PRO-DRUGS OF BETA-BLOCKING AGENTS

By KHALIFA ALI BELAID

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SUMMARY

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To study the design of liquid controlled-release dosage forms based upon ion-exchange resins a series of ester prodrugs of propranolol were synthesised. Compounds ranging from acetyl to decanoyl were prepared and structural assignments were confirmed by 1H n.m.r. spectroscopy and mass-spectrometry. The physico-chemical properties including solubility, pKa and partition coefficient were studied and chemical and enzymatic stability kinetics of these esters were also investigated using high-performance liquid chromatography (HPLC) as the analytical technique. HPLC methods were developed for the quantitative analysis of the pro-drugs and their degradation products during kinetic assessments of stability and release profiles.

The degradation of O-n-acyl propranolols under alkaline conditions was found to proceed via competing hydrolysis, to give the required propranolol and rearrangement to yield the corresponding N-acyl derivatives. The N-acyl derivatives were stable under these conditions and reaction did not proceed further. The formation of N-acyl derivatives is pH dependent with reaction being largely inhibited under acidic conditions. The hydrolysis rate is also dependent upon pH with a maximum stability demonstrated at pH 3 - 3.5. The rearrangement reaction was much suppressed with ester chain lengths higher than the O-hexanoyl derivative. In enzymatic systems using isolated esterases and 90% rabbit serum, the reactivity profiles were somewhat different. The specifity of the enzyme was such that the competing rearrangement reaction was much reduced and with ester homologues larger than O-propionyl, no rearrangement was detected at all. The generation of propranolol under these conditions was found to occur at a maximal rate with the O-hexanoyl derivative but all compounds showed acceptable release rates for propranolol.

These pro-drugs have been incorporated into controlled-release delivery systems suitable for formulation as liquid dosage forms using cationic ion-exchange resin. The effect of drug structure and the properties of the resin on the release profiles were studied to expose the factors controlling drug availability from these complexes. Better control of release rates was achievable when the drug-resin complex was coated with gelatin-acacia co-acervate and the liberation of the drug was further reduced when beads were double coated. The biovailability of the sustained-release formulation was studied following administration of an oral single-dose using aqueous propranolol as the standard reference.

KEY WORDS :

Propranolol pro-drugs
High-performance liquid chromatography
Stability
Ion-exchange resins
Sustained-release formulations
Bioavailability

DEDICATION

I would like to dedicate this project to my wife Drissia and my children Amal, Sami, Mohammed and Hana for their relentless spirits during the preparation of this work and throughout the three years of this course.

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CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

1.1 CHEMICAL STRUCTURES OF BETA-ADRENORECEPTOR BLOCKING AGENTS

Beta-adrenoreceptor blocking agents are derivatives of beta-amino alcohols. They are characterised by the presence of both a secondary alcohol and a secondary amine in the aliphatic side chain.

Proprietory names and chemical structures of some beta-adrenoreceptor blocking agents are summarised in Fig 1.1.

1.2 HISTORICAL CONCEPT

The modern concept of adrenergic receptors was first described by Ahlquist 1948 (1). Ahlquist studied the structure-activity relationships of a small group of sympathomimetic amines using a variety of tissue responses (in both isolated organ and intact animal) and he observed two distinct orders of potency. It was proposed that these responses were due to the presence of two different types of adrenergic receptors; designated alpha (α) and beta (ß).

The first beta-adrenoreceptor blocking agent Dichloroisoproterenol (DCI) was synthesised in 1958 [*Powell and Slater 1958 (2)*], it had some intrinsic beta-adrenergic stimulatory activity but it was not clinically used. In 1962 another beta-adrenergic blocking agent was synthesised (pronethalol), which was pharmacologically more active than DCI but it was withdrawn quickly because it was shown to produce tumours in animals. A further beta-blocker was synthesised (propranolol), which was introduced and approved for the treatment of hypertension in 1964, for cardiac arrhythmias in 1968 and for the treatment of angina pectoris in 1973.

Many beta-blockers have been synthesised in addition to propranolol and differ in their effects on cardiac and bronchial beta-receptors and direct membrane depressant properties.

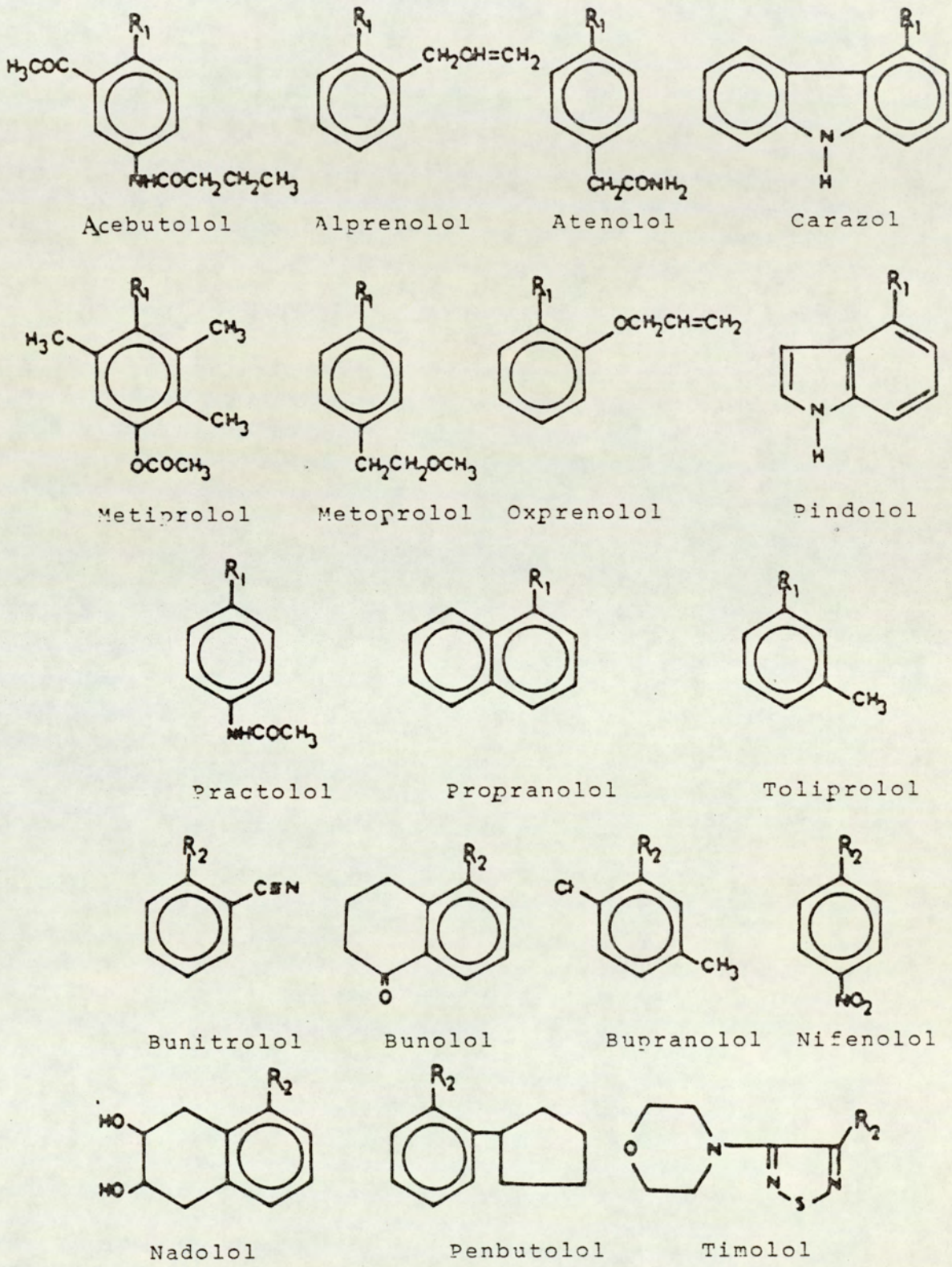
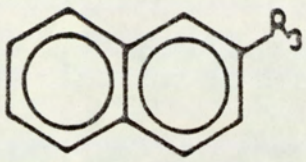
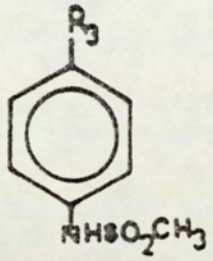


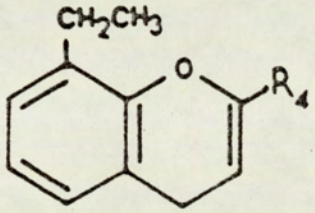
Fig. 1.1. Chemical Structures of Beta-blockers.



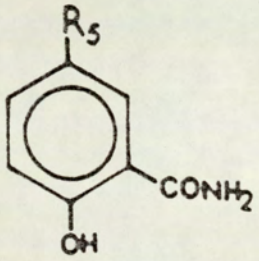
Pronethalol



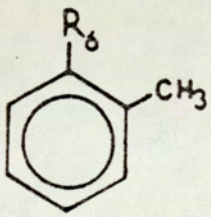
Sotalol



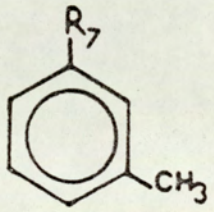
Bufuralol



Labetalol



Tolamolol



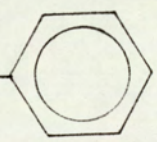
Bevantolol

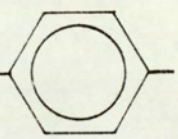
R_1 = -$OCH_2CH(OH)CH_2NHCH(CH_3)_2$

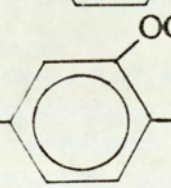
R_2 = -$OCH_2CH(OH)CH_2NHC(CH_3)$.

R_3 = -$CH(OH)CH_2NHCH(CH_3)_2$.

R_4 = -$CH(OH)CH_2NHC(CH_3)_3$.

R_5 = -$CH(OH)CH_2NH(CH_3)CH_2CH_2$- 

R_6 = -$OCH_2CH(OH)CH_2NHCH_2CH_2O$-  -$CONH_2$.

R_7 = -$OCH_2CH(OH)CH_2NHCH_2CH_2$-  -OCH_3.

Cont'd Fig. 1.1.

1.3 PHARMACOLOGY

Beta-adrenoreceptor blocking agents competitively inhibit the action of the adrenergic agonist on beta-receptors.

Excitation of Beta-receptors is known to trigger the formation of adenosine cyclic monophosphate "cAMP" (Fig 1.2) by adenylate cyclase. The receptor and the enzyme are situated in close proximity, with the receptor on the outside of the membrane and the enzyme on the inside. As soon as the receptor binds with the neurotransmitter, the enzyme produces cAMP which is the intracellular mediator for the action of cathecholamines [*Sutherland and Rall 1960 (3) and Rosen 1970 (4)*].

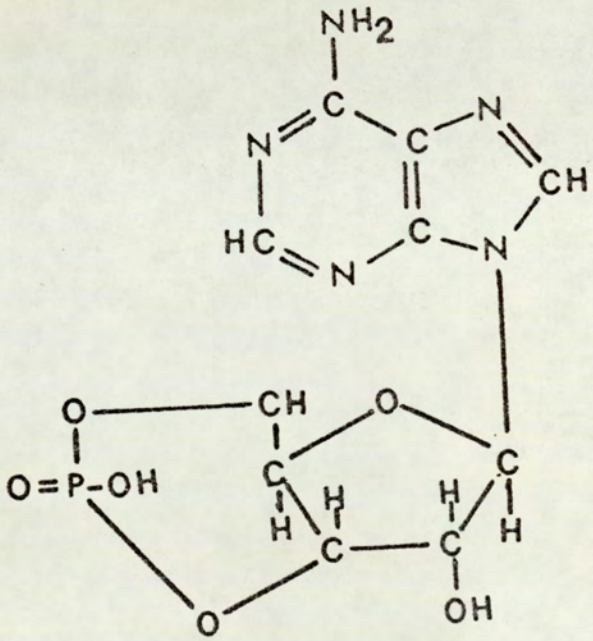


Fig 1.2. Adenosine Cyclic Monophosphate (cAMP)

Two types of beta-receptors have been distinguished [*Lands et al 1967 (5)*], $ß_1$ and $ß_2$ receptors. $ß_1$ receptors increase the rate of muscle contraction of the heart, dilatation of the coronary blood vessels and cause relaxation of the smooth muscles of the gastro-intestinal tract. The $ß_2$ receptors cause relaxation of the smooth muscles in the bronchi, uterus and arteries which supply skeletal muscles. Thus, the effect of beta-adrenoreceptor blocking

agents is to decrease the heart rate, myocardial contractility, cardiac output and arterial blood pressure and also to inhibit the action of other ß-adrenergic agonists on beta-receptors. Evans et al 1979 (6) and Scriabene 1979 (7) classified beta-adrenoreceptor blocking agents according to their cardio-selectivity and membrane stabilizing activity into three divisions as shown in Table 1.1.

The cardio-selectivity of beta-blockers is attributed to their inhibition of the cardiac beta-receptors ($ß_1$) with little influence on the bronchial and vascular beta-receptors ($ß_2$). The overall response to a beta-adrenergic blocking agent may be modified by other properties of drug in question.

The ß-receptor stimulation (intrinsic sympathomimetic activity) is an important component of the response to some beta-blockers. Some beta-blockers also have an important direct action on the cell membranes which is commonly described as membrane stabilizing, local anaesthetic and quinidine-like. The local anaesthetic potencies of pronethalol and propranolol are about equal to that of lidocaine, oxprenolol is about half as potent and sotalol and practolol are almost devoid of this property. The membrane stabilizing action of propranolol has contributed to reported cardiac effects, but it appears to be of little clinical importance with the usual therapeutic doses.

Other results of this action include inhibition of osmotic lysis of erythrocytes [*Langstet 1970 (8)*], inhibition of 5HT (5-hydroxytryptamine) uptake by the blood platelets [*Lemmer et al 1972 (9)*] and inhibition of norepinephrine release from and uptake by adrenergic nerves [*Mylecharane and Raper 1973 (10)*].

1.3.1. THERAPEUTIC USES AND DOSAGE FORM OF BETA-BLOCKERS

In general, beta-adrenoreceptor blocking agents are used clinically in the treatment of, hypertension [*Prichard 1978 (11), Waal-Manning 1979 (12) and McDevitte 1979 (13)*], cardiac arrhythmias [*Foex 1977 (14)*], angina pectoris [*Prichard 1978 (11)*], myocardial infarction [*Singh 1978 (15) and Lee 1978 (16)*] and other diseases characterised by excess

Table 1.1 Classification of B-adrenoreceptor Blocking Agents

No	Division	Drugs	Membrane Stabilizing Activity	Intrinsic Sympathomimetic Action
1	Non cardio-Selective Drugs	Alprenolol Oxprenolol Bunithalol Pronethalol Pindolol	+	+
		Propranolol	+	-
		Sotalol Timolol Nadolol Bunolol	-	-
2	Cardio-Selective Drugs	Acebutolol Metroprolol Tomalol	+	-
		Practalol	-	-
		Atenolol	-	-
3	Drugs which Blocks μ and ß Receptors	Labetalol	+	+

sympathetic activity such as thyrotoxicosis [*Rubenfield et al 1979 (17)*].

Beta-blockers have also been reported to be useful in the treatment of migrane [*Anthony 1978 (18) and Nanda 1978 (19)*]. anxiety [*Suzman 1976 (20)*], tremor [*Fitzgerald 1976 (21)*] and glaucoma [*Katz 1978 (22)*]. Timolol has been reported to reduce intraocular pressure in patients with open angle glaucoma or ocular hypertension [*Heel et al 1979 (23)*]. Dosage forms are usually tablets or capsules or injections of aqueous solutions of the acidic salt pH 3 - 4. Some marketed drugs and their trade names are listed in Table 1.2

1.3.2. MECHANISM OF ACTION OF BETA-BLOCKERS

Despite extensive investigations, the mechanism of the antihypertensive action of beta-blockers is not known [*Hummond and Kirkendall 1978 (24)*]. A single statement explaining the mechanism of action of all beta-blockers in all hypertensive patients cannot be made. Main theories for this drug class centre on : (a) central nervous system (CNS) effect, (b) decreased renin release, (c) reduced cardiac output. One or more of these actions are probably relevant in individual patients. Propranolol is the most widely used beta-blocker in hypertension. Its mechanism of action is still incompletely understood. Although the drug blocks renin release and reduces cardiac output [*Holland and Kaplan 1976, (25)*], these effects cannot explain the usefulness of the drug as an antihypertensive agent.

Beta-blockers improve exercise tolerance and relieve symptoms in anginal patients. This effect is caused by reduction of the cardiac output. The hypothesis has been put forward that one mechanism contributing to the efficacy of propranolol in angina pectoris may be due to the enhancement of oxygen availability [*Nevins 1974 (26)*]. In the in-vitro situation, high concentrations of propranolol (0.5 mM) reduce oxygen binding to haemoglobin in intact red cells [*Lichtman et al 1974 (27)*]. The antiarrhythmic activity of propranolol was related to its quinidine - like properties that are independent of beta-adrenergic blockade (Remington's Pharmaceutical Science, 16th Edition, P846).

Table 1.2 Trade Names of Some Beta-Blocker

DRUG	TRADE NAME	DRUG	TRADE NAME
Propranolol	Inderal	Metopronol	Lopressol
Acebutolol	Sectral	Nadolol	Corgarol
Alprenolol	Aptin	Oxprenolol	Trasicol
Atenolol	Tenormin	Pindolol	Visken
Bevantolol	Bevantol	Practalol	Eraldin *
Bupranolol	Betadrenal	Pronethalol	Alderlin @
Bunitrolol	Stresson	Sotalol	Sotacol
Labetalol	Trandate	Timolol	Blocadren
Metiprolol	Disolol		

* Marketed with restricted use.

@ Withdrawn from study and use

The mechanism of a reduction in the recurrence rate of myocardial infarction involves the prevention of the development of malignant arrhythmias during the initial phase of infarction and an influence on the area of ischaemia.

1.3.3. SIDE EFFECTS

The most severe side effects of beta-blockers include heart failure, bronchospasm and atrioventricular conduction [*MacDonald and McNeill 1968 (28)*]. Other common side effects reported include nausea, vomiting, diarrhea, fatigue, cold extremities, vivid dreams and skin rashes and rarely it may cause psoriasis [*Scriabene 1979 (7)*]. Other serious adverse effects on the skin, eyes, oral and nasal mucous membrane and ear have been associated with practolol therapy [*Michael et al 1979 (29)*]. Nevertheless, side effects of beta-blockers have been reported to be lower than other hypotensive drugs [*Bulpitt and Dollery 1973 (30)*]. Side effects may be minimised by an appropriate strategy to minimise initial dosage.

1.3.4. STRUCTURE-ACTIVITY RELATIONSHIPS

A great deal is known about the relationships between structure and activity of beta-blockers. Such knowledge in general is important to the pharmaceutical chemist as a guide in synthetic work on new drugs. Structure-activity relationships (S.A.R) are also of fundamental importance in that they should reflect basic characteristics of receptor mechanisms.

Pharmacological actions of beta-adrenergic drugs have been shown to be linked to relatively strict structural requirements. Hinderling et al 1984 (31) have considered in detail the quantitative structure-activity relationships of beta-blockers.

Howe et al 1968 (32) reported that the alcoholic hydroxyl group on the beta-carbon atom and the secondary amine in the aliphatic side chain are essential for the activity of beta-blockers through the formation of hydrogen bonds with receptor functional groups.

Ariens 1967 (33) and Burns 1967 (34) studied the S.A.R. of beta-blockers and reported that the presence of an isopropyl or t-butyl group on the nitrogen atom in the aliphatic side chain increased the affinity for beta-receptors. This hypothesis was supported by Howe et al 1968 (32) and Crowther et al 1969 (34) when they reported that the highest activity was associated with branched alkyl chain group on the nitrogen atom. The activity was maintained when a phenoxymethyl group extended the main alkyl chain.

The insertion of an oxymethylene (-OCH_2-) bridge between the aromatic nucleus and the ethanolamine side chain enhanced the activity by separation of the aromatic moiety from the rest of the molecule [*Black et al 1965 (35) and Rosen 1970 (4)*].

The aliphatic hydroxyl appears to be essential for the activity, it gives the molecule optical activity, and the (-) forms of the beta-blockers are much potent than (+) forms. This difference is useful in distinguishing the effect of ß-receptor blockade from those of other pharmacological actions of the molecule, for example, the (+) form of propranolol has less than 1% of the potency of the (-) form of propranolol in blocking beta-adrenergic receptors, but the two isomers are equipotent as Local anaesthetics [*Howe and Shank 1966 (36), Barrett and Cullum 1968 (37) and Buckner and Patil 1971 (38)*].

Alkenyl and alkenyloxy substitution on the ortho-position of the aromatic nucleus confers a high level of beta-adrenoreceptor blocking activity (for example; oxprenolol and alprenolol). Larsen and Lish 1964 (39) reported that a methyl sulfonamido substituent at the para position of the aromatic ring increased beta-blocking activity (e.g: sotalol).

With regard to cardio-selectivity, the most significant feature common to $ß_1$- selective compounds is the presence of a substituent group in the para-position of the aromatic ring (Fig 1.1). Thus practolol [*Dunlop and Shank 1968 (40)*], Metoprolol [Ablad et al 1973 (41)], acebutolol [Basil et al 1973 (42)], tolamolol [*Augslein et al 1973 (43)]* and atenolol *[Barrett et al 1973 (44)*] have all been reported to be cardio-selective. In this regard, it is interesting to note that the ortho and meta isomers of practolol, alprenolol and oxprenolol are

non-selective beta-blockers, whereas the para isomers have a cardio-selective action [*Ablad et al 1970 (45)*].

Levy 1973 (46) and Vaughan et al 1973 (47) have confirmed the importance of para substitution in the cardio-selectivity of this group of drugs.

1.3.5 PHARMACOKINETICS OF BETA-BLOCKERS

Pharmacokinetics is the study of the time course of absorption, distribution, metabolism and excretion of drugs and their metabolites in the intact organism.

1.3.5.1. ABSORPTION

The process whereby a drug is made available to the fluids of distribution is referred to as absorption. Beta-blockers are rapidly and completely absorbed from the gastro-intestinal tract. They reach the maximum blood concentration in a range from one to four hours (Table 1.3) [*Johansson and Regardh 1976 (48), Gugler et al 1974 (49) and Shand 1976 (50)*]. An exception is atenolol, for which approximately 50% of an oral dose is absorbed [*McAinsh 1977 (51) and Reeves et al 1978 (52)*]. Similar findings have been reported for nadolol [*Dreyfuss et al 1978 (53)*].

Hicks 1973 (54) and Taylor et al 1985 (55) found that the pH of the gastro-intestinal fluids affects the rate of absorption of some beta-blockers (e.g. propranolol) while this effect is less with others.

Absorption may be retarded by the use of sustained release formulations including alprenolol [*Johansson et al 1971 (56)*], metoprolol [*Regardh et al 1975 (57)*], oxprenolol [*West et al 1976 (58)*] and propranolol [*McAinsh et al 1978 (59)*].

1.3.5.2. DISTRIBUTION

When the drug reaches the plasma, its main fluid of distribution, it must pass across various barriers to reach the final site of action. The first of these barriers is the capillary

Drug	Absorption half-life (min)	max (h)	Extent of absorption (% of dose)	Extent of bioavailability (% of dose)	Dose dependent bioavailability
Acebutolol	-	3 - 4	-	-	-
Alprenolol	-	0.5 - 1.5	> 90	≈ 10	Yes
Altenolol	-	2 - 4	-	≥ 40	No
Metroprolol	12	0.5 - 1.5	> 95	≈ 50	No
Oxprenolol	-	0.5 - 1	70 - 95	24 - 60	No
Pindolol	25	1.5 - 2	> 90	≈ 100	No
Practolol	-	1 - 3	> 95	≈ 100	No
Propranolol	-	1 - 3	> 90	≈ 30	Yes
Sotalol	-	2 - 3	-	≥ 60	-
Timolol	-	1 - 3	-	≈ 30	-
Tolamolol	-	1 - 3	-	≈ 30	-

Table 1.3 Gastro-intestinal Absorption Characteristics of some β-adrenoreceptor Blocking Drugs (Ref. 48)

Drug	Distribution half-life (min)	Distribution volume (VdB) (L/kg)	Distribution Ratio octanol/water pH = 7.0	Distribution Ratio erythocrytes/ Plasma	Per Cent Bound	
					Serum Proteins	HSA[1]
Alprenolol	-	3.3	3.27	-	85	38
Altenolol	20 - 30	-	-	1.2	-	-
Metropolol	12	5.6	0.18	1.2	1.2	12
Oxprenolol	-	-	0.43	-	-	-
Pindolol	-	2.0	0.12	-	-	57
Practolol	5	1.6	0.009	-	-	32
Propranolol	10	3.6	5.39	1.72	93	62
Sotalol	-	-	-	-	-	54
Tolamolol	7	3.2	-	0.46	91	-

1 Human Serum Albumin.

Table 1.4 Distribution Characteristics of some β -adrenoreceptor Blocking Drugs (Ref 48)

Drug	Elimination half-life (h)	Total body clearance (L/min)	Urinary recovery of unchanged drug (% of dose)	Total urinary recovery (% of dose)	Active metabolites of clinical importance
Alprenolol	2 - 3	1.2	< 1	> 90	Yes
Atenolol	6 - 9	-	≈ 40	-	-
Metoprolol	3 - 4	1.1	≈ 3	> 95	No
Oxprenolol	1 - 2	0.6	-	70 - 95	-
pindolol	3 - 4	0.4	≈ 40	> 90	No
Practolol	5 - 13	0 - 14	> 90	> 90	No
Propranolol	2 - 3	1.0	< 1	> 90	Yes
Sotalol	5 - 6	-	≈ 60	-	-
Tomolol	4 - 5	-	≈ 20	65	-
Tolamolol	≈ 2	1.0	-	-	-

Table 1.5 Elimination Characteristics of some Orally Administered β -adrenoreceptor Blocking Drugs (Ref 48)

wall. The lipid-soluble substances diffuse through the entire capillary endothelium where-as lipid-insoluble drugs pass through the pores which represent a fraction of the total capillary surface.

A schematic representation of the distribution of drugs into different compartments are shown in Fig 1.3 [*from Klotz 1976 (60)*].

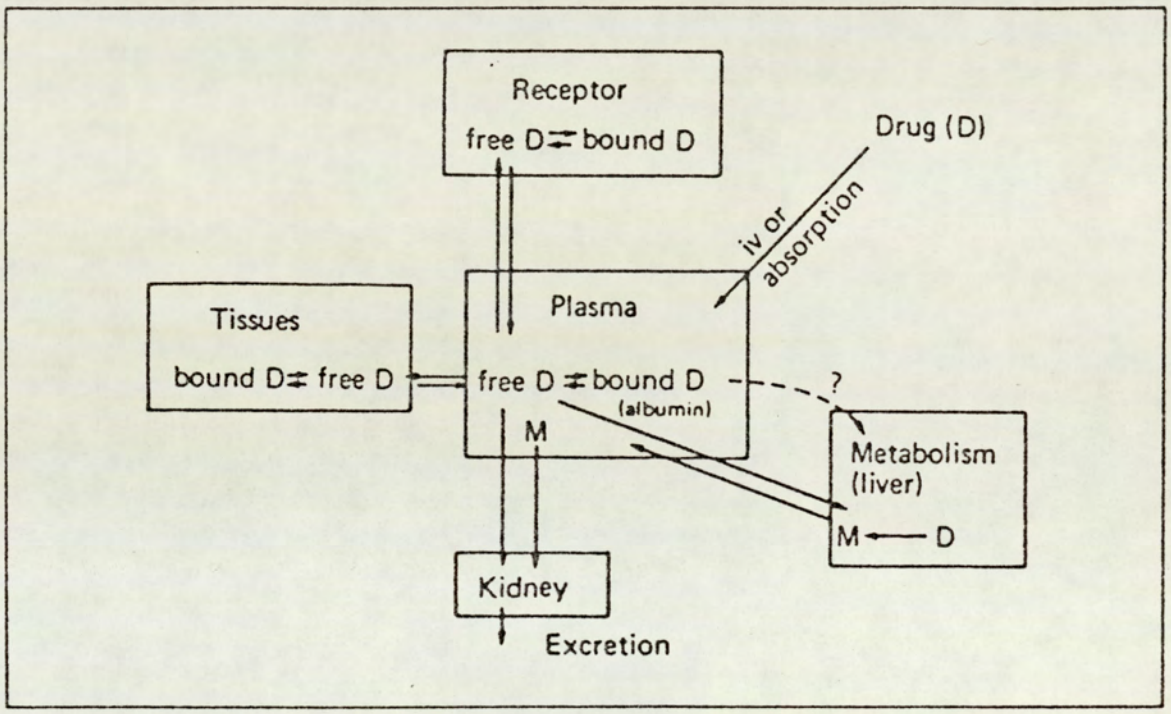


Fig 1.3 Schematic representation of the distribution of drugs into different compartment (from Ref. 60)

Fung et al 1971 (61) suggested that the rate of distribution of drugs from the circulations to various tissues of the body depends upon:

a) The degree of ionization of the drug

b) Lipophilicity and tissue lipid content

c) Plasma protein and tissue binding

d) Tissue perfusion

Ritchel 1980 (62) and Johansson and Regardh 1976 (48) reported that beta-blockers are widely distributed to the peripheral tissues with a half-life ranging between

5 to 30 minutes (Table 1.4) and the apparent volume of distribution (V_d) of the beta-phase is in all cases greater than the body weight in ranges between 1.6 - 5.6L/Kg (Table 1.4).

PROTEIN BINDING

Binding to various protein fractions in the blood has a significant effect on the pharmacokinetic and pharmacodynamic properties of drugs. Generally, only the unbound fraction of the drug is considered to be pharmacologically active (Fig 1.3). The binding of beta-blockers to various serum proteinshas been studied as the degree of protein binding of the beta-blockers can have an effect on their distribution and elimination kinetics, particularly, those with a higher affinity for proteins (Table 1.4).

A compilation of pharmacokinetic parameters of beta-blockers is illustrated by Ritchel 1980 (62).

1.3.5.3. METABOLISM

Bodin et al 1974 (63) and 1975 (64) reported that the metabolic fate of beta-blockers has been studied and the number of the metabolites varies significantly. Common metabolic pathways for the beta-blockers include oxidative deamination and O-dealkylation with oxidation of the side chain, aromatic and aliphatic hydroxylation and conjugate formation between the secondary alcoholic OH group of the side chain and glucuronic acid. Some of the beta-blockers have a low extent of bioavailability due to first-pass metabolism. These include alprenolol and propranolol while other beta-blockers are affected very little by this effect [*Ablad et al 1974 (65)*].

In spite of the intensive first-pass metabolism of alprenolol and propranolol, the dosage is not increased on oral administration as the 4-hydroxy metabolites are pharmacologically active [*Shand et al 1970 (66)*]. It has been shown that 4-hydroxy propranolol is equipotent with propranolol as beta-blocker but is eliminated more rapidly [*Fitzgerald and Donnell 1971 (67) and Holland and Kaplan 1976 (25)*]. Fig 1.4 shows the major metabolites of propranolol.

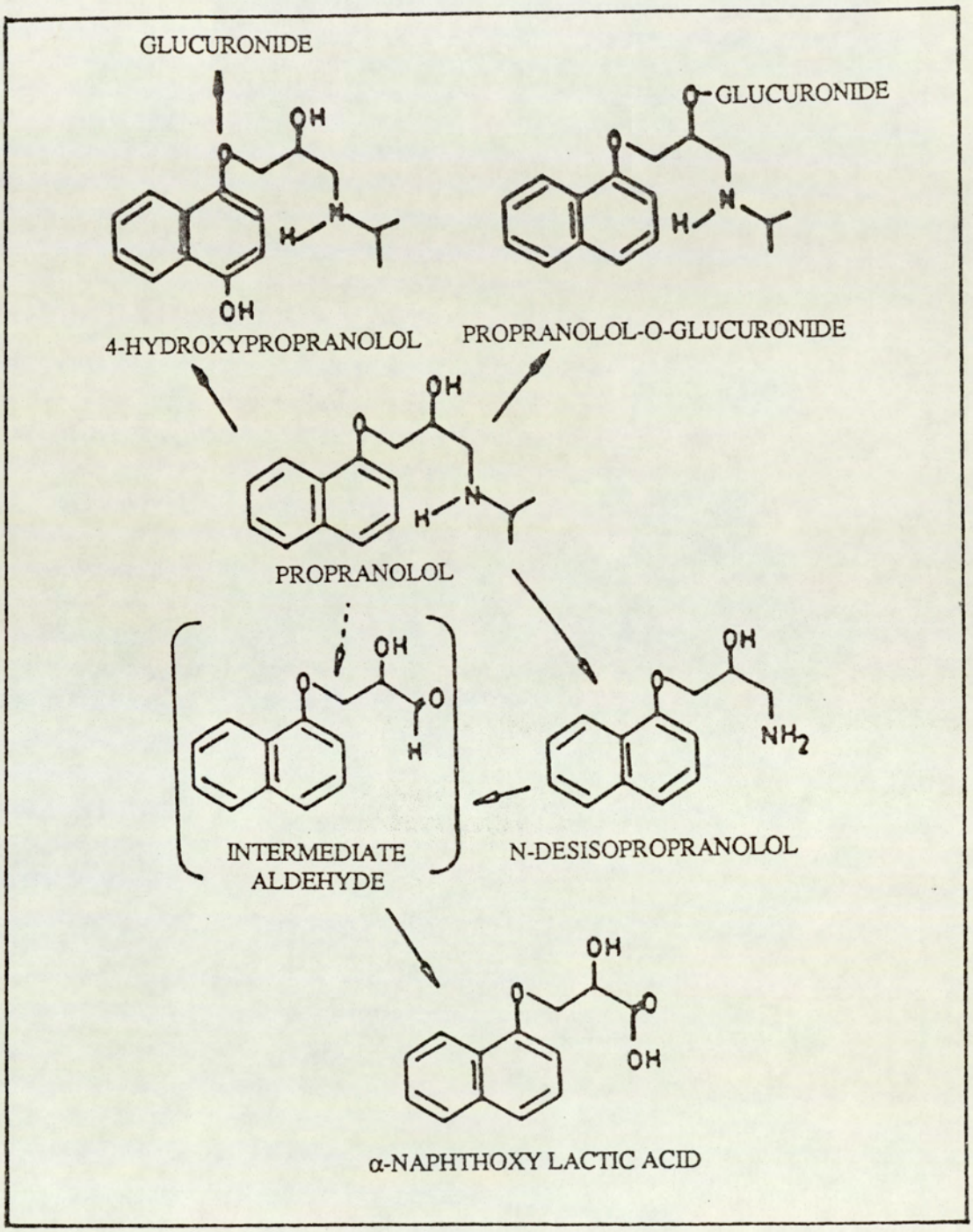


Fig 1.4 The major metabolites of propranolol

1.3.5.4. ELIMINATION

Beta-adrenoreceptor blocking agents show an elimination pattern which is associated with the lipophilicity of the drugs. Highly lipid-soluble beta-blockers such as alprenolol and propranolol are almost completely eliminated by various metabolic systems in the liver [*Evans and Shand 1973 (68)*]. As the lipophilicity decreases, renal excretion becomes more important (for example, practolol) [*Bodem and Chidsey 1973 (69)*]. The rate of excretion of most of the beta-blockers is directly proportional to the plasma concentration of the free drug [*Kaye et al 1973 (70)*]. There are several beta-blockers for which both biotransformation and renal excretion significantly effect elimination as shown by the ratio of unchanged drug and metabolites in the urine (Table 1.5).

1.3.6. CORRELATION OF PROPRANOLOL DOSE, PLASMA CONCENTRATION AND THERAPEUTIC RESPONSE

Propranolol shows a large interindividual variation in plasma concentrations after a given oral dose. This is because of the first-pass effect and genetic differences in the rate of drug metabolism.

A wide variation also exists between plasma concentration and any therapeutic effect. There have been many explanations posed to explain this phenomenon. Firstly, patients may have different levels of "Sympathetic tone", therefore, a greater concentration of propranolol would be required to achieve the desired therapeutic result [*Frishman et al 1975 (71)*]. Secondly, many beta-blockers have flat plasma-response curves. This means that the plasma concentration may vary considerably within a very narrow effect interval [*Johansson and Regardh 1976 (48)*]. Another reason may lie in the formation of varying amounts of active metabolites in different individuals.

1.4. PHYSICOCHEMICAL PROPERTIES OF BETA-BLOCKERS

The biological activity of a compound depends not only on its effect at the receptor site, but also on its ability to reach the locus of action in sufficient concentration to exert that effect.

In order to achieve its action in-vivo, the drug must first be absorped from its site of administration and then transported to the site of action. Absorption and distribution of a drug depend predominantly upon its physicochemical properties such as lipid solubility, ionization and partition and distribution coefficients.

1.4.1. pKA AND SOLUBILITY

Beta-adrenoreceptor blocking agents are weak bases with pKa values in the region of 8.97 - 9.65 [*Avery 1976 (72)*].

Schoenwald and Huang 1983 (73) suggested that, although the aromatic substituents varied substantially in beta-blockers, these are too far removed from the amino group in the aliphatic side chain to exert much effect on the pKa values.

The pKa value of bevantolol is quite low (8.38) because of the electron withdrawal effect of the dimethoxy benzyl ethyl substitution in the side chain. Sotalol has two pKa values 8.3 and 9.8 [*Schoenwald and Haung 1983 (73) and Garett and Schnelle 1971 (74)*]. The lower value is due to the sulphonamido group acting as a weak acid and the higher pKa (9.8) is due to the protonation of the amino group in the side chain. Beta-blockers have been divided into three groups; (a) very lipophilic (b) lipophilic and (c) hydrophilic [*Cruickshank 1980 (75)*], as shown in Table 1.6. The hydrophilic group is characterised by a polar substituent on the aromatic nucleus which can form hydrogen bonds with water to increase its aqueous solubility. They include atenolol and acebutolol which have an amido group at the para position of the aromatic nucleus. The hydrophilicity of sotalol and nadolol is due to the presence of a sulphonamido group (sotalol) and two hydroxy groups (nadolol) on the aromatic nucleus.

1.4.2. PARTITION AND DISTRIBUTION COEFFICIENTS

The term partition coefficient [P] is related to the chemical characterization of an organic molecule and is strictly defined as the ratio of concentration for the same molecular species between two liquid phases at equilibrium [Leo et al 1971 (76)]. i.e. $P = [B]_{org.} / [B]_{aq.}$. where $[B]_{org.}$ and $[B]_{aq.}$ represent the concentrations of the species B in organic and aqueous phases respectively. For compounds that do not ionize, no ambiguities arise in applying results from this in-vitro system to biological systems.

In contrast, the term distribution coefficient [D] is more applicable to biological systems because it takes into account the possible effect of pH and temperature. This is especially important if the organic species becomes ionized in solution. Thus D is pH-dependent quantity having a maximum value of P. The logarithmic forms are quantitatively related by equation 1.1.

$$\log D = \log P - \log [1 + \text{antilog} (pKa - pH)] \qquad 1.1$$

This equation can be used to calculate D from P at any pH, given the pKa (assuming Pi = 0). According to the previous classification (Table 1.6). The very lipophilic compounds which contain hydrophobic substituents such as a cyclopentyl group for penbutolol, ethoxybenzyl group for bevantolol, furanyl group for bufuranol and naphthalene ring for propranolol, have high partition and distribution coefficients which make this group associated with a higher incidence of CNS side effects [*Westerlund 1982 (77)*].

The lipophilic compounds have intermediate partition and distribution coefficients. As the polarity increases the partition and distribution coefficients decrease. For example; hydrophilic compounds such as acebutolol, sotalol, nadolol and atenolol are considered to possess clinically relevant advantages of which low penetration of the CNS is the most important [*Cruickshank 1980 (75) and Neil-Dwyer et al 1981 (78)*].

DRUG	CLASSIFICATION	pKa	LOG P	LOG D.C	LOG PT cm/sec
Penbutolol	VERY LIPOPHILIC	9.26	4.15	2.53	-4.22
Bufuralol		8.97	3.65	2.31	-4.14
Bevantolol		8.38	3.00	2.19	-4.17
Propranolol		9.23	3.21	1.62	-4.24
Levbunolol	LIPOPHILIC	9.32	2.40	0.72	-4.76
Oxprenolol		9.32	2.37	0.69	-4.56
Timolol		9.21	1.91	0.34	-4.91
Metiprolol		9.24	1.88	0.28	-4.62
Acebutolol	HYDROPHILIC	9.20	1.77	0.20	-6.07
Sotalol		8.3/9.3	0.93	-0.82	-5.99
Nadolol		9.39	-0.62	-1.25	-5.79
Atenolol		9.32	0.16	-1.52	-6.17

"(These data were obtained from Reference 73)"
Where: P = Partition coefficient
D.C = Distribution coefficient
P.T = Permeability coefficient

Table 1.6 Permeability coefficient and physical constants of ß-blockers

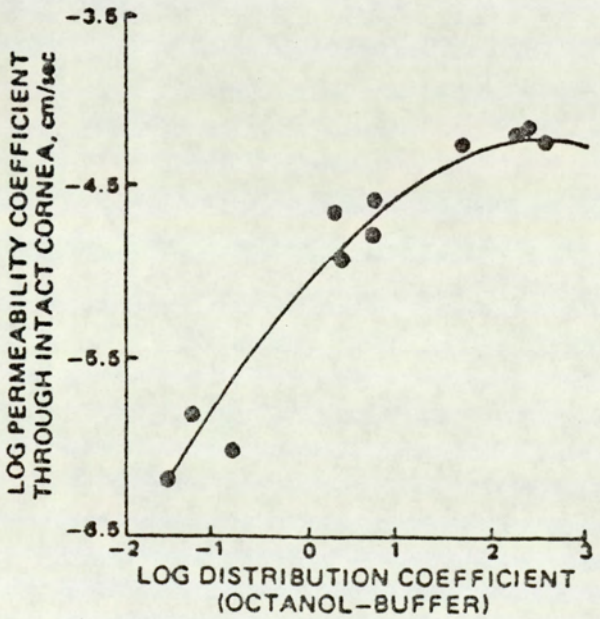


Fig 1.5 Log-log plot of permeability coefficient (pH 7.65) and distribution coefficient (pH 7.65) of beta-blockers (acebutolol excluded) from Reference 73.

Taylor et al 1985 (55) studied the effect of the distribution coefficient on the absorption of eleven beta-blockers by monitoring their rates of disappearance ($K_{dis.}$) from in-situ intestinal loops (Jejunum and ileum) in the anaesthetised rat (Fig 1.6) where a roughly linear relationship for the lipophilic compounds was obtained. In contrast, the rate of disappearance (K_{dis}) for the hydrophilic compounds is log D-independent.

Schoenwald and Huang 1983 (73) studied the penetration coefficient of some beta-blockers across the rabbit cornea and found that there is a direct relationship (non-linear) between the penetration and distribution coefficients (Table 1.6 and Fig 1.5)

Wood and Robinson 1981 (79) have investigated the comparative lipophilicity of ten beta-blockers by determining their distribution between n-octanol and aqueous buffer (distribution coefficient) where the results at room temperature (20°C), pH7 and at 37°C, pH7 and pH7.4 were comparable to those obtained by Coombs et al 1980 (80), Hellenbrecht et al 1973 (81) and Appelgren et al 1974 (82) [Table 1.7].

Taylor and Cruickshank 1984 (83) reported that the distribution coefficient of atenolol is lower than that of sotalol (Table 1.8), despite the latter's lower partition coefficient, because of the lower pKa value of sotalol. These results were in close agreement to those determined by Wood and Robinson 1981 (79).

Criticism of Taylor and Cruickshank's work 1984 (83) was made by Day and Barr 1984 (84) who complained that the results were analysed incorrectly as only the acidic pKa of sotalol was taken and this was mistakenly treated as a base.

1.4.3. STABILITY OF BETA-BLOCKERS

Most beta-adrenoreceptor blocking agents are stable to heat and unstable in light. In aqueous solution they decompose with oxidation of the alkylamine side chain accompanied by a reduction in pH [*Martindale (85)*]. Solutions are most stable at pH 3 - 3.5 and decompose rapidly when alkaline.

Drug	Previously published* distribution coefficients		Distribution coefficients at: pH 7.0 and 20 °C	pH 7.0 and 37 °C	pH 7.4 and 37 °C
Atenolol	—		0.003	0.008	0.015
Nadolol	—		0.008	0.022	0.066
Sotalol	0.011		0.011	0.012	0.039
Pindolol	0.12		0.20	0.29	0.82
Acebutolol	0.62	(pH 7.4)	0.17	0.35	0.68
Metoprolol	0.18		0.15	0.37	0.98
Timolol	—		0.28	0.51	1.16
Oxprenolol	0.43		0.51	1.01	2.28
Labetalol	—		4.6	8.3	11.5
Propranolol	5.4		5.4	8.6	20.2

*Coombs et al 1980 (80); Hellenbrecht et al 1973 (81); Appelgren et al 1974 (82).

Table 1.7 Distribution coefficients n-octanol/buffer (from Reference 79).

Drug	log P (25°C)	pK (25°C)	Distribution Coefficients (D): pH 7.4 and 25°C (calc)*	pH 7.4 and 37°C (this work)	pH 7.4 and 37°C (lit.)**
Atenolol	0.23	9.55	0.012	0.018	0.015
Sotalol	-0.79	8.37	0.016	0.030	0.039

* From equation 1.1
** Woods & Robinson 1981. (79)

Table 1.8 Partitioning data on atenolol and sotalol (from Reference 83).

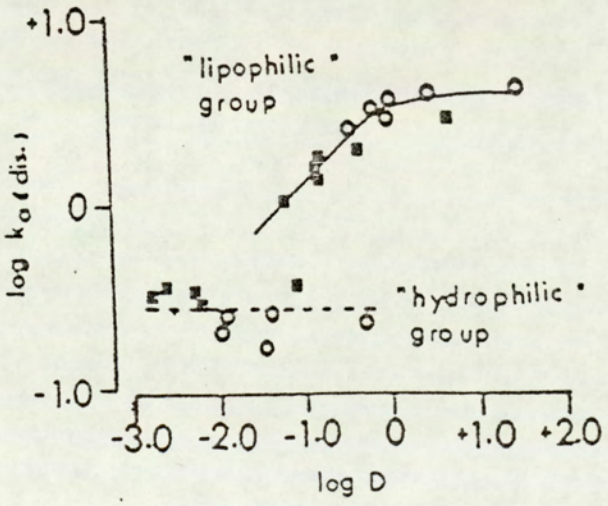


Fig 1.6 Correlation between log k_a (dis) and log D for eleven ß-blockers in the rat in-situ jejunum (-■-) and ileum (-O-) [from Reference 55].

1.5 DERIVATIVES AND ANALOGS OF PROPRANOLOL (literature review)

There are two major types of chemical modifications of a drug (a) analogs and (b) prodrugs.

(a) An analog generally involves a modification of the carbon skeletal arrangement or variation in substituents. Homologation and replacement of a hydroxyl group by halogen are examples of analogs.

(b) A prodrug is a derivative of a drug which undergoes in-vivo degradation to the parent compound (e.g. esters such as chloramphenicol palmitate) or an analog which is metabolically transformed to the biologically active drug. For example, phenyl butazone is converted in-vivo to the antirheumatic agent oxyphenbutazone through hydroxylation [*Riegelman and Sadee 1974 (86) and Ariens 1966 (87)*].

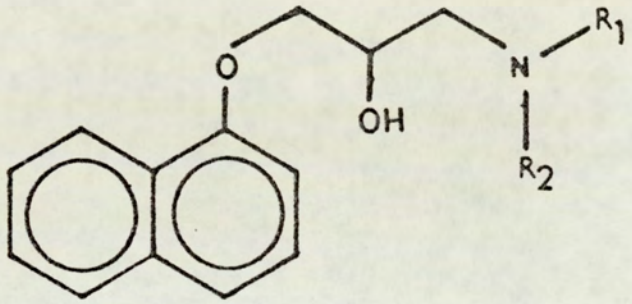
Not all drug derivatives are prodrugs since some are active without undergoing cleavage for elliciting pharmacological activity. The utility of analogs is distinctively different from that of prodrugs. Analogs are primarily employed to increase potency and to achieve specificity of action while prodrugs are employed to improve the biopharmaceutical properties such as solubility, melting point, stability , and their related physicochemical properties (pharmaceutical) and absorption, pharmacodynamic and other delivery related properties (biological).

In this section, propranolol will be considered the prototype compound, because it has been much more extensively studied than any other beta-blocker, and a review of its derivatives and analogs will be illustrated.

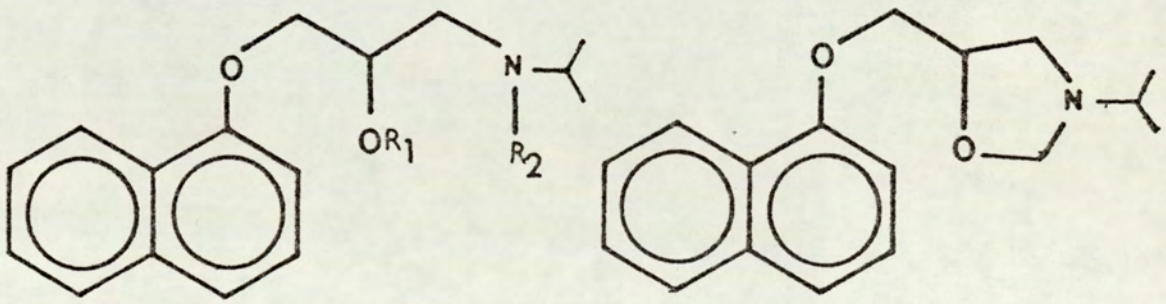
Several derivatives and analogs of propranolol had been synthesised for the most part by well-established methods and their pharmacological activity studied to assess their suitability as beta-blockers.

1.5.1.

Crowther and Smith 1968 (88) synthesised several 1-amino-3-napthoxy-2- propanols with different substituents on R_1 and R_2 and studied the structure-activity relationships concerned with the increase of the carbon atomsin the substituents and presence or absence of a functional group attached to it. They also synthesised the acetyl ester (II) and the oxazolidine derivative (V) of propranolol, both of which were readily hydrolyzed to the amino alcohol [propranolol (I)] in-vitro and might therefore be expected to give the parent



compound in-vivo. Their activity was only slightly less than that of the alcohol. The acetyl ester of propranolol has also been synthesised by Nelson and Walker 1978 (89) in addition to the acetylamide (III) and the diacetyl (IV) of propranolol.



I) $R_1 = R_2 = H$ Propranolol

II) $R_1 = COCH_3$, $R_2 = H$ O-acetylpropranolol

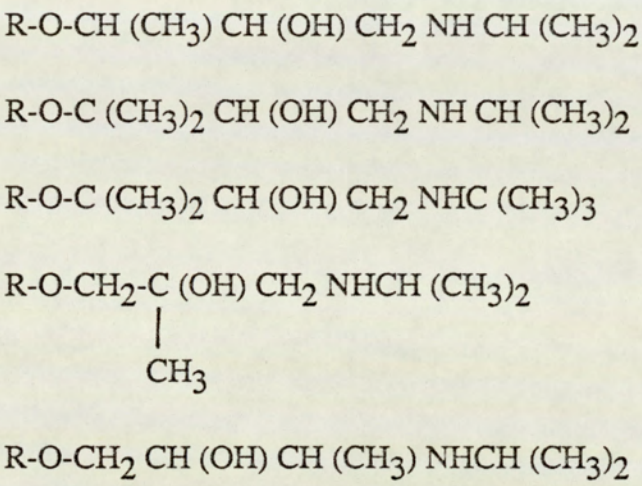
III) $R_1 = H$, $R_2 = COCH_3$ N-acetylpropranolol

IV) $R_1 = R_2 = COCH_3$ N, O-diacetylpropranolol

V) Oxazolidine derivative of propranolol

1.5.2.

Howe 1969 (90) synthesised propranolol analogs having methyl-substituents in the amino hydroxy propoxy side chain.



where R = α - naphthyl

He reported that the introduction of substituents into the side chain of propranolol analogs, generally, gave compounds which were less potent than the unsubstituted analog.

1.5.3.

Sinha and Chingnell 1975 (91) synthesised spin-labelled analogs of propranolol by substitution of the isopropyl group with a 4-(2,2,6,6 - tetramethyl-1-piperidinyloxy) group.

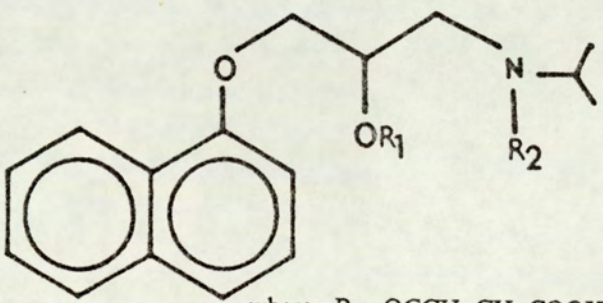
The activity of this compound was less than that of propranolol by about 50% due to the decreasing of its affinity to the ß-receptors caused by the introduction of the nitroxide moiety in place of the isopropyl group.

1.5.4.

Yamamura and Rodbell 1976 (92) synthesised hydroxy benzyl propranolol and found that it exerts a partial agonist effect about equivalent in intrinsic activity to pindolol, and its affinity to the ß-adrenoreceptors is some ten-fold higher than that of propranolol.

1.5.5.

Kawashima et al 1976 (93) described the conjugation of propranolol and its (-)-2S-isomer by succinic anhydride and subsequent acylation of the terminal carboxyl group of the hemisuccinyl conjugate with bovine serum albumin to form an immunogen. The immunogen proved suitable for stimulation of antibody formation in the rabbit and led to the development of a sensitive radioimmunoassay method for racemic and optically active propranolol.

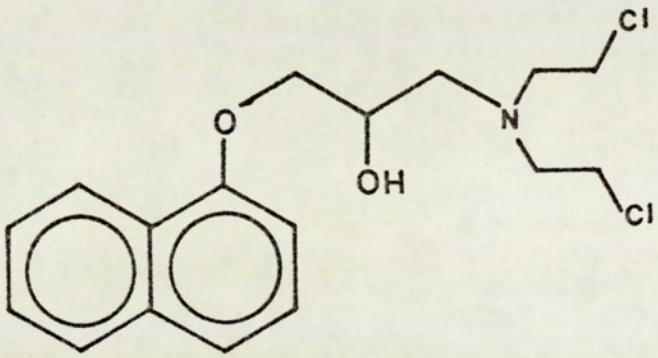


where R_1=$OCCH_2CH_2COOH$ O-hemisuccinylpropranolol
R_2=$OCCH_2CH_2COOH$ N-hemisuccinylpropranolol

Propranolol hemisuccinate derivatives was also then prepared by Garceu 1978 (94) and Nelson and Walker 1978 (89).

1.5.6. SYNTHESIS OF PROPRANOLOL MUSTARD

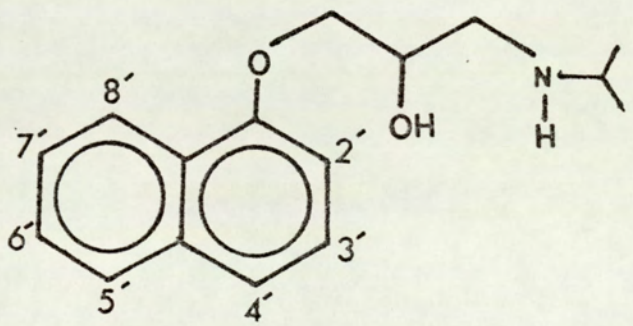
Feiyns et al 1980 (95) synthesised the nitrogen mustard analog of propranolol by replacement of the isopropylamino substituent of propranolol with a bis (2-chlorethyl) amino group which found as a potential lung specific antitumour agent.



1.5.7 RING HYDROXYLATED PROPRANOLOL

Walle et al 1980 (96) and Oatis et al 1981 (97) synthesised all seven isomers of the ring hydroxylated propranolol and reported that the potency strongly depends upon the position of the hydroxyl group i.e; 1e is 4 times as potent as 1 as a beta-receptor antagonist, where as 1a, 1b and 1g are all significantly less potent than 1.

For direct vasodilation, 1a and 1g are equipotent to 1, while 1b - f are much less potent.



1, R = H

1a, R = 2' - OH

1b, R = 3' - OH

1c, R = 4' - OH

1d, R = 5' - OH

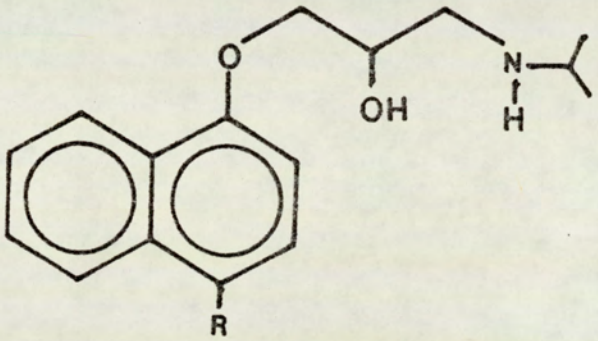
1e, R = 6' - OH

1f, R = 7' - OH

1g, R = 8' - OH

1.5.8.

Rossel et al 1981 (98) reported that the substitution of methoxy, methylthio, ethylthio and propylthio groups at position 4 of the naphthalene ring of propranolol increase lipid solubility and enhanced non beta-receptor mediated vasodilation in contrast to beta-adrenergic receptor antagonists where the potency decreased.

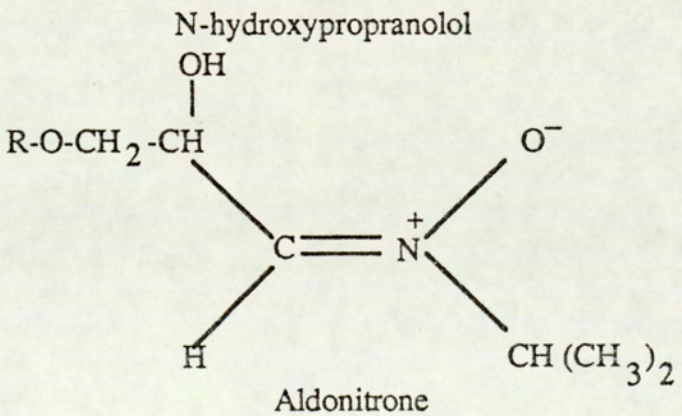


R	Compound
OCH_3	Methoxypropranolol
SCH_3	Methylthiopropranolol
SC_2H_5	Ethylthiopropranolol
SC_3H_7	Propylthiopropranolol

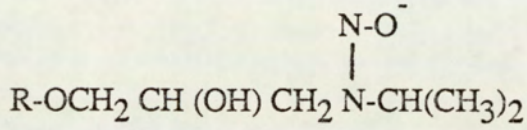
1.5.9.

N-nitrosopropranolol and N-hydroxypropranolol and its aldonitrone were synthesised and their mutagenic activity in the Ames salmonella test system were reported [*Zhang et al 1983 (99)*].

$$R\text{-}OCH_2\ CH\ (OH)\ CH_2N\ (OH)\ CH\ (CH_3)_2$$



Aldonitrone

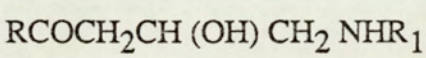


N-nitrosopropranolol

1.5.10.

Rzeszotarski et al 1983 (100) synthesised a series of 1-(aralkylamine)-3- (aryloxy) propan-2-ols and reported that the introduction of a methoxyethyl group in place of the usual isopropyl residue of propranolol (I) leads to a twenty-fold loss in affinity to both $ß_1$ and $ß_2$ receptors. The replacement of the isopropyl group in propranolol with a 3', 4' dimethoxyphenethyl (Homoveratryl) group (II) improves the lipophilicity and enhances the affinity to the $ß_2$ - receptors by about ten-fold. This compound has been also synthesised by Hoefle et al 1975 (101) and examined for cardio-selective ß-blockade where the introduction of the 3', 4' -dimethoxyphenethyl group led to the most cardio-selective agent.

Introduction of the 4-acetamido substituent in the naphthalene part of the antagonist (III) leads to a far greater loss in affinity to $ß_1$-receptor and the loss of affinity to the $ß_2$-receptor is less pronounced. Elongation of the acyl chain (IV) improves the affinity to both receptors and renders the compound non-selective.



Compound	R	R_1
I	1- $C_{10}H_7$	$CH_3\ OCH_2CH_2$
II	1- $C_{10}H_7$	3',4' $(CH_3)_2\ C_6H_3CH_2CH_2$
III	1- (4- $CH_3\ CONHC_{10}H_6$)	3', 4' $(CH_3)_2\ C_6H_3CH_2CH_2$
IV	1- (4- $CH_3(CH_2)_4\ CONHC_{10}H_6$)	3',4' $(CH_3)_2\ C_6H_3CH_2CH_2$

1.6 METHODS OF DETERMINATION OF BETA-BLOCKERS

Several chemical and biological methods of analysis were developed and used for the determination of beta-blockers. These are listed in Table 1.9.

No.	Method	Reference
1	Titrimetric:	
	a) Non aqueous titration	102
	b) Potentiometric titration	103,104
2	Polarographic	105,106
3	Spectrometric:	
	a) U.V. spectrophotometric	107 - 109
	b) Fluorimetric	110 - 112
	c) Colorimetric	113
4	Radioimmunoassay	114 - 116
5	Chromatographic:	
	a) Thin-layer chromatography	117 - 120
	b) Gas chromatography	103, 121 - 123
	c) High-performance liquid chromatography (HPLC.)	124 (Review)

Table 1.9 Methods of Determinaton of Beta-blockers

All these methods have been used extensively in determinations or separations of beta-blockers. However, each method has limitations:

(i) Titrimetric and polarographic methods have limitations in that

a) they require a large sample and high concentration

b) they are not applicable for the analysis of multi-component samples

c) they are time consuming

(ii) Colorimetric analysis cannot generally be used for the analysis of pharmaceuticals because of the lack of specificity and the interference of excipients with the analysis.

(iii) The limitation of radioimmuno assay is that it cannot be used for simultaneous determination of beta-blockers and requires a number of samples, additionally, the time consumed limits its usage in pharmacokinetic studies.

(iv) Thin-layer chromatography has a limitation that it is inapplicable in kinetic studies as it lacks the quantitative precision necessary for monitoring the rate of formation of the degradation products.

(v) Gas chromatography is a rapid method with a relative ease of qualitative and quantitative analysis of multicomponent samples in one separation whereas in chemical and spectrometric analysis a number of determination are used. Small samples may be readily handled and low concentrations of components can be detected. The major limitation of gas chromatography is that most of the beta-blocker derivatives are either of low volatility or are too thermolabile for direct chromatographic analysis.

High- performance liquid chromatography (HPLC), where heat and derivatization are not required, has been developed to overcome these problems. The ease of sample preparation , the speed of the analysis and the specificity, sensitivity, accuracy and precision associated with this method are obtained by using a reusable narrow bore column, small diameter support, low dead volume injector and pressurised eluent delivery with a fixed flow rate. HPLC allows the use of almost all type of solvent and fast separation of closely related compounds can be achieved.

The advantages of HPLC over the other analytical techniques such as colour based procedures and other chromatographic techniques, have led to HPLC being the analytical method of choice in many situations. It has wide application in the monitoring of therapeutic drugs in body fluids [*Altshular et al 1979 (125)*]. Using HPLC; the analysis of numerous drugs and metabolites can be performed simultaneously during a single chromatographic run.

Several useful reviews [*Hawk et al 1977 (126), Wessely 1979 (127), Li Wan Po and Irwin 1980 (128) and Irwin and Scott 1982 (129)*] are available which outline the principles and applications of HPLC.

1.6.1 APPLICATION OF HPLC TO BETA-BLOCKERS

1.6.1.1 DETERMINATION OF BETA-BLOCKERS IN BIOLOGICAL FLUIDS AND IN PHARMACEUTICAL PREPARATIONS

High performance liquid chromatography (HPLC) enables the specific, sensitive and precise separation and determination of beta-blockers. HPLC systems have been developed and used for the assay of beta-blockers in biological fluids such as urine , plasma , and blood and in pharmaceutical preparations such tablets, capsules, suspensions and injections. Some of these systems are summarised in Table 1.10.

1.6.1.2 SEPARATION OF THE ENANTIOMERS OF BETA-BLOCKERS

The progress in asymmetric synthesis of chiral compounds in recent years [*Meffin et al 1976 (156)*], has simulated the development of new analytical techniques for evaluating enantioselectivity and stereoselectivity of their disposition. In the case of a racemic drug, where only one of the enantiomers is pharmacologically active, it is important that pharmacokinetics and metabolism studies be directed to the active species. There are other reasons for this, for example; an efficient method for determining optical plurity would be highly beneficial in many scientific disciplines including: organic and inorganic synthesis, kinetics and pharmacology.

The traditional method of resolving racemic mixtures (i.e. fractional recrystallization of diasteromeric salts) is relatively difficult, insufficient and limited in applicability [*Eliel 1962 (157)*], immunological techniques of uncertain specifity [*Kawashima et al 1976 (93)*] and gas-liquid chromatographic techniques which are not sensitive enough for studies of concentration - effect relationships in man [Cacia et al 1979 (158)], had greatly increased the

Table 1.10 HPLC Systems for Determination of Propranolol and Other Beta Blockers in Biological Fluids and in Pharmaceutical Preparations

DRUG	SAMPLE	COLUMN	STATIONARY PHASE AND SUPPORT	MOBILE PHASE	DETECTOR & WAVE LENGTH	RATE OF FLOW	INTERNAL STANDARD	REF NO.
Propranolol Alprenolol	Serum	25 cm x 8.0 mm	Partisil-10 O.D.S.	MeCN: Phosphate buffer pH 2.7 (1:3)	Fluorometric 300 nm	2.5 ml/min	Pronethalol	130
Propranolol Alprenolol	Plasma	12 cm x 4.6 mm	Zorbax S.I.L.	MeOH: H_2O: 0.2M Phosphate buffer pH8 (14:5:1) Containing 2.5mM Hexdecyl-Trimethyl-Ammonium Bromide	U.V. 290 nm	1.0 ml/min	-	131
Propranolol Metroprololol Atenolol	Serum	25 cm x 4.6 mm	LiChrosorb RP 18	H_2O: MeOH (13:7)	Fluorometric 280 nm	2.0 ml/min	-	132
Propranolol and its metabolites	Plasma	(at 55°C) 25 cm column	LiChrosorb CN	MeOH, 0.03M Acetate Buffer at pH 5.5 (13:7)	Fluorometric 405 nm	1.5 ml/min	-	133
Propranolol and its metabolites	Plasma	15 cm x 4.6 mm	Ultrasphere O.D.S.	21M : MeOH : H_3PO_4: MeCN : (14:7:4)	Fluorometric 340 nm	2.5 ml/min	Pronethalol	134

Table 1.10 Contd...

DRUG	SAMPLE	COLUMN	STATIONARY PHASE AND SUPPORT	MOBILE PHASE	DETECTOR & WAVE LENGTH	RATE OF FLOW	INTERNAL STANDARD	REF NO.
Propranolol and its Metabolites	Plasma	30 cm x 3.9 mm	µ Bondapack C 18	50% MeOH solution in 10 mM KH_2PO_4 Buffer pH 3.4 (µ 0.5M)	Fluorometric 380 nm	2.0 ml/min	Labetalol	135
Propranolol	Urine	25 cm x 4.6 mm	Cyanoamino--bonded Partisil-10 P.A.C.	0.01 M NaAc. (pH 6): MeCN (3:17)	U.V. 217 nm	4.0 ml/min	Protriptylene	136
Propranolol and 4-OH Propranolol	Plasma	(at 40°C) 25 cm x 4.6 mm	LiChrosorb RP - 18	Solvent (A) 17.33 Heptane Sulphonic acid in 2.5% acetic acid solvent A CH_3OH (1:1)	Fluorometric 340 nm	1.0 ml/min	4-Me-Propra-n nolol	137
Propranolol	Plasma	24 cm x 4 mm	Hitachi-Gel 3013 (µm)	Ethanol - 0.02M $HClO_4$ - $NaClO_4$ (65:35) pH 2.0	Fluorometric 340 nm	2.0 ml/min	Penbutolol	138
Propranolol and its metabolites without solvent Extraction	Plasma	Precolumn 4 cm x 3.2 mm column 25 cm x 4.6 mm	Precolumn LiChrosorb RP-2 (10µm) Column LiChrosorb RP-8 (10 µm)	MeCN: MeOH: 0.08M H_3PO_4 (50:15:11)	Fluorometric 430 nm	1.5 ml/min	N-Ethyl-pro-pranolol	139

Table 1.10 Contd.

DRUG	SAMPLE	COLUMN	STATIONARY PHASE AND SUPPORT	MOBILE PHASE	DETECTOR & WAVE LENGTH	RATE OF FLOW	INTERNAL STANDARD	REF NO.
Propranolol and its metabolites	Plasma	30 cm x 4.0 mm	Bondapack C 18	MeCN: Acetic acid: H_2O (36:1:53)	Fluorometric detection	2.0 ml/min	2-naphthyloxy acetic acid	140
Propranolol and other Beta-blockers	Serum	-	µ Bondapack CN	MeOH, 0.174M Acetate Buffer pH 5.5 (1:1)	Fluorometric 340 nm	1.4 ml/min	Protriptylene HCl	141
Propranolol	Plasma	30 cm x 4.0 mm	µ Bondapack C-18	35% MeCN solution in 45mM KH_2PO_4 at pH3	Fluorometric detection	1.5 ml/min	Promazine	142
Propranolol	Plasma	25 cm x 4.6 mm	Partisil (10) O.D.S.	MeCN: Phosphate Buffer pH 2.7 (1:3)	Fluorometric 350 nm	1.5 ml/min	Pronethalol	143
Propranolol	Plasma	30 cm x 3.9 mm	µ Bondapack C18	MeOH: H_2O: Acetic acid (60:39:1) with 5mM Heptanesulphonic acid	Fluorometric 360 nm	1.0 ml/min	Pronethalol	144
Propranolol and some other Beta-Blockers	Plasma	-	µ Bondapack CN	0.02M Acetate Buffer pH7 and MeCN (3:7)	Fluorometric 340 nm	2.0 ml/min	Cyclomethy-caine	145
Propranolol 4-OH-Propranolol	Plasma	-	µ Bondapack	MeCN: 0.06M H_3PO_4 (27:73)	Fluorometric detection	2.0 ml/min	4-Methyl-propranolol	146

Table 1.10 Contd.

DRUG	SAMPLE	COLUMN	STATIONARY PHASE AND SUPPORT	MOBILE PHASE	DETECTOR & WAVE LENGTH	RATE OF FLOW	INTERNAL STANDARD	REF NO.
Propranolol and other Beta-Blockers	Serum	25 cm x 2.6 mm	O.D.S. SIL-x-I	MeOH: H_2O : H_3PO_4 (37:62:1)	Fluorometric 375 nm	1.0 ml/min	Protriplylene	147
Propranolol & 4-OH-propranolol	Serum	-	µ Bondapack CN	250ml MeOH and 250ml isopropylalcohol and 2.9M (0.4ml) NH_3 then complete to litre with Hexane	Fluorometric 370 nm	2.0 ml/min	-	148
Propranolol & six metabolites	Plasma	30 cm x 4.0 mm	µ Bondapack C-18 (10µm)	MeCN:MeOH:HAC: H_2O 35: 5: 1: 59 36: 0: 1: 63 (1) for acidic extraction (2) for basic extraction	Fluorometric detection	2.0 ml/min	Procainamide HCl	149
Sotalol, Propranolol, Metoprolol, Oxprenolol and Atenolol	Serum	-	Spherosorb 5 Silica	MeOH: Hexane (85:15)	U.V. 215 nm	2.0 ml/min	Benzimidazole	150
Propranolol and another 10 ß -Blockers	Tablet	25 cm x 4.6 mm	Ethylsilane RP-2 10µm	35% MeOH - 65% of aqueous (0.0005M HCl and 0.05M NaCl) pH 4.5	U.V. 254 nm	1.2 ml/min	Atenolol	151

Table 1.10 Contd.

DRUG	SAMPLE	COLUMN	STATIONARY PHASE AND SUPPORT	MOBILE PHASE	DETECTOR & WAVE LENGTH	RATE OF FLOW	INTERNAL STANDARD	REF NO.
Propranolol Hydrochloride	Capsule, tablet & injection	30 cm x 4mm	µ - Bondapack C-18	45% MeCN: 0.5% HOAC and 0.02M HCO_2 NH_4 in water	U.V. 270 min	2.0 ml/min	Verapamil	152
Propranolol ≈-naphthoxy-lactic acid	Plasma	15 cm x 4mm	C-18 Nova-Pak (5 µm)	MeCN - 0.1M Phosphate buffer pH7 (25:75)	Fluorometric Excitation 220 nm	2.0 ml/min	-	153
Propranolol	Plasma blood	30 cm x 4 mm	µ - Bondapack-Phenyl (10µm)	MeCN: H_2O: HOAC: Et_3N (450:600:10:0:15) pH 3.5	Fluorometric Excitation 228 nm	2.0 ml/min	N-ethyl propranolol	154
Propranolol and its neutral and basic metabolites	Plasma	30 cm x 3.9 mm	C18, µ Bondapack 10 µm	MeCN:0.1% H_3PO_4 (23:77) MeCN: H_2O : HOAC (30:69.8:0.2) MeCN: H_2O (30:70)	Fluorometric Excitation 217 nm Emission 360 nm	2.0 ml/min	-	155

interest in alternative techniques and HPLC is now the method of choice for most of the recently reported enantiomeric separations.

Initial studies with propranolol demonstrated that the l-isomer is about 100 times as potent as the d-isomer [*Howe and Shank 1966 (36), Barrett and Cullum 1968 (37) and Buckner and Patil 1971 (38)*] and has a longer plasma half-life [*Kawashima et al 1976 (93)*]. These findings sparked a great deal of interest in the pharmacokinetics of propranolol enantiomers and resulted in a number of studies that measured the concentration of the enantiomers in biological fluids [*Cacia et al 1979 (158), Sibler and Riegelman 1980 (159), Hermansson and Bohr 1980 (160), Hermansson 1982 (161), Wainer and Doyle 1984 (162), Wilson and Walle 1984 (163) and Gal and Sedman 1984 (164)*].

These studies faced problems, for example; Sibler and Riegelman 1980 (159) found that the commercial derivitizing agent [N-trifluoroacetyl-L-propyl chloride (TPC)] was contaminated with between 4 and 15% of the (+)-enantiomer and that the reagent rapidly racemized during storage. Efforts to synthesize optically pure TPC were unsuccessful [*Wainer and Doyle 1984 (162)*]. An additional complication is that the enantiomers may have quite different rates and/or equilibrium constants when they react with another Chiral molecule providing two diastereoisomer products which may be different in properties from the original enantiomers [*Krull 1977 (165)*].

Separations of the enantiomers of propranolol and other beta-blockers by reversed-phase high-performance liquid chromatography are summarised in Table 1.11.

1.7 CONTROLLED RELEASE WITH ION-EXCHANGE RESIN DRUG SYSTEMS

An oral formulation in which the active ingredients are liberated into the gastro-intestinal tract lumen more slowly than from conventional tablets or capsules are known as "sustained" or "delayed" release preparations, and are produced for three reasons:

a) To prevent adverse effects which may result from local irritation of the gastro-intestinal mucosa due to high concentrations of drug [e.g. KCl (slowK)].

