to 100 ml NaOH 1-N, add 3.54 g crystalline orthoboric acid and 343 ml NaOH 1-N, and make up the mixture to 1 l.

Stock Solution B: Hydrochloric acid 0.1 N.

Composition of the Buffer: 20 ml A + x ml B made up to 100 ml.

The table gives the amounts (x ml) of the stock solutions listed above required to make up a buffer solution of the desired pH value.

pH	x (ml)	pH	x (ml)
2	74.4	7.0	32.9
2.2	68.8	7.2	31.7
2.4	64.6	7.4	30.6
2.6	61.3	7.6	29.6
2.8	58.9	7.8	28.8
3.0	56.9	8.0	28.1
3.2	55.2	8.2	27.6
3.4	53.9	8.4	27
3.6	52.9	8.6	26.3
3.8	51.8	8.8	25.2
4.0	50.7	9.0	24
4.2	49.7	9.2	22.6
4.4	48.6	9.4	21.4
4.6	47.5	9.6	20.2
4.8	46.4	9.8	19
5.0	45.4	10.0	18.1
5.2	44.3	10.2	17.1
5.4	43.2	10.4	16.5
5.6	42	10.6	16.0
5.8	40.8	10.8	15.5
6.0	39.7	11.0	14.7
6.2	38.4	11.2	13.5
6.4	37	11.4	11.7
6.6	35.6	11.6	9.1
6.8	34.2	11.8	5.5
		12.0	1.3

(From Biochem. Z., 1938, 299, 416-419)

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