Table 1.11 Separation of the enantiomers of propranolol and other beta-blockers by reversed-phase HPLC (α = resolution factor)

COLUMN	STATIONARY PHASE AND SUPPORT	DERIVATIZING AGENT	MOBILE PHASE	FLOW RATE	DETECTOR WAVELENGTH	COMPOUND	α	REF.
25 cm x 4.6 mm I.D.	Ultrasphere ODS (5µm)	N-trifluoro-acetyl- (-) - prolyl chloride	0.0122M H_3PO_4 & 0.0048N KCl in acetonitrile: tetra-hydrofuran (49:3)	2ml/min	Fluorometric Excitation Emission 290 - 404 nm	Propranolol		159
25 cm x 3.2 mm I.D.	Lichrosorb RP-18, RP-8 (5µm)	N-trifluoro-acetyl - (-) - prolyl chloride	45% acetonitrile in phosphate buffer pH 2.2 (µ 0.05)	1ml/min	Fluorometric Excitation 210 nm Emission 340 nm.	Propranolol	< 1.5	160
25 cm x 4.5 mm I.D.	LiChrosorb RP-18 (5µm)	Tert-butoxy-Carbonyl-L-analine and tert-butoxy-carbonyl-L-leucine	Sodium phosphate buffer pH 3 containing 30% v/v acetonitrile and 0.0194 M N,N-dimethyl-octylamin.	1ml/min	Fluorometric Excittation 210 nm, Emission 340 nm	Propranolol-analine Propranolol-leucine	1.7 1.32	161
25 cm x 4.6 mm I.D.	y-aminopropyl silanized silica	(R)-N-(3,5-dinitro-benzoyl)-phenyl glycine	Hexane : isoprop-anol : acetonitrile (97 : 3 : 1)	2ml/min	Fluorometric Excitation 290 nm Emission 335 nm	Propranolol as oxazolidine derivative (by reacting with phosgene)	< 1	162

Table 1.11 Contd..

COLUMN	STATIONARY PHASE AND SUPPORT	DERIVATIZING AGENT	MOBILE PHASE	FLOW RATE	DETECTOR WAVELENGTH	COMPOUND	α	REF.
25 cm x 4.6 mm I.D.	5 µm C18 Silica	R-(+)-phenyl-ethyl isocyanate	Methanol : water (70 : 30)	1 ml/min	U.V. 313 nm	Propranolol 4-OH-propra-nolol	< 1 < 1	163
15 cm x 4.6 mm I.D.	Ultrasphere ODS (5µm)	R-α-Methylbenzyl isothiocyanate	65% aqueous acetonitrile	1 ml/min	U.V. 254 nm	Propranolol	1.15	164
25 cm x 4.6 mm I.D.	y-aminopropyl silanized silica	(R)-N-(3,5-dinitro benzylphenyl glycine (to saturate the column prior to the analysis).	5% isopropanol in hexane	2 ml/min	-	Propranolol	1.7	166
10 - 15 cm x 3.2 mm I.D.	LiChrosorb - DIOL (10 µm)	-	(+)-10-Comphor-sulphonate 2.2×10^{-3} M in methy-lene chloride-1-pentanol (199 - 1)	3.2 mm / sec.	U.V. 254 nm	Alprenolol Metoprolol Oxprenolol Propranolol	1.10 1.11 1.00 1.12	167

Table 1.11 Contd....

COLUMN	STATIONARY PHASE AND SUPPORT	DERIVATIZING AGENT	MOBILE PHASE	FLOW RATE	DETECTOR WAVELENGTH	COMPOUND	α		REF.
10 cm x 4.5 mm I.D	μ-Bondapack C 18 (10 μm)	Tert-butoxycarbonyl-Carbonyl-L-Leucine	35% acetonitrile in phosphate buffer pH3	0.5 ml /min	Fluorometric Excitation	Alprenolol Metoprolol	1.27 1.45		168
25 cm x 4.6 mm I.D.	Spherosorb C18 (μm)	R-(+)-1-Phenyl-ethyl isocynate	Methanol:Water (65:35 v/v)	3.2 ml/ min	Fluorometric Excitation 220 nm Emission > 340 nm.	Propranolol	Well-resolved		169
							AITC	GITC	
15 cm x 4.6 mm I.D	Ultrasphere ODS (5μm)	2,3,4, tri-O-acetyl-α-D-arabino-pyranosyl isothio-cynate (AITC) and 2,3,4,6-tetra-O-acetyl-ß-D-gluco-pyranosyl-isothio-cyanate (GITC).	Acetonitrile in 0.02M aqueous ammonium phos-phate. The % of CH_3CN in the mobile phase ranging from 37-75 (depend on the separation of the Drugs)	-	U.V. 254 nm.	Propranolol Pindolol Alprenolol Bupranolol Atenolol Pronethalol Metoprolol 4-OH-propra-nolol Practolol Deacetyl-Practolol Sotalol	1.28 1.24 1.24 1.21 1.26 1.28 1.24 1.26 1.28 1.28 1.22	1.14 1.09 1.15 1.10 1.09 1.22 1.11 1.10 1.12 1.13 1.11	170
25 cm x 4.6 mm I.D.	ODS (5μm)	S-(-)-and R-(+)-1-phenyle-hyl isocyanate	Water-methanol-triethylamine (50:50:0.05)	1.2 ml/ min	Fluorometric Excitation 238 nm. Emission 450 nm	Acebutolol Diacetolol	1 1		171

b) To prolong the pharmacological and therapeutic effects of the drug and to provide more uniform plasma levels (e.g. glyceryl trinitrate).

c) To cut down the dosage frequency thereby improving compliance.

In addition to these advantages, however, sustained-release preparations also have certain drawbacks which may be summarised as follows:

a) Inadequate plasma concentration which maybe due to unexpectedly low absorption of the active ingredients. This is particularly liable to occur in patients with abnormal gastro-intestinal motility.

b) Sustained-release preparations contain a larger dose of the drug than is usually administered at one time. If the rate of release of the active ingredient is unexpectedly high, a toxic reaction may be produced.

c) The preparations are more complicated to manufacture and require more extensive quality control.

A number of methodologies for controlled-drug release have been developed. These include, repeat-action, prolonged-action and controlled-release systems.

a) Repeat-action preparations provide for the usual initial dose and are constructed to deliver a second dose after a period of time. Repeat action tablets with slow release cores are prepared by mixing the active drug with substances that are nonabsorbable from the gastro-intestinal tract (e.g. cellulose esters). Drug delivery is dependent upon leaching of the active substance from the inner carrier. An initial loading dose can be formulated in the outer layer of the tablet. Examples of such preparations were described by Guley et al 1982 (172) and Deneale et al 1982 (173) for sustained-release preparations of propranolol hydrochloride.

b) Prolonged-action products also provide an initial dose followed by replacement delivery, thus prolonging the therapeutic effect when compared to the usual single dose.

c) Controlled-release systems, where the initial dose is superimposed on a constant rate of delivery to maintain the desired drug level over a period of time.

More sophisticated systems are based on technology that uses porous inert carriers and ion-exchange polymers. In the former, an inert plastic containing thousands of minute pores is filled with drug and channelling agent. The agent attracts fluids from the gastro-intestinal tract, which dissolve the drug [*Sjogren 1971 (182)*]. In the latter case the active drug is bound to an ion-exchange polymer. Drug release is affected when ions within the gastro-intestinal tract displace the drug from the matrix.

1.7.1. RATIONALE FOR DRUG RESINATES AS AN ORAL SUSTAINED-RELEASE SYSTEM

In recent years ion-exchange resins have found a number of uses in medicine, but the possibility of using ion-exchange resins as a means of obtaining sustained-release systems has not been thoroughly explored. The reported work in this area, however suggests that the principles of ion-exchange presents a potentially useful method of sustained-release control.

Resinate formulations also offer an additional advantage. Drug release is initiated by exchange of bound drug, in a charged form, with ions in the bathing medium. This process is reliable since the release depends upon the ion concentration in the environment and in the gastro-intestinal tract which is relatively consistent from one person to another. Drug release is also unaffected by pH, temperature, enzymes or fluid volume in the gastro-intestinal tract. Other advantages of ion-exchange resins are that complexation may be a route to taste-mask unpleasant compounds and also enhancement of stability of labile drugs may be achieved. The main area of application of this type of formulation is in the field of oral delivery.

Procedures are based upon loading of either positively or negatively charged drugs onto the appropriate ion-exchange resins (cation or anion exchangers) to yield insoluble, unabsorbable drug-resin complexes.

$$R - \overset{-}{SO_3}\overset{+}{H} + H_2N - B \longrightarrow R - \overset{-}{SO_3} \text{ ----- } \overset{+}{H_3N} - B$$

$$R - NH_2 + HOOC - A \longrightarrow R - \overset{+}{N}H_3 \text{ ------ } \overset{-}{O}OC - A$$

$$R - \overset{+}{N}(CH_3)_3Cl^- + Na^+\overset{-}{O}OC - A \longrightarrow R\overset{+}{N}(CH_3)_3 \text{----} \overset{-}{O}OC - A + NaCl$$

Where: H_2N - B and HOOC - A are the basic and acidic drug respectively.

$R - \overset{-}{SO_3}\overset{+}{H}$ and $R - NH_2$ or $R - \overset{+}{N}(CH_3)_3$ are the cationic and anionic resins respectively

Drug release takes place when the drug-resinate contacts gastro-intestinal fluids and ionic species therein. The displacement reaction from drug-resinates could be explained by the following equations.

In the stomach:

$$R - SO^-_3 - - -H_3N^+ - B + HCl \longrightarrow R - SO^-_3H^+ + B - \overset{+}{N}H_3\ Cl^-$$

$$R - \overset{+}{N}H_3 - - \overset{-}{O}OC - A + HCl \longrightarrow R - \overset{+}{N}H_3Cl^- + A - COOH$$

$$R - \overset{+}{N}(CH_3)_3 \text{ ----- } \overset{-}{O}OC - A + HCl \longrightarrow R - \overset{+}{N}(CH_3)_3\ \overset{-}{Cl} + A - COOH$$

In the intestine:

$$R - SO^-_3 - - -H_3N - B + NaCl \longrightarrow R - SO^-_3\ Na^+ + B - \overset{+}{N}H_3\ Cl^-$$

$$R - \overset{+}{N}H_3 - - - \overset{-}{O}OC - A + NaCl \longrightarrow R - \overset{+}{N}H_3Cl^- + A - COONa^+$$

$$R - \overset{+}{N}(CH_3)_3 \text{ --- } ^-OOC - A + NaCl \longrightarrow R - \overset{+}{N}(CH_3)_3\ \overset{-}{Cl} + A - \overset{+}{COONa}$$

Both exchange and diffusion undoubtedly play roles in controlling the release of drug from its resinate, but there is no definitive work to separate the two factors although it might be expected that diffusional factors will be of increasing importance as particle size increases.

1.7.1.1. SOME IMPORTANT RESIN PROPERTIES

Various resin characteristics such as available capacity, acid-base strength, particle size and degree of cross-linkage can be chosen to accomplish the intended sustained properties.

a) Available Capacity

The capacity of the ion-exchange resin determines the ability of the resins to combine with exchangeable counter-ions. Experimentally, the actual capacity of the resin will depend on the affinity of the functional group to combine with the drug under investigation. Typical values for polystyrene sulphonate resins are in the range of 4.4 mEq. g^{-1} and for strong anion exchangers are 3.8 mEq.g^{-1} .

b) Acid-Base Strength

The release of drug from its resinates is also dependent upon the pKa value of resin. The acid strength of a cation exchange resin depends upon the various acidic groups incorporated into the resin itself. If a weakly cationic resin has a pKa value of 6, below pH 4 virtually no complexation would result. Above this value, the exchange capacity would increase with pH, as more ionisation of the pendant groups resulted. In contrast strong cationic resins would be highly dissociated at all pH values found in the gastro-intestinal tract, and their exchange capacity would be independent of pH.

c) Particle Size, Porosity and Swelling

Several studies have shown that the particle size of the resin exerts considerable influence on the release rate of the drug from its resinate .Decreasing the resin particle size leads to a reduction in the time required for equilibrium to be reached, [*Helfferich 1962 (174) and Boyd et al 1947 (175)*].

The rate of the ion-exchange reaction for the resin is also very much influenced by the porosity. This is dependent upon the degree of cross-linking controlled by the proportion of cross-linking agent (e.g. divinyl benzene) and on the actual polymerization procedure [*Seidl et al 1967 (176)*].

The pore size has been found to affect both the uptake and release of large organic ions and exhibits a sieve-like effect. Ion-exchange resins also swell to a limited extent in aqueous environments. This also is dependent upon the degree of cross-linking.In some circumstances reversible swelling characteristics could be used advantageously, for example, it has been reported that when cationic exchange resins

in the salt-form are brought in contact with acid, they shrink [*Samsonov and Pasechnik 1962 (177) and 1964 (178)*]. This shrinkage produces a decrease in the pore size in the periphery of the resin particles which causes entrapment of large ions [*Boyd et al 1947 (175)*].

For these reasons, the relative pore diameter in acidic or basic environments should be considered when the uptake of drugs by an ion-exchange resin is contemplated.

1.7.1.2 PREPARATION AND EVALUATION OF DRUG RESINATE

A) Preparation of Drug Resinate

A drug-resinate can easily be prepared using either of two methods: a) column process and (b) batch process:

(a) Column Process:

In this process a highly concentrated drug solution is passed through a bed or column of the resin several times until equilibrium is reached.

(b) Batch Process:

Loading of drugs onto the ion-exchange resin using this process can be established by stirring the resin with a large volume of a concentrated solution of the drug to be loaded. Chaudhry and Saunders 1956 (179) reported that the column process yields higher loading of the resin than the batch process. This was in agreement with Borodkin and Sundberg 1971 (180), who suggested that the drug-resin complexes prepared with amine drugs and polymethacrylate resin using the column process gave higher drug content in the resinate.

B) Evaluation of Drug Resinate Preparations

Although some attempts were made [e.g. Lang 1971 (184)] to develop standard specific dissolution methods to be used in the evaluation of drug-resin complexes, there is no such procedure yet. Instead, several conventional in-vitro dissolution tests that are already described in the literature [Lazarus and Cooper 1959 (181), Hersey 1969 (185), Sjorgren 1971 (183) and Barr et al 1972 (184) have been applied to the in-vitro testing of drug-resin complex preparations.

Due to this lack of standard procedure, comparison of different authors' results sometime proves difficult. In particular, the variables used in the tests, including agitation rate, dissolution medium composition and sampling time, are often quite different or inadequately described. Mainly two methods are employed in order to carry out the kinetic studies in release of drug from drug-resinates. These consist of either packing the resinate particles into a column and circulating a suitable fluid (simulated gastric or intestinal fluid) through the column or suspending the resinate particles in simulated gastric or intestinal fluids with appropriate speed of agitation. Such a system was described by Kressman and Kitchenere 1949 (186).

1.7.1.3 KINETIC INTERPRETATION OF IN-VITRO DISSOLUTION TESTS

Quantitative studies involving the release of a drug molecule from its resinate have been carried out with a concern for equilibria rather than kinetics, especially when the studies dealt with small ions where the equilibrium is rapidly established. This is in contrast to the case of large organic ions where equilibria is reached more slowly and the the kinetic processes are more important.

Hamlow et al 1956 (206) studied the release rate of drug from a resinate and found that the release can be controlled by diffusion in either the resin particle (particle diffusion) or in the adherent stagnant film (film diffusion).The more predominant is the rate controlling factor.

The release of the drug molecule from an ion-exchange resin in simulated gastric or intestinal fluids has been found to be controlled mainly by particle diffusion.

Boyd et al 1947(175) reported that when the particle diffusion is the rate controlling step (considering the resin particle as uniform spheres), equation 1.2 can be used for calculating the fraction of the drug released (F) at any time (t).

$$F = \frac{Qt}{Q\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{e^{-n^2Bt}}{n^2} \qquad 1.2$$

Where Q_t and Q_∞ are the amount released at time t and at time ∞, respectively,

Equation 1.2 operates only when a thin layer of resinate particles are exposed continuously to an infinite volume of fluid having a constant composition. For F values less than 0.85 Reichenberg 1956 (187) reported the following equation.

$$F = \frac{6}{\pi^{3/2}}\sqrt{Bt} - \frac{3}{\pi^{2}}(Bt) + \frac{6}{\pi^{3/2}}\int_{0}^{Bt}\frac{\sum_{n=1}^{\infty} e^{\frac{-n^2\pi^2}{Bt}}}{\sqrt{Bt}}.d(Bt) \qquad 1.3$$

Where Bt for each F value can be calculated using equation 1.4.

$$Bt = 2\pi - \frac{\pi^2 F}{3} - 2\pi\left(1 - \frac{\pi F}{3}\right)^{1/2} \qquad 1.4$$

A plot of Bt-values versus time yields a straight line with an intercept equal to zero and slope equal to (B).Using the calculated B value ,the effective diffusion coefficient (D) can be also calculated using the following equation.

$$B = \pi^2 D / r^2 \qquad 1.5$$

where r is the resin particle radius.

B and D values are important parameters which have been often used for studying of the resinate behaviour [*Chaudhry and Saunders 1956 (179) and Gyselinck et al 1981 (188)*].

Motycka and Nairn 1979 (189) and 1985 (190) studied the release of the drug ions from the resinates coated with various encapsulation techniques and observed a further delay in the drug release, but the release data still gave linear relatioship of Bt - t plots.

1.7.1.4 DRUG RESINATE AS ORAL LIQUID DELIVERY SYSTEMS

Drug-resin complexes may be tableted or encapsulated by standard procedures [*Motycka and Nairn 1979 (189) and Janusz and Zdzislow 1981 (191)*], but a further

important feature is that they can be suspended in a low ion content vehicle [*Amsel 1981 (192)*]. In liquid systems of low ionic strength they do not elute drug to the vehicle, in contrast to other (coated or matrix) forms of sustained action formulations. Such liquid preparations would be ideal for geriatric and paediatric patients.

Increased stability by protecting the drugs from hydrolysis or other degradative changes in the gastro-intestinal tract is also an advantage of complexing drugs with ion-exchange resins [*Keating 1961 (195)*]. Additionally, several authors have pointed out the reduced toxicity of drugs when used as a combination with ion-exchange resins. As a result of extensive investigations Keating 1956a (193), 1956b (194), 1961 (195) and 1964 (196) showed that with resin systems, the therapeutic effect is much more prolonged without an increase in toxicity or deleterious side effects.

1.7.1.5 PROBLEMS WITH THE CURRENT ION-EXCHANGE SYSTEMS

Ion-exchange resin-drug complexes have been used to formulate sustained-release products. However, sufficient sustained effect is usually difficult to achieve and improvements have been effected in some cases by coating the resin beads to provide an additional barrier. The encapsulation of solid particles is well known, but few workers have applied this process to ion-exchange resins [*Borodkin and Sundberg 1971 (180), Phares and Sperandio 1964 (197)*].

Direct application of an automized polymer solution to the fluidized drug-resinates was ineffective in controlling drug release since the coating came off in the dissolution medium due to swelling and fracturing of the resin particles. A novel technique of pretreatment of the drug-resin complex particles with a solvating agent such polyethylene glycol (PEG) was reported to overcome this problem and gave better results due to the retainment of the geometry of the resin particles during dissolution [*Amsel 1981 (192) and Raghunathan et al 1981 (198)*]. This approach has been utilized in other patentable developments [*Koff 1964 (199), Raghunathan 1979 (200) and Raghunathan et al 1980 (201)*], and a rank order

correlation between the rate of dissolution and blood levels has been obtained. This indicates the need for a better and simpler method for coating ion-exchange resin-drug particles.

1.7.2 SUSTAINED RELEASE PREPARATIONS OF PROPRANOLOL (review)

Several sustained release preparations containing propranolol have been developed and published in the recent years. In 1977 the Imperial Chemical Industires Limited (I.C.I. London) invented a sustained-release preparation containing propranolol hydrochloride [*British Patent 1980 (202)*] . A hard gelatin capsule containing film coated spheroids which consisted of propranolol hydrochloride and non-water swellable micro-crystalline cellulose (60:40), film coated with a mixture of ethyl cellulose and hydroxy propyl methyl cellulose (90:10), to about one-tenth of the weight of the coated spheroid. Each capsule contained 160 mg of propranolol HCl.

Jayaswal and Bedi 1980 (203) prepared a sustained-release solid dosage form containing propranolol hydrochloride by using cation-exchange resins. Weak and strong exchangers, both individually and in combination and of different particle size were used. Some of the in-vitro release data of these preparations are shown in Table 1.12.

Gyselinck et al 1981 (188) prepared sustained-release propranolol by combination with ion-exchange resins (DOWEX) and studied the effect of cross-linking, particle size and temperature on the loading and release of propranolol from these preparations. A summary of these results is shown in Table 1.13 a and b.

Zalani and Upadhyyaya 1981 (204) prepared prolonged acting beads of propranolol hydrochloride. Beads were prepared using this drug and Avicel (70:30) and were coated with different coating material such as Eudragit-S100, shellac, ethyl cellulose, cellulose acetate phthalate and combinations of hydrogenated castor wax, hard paraffin and stearic acid. They found that beads coated with 12% wax solution gave a satisfactory release pattern suitable for formulating a twelve hour prolonged action product. The formulation and in-vitro release data (in simulated gastric juice pH 1.2) of propranolol are listed in Table 1.14 a and b.

Batch No.	Resin	Particle size (mesh)	Amount of propranolol HCl bound/gm of dry resin (mg) (conc. of drug used for treatment 600 mg/30 ml)	Average cumulative per cent Release								
				pH 1.5	1.5	3.0	3.0	4.5	6.4	7.2	7.2	7.6
				Hr. 0.5	1	1.5	2	3	4	5	6	7
A_1	Amb. IRC-50	10/20	35.00	77.07	87.65	90.72	91.85	93.63	94.45	95.23	96.10	97.28
A_2	—do—	20/40	40.00	74.12	91.56	94.63	96.71	98.48	99.11	100.23	101.35	101.92
A_3	—do—	40/80	69.00	81.20	93.09	95.70	97.18	98.51	99.54	101.07	102.10	102.92
A_4	Amb. IR-120	10/20	77.00	3.96	8.79	—	11.29	14.61	17.46	20.12	23.80	26.96
A_5	—do—	20/40	78.25	1.97	3.79	—	5.54	7.80	9.55	11.38	13.64	15.58
Z_1	Zeroit 225	10/20	79.50	4.63	9.37	—	12.35	15.91	19.53	19.78	23.19	26.28
Z_2	—do—	20/40	114.00	4.40	7.33	—	10.19	12.83	16.19	19.91	24.34	27.77
Z_3	—do—	40/80	118.25	3.03	6.80	—	10.40	15.63	20.85	27.37	33.60	38.71

Table 1.12 Summary of in-vitro release data of propranolol HCl from 250 mg of various drug-resin complexes made with ion-exchange resins of different particle size (from Reference 203).

Propranolol Content in Different Propranolol Resinates [%]

Preparation [°C]	Ion exchanging resin	50 – 100 mesh	100 – 200 mesh	200 – 400 mesh
25	Dowex 50 WX 2	68.3	73.2	66.9
25	Dowex 50 WX 4	63.5	70.9	69.7
25	Dowex 50 WX 8	37.7	53.4	59.9
25	Dowex 50 WX 12	43.6	38.1	41.3
70	Dowex 50 WX 2	—	—	70.7
70	Dowex 50 WX 4	—	—	67.2
70	Dowex 50 WX 8	—	—	53.1
70	Dowex 50 WX 12	52.4	47.0	49.4

B-Values for the Release Curves of Different Propranolol Resinates

Preparation [°C]	Ion exchanging resin [min-1]	50 – 100 mesh	100 – 200 mesh	200 – 400 mesh
25	Dowex 50 WX 2	0.0015	0.0047	0.0069
25	Dowex 50 WX 4	0.0014	0.0036	0.0050
25	Dowex 50 WX 8	0.0005	0.0014	0.0023
25	Dowex 50 WX 12	0.0008	0.0010	0.0014
70	Dowex 50 WX 2	—	—	0.0044
70	Dowex 50 WX 4	—	—	0.0043
70	Dowex 50 WX 8	—	—	0.0020
70	Dowex 50 WX 12	0.00049	0.0009	0.0013

Table 1.13 The effect of cross-linking, particle size and temperature on the loading and release of propranolol from its resinates (from Reference 188).

Product	Coating	% Coating material applied	Drug content* per 100 g
Uncoated beads A	—	—	62.68
AI	Eudragit-S100	7.87	47.71
AII	Eudragit-S100	10.68	47.34
BI	Shellac	5.27	54.31
BII	Shellac	7.00	53.34
CI	CAP	6.00	54.82
DI	E.C.	10.00	49.13
EI	Shellac + Wax	3.5 + 12	50.00
EII	Shellac + Wax	3.5 + 48	41.81

*The Drug content in coated beads is less than expected due to lubricant (e.g. talc. mag. sterate etc.) dusting during coating in the pan.
CAP = Cellulose Acetate phthalate.
E.C. = Ethyl cellulose.

Table 1.14 (a) Formulation of propranolol hydrochloride coated beads (from Reference 204).

Product	Cumulative per cent released				
	1 hr.	2 hr.	3.5 hr.	5 hr.	7 hr.
AI	38.49	71.13	86.67	90.83	99.65
AII	33.26	58.89	73.52	79.50	88.88
BI	31.07	54.86	65.55	84.74	100.41
BII	20.93	30.14	48.36	64.07	74.41
CI	76.44	93.66	96.99	99.31	99.74
DI	82.32	92.45	95.01	95.89	98.58
EI	21.98	37.61	42.20	82.42	99.05
EII	1.66	3.91	5.91	18.01	75.40

Table 1.14 (b) Dissolution of propranolol hydrochloride beads. (from Reference 204).

Janusz and Zdzislaw 1981 (191) prepared prolonged-action propranolol and some other beta-blockers by combination with some sulphonated polystyrene cation exchange resins of the Dowex or Zerolite type and compression of the final resinate into tablet form. In-vitro dissolution tests showed that the action of practolol and propranolol, unlike oxprenolol was substantially prolonged and independent of the composition of the tablet excipients. The same authors in 1983 (205) studied the release of propranolol from texturised tablets into artificial gastric juice to determine the optimum tablet compositions. From tablets containing equal amounts of propranolol, NaCl and polyvinyl chloride - ethyl cellulose mixture (9:1), the propranolol released within the first hour was equivalent to the initial therapeutic dose while about 90% of the drug was released within 12 hours.

A patent publication by Guley et al 1982 (172) demonstrated the preparation of sustained-release propranolol tablets. The tablet consisted of a compressed core containing the drug and hydroxy propyl methyl cellulose, ethyl cellulose and carboxy vinylpolymer sealed with a mixture of cellulose acetate phthalate and ethyl cellulose by film coating. Finally the sealed compressed core was coated with a sugar coat with a coat to core ratio of 1:7. The same authors published another patent in 1982 (173) for the same purpose where they changed the tablet-core composition. The core consisted of the drug and Carbopol 934 (2.5 - 3.7:1). the other substances employed in the core were stearic acid, microcrystalline cellulose (8 - 25%), mannitol and ZnO (ratio of Carbopol 934 to ZnO was 2.2 - 12:1). The authors reported that the addition of stearic acid decreased the electrostatic effect of carbopol particles, cellulose facilitate the disintegration of the core, mannitol decreased the pH sensitivity of the drug release and ZnO decreased the release rate of the drug by forming a chelate with the Carbopol.

Elan Corporation PLC (Belgium) 1984 have published a patent (207) for the preparation of sustained-release propranolol granules. The granules were prepared from a mixture of propranolol HCl (100 mesh; 100 g.), talc (100 g.) and citric acid (500g) with 8 parts of 10% hydroxy propylmethyl cellulose and 2 parts of 10% ethyl cellulose in

methanol-methylene chloride mixture (60:40). The in-vitro dissolution rate of propranolol was determined and 85 - 100% was dissolved in 10 hours.

Bottini et al 1984 (208) examined the in-vitro dissolution and in-vivo pharmacokinetics of two market long acting propranolol formulations such as Duranol (Elan Corporation) and Inderal L.A. (Ayrest, ICI), and found that both Inderal LA and Duranol demonstrated pH-independent dissolution profiles with T_{50} values of 4 and 6 hours, respectively. The dissolution profile for Duranol was sigmoidal and complete in 12 hours whereas that of Inderal showed only 80% dissolution in 12 hours (Fig 1.7). The pharmacokinetic studies showed that the t_{max} of both products was achieved in 7 to 8 hours and C_{max} values for Duranol were higher than that of Inderal LA either after a single dose or during steady-state drug administration (Fig 1.8).

Ford et al 1985 (209) studied the effect of various types of hydroxypropylmethyl cellulose (HPMC), drug:HPMC ratio and drug particle size on the release rate of amino phylline and propranolol from sustained-release tablets and found that the major factor controlling drug release was the drug:HPMC ratio, increasing the polymer content decreased the dissolution rate of the drug (Fig 1.9).

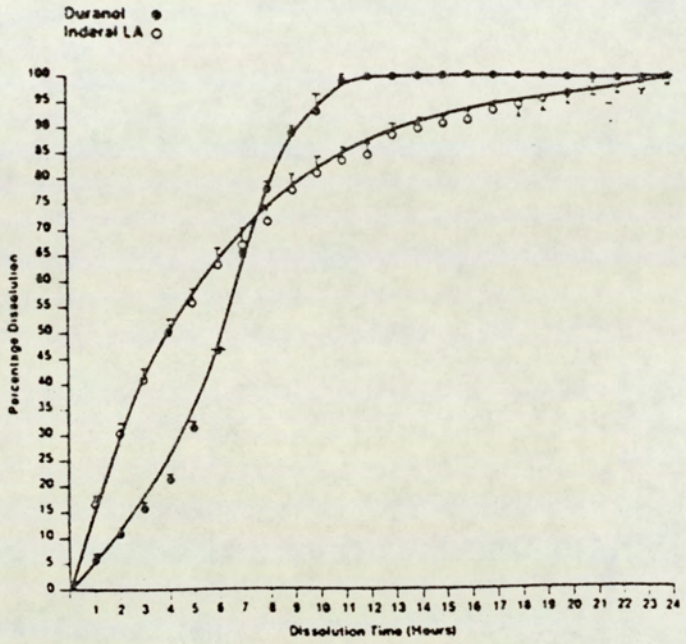


Fig 1.7 In-vitro dissolution profiles for Inderal LA and Duranol (mean ± s.d). (from Reference 208).

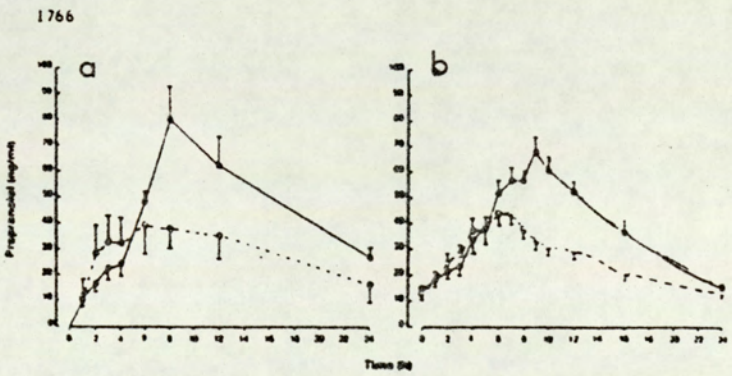


Fig 1.8 Plasma levels of propranolol following administration of Inderal LA 160 mg (O--O) and Dural 160 mg (●--●) (a) after a single dose and (b) at steady-state drug administration. (from Reference 208).

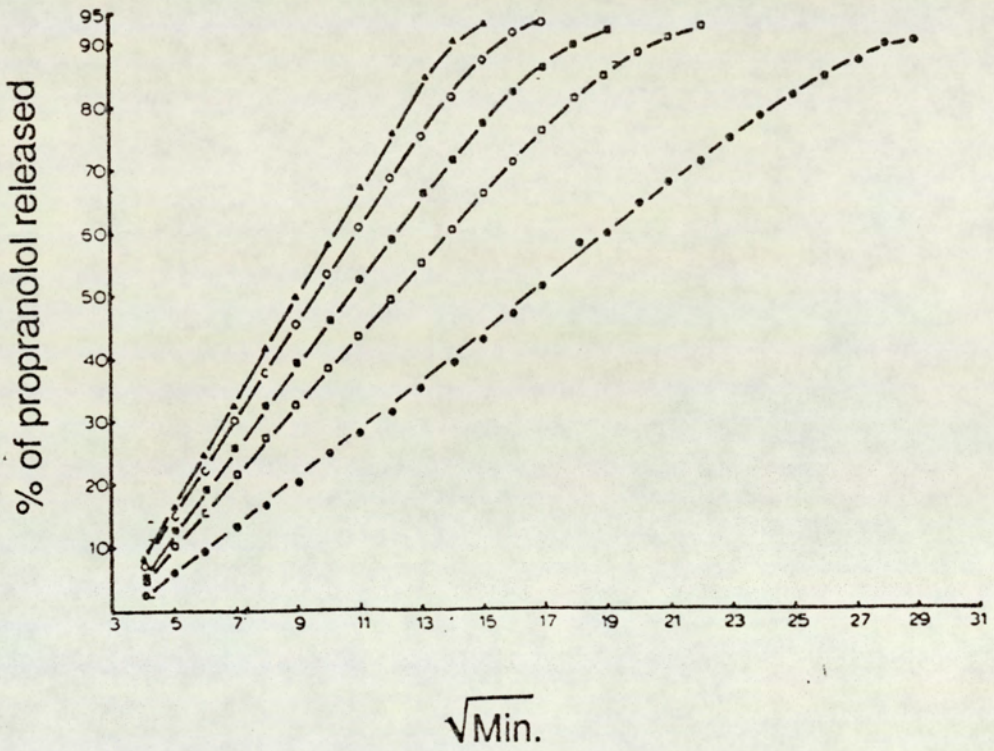


Fig 1.9 The effect of propranolol: hydroxypropylmethyl-cellulose K4M variation on the release of 160 mg propranolol hydrochloride into 1000 ml water at 37°C from tablet containing (mg of HPMC K4M) ▲. 57 ;○.71 ; ■. 95; □ .140 ; ● . 285, Ordinate: % propranolol HCl dissolved. Abscissa : $\sqrt{\text{time}}$ (min. $^{1/2}$) (from Reference 209).

CHAPTER TWO

SYNTHESIS OF PROPRANOLOL PRO-DRUGS

CHAPTER 2

2. SYNTHESIS OF PROPRANOLOL PRO-DRUGS

2.1 INTRODUCTION

The expression "pro-drug" [*Harper 1964 (206), Albert 1973 (210), Stella 1975 (211)*] denotes an inactive derivative of a medicinal compound which enhances its delivery characteristics and therapeutic effectiveness.The pro-drug is converted into the active molecule by enzymic or chemical activation within the body. It is well recognized that pharmacokinetic patterns and, ultimately, therapeutic success can be influenced by relatively minor structural modification [*Notari, 1973 (212)*].

The bioreversible derivatisation of a drug substance to produce a pro-drug with altered physicochemical properties can improve substantially both drug efficacy and safety [*Stella, 1975 (211), Sinkula and Yalkowsky 1975 (213) and Stella et al 1980 (214)*].

Most pro-drug research [*Notari 1977 (215), 1980 (216), 1981 (217)*] had been aimed at prolonging duration, improving bioavailability, solving a formulation problem such as solubility, decreasing pain on injection, improving taste or increasing stability.

One of the most promising pro-drug design concepts is the "inactive metabolite approach" [*Bodor 1982 (218) and Bodor et al 1984 (219)*] which uses an active derivative of inactive metabolite of a drug which can readily convert into the starting inactive metabolite by enzymic or chemical activation within the body without going through reactive, toxic intermediates.

As a part of current studies involving new chemical approaches, an investigation was carried out to obtain pro-drug candidates for propranolol.

The alcoholic functional group in propranolol is readily esterifiable and, therefore, it appeared to be possible to obtain ester derivatives providing improvement in lipophilicity

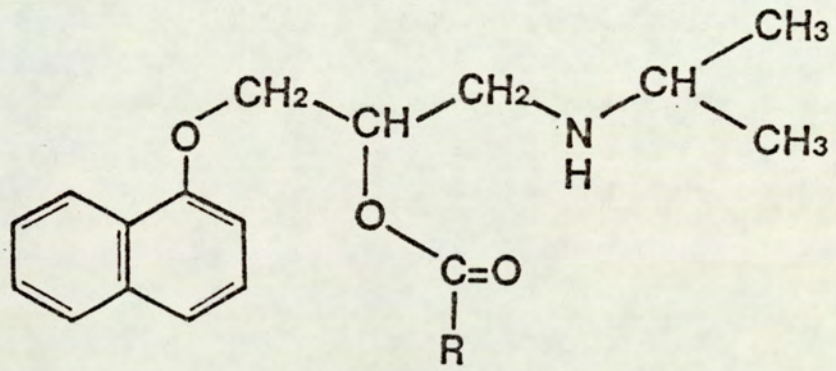
and at the same time, possessing a high susceptibility to undergo enzymatic hydrolysis in the body.

Esterifications of propranolol with the acid chlorides were undertaken under acid conditions to eliminate the competing N-acylation reactions. The preparations of N-acyl and N,O-diacyl derivatives were carried out in alkaline media using triethylamine. The triethylamine would be expected to release the free base and also act as a catalyst in activating acyl chloride to acylation reactions via a complex $[RCO\text{-}NEt_3]^+Cl^-$.

2.2 SYNTHESIS OF O-ACYL PROPRANOLOLS

Synthesis was performed according to the method reported by Crowther and Smith 1968 (88) and Nelson and Walker 1978 (89). Propranolol HCl 1g (3.4 m.moles) was refluxed for three hours with 15 m.moles of the corresponding acid chloride in 40ml chloroform. Excess acid chloride was removed in vacuo using a high vacuum pump and a water bath, the temperature of which was fixed according to the Nomogram which is presented in Appendix III.

The residues for the short chain esters were evaporated twice with 50 ml benzene for complete removal of the acid chloride, while the residues for the longer chain ones were left with 50 ml diethyl-ether in the fridge for a few hours, during which time the esters precipitated as hydrochlorides which were washed twice with 50ml of ether. The final solid ester hydrochlorides were recrystallized from isopropanol, except O-decanoyl ester which isolated only as an oily liquid.The yields and melting points (m.p) of the ester hydrochlorides synthesized are listed in Table 2.1.



R	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_7H_{15}	C_9H_{19}	t.C_4H_9
Yield %	80	79	60	60	52	28	45	65
m.p ^{o}C	171-173	144-145	135-137	127-129	120-122	101-102	-	145-147

Table 2.1. The yields and melting points (m.p.) of propranolol esters.

The infrared absorption spectra of these esters, obtained with a Unicam SP 200 infrared spectrophotometer or with a Perkin-Elmer 1310 infrared spectrophotometer (as Nujol mulls), all show a sharp peak in the 1720 - 1760 cm^{-1} region confirming the presence of the ester carbonyl function.The infrared spectra of propranolol and O-acetylpropranolol as hydrochlorides, are presented in Fig 2.1.

The proton nuclear magnetic resonance (^1H NMR) spectra of the esters were measured using $CDCl_3$ as solvent and $CHCl_3$ as internal standard (δ 7.25) at ambient temperature (295°K) with a 360 MHz Bruker spectrospin n.m.r spectrometer.The chemical shifts, multiplicities, numbers of protons and spectral assignments for all the esters are similar except in the upfield resonances (δ 1.2 - 2.2) where differences due to the increase in chain length were apparent.

The ^1H n.m.r. assignments of these esters are shown in Fig 2.2, and the spectra for the O-acetyl, O-hexanoyl and O-pivaloyl propranolol hydrochlorides are displayed in Fig 2.3 a,b and c.

The mass spectral analyses of the esters were carried out on Micromass-12 mass-spectrometer operated with an ionisation energy of 70 ev, an accelerating voltage of 3.5 kv and a source temperature of 300 °C.

The Spectra obtained by the fragmentation of these esters are displayed in Appendix IV. 1 - 5. These show that many of the fragments are similar in all of the derivatives (Fig 2.4) but specific fragments for each compound are apparent. A fragment (m/z 296) appeared in those esters in which the side chain contained two or more methylene groups. The mass spectra of neither the acetyl nor the pivaloyl esters contained this fragment due to the lack of the appropriate structural feature. The fragmentation pathway to produce the ion at m/z 296 is illustrated in Fig 2.5. These analyses confirm that the synthesised compounds were the expected O-acyl derivatives of propranolol.

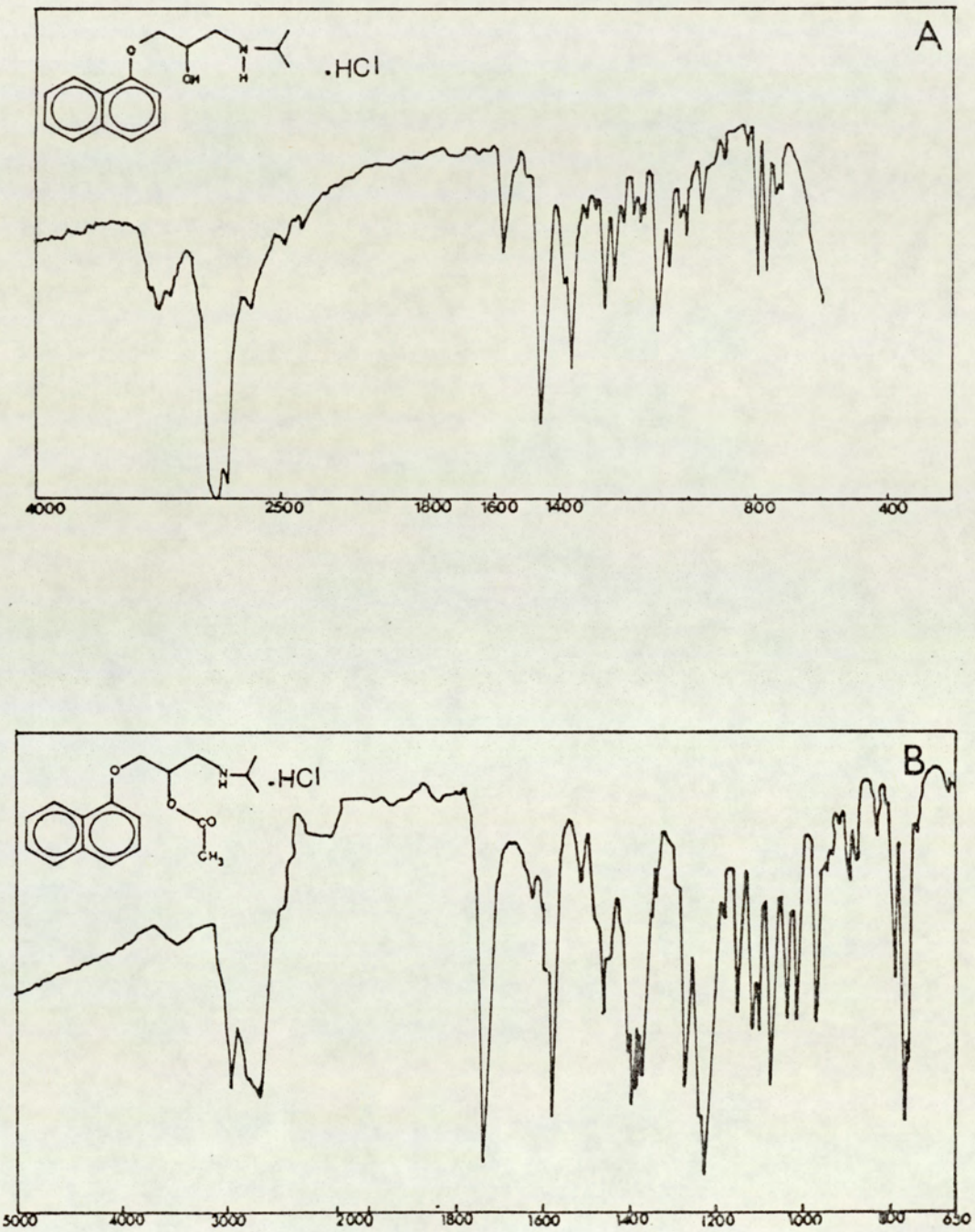


Fig 2.1 Infrared Spectrum of Propranolol HCl (A) and O-acetylpropranolol HCl (B).

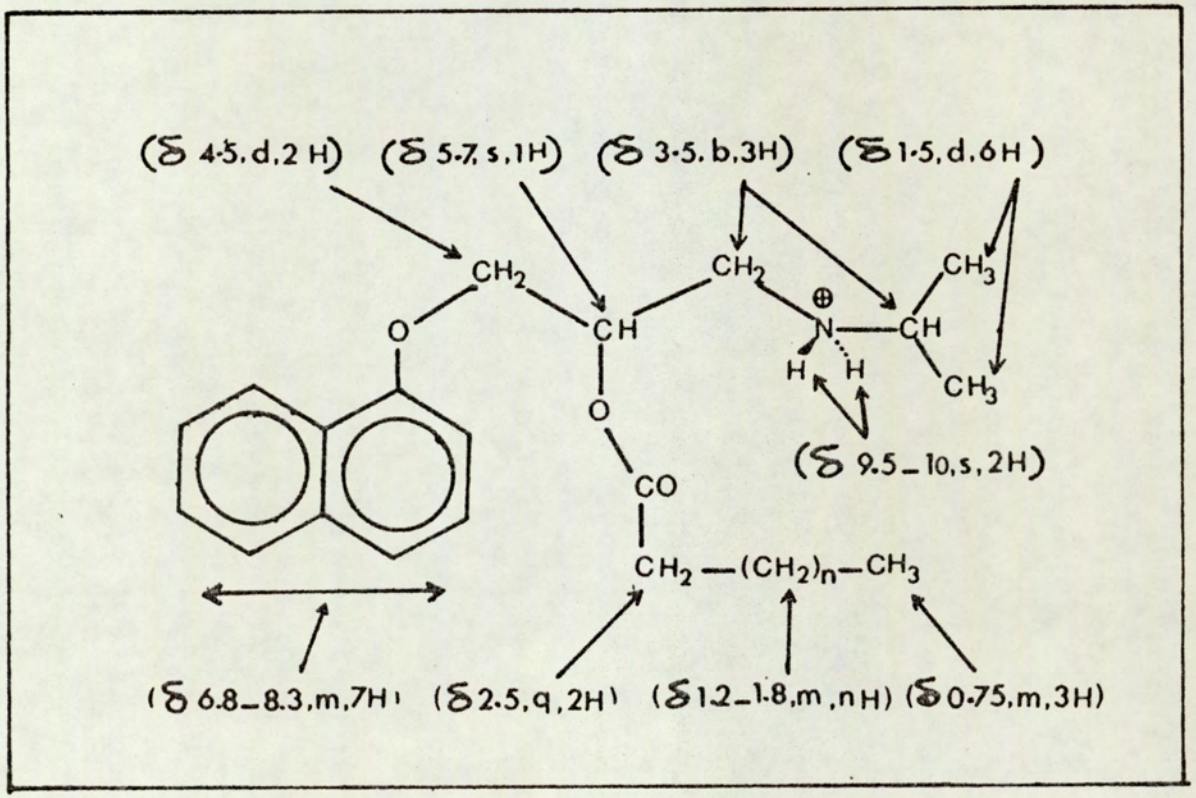


Fig 2.2 ^{1}H NMR Assignments of O-acylpropranolol HCl's

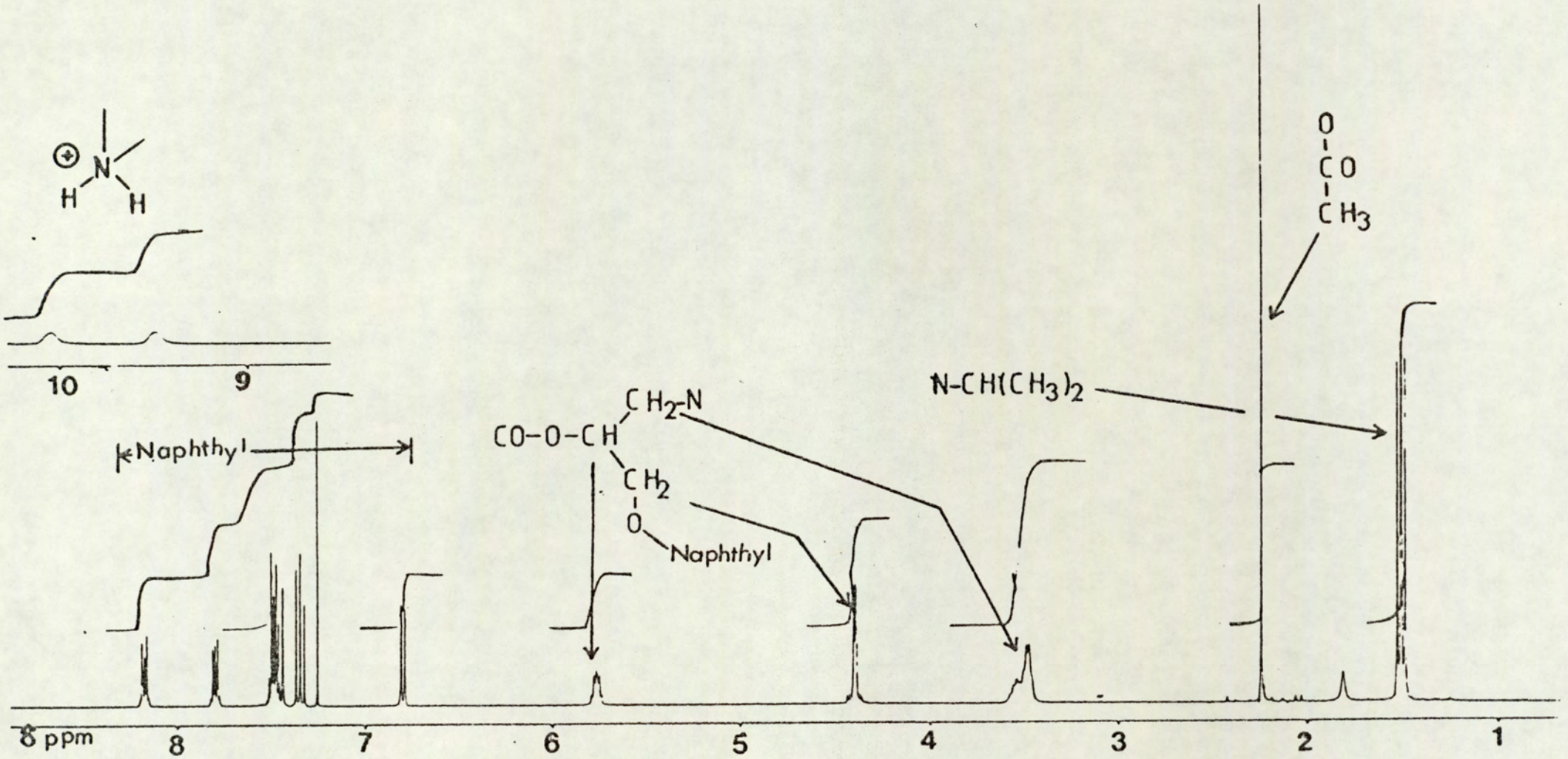


Fig 2.3a 360 MHz ^{1}H NMR Spectrum of O-acetylpropranolol HCl

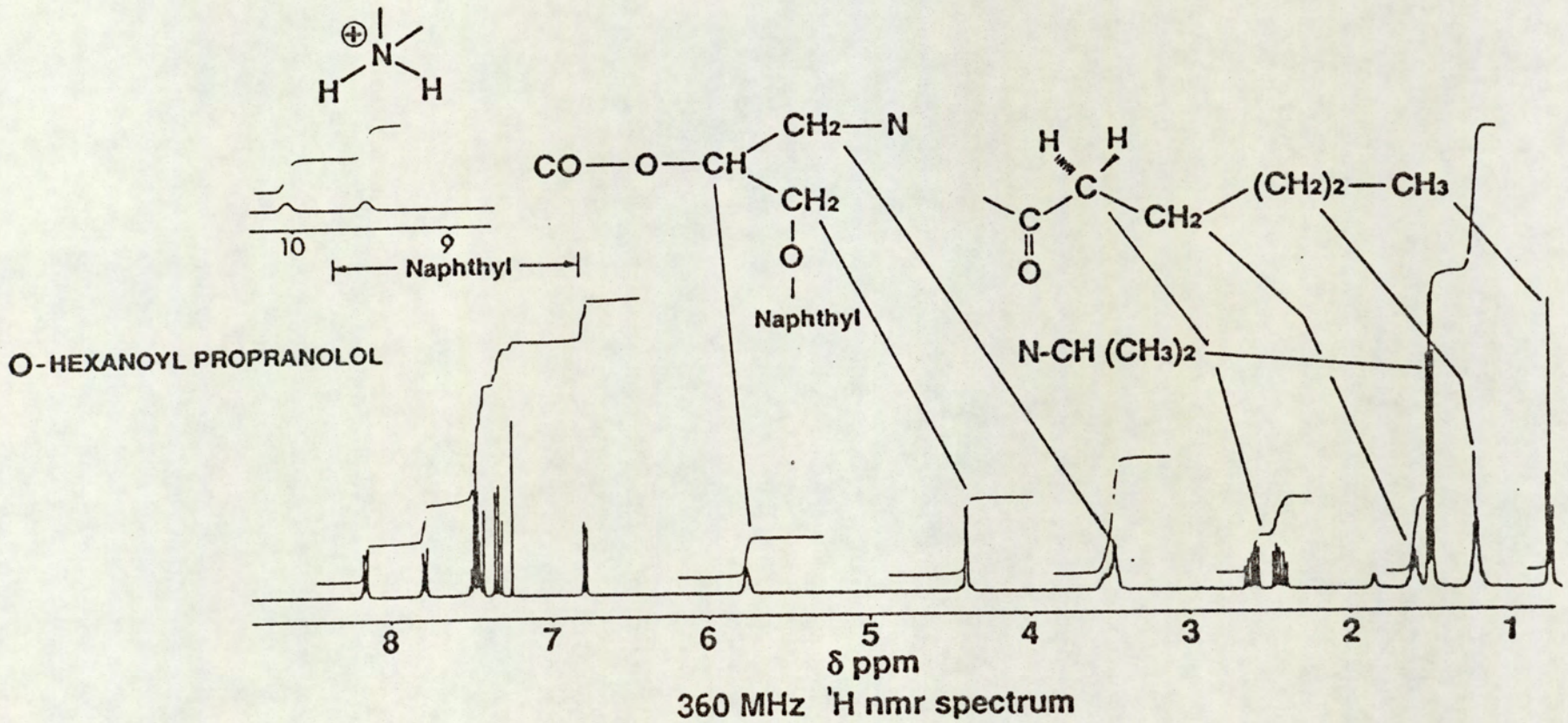


Fig 2.3b 360 MHz ^{1}H NMR Spectrum of O-hexanoly_propranolol HCl

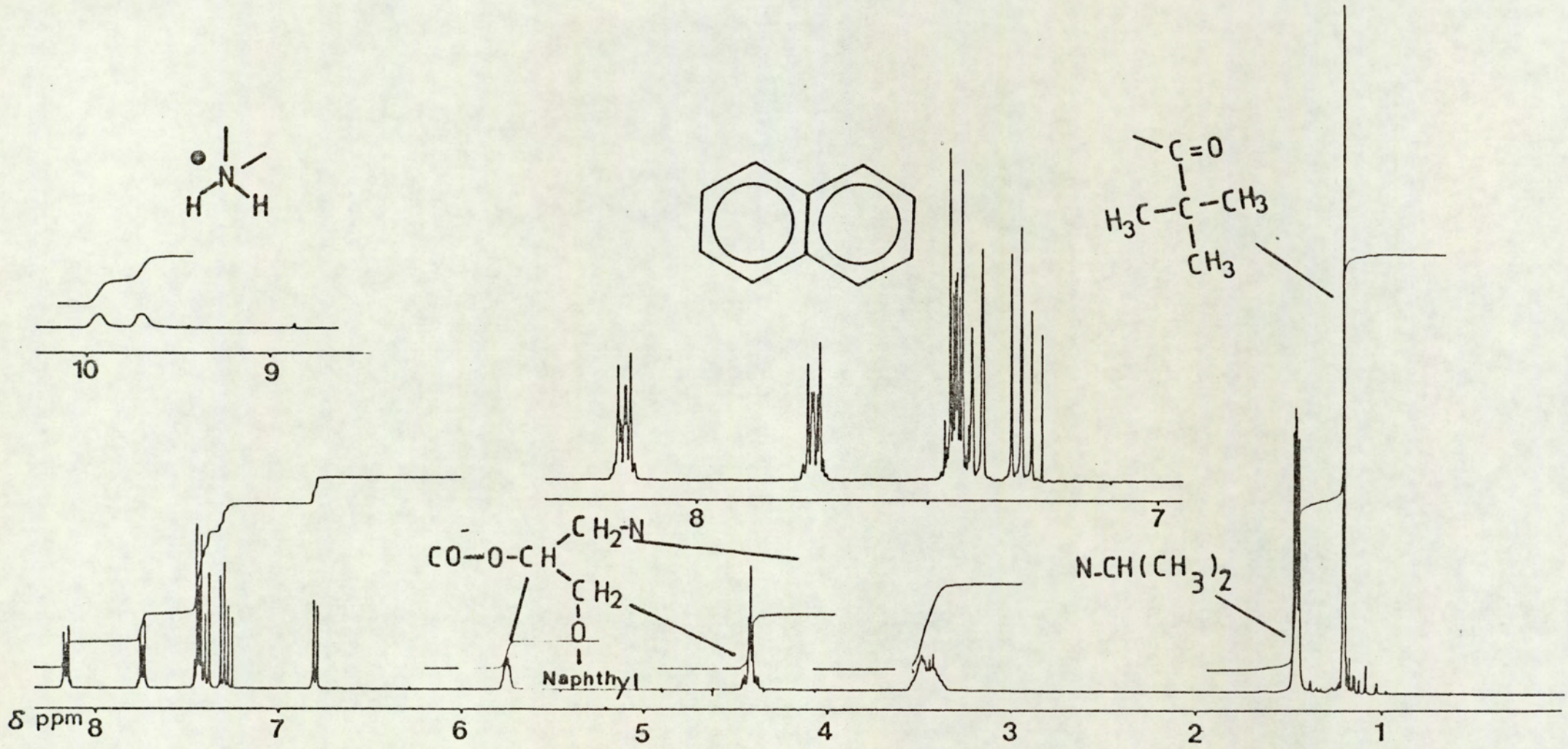


Fig 2.3c 360 MHz ^{1}H NMR Spectrum of O-pivaloylpropranolol HCl

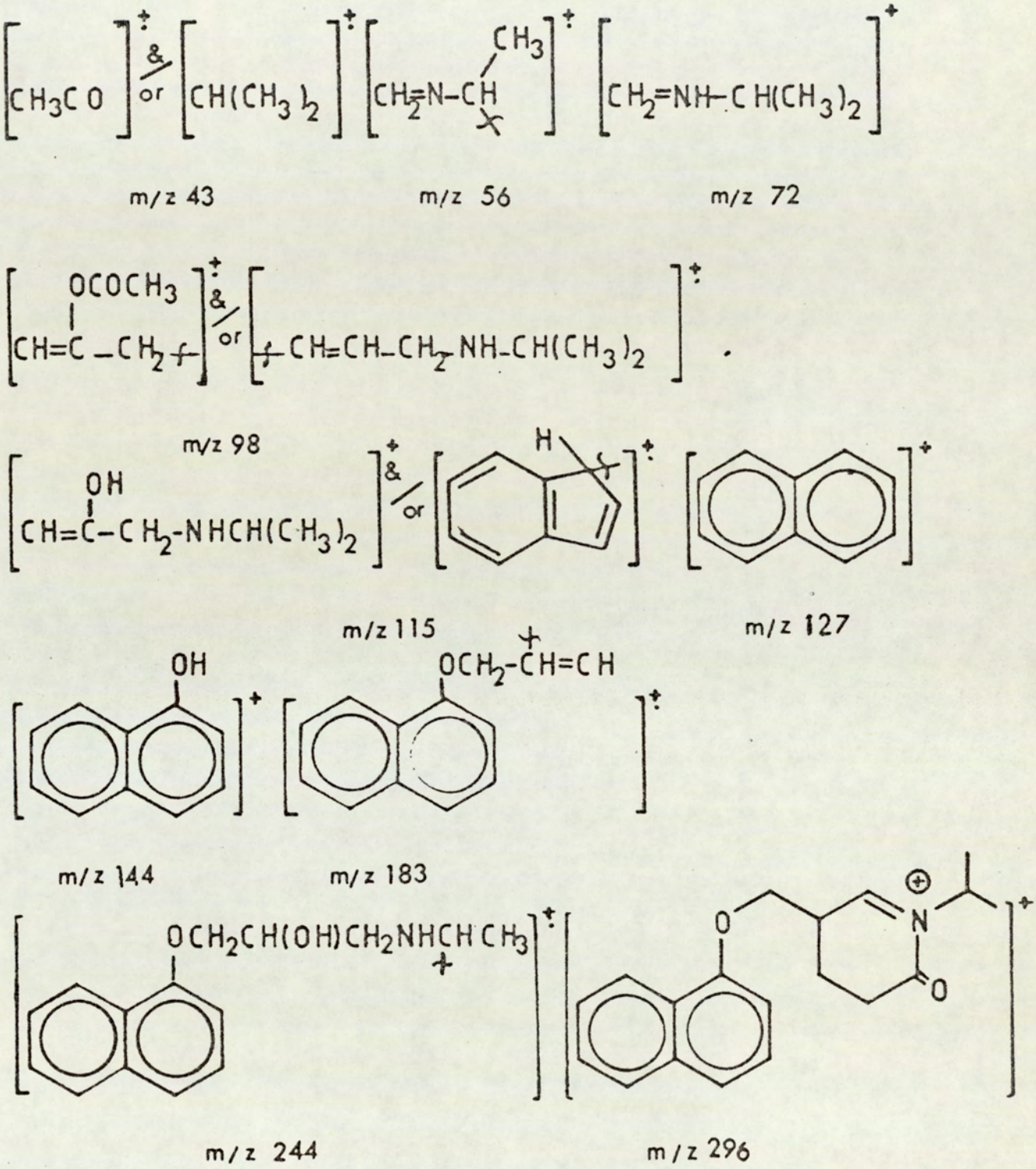


Fig 2.4 Major Fragments in Mass Spectra of Propranolol Derivatives.

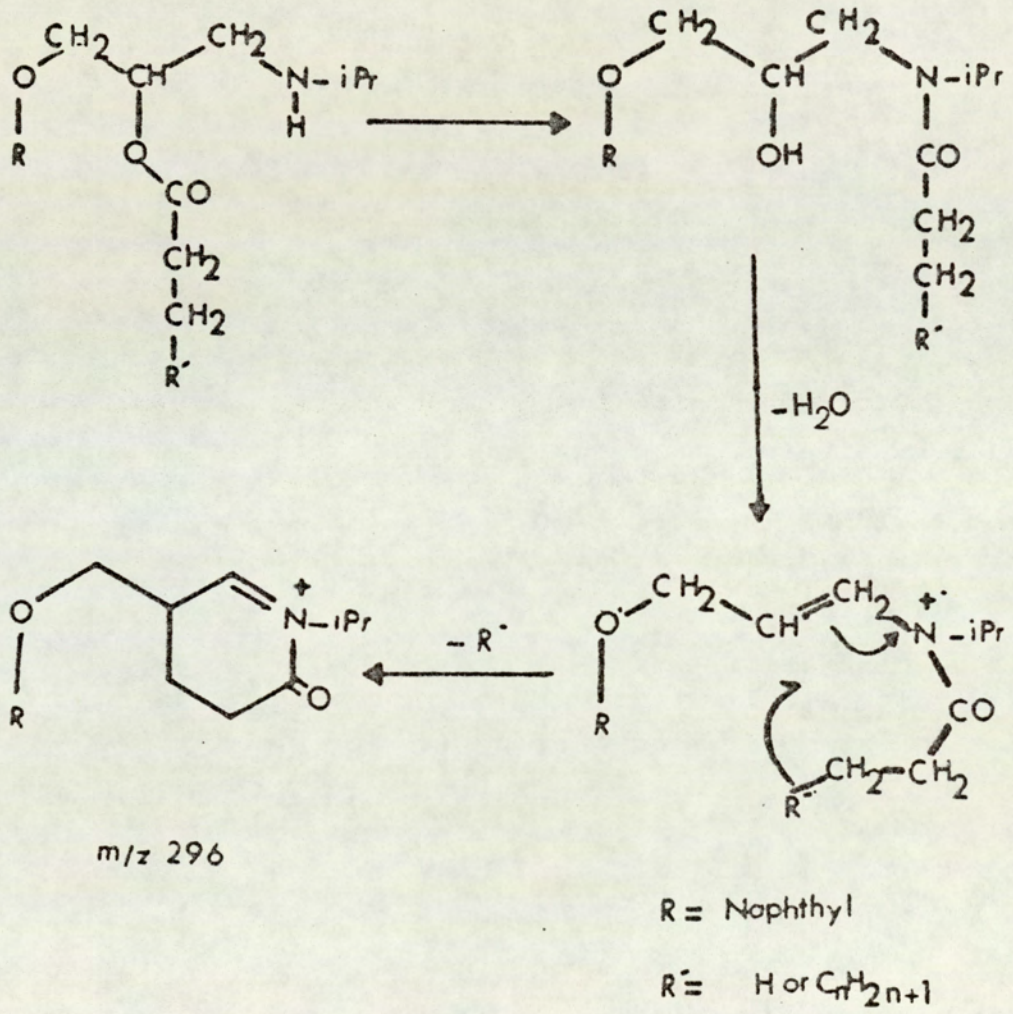


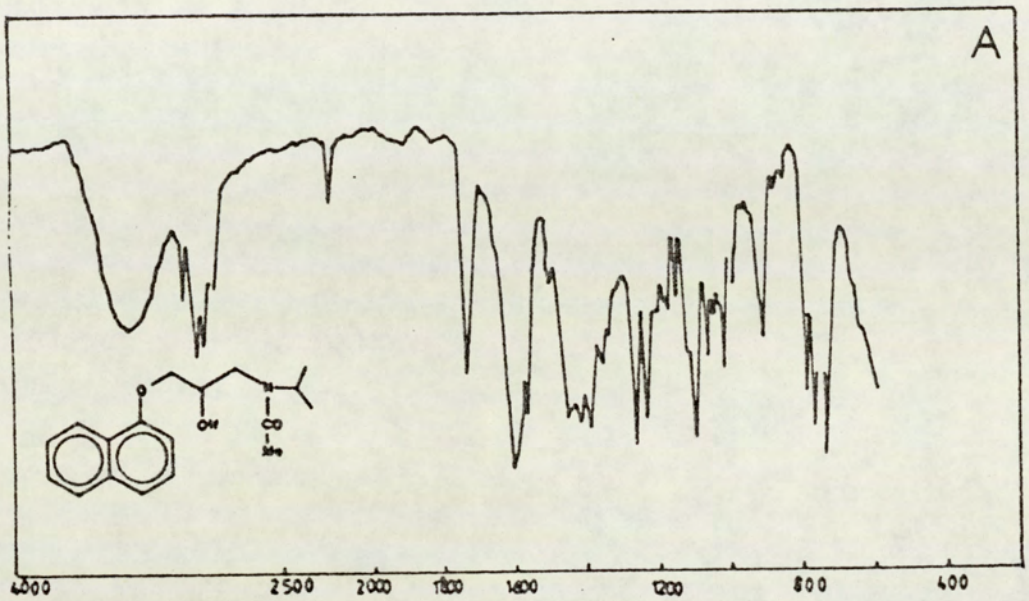
Fig 2.5 Fragmentation Pathway to produce the ion at M/z 296

2.3 SYNTHESIS OF N, O-DIACETYLPROPRANOLOL

Synthesis was similar to that reported by Nelson and Walker 1978 (89) with minor modifications. 1.5g Propranolol HC1 (5.08 m.moles) was heated at 45° C for four hours in a mixture of 30 m.moles acetyl chloride and 100 m.moles triethylamine in 60 ml chloroform.The mixture was washed with water, once with aqueous 0.2M HC1 to remove excess triethylamine, with 5% aqueous sodium carbonate to neutralize residual acid and finally with water.The organic phase was dried over anhydrous sodium sulphate overnight and evaporation in vacuo yielded a yellow oil which recrystallized from benzene-hexane (charcoal) to give 1.2g (73%) of white crystals (m.p 100 - 102°C; literature value 101 - 103°C).

The infrared absorption spectrum of N,O-diacetylpropranolol obtained with a Unicam SP 200 infrared spectrophotometer (KBr disc) showed two strong peaks at 1615 cm^{-1} (N - C = O) and at 1735 cm^{-1} (O - C = O), Fig 2.6.B revealing the diacetylated nature of the product. This was confirmed by the ^{1}H NMR spectral assignments of N,O-diacetyl-propranolol using $CDC1_3$ as solvent and tetramethylsilane (TMS) as internal standard which are displayed in Fig 2.7.B.

The mass spectral analysis of N, O-diacetylpropranolol gave the expected molecular ion (M^+) and showed many of the fragments listed in Fig 2.4 together with other specific fragments which are shown in Appendix IV.5.A.



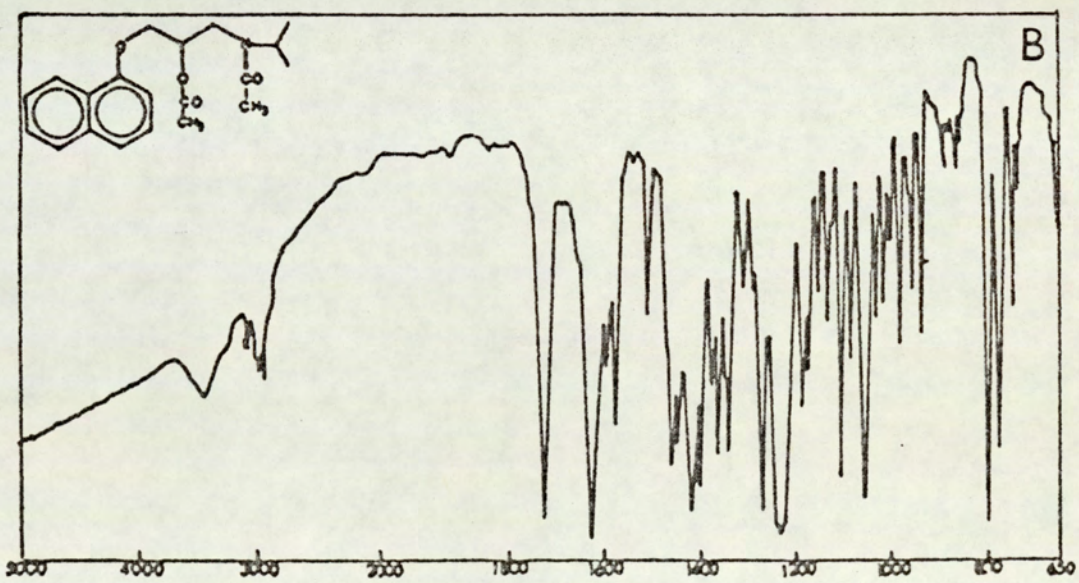


Fig 2.6 Infrared Spectrum of N-acetylpropranolol (A) and N,O-diacetylpropranolol (B).

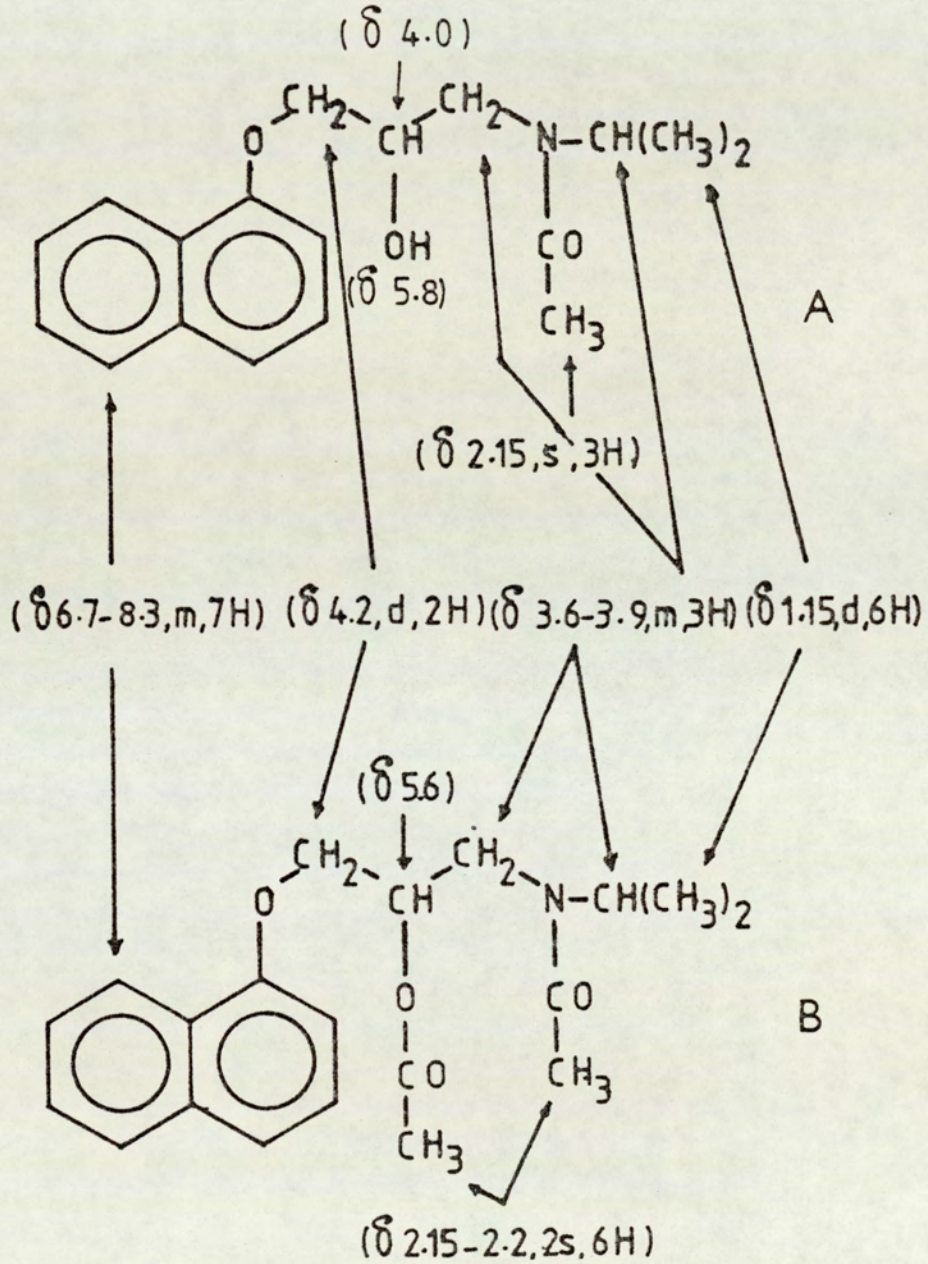


Fig 2.7 ^{1}H NMR Assignments of N-acetylpropranolol (A), and N,O-diacetylpropranolol (B).

2.4 SYNTHESIS OF N-ACETYL AND N-VALERYL PROPRANOLOLS

N-acetyl and N-valeryl propranolol were prepared by an adaptation of the method reported by Nelson and Walker 1978 (89) for N-acetyl propranolol. 1.25g Propranolol HC1 (4.3 m. moles) was heated under reflux for one hour in a mixture of 4.3 m.moles of acid chloride (0.34 g acetyl chloride in case of N-acetylpropranolol and 0.54 g valeryl chloride in case of N-valerylpropranolol) and 2g triethylamine (19.7 m.moles) in 20 ml methylene chloride. The mixture was washed similarly to N,O-diacetylpropranolol and then dried ($Na_2 SO_4$). The organic phase was evaporated in vacuo to give a yellow oil. Traces of unreacted propranolol were removed by dissolving the oil in freshly prepared ethereal HC1 obtained by bubbling a dry hydrogen chloride, prepared according to the method described in Reference (220) through dry ether. Propranolol HC1 precipitated out leaving a clear organic layer which was evaporated in vacuo to give an uncrystallizable oil of pure amide. (The yields for N-acetyl and N-valeryl propranolol were 0.73g (56%) and 0.88g (60%) respectively).

The infrared absorption spectrum of each amide revealed a strong peak at 1740 - 1750 cm^{-1} for the amide carbonyl group. The N-acetylpropranolol spectrum is displayed in Fig 2.6.A.

The ^1H n.m.r. spectrum of N-valerylpropranolol (Fig 2.8) was obtained with a 360 MHz spectrospin n.m.r spectrometer under the same conditions as described in Section 2.2. for O-acyl propranolol, and the assignments of N-acetylpropranolol which obtained with a Varian EM 360A, 60 MHz n.m.r spectrometer (using $CDC1_3$ as solvent and tetramethylsilane (TMS) as internal standard) are presented in Fig 2.7.A.

The mass spectral analysis of N-acetyl and N-valeryl propranolol shows in addition to the common fragments listed in Fig 2.4 , some other fragments characteristic for each amide which are presented in Appendix IV. 1(B).

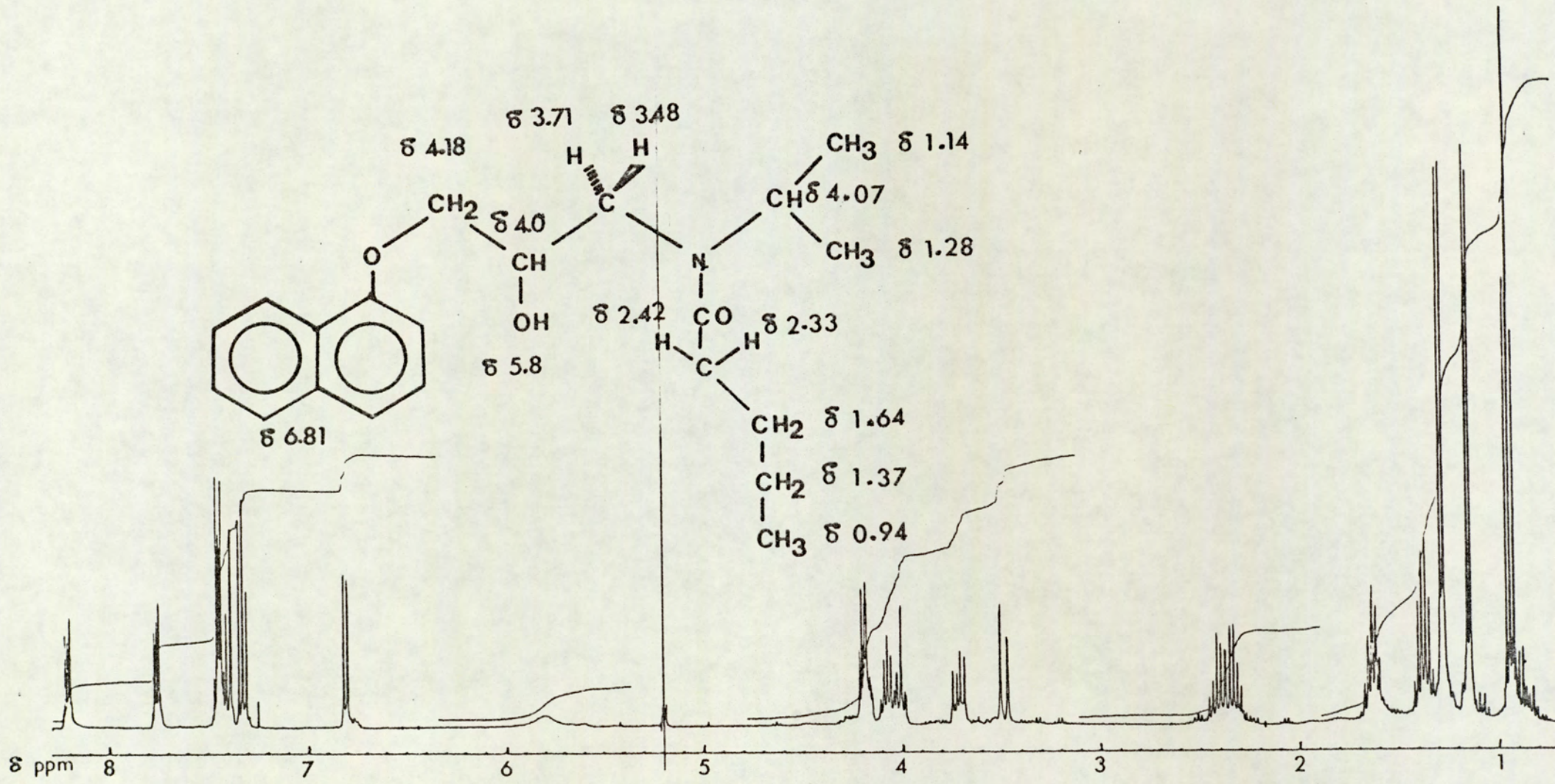


Fig 2.8 360 MHz ^{1}H NMR Spectrum of N-valerylpropranolol

2.5 SYNTHESIS OF N-ETHOXYCARBONYL-O-ACETYLPROPRANOLOL

N-ethoxycarbonyl-O-acetylpropranolol was prepared by adding with stirring 2 g O-acetylpropranolol HC1 (5.9 m.moles) to a cold mixture of 0.65g ethylchloroformate (5.9 m.moles) and 2 g triethylamine (19.7 m.moles) in 40 ml methylene chloride over a period of 30 minutes. The mixture was then heated at 45°C for 2 hours and treated in the same way as described in Section 2.4 for N-acetyl and N-valeryl propranolols. This yielded 1.2g of a yellow oil.

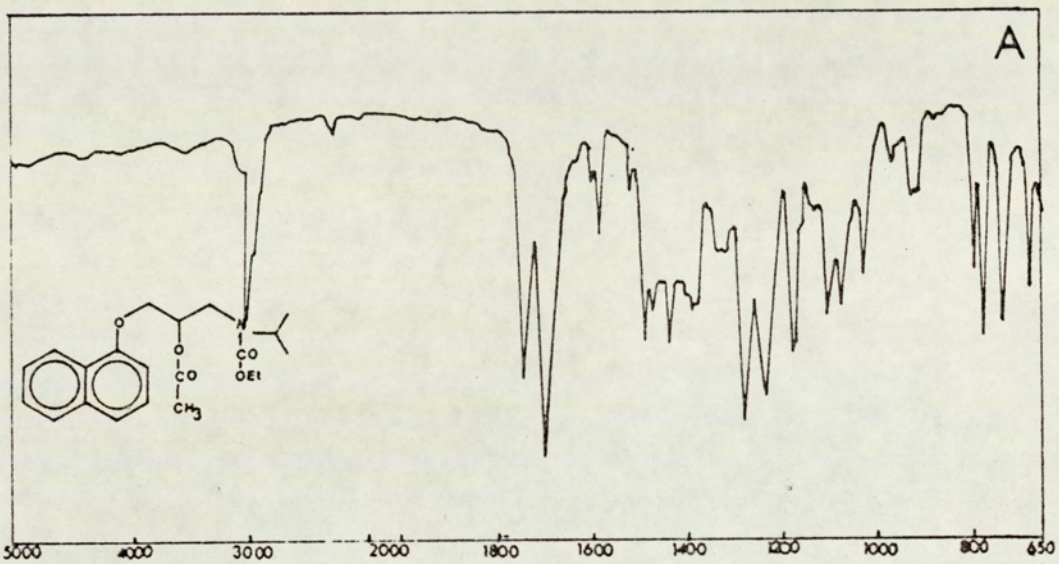
The infrared spectrum of N-ethoxycarbonyl, O-acetylpropranolol obtained a Unicam SP200 infrared spectrophotometer (using Nujol mulls), is presented in Fig 2.9 (A) and shows two strong peaks at 1720 cm^{-1} (N - C = O) and 1780 cm^{-1} (O - C = O).

The chemical shifts, multiplicities, numbers of protons and spectral assignments from the ^1H n.m.r spectral analysis of N-ethoxycarbonyl, O-acetylpropranolol are presented in Fig 2.10. (B).

The low resolution mass spectrum of N-ethoxycarbonyl-O-acetylpropranolol is shown in Fig 2.11.(B) and confirms that the compound had the expected structure.

2.6 SYNTHESIS OF 1-ISOPROPYL- 4 -NAPHTHYLOXYMETHYL OXAZOLIDIN - 2 - ONE

1-Isopropyl-4-naphthoxymethyl oxazolidin-2-one was prepared by refluxing 1.5g propranolol HC1 (5.08 m.moles) for one hour in a mixture of 30 m.moles ethyl-chloroformate and 80 m.moles triethylamine in 40 ml chloroform. The mixture was washed with water, once with aqueous 0.5M HC1 to remove excess triethylamine and then with aqueous 5% sodium carbonate to neutralize the excess HC1 and finally with water. The organic layer was dried ($Na_2 SO_4$) and evaporation under vacuo yielded a dark residue which on standing with 50ml of ether for a few hours yielded white crystals. The solid was washed twice with 50 ml of dry ether to yield 0.72 g of white crystals (m.p. 127 - 129 °C).



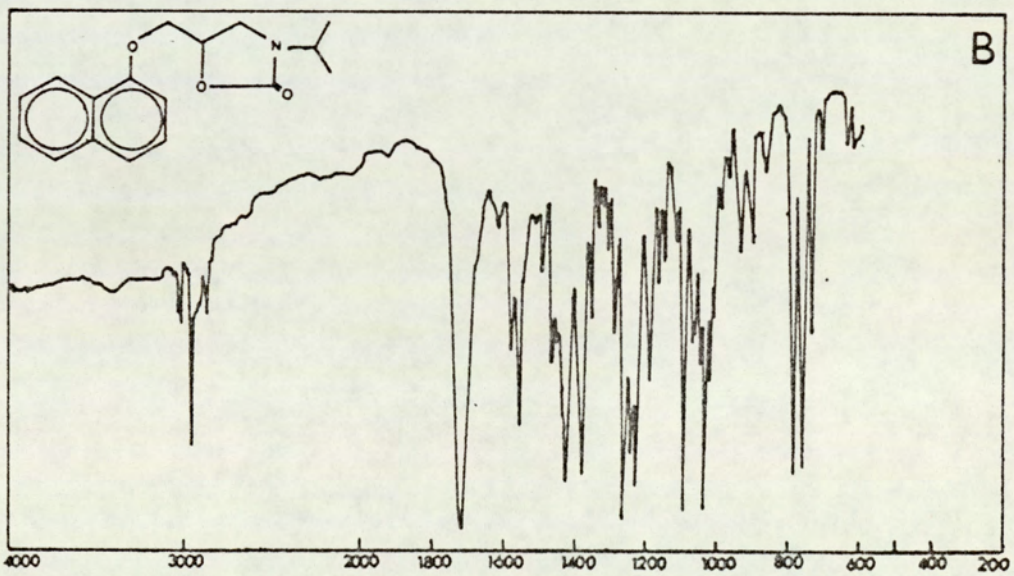


Fig 2.9 Infrared Spectrum of N-ethoxycarbonyl, O-acetylpropranolol (A) and 1-isopropyl-4-naphthyloxymethyl, Oxazolidin-2-one (B).

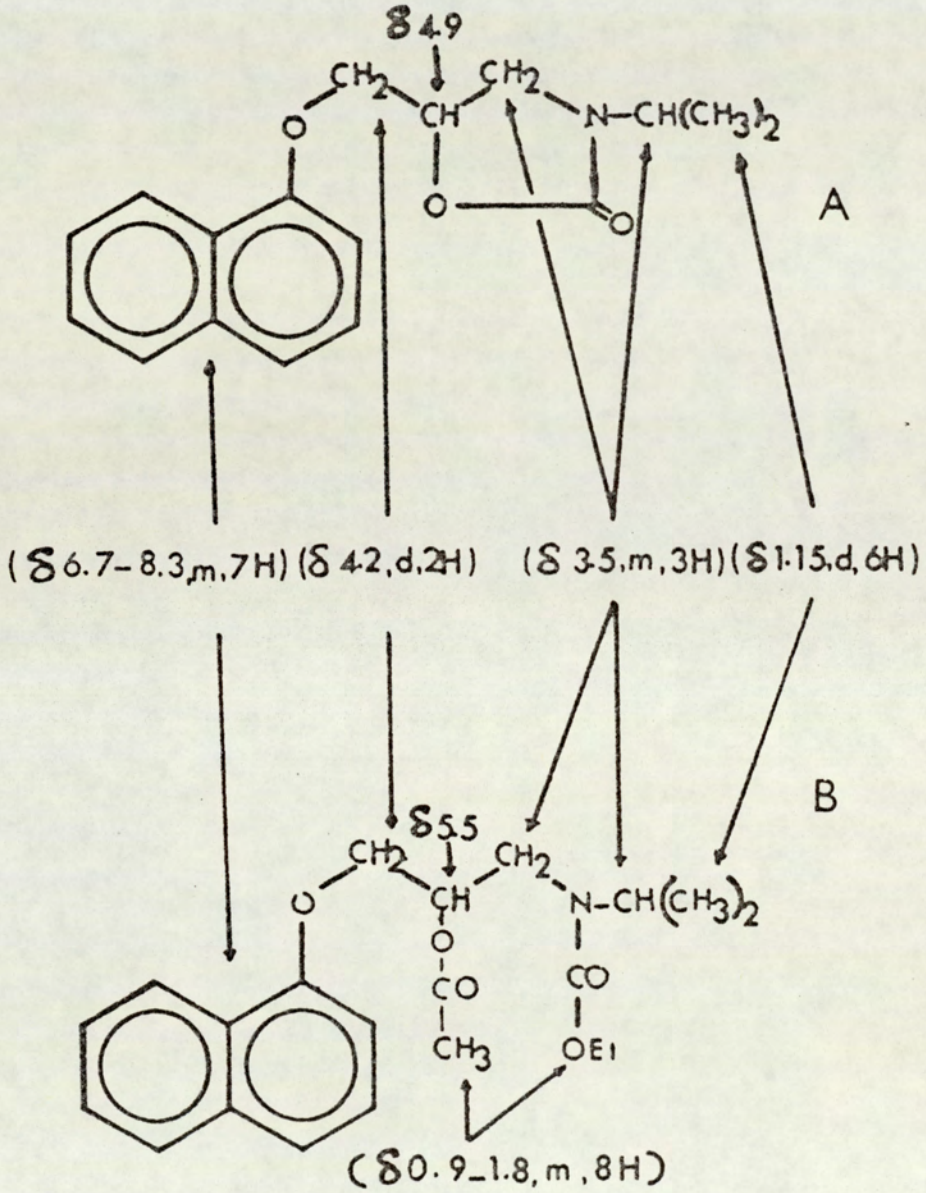


Fig 2.10 ^{1}H NMR Assignments of 1-isopropyl-4-naphthyloxy-methyl oxazolidin-2-one (A) and N-ethoxycarbonyl, O-acetylpropranolol (B).

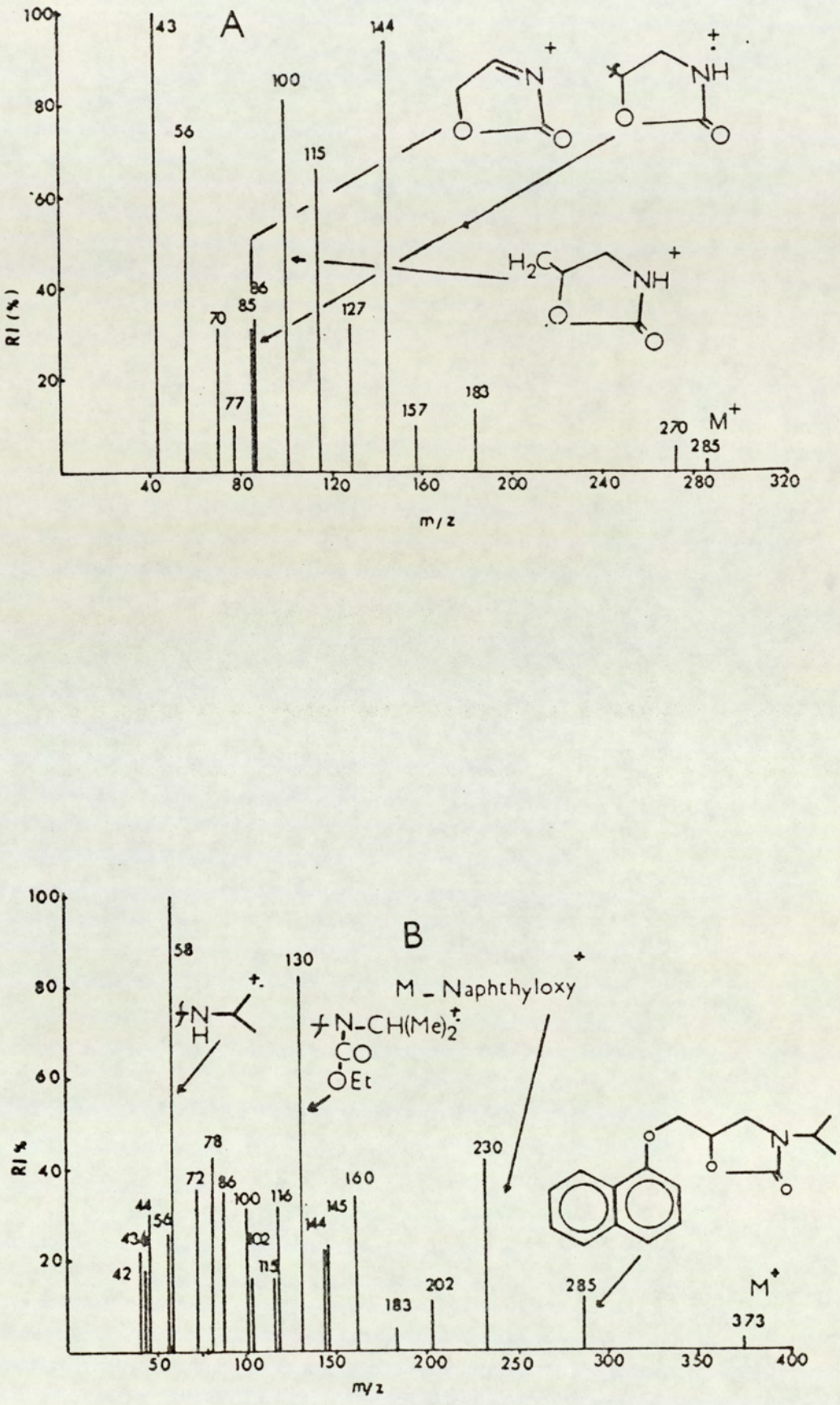


Fig 2.1] Mass Spectral Fragmentation of 1-isopropy-4-naphthyloxymethyl Oxazolidin-2-one (A) and N-ethoxycarbonyl, O-acetylpropranolol (B).

The infrared spectrum of 1-isopropyl - 4 - naphthyloxymethyl oxazolidin - 2 - one, which is presented in Fig 2.9.(B) shows a strong peak at 1760 cm^{-1} for the carbonyl group in the oxazolidine ring.

The ^{1}H n.m.r assignments of this compound are shown in Fig 2.10. (A). The mass spectrum obtained by fragmentation of 1-isopropyl - 4 - naphthoxymethyl oxazolidin - 2 - one is displayed in Fig 2.11.(A). This shows in addition to many of the common fragments (Fig 2.4) some other fragments. These include the fragment m/z 285 which confirms the degradation pathway of the oxazolidinone derivative which will be discussed in depth later in the chemical stability section.

CHAPTER THREE

DEVELOPMENT OF HPLC SYSEMS FOR PROPRANOLOL PRO-DRUGS

CHAPTER 3

3. DEVELOPMENT OF HPLC SYSTEMS FOR PROPRANOLOL PRO-DRUGS

3.1 INTRODUCTION

In this chapter the development of high-performance liquid chromatography (HPLC) systems for the quantitative estimation of propranolol pro-drugs to be used in the study of their kinetic and physicochemical properties is described.

Work described elsewhere in this thesis shows that O-acyl propranolol derivatives undergo competitive hydrolysis and intramolecular rearrangement, particularly in alkaline media, to yield propranolol and the corresponding N-acyl compound. The HPLC systems reported here are able to discriminate between these compounds and the parent esters and are useful for the simultaneous assay of these components to enable full kinetic profiles to be obtained.

Another advantage of HPLC is that thermolabile beta-blockers such as the propranolol esters described here can be analysed without derivatization or exposure to heat, light and air. Reversed-phase columns with buffered methanol or acetonitrile are widely used (Table 1.10). The only disadvantage of using methanol is its viscosity which increases the pressure on the column which may shorten the column life. However, buffered acetonitrile activated with acid can resolve beta-blockers effectively [*Hackett and Dusci 1979 (142), Hermansson and Von-Bohr 1980 (160), Cooper and Midha 1981 (136) and Terao and Shen 1982 (130)*].

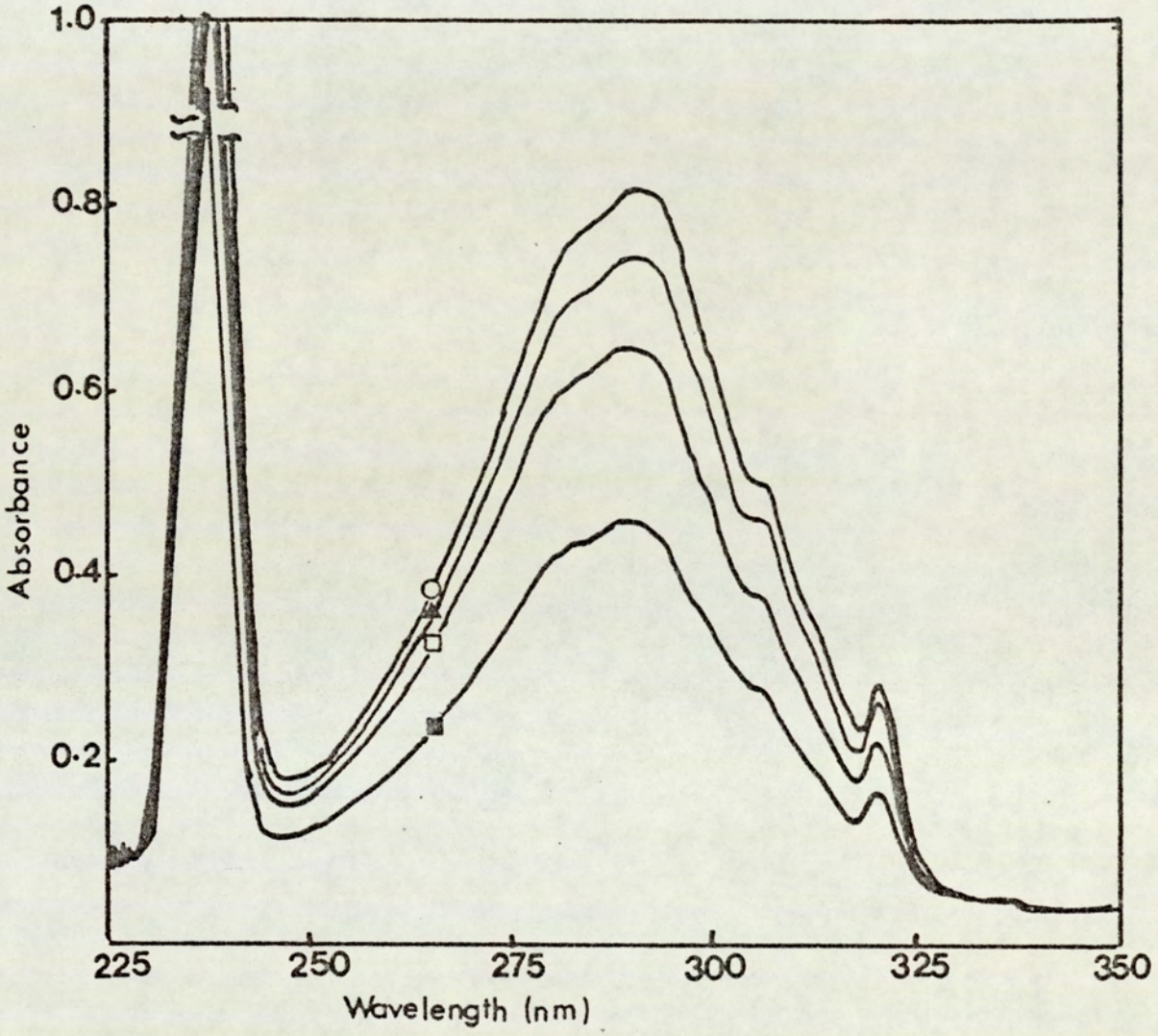


Fig 3.1 Ultraviolet Spectra for N-O-diacetylpropranolol (○), propranolol (▲), O-acetylpropranolol (□) and O-hexanoylpropranolol (■).

Concentration : 0.15 mM in 30% buffered DMF pH3
Reference : 30% buffered DMF pH3

3.2 INSTRUMENTATION

The high-performance liquid chromatography (HPLC) consisted of an Altex 100A double piston constant flow solvent - metering pump, a Rheodyne 7120 injector fitted with a 20µl loop, a Pye Unicam LC3 variable wavelength detector equipped with an 8µl flow cell and operated at 290 nm. and a J.J. chart recorder (J.J. Instruments). The columns used in these studies were 10 cm x 4.6 mm. I.D. Shandon stainless steel columns which were packed in the laboratory with the appropriate packing material (Hypersil-ODS. 5µm in most cases) using a Shandon column packing machine. A Unicam SP8000 ultraviolet recording spectrophotometer was used for obtaining the u.v. spectra for drugs under investigation.

A Radiometer PHM64 Research pH meter with three decimal place display of pH was used in measuring the pH. A Fisons whirlimixer was used throughout the experiments. An ultrasonic water bath (Kerry, Pulsatron 125) was used to ensure complete removal of gases from the mobile phases and to aid the solubilizing of the drugs in aqueous systems.

3.3 ULTRAVIOLET ABSORPTION OF BETA-BLOCKERS

A solution of 0.15 mM of N,O-diacetylpropranolol, propranolol HCl, O-acetyl-propranolol HCl and O-hexanoylpropranolol HCl were prepared separately in 30% buffered diamethylformamide (DMF) at pH 3.5 (to avoid degradation of the esters) and scanned over the wavelength range 190-450 nm. using a matched pair of 1 cm quartz cells for the sample and the blank (30% buffered - DMF pH 3.5).

The ultraviolet traces (Fig 3.1) shows 2 peaks, one sharp peak with λ_{max} of 233 - 239 nm. and another peak of λ_{max} of 288 - 293 nm.

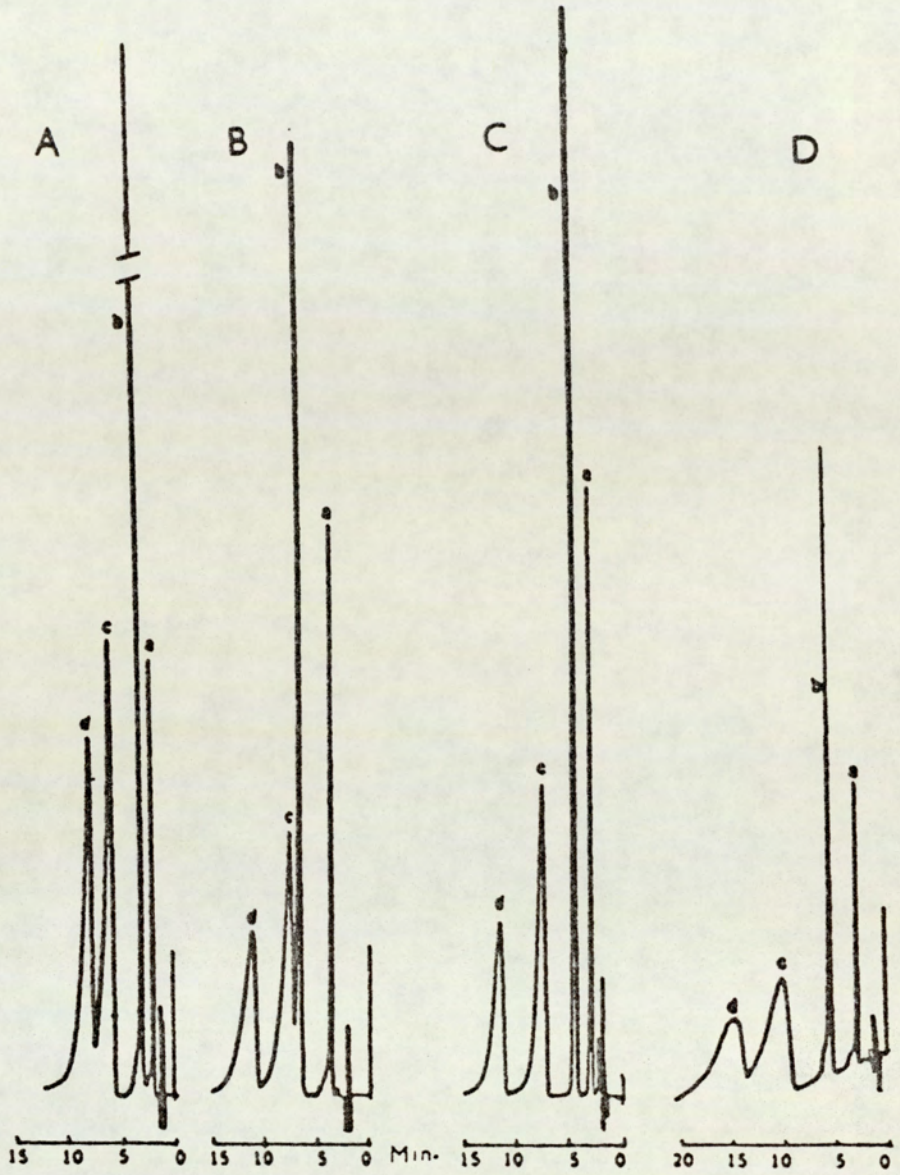


Fig 3.2 Development of the HPLC Separation for Ethyl Paraben as Internal Standard (a), N-acetylpropranolol (b), Propranolol HCl (c) and O-acetylpropranolol (d).

Mobile Phase Composition (per volume)

System	Acetonitrile	0.8% H_3PO_4	0.3M KH_2PO_4	Water	pH
A	54	3	23	20	2.0
B	70	1	-	29	≈ 2.8
C	54	1	23	22	≈ 2.8
D	54	1	-	45	≈ 2.8

Column: Hypersil - ODS, 5µm, 10 cm x 4.6 mm i.d.
Flow Rate: 1 ml / min Detection at 290 nm.

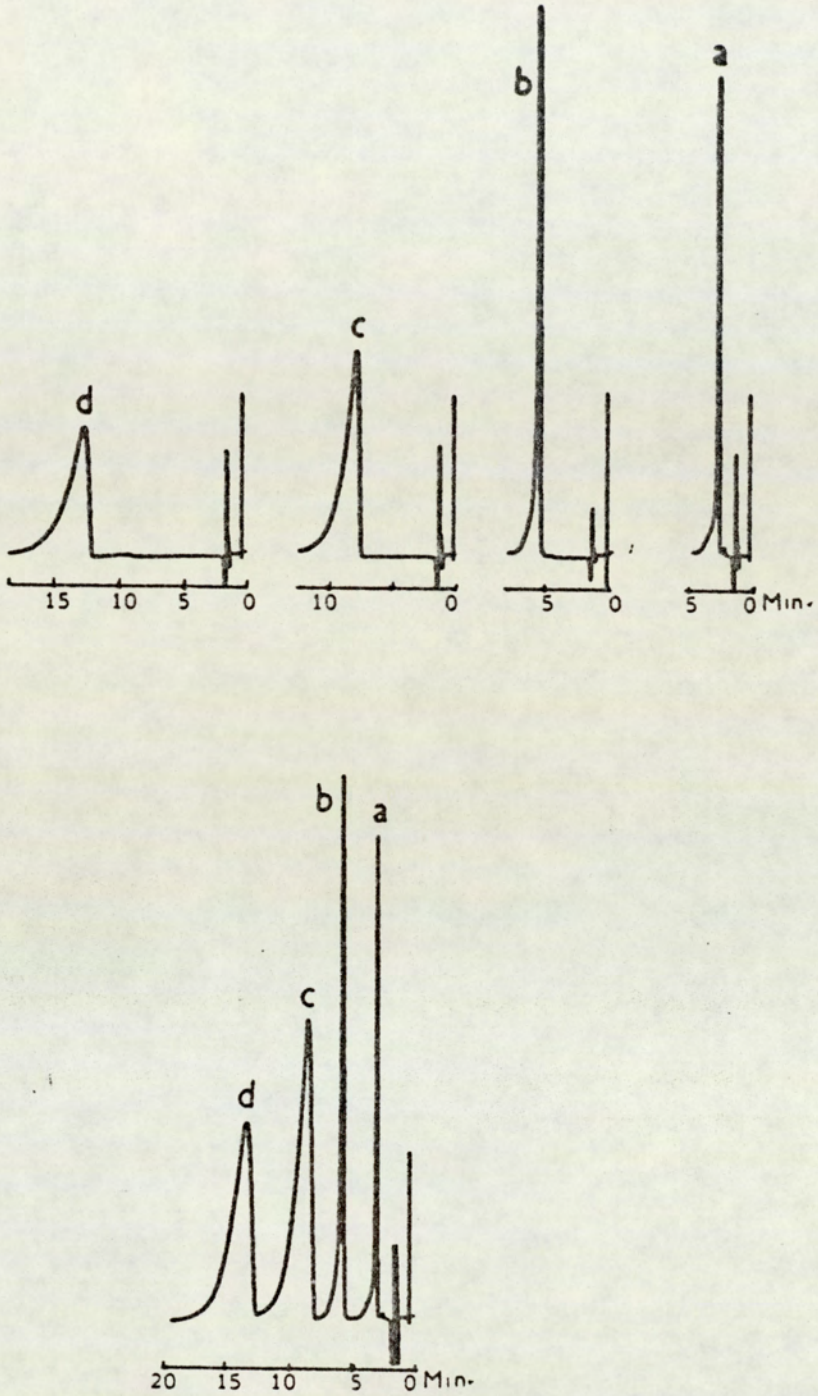


Fig 3.3 HPLC Separation of Ethyl Paraben as an Internal Standard (a), N,O-diacetylpropranolol (b), Propranolol (c) and O-acetylpropranolol (d).

Mobile Phase: Acetonitrile: 0.8% H_3P_{04}: 0.03 MKH_2 P_{04}: Water (54: 0.3: 23: 22.7) pH = 3.4

Column : Hypersil-ODS 5um, 10cm x 4.6 m.m.i.d.

Flowrate : 1 ml/min

Detection Wavelength: 290 nm

3.4 DEVELOPMENT OF THE HPLC SYSTEMS

3.4.1 HPLC SYSTEM (I) FOR PROPRANOLOL AND ITS ACETATE DERIVATIVES

This mobile phase was an adaptation of those used previously [*(Hackett and Dusci 1979 (142), Hermansson and Von-Bohr 1980 (160), Cooper and Midha 1981 (136) and Terao and Shen 1982 (130)]* but satisfactory separation required some modification of the method. The acetonitrile proportion was selected and found to be in the region of 50 - 55%.

Fig 3.2.C shows a typical separation of N-acetylpropranolol, propranolol, O-acetyl-propranolol and ethyl paraben as internal standard with a mobile phase consisting of acetonitrile : 0.03 M KH_2PO_4 : 0.8% orthophosphoric acid: water (54:23:1:22 v/v/v/v) pH ≈ 2.8.

Fig 3.2A shows the effect of the eluent pH on the resolution of these compounds with a lowering of the mobile phase pH to 2 where a poor resolution was obtained due to the overlapping of propranolol and O-acetylpropranolol peaks. Fig 3.2.B and D shows their resolution by 70 and 54% acidified aqueous acetonitrile (pH 2.8) without incorporation of any buffer into the mobile phase.

N,O-diacetylpropranolol may also be resolved from a mixture containing ethyl-paraben, N,O-diacetylpropranolol, propranolol and O-acetylpropranolol by decreasing the orthophosphoric acid proportion from 1% to 0.3% which raises the pH of the mobile phase from 2.8 to 3.4. Chromatograms are shown in Fig 3.3.

3.4.2 HPLC SYSTEM (II) FOR PROPRANOLOL AND ITS ACETATE PROPIONATE, BUTYRATE, VALERATE AND HEXANOATE ESTERS.

3.4.2.1. EFFECT OF ACETONITRILE PROPORTION IN THE MOBILE PHASE ON THE SEPARATION OF PROPRANOLOL DERIVATIVES

A series of mobile phases were prepared over an acetonitrile concentration of 45 - 70% in a mixture of 0.1% v/v orthophosphoric acid (88%), 0.1% v/v diethylamine and water (to 100%) and the final pH of the mobile phases were around pH 2.5.

A four component mixture of N-acetylpropranolol (0.05 mM), propranolol (0.1 mM), O-acetylpropranolol (0.1 mM) and O-propionylpropranolol (0.1 mM) in 30% aqueous acidified DMF pH 3.5 (the lower pH to avoid the degradation of the esters) were analysed after equilibration of the HPLC system with various mobile phases.

The chromatograms are shown in Fig 3.4. Plots of retention time against acetonitrile % in the mobile phase are displayed in Fig 3.5.

The chromatographic parameters such as retention time (t_1), column capacity factor (K´), number of theoretical plates (N) and the resolution between the components (R_S) are summarised in Table 3.1.

This study enables the selection of the optimum acetonitrile proportion in the mobile phase in which all the components could be analysed within 10 minutes. A mobile phase containing 45 - 55% of acetonitrile was not suitable for routine analysis because of the broadening and tailing of the peaks. In contrast, with a concentration > 65% acetonitrile in the mobile phase the peaks overlapped. A mobile phase containing 65% acetonitrile was found to be the best where the chromatogram was satisfactory with respect to retention time, peak width and resolution.

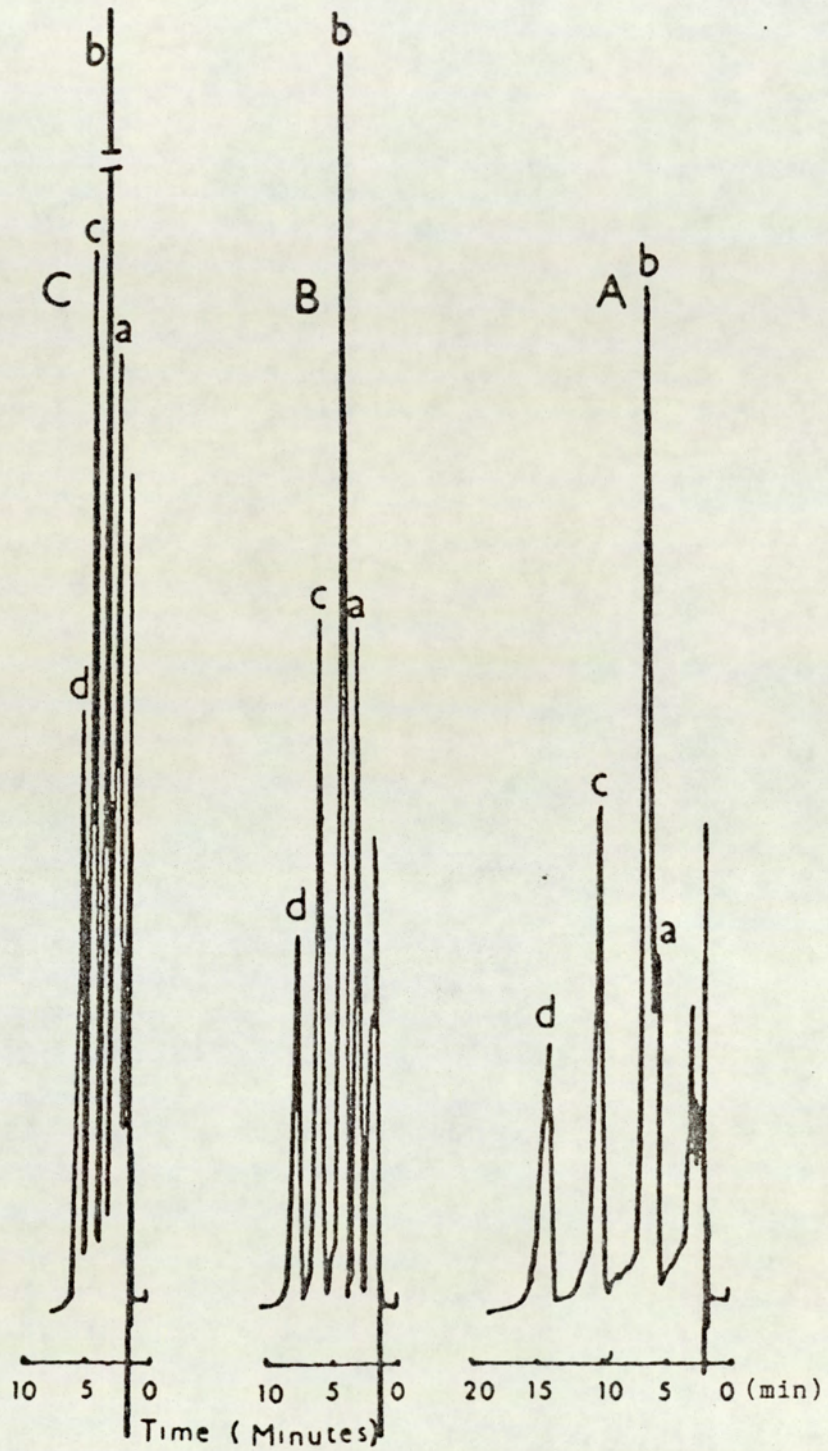


Fig 3.4 Chromatograms showing the effect of the Acetonitrile proportion in the mobile phase on the separation of N-acetylpropranolol (a), Propranolol (b), O-acetyl-propranolol (c) and O-propionylpropranolol (d).

% of Acetonitrile in the mobile phase: A : 45 B : 65 C : 70

Column : Hypersil-ODS (5µm), 10 cm x 4 x 6 mm i.d.
Flow Rate : 1 ml. min^{-1}
Detection : 290 nm. 0.08 AUFS

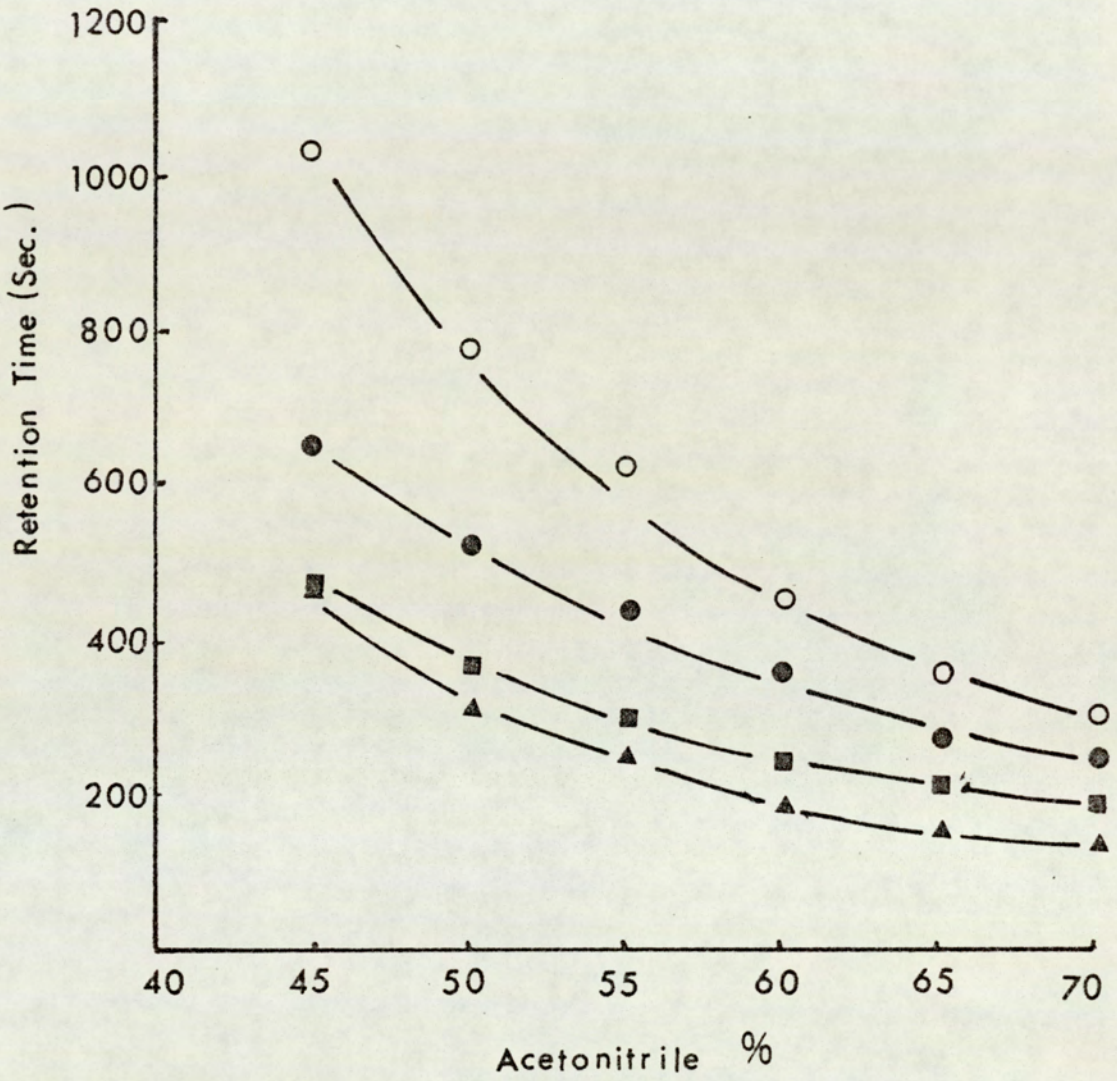


Fig 3.5 Effect of Acetonitrile % in the Mobile Phase on the Retention Time of N-acetylpropranolol (▲), Propranolol (■), O-acetylpropranolol (●) and O-propionylpropranolol (O).

Table 3.1 Effect of the proportion of acetonitrile in the mobile phase on the chromatographic parameters for the separation of N-acetylpropranolol (N-Ac), Propranolol (Prop), O-acetylpropranolol (O-Ac), and O-propionylpropranolol (O-prop).

% Acetonitrile	Retention Time (Sec)				Capacity Factor K'				No. of Theoretical Plates (N)				Resolution between		
	N-Ac.	Prop.	O-Ac	O-Prop.	N-Ac.	Prop.	O-Ac.	O-prop.	N-Ac	Prop.	O-Ac.	O-prop.	N-Ac & Prop.	Prop & O-Aç	O-Ac & O-prop.
45	480	480	660	1035	7.00	7.00	10.00	16.25	-	113.8	310.0	297.6	-	1.10	1.96
50	300	360	525	780	4.00	5.00	7.75	12.00	-	144.0	306.3	534.1	-	1.40	2.00
55	240	300	439	634	3.00	4.00	6.32	9.57	256	100.0	279.7	446.6	0.700	1.24	1.73
60	180	240	360	459	2.00	3.00	5.00	6.65	256	113.8	225.0	416.2	0.900	1.29	1.10
65	150	213	270	360	1.50	2.55	3.50	5.00	400	201.6	207.4	256.0	1.400	1.05	1.09
70	135	186	249	300	1.25	2.10	3.15	4.00	328	153.8	275.6	256.0	1.133	0.85	0.96

Where $K' = \frac{t_A - t_o}{t_o}$ $\quad N = 16\,\frac{t_A^2}{W_A}$ or $5.54\,\frac{t_A^2}{W_{\frac{1}{2}A}}$ $\quad R_s = \frac{2t_B - t_A}{W_A + W_B}$

Where t_o = Retention time of the unretained compound
t_A, t_B = Retention time of compounds A and B respectively
W_A, W_B = Peak width of compounds A and B respectively
$W_{\frac{1}{2}}$ = Peak width at half of the peak height.

3.4.2.2. EFFECT OF DIETHYLAMINE PROPORTION IN THE MOBILE PHASE ON THE SEPARATION OF PROPRANOLOL DERIVATIVES

Various mobile phases were prepared to cover a concentration range of diethylamine of 0.04 - 0.24% v/v in a mixture of 65% v/v acetonitrile, 0.1% v/v orthophosphoric acid (88%) and water to (100%) and the pH was about 2.5. A six component mixture of propranolol and its acetate, propionate, butyrate, valerate and hexanoate esters (0.1 mM each) was prepared in 30% acidified aqueous DMF pH 3.5 and analysed after equilibration of the HPLC system with various mobile phases containing different concentrations of diethylamine.

The chromatograms which are presented in Fig 3.6 show the effect of diethylamine concentration in the mobile phase on the elution of propranolol and its esters. At low concentration of diethylamine (0.04%) the chromatogram was very poor with respect to the retention time and the peak shape which may be due to the interaction of the basic amino group in propranolol or its esters with the free surface silanol group in the stationary phase.

When the concentration of diethylamine was increased to 0.2% the chromatogram improved dramatically, perhaps due to blocking of the free surface silanol group of the stationary phase with diethylamine with a reduction in the absorbtive behaviour of the analytes. A mobile phase containing a 0.24% diethylamine caused propranolol and the lower homologues O-acetyl and O-propionyl propranolol to overlap. This mobile phase may be useful for the analysis for longer chain esters (O-butyryl , O-valeryl and O-hexanoyl propranolols) but for the current analysis,a concentration of 0.2% v/v diethylamine in the mobile phase was found to be the best. At this level the chromatogram was satisfactory with respect to retention time, peak width and resolution.

A plot of retention times against the proportion of diethylamine in the mobile phase are displayed in Fig 3.7. The retention characteristics of the eluted components in various mobile phases are recorded in Table 3.2.

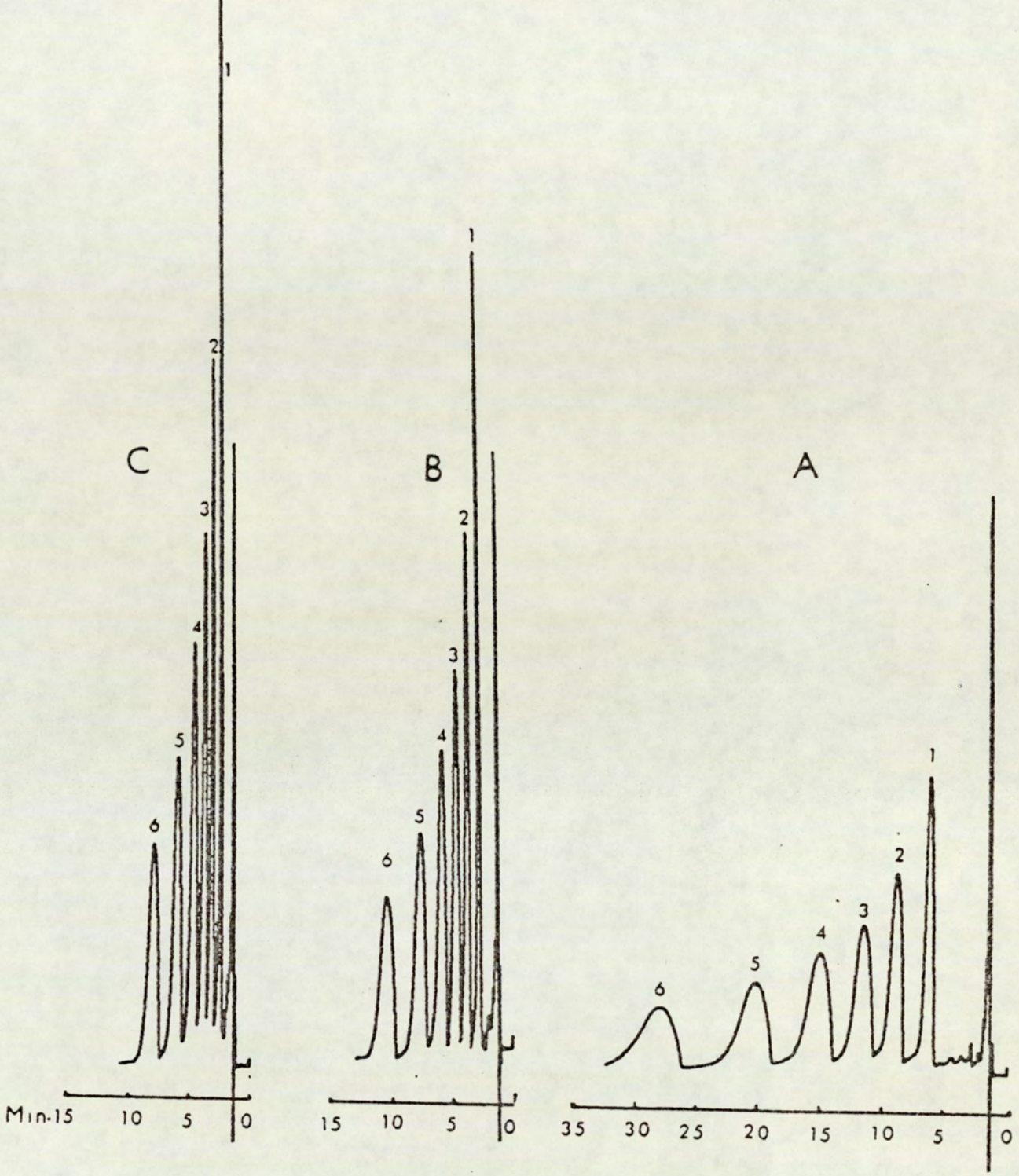


Fig 3.6 Chromatogram showing the effect of the Diethylamine proportion in the mobile phase on the elution of Beta-blockers.

Peak	1	2	3	4	5	6
Compound	Propranolol	O-Acetyl	O-Prop-ionyl	O-Buty-ryl	O-Vale-ryl	O-Hexan-oyl

Column: Hypersil ODS (5µm), 10cm x 4.6 m.m.i.d.

Flow Rate: 1ml/min
Detection: 290 nm., 0.08 AUFS
% of Diethylamine: A= 0.04 B= 0.20 C=0.24
in the Mobile Phase

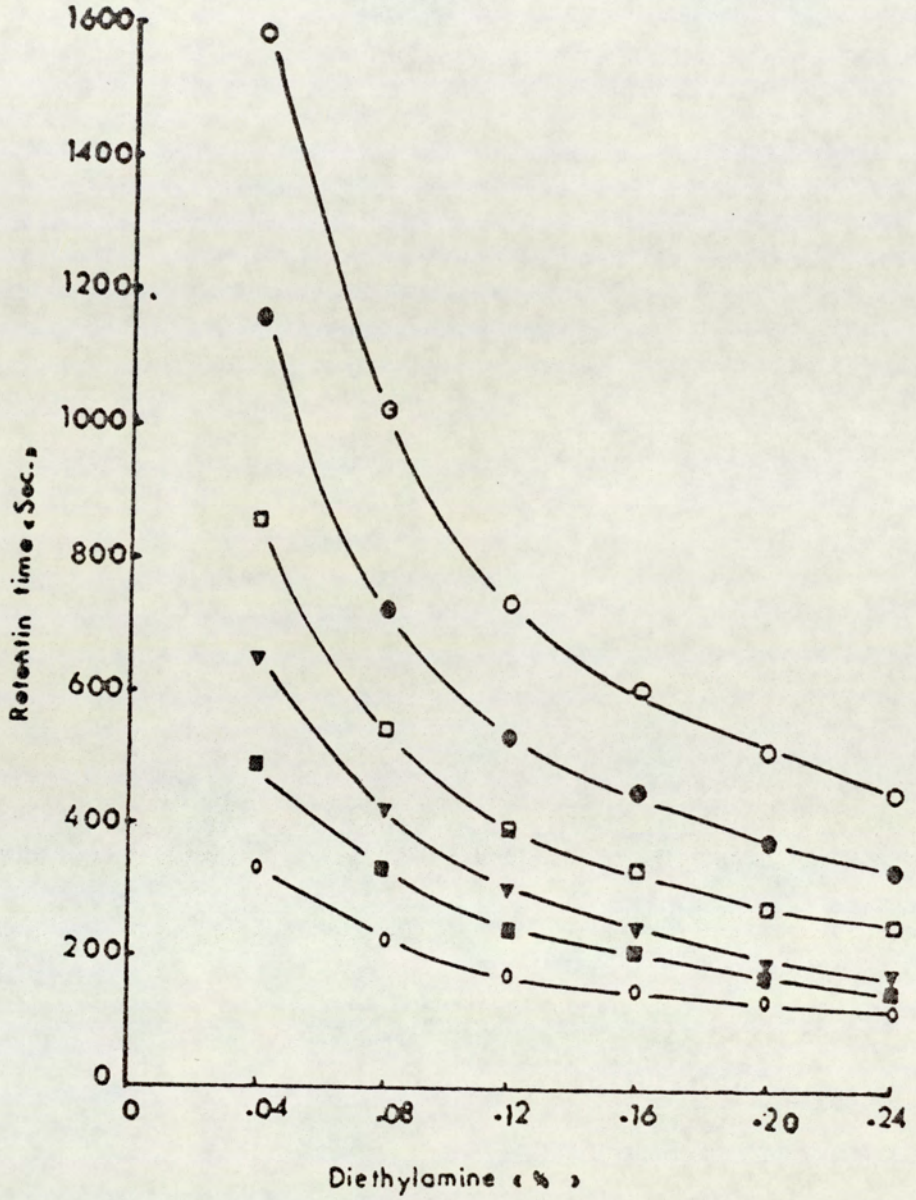


Fig 3.7 Effect of Diethylamine % in the Mobile Phase on the Retention Time

Symbols	0	■	▼	□	●	○
Compound	Propran-olol	O-Acetyl	O-propio-nyl	O-Butyryl	O-Valeryl	O-Hexanoyl

Table 3.2 Effect of the Proportion of Diethylamine in the Mobile Phase on the Chromatographic Parameters Measured from the Chromatograms obtained from the Analysis of Beta-Blockers by Various Mobile Phases

Diethylamine %	Retention Time (Sec.) ← Propranolol Ester →						Column Capacity Factor K' ← Propranolol Esters →					
	Propran-olol	Acetyl	Propionyl	Butyryl	Valeryl	Hexanoyl	Propran-olol	Acetyl	Propionyl	Butyryl	Valeryl	Hexanoyl
0.04	330	480	645	855	1155	1580	4.50	7.00	9.75	13.25	18.25	25.3
0.08	225	330	420	540	720	1020	2.75	4.50	6.00	8.00	11.00	16.00
0.12	171	240	300	390	525	720	1.85	3.00	4.00	5.50	7,75	11.00
0.16	150	210	255	336	450	600	1.50	2.50	3.25	4.60	6.50	9.00
0.20	135	171	195	276	375	510	1.25	1.85	2.25	3.60	5.25	7.50
0.24	120	150	180	255	336	450	1.00	1.50	2.00	3.25	3.60	6.50

3.4.2.3 EFFECT OF THE ELUENT pH ON THE RETENTION TIME

A knowledge of some of the important properties of beta-blockers such as solubility and pKa are required for the development of a reversed-phase HPLC separation.

Theoretically, the beta-blockers are weak bases with a pKa in the region of 8.97 to 9.65. Changing the pH of the mobile phase therefore affects the ionization of the solute on which the reversed-phase HPLC analysis is strongly dependent.

a) Effect of pH of System I on the Retention Time

A three-component mixture of N,O-diacetylpropranolol, propranolol and O-acetylpropranolol (0.1 mM each) in 30% acidified aqueous DMF pH 3.5 was analysed after equilibration of the HPLC system with various mobile phases prepared from system I (Section 3.4.1) by changing the pH within the range of 1.4 - 4.5 by varying the amount of orthophosphoric acid (0.8%) in the mobile phase (from 0.2 to 4%).

As shown in Table 3.3, it has been found that increasing the mobile phase pH had a direct effect on the resolution as a result of increasing the retention time of propranolol and O-acetylpropranolol but not N,O-diacetylpropranolol (neutral) and consequently affects the column capacity factor (K´), number of the theoretical plates (N) and the resolution between the components (R_S). The optimal pH for a good separation of this mixture was found to be pH 3.4 (Fig 3.3).

b) Effect of pH of System II on the Retention Time

Five mobile phases consisting of 65% v/v acetonitrile, 0.2% diethylamine, water (to 100%) and orthophosphoric acid 88% (0.02 to 0.2% v/v) to provide a pH range of 2.2 - 4.6 were prepared.

Table 3.3 Effect of the pH of the HPLC system (I) on the separation of N, O-diacetylpropranolol (D.A.P), Propranolol (P), and O-acetylpropranolol (O.A.P).

pH	Retention Time (Minutes)			Capacity Factor (K')			No. of Theoretical Plates (N)			Resolution between	
	D.A.P.	P	O.A.P	D.A.P.	P	O.A.P.	D.A.P.	P	O.A.C.	D.A.P. & P	P & O.A.P
1.9	5.45	5.45	8.50	4.45	4.45	7.50	5698	257	231	0.00	0.80
2.2	5.40	6.10	9.80	4.40	5.10	8.80	5585	322	315	0.30	0.90
2.4	5.50	6.70	11.00	4.50	5.70	10.00	5780	388	397	0.40	1.08
2.6	5.60	7.30	12.00	4.60	6.30	11.00	5670	461	472	0.70	1.28
3.0	5.60	7.60	12.60	4.60	6.60	11.60	6004	500	520	0.80	1.25
3.4	5.40	7.90	12.90	4.40	6.90	11.90	5590	540	546	1.00	1.25
4.0	5.30	8.05	13.05	4.30	7.05	12.05	5385	561	558	1.10	1.25
4.5	5.50	8.25	13.25	4.50	7.25	12.25	5798	589	584	1.10	1.30

A six-component mixture of propranolol and its first five acyl esters (mentioned in section 3.4.2.2.) was prepared (0.1 mM each) in 30% acidified aqueous DMF pH 3.5 and analysed after equilibration of the HPLC system with the various mobile phases . The retention time plots as a function of the mobile phase pH are shown in Fig 3.8. In contrast to table 3.3, the change in the retention time with the pH of the mobile phase varies only slightly in the presence of diethylamine.

The addition of this moderator makes the separation much less sensitive to the pH of the mobile phase and it is the level of diethylamine which exerts the major influence.The chromatograms showing these effects are displayed in Fig 3.9 and the retention parameters are summarised in Table 3.4.

3.4.3 HPLC SYSTEM (III) FOR O-OCTANOYL AND O-DECANOYL PROPRANOLOL

A reversed-phase system was also developed for the assay of O-octanoyl and O-decanoyl propranolols. It was necessary to increase the proportion of acetonitrile in the mobile phase to prevent precipitation of the esters on the column and also an increase in the amount of diethylamine improved the resolution of the esters and shortened the retention time of the decanoyl ester.

The developed mobile phase consisted of acetonitrile: diethylamine: orthophosphoric acid (88%): water (85:0.4:0.2:14.4 v/v/v/v) pH ≈ 2.8. The mobile phase was delivered at a flow rate of 1ml/min into an ODS-Hypersil (5 μm) column and the analytes were detected at 290 nm. The chromatogram obtained are shown in Fig 3.15.

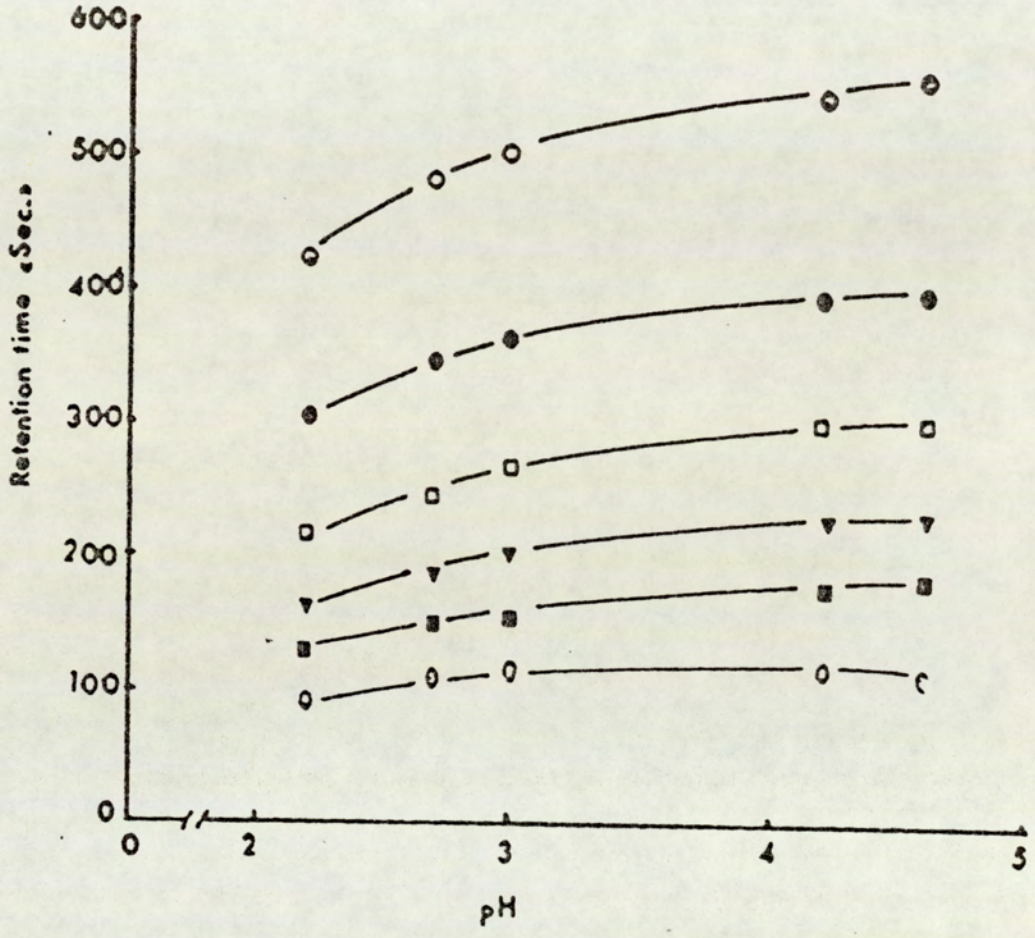


Fig 3.8 Graphical Presentation showing the Effect of the Mobile Phase pH on the Retention Time

Beta-blockers	Propran-olol	Propranolol Esters				
		Acetyl	Propionoyl	Butyryl	Valeryl	Hexanoyl
Symbol	0	■	▼	□	●	○

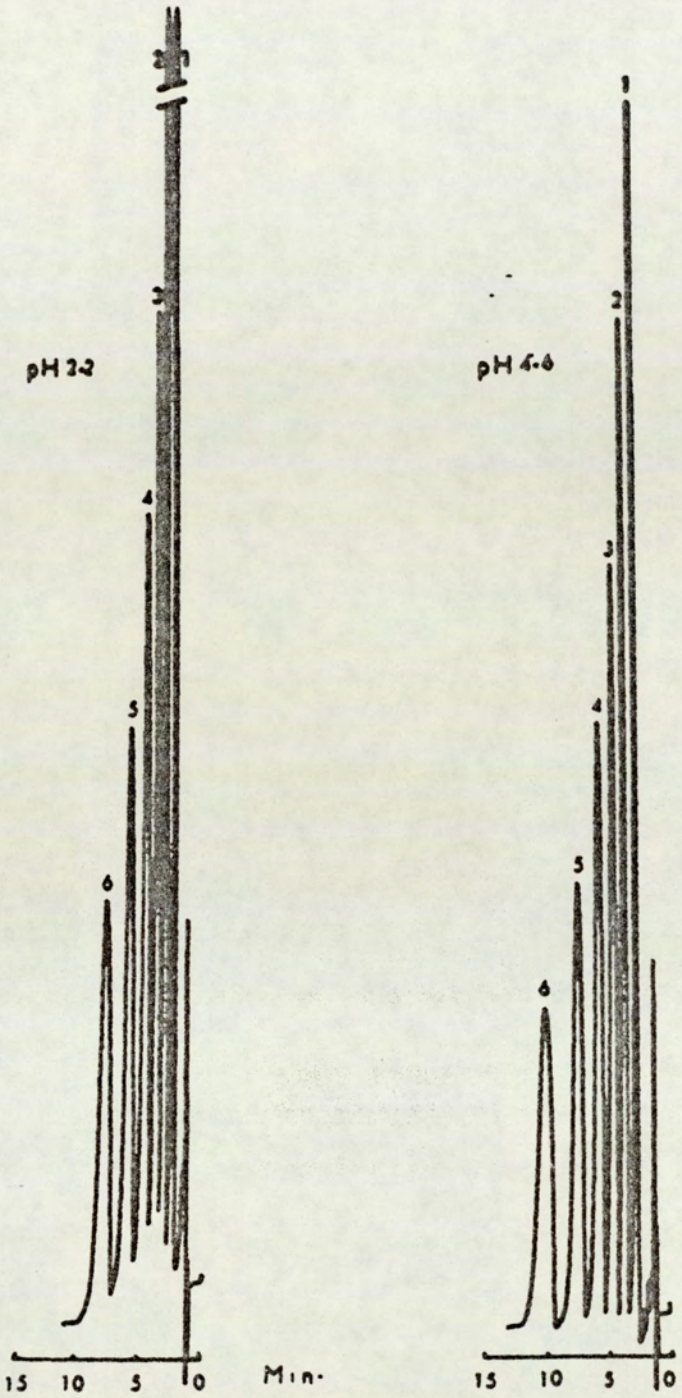


Fig 3.9 Comparative Chromatograms showing the effect of the Mobile Phase pH on the Elution of Beta-blockers.

Beta-blockers	Proprano-lol	Propranolol Esters				
		Acetyl	Propionoyl	Butyryl	Valeryl	Hexanoyl
Peak	1	2	3	4	5	6

Column: Hypersil-ODS (5µm), 10cm x 4.6 mm.i.d.
Mobile Phase: Acetonitrile: diethylamine: water (65: 0.2: to 100%) pH adjusted with H_3PO_4

Flow Rate: 1ml/min
Detection: 290 nm., 0.08 AUFS

Table 3.4 Effect of the pH of the HPLC System (II) on the Separation of Propranolol (Prop), and its Acetyl (O-Ac), Propionyl (O-Prop), Butyryl (O-But), Valeryl (O-Val), and Hexanoyl (O-Hex) Esters.

pH	Retention Time (Sec.)						Column Capacity Factor K′					
	Prop.	O-Ac.	O-Prop.	O-But.	O-Val.	O-Hex.	Prop.	O-Ac.	O-Prop.	O-But.	O-Val.	O-Hex.
2.2	90	126	165	215	300	420	0.50	1.10	1.75	2.58	4.00	6.00
2.7	105	150	186	246	345	480	0.75	1.50	2.10	3.10	4.75	7.00
3.0	110	160	204	265	360	495	0.83	1.67	2.40	3.42	5.00	7.25
4.2	110	174	220	294	390	540	0.83	1.90	2.67	3.90	5.50	8.00
4.6	110	180	225	300	396	555	0.83	2.00	2.75	4.00	5.60	8.25

3.5 SEARCH FOR THE ANALYTICAL WAVELENGTH

A six component mixture was prepared as described in Section 3.4.2.2. The solutes were eluted with a mobile phase consisting of acetonitrile, orthophosphoric acid (88%), diethylamine and water (65:0.1:0.2:34.7 v/v/v/v) pH 2.5 at a flow rate of 1 ml/min with a pressure of 70 bar into an ODS-Hypersil (5 μm) column (10 cm x 4.6 mm.I.D.) using a range of detection wavelengths from 265 to 305 nm with a detector sensitivity of 0.08 AUFS.

The relative peak heights were measured from the chromatograms at each detection wavelength and are summarized in Table 3.5. Plots of these peak heights as a function of wavelength are displayed in Fig 3.10 and the chromatograms showing examples of the effect of the wavelength on the relative peak height of the component are shown in Fig 3.11 from which preference was given to 290 nm to be as the analytical wavelength for subsequent analyses.

N.B. It was found that the use of the lower λ_{max} such as 235 nm caused an interference with first eluted peaks due to the solvent front from DMF being enhanced in intensity.

3.6 EFFECT OF SAMPLE SOLVENT ON THE LINEARITY OF THE CALIBRATION CURVES

A stock solutionsof 60 mM of O-pivaloylpropranolol (569.7 mg) and O-hexanoyl-propranolol (590.7 mg) were prepared separately in 25 ml DMF.

1ml of each stock solution was diluted in combination to 100 ml with an acidified aqueous DMF pH3, to provide a concentration range of DMF in the solvent mixture from 5 - 30%.

The final concentration was 100 ml, 0.6 mM which was considered as the 100% standard preparation of the analytes and this was diluted with the same solvent containing the same proportion of DMF to give a concentration range (10 - 100%) of the propranolol esters.

Compound / Wave length (nm)	Peak Height (m.m)					
		Propranolol Esters				
	Propranolol	Acetyl	Propionyl	Butyryl	Valeryl	Hexanoyl
265	76.0	49.0	34.0	28.0	20.5	15.0
270	102.5	67.2	49.5	39.2	30.0	21.5
272	112.5	73.0	52.5	43.0	32.0	23.0
275	133.5	87.0	66.0	52.0	38.5	27.5
277	146.0	95.5	72.0	57.0	42.0	30.5
279	160.0	104.0	79.0	63.0	46.5	33.5
281	173.0	113.0	87.0	68.5	51.0	37.0
285	193.0	127.5	98.5	78.0	57.6	43.0
290	212.0	144.0	111.0	88.0	67.0	50.0
295	205.0	140.0	108.0	85.0	63.0	46.0
300	194.0	125.0	97.5	76.5	56.0	41.0
305	165.0	100.0	77.0	59.0	44.0	32.0

Table 3.5 Effect of the Analytical Wavelength on Peak Height

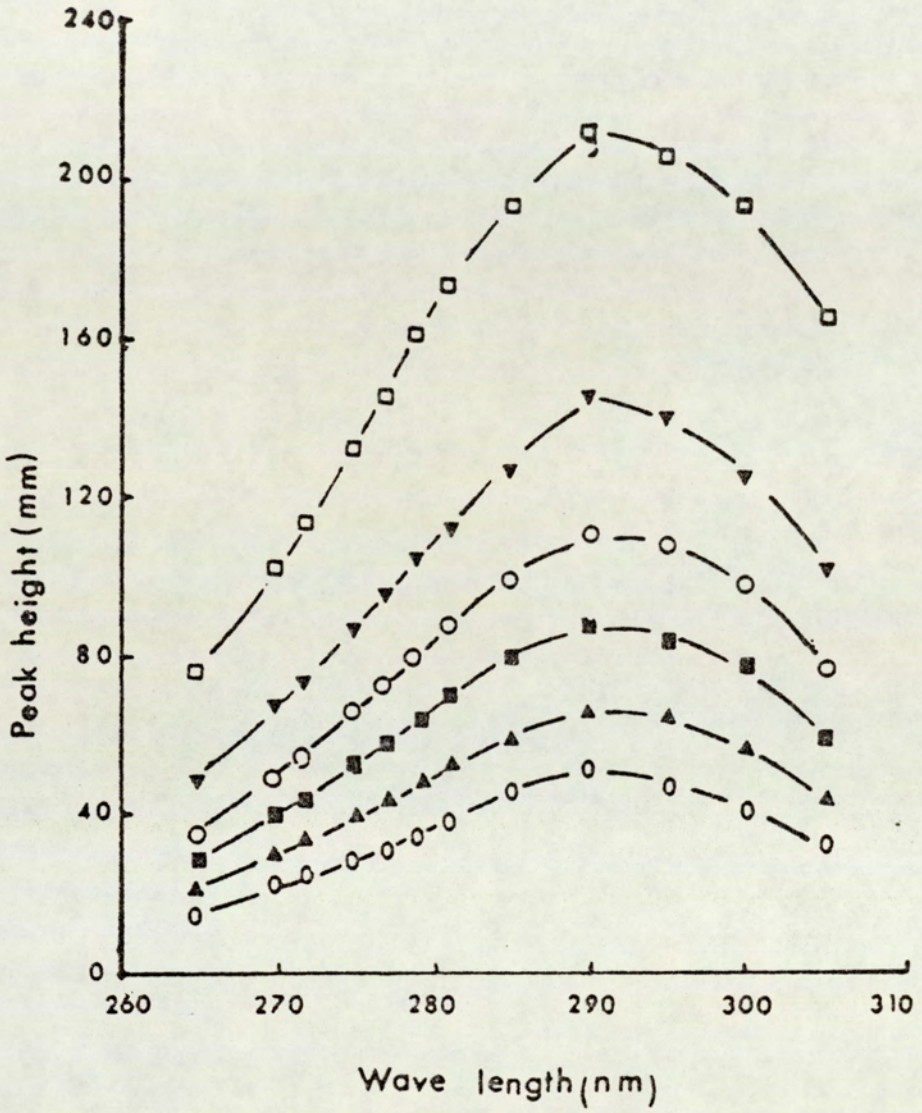


Fig 3.10 Effect of the Analytical Wavelength on the Peak Height

Compound	Propran-olol	Propranolol Esters				
		Acetyl	Propionoyl	butyryl	valeryl	hexanoyl
Symbol	0	Δ	■	○	▼	□

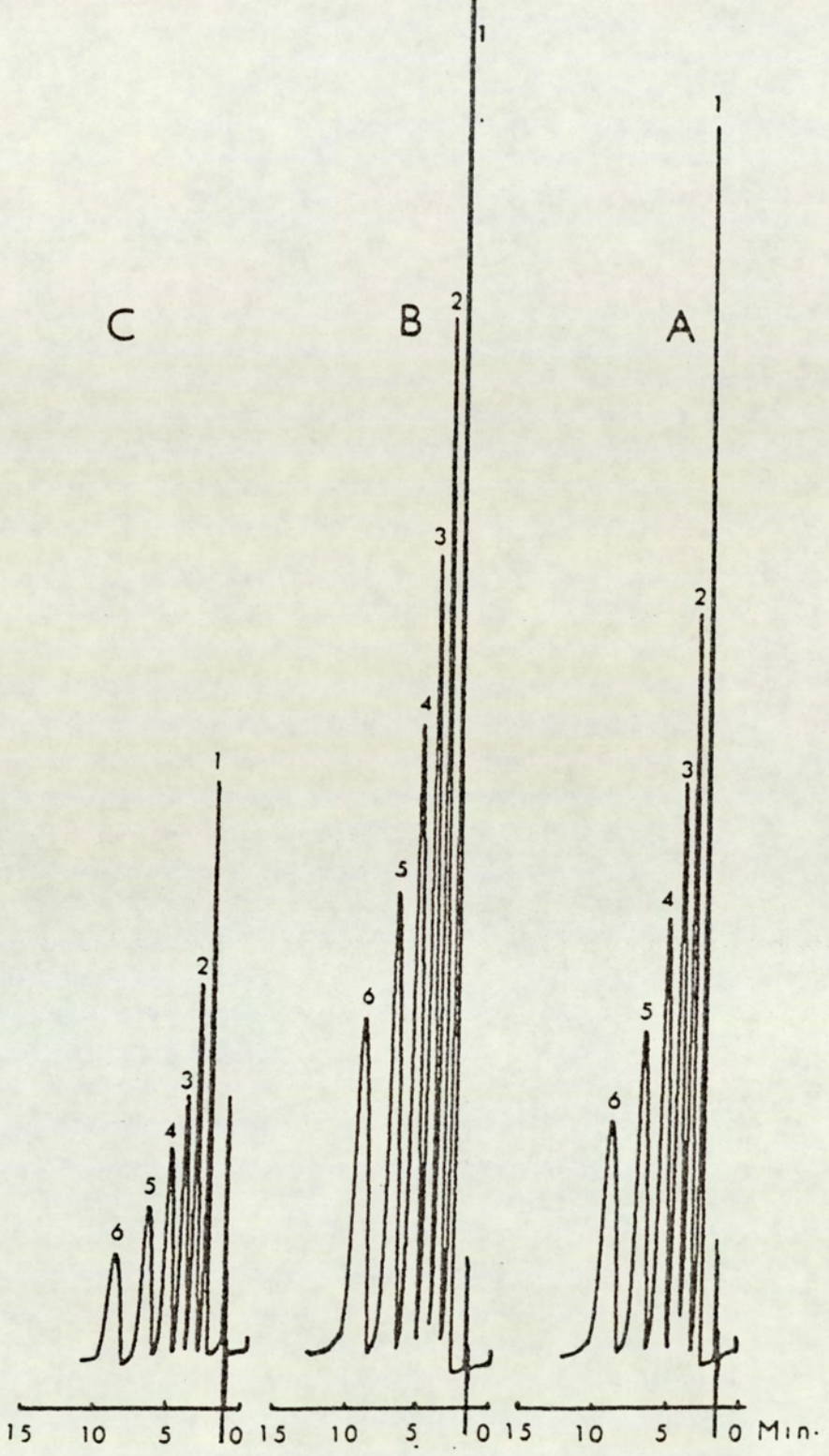


Fig 3.11 Chromatogram showing the effect of the analytical wavelength on the peak heights.

Compound	Propran-olol	Propranolol Esters				
		Acetyl	Propionoyl	Butyryl	Valeryl	Hexanoyl
Peak	1	2	3	4	5	6

Column: Hypersil ODS (5µm) 10cm x 4.6mm.i.d.
Mobile Phase: Acetonitrile: H_3PO_4 (88%): diethylamine:water (65:0.1:0.2:34.7
Flow Rate: 1 ml/min.
Detection: A: 305 nm. B: 290 nm. C: 265 nm. at 0.08 AUFS.

A 20 µl aliquot of each standard solution was injected onto an ODS-Hypersil (5 µm) column (10 cm x 4.6 mm I.D.) and eluted with mobile phase consisting of acetonitrile, orthophosphoric acid (88%), diethylamine and water (65: 0.1: 0.2: 34.7 v/v/v/v) of pH 2.5 which was delivered at flow rate of 1 ml/min. The analytes were detected at 290 nm, 0.08 AUFS.

The calibration curves which were constructed by plotting the peak heights against concentrations for each solvent composition are shown in Fig 3.12.

The statistical parameters of the lines were calculated from the calibration curves by a linear regression analysis program (using a CASIO fx 180P Scientific Calculator) and are recorded in Table 3.6. This shows that at lower concentrations of DMF in the sample solvent , the solubility of both esters was not enough to produce a linear relationship of the calibration curve while at a higher concentration of DMF, the solubility of the esters improved and consequently affected the linearity of the calibration curve. A chromatogram for the separation of both esters together with propranolol are shown in Fig 3.13.

3.7 APPLICATIONS OF THE DEVELOPED HPLC SYSTEMS

3.7.1 SEARCHING FOR THE INTERNAL STANDARDS

The term internal standard here means an incorporation of a known concentration of a pure stable compound into the sample to minimize the errors caused by the fluctuations in the column performance resulting in variation in peak height.

The ratio of the peak heights (analytes/internal standard) would then be directly proportional to the concentration of the analytes assuming both peaks suffer similar distortion.

The concentration of the internal standard must be adjusted to give a reasonable peak height with respect to that of the analytes (about half maximum height) under the same chromatographic condition. A compound which is suitable for use as an internal standard must have the following characteristics:-

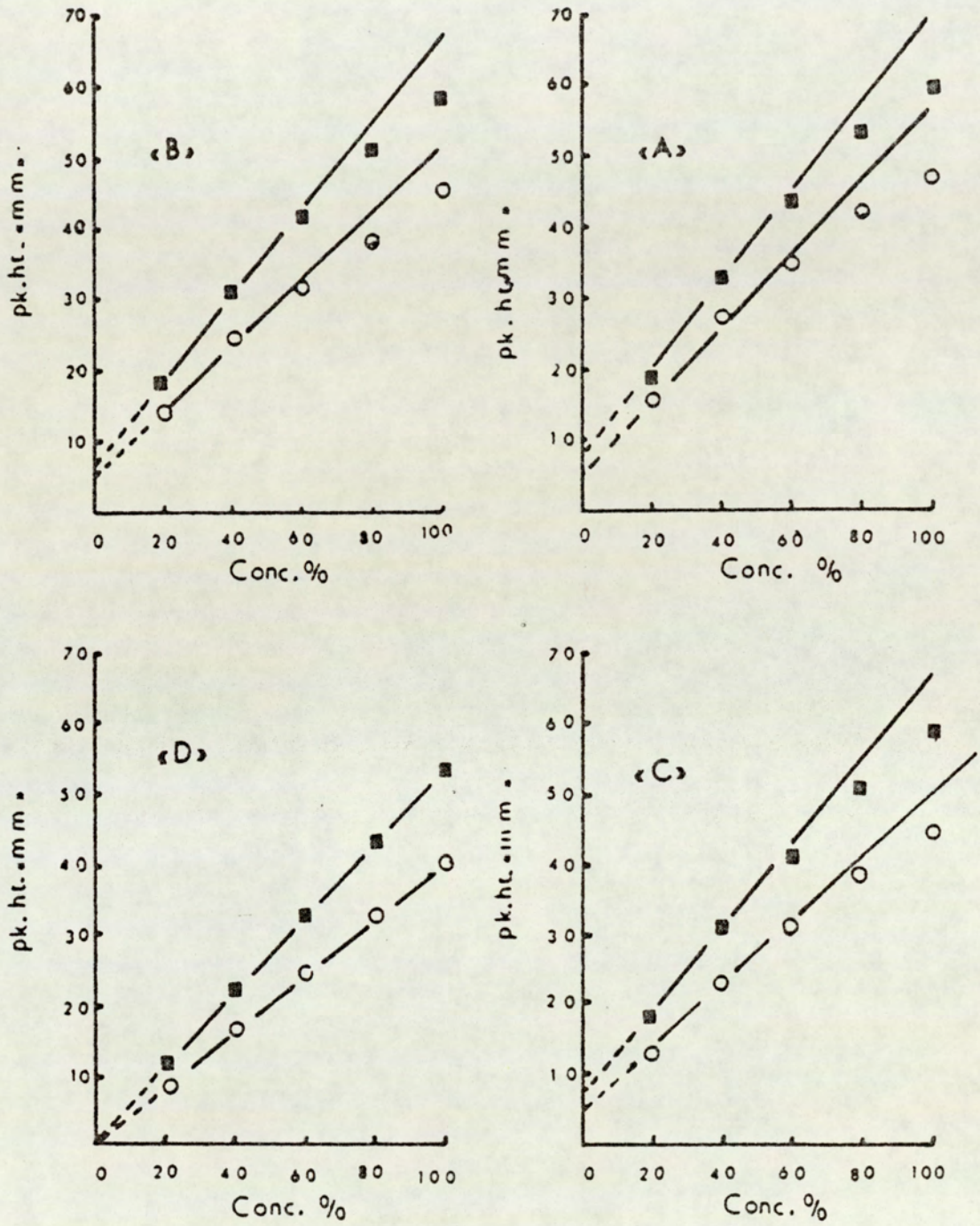


Fig 3.12 Effect of DMF Concentration on the linearity of the Calibration Curve for O-pivaloylpropranolol (■) and O-hexanoylpropranolol (0).

Graph	A	B	C	D
% DMF in Sample Solvent	5	10	15	30

D.M.F (%)	O-Pivialoylpropranolol			O-Hexanoylpropranolol		
	Correlation Coefficient	Slope	Intercept	Correlation Coefficient	Slope	Intercept
5	0.9911	0.5225	10.05	0.9881	0.3951	9.58
10	0.9941	0.4910	10.70	0.9941	0.3701	8.00
15	0.9950	0.4985	9.83	0.9954	0.3845	6.85
30	0.9997	0.5205	1.11	0.9993	0.3721	2.60

Table 3.6 Statistical Parameters for the Calibration Curves at Various DMF Concentration in the Sample Solvent

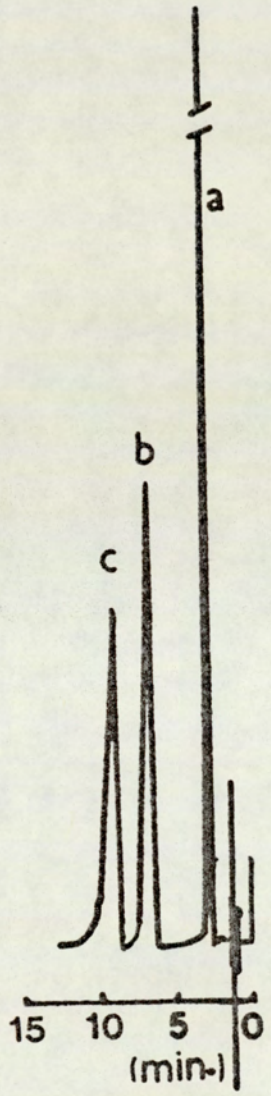


Fig 3.13 Chromatogram Showing the Separation of Propranolol (a), O-pivaloylpropranolol (b), and O-hexanoylpropranolol (c), using a Mobile Phase (System II) at Flow Rate 1ml/min, Detection at 290 nm.

a) It must be chemically stable under the condition of the analysis

b) It does not interact with other components in the sample

c) It will not interfere with the chromatographic resolution of the other compounds in the same run.

d) It should give an ultraviolet absorption at the analytical wavelength used.

e) It should not precipitate out after its addition to the sample.

The preliminary search showed that ethylparaben is a satisfactory internal standard for the analysis of propranolol and its esters using both mobile phases (I and II).
HPLC system I is also applicable to other beta-blockers and parabens where one agent can be used as internal standard for another. Their retention time and the column capacity factor are listed in Table 3.7.

Test chromatograms for the optimum mobile phases are displayed in Fig 3.14 and 3.15 and the details of these mobile phases are summarised in Table 3.8.

3.7.2 CONSTRUCTION OF CALIBRATION CURVES

Stock solutions of 10 mM propranolol, O-acetylpropranolol and O-pivaloyl-propranolol were prepared separately in 25 ml 50% acidified aqueous DM pH3.

1 ml of each of these stock solutions was diluted in combination to 100 ml with 50% acidified aqueous DMF pH3 to produce a final concentration of 100 ml, 0.1 mM which was considered as the 100% standard preparation of the analytes. This solution was diluted with the same solvent to provide calibration solutions with a concentration range of 10 - 100%.

Two ml of the standard solution and 2 ml of the internal standard (7 mg of ethylparaben in 100 ml 0.01 M HCl) were mixed together (whirlimixed) in a test tube. A 20 µl aliquot was injected onto the column and eluted with mobile phase (System II) at rate of 1 ml/min at 290 nm., 0.08 AUFS.

No.	Compound	Retention Time (Minutes)	Column Capacity Factor K´
1	Ethyl } Paraben	1.85	0.85
2	Propyl } Paraben	2.35	1.35
3	Butyl } Paraben	3.00	2.00
4	Sotalol	1.75	0.75
5	Pindolol	2.50	1.50
6	Acebutolol	2.60	1.60
7	Labelalol	2.90	1.90
8	Timolol	3.00	2.00
9	Metiprolol	3.20	3.20
10	Alprenolol	4.40	3.40
11	Oxprenolol	4.70	3.70
12	Bevantolol	5.40	4.40
13	Penbutolol	11.30	10.30

Table 3.7 Relative Retention Time and Column Capacity Factor of Different Compounds which can be used as Internal Standard in Hypersil-ODS Column (10 cm) Eluted by Acetonitrile, 0.03M KH_2PO_4 (0.8%) and water (54:23:1:22v/v/v/v) at Flow Rate of 1ml/min. Detected at 290 nm.

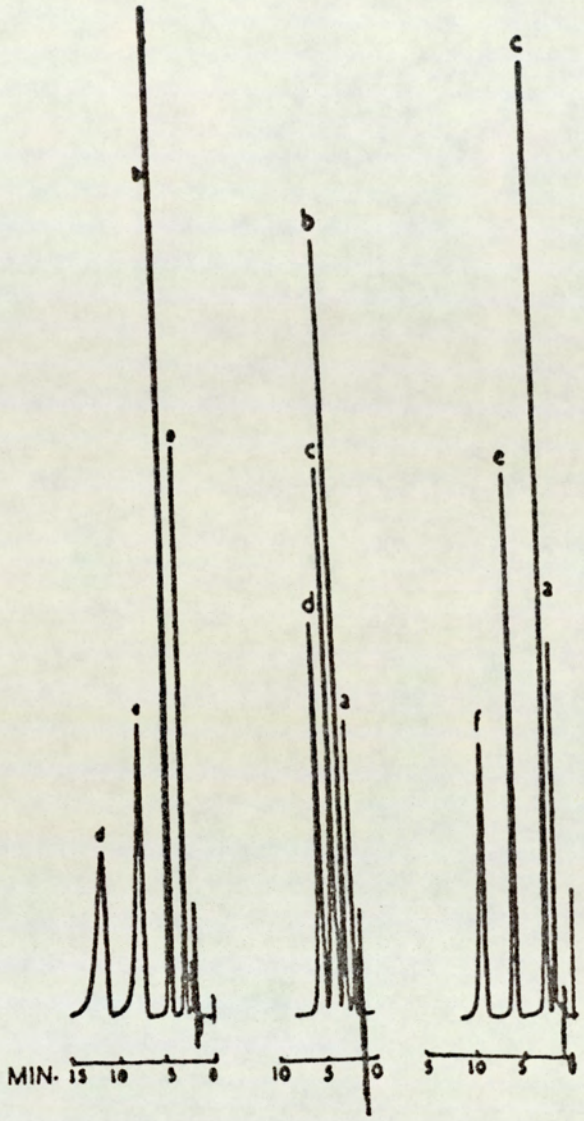


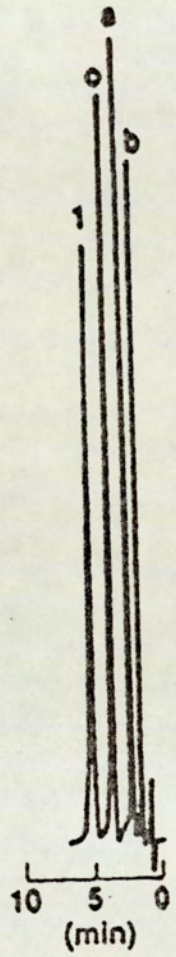
Fig 3.14 Test Chromatograms

Mobile Phase	Chroma-togram	Symbol	Component
CH_3CN: 0.03M KH_2PO_4: 0.8% H_3PO_4: H_2O (54: 23: 1: 22) pH2.8	A	a	Ethylparaben
		b	N-acetylpropranolol
		c	Propranolol
		d	O-acetylpropranolol
CH_3CN: Et_2NH: H_3PO_4 (88%) : H_2O (65: 0.15: 0.1: 34.75) pH2.8	B	a	Ethylparaben
		b	N-acetylpropranolol
		c	Propranolol
		d	O-acetylpropranolol
CH_3CN: Et_2NH: H_3PO_4 (88%) : H_2O (65: 0.2: 0.1: 34.7) pH2.8	C	a	Ethylparaben
		c	Propranolol
		e	N-hexanoylpropranolol
		f	O-hexanoylpropranolol

Column: Hypersil 1 - ODS (5µm), 10 cm x 4.6 mm i.d.
Flow Rate: 1ml / min Detection at 290 nm.

No.	Compound	Retention Time (Minutes)	Column Capacity Factor K′
1	Ethyl } Paraben	1.85	0.85
2	Propyl } Paraben	2.35	1.35
3	Butyl } Paraben	3.00	2.00
4	Sotalol	1.75	0.75
5	Pindolol	2.50	1.50
6	Acebutolol	2.60	1.60
7	Labelalol	2.90	1.90
8	Timolol	3.00	2.00
9	Metiprolol	3.20	3.20
10	Alprenolol	4.40	3.40
11	Oxprenolol	4.70	3.70
12	Bevantolol	5.40	4.40
13	Penbutolol	11.30	10.30

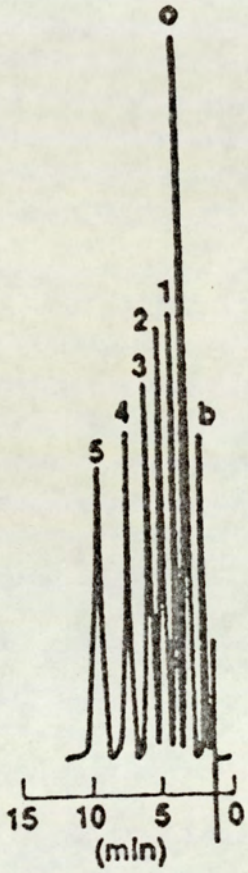
Table 3.7 Relative Retention Time and Column Capacity Factor of Different Compounds which can be used as Internal Standard in Hypersil-ODS Column (10 cm) Eluted by Acetonitrile, 0.03M KH_2PO_4 (0.8%) and water (54:23:1:22v/v/v/v) at Flow Rate of 1ml/min. Detected at 290 nm.



Acetyl Derivatives

65% acetonitrile

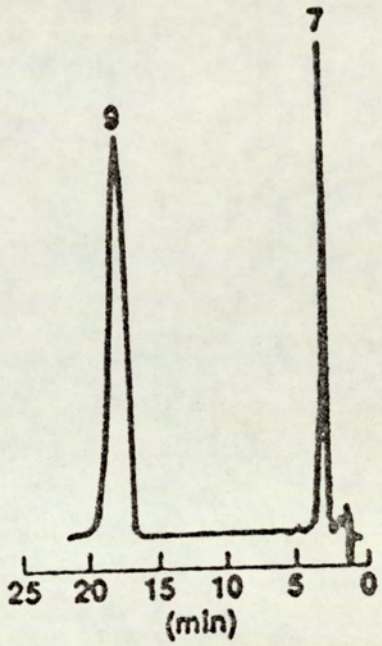
0.15% diethylamine



Shorter Esters

65% acetonitrile

0.2% diethylamine



Longer Esters

85% acetonitile

0.4% diethylamine

0, Propranolol; 1, acetyl; 2, propanoyl; 3, butanoyl; 4, valeryl; 5, hexanoyl;

7, octanoyl; 9, decanoyl; a, N-acetylpropranolol; b, ethyl paraben (internal standard)

PUMP:	Altex 100A dual-reciprocating
INJECTOR:	Rheodyne 7120 with 20µL loop
STATIONARY:	ODS-Hypersil (5 µm)
PHASE:	Reversed phase system
COLUMN:	10 cm x 4.6 mm ID (SS)
DETECTOR:	Pye LC3
WAVE LENGTH:	290 nm
FLOW RATE:	1 ml min^{-1}
MOBILE PHASE:	Adjusted to pH = 2.8 with H_3PO_4

Fig. 3.15

Test Chromatograms

Table 3.8 HPLC systems for propranolol pro-drugs and their degradative products.

Mobile Phase					Solute Components	Internal Standard	Approximate Elution Time (min)
CH_3CN	0.03M KH_2PO_4	0.8% H_3PO_4	Water	pH			
54	23	1	22	2.8	N-acetylpropranolol + Propranolol + O-acetylpronolol	Ethyparaben	15
54	23	0.2	22.8	3.4	N-acetylpropranolol + N, O-diacetylpropranolol +	"	16
					Propranolol + O-acetylpropranolol	"	12
					N-acetylpropranolol + N, O-diacetylpropranolol + Propranolol	"	7
					N-acetylpropranolol + Propranolol	"	12
CH_3CN	diethyl-mine	88% H_3PO_4	Water	pH	Solute Components	Internal Standard	Approximate Elution Time (min)
65	0.2	0.1	34.7	2.8	Propranolol + The First Five O-acyl esters	Ethylparaben	11
					Propranolol + N-butyryl propranolol + O-butyryl propranolol	"	7
					Propronaolol + N-valerylpropranolol + O-valeryl-propranolol	"	9
					Propranolol + N-hexanoylpropranolol + O-hexanoylpropranolol	"	11
65	0.15	0.1	34.75	2.5	N-acetylpropranolol + Propranolol + O-acetylpropranolol	"	7
					N-propionylpropranolol + propranolol + O-propionyl-propranolol	"	9

Table 3.8 Contd.

Mobile Phase					Solute Components	Internal Standard	Approximate Elution Time (min)
CH_3CN	diethyl-mine	88% H_3PO_4	Water	pH			
65	0.1	0.1	34.8	2.5	N-acetylpropranolol + N, O-diacetylpropranolol + Propranolol	Ethyparaben	6
					I-isopropyl-4-naphthyloxymethy Oxazolidin-2-one + Propranolol + N-ethoxy carbonyl, O-acetylpropranolol	"	8
65	0.02	0.08	34.9	3	4-OH-Propranolol + Propranolol (extracted from Plasma)	Desimipramine	≈15
85	0.4	0.2	14.4	2.8	Propranolol was washed off the column with the solvent front		
					N-decanoylpropranolol + O-decanoylpropranolol	-	20
					O-octanoylpropranolol + O-decanoylpropranolol	-	20
85	0.2	0.2	14.6	2.5	N-octanoylpropranolol + O-octanoylpropranolol	-	≈12

The calibration curves were constructed by plotting the peak height ratio against the concentrated % of the analytes and are shown in Fig 3.16.The statistical parameters of the lines are recorded in Table 3.9.

Component in the Mixture	Statistical Parameters		
	r	y	x
Propranolol	0.9999	0.02962	0.01315
O-acetylpropranolol	0.9999	0.01781	0.00296
O-pivaloylpropranolol	0.9996	0.00670	0.01528

Where r = Correlation coeffecient

y = Slope

x = intercept

Table 3.9 Statistical Parameters for Calibration Curves

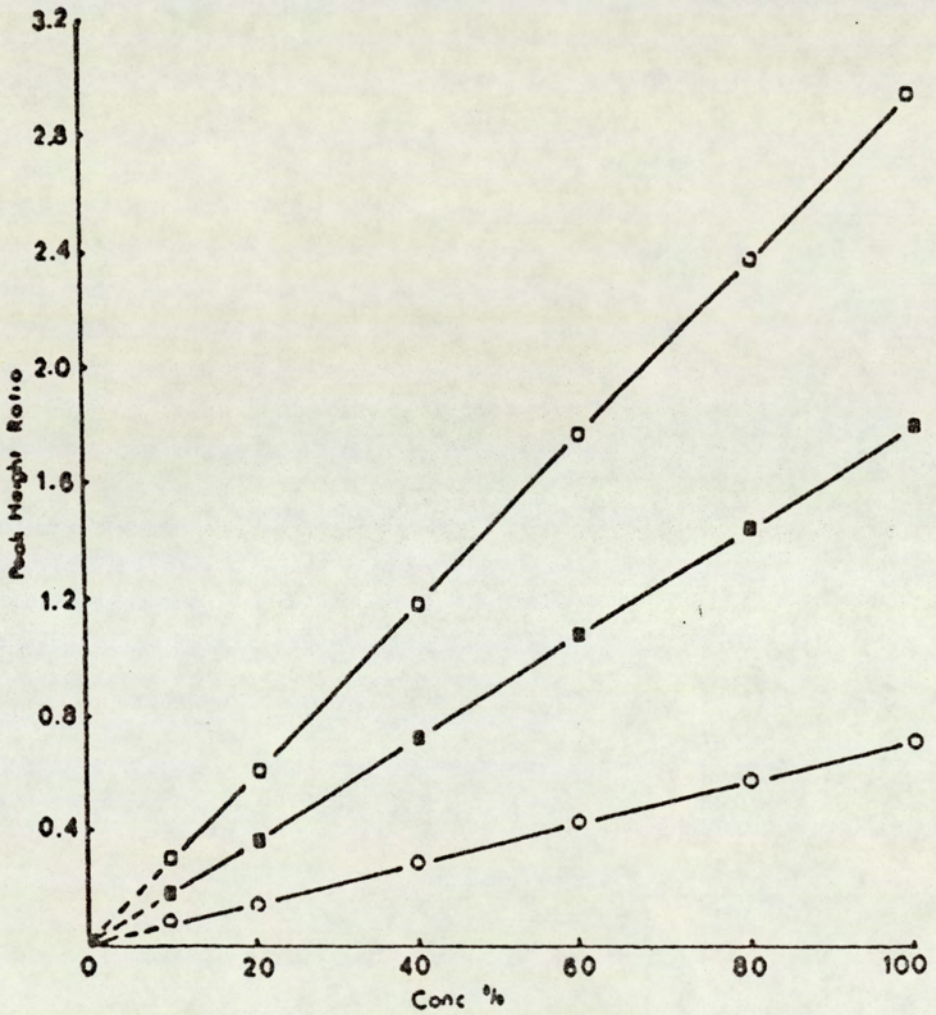


Fig 3.16 Calibration Curve for Propranolol (□), O-acetylpropranolol (■), and O-pivaloyl-propranolol (O) in Multi-component Solution.

CHAPTER FOUR

PHYSICO-CHEMICAL PROPERTIES OF PROPRANOLOL PRO-DRUGS

CHAPTER 4

4. PHYSICOCHEMICAL PROPERTIES OF PROPRANOLOL PRO-DRUGS

4.1 INTRODUCTION

A knowledge of the physicochemical properties of a drug such as a dissociation constant (pKa), solubility and partition coefficient (P) is useful in the optimisation of drug formulation and delivery. Additionally, the pharmacokinetic profile of the drug will be affected by these parameters [*Hinderling et al 1984 (31)*]. Ritschel 1972 (221) has reviewed some clinical effects of pKa values on the absorption, distribution and elimination of a drug. One of the more important applications of the ionization equilibria of weak acids and bases involves adjusting the pH of urine to enable reabsorption of unionized species for therapeutic reasons or to enhance excretion of ionized species in toxicological emergencies such as overdoses of barbiturates by alkalinizing the urine pH with sodium citrate or bicarbonate. In contrast, amphetamine overdoses may be treated by acidification [*Ritschel 1972 (221), Milne 1965 (22), Diechman and Gerarde 1969 (223), Peters 1960 (224) and Portnoff et al 1961 (255)*].

Stella 1973 (226) reported the adjustment of the pH of parenteral solutions to prevent precipitation, in admixture. This treatment relied upon the observation that the unionized form of most drugs is poorly water-soluble compared to the ionized form, the relationship may be quantified [*Albert and Serjeant 1971 (227), Gill et al 1970 (228) and Martin et al 1969 (229)*] so that the pKa of a drug may be used to predict the solubility of weak electrolytes in equeous solutions using the following equations:

For acids:

$$S_o = S_i \, [1 + \text{antilog (pH - pKa)}] \qquad 4.1$$

For bases:

$$S_o = S_i \, [1 + \text{antilog} \, (pKa - pH)] \qquad 4.2$$

where S_o and S_i are the observed and intrinsic solubilities respectively. Albert and Serjeant 1971 (227), Cookson 1974 (230), Connors 1975 (231) and Benet and Goyan 1967 (232) have reviewed several techniques for determination of the dissociation constant (pKa). These methods include potentiometric titration, the ultravoilet spectrophotometric technique which requires significant pH-dependent changes in the absorption spectra of a compound, and other techniques using the pH-dependence of the partition coefficient and the aqueous solubility of a compound.

A precise and rapid micro-determination of pKa values for soluble or insoluble bases has been reported by Li Wan Po and Irwin 1980 (233) which is based upon the potentiometric titration of a base hydrochloride with alkali.

Two cases may be observed:

a) A non-logarithmic titration method where precipitation of the insoluble base during titration takes place [*Irwin and Li Wan Po 1980 (233), Levy and Rowland 1971 (235) and Benet and Goyan 1967 (232)*].

b) A logarithmic method for the determination of pKa values of poorly soluble compound using a solvent system composed of water and miscible solvent (e.g. methanol or ethanol) where the ionized and unionized species of the compound must remain in solution during the titration.

The octanol/water partition coefficient is a measure of the lipophilicity of a drug and this parameter may indicate the level of drug distribution into extravascular compartments and a-dipose tissue. It may also be a measure to indicate how well a drug may be absorbed by permeation through lipid membranes [*Lien 1981 (236)*].

Lipophilicity tends also to be positively correlated with drug protein binding, with the more lipophilic drug being more extensively protein bound [*Johansson and Regardh 1976 (48), Dawes and Kendell 1978 (237) and McDevitt et al 1976 (238)*].

Attempts have been made to calculate the partition coefficient of a given compound [*Hansch and Fujita 1964 (239), Rekker 1977 (240), and Nys and Rekker 1973, 1974 (241 & 242)*] by summation of the hydrophobic constants for individual molecular fragments. These theoretical approaches cannot take into account all of the physicochemical properties of the molecule,especially, the stereochemistry.

It is thus advantageous to experimentally determine the lipophilicity of the compound of interest. For this purpose, several methods have been proposed of which the shake-flask method [*Hansch 1969 (243)*] and thin-layer chromatography (TLC) method [*Boyce and Milborrow 1965 (244)*] are the most widely used.

High-performance liquid chromatography (HPLC) has been proposed for this purpose [*Mirrless et al 1976 (245), Unger et al 1978 (246), Unger and Feuerman 1979 (247) and Carlson et al 1975 (248)*] by running solutes in octanol-saturated water or octanol-saturated buffer as the mobile phase against water-saturated octanol entrained on an inert support, where the logarithmic value of the column capacity factor (log K′) correlates with that of the partition coefficient.

$$\log K' = \log [(t_R - t_o) / t_o]$$

where t_R = retention time of the compound and t_o = retention time of the unretained compound (solvent front).

The major disadvantage of this technique observed in our laboratory is that the increase in the pressure on the column (900 bar) caused by the higher viscosity of the octanol mobile phase was unacceptable.

The methodology, values and applications of solubility data and of partition or distribution coefficientsare listed in a review by Leo and Hansch 1971 (76).

4.2 EXPERIMENTAL

4.2.1. pKA DETERMINATION

The determination of the pKa of propranolol and its ester derivatives was similar to that reported by Li Wan Po and Irwin 1980 (233) but with some modification.

4.2.1.1. APPARATUS

The apparatus (Fig 4.1) which used to determine the pKa of propranolol and its esters consisted of a jacketed titration cell (25 ml capacity) fitted into a Radiometer TTA60 titration assembly. The cell was maintained at 25°C or 37°C using a Churchill recirculating thermostatic bath. A flow of nitrogen gas was maintained over the sample to prevent carbon dioxide (CO_2) absorption from the air. A pH meter, type PHM64 radiometer utilizing a combined glass electrode, was used for pH measurement. Titrant (freshly prepared 0.1 M or 1M NaOH) was delivered to just under the surface of the cell contents by means of a microburette to which was attached a microdelivery tip to enable the accurate addition of small quantities of the titrant.

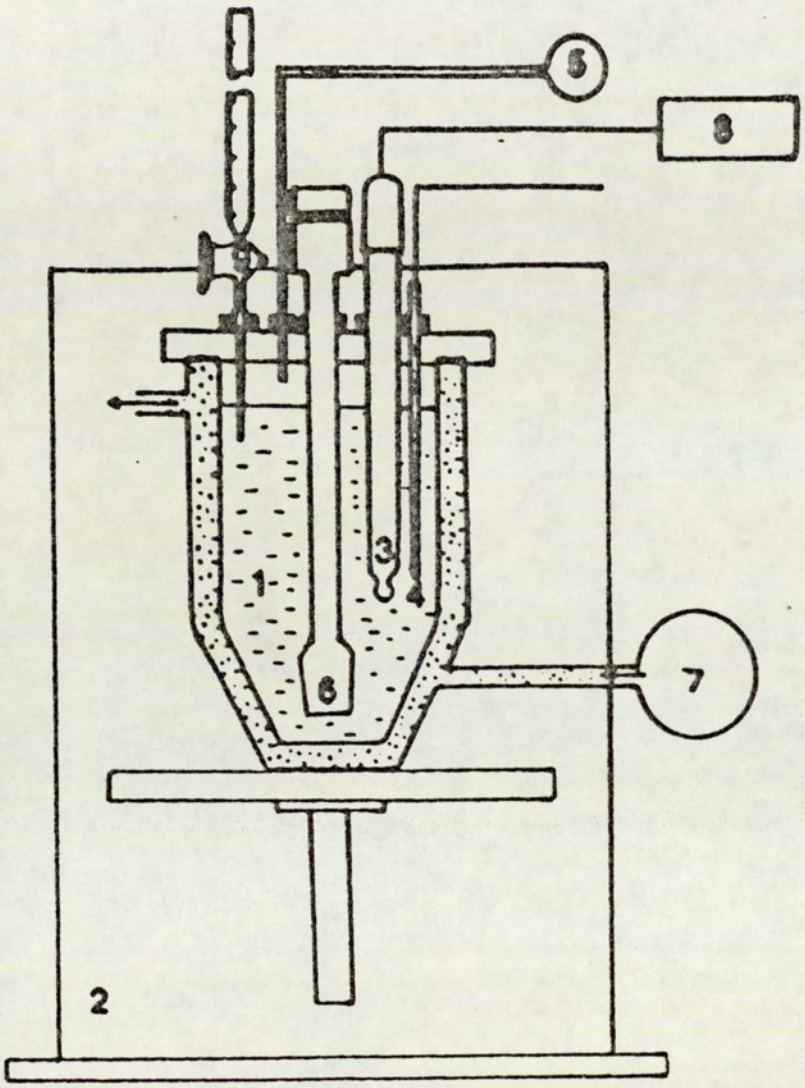


Fig. 4.1 Block diagram of titration equipment . The numbered features are:

1. Jacketed titration cell
2. Radiometer TTA 60 titration assembly
3. Glass pH meter electrode
4. Thermometer electrode (Digital Thermometer)
5. Flushing source of nitrogen gas
6. Paddle stirrer
7. Churchill recirculating thermostatic bath
8. Radiometer PHM 64 research pH meter
9. Microburet

4.2.1.2. PROCEDURES

Two different potentiometric titration procedures were carried out to determine the pKa of propranolol HC1.

a. Non-Logarithmic Titration Method

The non-logarithmic titration method described by Levy and Rowland 1971 (235) and Li Wan Po and Irwin 1980 (233) was used to determine the pKa of propranolol as the hydrochloride at 37°C.

An accurately measured 25 ml (5.4 x 10^{-3} M) of the stock solution were added to the titration cell and titrated with 1M NaOH at 37°C (usually 10 µl aliquots, end-point 0.135 ml). The aqueous solubility of the free base was determined in 0.01 M NaOH (see the section on the procedure in solubility).

b. Logarithmic Titration Method

The logarithmic titration method reported by Li Wan Po and Irwin 1980 (233) was used to determine the pKa of propranolol and its acyl esters (as hydrochlorides) in a solvent system consisting of water and methanol.

Stock solutions of propranolol HC1 and its acetyl, propionyl, butyryl, valeryl, hexanoyl and pivaloyl esters (as hydrochlorides) were prepared separately by dissolving 800 mg of each in 100 ml methanol.

5ml (40 mg) of each stock solution was added separately to the titration cell and diluted to 25 ml with water or aqueous methanol to provide a methanol concentration range in the solvent mixture between 80 - 20% v/v (for propranolol and its acetyl and propionyl esters), 80 - 40% v/v (for O-butyryl and O-valeryl propranolol) and 90 - 50% (for O-hexanoyl and O-pivaloyl propranolol). Solutions were titrated with freshly prepared 0.1 M NaOH at 25°C. The titrations were carried out quickly to avoid degradation of the esters during titration and only data obtained below pH9 were used in calculation for the same reason.

4.2.1.3. EFFECT OF SURFACTANTS ON THE pKA OF O-ACETYL-PROPRANOLOL

The determinaton of the pKa of O-acetylpropranolol in aqueous surfactant was carried out using the logarithmic titration technique. A stock solution of O-acetylpropranolol HC1 was prepared by dissolving 40 mg of the pro-drug separately in 100 ml 0.02 M aqueous dodecyltrimethylammonium bromide (D.T.A.B) and in 100 ml 0.02 M aqueous sodium lauryl sulphate (S.L.S.). The concentration of the surfactants (0.02M) were above their critical micelle concentration (C.M.C).

25 ml (1.184×10^{-3}M) aliquots of each stock solution were placed separately in the titration cell and titrated quickly with 0.1M NaOH at 25°C. All the data and statistics for the determination of pKa in all cases were calculated on an IBM-PC computer using PKA Basic program, written by Dr W.J. Irwin (Aston University, Department of Pharmaceutical Sciences, Birmingham .U.K.).

4.2.2. PARTITION DETERMINATIONS

The apparent partition coefficient (Papp) of propranolol and its pro-drugs in 1-Octanol/buffer has been measured using the shake-flask method described by Hansch 1969 (243).

4.2.2.1. PREPARATION OF BUFFER SOLUTIONS

McIlvaine buffer (Appendix V) (250) solutions at different pH's were prepared by mixing together suitable amounts of sodium dihydrogen orthophosphate.12 H_2O and citric acid mono hydrate (ionic strength of 0.5M was adjusted with potassium chloride). The final pH of these buffer solutions was checked at 37°C after calibration of the pH meter (Radiometer PHM64 pH meter) using standard buffers of pH4, 7 and 10.

4.2.2.2. PREPARATION OF OCTANOL-SATURATED BUFFER AND BUFFER-SATURATED OCTANOL

Octanol-saturated buffer and buffer-saturated octanol for use in the partitioning experiments were prepared by adding approximately 200 ml octanol (spectral grade) to 500 ml of buffer solution. The mixture was stirred vigorously by means of a magnetic stirrer for one hour and then agitated in a mechanical shaker (5B - 15 Techam-Water temperature control, set to 37°C) at maximum rate for four hours to ensure saturation. The phases were left standing overnight to separate and the pH of buffer phases were remeasured at 37°C.

4.2.2.3 PREPARATION OF STANDARD SOLUTIONS FOR THE CALIBRATION CURVES

Concentrated standard solutions for the compound under investigation (propranolol or its esters) were prepared at a concentration equivalent to those of experimental solutions in either octanol-saturated buffer or buffer-saturated octanol (for solubility reasons) and each was diluted with the same solvent over a concentration range of 10-100% and the absorbances were measured with a Unicam SP8000 ultravoilet recorded spectrophotometer using a matched pair of 1 cm quartz cells. Calibration data were collected over the same range for each experiment separately and concentrations were estimated by interpolation onto the line from a plot of the corrected absorbance (total absorbance of sample minus reference blank absorbance, base line) against the compound concentration using a linear regression program (CASIO Scientific Calculator fx - 180P).

4.2.2.4 THE APPARENT PARTITION COEFFICIENT OF PROPRANOLOL AS A FUNCTION OF pH

Stock solutions (200 mg; 6.76 x 10^{-3}M) of propranolol HC1 in 100 ml octanol-saturated buffer at different pH values (6.5, 6.82, 7.29, 7.7 and 8.09) were prepared.

50ml of each of these solutions and 50 ml of the corresponding buffer-saturated octanol were pipetted into stoppered bottles. Each bottle was then shaken vigorously by hand for one minute before agitation in a mechanical shaker (5B - 15 Techam-water temperature control, set at 37°C), at medium rate for 12 hours.

After complete phase separation, a sample was withdrawn from the aqueous phase at each pH value and a 1 ml aliquot was diluted to 5 ml with the same octanol-saturated buffer. Absorbances (at 290 nm) of the diluted samples were recorded using octanol-saturated buffer as a blank. The concentrations of propranolol in the diluted samples were calculated from the corresponding calibration curves. Shaking was repeated for a further 12 hours and phases were re-assayed to ensure equilibrium. In all cases equilibrium was established at the first sample. The U.V. measurements were carried out at room temperature (22°C).

4.2.2.5. DETERMINATION OF THE TRUE PARTITION COEFFICIENTS OF O-ACYL PROPRANOLOLS

The partition coefficients of O-acyl propranolols were determined in an octanol-buffer (pH 2.56, 3.5 and 4.4) system. The solute concentration in the octanol phase was determined spectrophotometrically at 290 nm before and after partition, the equilibrium being obtained after mixing (Whirlimixer) the phases for 3 minutes. This period was chosen to minimize degradation of the esters. The initial concentration in the octanol phase was about 2.96×10^{-3}M and the partitioning system consisted of 10 ml of buffer-saturated octanol and 100 ml of octanol-saturated buffer. For each compound, determinations were carried out in triplicate, the log P values thereby obtained being reproducible to within ± 4%.

4.2.2.6. EFFECT OF ION-PAIRING ON THE Papp OF PROPRANOLOL IN pH 6.5 AT 37°C

A stock solution 1g (6.76×10^{-3}M of propranolol HC1 in 500 ml octanol-saturated buffer pH 6.5 was prepared.

Fifty ml of this solution was pipetted into each of six 250 ml stoppered bottles. Different amounts of sodium hexanesulphonate were added to the six bottles containing the 50 ml propranolol solution to provide an ion-pair concentration range of 0 -10 mM. 50 ml of the corresponding buffer-saturated octanol was then added to each bottle, which were then shaken vigorously by hand for one minute before placing in the shaking water bath at 37°C. Partition coefficients were obtained as described for the determination of Papp of propranolol at various pH values (Section 4.2.2.4).

4.2.3. SOLUBILITY DETERMINATION

4.2.3.1. DETERMINATION OF THE AQUEOUS SOLUBILITY OF PROPRANOLOL BASE

Excess propranolol HCl was dispersed ultrasonically in 10 ml of 0.01 M NaOH for 15 minutes, and the suspension was stored at 25°C in a water bath with gentle agitation for 24 hours. The mixture was centrifuged and replaced in the water bath for several hours to readjust for any changes arising from the centrifugation. One ml aliquot of the supernatant was diluted to 10 ml with methanol and analysed spectrophotometrically at 290 nm using a mixed solvent similar to that of the sample [0.01M NaOH and methanol (1:9)] as a reference. Standard solutions of propranolol were prepared in the same solvent mixture (over a concentration range of 1-10 mM for construction of the calibration curve.

Determination were carried out in triplicate with the coefficient of variation from the mean never exceeding 2%.

4.2.3.2. DETERMINATION OF THE AQUEOUS SOLUBILITY OF THE FREE BASE OF O-ACYL PROPRANOLOLS

The aqueous solubility of the free base of O-acyl propranolol hydrochlorides cannot be measured in alkali due to instability of the esters under these conditions. As a consequence, an attempt was made to calculate the aqueous solubility of the free base using non-logarithmic potentiometric titration method described earlier (Section 4.2.1.2.).

Aqueous solutions of propranolol HCl and its acetyl, propionyl, butyryl, valeryl, hexanoyl and pivaloyl esters (as hydrochlorides) were prepared by dissolving 40 mg of each compound separately in 100 ml cooled, boiled distilled water. 25 ml of each of these solutions were then separately titrated (in triplicate) with 0.1 M NaOH at 25°C.

The pH during the titration was not allowed to exceed pH 9 and the titration time was limited to 10 minutes.

4.2.3.3. SOLUBILITY OF N, O-DIACETYLPROPRANOLOL IN BUFFERED-DIMETHYL FORMAMIDE (DMF) AND IN BUFFERED-PROPYLENE GLYCOL (P.G.) AT 25°C

Solvents were prepared by adding separately different volumes of dimethyl formamide (DMF) and propylene glycol (P.G) in 0.5 M McIlvaine buffer solution (Appendix V) pH 7.4 to cover a concentration range of each from 0 - 40% v/v.

The addition of DMF to the buffer solution produced an increase in the pH greater than that produced by the addition of P.G. Table 4.1 shows the effect of addition of DMF and P.G on the buffer pH.

	pH								
Co-solvent % v/v	0	5	10	15	20	25	30	35	40
DMF	7.4	7.63	7.78	7.91	8.06	8.24	8.41	8.62	8.71
P.G.	7.4	7.47	7.54	7.59	7.67	7.74	7.80	7.88	7.96

Table 4.1 Effect of Addition of the Co-solvent on the pH

5 ml of each solvent was transferred to a stoppered graduated test tube. Excess amount of N,O-diacetylpropranolol was added to each test tube. The systems were well mixed using a Whirlimixer for 10 minutes, and then stored at 25°C in water bath with gentle agitation for 2 hours. The mixtures were centrifuged and equilibrated as before (Section 4.2.3.1) for at least one hour. 2 ml of the supernatant solutions were taken out and diluted with the appropriate amount of buffer or the co-solvent (DMF or P.G) such that all test solutions contained the same proportion of the co-solvent (e.g. 20% co-solvent

in buffer). These solutions were then diluted with an equal volume of methanol containing the internal standard (ethyl paraben 0.07 mg/ml).

Care was taken to ensure that composition of the solvent in both standard and best solutions were the same.

The standard and the test solutions were then analysed by HPLC using the mobile phase for N, O-diacetylpropranolol (Table 3.8) at a flow rate of 1 ml/min and detection at 290 nm.

All the pKa, partition coefficient and solubility experiments were carried out in triplicate.

4.3 RESULTS AND DISCUSSION

4.3.1 DETERMINATION OF THE pKA OF PROPRANOLOL HYDROCHLORIDE USING NON-LOGARITHMIC TITRATION METHOD.

This method is based upon the potentiometric titration of a base hydrochloride (e.g. propranolol HCl) with alkali, where the insoluble base precipitates during the titration .

The change in the hydronium ion concentration as a function of added alkali is given by equation 4.3 reported by Li Wan Po and Irwin 1980 (233).

$$Z' = A_o - \frac{B_{Sol}}{K_a} [H_3O^+] \qquad 4.3$$

where Z' = $M^+ + H_30^+ - OH^-$

M^+ = moles of alkali added to the titration cell

H_30^+ = moles of hydronium ion in the titration cell

OH^- = moles of hydroxyl ion in the titration cell

A_o = moles of base hydrochloride added to the titration cell

B_{sol} = moles of unionized base in solution in the titration cell

$[H_30^+]$ = molar concentration of hydronium ions

K_a = dissociation constant of base hydrochloride

A plot of $[H_30^+]$ (after the precipitation point) against Z', yields a linear relationship with an intercept of A_o and a slope $-\frac{B_{sol}}{K_a}$ from which the pKa may be determined provided

the aqueous solubility of the free base is available. The aqueous solubility of the free base of propranolol HC1 has been determined in 0.01 M NaOH and was found to be 8.03×10^{-4} moles/litre, comparable to that calculated using equation 4.4 reported by Yalkowsky et al 1983 (251) which enables the estimation of the aqueous solubility (S_w in moles/litre) of an organic compound from melting point (m.p) and octanol/water partition coefficient (P.C.) data, this equation has the simple form.

$$\log S_w = -0.01\ m.p - \log P.C + 1.05 \qquad 4.4$$

Applying this equation to calculate the aqueous solubility of propranolol base where the melting point is 96°C (from manufacturer's specification) and the log octanol/water partition coefficient = 3.18 [*Johansson and Regardh 1976 (48)*].

$$\log S_w = -0.01\ (96) - 3.18 + 1.05$$

$$= 8.12 \times 10^{-4} \text{ moles/litre}$$

Fig 4.2 shows the change of the pH as a function of added alkali during titration. A sufficiently large portion of the titration curve before precipitation was obtained which permits a determination of the pKa value using the Handerson Haselbalch equation (equation 4.5). A quick estimation of the aqueous pKa from the titration curve by interpolation of the amount of alkali for half of the neutralization (half the end point) onto the titration curve where it was found to be about 9.33.

At the point where the precipitation occurred, a break occurred in the titration curve with a sudden drop in pH. Continuation of the titration at slow rate throughout its range of saturation provided data in which the hydrogen ion concentration is linearly related to the

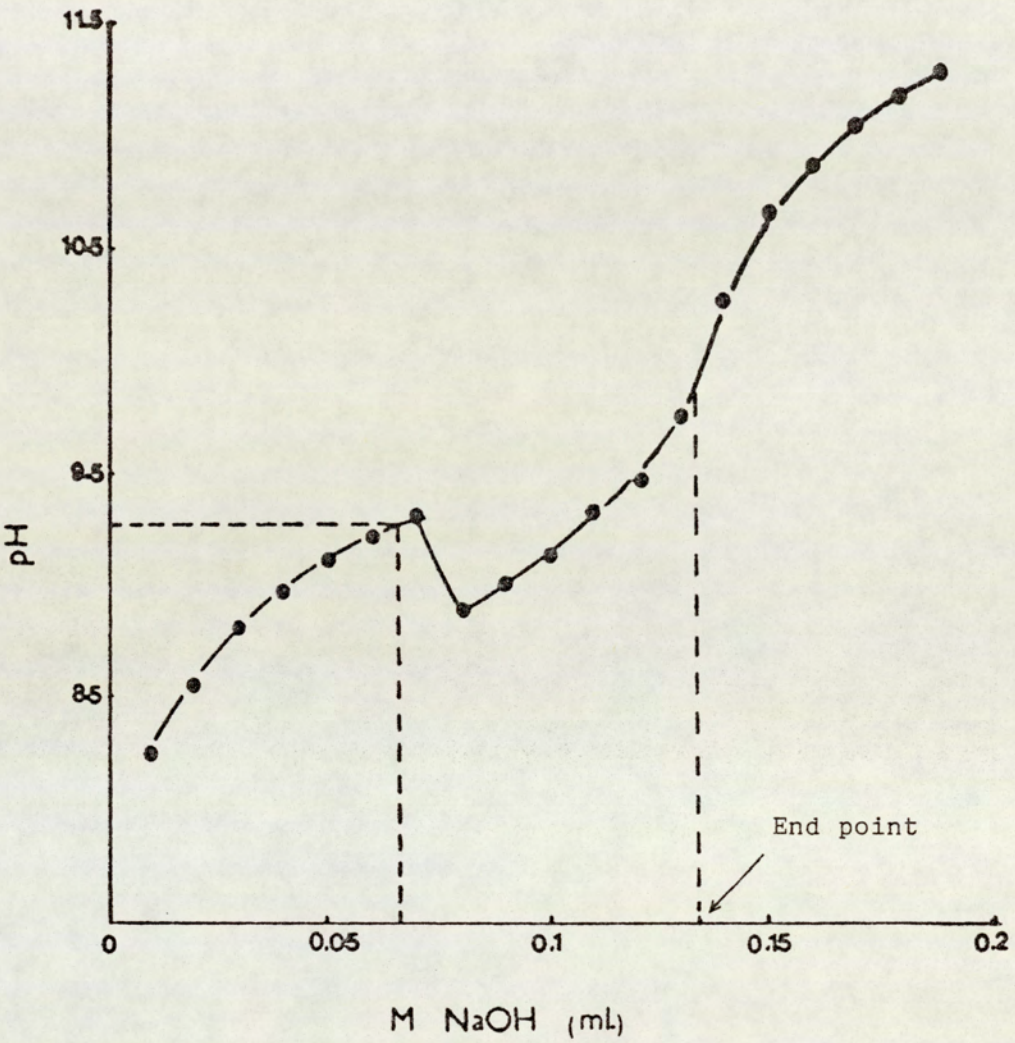


Fig 4.2 Non-logarithmic titration curve of propranolol HCl

amount of added base (Fig 4.3). The solubility estimates allow a further estimate of the pKa value (9.32).

Table (4.2.A.) shows the variation of the aqueous pKa of propranolol using measured and calculated basic aqueous solubility data together with the value determined from the initial part of the titration curve using equation 4.5.

4.3.2 DETERMINATON OF THE pKA OF PROPRANOLOL HYDROCHLORIDE AND ITS ACYL ESTERS HYDROCHLORIDES USING LOGARITHMIC TITRATION

Potentiometric titration of a base hydrochloride (propranolol or its esters) with alkali in which the ionized and unionized species of these compounds must remain in solution during the titration is modelled by the Handerson-Haselbalch equation (equation 4.5).

$$[H_30^+] = Ka \frac{a - [H_30^+] + [OH^-]}{b + [H_30^+] - [OH^-]} \cdot \frac{f\,BH^+}{f\,H_30^+.fB} \qquad 4.5$$

where $[H_30^+]$ = molar hydronium ion concentration

$[OH^-]$ = molar hydroxyl ion concentration

a = molar concentration of base hydrochloride

b = molar concentration of base

Ka = dissociation constant of base hydrochloride

f = activity coefficient

The low aqueous solubility of O-acyl propranolol derivatives precluded the direct application of this relationship. The technique which was used in this instance involved the determination of the pKa in a solvent system consisting of a mixture of water and another miscible organic solvent (methanol).

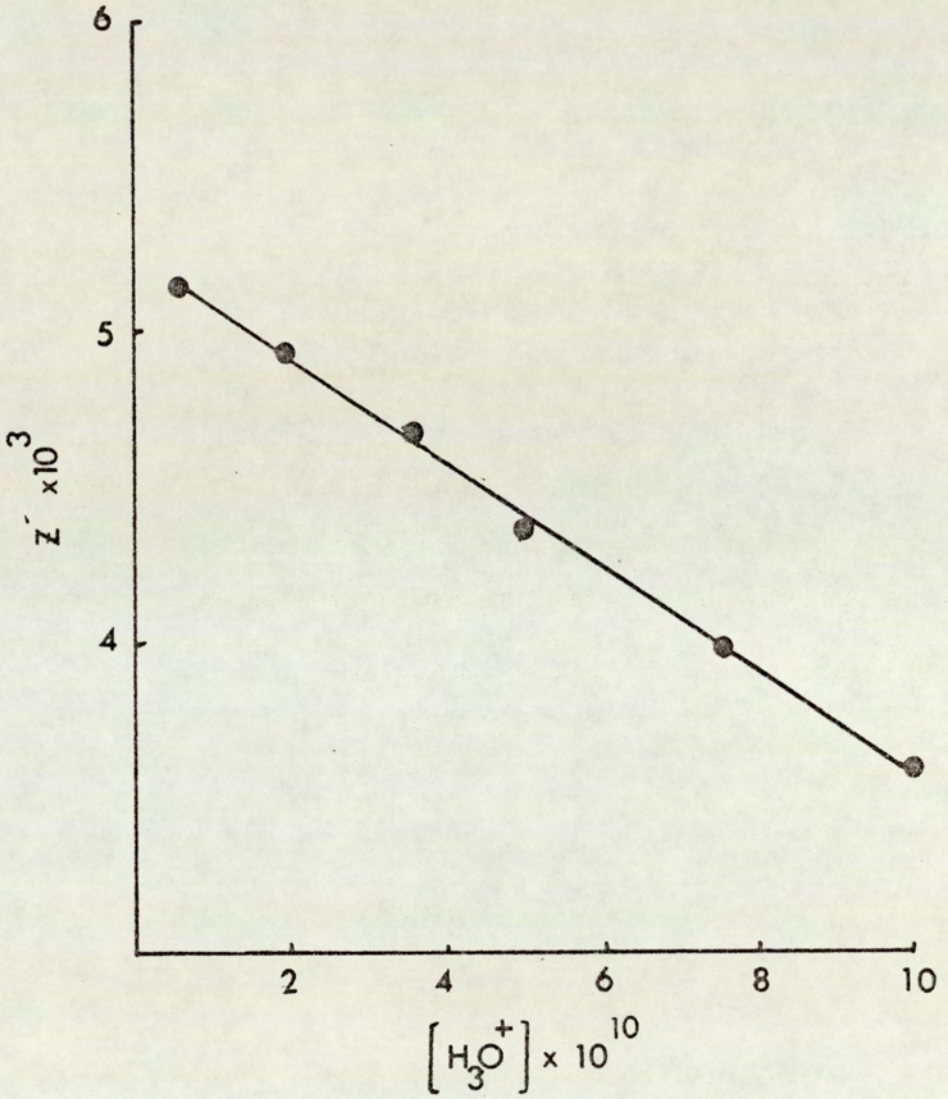


Fig 4.3 Non-logarithmic titration of propranolol·HCl

Table 4.2.A Aqueous pKa of propranolol HCl determined by non-logarithmic titration method.

Bsol (moles/liter)	Aqueous pKa	95% error limits	Mean pKa
8.03×10^{-4} [a]	9.31	9.29 - 9.33	
8.126×10^{-4} [b]	9.307	9.295 - 9.319	9.32
-	9.33 [c]	9.30 - 9.36	

(a), (b) The aqueous solubility of the free base of propranolol HCl determined in 0.01 N NaOH and calculated from equation 4.2.

(c) Calculated pKa from the titration curve before precipitation using equation 4.3.

Table 4.2.B Aqueous pKa of propranolol HCl determined by non-logarithmic and logarithmic titration methods.

Method	pKa	Mean pKa
Non-logarithmic titration	9.32	9.42
Logarithmic titration	9.51*	

* Calculated by extrapolation of the aqueous-methanol data

The delivery of an accurate, small volume (20µl) of the titrant (0.1M NaOH) gave many data points for calculation of the pKa before reaching the limiting pH where degradation of the esters was facilitated.

A typical batch of data together with the estimated aqueous pKa of propranolol and its o-acyl esters are recorded in Table 4.3. This shows that an increasing proportion of methanol resulted in a lowering of the observed pKa. This phenomenon is attributable to the depressed ionization, which occurs when the dielectric constant of the solvent system decreases with the increasing proportion of the water-miscible co-solvent (methanol). Extrapolation of this data to the pure aqueous system enables an estimation of the aqueous pKa to be obtained.

Fig 4.4 shows a linear relationship of the proportion of methanol in the solvent system and observed pKa. Several authors [*Hall and Sprinkle 1932 (252); Cavill et al 1944 (253)*] showed a "hockey-stick" shaped curve resulting from the plots of these parameters when partly aqueous solutions were used. When such a curve is produced successful extrapolation for finding pKa (pKa value at zero alcohol concentration) is not always possible, but extrapolation from the linear plot of P_sK_a + log [H_2O] v.s reciprocal of dielectric constant can be employed [*Benet and Goyan 1967* (232)].

Comparative results for the determination of the aqueous pKa of propranolol. HC1 by non-logarithmic and logarithmic titration methods are recorded in Table 4.2.B where the average estimated pKa (9.42) was close to that (9.45) reported by Avery 1976 (72).

The fall in pKa of the esters from that of propranolol may be due to the steric interaction between the ester carbonyl group and the secondary amine in the molecule's side chain.

Compound*	Measured pKa (Logarithmic) % Methanol							Correlation Coefficient (r)	Calculated pKa
	20	40	50	60	70	80	90		
Propranolol HCl	9.35	9.163	9.09	9.00	8.92	8.85		0.9991	9.51
O-Ac.P. HCl	8.37	8.23	8.13	8.04	7.988	7.92		0.997	8.52
O-Prop.P. HCl	8.38	8.24	8.17	8.11	8.02	7.93		0.997	8.54
O-But.P.HCl		8.25	8.19	8.09	8.00	7.95		0.994	8.57
O-Val.P. HCl		8.24	8.20	8.10	8.01	7.92		0.993	8.59
O-Hex.P. HCl			8.12	8.03	7.95	7.85	7.79	0.998	8.53
O-Piv.P. HCl			8.34	8.27	8.18	8.11	8.03	0.998	8.71

* Abbreviations:
O-Ac.P = O-acetylpropranolol
O-Prop.P = O-propionylpropranolol
O-But.P = O-Butyrylpropranolol
O-Val.P. = O-valerylpropranolol
O-Hex.P. = O-hexanoylpropranolol
O-Piv.P. = O-pivaloylpropranolol
HCl = Hydrochloride

Table 4.3 pKa values of propranolol HCl and its pro-drugs hydrochlorides in aqueous methanolic solution

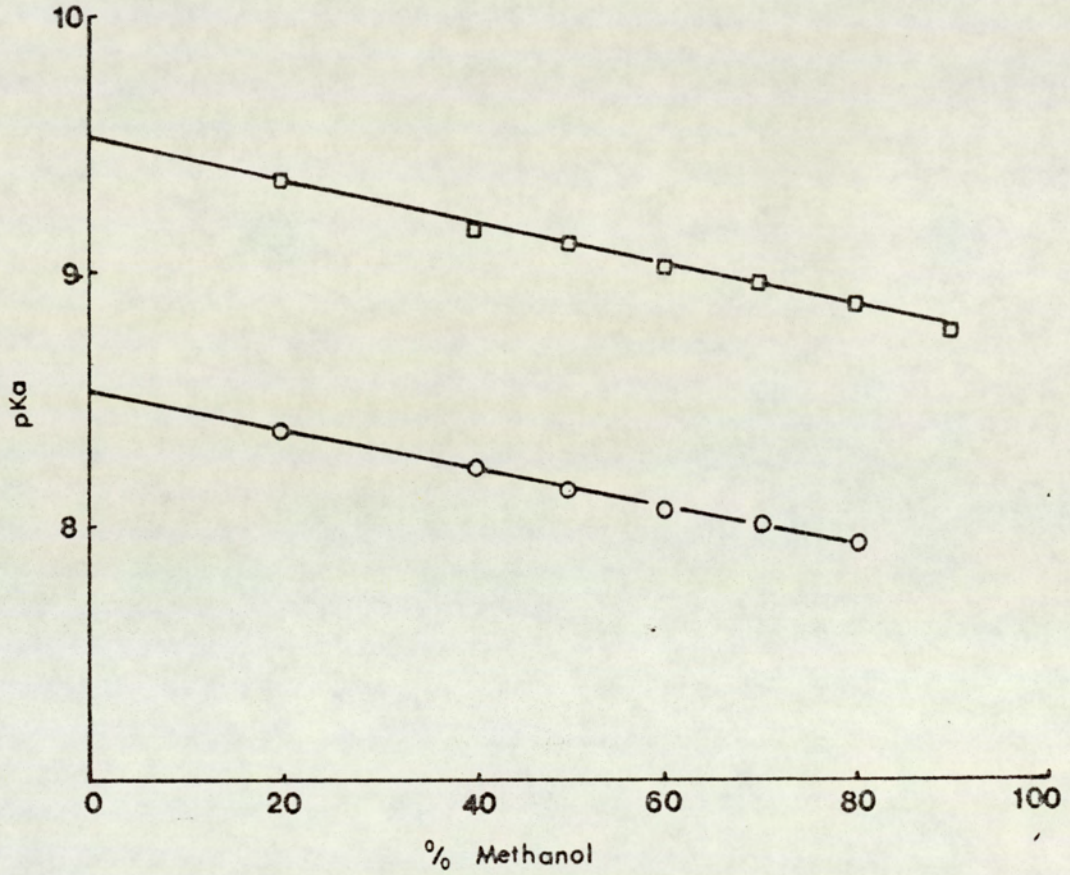
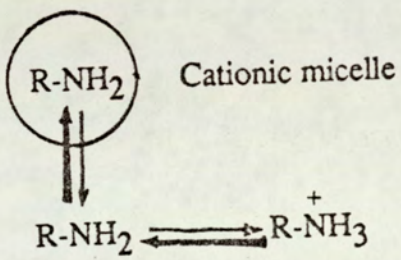


Fig 4.4 The dissociation constants (pKa) of propranolol HCl (□) and O-acetylpropranolol HCl (O) in aqueous methanol.

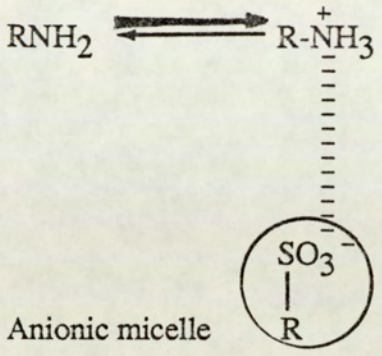
4.3.3. EFFECT OF MICELLIZATION ON THE pKA OF O-ACETYLPROPRANOLOL

The effect of the aqueous micellar systems on the apparent pKa value has been examined in some details by Mukerjee and Banerjee 1964 (254), Fendler and Fendler 1975 (255), Chainovich et al 1982 (256) and El Seoud and Vieira 1983 (257). The determination of the pKa of O-acetylpropranolol in 0.02 M aqueous dodecyltrimethylammonium bromide (D.T.A.B) using the logarithmic titration method showed a lower pKa value (7.664) than that predicted by extrapolation of the aqueous methanol data. This may be due to incorporation of the unionized species of the molecule (Scheme I) into the cationic micelle driving the equilibrium towards the neutral region which causes falling in the solution pH and thus provided a lower value of pKa.



Scheme 1

The pKa of O-acetylpropranolol in 0.02 M sodium lauryl sulphate (S.L.S) pH7 (the pH adjusted with 0.5M HC1) was determined by the logarithmic titration method. In contrast, this was found to be higher (10.18) than the estimated aqueous pKa. This apparent increase in basicity may be accounted for by ion-pairing between the ionized form of the ester molecule and the anionic group in the surfactant (Scheme II) leading to a shift in the equilibrium favouring ionization. This will raise the solution pH and thus provide a higher pKa value.



Scheme II

Typical batches of titration data for the determination of the pKa of O-acetyl -propranolol in the presence of cationic and anionic surfactants are recorded in Appendix VI. The pKa of O-acetylpropranolol in aqueous surfactants and that calculated in water are listed in Table 4.4.

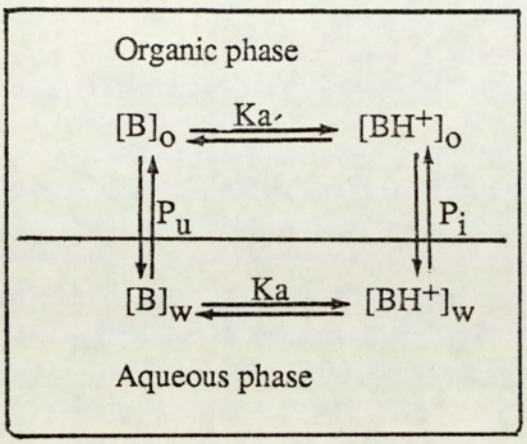
4.3.4 THE APPARENT PARTITION COEFFICIENT OF PROPRANOLOL AS A FUNCTION OF pH

During the partitioning of a weak base between organic and aqueous phases, the distribution of each species can be described by Scheme III: where P_u and P_i are the partition coefficients of unionized and ionized molecules respectively; $[B]_o$ and $[B]_w$ are the molar concentration of unionized molecules in the organic and equeous phases, respectively; $[BH^+]_o$, and $[BH^+]_w$ are the molar concentration of the ionized molecules in the organic and aqueous phases respectively, and Ka and Ka′ are the stoichiometric ionization constants of BH^+ in the aqueous and organic phases respectively.

Solvent	95% Range	pKa
Water	-	8.523*
0.02 M D.T.A.B	7.614 - 7.72	7.664
0.02 M S.L.S pH7	10.012 - 10.466	10.182
0.02 M S.L.S pH > 7	10.56 - 10.74	10.644

* Calculated by extrapolation of the aqueous-methanol data.

Table 4.4 The pKa of O-acetylpropranolol in water and in aqueous surfactants



Scheme III

The apparent partition coefficient (P_{app}) of propranolol was calculated by determining the concentration of propranolol in the octanol-saturated buffer and its concentration in the buffer saturated octanol using equation 4.7.

$$P_{app} = \frac{I - XD \,.\, Vw}{XD \,.\, Vo} \qquad 4.6$$

In these experiments Vw = Vo

$$P_{app} = \frac{I - XD}{XD} \qquad 4.7$$

Where P_{app} = the apparent partition coefficient of propranolol

I = the initial concentration of propranolol in the aqueous phase

X = concentration of propranolol in diluted sample at equilibrium

D = times of dilution

The apparent partition coefficient (P_{app}) of propranolol at various pH values are listed in Table 4.5. It is shown that the P_{app} of propranolol varies directly with the pH, due to an increase of the percentage of the unionized form of propranolol enhancing the affinity of propranolol to the octanol phase.

pH	Papp	log Papp
6.50	3.30	0.52
6.82	6.38	0.80
7.29	15.10	1.18
7.70	33.54	1.53
8.09	78.00	1.89

Table 4.5 The P_{app} of propranolol at various pH values at 37°C.

A plot of P_{app} and % unionized of propranolol as a function of pH are presented in Fig 4.5.a and b. Fig 4.6.a shows the linear relationship resulting from a plot of pH against log P_{app}. Several basic drugs such as propranolol and some other beta-blockers were not soluble enough in an alkaline pH for direct measurement of P_u. Hence, the true partition coefficient could not be determined by the conventional methodology. Consequently equation 4.8 derived by Irwin and Li Wan Po 1979 (234) has been used to calculate the partition coefficient of the unionized (P_u) and ionized (P_i) fraction of propranolol .

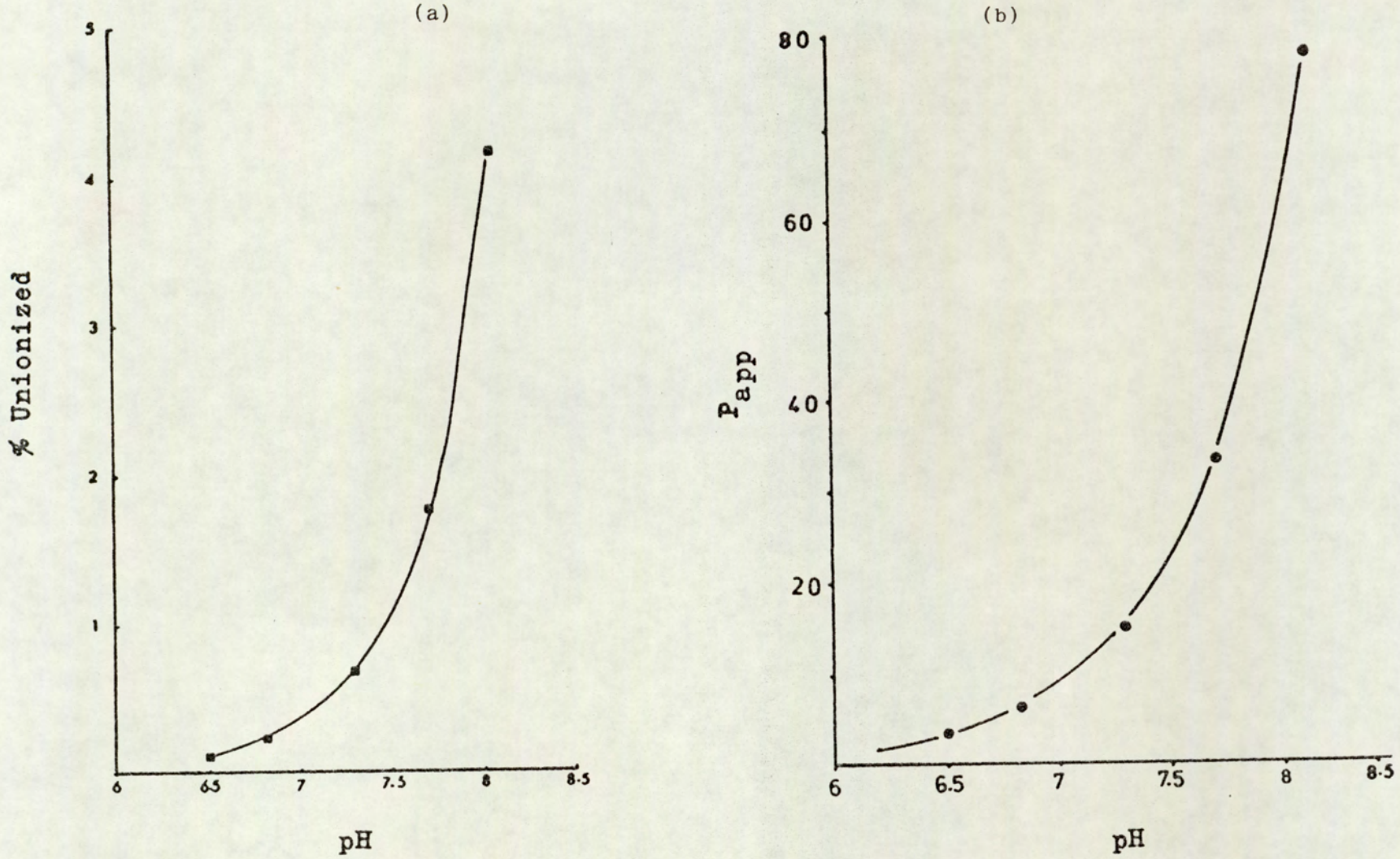


Fig 4.5 Effect of pH on the % unionized (a) and on the apparent partition coefficient (Papp) of propranolol(b)

(a)

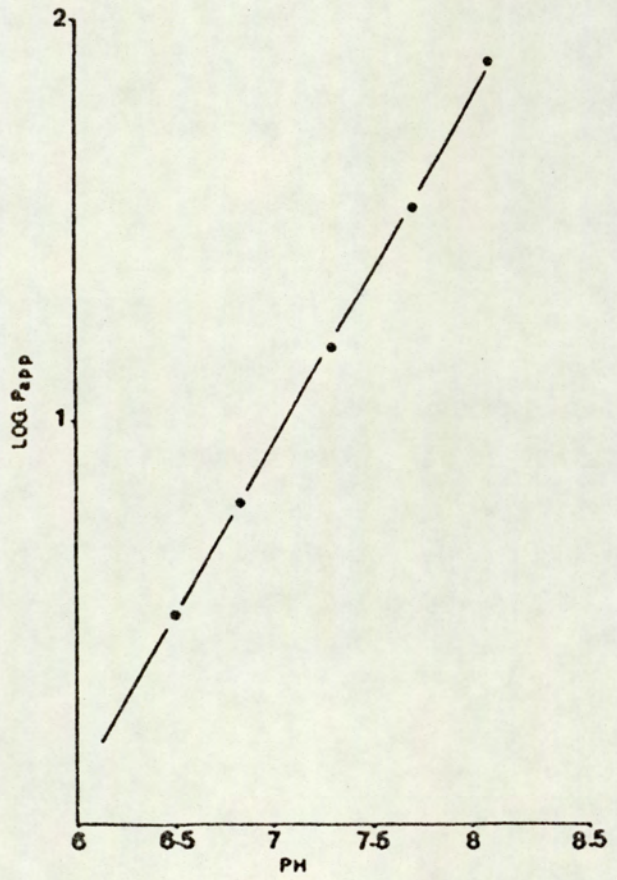


Fig 4.6.a The experimental log Papp of propranolol as a function of pH

(b)

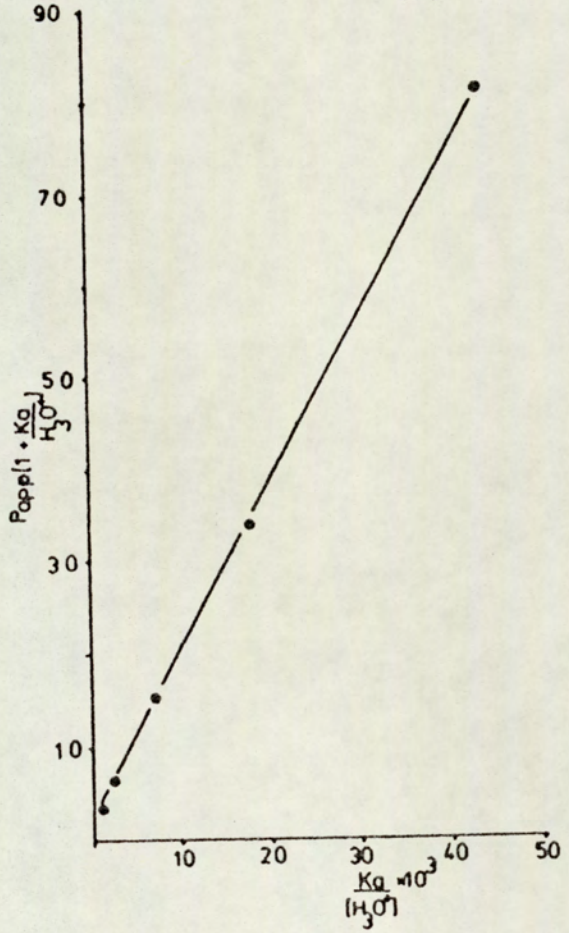


Fig 4.6.b The linear relationship of equation 4.8 for the partition of propranolol in Octanol/buffer at different pHs at 37°C.

$$P_{app}\left(1 + \frac{Ka}{[H_3O^+]}\right) = P_i + P_u\left(\frac{Ka}{[H_3O+]}\right) \qquad 4.8$$

A plot of $P_{app}\left(1 + \frac{Ka}{[H_3O^+]}\right)$ against $\frac{Ka}{[H_3O^+]}$ will yield a straight line with slope line with slope P_u and intercept P_i.

$P_{app}\left(1 + \frac{Ka}{[H_3O^+]}\right)$	$\frac{Ka}{[H_3O^+]}$ x 10^3
3.304	1.122
6.395	2.344
15.204	6.920
34.136	17.783
81.405	43.651

Such a plot using these data gave a straight line (r = 0.9999) with slope P_u = 1821.44 and intercept P_i = 1.9249 (Fig 4.6.b), values are close to those quoted by Wang and Lien 1980 (107). From these results the P_{app} of propranolol at any pH value can be determined by using the following equation.

$$P_{app} = x\,P_i + (1 - x)\,P_u \qquad 4.9$$

where x is the ionized fraction and (1 - x) the unionized fraction x can be determined by using 4.10 [*Wang and Lien 1980 (107)*].

$$x = \frac{1}{1 + \text{antilog}\,(pH - pKa)} \qquad 4.10$$

4.3.5. DETERMINATION OF THE TRUE PARTITION COEFFICIENT OF O-ACYL PROPRANOLOL DERIVATIVES

The P_{app} of O-acyl propranolols in octanol/buffer at pH 2.56, 3.5 and 4.4 at 25°C has been determined using equation 4.11.

$$Papp = \frac{C_a \cdot V_w}{(C_b - C_a)\, V_o} \qquad 4.11$$

where C_a = the solute concentration in the organic phase after partitioning.

C_b = the solute concentration in the organic phase before partitioning.

V_w and V_o = the volume of the aqueous and organic phases respectively.

The log P_u values were calculated from the P_{app} at various pH values (assuming P_i = 0) using equation 4.12 [*Wang and Lien 1980 (107)*].

$$P_{true} = P_{app} / (1 - x) \qquad 4.12$$

Table 4.6 and Fig 4.7 show the partition coefficient (log P) of the undissociated form of the esters indicating that lipophilicity is improved by esterification of propranolol.

No.	Compound	Log P	Log S_w
I	O-acetylpropranolol	4.505	- 3.17
II	O-propionylpropranolol	4.991	- 3.77
III	O-butyrylpropranolol	5.560	- 4.28
IV	O-valerylpropranolol	6.300	- 4.82
V	O-hexanoylpropranolol	6.850	- 5.27
VI	O-pivaloylpropranolol	6.440	- 5.07
VII	O-octanoylpropranolol	7.870	-

Table 4.6 Octanol-buffer partition coefficients (P) and the aqueuos solubility (S_w) of the free bases of O-acyl propranolols

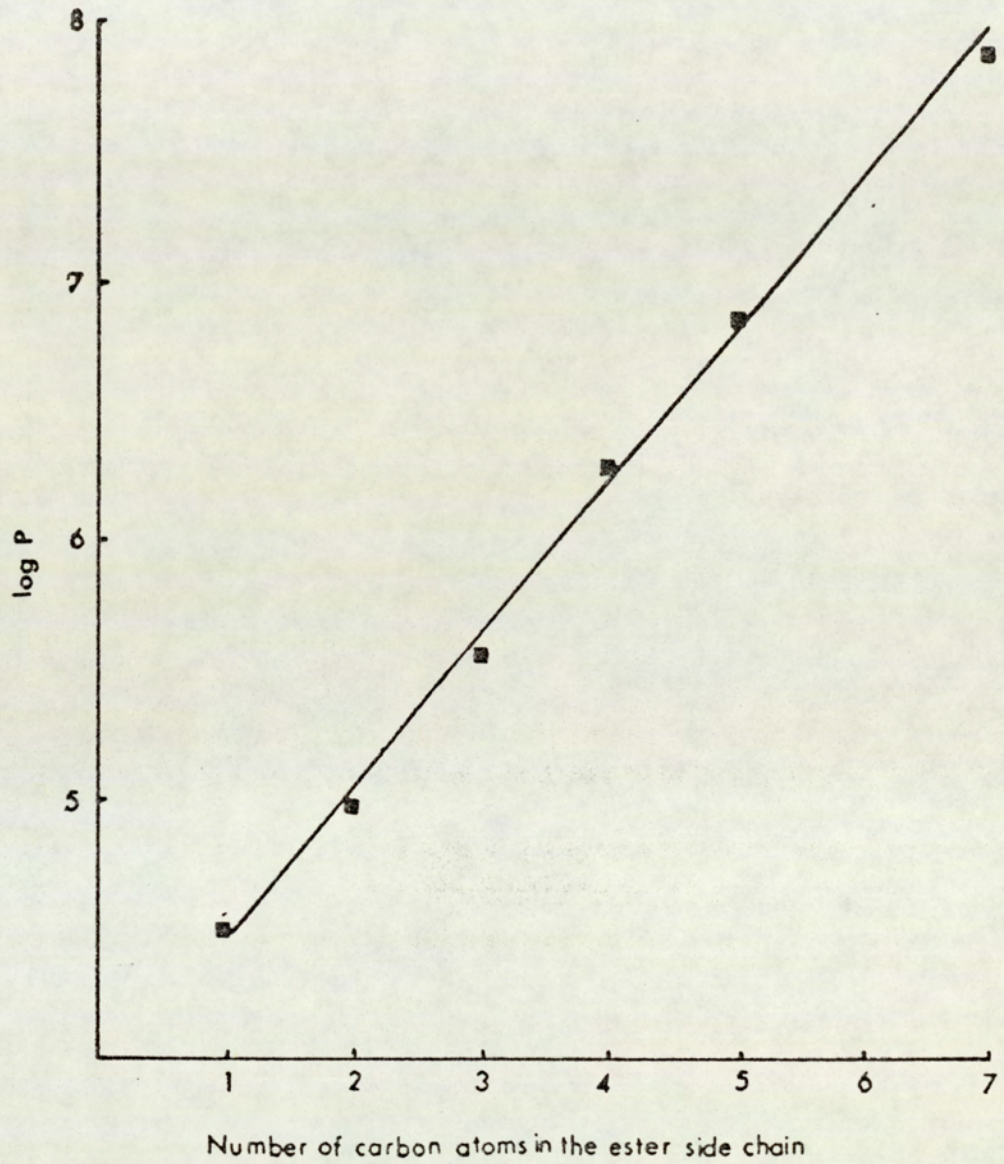


Fig 4.7 True partition coefficients of O-acylpropranolol

Lien 1975 (249) found that optimal logP values resulting in good absorption from the rat intestine were about 2 for several series of drugs. The increase in the lipophilicity may be useful for the formulation of sustained-release preparations containing these esters, by combination with ion-exchange resins.

4.3.6. EFFECT OF ION-PAIRING ON THE Papp OF PROPRANOLOL IN pH 6.5 AT 37°C

The P_{app} of propranolol between phosphate-citrate buffer and octanol phases in pH 6.5 at 37°C with different concentration of ion-pairing (sodium hexanesulphonate) were calculated as before using equation 4.7.

Table 4.7 and Fig 4.8 show the effect of adding sodium hexanesulphonate on the P_{app} of propranolol in pH 6.5 at 37°C. It was found that increasing amounts of ion-pairing agent increased the P_{app} of propranolol. This may be due to the ion-pair formation which increases the lipophilicity of propranolol-hexanesulphonic acid complex.

Sodium Hexanesulphonate Concentration (mM)	Papp
0	3.15
2	5.50
4	8.30
6	10.80
8	13.70
10	16.60

Table 4.7 The P_{app} of Propranolol as a Function of Ion-Pair Concentration

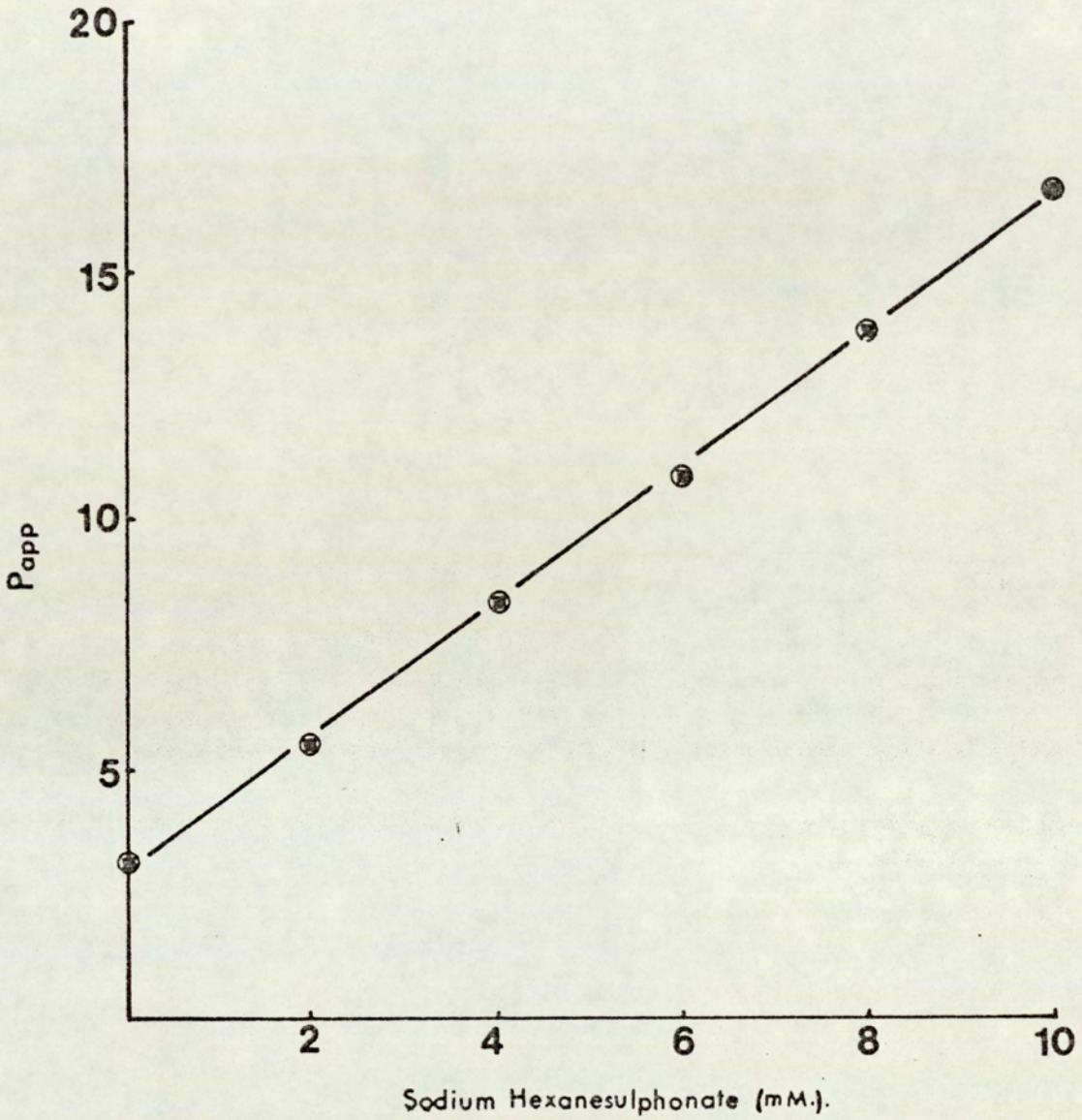


Fig 4.8 Effect of ion-pairing agent on the apparent partition coefficient (Papp) of propranolol in pH 6.5 at 37°C

4.3.7 DETERMINATION OF THE AQUEOUS SOLUBILITY OF THE FREE BASE OF O-ACYL PROPRANOLOLS

The aqueous solubility of the free bases of O-acyl propranolols was determined by the non-logarithmic titration technique. This produced good linearity of plots of $[H_3O^+]$ against Z′ (Fig 4.9) with straight lines with a slope $= -\frac{B_{sol}}{Ka}$ being obtained. the aqueous solubility of the free bases (B_{sol}) may be determined provided that the Ka term for each compound, previously calculated by extrapolation of the aqueous methanol data, is available.

The aqueous solubility of propranolol base calculated by this technique (7.714×10^{-4} moles/litre) was close to those measured in 0.01 M NaOH (8.03×10^{-4} moles/litre) and calculated using equation 4.4 (8.126×10^{-4} moles/litre).

Table 4.8 shows the estimated aqueous solubility of the free bases of O-acyl propranolols together with the statistical parameters of the non-logarithmic titration plot (H_3O^+ vs Z′), where the aqueous solubility of the esters decreases by increasing the number of carbon atoms in the ester side chain. Such a plot of these results are shown in Fig 4.10.

Batches of data for the determination of the solubility of O-acyl propranolols using the non-logarithmic titration method are recorded in Appendix VII. A plot of the true partition coefficient (logP) of O-acyl propranolols against the estimated aqueous solubility ($\log S_w$) showed a linear relationship and data are displayed in Fig 4.11.

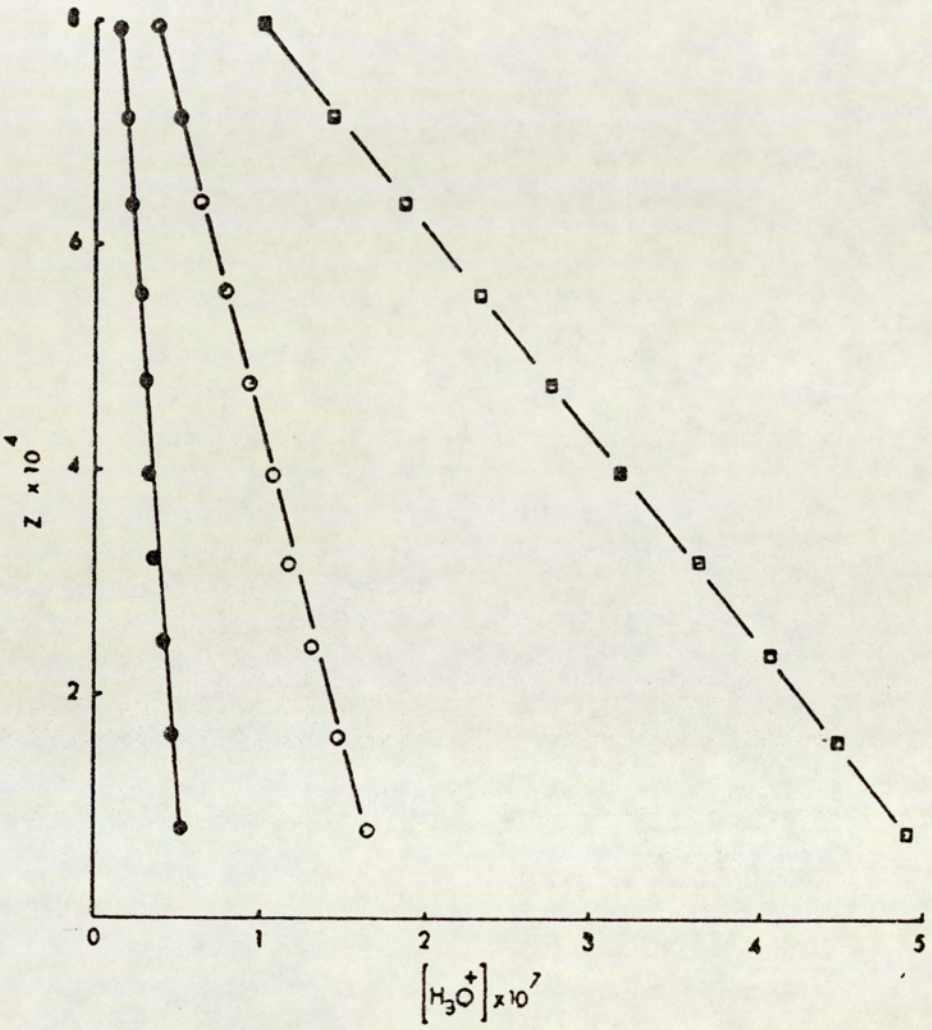


Fig 4.9 Non-logarithmic titration of O-hexanoylpropranolol (□), O-valerylpropranolol (○) and O-butyryl-propranolol (●).

Compound	pKa	Correlation Coefficient (r)*	Slope *	Basic Solubility 10^{-6} Mole/litre x 10^{6}
Propranolol	9.33	0.983	1649270.00	771.40
O-acetylpropranolol	8.52	0.998	225325.00	680.50
O-propionylpropranolol	8.54	0.994	59020.60	170.20
O-butyrylpropranolol	8.57	0.998	19603.10	52.76
O-valerylpropranolol	8.59	0.999	5934.57	15.25
O-hexanoylpropranolol	8.53	1.000	1832.42	5.40
O-pivaloylpropranolol	8.71	0.983	4329.02	8.44

* These parameters calculated using equation (4.1) assuming that the basic solubility for each compound = 1

Table 4.8 Determination of the basic solubility of propranolol HCl and its ester hydrochlorides using pKa.

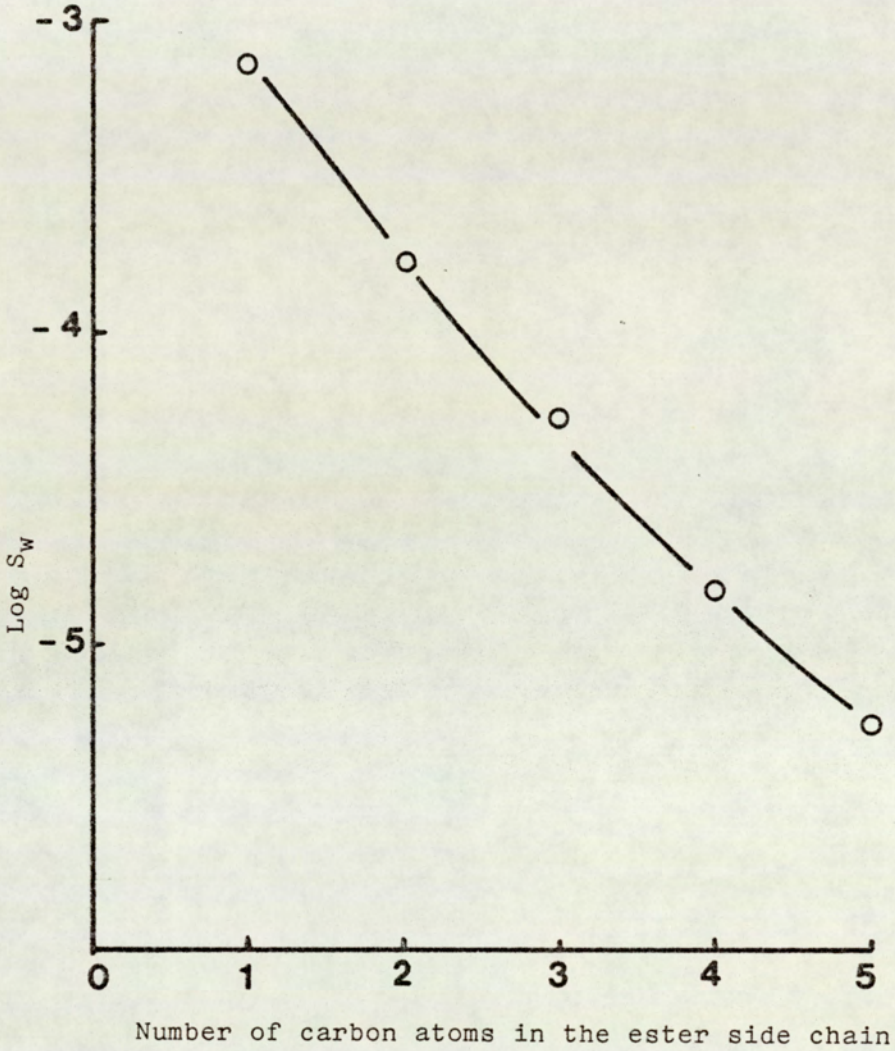


Fig 4.10 Aqueous solubility of the free base of O-acylpropranolol hydrochlorides.

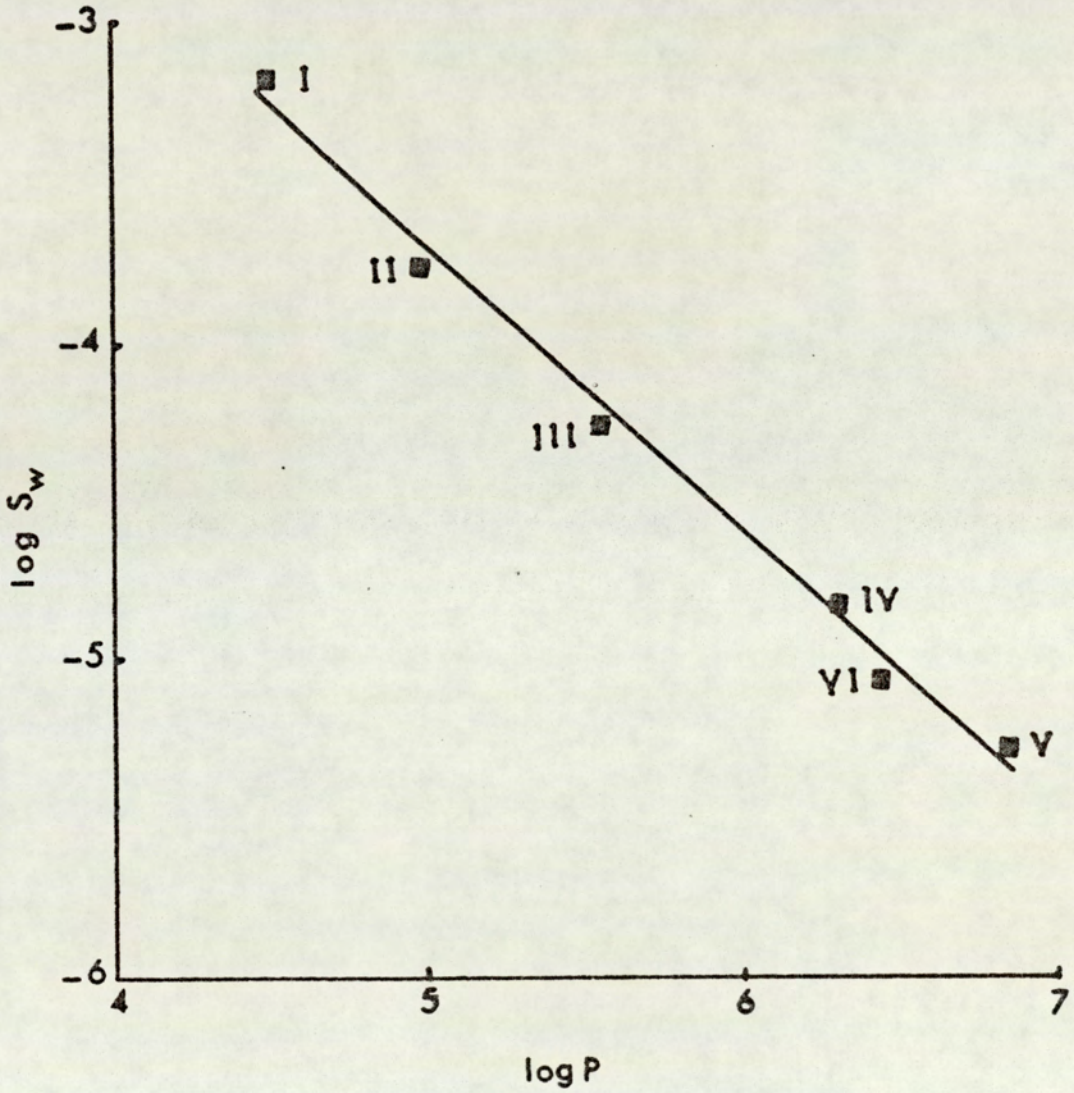


Fig 4.11 Graphical presentation of the aqueous solubility and partition coefficient of O-acyl propranolol bases

Propranolol esters	Acetyl	Propionyl	Butyryl	Valeryl	Hexanoyl	Pivaloyl
No.	I	II	III	IV	V	VI

4.3.8 EFFECT OF DMF AND P.G. CONCENTRATION ON THE SOLUBILITY OF N, O-DIACETYLPROPRANOLOL IN 0.5M BUFFER pH 7.4 AT 25°C

In order to improve the aqueous solubility of N, O-diacetylpropranolol , it is necessary to find a means of increasing its aqueous solubility. One of the most fruitful means is to use one or more solubilizing agentssuch as a co-solvent (e.g. DMF and P.G). This may often provide an effective means of increasing aqueous solubility. Literature data indicate that the solubility of many drugs in such binary aqueous systems can be represented by the following equation [*Yalkowsky et al 1972 (258) and Yalkowsky and Rubina 1985 (259)*].

$$S_f = S_w \cdot e^{af} \qquad 4.13$$

or written in logarithmic form, equation 4.13 becomes

$$\log S_f = \log S_w + af \qquad 4.14$$

where S_f is the solubility of the solute in a binary aqueous system consisting of volume fraction f of non-aqueous co-solvent and S_w is the solubility in water. The value of the constant a is characteristic of the system under study.

The solubilization curves for DMF-buffer and P.G-buffer systems are given in Fig 4.12, where a direct but non-linear relationship between the solubility and the proportion of the co-solvent in the solvent mixtures is demonstrated.

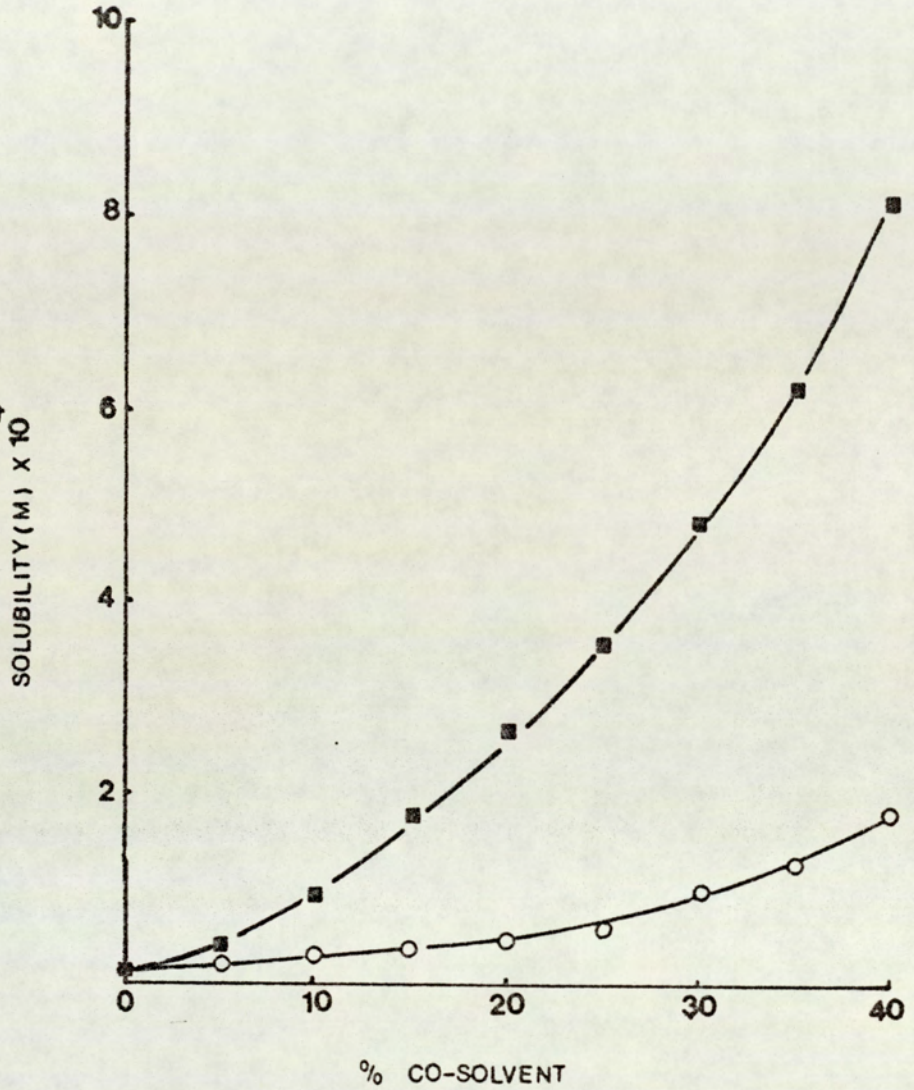


Fig 4.12 Solubility of N,O-diacetylpropranolol in DMF–buffer mixtures (■) and in P.G–buffer mixtures (O).

% Co-solvent	Concentration of N, O-diacetylpropranolol ($M \times 10^4$)	
	DMF	P.G.
0	0.14	0.14
5	0.46	0.20
10	0.93	0.29
15	1.81	0.35
20	2.65	0.44
25	3.53	0.61
30	4.78	0.93
35	6.18	1.25
40	8.16	1.81

Table 4.9 Effect of DMF and P.G. concentration on the solubility of N,O-diacetylpropranolol in 0.5 M Buffer pH 7.4 at 25°C

Table 4.9 shows the effect of the co-solvent systems on the solubility of N, O-diacetylpropranolol in 0.5M buffer at 25°C where the solubility at 40% DMF was found to be four-times greater than that at 40% P.G.

Fig 4.13 shows a roughly linear relationship between log S_f and f. These experiments also provides a means of estimating to what extent a particular drug can be solubilized and how much co-solvent would be requried to accomplish a particular degree of solubilization.

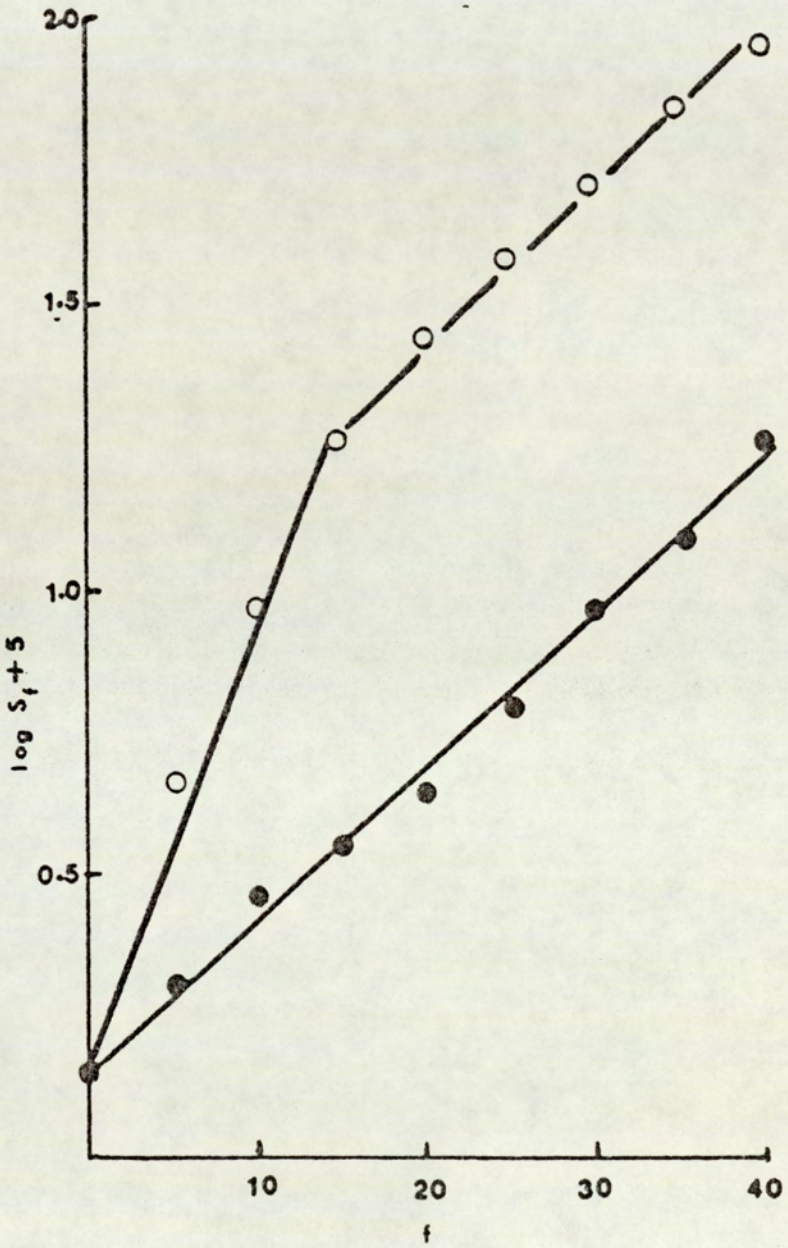


Fig. 4.13 Solubility of N,O,diacetylpropranolol in propylene glyco - buffer mixture (-●-) and in dimethylformamide - buffer mixture (-O-).

CHAPTER FIVE

CHEMICAL AND ENZYMATIC DEGRADATIONS OF PROPRANOLOL PRO-DRUGS

5. CHEMICAL AND ENZYMATIC DEGRADATIONS OF PROPRANOLOL PRO-DRUGS.

5.1 INTRODUCTION

Rate processes are of fundamental importance in pharmacy. The manufacturer must clearly demonstrate that his products are sufficiently stable during storage for a reasonable period of time without changing to an inactive or toxic form; the pharmacist must assess the potential instability of the drugs which he handles and physician and patient must be assured that the prescribed drug will eventually reach the site of action in sufficient concentration to exert the desired effect.

The prediction of the stability of drugs and pharmaceutical preparations may be based upon data obtained from accelerated decomposition studies, which depends on the quantitative relations between the degradative rate and the extent variables (concentration, pH, temperature, solvent kind and dielectric constant, ionic strength). Such relationships may aid the prediction of drug stability from a minimum number of experiments. These quantitative relations havebeen reviewed [*Garrett 1962 (260)*].

During the past decade studies on the effect of cationic and anionic surfactants on the decomposition rate have been studied by the hope that a micellar-cataylsed reaction might provide a basic model for interpretation of some aspects of enzymatic catalysis, since micelles can be regarded as a model for enzymes and biomembranes [*Fendler and Fendler 1975 (255), Cordes 1973 (261) and Mittal 1977 (262)*]. The effect of micelles on drug stability has been recently reviewed [*Linda et al 1981 (263)*].

In a prodrug approach, the ability to predict the in-vivo conversion rate of a prodrug to its parent compound is highly desirable in various pharmacokinetic models [*Rogers and Kwan 1979 (264); Stella and Himmelstein 1980 (265) and Notari 1981 (217)*].

Johanssen et al 1983 (266), Lee et al 1983 (267) and Babhair and Hussain 1983 (268) investigated the biological lability of many promoieties and suggest that not only the physico-

chemical properties but also the biological lability is important to control the regeneration of the parent drugs.

The present chapter focuses on a number of the factors which bear upon the proper formulation, stabilization and administration of the prodrugs. Concentration, temperature and catalysts are important in relation to the speed and the mechanisms of the reactions and will be discussed in turn.

5.2 EXPERIMENTAL

5.2.1 CHEMICAL DEGRADATION OF PROPRANOLOL PRODRUGS

5.2.1.1. PREPARATION OF THE REACTION MEDIUM

(a) Preparation of buffer solutions at different pH values

The buffer solutions used in this investigation were prepared from Britton-Robinson buffer solutions (AppendixVIII) at various pH values by mixing together suitable amounts of sodium hydroxide, citric acid monohydrate, boric acid, acetic acid, and orthophosphoric acid, the ionic strengths were adjusted to 0.5 with potassium chloride.

(b) Preparation of the buffered-DMF solutions at different pH values

50% buffered-DMF solutions at different pH values were used to study the influence of pH and temperature on the degradation rate constants of O-acyl propranolols to ensure complete solubilization of the esters and the degradative products during the reaction. The solutions were prepared by mixing together equal volumes of DMF and 0.25M Britton-Robinson buffers at different pHs. Buffer solutions of this strength (0.25M) were used to avoid precipitation of the buffer salts after addition of the DMF. As expected, addition of DMF to the aqueous diluted buffer solutions produced a large change in the pH. The pH values were readjusted with orthophosphoric acid.

The pH values quoted in the stability studies were the final values after addition of DMF to the buffer solutions at the experimental temperature, Measurements were made after calibration of the pH meter (Radiometer PHM64 pH meter) with the standard buffers at the experimental temperature.

5.2.1.2 PREPARATION OF THE STANDARD SOLUTIONS

All standard solutions for the stability studies were prepared with the ester concentration equivalent to that of the experimental solution (0.1- 1mM) together with an equivalent amount of propranolol in the same solvent. The pH was lowered to pH 3.5 to avoid degradation of the propranolol esters in the standard solutions. There was no need to prepare standard solutions at different pH values because it was shown experimentally that there was no effect on the peak height of propranolol and its prodrugs by changing the sample solvent pH. This was due to the reduction in the pH of the sample solvent on addition of the internal standard which was prepared in 0.1 M HCl. The standard solutions were diluted using the same solvent to provide a concentration range of 10 - 100% of the analytes for each experiment (assuming that the 100% equivalent to the initial concentration of the ester in the reaction mixture . 2ml of the standard solution and 2ml of the internal standard (7 mg ethylparaben in 100 ml 0.1M HCl in most cases) were mixed in a test tube using a whirlimixer. A 20 µl aliquot was injected onto the HPLC column (10cm x 4.6 mm.i.d , Hypersil-ODS 5µm) and eluted with the suitable mobile phase for each experiment (Table 3.8) at a flow rate of 1ml/min and a detection wavelength 290 nm. Calibration curves were constructed by plotting the peak height ratios against concentrations.

5.2.1.3. DEGRADATION OF O-ACYL PROPRANOLOLS IN BUFFERED-DMF pH10 at 37 °C

Stock solutions of O-acyl propranolols (acetyl, propionyl, butyryl, valeryl and hexanoyl) of 50 mM each were prepared separately in 100 ml DMF. 1ml of each stock solution was separately measured into a 100 ml three-necked round bottom flask and the volume was made to 100 ml by addition of 99 ml of 50% buffered-DMF solution pH 10 which had been preheated to 37°C, the temperature at which the particular experiment was to be conducted. The final pH of the mixture was measured immediately at that temperature. Losses due to evaporation were minimized by attaching a condenser to one of the necks (Fig 5.1.a). The flask was placed in a constant temperature water bath (5B-15 TECAM) that had been previously adjusted to 37°C ± 0.1. The mixture was stirred by means of a magnetic bar driven with an immersed magnetic stirrer (RANK BROTHERS). A 1 ml sample was withdrawn immediately after mixing (C_o). Samples (≈ 2 ml) were then withdrawn at appropriate intervals (5 to 60 minutes) by means of a 5 ml glass syringe using a 4 cm needle extended by a Teflon tube and fitted to the reaction vessel with a Quick-fit adaptor which was completely sealed by Teflon tape. The samples were immediately stored at -10°C in a dry-ice bath to quench the reaction. These conditions had previously been shown to inhibit further reactions . One ml of the sample (after melting at 20°C) and 1 ml of the acidic solution (pH 1.6) of the internal standard (7-14 mg ethyl paraben in 100 ml 0.1M HCl in most cases) were mixed together in a test tube. Twenty µl was injected onto the column and was eluted with the appropriate mobile phase (Table 3.8) at a flow rate of 1ml/min. The analytes were detected at 290 nm.

The concentrations of the esters and propranolol were determined from the calibration curve for each experiment by a linear regression analysis using an electronic calculator (CASIO fxP-180P) and the concentration of the formed amides were calculated by mass balance.

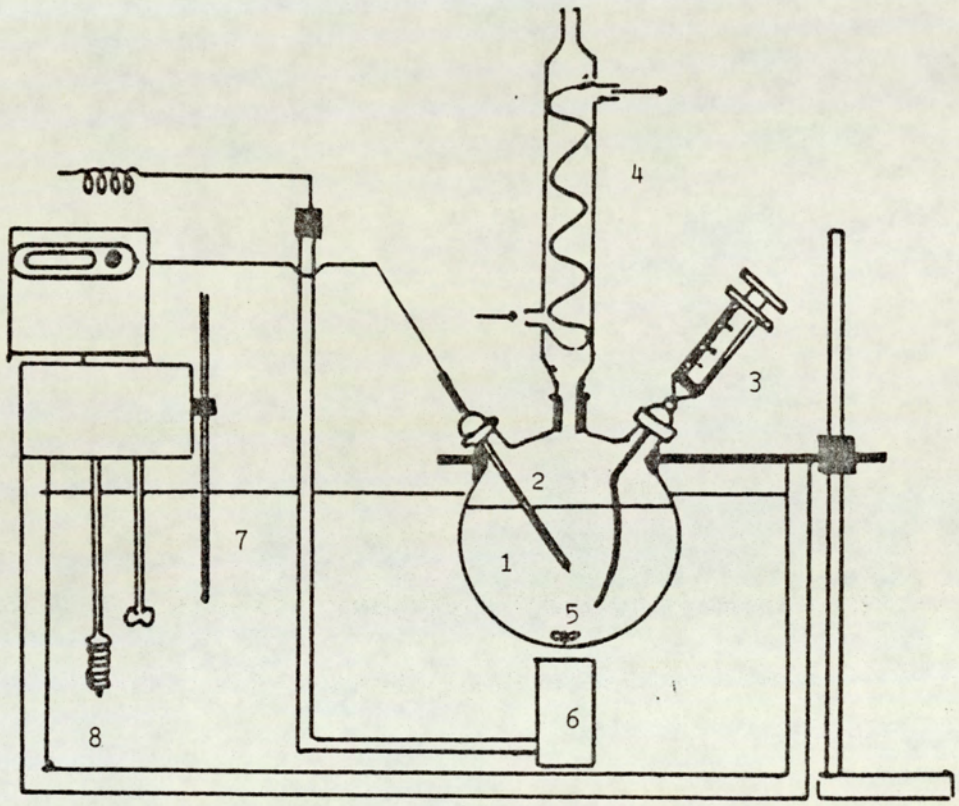


Fig 5.1 (a) Block diagram of the equipment used for the stability kinetics studies.

(1) Three necked round bottom flask

(2) Thermometer probe

(3) Glass syringe for sampling

(4) Condenser

(5) Magnet

(6) Electric magnetic stirrer

(7) Thermometer

(8) Thermostatic, circulating water bath

5.2.1.4 EFFECT OF pH ON THE DEGRADATION RATE CONSTANTS OF O-ACYL PROPRANOLOLS IN BUFFERED-DMF AT 80 °C

Accurately weighed quantities of O-acetylpropranolol (40 mg) and O-hexanoylpropranolol (10 mg) were added separately to the reaction vessels (Fig 5.1.a) and dissolved in 100 ml of 50% buffered-DMF at different pH values (2-9.45 in case of O-acetylpropranolol experiments and 2.5 - 8.8 in case of O-hexanoylpropranolol experiments) which had been preheated to 80°C. All the pH values quoted above were the final pH values after addition of the buffered-DMF solutions at 80°C at which the particular experiment was to be conducted. The initial concentrations of the esters were 1.184 mM for the acetyl and 0.255 mM for the hexanoyl. Samples (2ml) were withdrawn immediately (C_o) and at various intervals (5-10 minutes) in case of the high pH experiments and 1 - 6 hours in case of the lower pH ones). The rest of the procedure was performed as described in Section 5.2.1.3.

5.2.1.5 EFFECT OF TEMPERATURE ON THE DEGRADATION RATE CONSTANTS OF O-ACYL PROPRANOLOLS

Accurately weighed amounts of O-acetylpropranolol (40 mg) and O-hexanoylpropranolol (10 mg) were individually delivered to the reaction vessels and dissolved in 100 ml of 50% buffered-DMF at different pH values (pH 9.45 in case of O-acetylpropranolol experiments and pH 10.7 in case of O-hexanoylpropranolol ones) which had been thermally equilibrated at the temperature at which the particular experiment was to be conducted. The initial concentrations of the acetyl and hexanoyl esters in the reaction mixture were 1.184 mM and 0.254 mM in 100 ml, respectively. Reactions were carried out separately under isothermal conditions (40, 50, 60, 70 and 80°C). Samples were withdrawn immediately and at various intervals (5-10 minutes) and analysed as described earlier in Section 5.2.1.4.

5.2.1.6 EFFECT OF INITIAL CONCENTRATION OF THE REACTANT ON THE DEGRADATION RATE CONSTANTS

10, 20 and 50 mM stock solutions of O-acetylpropranolol in 10 ml neutralized-DMF were prepared. 1ml of each of the stock solutions were added separately to the reaction vessel and diluted to 100 ml with preheated Britton-Robinson buffer, pH 7.4, at 37°C. Reactions were carried out at 37°C with continuous stirring. The initial concentrations of the reactant were 0.1, 0.2 and 0.5 mM.

Aliquots (2ml) of the samples were withdrawn immediately and at various intervals (5-15 minutes), the samples were diluted 1:1 with 2ml of the internal standard solution (suitable concentration of ethylparaben in 0.1 M HCl) and 20 µl aliquots were injected onto the HPLC column. Elution was by means of a mobile phase consisting of acetonitrile, orthophosphoric acid (88%), diethylamine and water (65: 0.1: 0.15: 34.75 v/v/v/v), pH 2.6, delivered at 1ml/min with 290 nm as the analytical wavelength.

5.2.1.7 EFFECT OF BUFFER CONCENTRATION ON THE STABILITY OF O-ACETYLPROPRANOLOL IN pH 7.4 at 37 °C

A stock of a 1M Britton-Robinson buffer solution pH 7.4, ionic strength 0.5 was prepared according to the table shown in Appendix VIII. The ionic strength was kept constant and the concentration of the buffer was altered by dilution with distilled water while the ratio of the buffer salts was kept constant to maintain the pH. The final buffer concentrations ranged from 25 - 100% of the initial value. 1 ml aliquots of 50 mM O-acetylpropranolol stock solution, prepared by dissolving 168.9 mg of O-acetylpropranolol in 10 ml acetonitrile, were separately delivered to the reaction vessels containing various buffer concentrations (99 ml, 0.5 - 1M) to provide a reaction mixtures (0.5 mM, 100ml). Reactions were carried out at 37°C as described in Section 5.2.1.6.

5.2.1.8 EFFECT OF DMF CONCENTRATION ON THE STABILITY OF O-ACETYL-PROPRANOLOL IN pH 7.4 AT 37 °C

Aliquots of stock solution of O-acetylpropranolol in acetonitrile (1 ml, 50 mM) were delivered individually to the reaction vessels containing various concentrations of buffered-DMF (99 ml; 0 - 40% v/v) pH 7.4 (the final pH was adjusted to 7.4 with orthophosphoric acid) to prepare reaction mixtures (100 ml, 0.5 mM). Reactions were carried out at 37°C as described in Section 5.2.1.6. Samples were analysed by HPLC.

5.2.1.9 EFFECT OF PROPYLENE GLYCOL CONCENTRATION ON THE STABILITY OF O-ACETYLPROPRANOLOL IN pH 7.4 AT 37 °C

A 50 mM stock solution of O-acetylpropranolol in acetonitrile was prepared as described previously in Section 5.2.1.7. A range of 0 - 40% v/v propylene glycol (P.G.) in Britton-Robinson buffer solution pH 7.4, ionic strength 0.5 were also prepared. Aliquots of the ester stock solution (1 ml, 50 mM) were individually diluted to 100 ml with various concentrations of buffered -P.G. (0-40%) pH 7.4 at 37°C to provide a final ester concentration of 0.5 mM, 100 ml. Reactions were carried out at 37°C as described in Section 5.2.1.6.

5.2.1.10 EFFECT OF POLYETHYLENE GLYCOL 1000, CONCENTRATION ON O-ACETYLPROPRANOLOL DEGRADATION RATE AT pH 7.4 AT 37 °C

A concentration range (0 - 3% w/v) of polyethylene glycol (P.E.G.) 1000 in Britton-Robinson buffer solution pH 7.4, ionic strength 0.5 was prepared by weighing a suitable amount of P.E.G. 1000 (0 - 15g) and dissolving it in 500 ml buffer by stirring at room temperature for 2 hours. The viscosity of each mixture was measured on a Brookfield viscometer model LV using a spindle No. 2 at 30 rpm. 1 ml aliquots of 50 mM O-acetyl-propranolol in acetonitrile were separately delivered to the reaction vessels containing 99 ml

preheated (37°C) buffered - P.E.G. solutions (0-3% w/v) pH 7.4 to provide reaction mixtures (100 ml, 0.5 mM). Reactions, sampling and analyses were carried out similarly as described in Section 5.2.1.6.

5.2.1.11 EFFECT OF P.E.G. MOLECULAR WEIGHT ON THE DEGRADATION OF O-ACETYLPROPRANOLOL IN pH 7.4 AT 37 °C

2% w/v of PEG at various molecular weights (1000, 4000, 6000 and 20,000) in Britton-Robinson buffer solution pH 7.4, ionic strength 0.5 were prepared and their viscosities were measured on a Brookfield viscometer as described earlier in Section 5.2.1.11. Aliquots of stock solution of the ester (1 ml, 50 mM) were delivered individually to the reaction vessels and diluted to 100 ml with preheated buffered - P.E.G (with different M.Wt.), pH 7.4 to provide an ester concentration of 0.5 mM. Reactions sampling and analyses were carried out as before (Section 5.2.1.6).

5.2.1.12 EFFECT OF POLYVINYLPYRROLIDONE (PVP) WITH DIFFERENT MOLECULAR WEIGHTS ON THE DEGRADATION OF O-ACETYLPRO-PRANOLOL IN pH 7.4 AT 37 °C

2% w/v of PVP of various molecular weights (10,000; 40,000; 360,000 and 700,000) in 50% Britton-Robinson buffer solution pH 7.4, ionic strength 0.5, were prepared and their viscosities were measured as described earlier for P.E.G. (Section 5.2.1.11). The rest of the procedure was completed the same way as described in Section 5.2.1.6.

5.2.1.13 pH DEPENDENCE OF THE HYDROLYSIS OF O-PIVALOYLPRO-PRANOLOL IN BUFFERED-DMF AT 80 °C

Accurately weighed amounts of O-pivaloylpropranolol (19 mg) were added separately to the reaction vessels and dissolved in 100 ml of 50% buffered-DMF pH 9.6 which had been

thermally equilibrated at 80°C, to prepare reaction mixtures (100 ml) with initial reactant concentrations of 0.5 mM.

Reactions were carried out at 80°C as described earlier in Section 5.2.1.4. Samples were analysed by HPLC using the mobile phase shown in Table 3.8 and 8mg ethyl paraben in 100 ml 0.1 M HCl as the internal standard.

5.2.1.14 TEMPERATURE DEPENDENCE OF THE HYDROLYSIS OF O-PIVALOYL PROPRANOLOL IN BUFFERED-DMF pH 9.6

a) Non-isothermal Degradation

The reaction medium 199 ml of 50% buffered -DMF solution pH 9.6 was thermally equilibrated at 25°C in a thermostatic water bath (Fig 5.1.a) in which the heater was capable of increasing the temperature to 80°C in about 2 hours.

1 ml of neutralized-DMF containing 38 mg O-pivaloylpropranolol was delivered to the reaction vessel and immediately the heater was turned on. The solution was stirred continuously by a magnetic bar driven with an immersable magnetic stirrer.

Aliquots of the samples (3ml) were withdrawn immediately and at intervals of 2 - 5 minutes over a period of about 2 hours. Samples were stored for analysis in a dry-ice bath (-10°C). Both the reaction solution and the water bath temperatures were monitored during the run and it was found that a constant difference of about 1 degree was set up during the run. Only the test solution temperature was used in the calculation.

2ml of the test solution (after melting at 20°C) was diluted with 2ml of the internal standard (8 mg ethylparaben in 100 ml 0.1 M HCl). 20 µl aliquot was injected onto the column and this was eluted with a mobile phase consisting of acetonitrile, orthophosphoric acid (88%), diethylamine and water (65: 0.1: 0.2: 34.7 v/v/v/v) pH 2.7 at 1ml/min with a detection wavelength of 290 nm.

b) Iso-thermal Degradation

The hydrolysis of O-pivaloylpropranolol was conducted using the same reaction mixture (200ml, 0.5 mM) described earlier in the non-isothermal degradation experiment but various isothermal conditions (40, 50, 60 70 and 80°C) were chosen. Samples were withdrawn immediately and at various intervals (5 - 10 minutes) and were analysed as described earlier for the non-isothermal experiment.

5.2.1.15 EFFECT OF DODECYLTRIMETHYLAMMONIUM BROMIDE (D.T.A.B) CONCENTRATION ON THE DEGRADATION OF O-ACETYL AND O-HEXANOYL PROPRANOLOL

Stock solutions of 40 mM O-acetylpropranolol and O-hexanoylpropranolol were prepared separately in acetonitrile by dissolving 135.2 mg O-acetylpropranolol and 157.6 mg O-hexanoylpropranolol each in 10 ml acetonitrile. Aliquots of stock solutions (1 ml, 40 mM) of O-acetylpropranolol and O-hexanoylpropranolol were delivered individually to the reaction vessels containing various concentrations of D.T.A.B. below and above the critical micelle concentration (CMC) 1.42×10^{2}M in Britton-Robinson buffer solution pH 7.4 (199 ml, $0.52 - 1.82 \times 10^{-2}$M).

Buffers were previously thermally equilibrated at 37°C to prepare reaction mixtures (200 ml) with an ester concentration of 0.2 mM. Reactions were carried out at 37°C. Aliquots (2ml) were withdrawn from each reaction vessel immediately and at various intervals of 10 - 30 minutes depending upon the reaction rates. The samples (2ml) were treated with 2 ml of the acidified internal standard (7mg methyl paraben in 250 ml 0.1 M HCl for O-acetylpropranolol experiments and 10 mg butyl paraben in 250 ml 0.1 M HCl for the hexanoyl ester experiments). Samples were analysed by HPLC using mobile phases consisting of acetonitrile, orthophosphoric acid (88%) and water (42: 2: 56 v/v/v , pH 1.7 for O-acetylpropranolol experiments and 55 : 2.5 : 42.5 v/v/v , pH1.7 for O-hexanoylpropranolol experiments).

5.2.1.16 EFFECT OF TETRAMETHYLAMMONIUM CHLORIDE CONCENTRATION ON THE DEGRADATION OF O-ACETYLPROPRANOLOL

The same procedure described in Section 5.2.1.15 was followed, except the reaction media here were of various concentrations of tetramethylammonium chloride (TAC) in buffer pH 7.4 ($1.7 - 10 \times 10^{-2}$ M). Samples were analysed by HPLC using an eluent solution and internal standard as described earlier in Section 5.2.1.5.

5.2.1.17 EFFECT OF SODIUM LAURYL SULPHATE (SLS) CONCENTRATION ON THE DEGRADATION OF O-ACETYLPROPRANOLOL

Aliquots of the ester stock solution (1ml, 40 mM) in acetonitrile were individually delivered to the reaction vessels containing preheated (37°C) solutions of SLS at various concentrations above and below the CMC (8.5×10^{-3}M) in Britton-Robinson buffer solution pH 7.4 (199 ml, $0 - 17.5 \times 10^{-3}$M). Reaction mixtures (200 ml) of 0.2 mM initial concentration were thus obtained. Reactions were carried out at 37°C as described in Section 5.2.1.15.Samples were analysed by HPLC using a mobile phase consisting of MeCN:H_3PO_4:H_2O (48: 2.5: 49.5) pH 1.7 and internal standard of 15 mg ethylparaben in 500 ml 0.1M HCl.

5.2.1.18 EFFECT OF TWEEN 80 CONCENTRATION ON THE STABILITY OF O-ACETYLPROPRANOLOL

Reaction mixtures were prepared as described earlier in Section 5.2.1.17, except that the reaction media consisted of various concentrations of Tween 80 (CMC = 0.01% w/v) in buffer pH 7.4 (0 - 0.3% w/v). Reactions were carried out at 37°C and the samples were analysed by HPLC using an eluent solution consisting of MeCN: H_3PO_4: H_2O (45: 3: 52 v/v/v) pH 1.6 and internal standard of 17.5 mg propylparaben in 200 ml 0.1 M HCl.

5.2.2. ENZYMATIC DEGRADATION OF PROPRANOLOL PRODRUGS

5.2.2.1. DEGRADATION OF O-ACYL PROPRANOLOLS WITH ISOLATED ESTERASE

The studies of the degradation of O-acyl propranolols (acetyl, propionyl, butyryl, valeryl, hexanoyl, octanoyl and decanoyl) were carried out in 10% DMF in McIlvaine buffer, pH 7.4, ionic strength 0.5, at 37°C in the presence of a fixed amount of the active esterase enzyme. 1 ml esterase enzyme in phosphate buffer was purchased from Sigma (10 mg containing 120 units/mg) was diluted to 25 ml with McIlvaine buffer, pH 7.4, and stored in the fridge at -4 °C until used (within 30 hours for all experiments)

Stock solutions of 20 mM of the esters in 10 ml neutralized DMF were separately prepared. 1 ml aliquots from the esters stock solutions were accurately and individually delivered to the reaction vessels containing 99 ml of 9% buffered-DMF pH 7.4 which had been thermally equilibrated at 37°C. 1 ml samples were withdrawn immediately after mixing from each reaction vessel and replaced with 1 ml of the enzyme stock solution to prepare reaction mixtures (100 ml) with an ester concentration of 0.2 mM and 48 units of esterase activity. Reactions were carried out at 37°C. At appropriate time intervals (2 - 10 minutes) 2 ml samples were withdrawn from each reaction vessel and treated with 2 ml of the internal standard (ethylparaben in most cases), which was prepared in a mixture of acetonitrile and 0.1 M HCl (1:1) to deactivate the enzyme and to quench the degradation process. Samples were stored in a salt-ice bath until analysed.Analyses were carried out using HPLC with the mobile phase for O-acylpropranolols listed in Table 3.8.

5.2.2.2 DEGRADATION OF O-ACYL PROPRANOLOLS IN SERUM

a) Preparation of the Serum

A 250 ml sample of blood was collected from white New Zealand rabbits, the blood was left on the bench for about one hour to clot and then it was centrifuged at 4000 rpm at 4°C for 20 minutes. Serum layers were separated and divided into 9 ml portions in small vials which were sealed and stored frozen at -30°C until used.

b) Experimental Procedure

Stock solutions containing 2 mM of each propranolol ester (acetyl, propionoyl, butyryl, valeryl, hexanoyl and octanoyl) were prepared separately in 10 ml of neutralized DMF.

1 ml of the ester stock solutions (one ester each time) was added to the small vial containing 9 ml of serum, which was thermally equilibrated at 37°C in a thermostatic water bath. The vial was sealed and the mixture was stirred with a small magnetic bar driven with an immersible magnetic stirrer. The initial concentration of the reactant was 0.2 mM. Aliquots (0.5 ml) of the samples were withdrawn immediately and at various intervals (2 - 15 min) depending upon the degradation rate of each ester. The degradation processes were instantly quenched by adding the sample (0.5 ml) to a 2 ml of acetonitrile: 0.1 M HCl mixture (1:1). The mixture was mixed in a conical glass centrifuge tube using whirlimixer and centrifuged at 4000 rpm for 20 minutes. 1 ml of the supernatant layer and 1 ml of the internal standard (13 mg ethylparaben in most cases prepared in 200 ml of the mobile phase) were mixed together and stored in salt-ice bath until analysed by HPLC as described earlier in Section 5.2.2.1.

5.2.2.3 DEGRADATION OF O-ACETYLPROPRANOLOL BY RAT SMALL INTESTINE

Four male Wistar rats of average weight 370 grams were fasted overnight prior to the experiments but were allowed free access to water at all times.

Ten centimetre segments of everted small intestine were prepared as described by Crane and Wilson 1958 (269) and Levy et al 1967 (270) but with some modifications. The rats were anaesthetized with Halothane. Segments of intestine starting at the duodenum were stripped from the mesentery, rinsed and maintained as fast as possible in cold, oxygenated Krebs bicarbonate solution, pH 7.4.

10 centimetre segments were measured (when stretched slightly by attaching a small weight and suspending the segment vertically), the average weight of the segments was about 0.37 g, the segments were then sleeved on a glass rod and everted carefully.

Individual segments were placed in 100 ml capacity stoppered conical flasks containing 100ml of 1mM O-acetylpropranolol in 10% DMF in Krebs-bicarbonate solution of final pH = 7.4 which had been thermally equilibrated at 37 ^{o}C (the composition and method of preparation of krebs-bicarbonate solution are listed in Appendix IX. Ca Cl_2 and Mg SO_4 were omitted due to the formation of precipitates, these salts were also excluded from the solution used in the corresponding control experiments).

Other segments of everted small intestine were first immersed in boiling water for 5 minutes and placed in 1 mM O-acetylpropranolol solution (control). Other flasks with the drug solution but without intestine segments were also prepared.

All flasks were flushed with 95% oxygen and 5% carbon dioxide mixture, sealed with Quick-fit stoppers and transferred into a shaking water bath at 37^{o}C with medium shaking speed.Aliquots (2ml) were withdrawn immediately and at various intervals of 5 to 15 minutes. The degradation reaction was quenched by adding the sample to the internal standard (2ml, 12 mg ethylparaben in 200 ml acetonitrile and 0,1 M HCl mixture (1:1). Samples were analysed by HPLC as described in Section 5.2.1.6.

5.2.2.4 IN-VITRO METHOD FOR THE STUDY OF THE DEGRADATION OF O-ACYL PROPRANOLOLS DURING ABSORPTION

The degradation of O-acetylpropranolol and O-pivaloylpropranolol during absorption was studied using an everted sac of rat small intestine. The apparatus used in this study was similar to that used by Crane and Wilson 1958 (269) for the study of the rate of intestinal absorption of sugars. This is shown in Fig 5.1.b, and consists of two parts: one is a polyethylene test-tube of 15 ml capacity (c), the other is a one holed rubber stopper (B) through which was inserted a glass cannula (E), the hypodermic needles, (A) and (D), are forced through the rubber stopper with a slight angle and a length of a teflon tubing is attached to needle (D). Intestinal segments (about 10 cm each) were quickly removed from anaesthetized male Wistar rats, washed with Krebs-bicarbonate solution pH 7.4 and turned inside out as described earlier in Section 5.2.2.3. One end of the everted intestine segment was tied and the other end bound securely to the cannula with a thread.

10 ml of each of O-acetylpropranolol and O-pivaloylpropranolol (10 mM each) in 10% DMF in Krebs-bicarbonate solution of final pH of 7.4 were pipetted separately into the polyethylene test tube and were thermally equilibrated at 37°C (Mucosal fluid). 2 ml of 10% DMF in Krebs-bicarbonate solution pH 7.4 was placed into the intestinal sac (Serrosal fluid) through the cannula and then the intestinal was placed into the mucuosal fluid which was maintained at 37°C. The solution was saturated with oxygen-carbon dioxide mixture (95:5) by direct bubbling the O_2 and CO_2 into the mucosal fluid through needle (D) and leaving by way of needle (A).

After 40 minutes, the intestinal sac was removed from the mucosal fluid and was washed from the outside quickly with warm krebs-bicarbonate solution and transferred into another test-tube. This was placed into a water-bath at 37°C and immediately a sample (0.1 ml) was withdrawn and a 20 µl aliquot was injected directly onto the HPLC column. The concentration of the ester in this sample was considered as 100%.

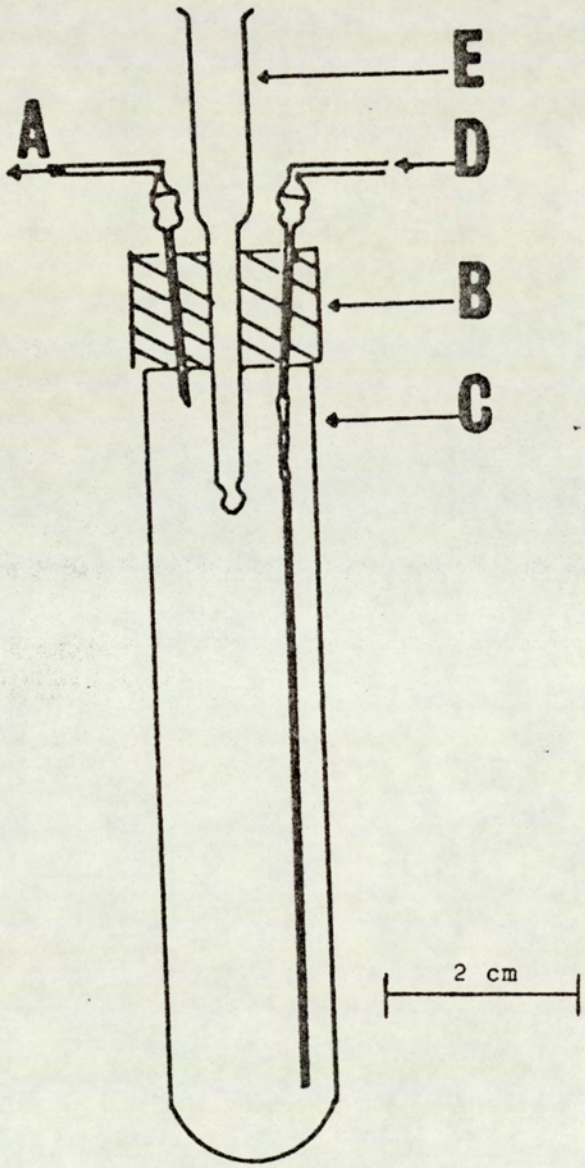


Fig 5.1 (b) Diagrammatic representation of the apparatus used for the study of the degradation of propranolol esters during absorption.

Further samples (0.1 ml) were withdrawn at various intervals of 2 - 5 minutes and 20 µl aliquots were directly injected onto the column to monitor the degradation of the ester in the serrosal compartment.

The concentration of the ester in the mucosal fluid after 40 minutes at 37°C was also considered as 100%. Aliquots (0.5 ml) were withdrawn immediately and at various intervals (2 - 5 min) and treated with 0.5 ml of the internal standard which was prepared in a mixture of acetonitrile and 0.1 M HCl (1:1) to deactivate the enzyme and quench the reaction. Samples were analysed by HPLC as described in Section 5.2.1.4.

5.2.2.5 EFFECT OF THE SUBSTRATE INITIAL CONCENTRATION ON THE ENZYMATIC DEGRADATION OF O-PIVALOYLPROPRANOLOL

A stock solution of 60 mM of O-pivaloylpropranolol in DMF was prepared by dissolving 228 mg of the ester in 10 ml neutralized DMF. Accurately measured volumes of this stock solution 0.125, 0.25, 0.3, 0.4 0.75 and 1 ml were individually placed in 100 ml capacity conical flasks and diluted to 100 ml with preheated 10% buffered-DMF pH 7.4 at 37°C to provide reaction mixtures (100 ml) with ester concentrations of 0.075 - 0.6 mM. 1ml samples were withdrawn from each flask and replaced with 1 ml of the esterase enzyme stock solution (10 ml, 1200 units (Fisons)). The reaction mixtures were transferred to a thermostatic water bath at 37°C and were continuously stirred by means of magnetic bars driven with electric magnetic stirrers. 1 ml aliquots were withdrawn from each flask immediately and at various intervals (10 - 20 min) and diluted with 1 ml of the internal standard (desimipramine) which was prepared in acetonitrile: 0.1M HCl (1:1).

The concentration of the internal standard [2-16 mg of Desimipramine in 100 ml MeCN:0.1M HCl (1:1)] was varied according to the variation of the initial concentration of the reactant. Samples were analysed by HPLC as described in Section 5.2.1.4.

5.2.3 DEGRADATION OF N, O-DIACYL PROPRANOLOLS

N, O-diacetylpropranolol (34.3 mg) and N-ethoxycarbonyl-O-acetylpropranolol (37.3 mg) were delivered separately to the reaction vessel and dissolved in 200 ml of preheated 50% DMF : 0.5M NaOH pH 13, mixture (37°C) to prepare reaction mixtures of 200 ml, 0.5 mM. Reactions were carried out at 37°C. Aliquots (3 ml) of the samples were withdrawn immediately and at various intervals of 0.25 - 50 hours depending on the rates of the reaction. Degradation processes were instantly quenched by pipetting aliquots (2 ml) of the withdrawn samples into an acidic solution of internal standard (2 ml, 9 mg ethyl paraben in 100 ml of 0.1 M HCl) and stored in an ice-bath until analysis.

The HPLC system for analysis for both compounds and their degradative products consisted of acetonitrile, diethylamine, orthophosphoric acid (88%) and water (65: 0.1: 0.1: 34.8%) pH 2.5 and is recorded in Table 3.8. Standard solutions for both experiment were prepared as follows:

(a) For N, O-diacetylpropranolol

Standard solutions of N,O-diacetylpropranolol with N-acetylpropranolol and propranolol (0.5 mM each) as the degradative products were prepared in combination in 50% DMF in 0.5 M HCl.

(b) For N-ethoxy carbonyl-O-acetylpropranolol

Standard solutions of N-ethoxycarbonyl-O-acetylpropranolol with 4-naphthoxy-methyl,N-isopropyloxazolidin-2-one (0.5 mM each) as the degradative product were prepared in combination in 50% DMF in 0.5 M HCl.

The standard solutions were then diluted with the same solvent over a concentration range of 0.05 - 0.5 mM (10 - 100%)of the analytes. Calibration curves were constructed for both experiments by plotting the peak height ratio against concentrations. The concentration of diacyl propranolols remaining and the formed degradation products were calculated from the corresponding calibration curve, except that the concentration of N-ethoxycarbonyl propranolol (the intermediate for the N-ethoxycarbonyl-O-acetylpropranolol experiment) was calculated by mass balance.

5.3 RESULTS AND DISCUSSION

5.3.1. CHEMICAL DEGRADATION OF PROPRANOLOL PRODRUGS

5.3.1.1 DECOMPOSITION PATHWAY OF O-ACYL PROPRANOLOLS

The experimental studies reveal that the degradation of O-acyl propranolols (acetyl, propionyl, butyryl, valeryl, hexanoyl, octanoyl and decanoyl) in buffered-DMF solution pH10 at 37^{o}C was found to proceed via competing reaction involving hydrolysis to give the required propranolol and intramolecular rearrangement to yield the N-acyl derivatives.

The intramolecular rearrangement was confirmed by the following:

(i) High field nmr studies which revealed the existence of a significant prochiral centre at the methylene group of the acyl substituent suggesting strong intra-molecular hydrogen bonding in solution (Fig 2.3.b, Section 2.2).

(ii) Mass-spectrometry studies which displayed an unusual rearrangement and elimination involving the acyl side chain (Fig 2.5, Section 2.2).

(iii) Computer modelling studies, using the CHEM-X program processed on a VAX 11/750 computer system and supported by Tektronix terminals, revealed the possibility of the intramolecular rearrangement by calculating the geometrical distance between the ester carbonyl group and the amino group in the molecule side chain ,which was found less than 1.3 Angstrom units.

The N-acyl derivatives were stable under the conditions of the experiment and reactions did not proceed further. The mechanistic presentation of the degradation of these esters is shown in Fig 5.2. The competitive hydrolytic and rearrangement processes maybe represented by the following kinetic model:

MECHANISM:

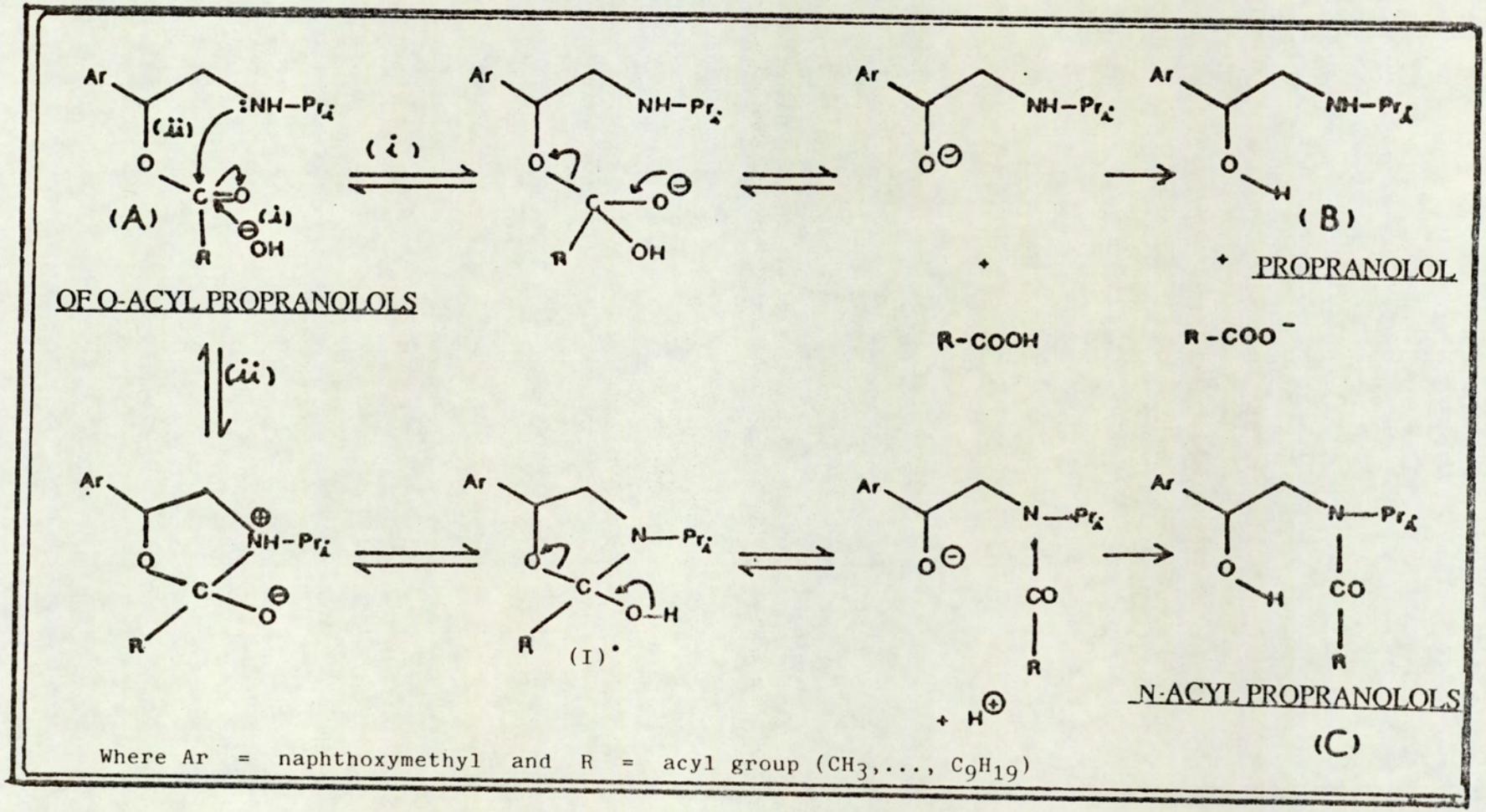
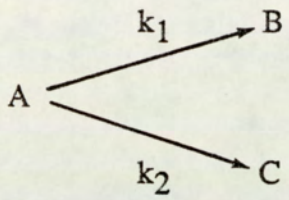


Fig 5.2 Mechanistic model for the degradation of O-acyl propranolols



Where : A - represents the ester

B - propranolol

C - N-acyl propranolol

k_1 & k_2 - hydrolysis and rearrangement rate constants respectively

This model was used to extract rate constants in all cases. The degradation pathway as suggested by the model are:

a) From A⟶B direct hydrolysis

b) From A⟶C intramolecular rearrangement

The mathematical model has been derived to determine the rate constants k_1 and k_2 and is shown in Appendix (X). The sum of the rate constants for the hydrolysis and rearrangement of the esters $(k_1 + k_2)$ was calculated by linear regression and from the graphical presentation of the data to the integrated form of the first-order rate equation (as shown in Fig 5.4.a).

$$A_t = A_o \cdot e^{-(k_1 + k_2)t}$$

or $$\ln A_t = \ln A_o - (k_1 + k_2)\, t \qquad 5.1$$

where A_o = initial concentration of the ester

A_t = concentration of the undecomposed ester at time t

$(k_1 + k_2)$ = overall degradation rate constant of the ester

k_1 = hydrolysis rate constant of the ester

k_2 = rearrangement rate constant of the ester

t = time

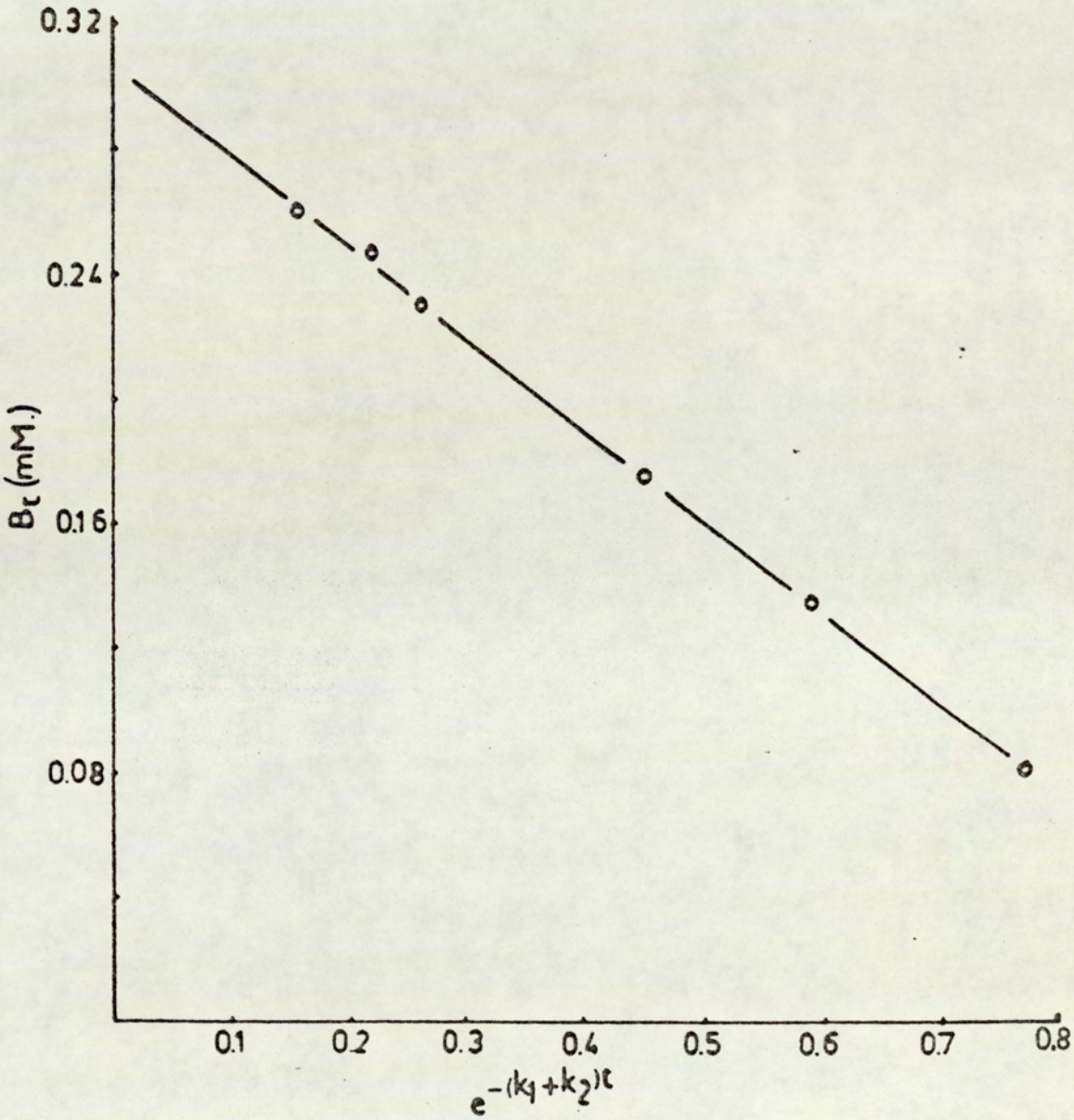


Fig 5.3 A linear plot of B_t against $e^{-(k_1+k_2)t}$

"Data obtained from the experiment of O-hexanoylpropranolol in buffered-DMF solution pH 8.8 at 80°C.
(r = 0.9997, y = 0.2976 and x = 0.3012).

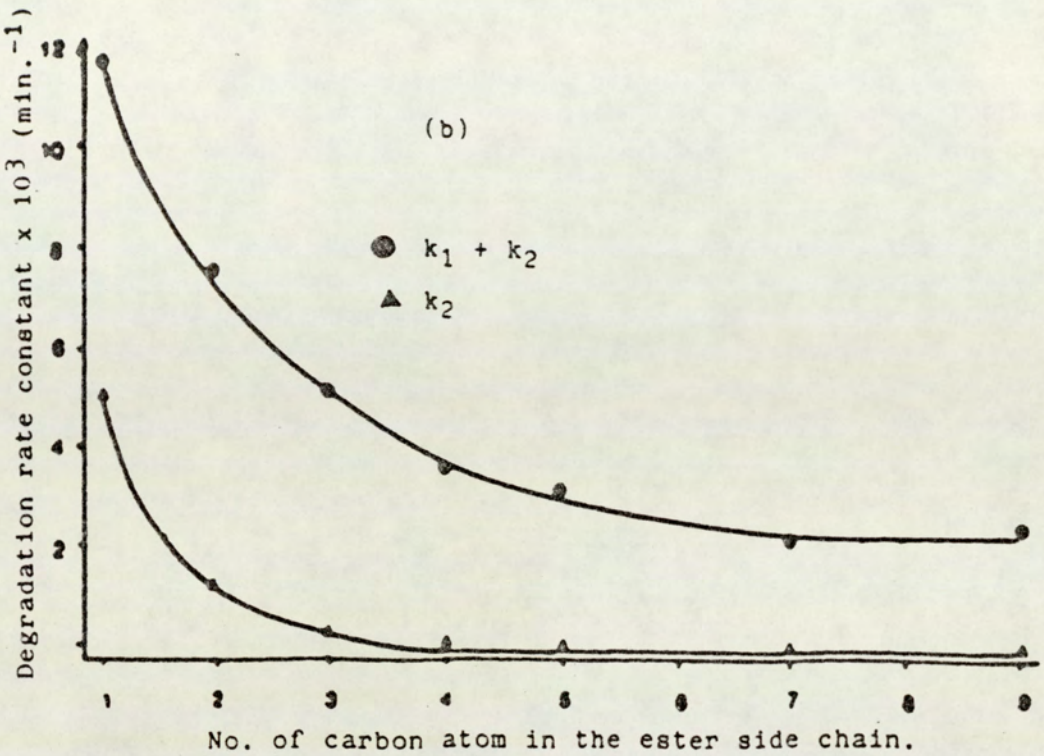


Fig 5.4 (b) Effect of the ester side chain on the degradation of propranolol esters in buffered-DMF pH 10 at 37°C.

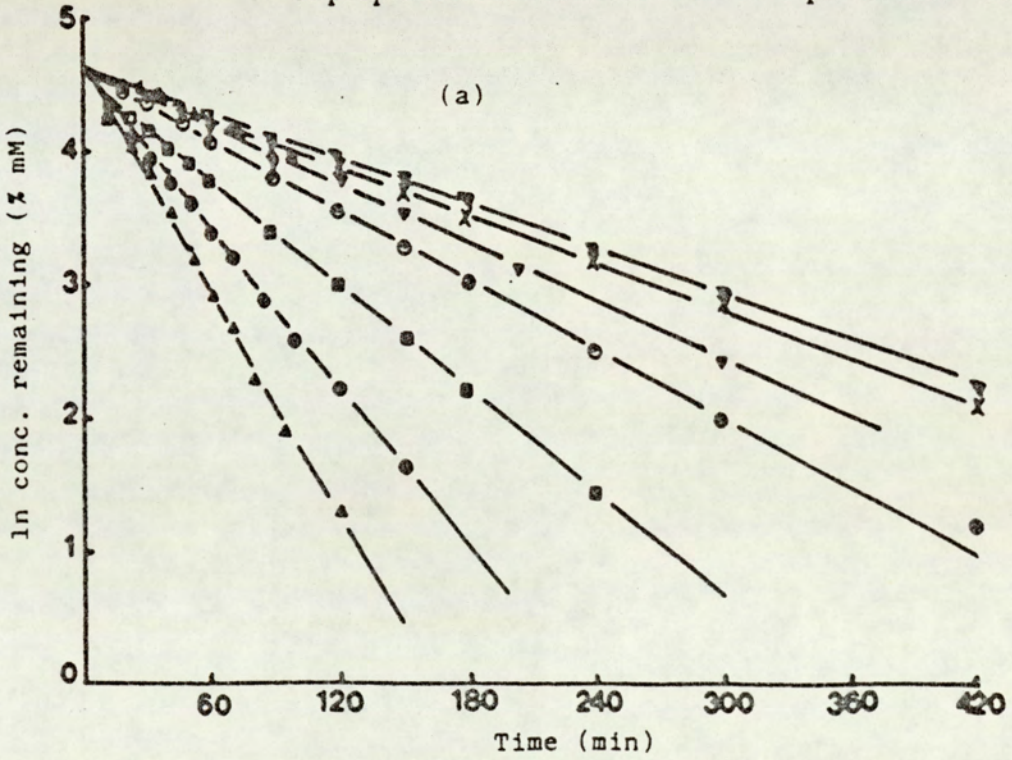


Fig 5.4 (a) In concentration time profile for the degradation of O-acyl propranolols in buffered-DMF pH 10 at 37° C.

Symbol	▲	●	□	○	▼	X	▽
No. of Carbon	1	2	3	4	5	7	9

The rate constants k_1 and k_2 were calculated from the following equation:

$$B_t = \frac{A_o k_1}{(k_1 + k_2)} - \frac{A_o k_1}{(k_1 + k_2)} \cdot e^{-(k_1+k_2)t} \qquad 5.2$$

where B_t = concentration of propranolol at time t (derivation, Appendix X.), linear plots of B_t against $e^{-(k_1 + k_2)t}$ were obtained with the negative slope equal to the intercept = $\frac{Ao\ k_1}{k_1 + k_2}$ (Fig 5.3). By knowing $(k_1 + k_2)$ from equation 5.1 and k_1 from equation 5.2, k_2 can be easily determined.

The transition state for intramolecular acyl rearrangement [Fig 5.2 (I)] appears rather bulky and it might be expected that when larger substitutents are present the rearrangement process may become less important. This was indeed found to be true when O-propionyl ester was monitored, the rearrangement rate falls demonstrably compared to that with the O-acetyl compound (Table 5.1 & Fig 5.4.b). The increased bulk of the substituent also reduces the hydrolysis rate, but overall, at pH = 10, the propionyl compound is a significantly better precursor of propranolol than the acetyl derivative. The hydrolysis pathway is favoured by a factor of about five times compared to the shorter chain compound. At pH values approaching those of tissue fluids, only low levels of N-propionyl product are detected.Higher chain homologues complement these results and show a somewhat reduced hydrolysis rate coupled with further suppression of the rearrangement reaction. Maximal effects are observed with the O-hexanoyl derivative and further increases in chain length has little effect upon the purely chemical degradation profiles.

In general, these results are in favourable agreement with the findings of Tablot 1976 (271), who found that the rate of the base catalysed hydrolysis of ethyl acetate was twice

Propranolol Ester	$k_1 + k_2 \times 10^3$ (min^{-1})	$k_1 \times 10^3$ (min^{-1})	$k_2 \times 10^3$ (min^{-1})	r	r_1	$t_{1/2}$ (min)
Acetyl	11.755	6.552	5.100	0.9997	0.9991	59
Propionyl	7.547	6.242	1.306	0.9993	0.9992	92
Butyryl	5.132	4.661	0.471	0.9995	0.9999	135
Valeryl	3.601	3.389	0.212	0.9996	0.9999	192
Hexanoyl	3.097	2.948	0.148	0.9996	0.9997	224
Octanoyl	2.199	2.938	0.010	0.9990	0.9980	315
Decanoyl	2.463	2.463	- -	0.9880	-	281

Where r = the correlation coefficient of plotting of ln A vs t

r_1 = the correlation coefficient of plotting B_t Vs $\exp^{-(k_1 + k_2)t}$

Table 5.1 Degradation rate constants and half-lives ($t_{1/2}$) of O-acyl propranolols in buffered-DMF solution , pH 10 , μ = 0.5M, at 37°C

that of ethyl butyrate. Higuchi et al 1983 (272) have determined the hydrolytic half-lives of acetominophen propionate and caproate at pH 7.0 and 25°C. The former ester derivative underwent hydrolysis 1.8 times faster than the caproate ester.

5.3.1.2 EFFECT OF pH ON THE DEGRADATION RATE CONSTANTS OF O-ACYL PROPRANOLOLS AT 80 °C

In order to study the pH-dependence of the degradation of O-acyl propranolols, the acetyl ester was chosen as an example for the short chain esters and the hexanoyl derivative for the longer chain ones. The reactions were carried out at 80°C in buffered -DMF at various pH values (2 - 9.45 for the acetyl ester and 2.5 - 8.8 for the hexanoyl derivative).

The calculated values of the degradation rate constants, half-life and shelf-life periods, and k_1 to k_2 ratios for O-acetyl and O-hexanoyl propranolol at various pH values at 80°C are summarized in Table 5.2. The rate-pH profiles for the degradation of both esters are shown in Fig 5.5 .a and b. Typical plots showing the degradation of these esters in various pH values at 80°C are displayed in Fig 5.6 a, b and c. These plots indicate that the reactions were first-order with respect to the two esters, since straight lines were obtained when ln A_t was plotted against time.

The concentration-time profile for the degradation of O-acetylpropranolol in 50% buffered - DMF pH 9.45 at 80°C is displayed in Fig 5.7 , which is also displayed by the HPLC chromatogram in Fig 5.8.

As shown from the rate pH profiles for the degradation of both esters (Fig 5.5 a & b), the optimum pH for maximum stability was found to be in the region of 3 - 3.5, and show also that the negative and positive slopes (<1) in these plots indicate that the degradation rate is not only due to the specific acid-base catalysis but may also be catalysed by the phosphate ions in the buffer solution.

Compound	pH	$k_1 + k_2 \times 10^2$ ($Hours^{-1}$)	$k_1 \times 10^2$ ($Hours^{-1}$)	$k_2 \times 10^2$ ($Hours^{-1}$)	$t_{1/2}$ (Hours)	$t_{10\%}$ (Hours)	$\frac{k1}{k_2}$
O-acetyl-	2.00	1.654	1.530	0.124	41.9	6.4	12.8
Propranolol	2.50	0.996	0.924	0.072	69.6	10.6	12.8
	3.20	0.764	0.698	0.066	90.7	13.8	10.6
	4.28	2.690	2.397	0.293	25.8	3.9	8.2
	5.30	7.820	6.960	0.860	8.9	1.4	8.1
	6.54	29.690	25.420	4.270	2.3	0.4	5.9
	7.80	76.200	-	-	0.9	0.1	-
	9.45	482.300	376.900	105.400	0.1 (min)	1.3	3.6
O-hexanoyl-	2.50	0.0136	-	-	5095.0	775.0	-
propranolol	3.60	0.0084	-	-	825.0	1254.8	-
	5.10	0.0423	0.0410	0.00022	1680.8	255.6	186.1
	6.20	0.4256	0.4200	0.00573	162.8	24.8	73.3
	7.40	4.4704	4.3316	0.13880	15.5	2.4	31.2
	7.70	4.8884	5.6584	0.23000	11.8	1.8	24.6
	8.30	13.2860	12.5520	0.73400	5.2	0.8	17.1
	8.80	25.4300	23.6390	1.79100	2.7	0.4	13.2

Table 5.2 Specific rate constants, half-life and shelf-life periods and k_1/k_2 ratio for the degradation of O-acetyl and O-hexanoyl propranolol in buffered-DMF solutions, $\mu = 0.5M$ at various pH values at $80^{o}C$.

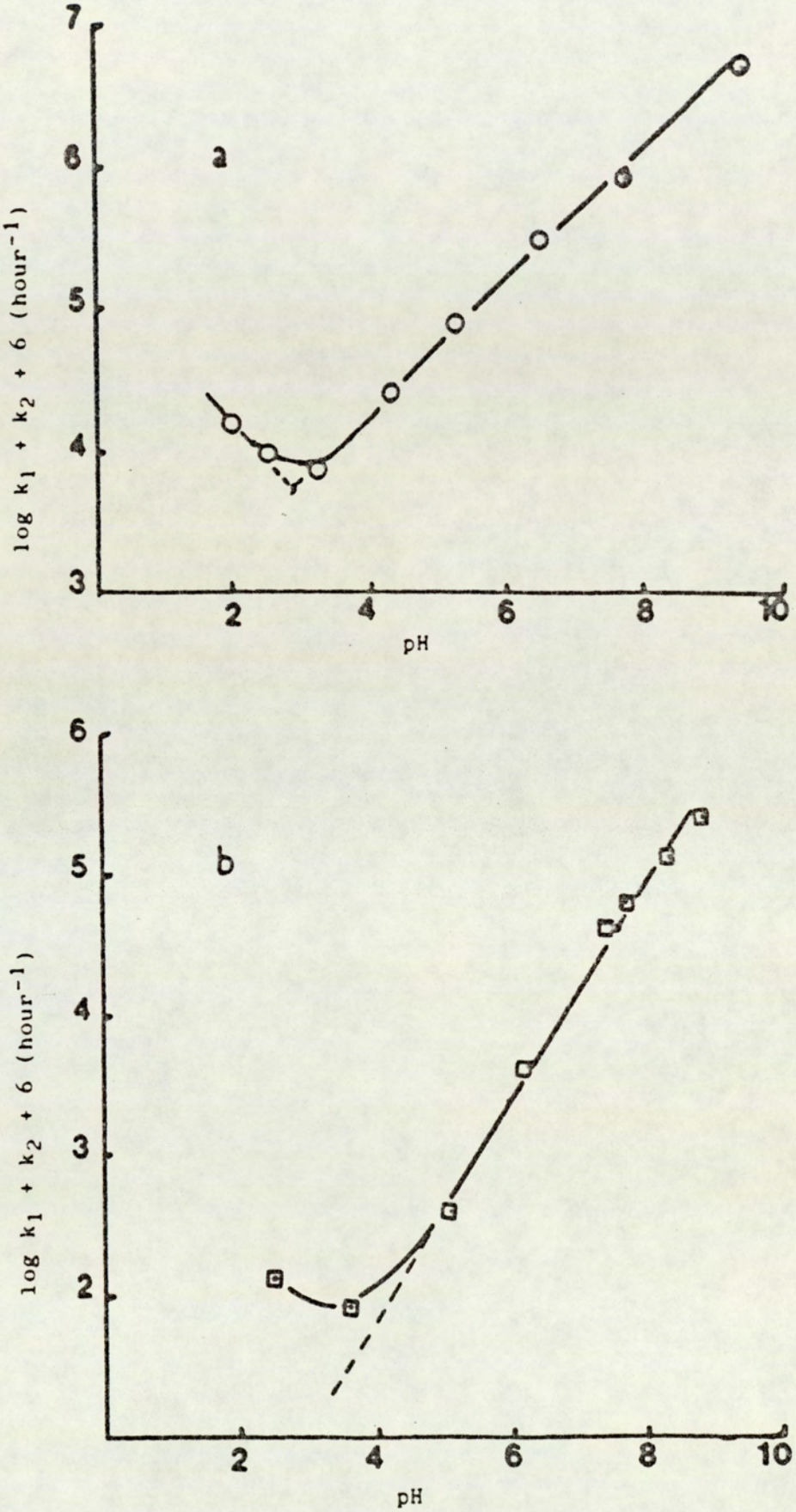


Fig 5.5 pH - rate profiles for the degradation of (a) O-acetyl and (b) O-hexanoyl propranolols in buffered-DMF solutions at various pH values at 80°C.

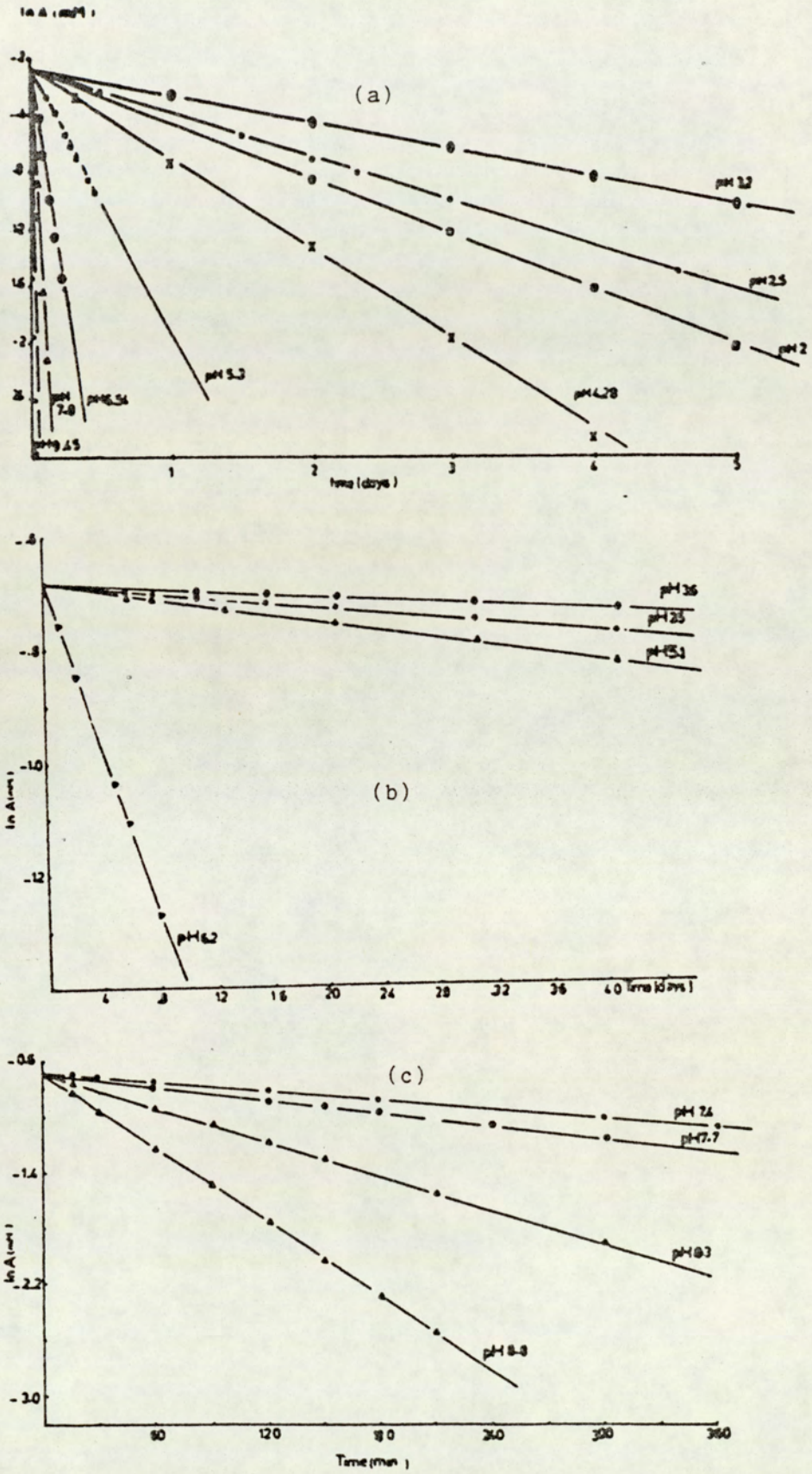


Fig 5.6 Ln concentration - time profiles for the degradation of O-acetylpropranolol (a) and O-hexanoylpropranolol (b + c) in buffered-DMF solutions of various pH values at 80°C.

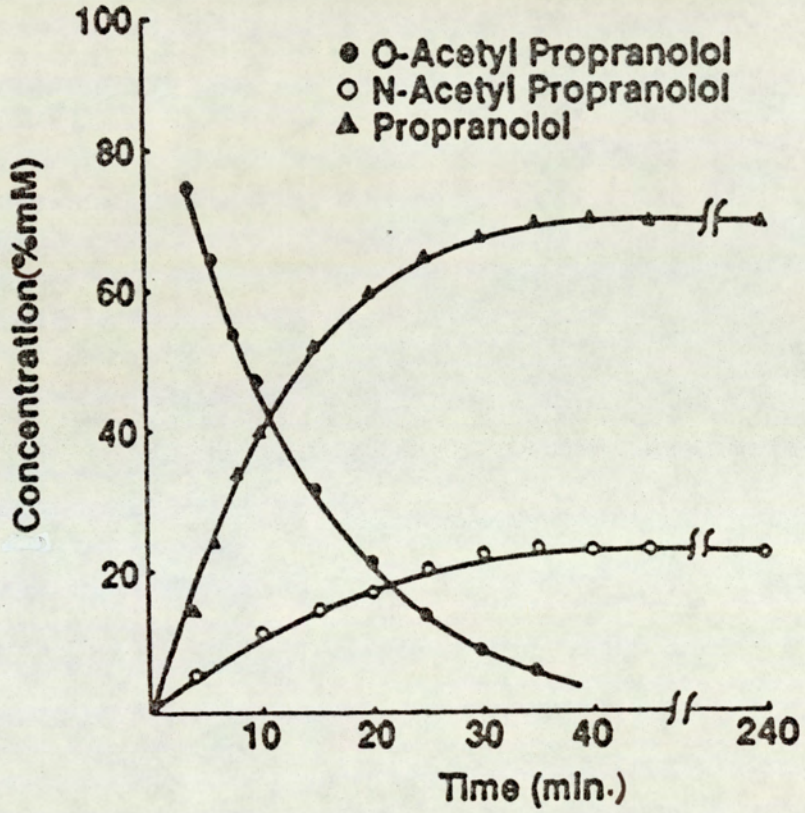


Fig 5.7 Concentration-time profile for the degradation of O-acetylpropranolol in buffered-DMF solution pH 9.45 at 80°C.

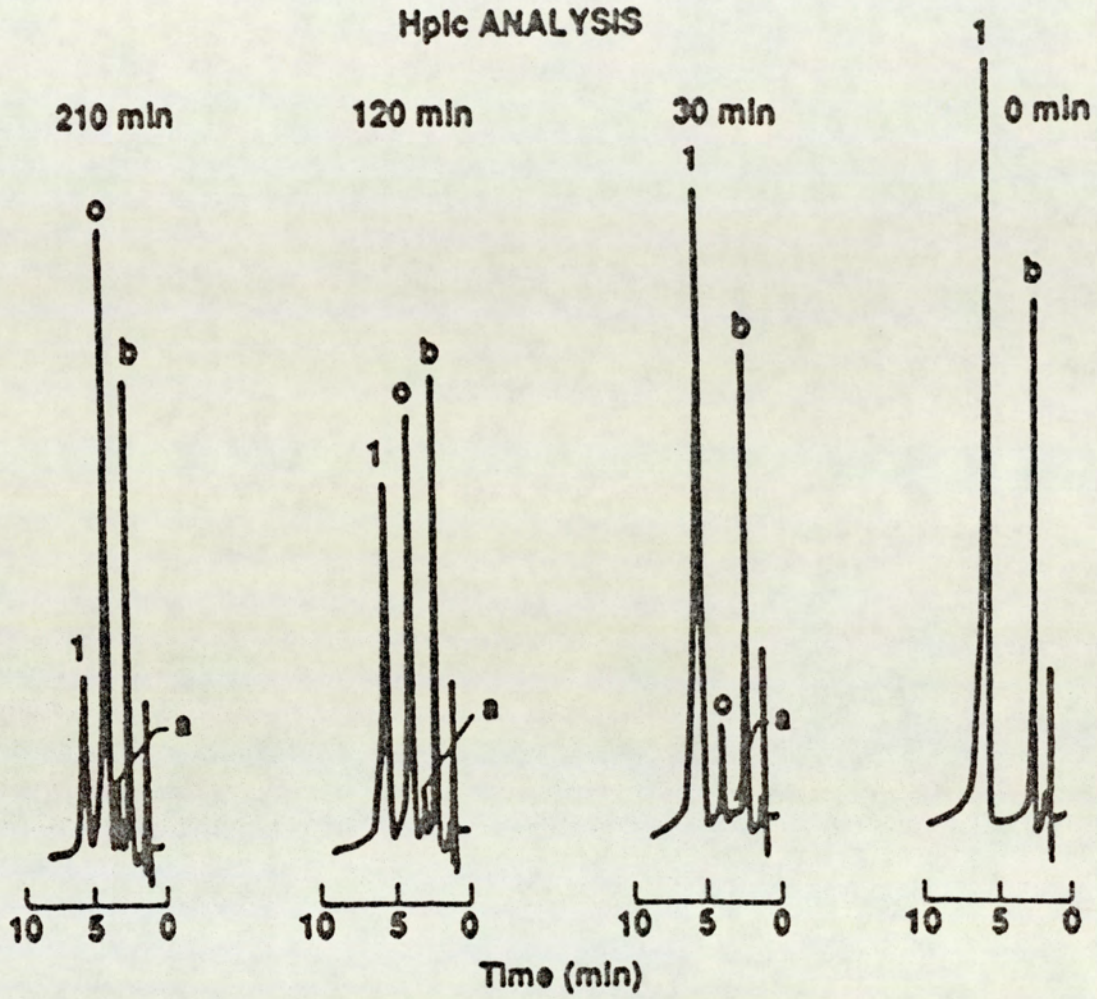


Fig 5.8 High-performance liquid chromatograms of the degradation of O-acetylpropranolol in buffered-DMF solution pH 9.45 at 80°C.

Column 10 cm x 4.6 mm, Hypersil-ODS
Mobile phase MeCN : H_3PO_4: Et_2NH : H_2O

(65 : 0.1 : 0.15 : 34.75) pH 2.7
Flow rate 1ml/min. Detection 290 nm.

Peak	Component
a	N-acetylpropranolol
b	Ethylparaben (internal standard)
1	O-acetylpropranolol
O	Propranolol

In this instance the observed rate constant (k_{obs}) becomes dependent upon the solvent, acid and hydroxyl rate processes for both ionized and unionized species of the ester which is represented in the following equation:

$$k_{obs} = (k_1 [H_3O^+] + k_2 [H_2O] + k_3 [OH^-]) \cdot x + (k_4 [H_3O^+] + k_5 [H_2O] + k_6 [OH^-]) \cdot (1 - x) \qquad 5.3$$

where

k_{obs} = the observed rate constant

k_1 & k_4 = specific rate constants for attack of acid

k_2 & k_5 = specific rate constants for attack of solvent

k_3 & k_6 = specific rate constants for attack of hydroxyl ion

x = fraction ionizied of the ester

$(1 - x)$ = fraction unionized of the ester

The data obtained in Table 5.2 show the following :

(a) as the pH increases the overall degradation rate constant ($k_1 + k_2$) as well as the hydrolysis rate constant (k_1) increased.

(b) the rate of formation of the N-acyl products is pH dependent. As the pH increases the rate of the amide formation increased as the intramolecular rearrangement was facilitated. This is observed as a decrease in the k_1/k_2 ratio and is probably a result of an increasing proportion of non-protonated base being available for anchimeric attack at the O-acyl group.

(c) direct hydrolysis of the hexanoyl ester to propranolol at pH 7.14 at 80°C has been found to be about 31 times faster than the formation of the corresponding amide , while with the acetyl derivative a difference of about five times (Fig 5.9 a & b) was observed.

5.3.1.3 EFFECT OF TEMPERATURE ON THE DEGRADATION OF O-ACYL PROPRANOLOLS

The calculated values of the specific rate constants and half lives for the degradation of O-acetyl and O-hexanoyl propranolol at various isothermal conditions are recorded in Table 5.3. This show that increases in the temperature caused acceleration in the overall degradation rates for both esters. The rearrangement rates were more affected by the temperature. This is observed as a decrease in the k_1/k_2 ratio. Typical plots showing the degradation of these esters at various temperatures in buffered-DMF pH 9.45 (O-acetylpropranolol) and pH 10.7 (O-hexanoylpropranolol) are shown in Fig 5.10 a and b.

The effect of temperature on the reaction rate may be expressed using the Arrhenius equation.

$$\log k = \log A - \frac{Ea}{2.303R} \cdot \frac{1}{T} \qquad 5.4$$

where

k = reaction rate constant

A = frequency factor

R = Universal gas constant (8.3143 joule/mole/K)

Ea = energy of activation

T = temperature (Kelvin)

Plots of log $k_1 + k_2$ versus 1/T yielded straight line relationships with negative slope equal to Ea / 2.303R as shown in Fig 5.11. The linearity of the lines indicates that the mechanism

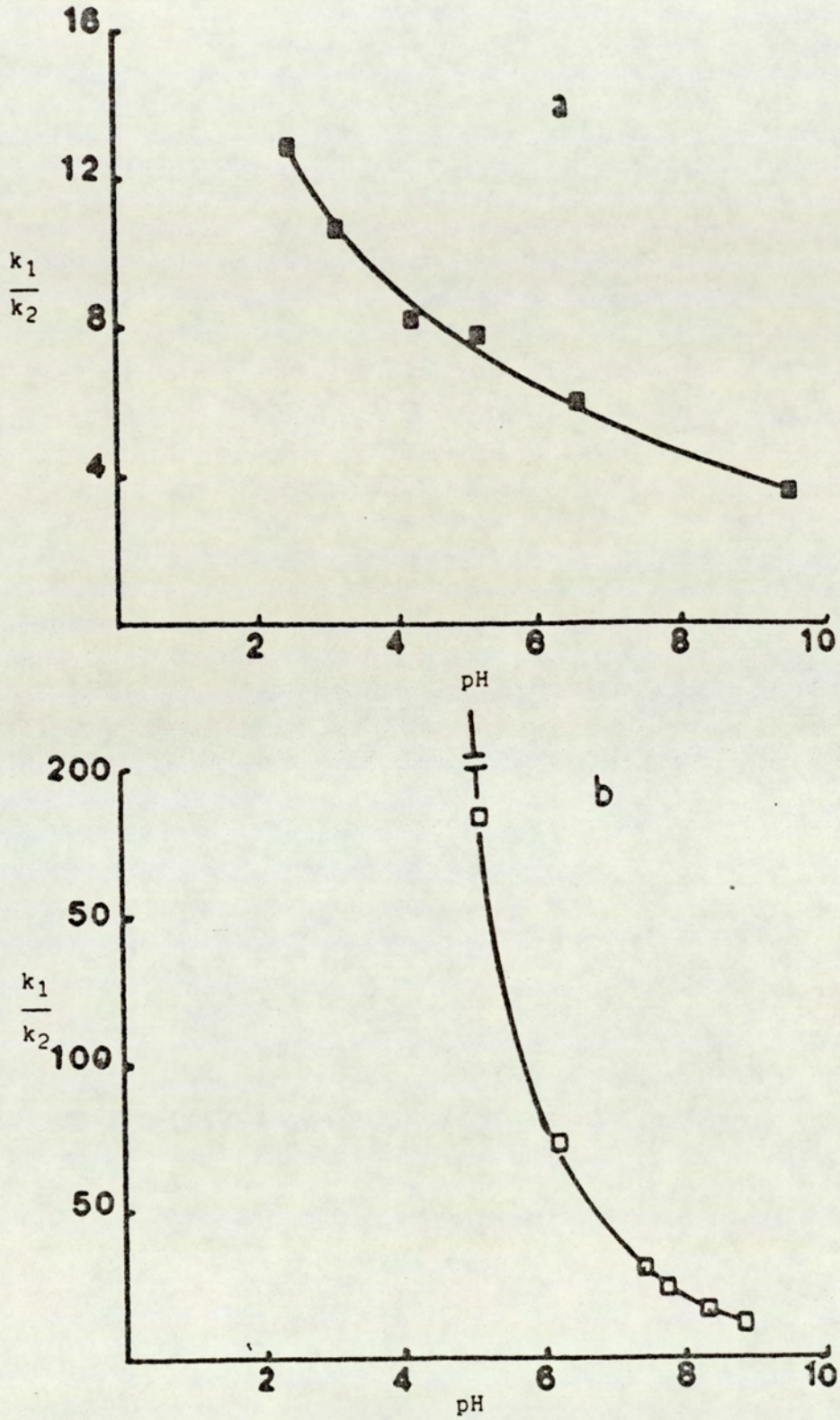


Fig 5.9 Effect of pH on k_1/k_2 ratios for the degradation of (a) O-acetyl and (b) O-hexanoyl propranolols in buffered-DMF solutions at various pH values at 80°C.

Compound	Temperature °C	$k_1 + k_2 \times 10^3$ (min^{-1})	$k_1 \times 10^3$ (min^{-1})	$k_2 \times 10^3$ (min^{-1})	$\frac{k_1}{k_2}$	$t_{1/2}$ (min)
O-acetyl-	80	80.350	62.816	17.570	3.60	8.6
propranolol	70	44.055	36.050	8.605	4.51	15.7
	60	22.188	18.670	3.520	5.30	31.2
	50	10.210	8.850	1.360	6.50	67.9
	40	4.395	3.868	0.477	8.10	157.7
O-hexanoyl-	80	44.775	32.854	11.920	2.76	15.5
propranolol	70	26.820	20.989	5.830	3.60	25.8
	60	15.100	12.278	2.822	4.35	45.9
	50	8.135	6.845	1.290	5.30	85.2
	40	4.112	3.538	0.574	6.20	168.5

Table 5.3 Specific rate constants, half-life periods and k_1/k_2 ratio for the degradation of O-acetyl and O-hexanoyl propranolol at various temperature in buffered-DMF solutions, pH 9.45 and 10.7, respectively, μ = 0.5M.

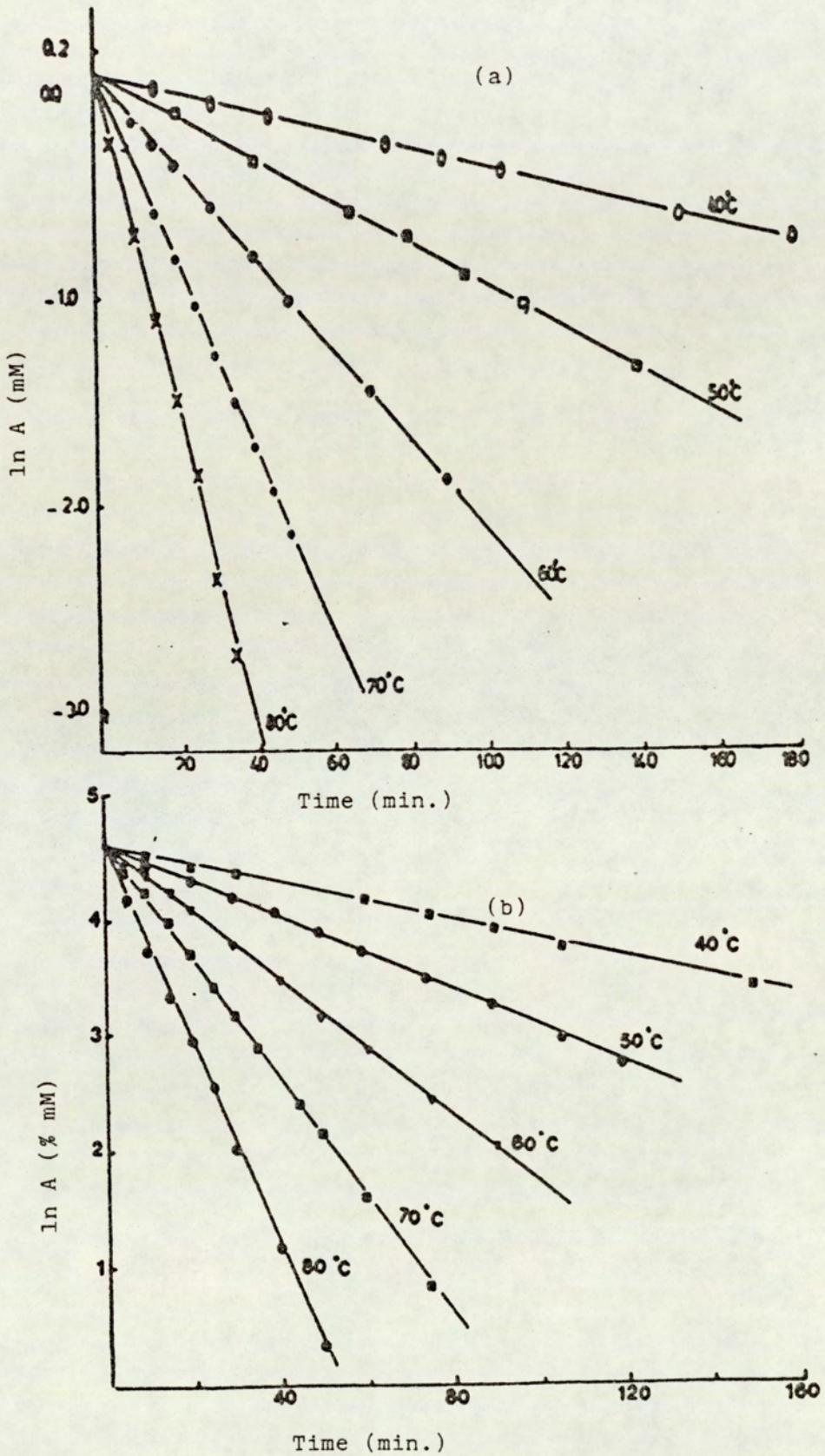


Fig 5.10 Ln of concentration of O-acetylpropranolol (a) and O-hexanoyl-propranolol (b) against time at various temperatures in buffered-DMF solutions pH 9.45 and 10.7 respectively.

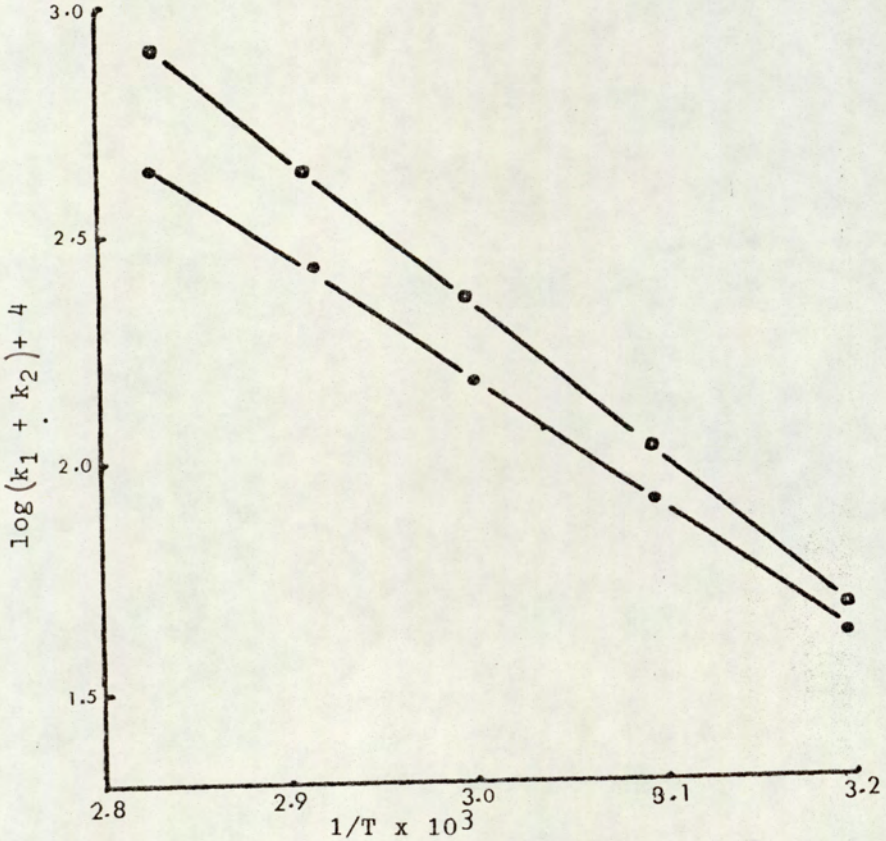


Fig 5.11 Arrhenius plots showing temperature dependence of O-acetyl (- □ -) and O-hexanoylpropranolol (- ● -) in buffered-DMF solutions pH 9.45 and 10.7 respectively.

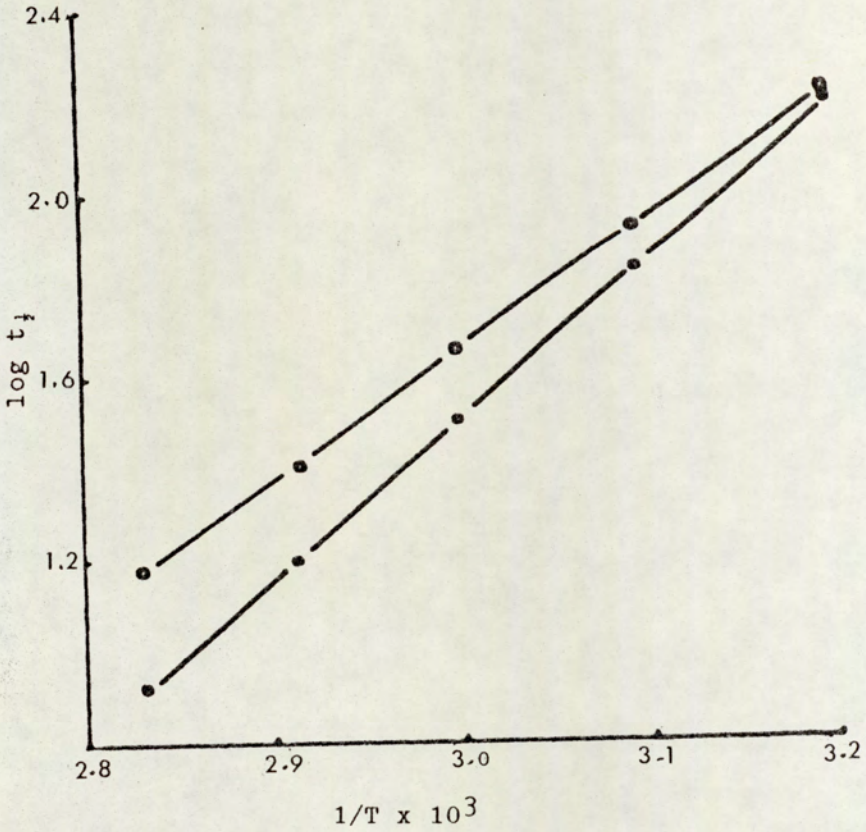


Fig 5.12 Plots of log $t_{1/2}$ Vs I/T for the degradation of O-acetyl (- ■ -) and O-hexanoylpropranolol (- □ -) in buffered-DMF solution pH 9.45 and 10.7 respectively at various temperatures.

for the degradation of both esters does not change with the temperature at the same pH. Energies of activation and the frequency factors which were calculated from Arrhenius plots are listed in Table 5.4.

It is sometimes useful to determine the energy of activation by plotting log $t_{1/2}$ versus 1/T (equation 5.5), the linearity of the plots were obtained (Fig 5.12) with a positive slope equal to Ea/2.303R.

$$\log t_{1/2} = \frac{Ea}{2.303R} \cdot \frac{1}{T} + \text{constant} \qquad 5.5$$

Half-life ($t_{1/2}$) and shelf-life ($t_{10\%}$) for the first order reaction are related to the reaction rate constant (k) by the fallowing equations:

$$t_{1/2} = 0.693 / k \qquad 5.6$$

$$t_{10\%} = 0.1054 / k \qquad 5.7$$

Specific rate constants, half-life and shelf-life periods for the degradation of O-acetylpropranolol in buffered-DMF pH 9.45 and O-hexanoylpropranolol in buffered -DMF pH 10.7 at 25°C were determined by extrapolation of the Arrhenius plots to 25°C and are recorded in Table 5.5.

5.3.1.4. EFFECT OF THE INITIAL CONCENTRATION ON THE DEGRADATION OF O-ACETYLPROPRANOLOL IN pH 7.4 AT 37 °C

Degradation of O-acetylpropranolol in Britton-Robinson buffer solution pH 7.4, μ = 0.5M at 37°C has been conducted using various initial concentrations of the ester (0.1, 0.2, and 0.5 mM).

Semilogarithmic plots of these degradation profiles were found to fit a first-order model (equation 5.1, Section 5.3.1.) as shown in Fig 5.13. The parallel lines signify equivalent rate constants for the degradation.

Compound	Ea (K.J.mole^{-1})			A (min^{-1}) x 10^{12}			Correlation Coefficient		
	$k_1 + k_2$	k_1	k_2	$k_1 + k_2$	k_1	k_2	r	r_1	r_2
O-acetyl-propranolol	67.11	64.23	82.61	7.06	2.10	30.1	0.9997	0.9994	0.9998
O-hexanoyl-propranolol	54.53	50.97	69.15	0.05	0.01	0.12	0.9996	0.9993	0.9999

Table 5.4 Energies of activation (Ea) and frequency factors (A) of O-acetyl and O-hexanoyl propranolol calculated from Arrhenius plots.

Compound	$k_1 + k_2 \times 10^5$ (min^{-1})	$k_1 \times 10^5$ (min^{-1})	$k_2 \times 10^5$ (min^{-1})	$t_{1/2}$ (min)	$t_{10\%}$ (min)
O-acetyl-propranolol in buffered-DMF pH 9.45, μ = 0.5M	122.4	116.2	6.216	568.0	86.4
O-hexanoyl-propranolol in buffered-DMF, pH 10.7 μ = 6.5M	146.0	135.0	11.000	474.6	72.2

Table 5.5 Specific rate constants, half-life and shelf-life periods for the degradation of O-acetyl and O-hexanoyl propranolol at 25°C as calculated from Arrhenius plots.

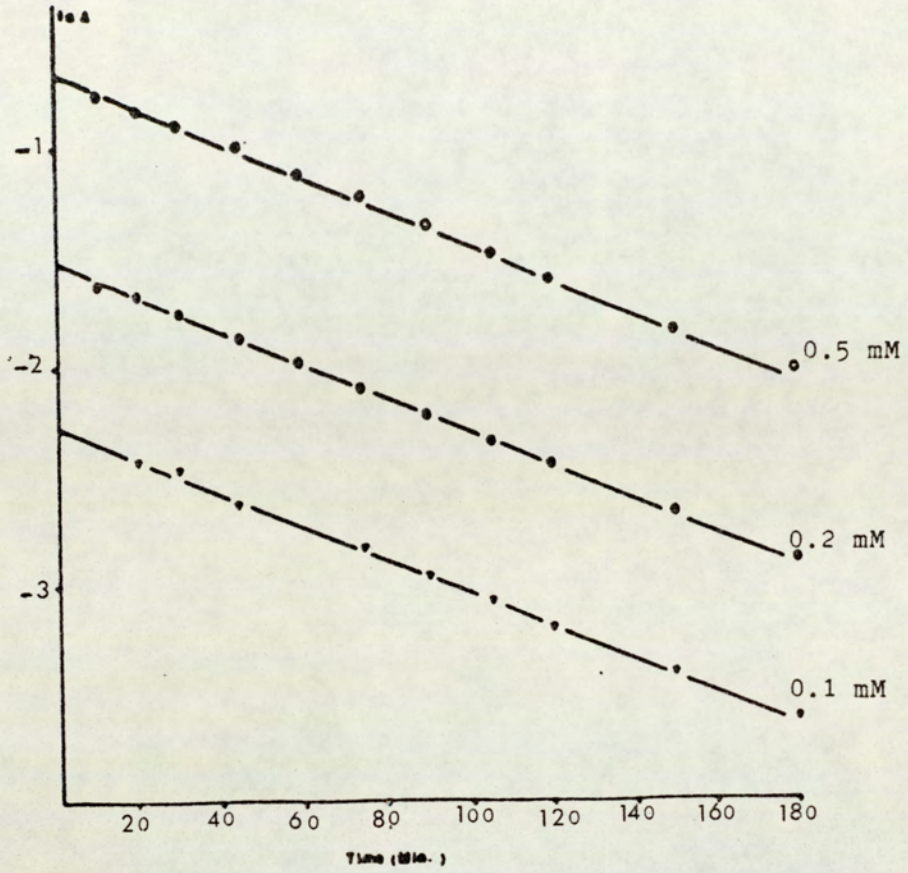


Fig 5.13 First-order plots showing the effect of initial concentration on the degradation of O-acetyl-propranolol in buffer pH 7.4, μ = 0.5M at 37°C.

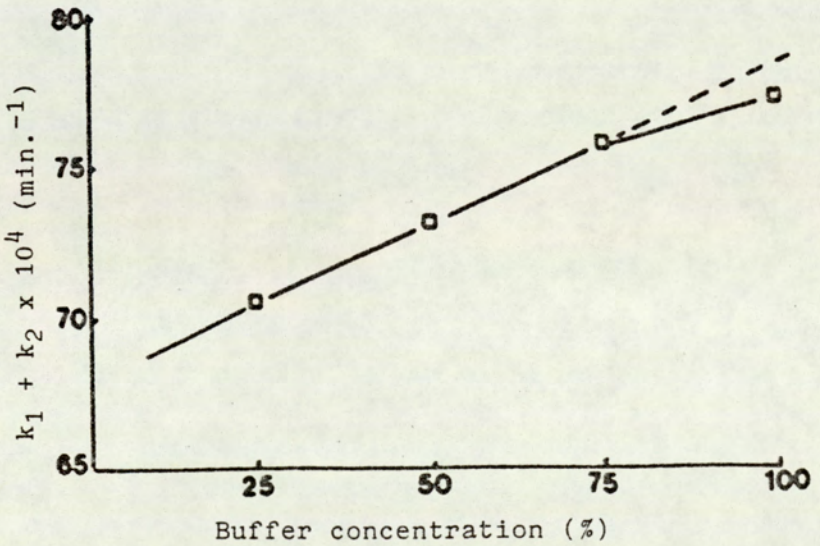


Fig 5.14 Effect of buffer concentration at pH 7.4, μ = 0.5 M on the degradation of O-acetyl-propranolol at 37°C.

The calculated rate constants and half-lives are in close agreement with each other as shown in Table 5.6. These results suggest that the degradation rate is independent of the initial concentration of the esters and confirm the first-order degradation under those conditions.

5.3.1.5 EFFECT OF THE BUFFER CONCENTRATION ON THE DEGRADATION OF O-ACETYLPROPRANOLOL

The effect of buffer concentration on the specific rate constants for the degradation of O-acetylpropranolol in Britton-Robinson buffer pH 7.4, μ = 0.5M at 37°C are summarized in Table 5.7.

The results reveal that a slight decrease in specific rate constants was observed with dilution of the buffer salts. This may correspond to the decrease in the amount of the buffer salts catalysing the reactions. As the buffer strength increases the rate of hydrolysis (k_1) increased as the intermolecular reactions was facilitated and the rate of rearrangement (k_2) decreased. This is observed as an increase of the k_1/k_2 ratio (from 9.6 - 12). A plot of such data is displayed in Fig 5.14.

5.3.1.6 EFFECT OF CO-SOLVENTS ON THE DEGRADATION OF O-ACETYL-PROPRANOLOL IN pH 7.4 AT 37 °C

The specific rate constants for the degradation of O-acetylpropranolol in the presence of different concentrations of DMF in Britton-Robinson buffer (final pH = 7.4, μ = 0.5M) at 37°C is equivalent to the absolute slope of the lines in Fig 5.16. These were determined by the linear regression analysis and parameters are recorded in Table 5.8. Half-lives and the rate constants for formation of propranolol and the amide (k_1 and k_2) were also measured and are similarly recorded.

Initial Concentration (mM)	$k_1 + k_2 \times 10^4$ (min^{-1})	$t_{1/2}$ (min)	r
0.1	75.37	92.0	0.9991
0.2	76.30	90.8	0.9993
0.5	75.86	91.4	0.9996

Table 5.6 Effect of the reactant initial concentration on the degradation of O-acetylpropranolol at pH 7.4 at 37°C

Concentration of buffer salt	pH	$k_1 + k_2 \times 10^4$ (min^{-1})	$k_1 \times 10^4$ (min^{-1})	$k_2 \times 10^4$ (min^{-1})	r
25%	7.37	70.67	63.99	6.67	0.9992
50%	7.39	73.26	67.16	6.10	0.9993
75%	7.40	75.90	69.98	5.92	0.9993
100%	7.41	77.23	71.38	5.85	0.9990

Table 5.7 Effect of the buffer concentration on the degradation of O-acetylpropranolol at pH 7.4 at 37°C

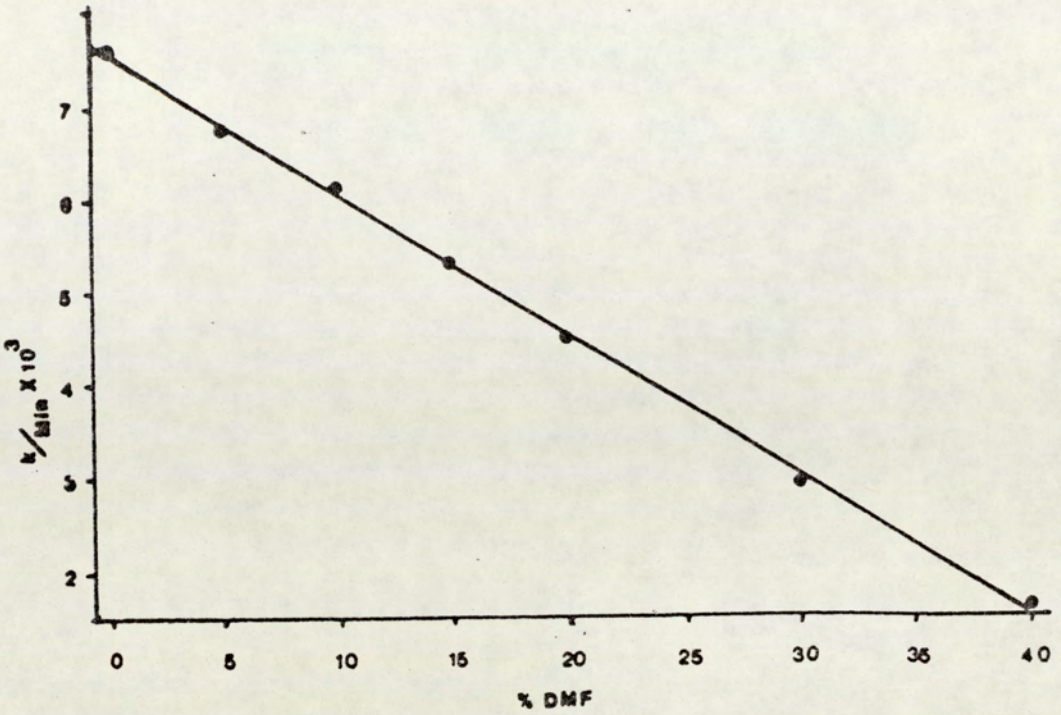


Fig 5.15 Effect of DMF concentration on the degradation of O-acetylpropranolol in buffer pH 7.4 at 37°C.

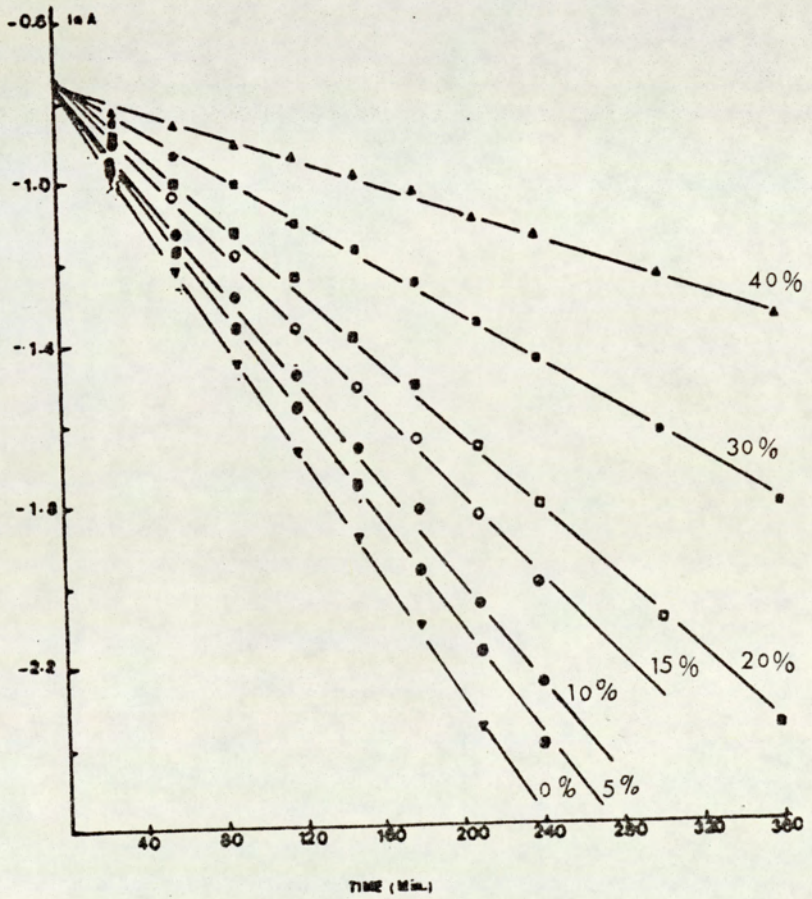


Fig 5.16 First-order plots showing the effect of DMF concentration on the degradation of O-acetyl-propranolol in buffer pH 7.4 at 37°C.

Co-solvent	% w/v	$k_1 + k_2 \times 10^4$ (min^{-1})	$k_1 \times 10^4$ (min^{-1})	$k_2 \times 10^4$ (min^{-1})	k_1/k_2	$t_{1/2}$ (min)	r
	0	73.20	67.10	6.10	11.0	94.7	0.9992
DMF	5	67.75	61.96	5.79	10.7	102.3	0.9993
	10	61.48	56.40	5.08	11.1	112.7	0.9987
	15	53.17	48.68	4.49	10.8	130.3	0.9986
	20	44.96	41.25	3.71	11.1	154.1	0.9990
	30	29.30	26.94	2.36	11.4	236.5	0.9991
	40	16.26	14.87	1.39	10.7	426.1	0.9991
	0	76.28	69.65	6.63	10.5	91.0	0.9980
P.G	5	89.21	81.96	7.25	11.3	77.7	0.9970
	10	102.20	95.30	6.90	13.8	67.8	0.9970
	15	108.99	102.32	6.67	15.3	63.6	0.9970
	20	112.84	107.03	5.81	18.4	61.4	0.9980
	30	113.44	108.54	4.90	22.1	61.1	0.9970
	40	113.29	109.25	4.04	27.0	62.2	0.9980

Table 5.8 Effect of DMF and P.G. Concentrations on the degradation of O-acetylpropranolol at pH 7.4 at 37°C

From Table 5.8 and Fig 5.15, as the concentration of DMF was increased in the reaction medium a significant reduction in the ester degradation rate was observed, which may be due to the depression of the ester ionization , which occurs when the dielectric constant of the reaction medium decrease by increasing the proportion of DMF in the solvent mixture. The increase of DMF proportion in the reaction medium did not alter the k_1 to k_2 ratios.

In contrast to the effect of DMF, increasing the proportion of propylene glycol (P.G) in the reaction medium has been shown to increase the overall degradation rate constant of the ester in pH 7.4 at 37°C (Fig 5.17 and Table 5.8).

The ratios k_1/k_2 are plotted as a function of the proportion of P.G. in the reaction medium as shown in Fig 5.18. The plots are linear and reveal that under the experimental conditions described, the ratio of k_1 to k_2 increases as the proportion of P.G in the mixed solvent increased by enhancing the hydrolysis rate rather than the rearrangement rate. This indicates that the P.G. plays as a catalyst in the hydrolysis process and also may indicate that the ions in the hydrolysis transition state have opposite charges in accord with the theory of ion-dipole reactions.

The effect of increasing the viscosity of the reaction medium on the degradation of O-acetylpropranolol at pH 7.4 at 37°C using PEG 1000 at different concentration (0 - 3% w/v), 2% PEG at different molecular weight (1000 - 20,000) and 2% PVP at different molecular weight (10,000 - 700,000) are summarized in Tables 5.9, 5.10 and 5.11.

The results reveal that the reaction rates were independent of the viscosity produced by either P.E.G. or PVP. These finding may have a considerable pharmaceutical significance. The usage of a mixed solvent is often employed in pharmacy as a means of solubilization or stabilizing the drug against hydrolysis. However, the results suggest that the use of solvent mixtures of lower dielectric constant may actually increase rather than decrease of the degradation rate (in the case of P.G-buffer system).

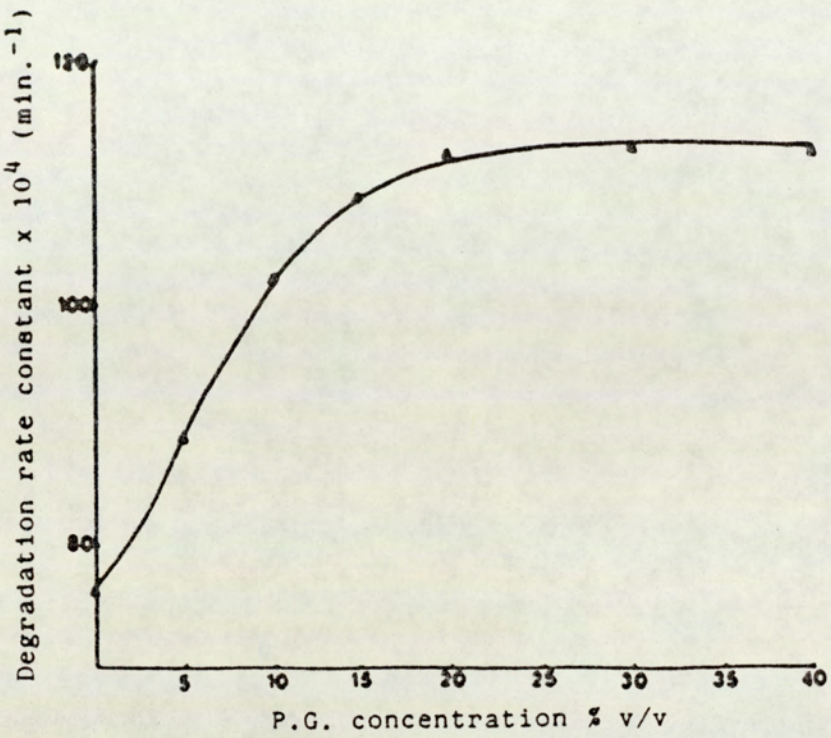


Fig 5.17 Effect of propylene glycol (P.G.) concentration on the degradation of O-acetylpropranolol in buffer pH 7.4 at 37°C.

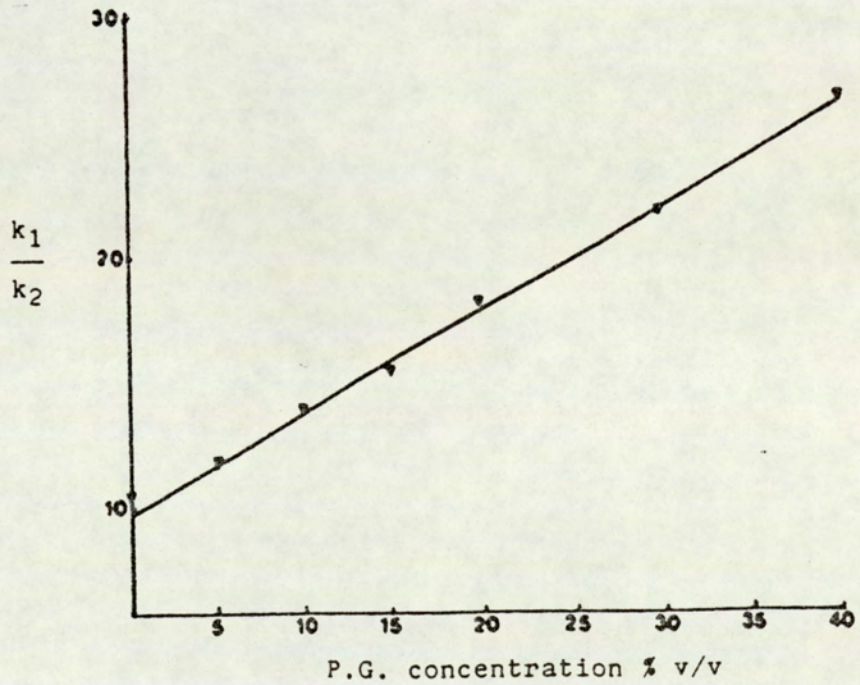


Fig 5.18 Effect of P.G. Concentration on k_1/k_2 for the degradation of O-acetylpropranolol in buffer pH 7.4 at 37°C.

P.E.G 1000 % w/v	Viscosity CPS	k x 10^4 (min^{-1})	r
0.0	-	73.76	0.9998
0.5	3	73.20	0.9998
1.0	3	74.06	0.9996
1.5	3	73.39	0.9992
2.0	3	73.42	0.9996
3.0	3	73.81	0.9996

Table 5.9 Effect of PEG 1000 concentration on the degradation of O-acetylpropranolol at pH 7.4 at 37°C

M.Wt	Viscosity CPS	k x 10^4 (min^{-1})	r
1000	3	74.25	0.9996
4000	3.5 - 4	74.59	0.9990
6000	4	74.53	0.9997
20000	5	75.24	0.9996

Table 5.10 Effect of PEG molecular weight on the degradation of O-acetylpropranolol at pH 7.4 at 37°C

M.Wt	Viscosity CPS	k x 10^4 (min^{-1})	r
10,000	3.5	74.47	0.9900
40,000	4.0	74.49	0.9974
360,000	8.0	74.39	0.9970
700,000	11.5	74.65	0.9998

Table 5.11 Effect of PVP molecular weight on the degradation of O-acetylpropranolol at pH 7.4 at 37°C

On the other hand, as pointed out by Marcus and Taraszka 1959 (273), a small increase in degradation rate due to the use of non-aqueous solvent may be outweighed by the enhancement of solubility of the drug in the solvent of lower dielectric constant.

Several studies relating to the dielectric constant of the solvent medium to the rate of reactions have been reported [*Noyes 1956 (274), Irwin et al 1984a (275) and 1984b (276)*].

5.3.1.7 DEGRADATION PATHWAY OF O-PIVALOYLPROPRANOLOL

O-pivaloylpropranolol is an example of an O-acyl propranolol which undergoes a simple hydrolysis to the parent compound without the involvement of competing intramolecular rearrangement. This is probably due to the steric hindrance involving the tertiary butyl group in the ester side chain, which prevents the interaction between the carbonyl group and the amino group in the side chain.The degradation of O-pivaloylpropranolol in buffered-DMF follows first-order kinetics,such that:

$$A \xrightarrow{k} B$$

where:

A : represents the ester

B : propranolol

k : the degradation rate constant

The residual concentration of the ester A_t at time t is dependent upon the initial concentration A_o and is given by:

$$A_t = A_o \cdot e^{-kt}$$

or

$$\ln A_t = \ln A_o - kt \qquad 5.8$$

A series of HPLC chromatograms showing the course of the reaction of O-pivaloylpropranolol (O.5mM) in buffered-DMF pH 9.6 at 80^{o}C together with standard chromatogram are shown in Fig 5.19.

The chromatograms reveal that under the experimental conditions, the ester gradually breaks down forming propranolol. It is also apparent in the figure that the column performance remains the same for both the experimental and the standard samples.

The concentration-time profile reflecting the hydrolysis of the ester and the subsequent formation of propranolol are shown in Fig 5.20. At any time, the mass balance of the residual ester and the generated propranolol corresponds to the initial concentration of the ester as shown in Fig 5.20.

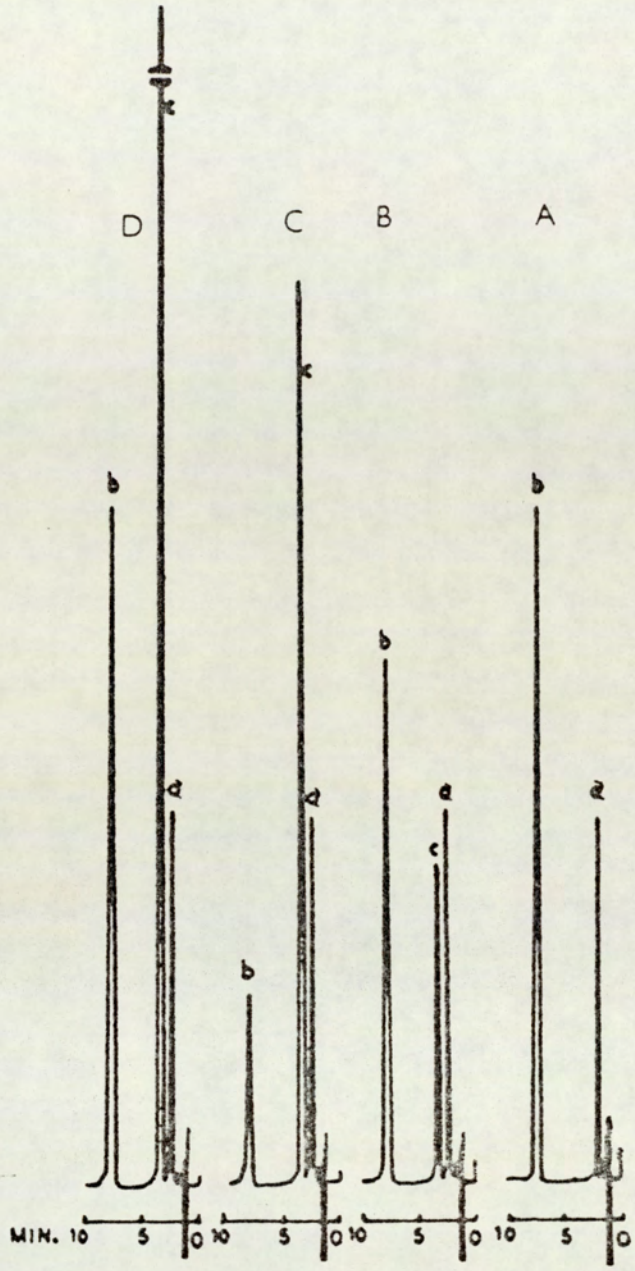


Fig 5.19 High-performance liquid chromatogram showing the hydrolysis of O-pivaloylpropranolol (0.5 mM) in buffered-DMF solution pH 9.6 at 80°C.

Column : 10 cm x 4.6 mm, Hypersil-ODS
Mobile phase : MeCN : H_3PO_4 : Et_2NH : H_2O
(65 : 0.1 : 0.2 : 34.7)
Flow rate : 1 ml/min. Detection : 290 nm.

A	B	C	D
t = 0 min	t = 30 min	t = 120 min	Standard chromatogram

a	b	c
Ethylparaben (I.S)	O-pivaloyl-propranolol	propranolol

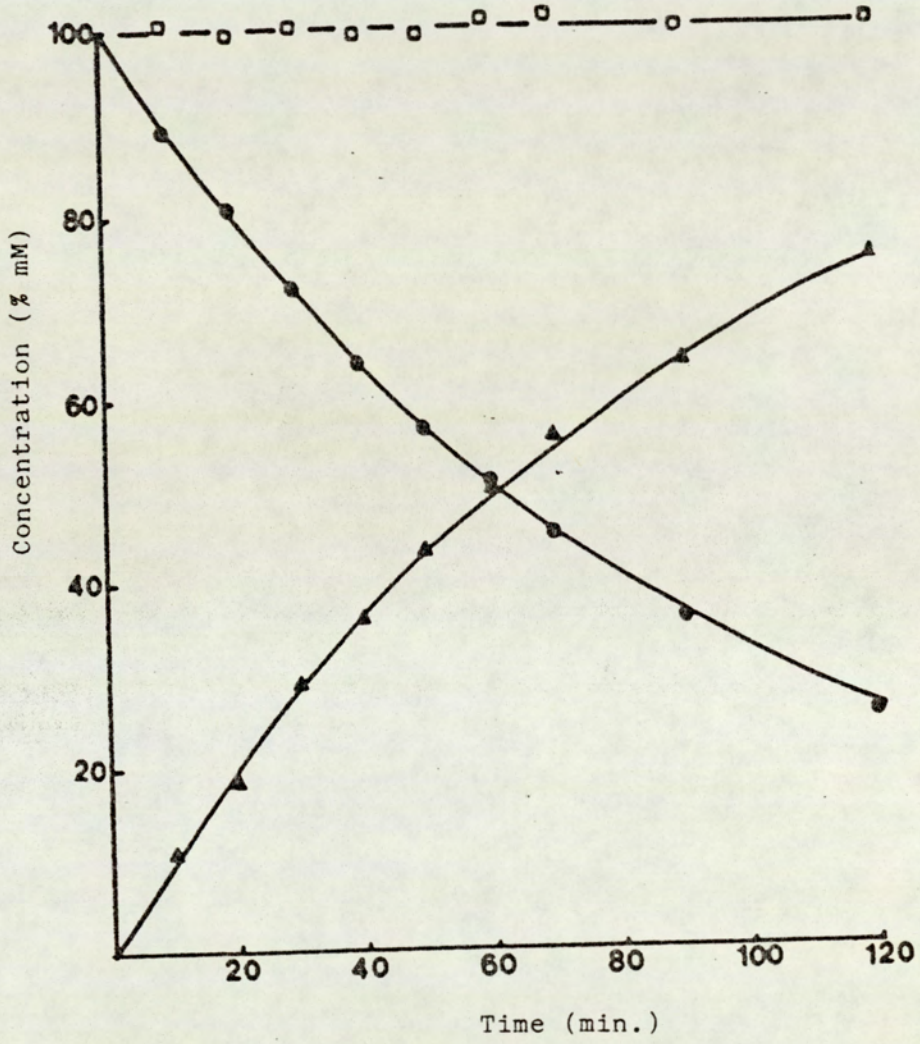


Fig 5.20 Concentration-time profile for the hydrolysis of O-pivaloylpropranolol in buffered-DMF pH 9.6 at 80°C.

● O-pivaloylpropranolol

▲ Propranolol

□ Mass-balance

5.3.1.8 EFFECT OF pH ON THE HYDROLYSIS OF O-PIVALOYLPROPRANOLOL IN BUFFERED-DMF AT 80 °C

The specific rate constants for the hydrolysis of O-pivaloylpropranolol at various pH values within the range 2.83 - 9.6 were determined from the slope of the first-order plots describing the disappearance of the ester, displayed in Fig 5.21. The effect of the pH on the hydrolysis rate constant and the half-life and shelf-life periods are summarized in Table 5.12.

pH-rate profile (Fig 5.22) shows that the rate of reaction is dependent upon the pH with a maximum stability demonstrated at pH3.

5.3.1.9 TEMPERATURE DEPENDENCE OF O-PIVALOYLPROPRANOLOL IN BUFFERED-DMF pH 9.6

A basic computer program NONISO reported by Hempenstall et al 1983 (277) has been applied in establishing the full temperature stability profile from a single non-isothermal experiment. This method was used to determine the temperature dependence of O-pivaloyl-propranolol in buffered-DMF pH 9.6.The rate constants at various experimental temperatures were determined by fitting the time-temperature-concentration data to the program NONISO and are recorded in Table 5.13. To check the validity of the method, hydrolysis of O-pivaloylpropranolol was conducted in the same reaction medium at various isothermal conditions.

The first-order plots of the concentration time profiles are shown in Fig 5.23 and the rate constants measured from the slopes are also recorded in Table 5.13. The 1/T against ln k plots for isothermal and non-isothermal results are shown in Fig 5.24; together with the Arrhenius parameter in Table 5.14. The specific rate constant, half-life and shelf-life periods at 25^{o}C were determined from extrapolation of the Arrhenius plot to 25^{o}C and are recorded in Table 5.15.

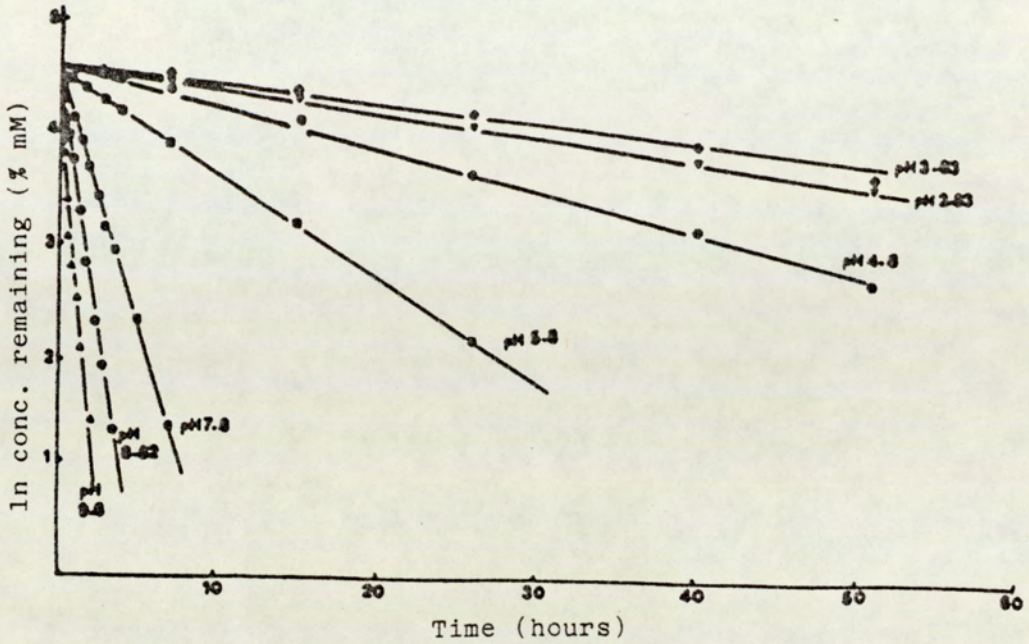


Fig 5.21 Ln concentration-time profile for the hydrolysis of O-pivaloylpropranolol in buffered-DMF solution at various pH values at 80°C.

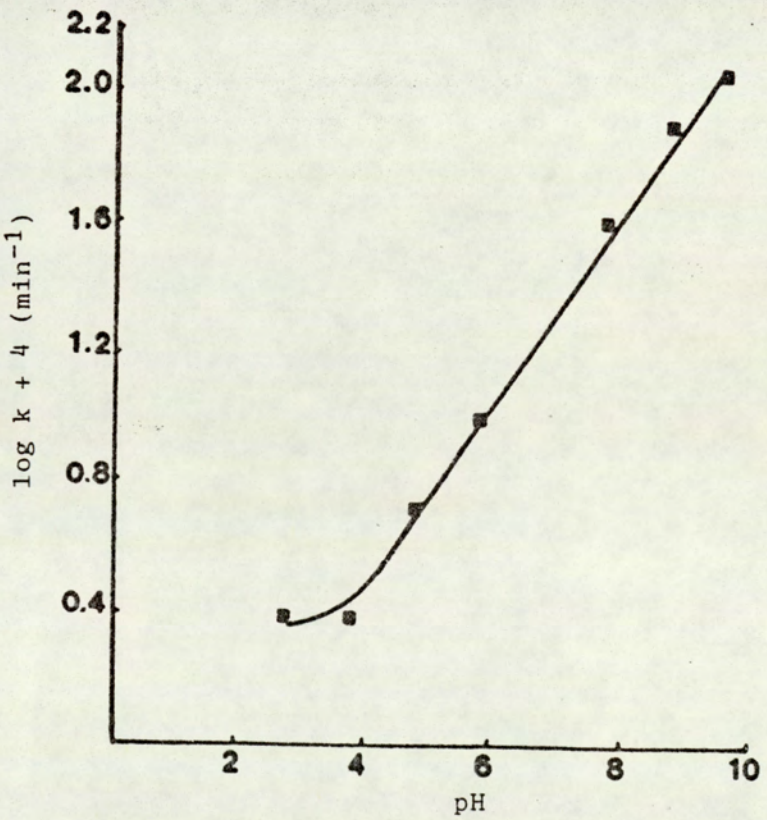


Fig 5.22 pH-Rate profile for the hydrolysis of O-pivaloylpropranolol in buffered-DMF solutions at 80°C.

pH	k x 10^2 ($hour^{-1}$)	$t_{1/2}$ (hour)	$t_{10\%}$ (hour)	r
2.83	1.556	44.5	6.80	0.9970
3.80	1.454	47.6	7.20	0.9980
4.80	3.061	22.6	3.40	0.9999
5.85	6.084	11.4	1.70	0.9994
7.80	24.666	2.8	0.40	0.9992
8.82	49.314	1.4	0.20	0.9992
9.60	69.120	1.0	0.150	0.9999

Table 5.12 Specific rate constants, half-life and shelf-life periods for the hydrolysis of O-pivaloylpropranolol in buffered-DMF at various pH values at 80°C

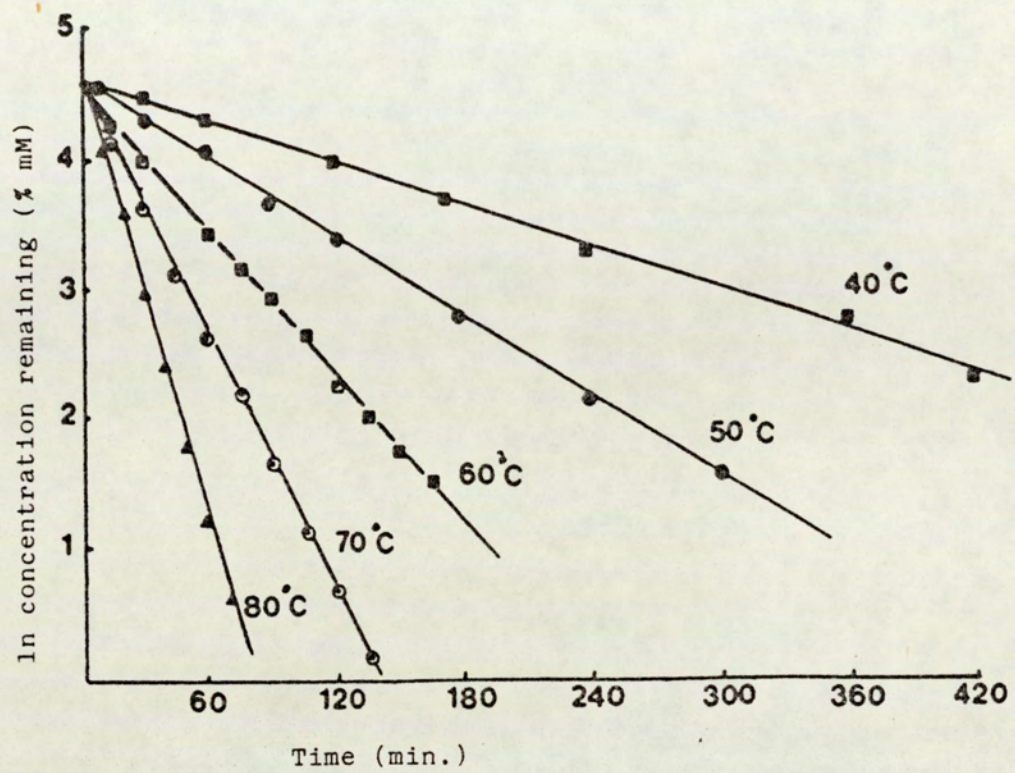


Fig 5.23 Ln concentration of O-pivaloylpropranolol at various temperatures in buffered-DMF pH 9.6.

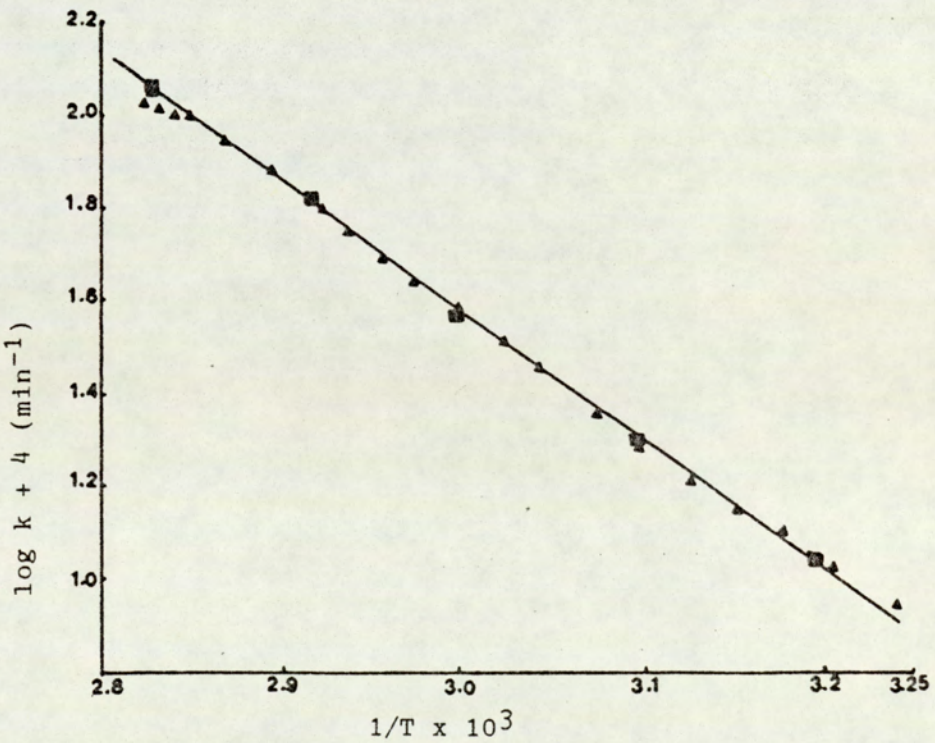


Fig 5.24 Arrhenius plots for O-pivaloylpropranolol in buffered-DMF pH 9.6

■ Isothermal rate constant

Δ Rate constants from the non-isothermal experiment.

Temperature	Isothermal rate constant x 10^3 (min^{-1})	Non-isothermal rate constant x 10^3 (min^{-1})
35.7		0.9334
39.1		1.040
40.0	1.100	-
42.0		1.318
44.2		1.496
47.0		1.585
49.9		2.044
50.0	2.026	-
52.2		2.239
55.5		2.871
57.6		3.162
60.0	3.740	-
60.7		3.885
63.1		4.467
65.3		4.898
67.5		5.623
69.3		6.309
70.0	6.627	-
72.6		7.611
75.7		8.892
78.0		10.088
79.2		10.116
79.9		10.399
80.0	11.52	-
81.3		10.765

Table 5.13 Rate constants for the hydrolysis of O-pivaloyl-propranolol in buffered-DMF pH 9.6 obtained from isothermal and non-isothermal experiments

Parameters	Values	
	Isothermal	Non-isothermal
E_a (K.J. mole $^{-1}$)	53.97	51.75
A (min^{-1})	9.645×10^9	4.82×10^9
r	0.9998	0.9984

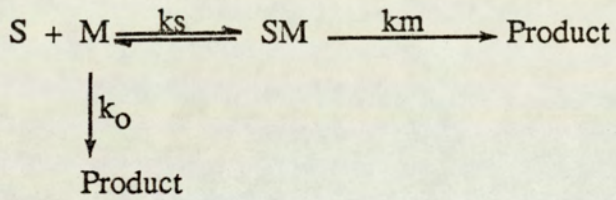
Table 5.14 Energies of activation (E_a) and frequency factor (A) calculated from isothermal and non-isothermal degradation of O-pivaloylpropranolol in buffered-DMF solution pH 9.6

Parameters	Values	
k	3.802×10^{-4}	min^{-1}
$t_{1/2}$	1822.7	min.
$t_{10\%}$	277.0	min.

Table 5.15 Specific rate constant, half-life and shelf-life periods for the hydrolysis of O-pivaloyl-propranolol in buffered-DMF solution pH 9.6 at 25°C as calculated from Arrhenius plot.

5.3.1.10 EFFECT OF SURFACTANTS ON THE DEGRADATION OF O-ACYL PROPRANOLOLS IN BRITTON-ROBINSON BUFFER pH 7.4, μ = 0.5M AT 37 °C

The effect of micelles on the rate of organic reactions are explicable in terms of the difference in reactivity of the substrate associated with the micellar pseudophase and the bulk solution, the distribution of the substrate between these two phases and hence, the degree and nature of substrate-micelle binding.



where S : represent the substrate

M : is the micellar surfactant

k_o & k_m : are the reaction rate constants in the aqueous and micellar phases respectively

k_s : is the binding constant

The quantitative treatment requires knowledge of the binding constant (k_s) of the substrate with the micelle. Unfortunately, in these studies k_s cannot be measured or estimated. However, the results confirm the importance of the distribution of the ionized and unionized species by the anionic and cationic micelles respectively causing a change in the pKa values. These effects of micelles on equilibria have been previously studied (Chapter 4, Section 4 .3.3).

In addition to the effect of the surfactant on the rates of the reaction, a pronounced effect on the high-performance liquid chromatography (HPLC) resolution of the esters under investigation is shown. In Fig 5.25, the high-performance liquid chromatograms show the effect of the cationic surfactant (DTAB, 1.42 x 10^{-4}M) on the resolution of a mixture consisted of N-acetylpropranolol, propranolol, O-acetylpropranolol and ethylparaben (as internal standard) using the previously developed mobile phase [CH_3CN: H_3PO_4: Et_2NH: H_2O, (65: 0.1: 0.2: 34.7)]. The figure shows that after successive injections of this mixture, the peaks overlap and little separation was observed. This phenomenon could be explained by the fact that the surfactant (DTAB) was acting as ion-pairing agent interacting with the protonated components and affecting the resolution. The neutral components such as N-acetylpropranolol and ethylparaben (the internal standard) were shown not to be affected by the surfactant. To overcome this effect, other mobile phases have been developed for each type of surfactant used and details are recorded in Table 5.16. High performance liquid chromatograms for the separation of the esters under investigation and their degradative products together with the appropriate internal standard in the presence of various surfactants are displayed in Figures 5.26 - 5.28.

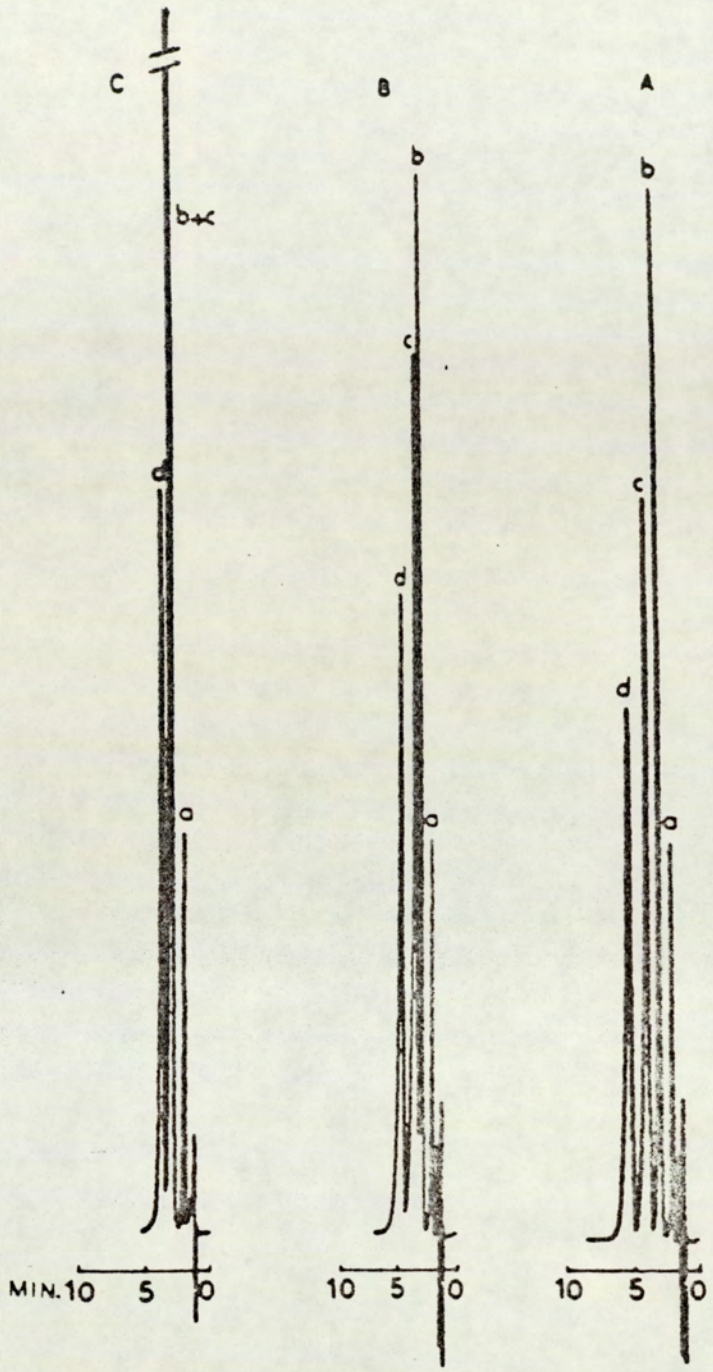


Fig 5.25 High-performance liquid chromatograms showing the effect of successive injection of a mixture of drugs in aqueous DTAB solution.

Rate of flow : 1 ml/min. Column 10 cm x 4.6 m.m. I.d.
Detection : 290 nm. Hypersil-ODS (5µm)

Mobile Phase			Peak	Solute Components
MeCN : H_3PO_4 : Et_2NH : H_2O 65 : 0.1 : 0.2 : 34.7 pH = 2.5			a	Ethylparaben (I.S)
			b	N-acetylpropranolol
A	B	C	c	Propranolol
1st injection	2nd injection	5th injection	d	O-acetylpropranolol

Solvent Composition	Solute Components	Mobile Phase $MeCN:H_3PO_4:H_2O$	Internal Standard	Elution Time (min)
2×10^{-2}M aqueous DTAB	Propranolol O-acetyl-propranolol N-acetyl-propranolol	42 : 2.0 : 56	Methylparaben	15
	Propranolol O-hexanoyl ester N-hexanoyl-propranolol	55 : 2.5 : 42.5	Butylparaben	16
2×10^{-2}M aqueuos SLS	Propranolol O-acetyl-propranolol N-acetyl-propranolol	48 : 2.5 : 49.5	Ethylparaben	8
2×10^{-2}% w/v aqueous Tween 80	Propranolol O-acetyl-propranolol N-acetyl-propranolol	45 : 3.0 : 52	Propylparaben	13

Table 5.16 HPLC condition for the analysis of O-acetyl and O-hexanoyl propranolols and their degradative products in aqueous surfactant solutions

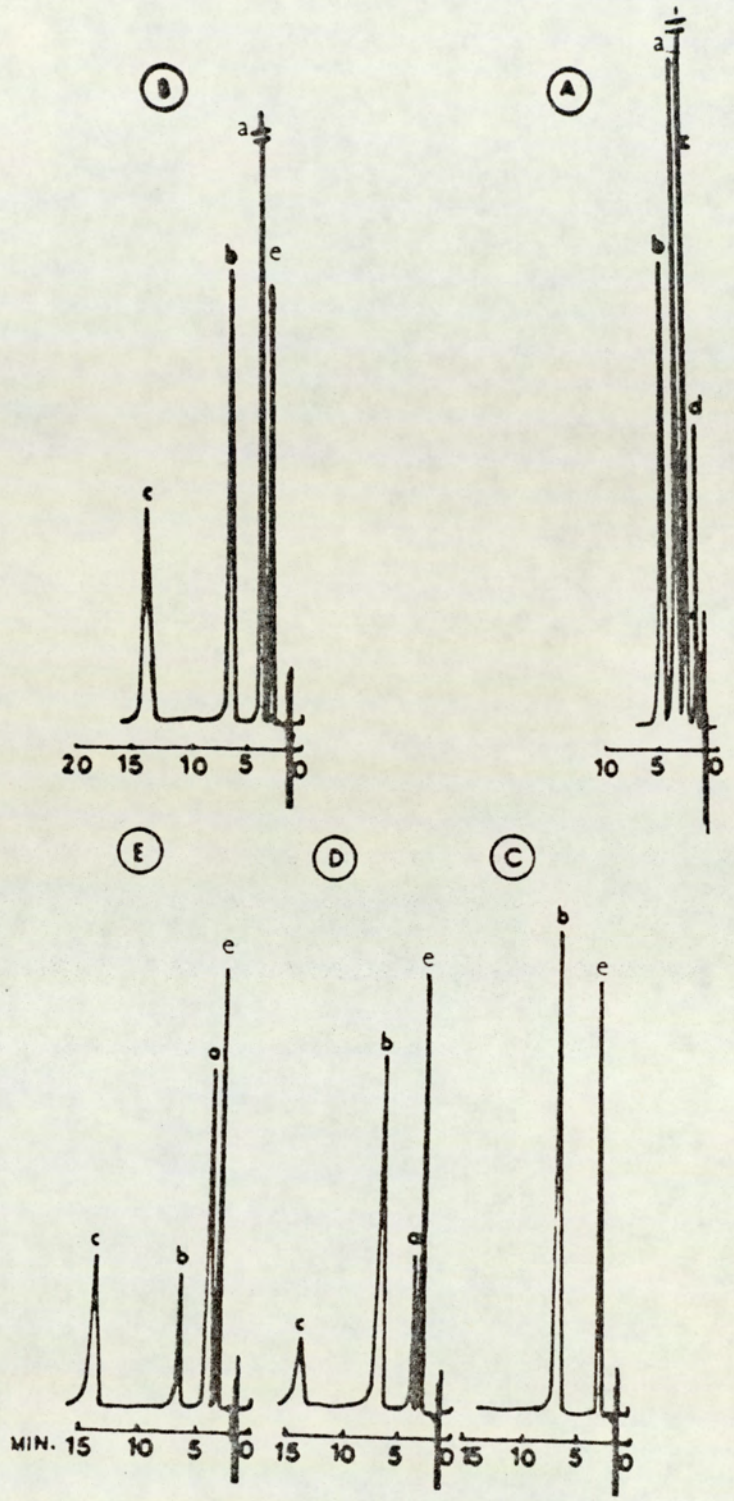


Fig 5.26 High-performance liquid chromatography of O-acetylpropranolol in buffered-DMF (A) and in 1.42×10^{-2} M buffered-DTAB (B-E) pH 7.4 at 37°C.

Mobile Phase				Peak	Solute Components
A	MeCN : H_3PO_4 Et_2NH : H_2O 65 : 0.1 : 0.2 : 34.7 pH = 2.5			a	Propranolol
				b	O-acetylpropranolol
B	MeCN : H_3PO_4 : H_2O 42 : 2 : 56 pH 1.7			c	N-acetylpropranolol
				d	Ethylparaben
C		D	E	e	Methylparaben
t = 0 min.		t = 30 min.	t = 120 min		

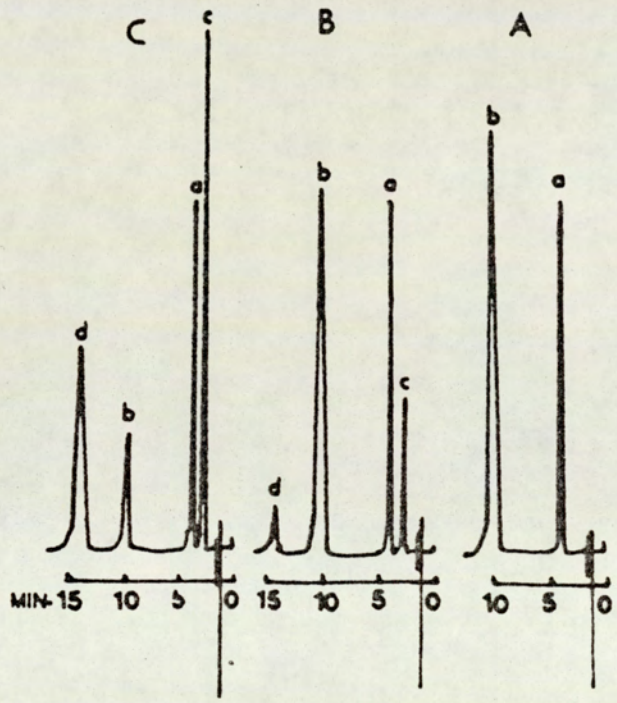


Fig 5.27 High-performance liquid chromatography of O-hexanoylpropranolol in 1.42×10^{-2}M DTAB in buffer pH 7.4 at 37°C (initial concentration 0.2 mM)

Mobile Phase			Peak	Solute Component
MeCN : H_3PO_4 : H_2O 55 : 2.5 : 42.5 pH 1.7			a	Butylparaben
A	B	C	b	O-hexanoylpropranolol
t = 0 min.	t = 60 min.	t = 300 min.	c	Propranolol
Column 10 cm x 4.6 mm Hypersil -ODS Flow rate 1 ml/min. Detection 290 nm.			d	N-hexanoylpropranolol

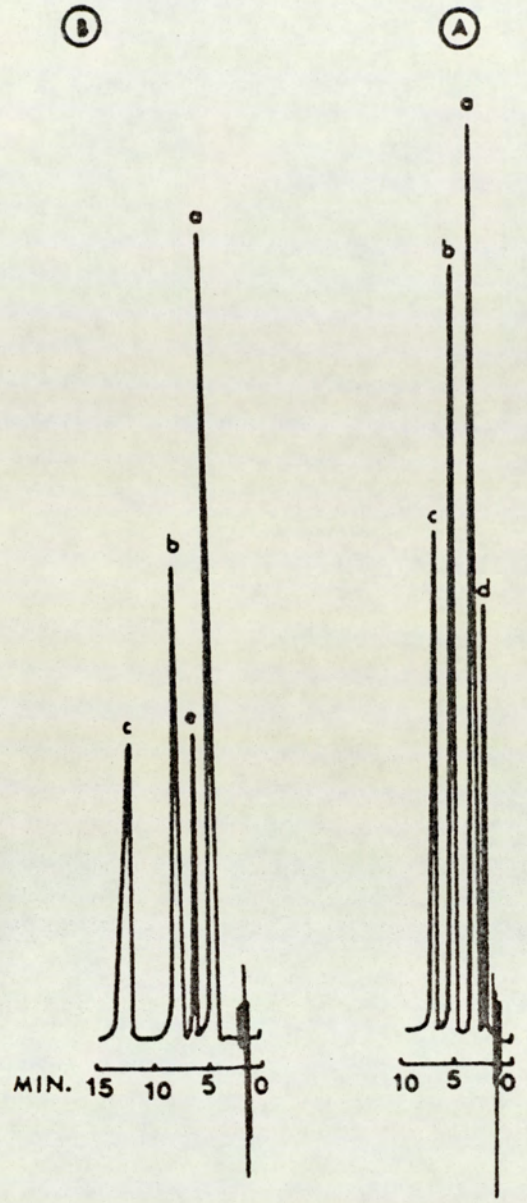


Fig 5.28 High-performance liquid chromatography of O-acetylpropranolol in buffered-surfactant pH 7.4 at 37°C. (initial concentration 0.2 mM)

Solvent Composition	Peak	Solute Component
A 2×10^{-2} M buffered - SLS pH 7.4	a b c d e	Propranolol O-acetylpropranolol N-acetylpropranolol Ethylparaben Propylparaben
B 2×10^{-2} M buffered - Tween 80 pH 7.4		

Mobile phase	MeCN : H_3PO_4 : H_2O
A	48 : 2.5 : 49.5 pH 1.7
B	45 : 3 : 52 pH 1.6

Column 10 cm x 4.6 mm, Hypersil-ODS
Flow rate 1ml/min. Detection 290 nm.

5.3.1.10.1 EFFECT OF DTAB CONCENTRATION ON THE DEGRADATION OF O-ACETYL AND O-HEXANOYL PROPRANOLOL AT pH 7.4 AT 37 °C

The catalytic effect of the cationic micelles of dodecyltrimethylammonium bromide (DTAB) on the degradation rate constants of O-acetyl and O-hexanoyl propranolol are summarized in Table 5.17, and the high-performance liquid chromatograms showing these effects are displayed in Figures 5.26 and 5.27.

The accelerated degradation rates of the two esters in the presence of micelles of DTAB in buffer pH 7.4, μ = 0.5 M at 37°C (Fig 5.29.a & b) are first-order reactions. The hydrolysis rate constant (k_1) of both esters is shown not to be affected by such influences, while the rearrangement rate constants (k_2) were considerably enhanced (Fig 5.30 a & b). Increases in the surfactant (DTAB) concentration increases the rearrangement rate constant (k_2) which results in decrease of the k_1/k_2 ratios until the surfactant concentration reaches the CMC value. Further increase in the surfactant concentration after the CMC appears to have little effect on both the reaction rate constants and on the k_1/k_2 ratio (Figures 5.29 a & b, 5.30 a & b and 5.31 a & b).

It has been postulated that the micellar stern layer is similar to a concentrated ionic solution [*Romsted 1977 (278)*]. The enhanced concentration of the buffer hydroxyl ion in the stern layer of the cationic micelle will favour formation of the free base. The tetrahedral intermediate for the rearrangement process [Fig 5.2 (I)] will thus proceed to product, even though the analytical concentration of the hydroxyl ion in the solution may be low [*Cipiciani et al 1983 (279)*].

Table 5.17 reveals that variations in the substrate structures have a profound influence on the magnitude of micellar catalysis. The degradation of O-hexanoylpropranolol is catalysed by the presence of micellar DTAB significantly more than is the acetyl ester.

DTAB Concentration M x 10^2	$k_1 + k_2 \times 10^3$ (min^{-1})		$k_1 \times 10^3$ (min)		$k_2 \times 10^3$ (min^{-1})		k_1/k_2	
	O.A.P.	O.H.P.	O.A.P.	O.H.P.	O.A.P.	O.H.P.	O.A.P.	O.H.P.
0.00	7.330	0.186	6.716	0.180	0.610	0.006	12.00	30.00
0.52	8.650	2.960	6.446	0.187	2.204	2.773	2.930	0.067
0.78	15.330	4.230	6.243	0.208	9.087	4.022	0.687	0.052
1.04	20.050	4.691	6.167	0.201	13.883	4.490	0.444	0.045
1.30	23.680	5.138	6.117	0.193	17.570	4.945	0.348	0.039
1.42	25.940	5.218	6.356	0.192	19.590	5.026	0.325	0.038
1.495	26.540	5.401	6.403	0.198	20.317	5.203	0.315	0.038
1.56	25.980	5.439	6.280	0.193	19.700	5.246	0.319	0.037
1.69	24.960	5.483	6.118	0.194	18.840	5.289	0.325	0.037
1.82	24.430	5.487	6.073	0.197	18.360	5.290	0.331	0.037

Table 5.17 Effect of dodecyltrimethylammonium bromide (DTAB) concentration on the degradation of propranolol esters [acetyl (O.A.P) and hexanoyl (O.H.P)] in buffer pH 7.4, μ = 0.5M at 37°C

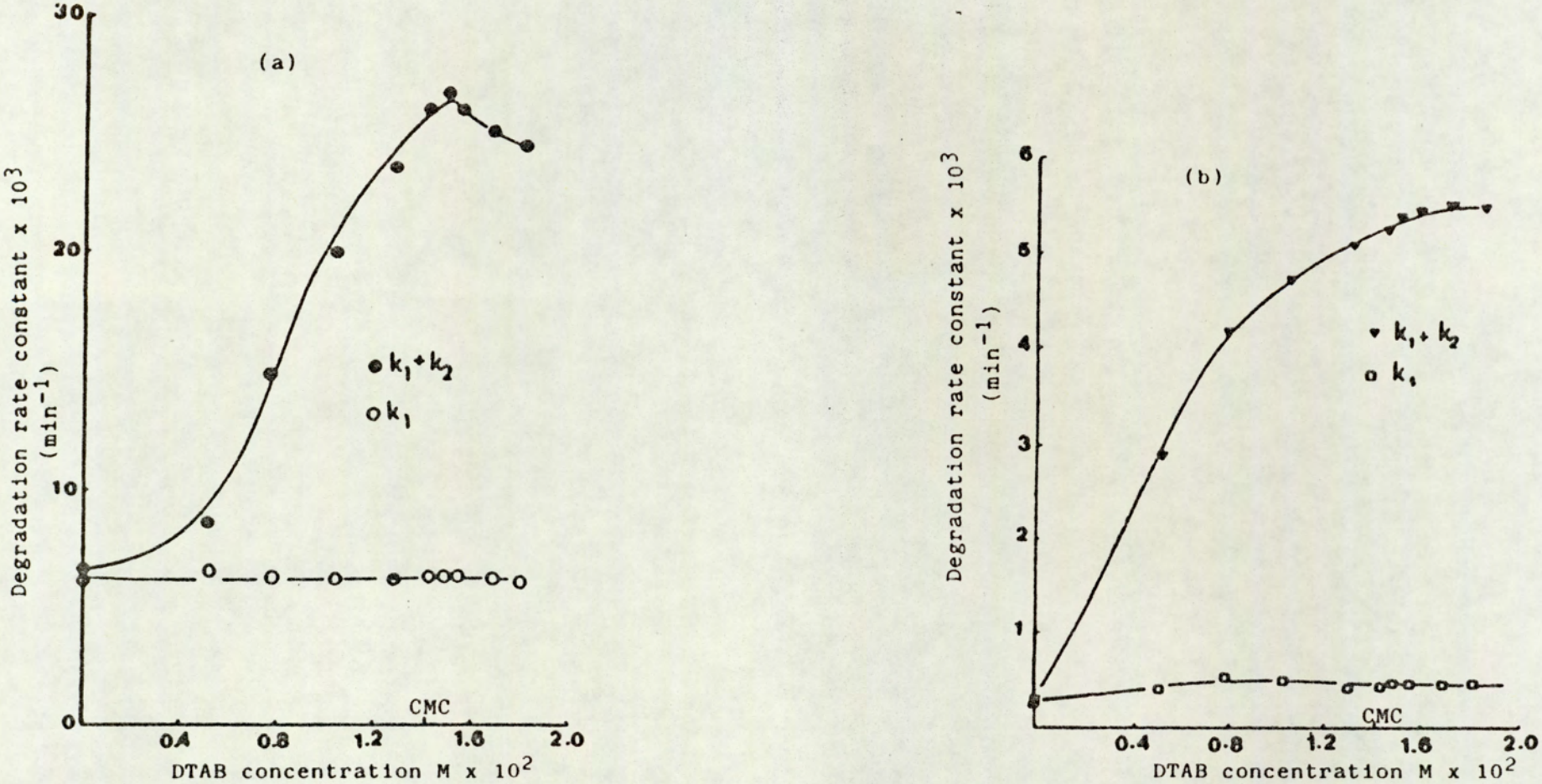


Fig 5.29 Effect of dodecyltrimethylammonium bromide (DTAB) on the degradation of O-acetyl (a) and O-hexanoyl propranolols (b) in buffer pH 7.4, μ = 0.5M at 37°C.

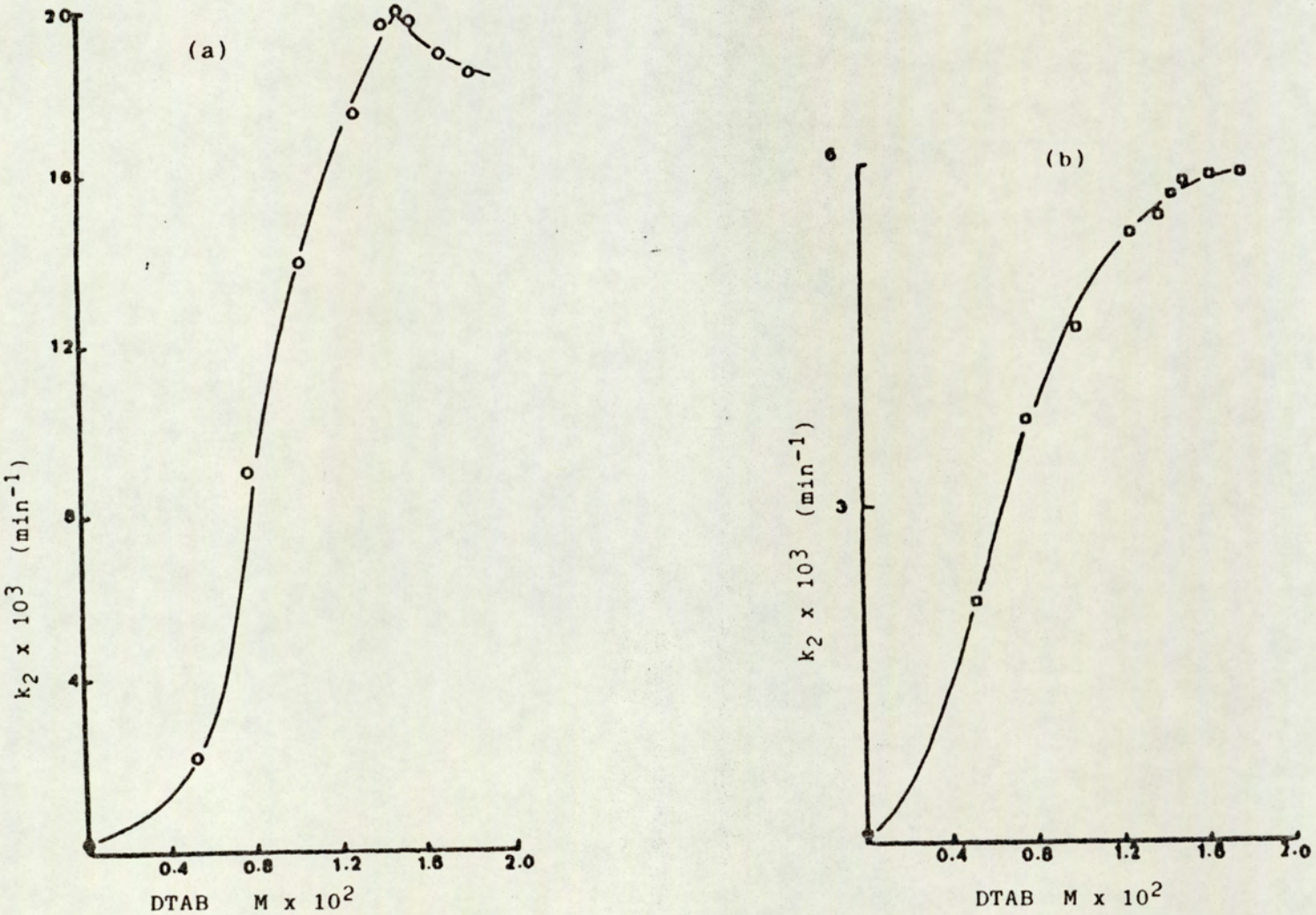


Fig 5.30 Effect of DTAB concentration on the rearrangement rate constants (k_2) of propranolol esters [acetyl (a) and hexanoyl (b)] in buffer pH 7.4 μ = 0.5M at 37°C.

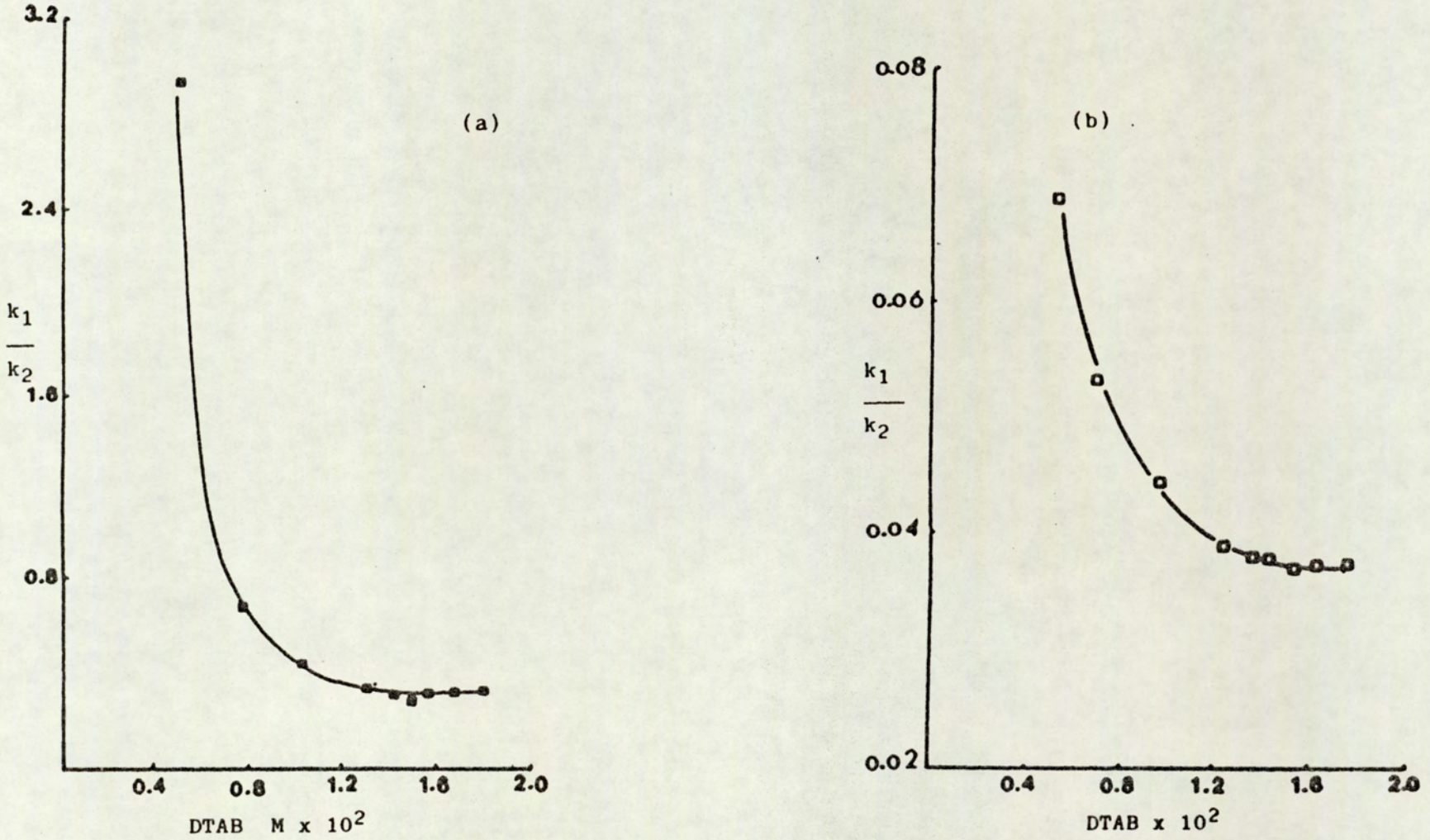


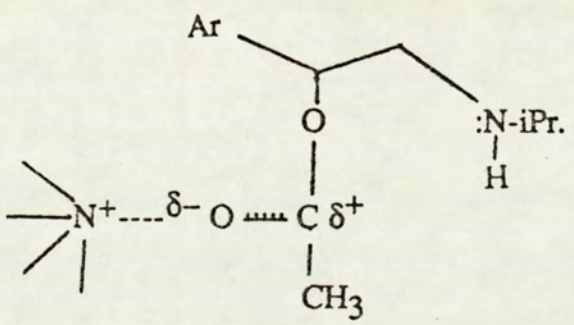
Fig 5.31 Effect of DTAB concentration on k_1/k_2 ratio of the degradation of propranolol esters [O-acetyl (a) and O-hexanoyl (b)] in buffer pH 7.4 μ = 0.5M at 37°C.

Thus at a DTAB concentration equivalent to the CMC (1.42×10^{-2}M), the ratio of the observed rate constant ($k_{obs.}$) to that at zero concentration of the surfactant (k_o) for the hexanoyl and acetyl esters were 28 and 3.5 respectively. This is probably due to an increase in the magnitude of substrate-micelle binding with the increasing chain length of the ester.

These results appear to obey the general rule that the more hydrophobic the substrate the more pronounced the micellar catalysis [*Behme et al 1965 (280); Menger and Portnoy 1967 (281); Romsted and Cordes 1968 (282)*].

5.3.1.10.2 EFFECT OF TETRAMETHYLAMMONIUM CHLORIDE CONCENTRATIONS ON THE DEGRADATION OF O-ACETYLPROPRANOLOL IN pH 7.4 AT 37 °C

The effect of tetramethylammonium chloride (TAC) concentrations on the specific rate constants for the degradation of O-acetylpropranolol in Britton-Robinson buffer solution pH 7.4, μ = 0.5M, at 37°C is summarized in Table 5.18. The results show a small increase in the overall degradation rate constant ($k_1 + k_2$) as the quaternary salt is added to the system, further additions caused negligible further changes due to the lack of micellar structure in those systems although the k_1/k_2 ratio continuously decreased. The initial increase in the overall degradation rate constant may be due to catalysis of the rearrangement process by the quaternary ammonium group of TAC, which can be illustrated as follows:



where Ar = Naphthoxymethyl

TAC Concentration M x 10^2	$k_1 + k_2 \times 10^3$ (min^{-1})	$k_1 \times 10^3$ (min^{-1})	$k_2 \times 10^3$ (min^{-1})	k_1/k_2
0.0	7.590	6.988	0.602	12.0
1.7	8.732	7.884	0.848	9.3
2.5	8.649	7.673	0.976	7.8
5.0	8.570	7.336	1.234	5.9
7.5	8.324	7.294	1.389	5.3
10.0	8.270	6.864	1.406	4.8

Table 5.18 Effect of tetramethylammonium chloride (TAC) concentration on the stability of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37°C

Fig 5.32 a & b shows the influence of TAC concentrations on the overall degradation rate constants and k_1/k_2 ratios of O-acetylpropranolol in pH 7.4 at 37°C.

5.3.1.10.3 EFFECT OF S.L.S. CONCENTRATION ON THE DEGRADATION OF O-ACETYLPROPRANOLOL IN pH 7.4 AT 37 °C

The significant retardation of the degradation rate constants of O-acetylpropranolol, both by hydrolysis and rearrangement caused by the anionic micelles of sodium lauryl sulphate (SLS) has been demonstrated by plotting the degradation rate constants against the surfactant concentration and results are illustrated in Fig 5.33.a.The decrease in the reaction rate (k_1+k_2) parallels the increase of the basicity of the ester in this system due to the increase of the ester pKa. This phenomenon had been previously discussed in Section 4.3.3 , for the effect of micellar SLS on the pKa of O-acetylpropranolol. Table 5.19 shows the effect of the anionic surfactant (SLS) concentration on the reaction rate constant of the degradation of O-acetylpropranolol in pH 7.4 at 37°C. Increasing the surfactant concentrations resulted in reducing the degradation rate constants for both the hydrolysis and rearrangement processes, although the rate of rearrangement is more sensitive and is largely responsible for the effect of the SLS concentration on the k_1/k_2 ratio which is shown in Fig 5.33.b.

5.3.1.10.4 EFFECT OF TWEEN 80 CONCENTRATION ON THE DEGRADATION OF O-ACETYLPROPRANOLOL IN pH 7.4 AT 37 °C

A relatively small acceleration in the degradation of O-acetylpropranolol by the neutral associations of Tween 80 molecules in Britton-Robinson buffer pH 7.4, μ = 0.5M, at 37°C was observed. These are recorded in Table 5.20 and are plotted in Fig 5.34.

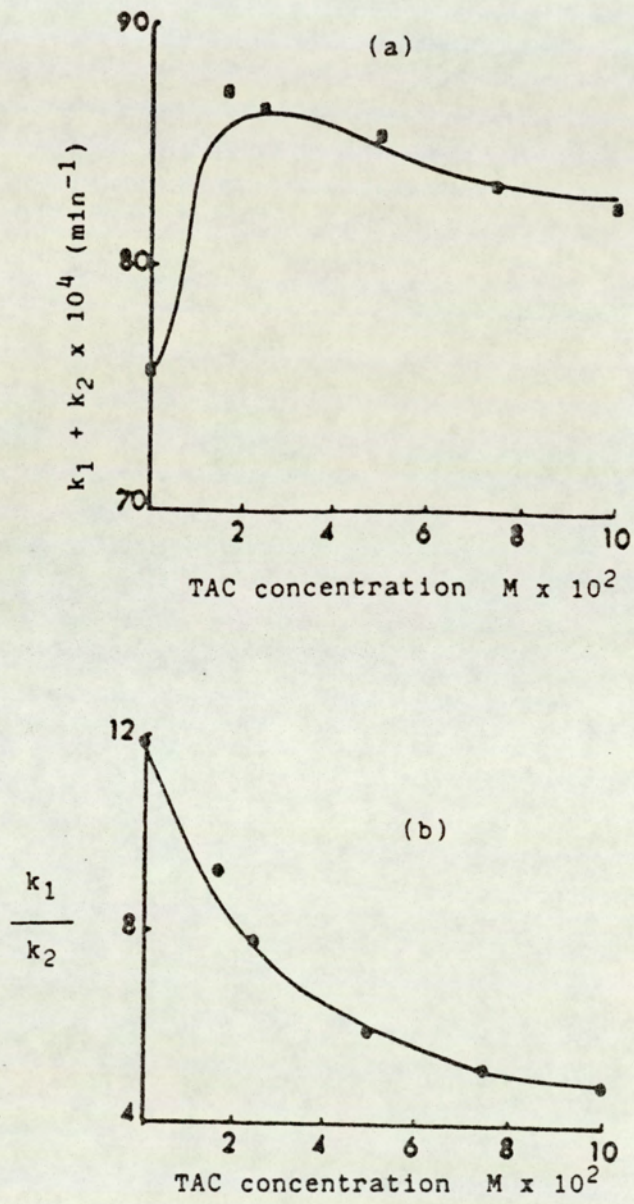


Fig 5.32 Effect of tetramethylammonium chloride (TAC) concentration on the degradation rate constant (a) and on the k_1/k_2 ratio of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37°C.

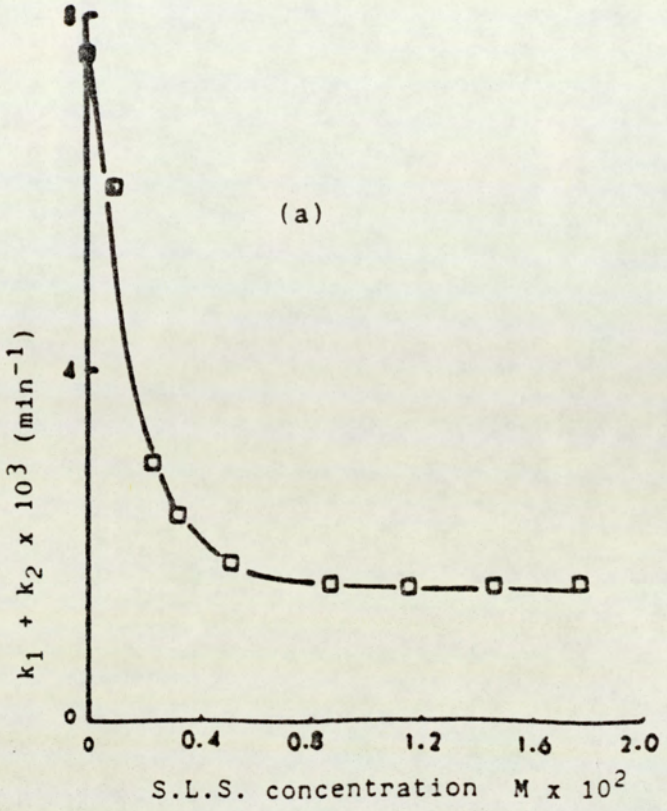


Fig 5.33 (a) Effect of sodium lauryl sulphate (SLS) concentration on the degradation of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37°C.

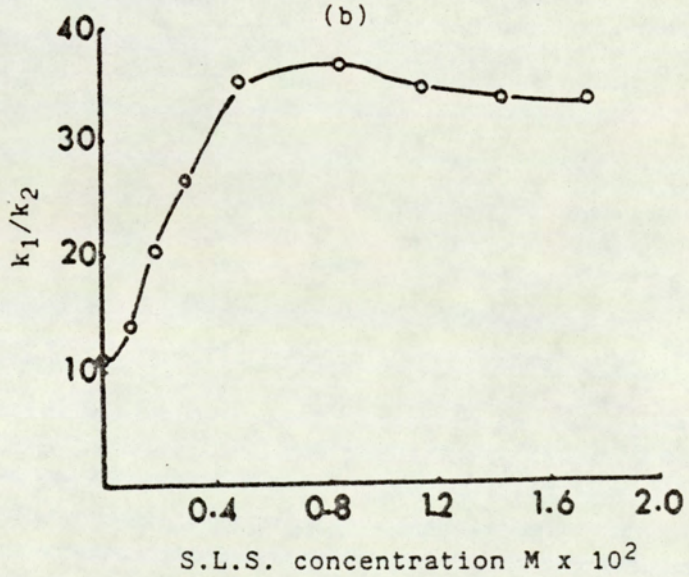


Fig 5.33 (b) Effect of S.L.S. concentration on the k_1/k_2 ratio of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37°C.

SLS Concentration M x 10^3	$k_1 + k_2 \times 10^3$ (min^{-1})	$k_1 \times 10^3$ (min^{-1})	$k_2 \times 10^3$ (min^{-1})	k_1/k_2
0.0	7.452	6.811	0.6410	11.0
1.0	5.994	5.599	0.3940	14.0
2.0	2.767	2.639	0.1275	21.0
3.0	2.260	2.178	0.0822	26.5
5.0	1.716	1.669	0.0478	35.0
8.5	1.525	1.484	0.0407	36.5
11.5	1.459	1.418	0.0405	35.0
14.5	1.454	1.412	0.0415	34.0
17.5	1.498	1.455	0.0428	34.0

Table 5.19 Effect of sodium lauryl sulphate (SLS) concentration on the stability of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37°C

Tween 80 Concentration w/v	$k_1 + k_2 \times 10^4$ (min^{-1})	$k_1 \times 10^4$ (min^{-1})	$k_2 \times 10^4$ (min^{-1})	k_1/k_2
0	76.286	69.652	6.630	10.5
0.005	79.089	71.704	6.385	9.7
0.010	78.753	72.028	6.725	10.7
0.020	77.854	71.614	6.240	11.5
0.030	76.436	70.034	6.402	11.0

Table 5.20 Effect of Tween 80 concentration on the degradation of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37°C

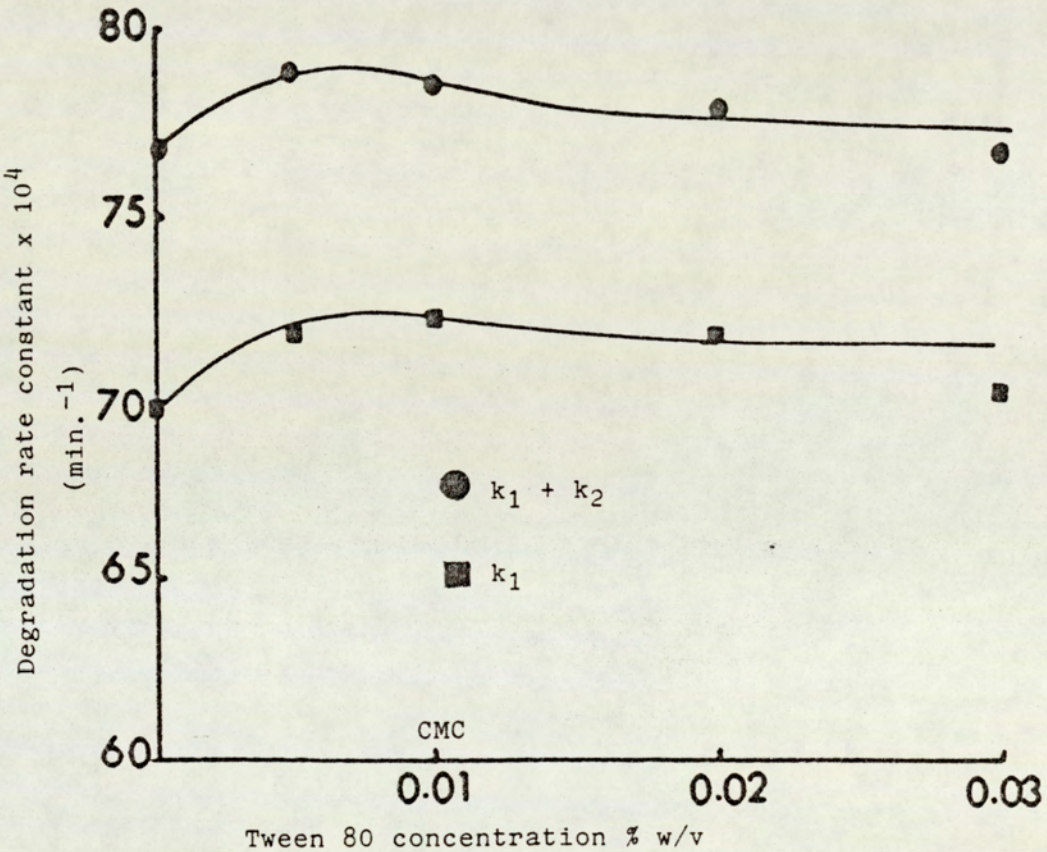


Fig 5.34 Effect of Tween 80 concentration on the degradation of O-acetylpropranolol in buffer pH 7.4, μ = 0.5M at 37^{o}C.

5.3.2. ENZYMATIC DEGRADATION OF PROPRANOLOL PRODRUGS

The rate of enzyme catalysed reactions is usually much faster than the same reaction catalysed by non-biological catalysts. Enzymes are usually very specific and one enzyme normally catalyses only one particular type of reaction under very limited chemical and physical conditions. There exists a vast number of enzymes each catalysing a specific reaction which frequently form links in a metabolic pathway.

All enzymatic reactions proceed in several steps. If the enzyme reacts with only one substrate, the mechanism can be represented as follows [*Briggs and Haldane 1925 (283)*].

$$S + E \underset{k_{-1}}{\overset{k1}{\rightleftharpoons}} ES \xrightarrow{k2} E + P$$

The enzyme E reacts with the substrate S to form an intermediate enzyme-substrate complex ES. The reaction proceeds within this complex, and the latter then decomposes into the enzyme and the reaction product P.

A number of simplifications are possible in the formulation of the mathematical expression for the rate of transformation of S in this reaction chain. The following expression in the notation of chemical reaction kinetics is valid for this reaction sequence.

$$\frac{dx}{dt} = k_2 \times e. \frac{(a - x)}{(a - x) + \frac{k_{-1} + k_2}{k_1}} \qquad 5.9$$

where a : is the initial concentration of the substrate to be converted

x : is the quantity of the substrate converted

k : is the rate constant of the reaction

e : is the enzyme concentration

dx/dt : is the rate of increase in the concentration of P

Using square brackets to denote the concentration of the enzyme and the substrate and substituting $(k_{-1} + k_2)/k_1 = k_m$, one can obtain the Michaelis - Menten equation [*Michaelis and Menten 1913 (284)*] for the reaction rate.

$$v = k \frac{[E] \times [S]}{[S] + k_m} \qquad 5.10$$

where k is the rate constant of the decomposition reaction, k_m is the Michaelis-Menten constant (which is simply defined as the concentration of the substrate that gives half of the maximum velocity) and v is the initial rate of a reaction (the reaction velocity) which must always be used in the determination of k_m because of the known dependence of the reaction velocity on the substrate concentration (which decreases in the course of the reaction).

At high substrate concentration where $[S] \gg k_m$, equation 5.9 becomes $V = k [E]$. The reaction rate therefore reaches a maximum value and hence equation 5.10 can be written as :

$$v = \frac{V [S]}{k_m + [S]} \qquad 5.11$$

k_m and V are two important parameters that are very useful for describing the properties of an enzyme-catalysed reaction. Several method for the determination of the Michaelis-Menten constant (k_m) and V (in one substrate reactions) have been reported [*Lineweaver & Burk 1934 (285), Hofstee 1959 (286) and Wilkinson 1961 (287)*].

One of the most commonly used methods is that of Lineweaver and Burk 1934 (285). The reciprocal form of equation 5.11 is:

$$\frac{1}{v} = \frac{k_m}{V} \times \frac{1}{[S]} + \frac{1}{V} \qquad 5.12$$

A plot of 1/v against 1/[S] yields a straight line with slope equal to k_m/V and intercept = 1/V. k_m is a measure of the substrate-enzyme interaction where the more firmly an enzyme is bound to its substrate, the smaller is the value of k_m.

5.3.2.1. DEGRADATION OF O-ACYL PROPRANOLOLS IN ISOLATED ESTERASE AND IN SERUM

In enzymatic systems, using isolated esterases, the reactivity profiles for O-acyl propranolols were somewhat different. The specificity of the enzyme, coupled with the moderate pH 7.4 used, was such that the competing rearrangement reaction was much inhibited ($k_1 \gg k_2$). Indeed, with ester homologues larger than O-propionyl, no rearrangement was detected at all. The generation of propranolol under these conditions was found to occur at a maximum rate with O-hexanoyl derivative (Fig 5.35) but all compounds showed acceptable release rates for propranolol.

The degradation rates of these esters in 90% rabbit serum were investigated at 37°C. Complete reversion to propranolol was observed with all esters larger than the acetyl derivative as determined by HPLC, and in all cases the degradations could be modelled by first-order kinetics (Fig 5.36 a & b). The susceptibility of the various ester derivatives to undergo enzyme catalysed hydrolysis in 90% rabbit serum was strongly influenced by the structure of the acyl moiety. This order of susceptibility is the same as that obtained for the isolated esterases.

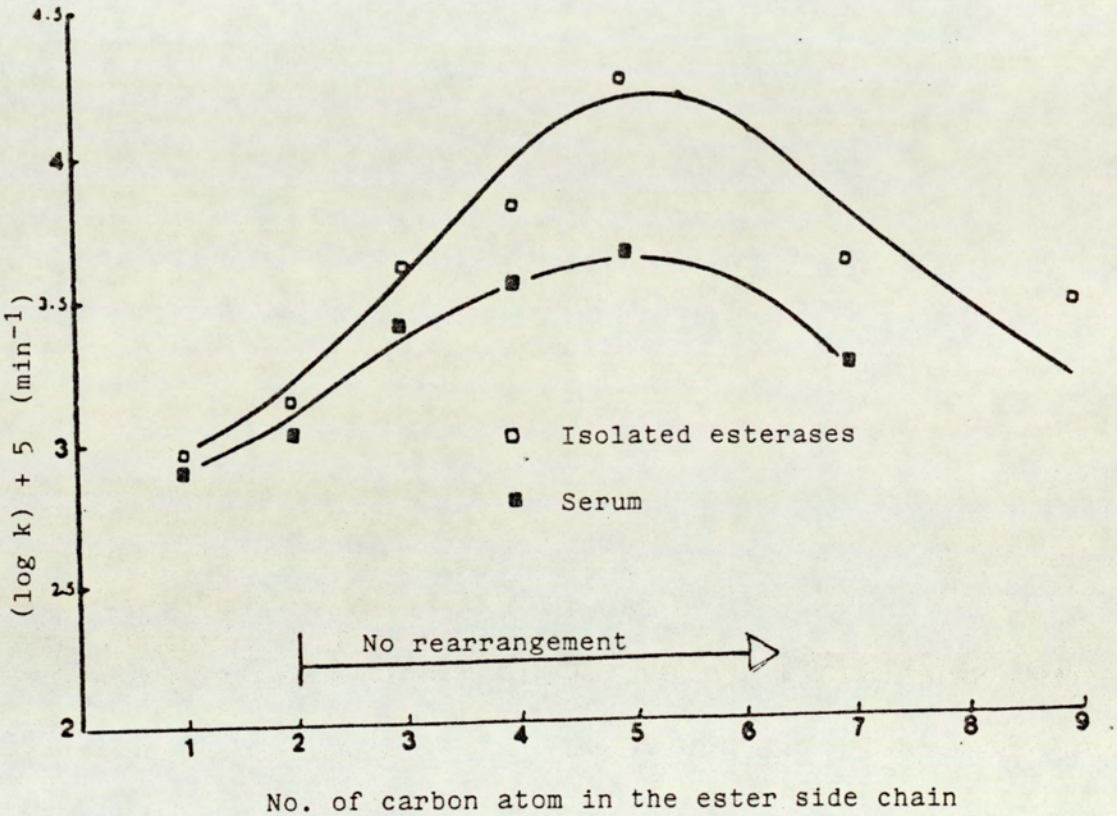


Fig 5.35 Effect of the side chain on the enzyme catalysed degradation of O-acyl propranolols.

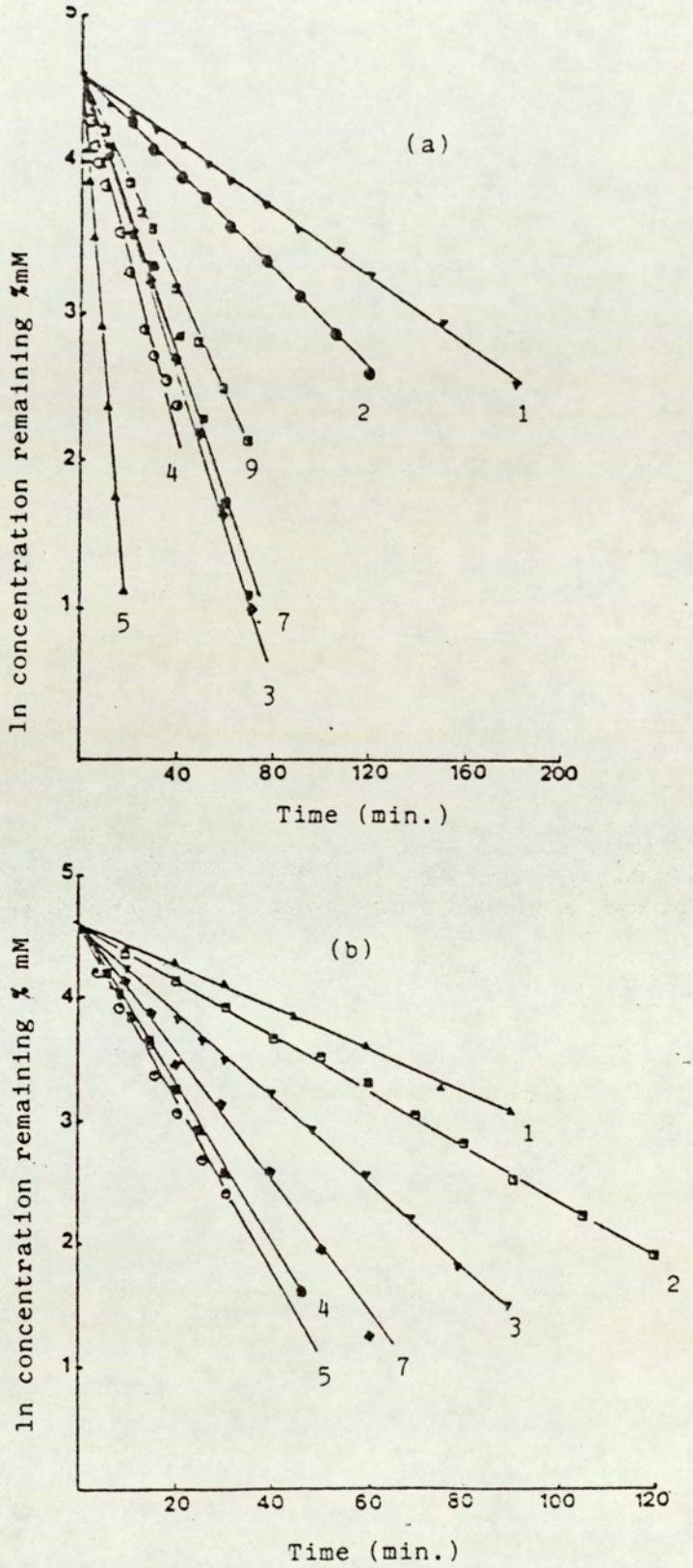


Fig 5.36 First-order plots showing the effect of the side chain on the enzymatic degradation of O-acyl propranolol (a) in isolated esterase (b) in serum. The numbers indicate the number of carbon atoms in the ester side chain.

The degradation rate constants of O-acyl propranolols in isolated esterases and in 90% rabbit serum at 37°C were calculated by linear regression analysis of semilogarithmic plots of concentrations against time (Fig 5.36 a & b) and the hydrolytic data are presented in Table 5.21, plots of such data are shown in Fig 5.35. These results were in agreement with the finding of Neill and Carless 1980 (288) who studied the influence of the side chain of hydrocortisone esters on the enzymatic hydrolysis by carboxylesterase and found that the rate of the ester cleavage varies with the chain length and indicated a similar optimum carbon number for maximum hydrolysis rate.

5.3.2.2. HYDROLYSIS OF O-ACETYLPROPRANOLOL BY RAT SMALL INTESTINE

Natural logarithm of concentration-time profiles for the degradation of O-acetylpropranolol by active and boiled everted small intestines segment of rat are shown in Fig 5.37, the degradation rate of the ester with the active segments was faster than that with the inactive segments. The latter did not differ measurably from that in solutions without intestinal segments. The enhanced degradation rate of the ester by the active segments is characteristic of enzymic process and suggests that the hydrolysis by intestinal tissue is due to esterase (s).

The presence of esterases in the human gastro-intestinal tract is well established [*Dawson and Pryse 1963 (289)*]. It was suggested that on this basis that appreciable hydrolysis of the ester may take place during the absorption of this drug from the gastrointestinal tract.

5.3.2.3 DEGRADATION OF O-ACETYL AND O-PIVALOYL PROPRANOLOLS DURING ABSORPTION

In view of the apparent enzymic nature of the hydrolytic process of O-acetylpropranolol using active intestine segments of rat (section 5.3.2.2.) it was of interest to study the degradations of O-acetyl and O-pivaloyl propranolols during absorption using the everted sac of rat small intestine.

No. of Carbon atoms in the ester side chain	O-acyl propranolols	Degradation rate constants log k + 5 (min^{-1})	
		Esterase 48 units	90% Rabbit Serum
1	Acetyl	2.9825	2.9230
2	Propionyl	3.1608	3.0500
3	Butyryl	3.6343	3.4120
4	Valeryl	3.8350	3.5620
5	Hexanoyl	4.2984	3.6307
7	Octanoyl	3.5984	3.2642
9	Decanoyl	3.4619	-

Table 5.21 Effect of side chain on the enzyme catalysed degradation of O-acyl propranolols.

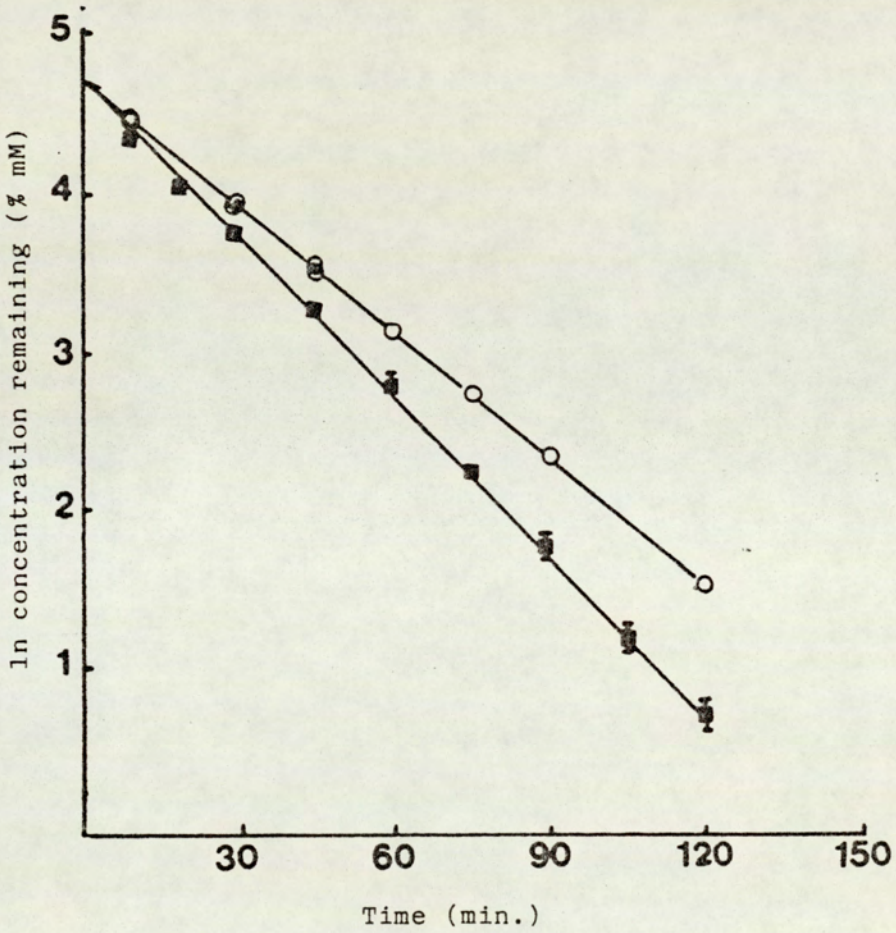


Fig 5.37 First-order plots of the degradation of O-acetylpropronolol by rat small intestine.

O with inactive segments
k = 0.008646 r = 0.9993

■ with active segments
k = 0.01471 r = 0.999

The time course reflecting the degradation of both esters in the mucosal and serrosal compartments of the everted sac of rat small intestine at 37°C are shown in Fig 5.38 a. Semilogarithmic plots of these degradation profiles were found to follow first-order reaction as shown in Fig 5.38 b. The measured rate constants and half-lives are recorded in Table 5.22. The significant increase of the hydrolysis rates of both esters in the mucosal and serrosal compartment (serrosal > mucosal) appear to be due mainly to the presence of esterases in the intestinal epithelium as well as in the gastric mucosa [*Lutwak-mann 1942 (290)*]. Due to the limited capacity of enzyme systems located in the gastro intestinal mucosa [*Levy et al 1967 (270)*], the extent of O-acetyl and O-hexanoyl propranolol hydrolysis is probably more pronounced when the drugs are administered in slowly absorbed form (e.g. sustained-release formulation using drug-ion exchange resin complex).

Parameters	O-acetylpropranolol ($k_1 + k_2$)		O-pivaloylpropranolol	
	Serrosal	Mucosal	Serrosal	Mucosal
$k \times 10^3$ (min^{-1})	44.343	11.6000	21.6670	2.4860
r	0.9990	0.9980	0.9960	0.9995
$t_{1/2}$	16	60	32	143

Table 5.22 Stability of O-acetyl and O-pivaloyl propranolol during absorption using everted sac of rat small intestine at 37°C

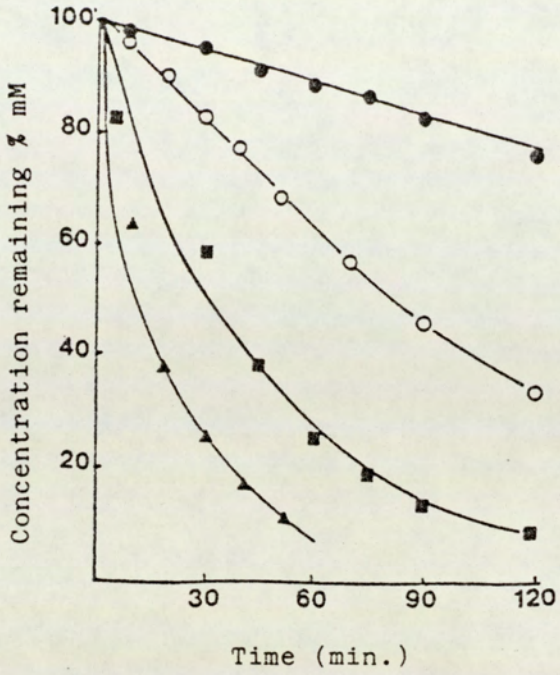


Fig 5.38 (a) Concentration-time profiles for the degradation of O-acetyl and O-pivaloylpropranolol in rat small intestine during absorption.

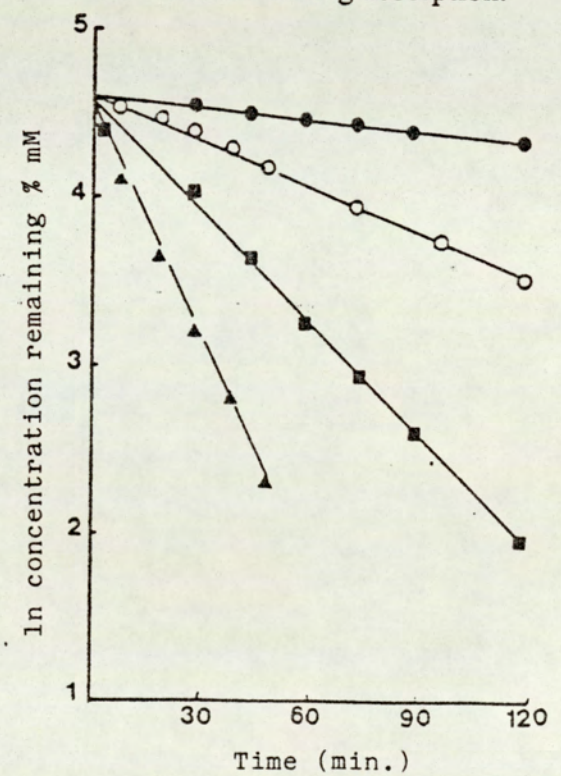


Fig 5.38 (b) First-order plot showing the degradation of the propranolol esters during absorption.

Compound	O-acetylpropranolol		O-pivaloylpropranolol	
	Serrosal	Mucosal	Serrosal	Mucosal
Symbol	▲	O	■	●

5.3.2.4 EFFECT OF THE SUBSTRATE INITIAL CONCENTRATION ON THE ENZYMATIC DEGRADATION OF O-PIVALOYLPROPRANOLOL

The effect of the substrate initial concentration on the enzymatic reaction velocity (v) of O-pivaloylpropranolol at 37°C is recorded in Table 5.23. At low substrate concentration, only small amounts of the enzyme are in the form of enzyme-substrate complex, but as the substrate concentration increases the reaction velocity increased until a point is reached when saturation of the enzyme occurs. Any further increase in the substrate concentration will not therefore lead to an increase in the velocity of the reaction (Fig 5.39 a). These data are plotted in accordance with the Lineweaver-Burk equation (equation 5.11) and are displayed in Fig 5.39 b. The parameters k_m and V are obtained and recorded in the following table.

k_m (mM)	V (min^{-1})
0.209	2.527×10^{-3}

5.3.3 DEGRADATION OF N,O-DIACYL PROPRANOLOLS

The degradation of N,O-diacetylpropranolol (I) and N-ethoxycarbonyl,O-acetylpropranolol (II) in alkaline-DMF pH 13 at 37°C follows first-order kinetics and can be represented by the following kinetic model:

$$A \xrightarrow{k1} B \xrightarrow{k2} C$$

where A - represents Diacyl propranolols (I and II)

B - the intermediate (N-acyl derivatives)

C - propranolol in case of I and 4-naphthoxymethyl,N-isopropyl oxazolidin-2-one in case of II

k_1 & k_2 - degradation rate constants

O-pivaloylpropranolol initial concentration [S] mM	Reaction Velocity v x 10^4 (min^{-1})	r
0.60	18.050	0.9999
0.45	17.860	0.9980
0.30	15.215	0.9960
0.24	13.467	0.9993
0.18	11.572	0.9996
0.15	10.500	0.9991
0.075	6.688	0.9990

Table 5.23 Effect of substrate concentration on the enzymatic reaction velocity of O-pivaloylpropranolol.

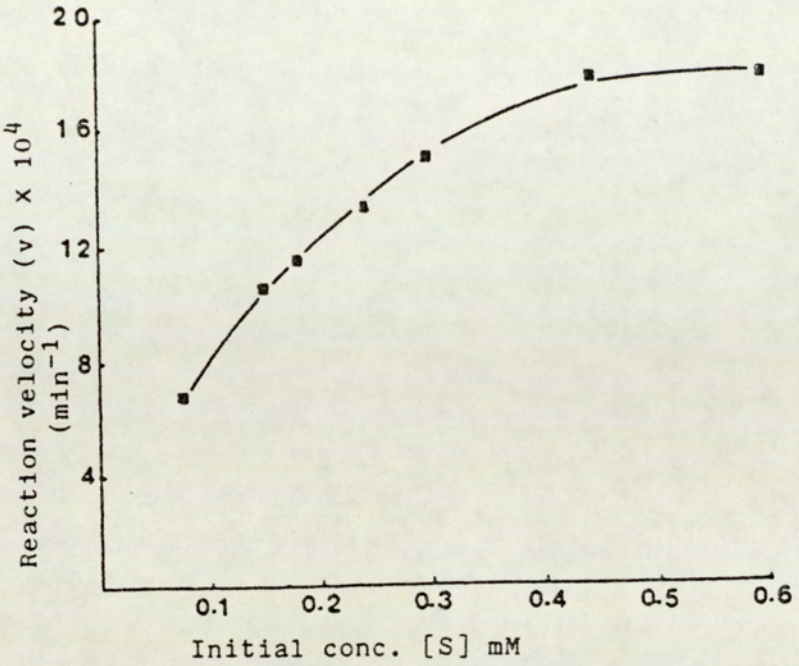


Fig 5.39 (a) Effect of the substrate concentration on the enzyme catalysed hydrolysis of O-pivaloylpropranolol.

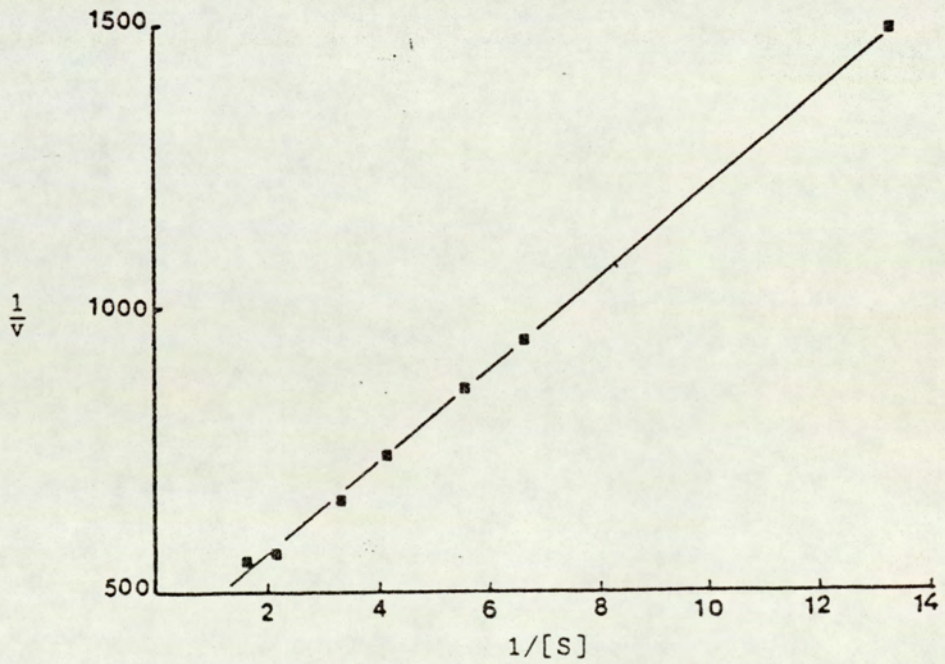


Fig 5.39 (b) Lineweaver-Burk plot for the hydrolysis of O-pivaloylpropranolol in presence of 120 units of esterase in 10% buffered-DMF pH 7.4 at 37°C.

This model was used to extract rate constants in both cases. The degradation pathway as suggested by the model are:

a) from A $\rightarrow$ B direct hydrolysis

b) from B $\rightarrow$ C direct hydrolysis to propranolol (in case of I and rearrangement to the oxazolidinone derivative (in case of II).

The rate constants k_1 and k_2 can be determined using the following equations:

$$At = Ao \cdot e^{-k_1 t}$$

or

$$\ln At = \ln Ao - k_1 t \qquad 5.13$$

and

$$Bt = \frac{A_o k_1}{k_1 + k_2} \cdot \left[e^{-k_1 t} - e^{-k_2 t} \right] \qquad 5.14$$

At later time points the exponential term representing the appearance of B falls to zero. Equation 5.14 becomes a mono-exponential decrease due solely to the loss of B from the system.

$$\ln B_t = \ln \left[\frac{Aok1}{k2 - k1} \right] - k_2 t \qquad 5.15$$

where A = initial concentration of the reactant (I and II)

At = concentration of the undecomposed I and II at time t

Bt = concentration of the intermediate (N-acetylpropranolol in case of I and N-ethoxy carbonylpropranolol in case of II) at time t.

k_1 = disappearance rate constants of I and II

k_2 = degradation rate constants of the intermediate (B)

t = time

High performance liquid chromatograms following the degradation of I and II (0.5mM each) in 50% DMF in 0.5M NaOH at 37°C are shown in Fig 5.40 a and b. The initial trace (t = o) indicates the presence of the starting compound (I or II) and the internal standard (ethylparaben). The chromatograms also reveal that under the experimental conditions, I and II gradually break down forming the N-acyl intermediate (N-acetylpropranolol and N-ethoxycarbonylpropranolol from I and II respectively) which in turn break down to yield the final stable product (propranolol and 4-naphthoxymethyl-N-isopropyl oxazolidin-2-one in case of I and II respectively). The degradation schemes for I and II are shown in Fig 5.41a and b.

The reaction profiles showing the fate of the reactants (I and II), the intermediate N-acyl propranolols (N-acetyl propranolol and N-ethoxy carbonylpropranolol) and the products (propranolol and the oxazolidinone derivative) are shown in Fig 5.42 a and b.

It is apparent in the profile that under the experimental conditions of alkaline-DMF (50% v/v, 0.25 M NaOH, 37°C) maximum levels (94 - 97%) of N-acetylpropranolol and N-ethoxycarbonylpropranolol (the intermediates) are formed in 5 and 30 hours respectively. The disappearance of both I and II is presented as a semi-logarithmic plot in Fig 5.43, the linearity confirms the overall first-order degradation expected by equation 5.12.

The measured slopes thus yield the disappearance rate constants of I and II. The degradation rate constants for the degradation of I and II are recorded in Table 5.24.

The slower rate of disappearance of II compared to that of I can possibly be explained by the effect of the more bulky group (ethoxy group) in the carbamate chain which may inhibit the formation of the tetrahedral intermediate during the hydrolysis process of the ester.

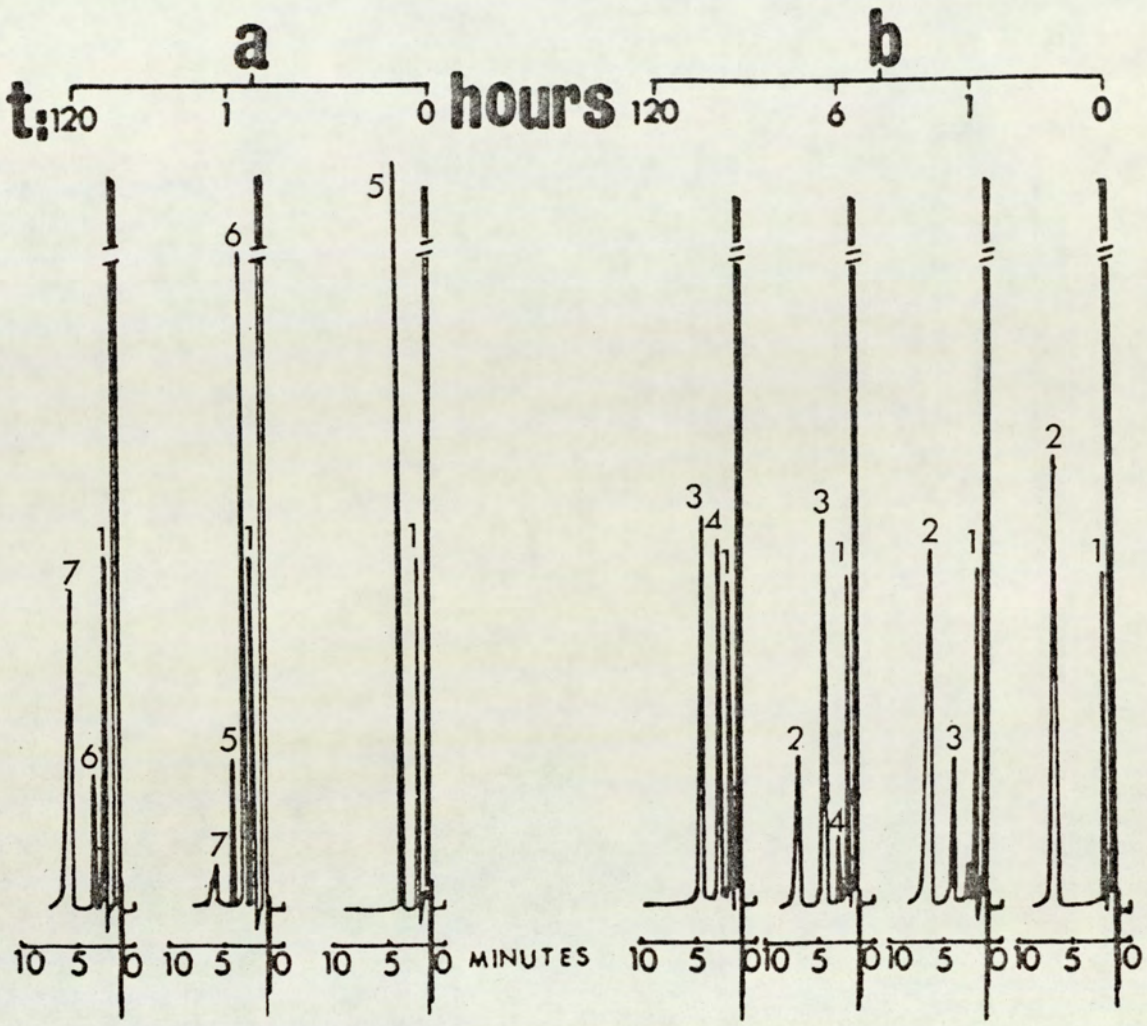


Fig 5.40 High-performance liquid chromatograms showing the degradation of N, O-diacetylpropranolol (a) and N-ethoxycarbonyl, O-acetylpropranolol (b) in alkaline DMF (50% v/v, 0.25 M NaOH) at 37°C.

Peaks	Component	Mobile Phase
1	Ethylparaben (I.S)	MeCN : Et_2NH : H_3PO_4:H_2O
2	N-ethoxycarbonyl, O-acetylpropranolol	(65 : 0.1 : 0.1 : 34.8) pH 2.5
3	N-ethoxycarbonyl-propranolol	Flow rate : 1 ml/min.
4	4-naphthoxy, N-isopropyl-oxazolidinone	Column : 10 cm x 4.6 mm Hypersil-ODS
5	N,O-diacetylpropranolol	Detection : 290 nm
6	N-acetylpropranolol	
7	Propranolol	

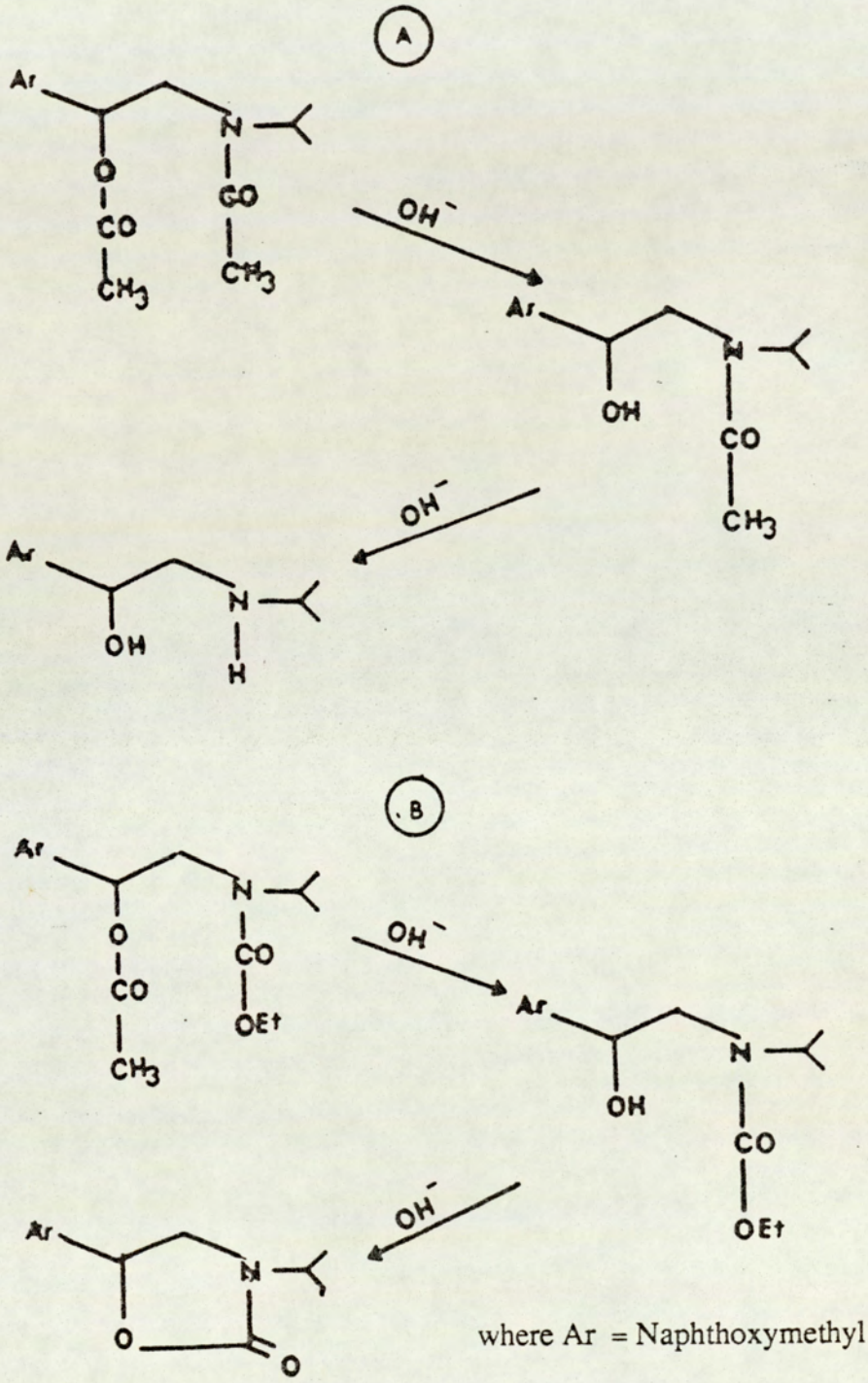


Fig 5.41 Models showing the possible route of degradation of N, O-diacetylpropranolol (A) and N-ethoxy-carbonyl, O-acetylpropranolol (B) in alkaline DMF (50% v/v, 0.25M NaOH) at 37°C.

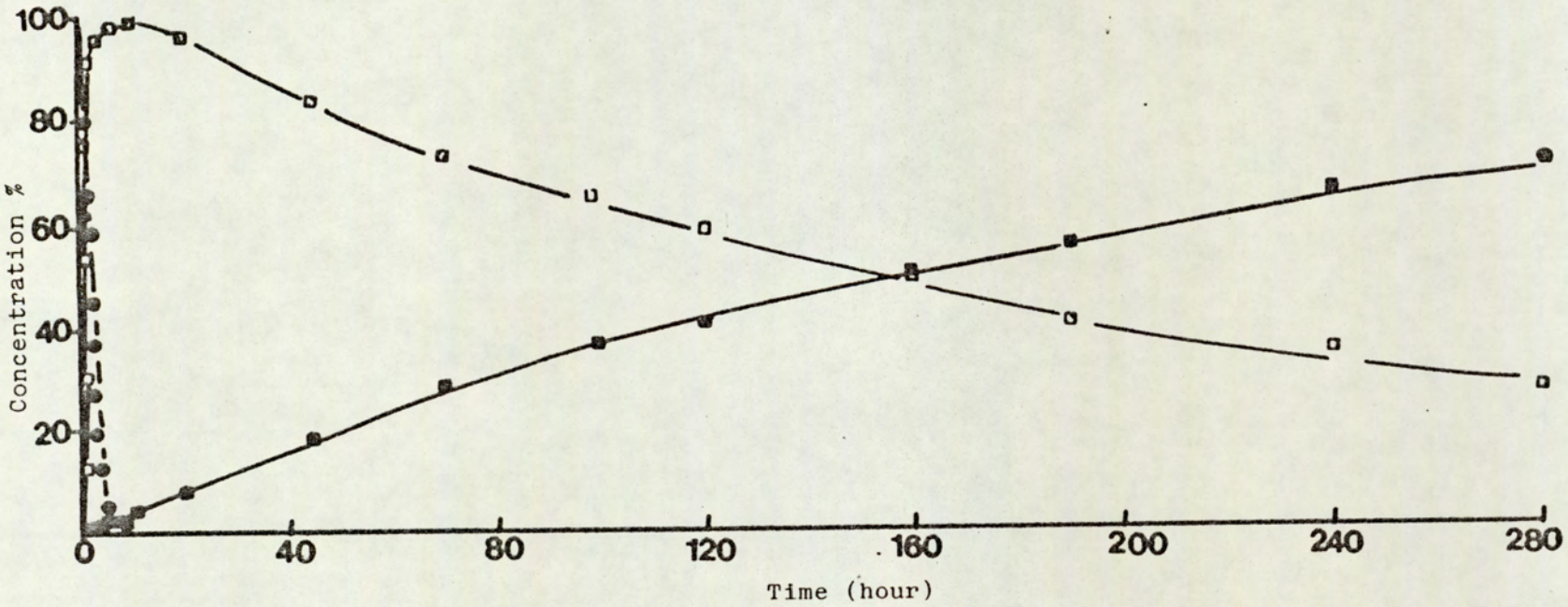


Fig 5.42 (a) Concentration-time profiles for the degradation of N, O-diacetyl-propranolol in alkaline DMF (50% v/v, 0.25 M NaOH) at 37°C.

Symbol	Compound
O	N, O-diacetylpropranolol
□	N-acetylpropranolol
■	Propranolol

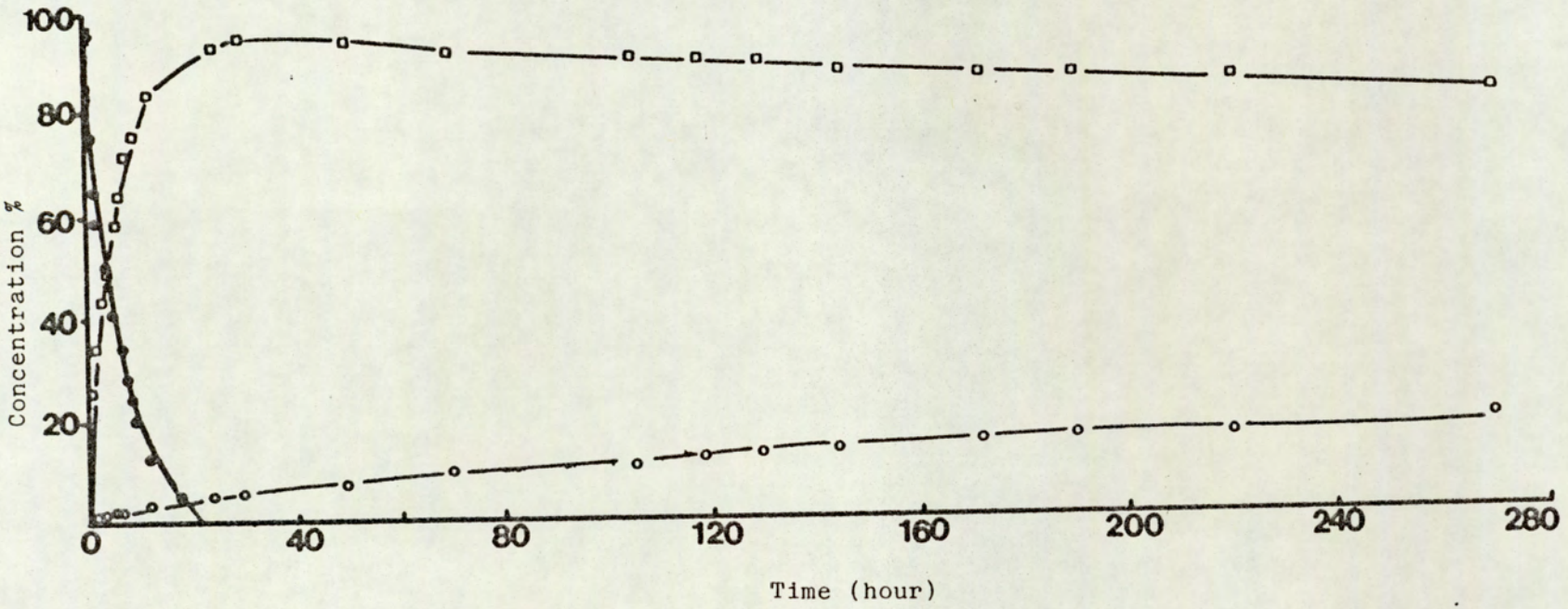


Fig 5.42 (b) Concentration-time profiles for the degradation of N-ethoxycarbonyl - O-acetylpropranolol in alkaline-DMF (50% v/v, 0.25 M NaOH) at 37°C.

Symbol	Compound
●	N-ethoxycarbonyl_O-acetylpropranolol
□	N-ethoxycarbonylpropranolol
O	4-naphthoxy, N-isopropyl, oxazolidin-2-one

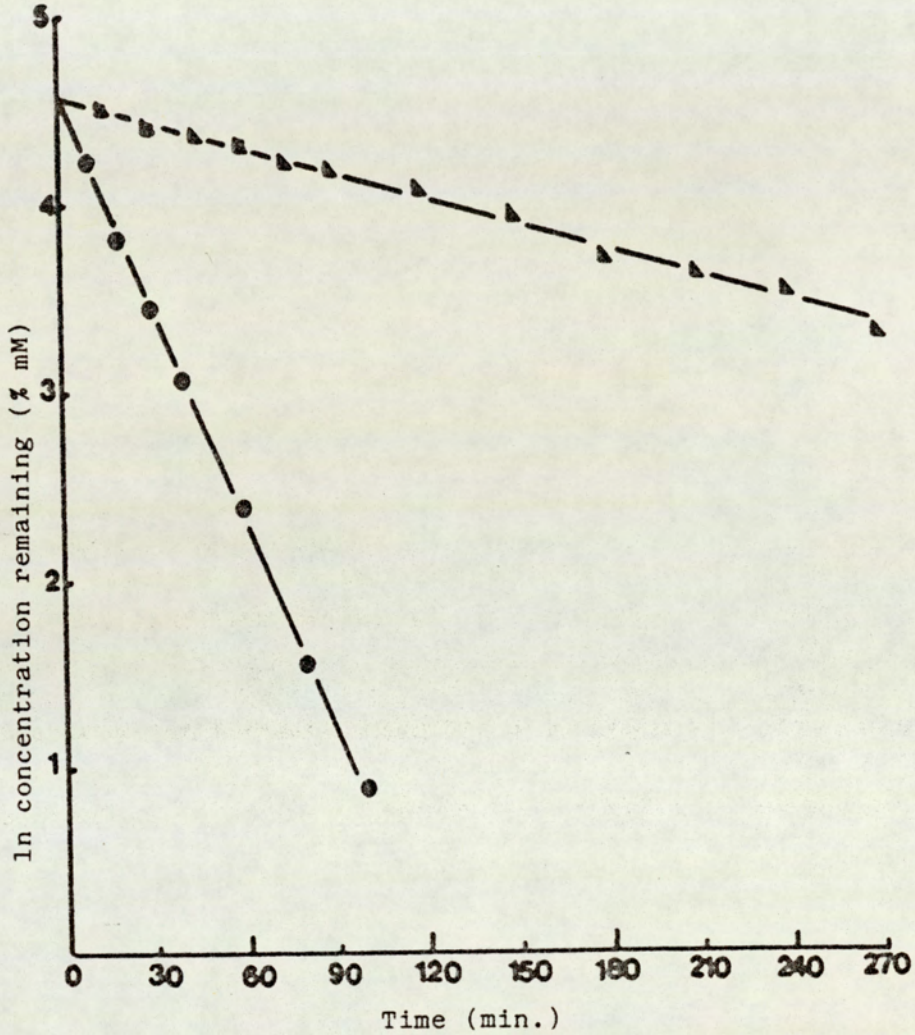


Fig 5.43 First-order plots of the degradation of N,O-diacetylpropranolol (-●-) and N,ethoxycarbonyl, O-acetylpropranolol (-▲-) in alkaline DMF (50%, 0.25 M NaOH) at 37°C.

Compound	k_1 (hour $^{-1}$)	$k_2 \times 10^4$ (hour^{-1})	r_1	r_2
N, O-diacetylpropranolol	2.2480	43.0300	0.9997	0.9993
N-ethoxy carbonyl - O-acetylpropranolol	0.1611	6.0212	0.9980	0.9970

Table 5.24 Specific rate constants for the degradation of N,O-diacyl propranolols in 50% DMF in 0.5M NaOH pH13 at 37^{o}C.

CHAPTER SIX

CONTROLLED-RELEASE WITH ION-EXCHANGE RESIN-DRUG SYSTEMS

6. CONTROLLED-RELEASE WITH ION-EXCHANGE RESIN-DRUG SYSTEMS

6.1 INTRODUCTION

Controlled-release drug delivery systems hold considerable promise for improving the administration of drugs. Although the convenience of these preparations is often stressed a greater understanding of the pharmacokinetics of drug absorption and metabolism suggests that controlled-release delivery systems can also offer therapeutic benefit [*Ariens 1966 (87)]*. The controlled-release drug delivery system is defined as a preparation which is capable of delivering a sufficient initial dose and then sustaining adequate drug levels over a period of time or for at least two intervals of the conventional preparation (non-controlled -release). For safety and efficacy, the controlled-release drug delivery system must not generate drug-blood levels above those of the conventional systems nor drop below the therapeutic minimum.

There are several sustained-release preparations containing propranolol which are available in tablet or capsule forms, eg. Inderal LA (ICI) and Duranol (Alan Corporation).

The purpose of the work discussed here is to prepare a sustained-release product of propranolol in the form of coated and/or uncoated ion-exchange-drug complexes. This product may then be considered for use in the form of capsules or in an aqueous suspension. This formulation could be easily adjusted for dose to provide prolonged-action characteristics. One method of achieving this objective is to prepare a drug-resin complex by the use of ion-exchange resin [*Gyselinck et al 1981 (188)*] and further controlling the rate of release by various types of coating [*Motycka and Nairn 1979 (189), Borodkin and Sundberg 1971 (180) and Raghunathan et al 1981 (198)*]. One effective and versatile method of coating small resin particles is microencapsulation, which can be accomplished with various procedures [*Luzzi 1970 (292) and Bakan and Sloan 1972 (293)*].

Many researchers have discussed the encapsulation of solid particles [*Nixon and Walker 1971 (294), Merkle and Speiser 1973 (295), Madan et al 1976 (296) and Takenaka et al 1980 (297)*] but few have applied this process to ion-exchange resins [*Motycka and Nairm 1979 (189) and Motycka et al 1985 (190)*].

6.2. EXPERIMENTAL

6.2.1. PREPARATION OF THE DRUG RESINATES

The method of preparing the drug resinates was an adaptation of that reported by Gyselinck et al 1981 (188), but with some modification.

6.2.1.1. PURIFICATION OF THE RESINS

The purification of the resin has been carried out with two different techniques (a) column process or (b) batch process.

a) Column Process

This technique was used for the large particle size resin (50-100 mesh). A plug of glass wool was introduced into a glass column fitted at one end with a tap. A slurry of 2g of ion-exchange resin of well defined crosslinking, particle size and moisture content in about 20 ml double distilled water was poured onto the column. The resin was washed subsequently three times with each of the following liquids; methanol (50 ml), bezene (50 ml), methonol (50 ml). Purification was achieved by reactivating the resin three times with IM NaOH and IM HCI and finally the resin was washed with double distilled water to remove any acid left on the surface.

b) Batch Process

This method was used in case of the fine particle size ion-exchange resin (200-500 mesh) to which the column process can not be applied, due to the difficulty in passing the solution through the resin bed. The resin was purified and activated with the same liquids mentioned in the column process, by stirring the resin and each liquid in a beaker using a magnetic stirrer for at least 5 minutes. The supernatant liquids were separated by centrifugation and discarded.

6.2.1.2. PREPARATION OF THE DRUG-RESIN COMPLEXES

a) Column Process

An accurately weighed amount of drug (2g in most cases) in 10 ml of 50% acidified aqueous DMF, pH 3, was passed through a column containing a known weight (1 g in most cases) of dry purified resin (calculated from the water content determination after purification). The elute was recycled through the column several times (about 20 times) until the resin was completely saturated (ie. when the UV absorbance of the drug solution before and after passage were identical and the pH is reduced to about 1.7). The drug-resin complex formed was filtered and the filtrate was collected to allow the estimation of the amount of drug remaining uncomplexed. The resinate was washed three time with 20 ml of cooled, boiled double-distilled water and finally washed once with 25 ml acetone and dried at room temperature overnight.

b) Batch Process

A known weight (1g in most cases) of purified ion-exchange resin of well-defined cross-linking degree, particle size and moisture content was stirred in a beaker (using a

magnetic stirrer) with a concentrated solution (2g in 10 ml) of the drug in 50% acidified aqueous DMF pH3 until complete saturation of the resin was achieved [ie until there was no change in the U V absorption of the drug solution (it takes about 3 hours)]. The drug-resin complex formed was separated by centrifugation (4000 rpm for 10 minutes) and the supernatant layer was collected to allow the estimation of the amount of unreacted drug. The drug-resin complex was then washed and dried as described earler for the column process.

c) Water-content determination

The water-content of the purified resins and the drug resin complexes obtained by both methods (column and batch processes) was determined by the Karl-Fisher method using an auto metering and titration unit (Baird & Tatlock AF3).

6.2.2. PREPARATION OF THE DISSOLUTION MEDIA

A modified simulated gastric juice, pH 1.6, was prepared by dissolving 21.6 g of sodium chloride in one litre of 0.16 M hydrochloric acid. The values of free and total acid and sodium ions in this solution were equivalent to that in the gastric juice which was reported by Harrison 1957 (298).

Phosphate-citrate buffer (McIlvaine, Appendix V.) solution pH 7.4, ionic strength 0.1M (adjusted with potassium chloride) was used as the dissolution medium to simulate the pH of the intestinal fluids.

6.2.3. IN-VITRO DISSOLUTION TESTS

The dissolution of the drug from its resinate or from microcapsules was carried out (in triplicate) in simulated gastric juice, pH 1.6, or in phosphate-citrate buffer solution pH 7.4 of ionic strength 0.1 M at 37 °C by either (a) continuous-flow spectrophotometric or (b) HPLC analysis.

a) Continuous-flow spectrophotometric method

This method was used to study the dissolution of chemically stable drugs in which no degradation during dissolution takes place.

1000 ml of the selected dissolution medium was placed in a one litre jacketed cylindrical glass beaker which was maintained at 37°C by a Churchill recirculating thermostatic bath. A three-bladed stainless steel stirrer was placed centrally and rotated at 300 rpm by means of an electric motor (Heidolph). The stirrer blades were about 2 cm from the bottom of the beaker. A continuous-flow analysis for the release of the drug from the uncoated or coated resinates (250-500 mg) was carried out using an electric pump (FMI model, Fluid metering Co.) fitted with teflon tubes (1 mm ID) for sampling and replacing. A sintered glass sparger terminated the teflon tube (the sampler) to avoid withdrawing any solid particles into the UV cell (1 cm Quartz continuous-flow cell). The absorbances were measured continuously using a CECIL CE272 Linear readout ultraviolet spectrophotometer at 290 nm at 1-2 AUFS and the absorbances were recorded on a J J recorder (J J Instruments Ltd). A diagram of this system is displayed in Fig 6.1.

b) HPLC Method

This method was mainly used for the determination of the release of the propranolol esters from their resinates at high pH (pH 7.4) to monitor degradation during dissolution. 1000 ml of the selected dissolution medium was placed in the dissolution vessel which was maintained at 37 °C. A known amount of the dry drug-resin complex or microcapsules (250-500 mg) was suspended in the dissolution medium and stirred at 300 rpm by means of a three bladed stainless steel stirrer. Samples (1 ml) were withdrawn every 5-10 minutes by means of a pipette using a coarse cotton wool plug on the end of the pipette to prevent the entrainment of any solid particles. After sampling , the cotton wool plug was returned to the dissolution medium which was maintained constant by replacing the withdrawn sample with 1 ml of the same solvent equilibrated at 37 °C.

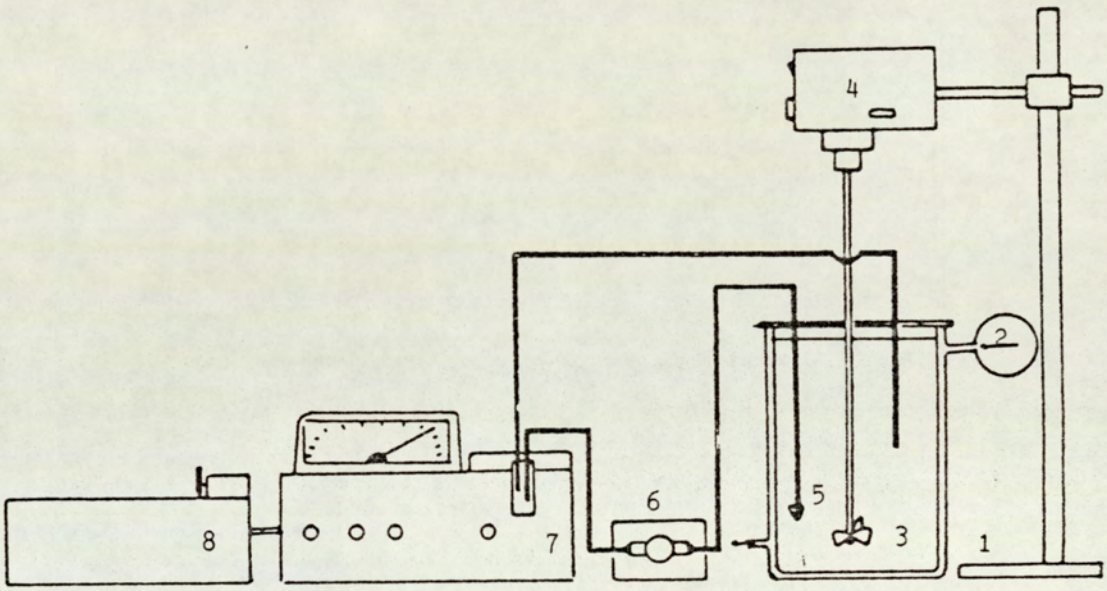


Fig 6.1 Block diagram of dissolution equipment.
The numbered features are:

(1) Jacketed cylindrical glass beaker
(2) Churchill recirculating thermostatic bath
(3) Three bladed stainless steel stirrer
(4) Electric motor
(5) Sintered glass sparger
(6) Electric pump
(7) U.V. spectrophotometer
(8) Chart recorder

1 ml sample and 1 ml of the internal (8 mg ethyl paraben in 100 ml 0.1 M HCl) standard were mixed together in a test tube. 20 µl aliquots were injected onto the HPLC column and eluted with the specific mobile phase (Table 3.8) .UV detection of the analytes was carried out at 290 nm. All standard solutions for both methods were prepared in a solvent similar to that of the dissolution medium. The calibration curves were constructed by plotting either the peak height ratio (in case of the HPLC method) or the absorbance (in case of the spectrophotometric one) against concentration. The concentration of the drug released at equilibrium was calculated from the calibration curve using a linear regression analysis program (CAS10 Scientific Calculator Fx 180P).

6.2.4. EFFECT OF THE pH OF THE REACTION MEDIUM ON THE ADSORPTION OF THE DRUG ONTO THE RESIN

Concentrated solutions of propranolol HCl (500 mg) in 10 ml 50% acidified aqueous DMF at different pH values (2.03, 3.03, 4.00, 4.64 and 5.96 adjusted with IM HCl) were prepared.

Each of these propranolol solutions were loaded separately onto 1 g of cationic ion-exchange resin [Dowex 50 WX8 (100-200 mesh)], moisture content 55%) using the batch method at room temperature. The amount of the unreacted drug was determined from the supernatant layer after equilibrium. 250 mg of each of the formed drug-resin complex (with a known moisture content) were placed separately into the dissolution vessel containing 1000 ml of simulated gastric juice pH 1.6 at 37 °C. The percentage of propranolol released from the resinate at 60 minutes was measured spectrophotometrically at 290 nm.

6.2.5. EFFECT OF STIRRING SPEED ON THE RELEASE OF PROPRANOLOL FROM ITS RESINATE

2 g of propranolol HC1 in 10 ml of 50 % acidified aqueous DMF pH3 was loaded onto 1 g of dry purified cationic ion-exchange resin [Dowex-50WX8 (50-100 mesh)] using the column technique, the amount of the drug adsorbed onto the resin was estimated from the filtrate after equilibration.

250 mg of dry propranolol-resin complex was placed in the dissolution vessel containing one litre of simulated gastric juice, pH 1.6, at 37 ^{o}C. The release of propranolol from its resinate complex was monitored using the continuous-flow spectrophotometric method at 290 nm. The stirring speed was changed after each run (100, 150, 200, 250 and 300 rpm).

6.2.6. EFFECT OF THE RESIN CROSS-LINKAGE ON THE LOADING AND RELEASE OF O-PIVALOYLPROPRANOLOL

1 g of dry purified reactivated resin [Dowex (100-200 mesh)] with different cross-linking degrees (1, 2, 4, 8 and 12 %) were allowed to react separately with 500 mg of 0-pivaloylpropranolol HC1 in 5 ml of 50% acidified aqueous DMF pH 3 using the batch technique at room temperature.

250 mg of each of the formed drug-resin complexes were assayed separately for the release of 0-pivaloylpropranolol in simulated gastric juice pH 1.6 at 37 °C using HPLC as the analytical technique. The mobile phase was a mixture of acetonitrile, orthophosphoric acid (88%), diethylamine and water (65 : 0.1 : 0.2 : 34.7) pH 2.8 delivered at 1ml/min, detection at 290 nm and ethylparaben (7 mg/100 ml 0.1 M HC1) was used as the internal standard. The chromatogram showing the separation of propranolol and 0-pivaloylpropranolol together with the internal standard using this mobile phase is shown in Fig 6.2.

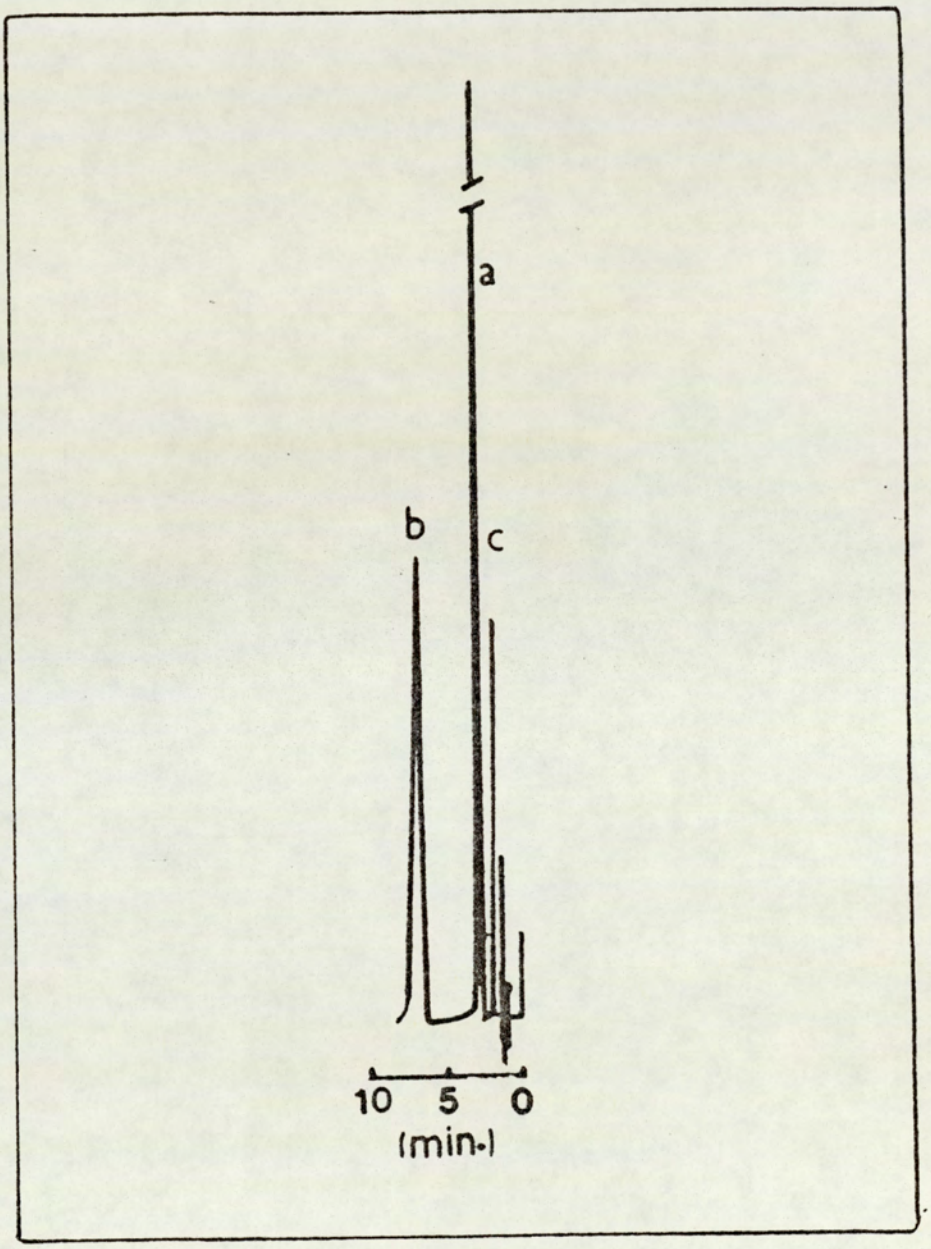


Fig 6.2 Chromatogram showing the separation of propranolol (a) O-pivaloylpropranolol (b)and ethyl paraben as internal standard (C) using a mobile phase consisted of acetonitrile, orthophosphoric acid (88%), diethylamine and water (65:0.1:0.2:34.7) at flow rate 1 ml/min, detection at 290 nm.

6.2.7. EFFECT OF THE RESIN PARTICLE SIZE ON THE LOADING AND RELEASE OF O-PIVALOYLPROPRANOLOL

Weighed amounts of wet purified reactivated cationic ion-exchange resin containing 1 g of the dry resin with different particle sizes (100-200 mesh and 200-400 mesh) were loaded separately with O-pivaloylpropranolol as described earlier in section 6.2.6. 250 mg of the dry drug-resin complex was used from each batch to investigate the effect of the resin particle size on the release of O-pivaloylpropranolol from its resinate complex in simulated gastric fluid pH 1.6 at 37 °C using HPLC as the analytical technique (as described in section 6.2.6.)

6.2.8. EFFECT OF pH OF THE DISSOLUTION MEDIUM ON THE RELEASE OF O-PIVALOYLPROPRANOLOL FROM STRONG AND WEAK CATIONIC RESINS

1 g of each of strong cationic resin [Dowex 50WX8 (200-400 mesh)] and 55% moisture content] and weak cationic resin [Amberlite IR (200-400 mesh) and 10% moisture content] was reacted separately (batch method) with 500 mg of O-pivaloylpropranolol in 5 ml of 50% aqueous DMF pH 3 (for about 3 hours) until equilbrium was achieved.

A weighed amount of each formed drug-resin complexes containing 250 mg dry resinate was used separately to study the release of O-pivaloylpropranolol in simulated gastric fluid, pH 1.6, and in phosphate-citrate buffer solution pH 7.4 (0.1 ionic strength) at 37 °C using the previously mentioned HPLC method as the analytical technique (Section 6.2.6).

6.2.9. EFFECT OF THE BUFFER IONIC STRENGTH ON THE RELEASE OF O-PIVALOYLPROPRANOLOL

A weighed amount of O-pivaloylpropranolol-Ambertile IR complex previously prepared (Section 6.2.8) containing 250 mg dry resinate was used to study the release of O-pivaloylpropranolol in phosphate-citrate buffer solution, pH 3, at different ionic strength values (1, 0.5 and 0.0771 M) at 37 °C.

The ionic strength of the buffer solution was adjusted to the desired value by addition of a calculated amount of potassium chloride (Appendix V.). The analysis was performed by HPLC as described earlier (Section 6.2.6)

6.2.10. EFFECT OF TREATMENT OF THE DRUG-RESIN COMPLEX WITH POLYETHYLENE GLYCOL 4000 ON THE RELEASE OF O-PIVALOYLPROPRANOLOL

1 g of dry O-pivaloylpropranolol-Dowex 50WX2 (100-200 mesh) complex was prepared as described earlier in Section 6.2.6 and was placed in a suitable jacketed cell. The polyethylene glycol (P.E.G.) 4000 (100 mg) was added and gently mixed by means of a glass rod with the application of gentle heat. When the P.E.G. 4000 had completely melted (56 °C), the heat was removed. The mixing was continued until the temperature returned to room temperature, the coated drug-resin complex particles were gently passed through a suitable mesh sieve (100 mesh) to remove any agglomerates, and then 250 mg of this coated resinate was transferred to the dissolution cell containing one litre of simulated gastric fluid, pH 1.6, at 37 °C. The release of O-pivaloylpropranolol from its coated and uncoated resinates was monitored by the previously mentioned HPLC method (Section 6.2.6).

6.2.11. EFFECT OF TEMPERATURE ON LOADING AND RELEASE OF PROPRANOLOL

The influence of increased temperature on the quantity of propranolol bound to the strongly cationic resin [Dowex 50 WX8 (50-100 mesh)] and on the release of propranolol from its resinate in simulated gastric fluid pH 1.6 at 37 °C was checked.

For this purpose, resinates were prepared in the same manner as described before (Secion 6.2.1.2 b) with the batch method at room temperature (22 °C), at 90 °C (using a thermostatic water bath) and by refluxing the resin with a concentrated aqueous solution of propranolol hydrochloride (1 g/20 ml double distilled water) until equilibrium was achieved.

6.2.12. LOADING AND RELEASE OF O-ACYL PROPRANOLOL-RESINATES

O-acyl propranolol (acetyl, propionyl, butyryl, valeryl and hexanoyl) resinates were prepared by the column method (Section 6.2.1.2 a) at room temperature, by passing a concentrated solution of the ester hydrochlorides in 50% acidified aqueous DMF, pH 3, down a column containing 1 g of cationic ion-exchange resin [Dowex 50 WX8 (50-100 mesh)] several times until equilibrium was established. The release of the esters from their resinates was performed in simulated gastric juice, pH 1.6, at 37 °C using the continuous-flow spectrophotometric procedure (at 290 nm) as the analytical technique. At the end of the experiments 1 ml aliquots from the dissolution medium were analysed by HPLC where no degradation was noticed. All the percentage content of drugs in their resinates were estimated from the supernatant after loading and from the in-vitro dissolution tests.

6.2.13. MICROENCAPSULATION OF THE DRUG-RESIN COMPLEXES

Ion-exchange resin-drug complexes have been used to formulate sustained-release products of acidic and basic drugs. However sustained-release may be difficult to achieve due to many variables. A technique which minimizes these variables is to provide a polymeric coat over the ion-exchange resin-drug complex particles. Here, this technique has involved microencapsulation of the drug resinate particles with gelatin-acacia co-acervate.

6.2.13.1 MICROENCAPSULATION PROCEDURE

The microencapsulation method for coating the drug resinate used was a modification of that reported by Takenaka et al 1980 (297) and the recovery process used was a slight modification of that used by Madan et al 1976 (298).

a) Preparation of the Coacervate

250 ml of 2% w/v (ie 5 g) of aqueous gelatin and acacia were prepared separately, their temperature was maintained at 50 °C in a thermostatic water bath. A known weight (1-2 g) of drug-resin complex (the weight depending on the core to wall ratio) was triturated with a minimum amount of glycerol to produce a paste. The trituration was done efficiently to ensure that the resinate particles are individually separated in a uniform manner, more glycerol was added to make the paste more flexible, and the mass was transferred into a one litre glass beaker. About 30 ml of the acacia solution maintained at 50 °C was triturated with the resin mass by means of a glass rod to make a uniform suspension , care being taken to ensure that any aggregates were dispersed. The remaining acacia solution was than added gradually after transferring the beaker into a thermostatic water bath (50 °C) with stirring (400 rpm) by using a double bladed glass stirrer driven with an electric motor. The 250 ml of the aqueous gelatin solution (at 50 °C) was adjusted to pH 3.9 with 1M HCl and this was added gradually to the beaker containing the drug-resin complex, suspended in the aqueous

acacia solution, with continuous stirring (400 rpm). The pH of the mixture was re-checked and readjusted to pH 3.9 with 1M NaOH by means of a pH meter (Radiometer PHM64 pH meter) which was calibrated at 50 °C by the standard buffer solutions pH 4 and 7. The stirring was continued at 400 rpm for about 30 minutes.

b) Rigidification of the capsule wall

The beaker was transferred to an ice bath with continuous stirring (400 rpm). The temperature was allowed to drop to about 5 °C over about 1 hour, which caused gelling of the capsule wall (the coacervate). Microscopic examination was made at every 10 °C drop in temperature to ensure whether microencapsulation had occured or not. When the temperature had dropped to 5 °C, the stirring was stopped and the formed microcapsules were allowed to settle down. The supernatant fluid was separated and checked for the presence of the drug which may be released into the weakly ionic solution of the acacia where it was found that only a very small amount of the drug (< 1 %) was leached. After separation of the supernatant fluid, about 50 ml of ice-cold isopropyl alcohol was added to the capsules with stirring, the particles were allowed to settle down and the supernatant layer was decanted and the procedure was repeated with another portion of isopropanol with stirring. The mother liquor was then decanted and 50 ml of chilled 19% formaldhyde in isopropanol was added with stirring. This procedure was to harden the capsule wall. The microcapsules were allowed to settle down and the hardening procedure was repeated. The mixture was left stirring for about 14 hours (in most cases) in an ice-bath.

c) Recovery of the microcapsules

At the end of the hardening time, the mother liquor was decanted and the microcapsules were washed twice with 50 ml of chilled isopropanol. The mother liquor was

decanted each time and the slurry of the microcapsules was transferred to a crystallizing dish covered with a filter paper, the microcapsules were allowed to dry at room temperature overnight. The dried microcapsules were then sieved through a nest of B S 1796 sieves by shaking manually and stored in labelled sample bottles for analysis.

6.2.13.2. CONTROLLED RELEASE OF O-PIVALOYLPROPRANOL-RESIN COMPLEX ENCAPSULATED BY GELATIN-ACACIA

O-Pivaloylpropranolol-resin (Dowex 50 WX2, 100-200 mesh) complex was prepared as described in Section 6.2.6. 2 g of this drug-resin complex (calculated as dry weight) was encapsulated by gelatin-acacia following the procedure described in Section 6.2.13.1. the total weight of the polymers (gelatin and acacia) was 10 g making a core to wall ratio of 1:6.

The evaluation of the microencapsulated drug resinate was carried out by the in-vitro dissolution test in simulated gastric juice, pH 1.6, at 37 °C using the HPLC method as the analytical technique. To obtain a controlled-drug delivery system, mixtures of coated and uncoated drug resin complexes with different ratios of (100:0, 95:5, 90:10, 85:15 and 0:100) were also assayed.

6.2.13.3. EFFECT OF THE CORE TO WALL RATIO ON THE RELEASE OF DRUGS FROM THEIR MICROENCAPSULATED RESINATES

Propranolol and O-pivaloylpropranolol resinates were prepared by reacting both drugs with the pharmaceutical grade resin IRP69* using the batch process method as described earlier in Secion 6.2.1.2.b. The resinates were encapsulated by the gelatin-acacia method. The ratio of the core to wall was 1:6 and 1:12 by weight in the case of the propranolol resinate and 1:4, 1:5 and 1:6 by weight in the case of the O-pivaloylpropranolol complex. Sieve analysis was carried out manually using a nest of B.S. 1796 sieves and the fractions between 53-90 μm were used for the in-vitro dissolution tests. The in-vitro dissolution tests

were performed in simulated gastric fluid, pH 1.6, at 37°C using the continuous-flow spectrophotometric method at 290 nm as the analytical technique

*IRP69 Pharmaceutical grade strongly cationic ion-exchange resin (functional group SO_3-Na^+), particle size 100-500 mesh, parent resin IR-120, 10% moisture content, pH range 0-14 and maximum operating temperature 120 °C.

6.2.13.4. EFFECT OF DOUBLE COATING OF THE DRUG RESINATES ON THE RELEASE RATE

The encapsulated batches of propranolol and O-pivaloylpropranolol-IRP69 resin complexes which were prepared earlier in Section 6.2.13.3 with a core to wall ratio of 1:6 and particle size 53-90 mesh were re-encapsulated with the same procedure (gelatin-acacia) with a core to wall ratio of 1:1. The release of the drugs from the formed double coated microcapsules (125-150 μm) were carried out in simulated gastric juice, pH 1.6, at 37°C as before (Section 6.2.13.3.).

6.2.14 APPLICATION OF THE ION-EXCHANGE RESIN SYSTEMS FOR A SUSTAINED-RELEASE FORMULATION OF SOME OTHER DRUGS

Concentrated solutions of nadolol, doxipen HCl, promazine HCl, nortriptyline HCl and amitriptyline HCl (Fig 6.3) were prepared separately by dissolving 2 g of the drug in 20 ml of 50% acidified aqueous DMF, pH 3.

Each of these solutions was passed separately through a column containing 1 g dry resin (Dowex 50 WX8, 50-100 mesh) several times at room temperature (22 °C) until equilibrium was achieved. The release of these drugs from their resinates was carried out in simulated gastric fluid, pH 1.6, at 37 °C using the continuous-flow spectrophotometric method as the analytical technique. The analytical wavelength for each compound was determined from their U V spectra displayed in Fig 6.4.

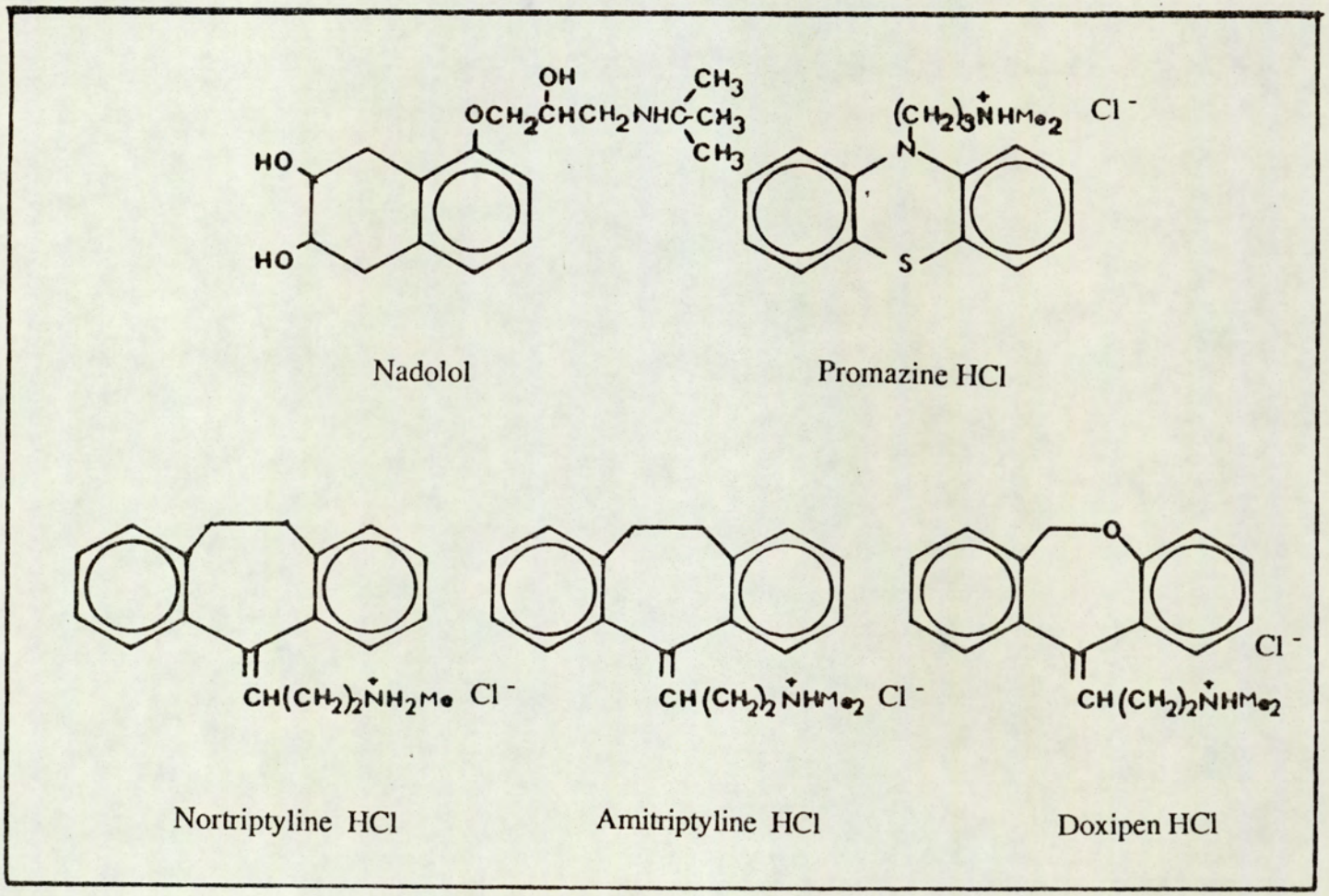
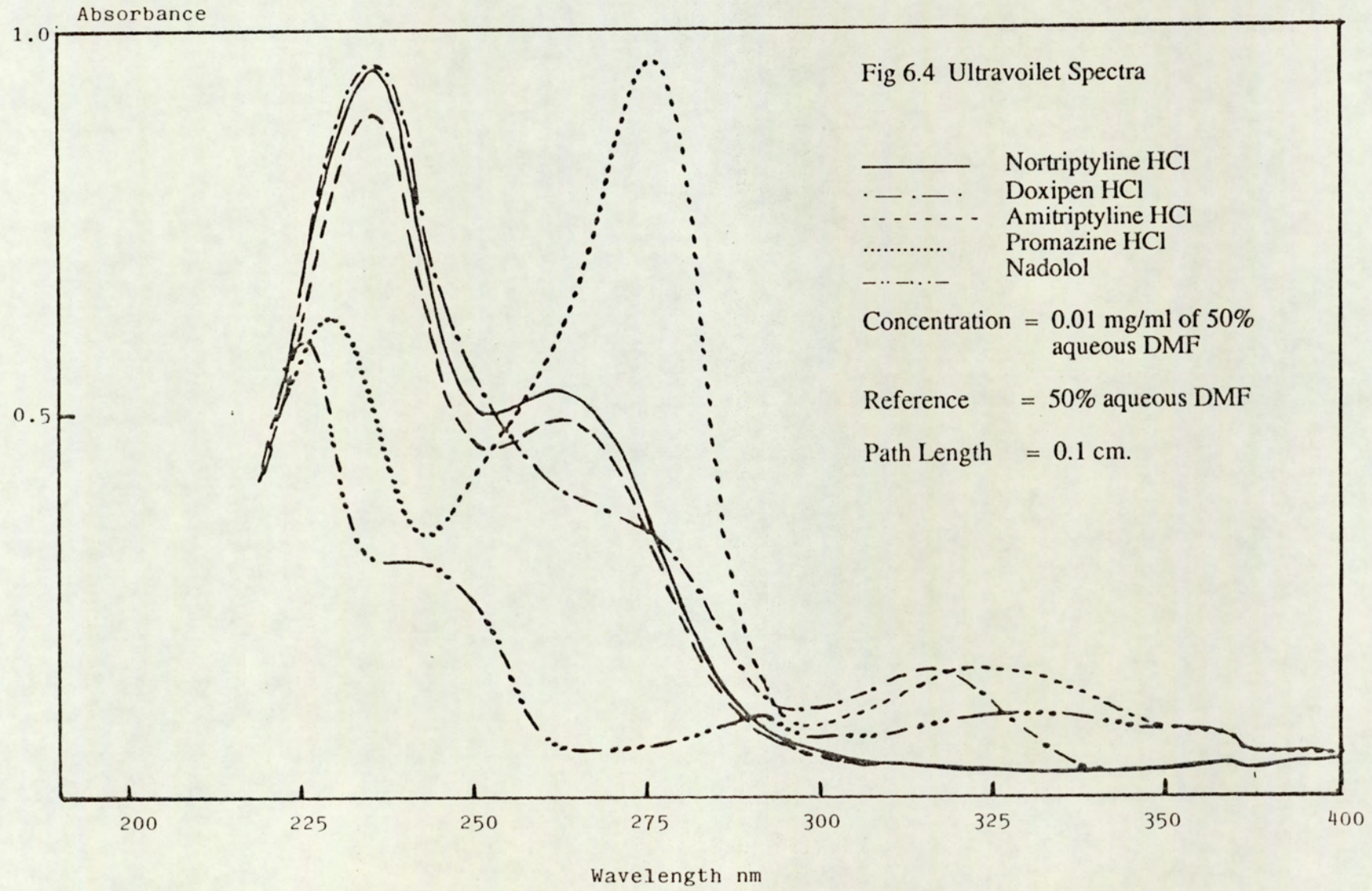


Fig 6.3 Chemical Structures of Basic Drugs


Absorbance
1.0
0.5
200
225
250
275
300
325
350
400
Wavelength nm
Fig 6.4 Ultravoilet Spectra
Nortriptyline HCl
Doxipen HCl
Amitriptyline HCl
Promazine HCl
Nadolol
Concentration = 0.01 mg/ml of 50% aqueous DMF
Reference = 50% aqueous DMF
Path Length = 0.1 cm.

The effect of temperature on the loading and release of amitriptyline HCl has been investigated by loading the drug onto the resin by reflux as described earlier in Section 6.2.11. and the dissolution test was carried out similarly to that prepared at room temperature.

6.3 RESULTS AND DISCUSSION

To compare the effect of certain variables on the release of drugs from the drug resin complex during the dissolution procedure as a function of time, the percentage of drug released at time t can be calculated from the following equation.

$$U_t = \frac{C_t}{C_\infty} \times 100 \qquad 6.1$$

where U_t = % of drug released at time t

C_t = the concentrations of drug released at time t

C_∞ = the concentration of drug released at equilibrium

The drug content in the drug-resin complex can also be determined from the in-vitro dissolution test using equation 6.2.

$$\% \text{ of drug loaded} = \frac{D_\infty}{R_o} \times 100 \qquad 6.2$$

Where D_∞ = the total amount of drug release at equilibrium

R_o = the weight of the dry resin used for dissolution

6.3.1. EFFECT OF THE REACTION MEDIUM pH ON THE ABSORPTION OF THE DRUG ONTO THE RESIN

Table 6.1 shows the loading and release of propranolol HCl in simulated gastric juice, pH 1.6, at 37° from drug-resin (Dowex 50 WX8, 100 - 200 mesh) complex made at different pH values of the reaction medium. Changing the pH of the reaction medium (50% aqueous DMF) from pH 2.03 to pH 5.96 exerted a modest effect on the loading of propranolol HCl onto the resin. As the pH increases the fraction protonated will decrease reducing the interaction with the resin. However, these pH values still favour large degrees of protonation (for pKa = 9.45, α (5.96) = 99.97%) so only marginal effects are to be expected. No difference in release profiles were noted from any of these resins.

pH of the Reaction Medium	Concentration of Propranolol Solution		% of drug Released in 60 min.	% of Moisture Content in the Resinates
	Before resin Treatment mg/10 ml	After Resin Treatment mg/10 ml		
2.03	500	200.7	86.2	15.20
3.03	500	199.4	89.0	11.14
4.00	500	197.1	88.7	11.57
4.64	500	190.0	88.4	12.20
5.96	500	177.0	87.0	16.33

Table 6.1 Summary of in-vitro release of propranolol HCl in simulated gastric juice pH 1.6, at 37°C from propranolol-resin complexes made at different pH values of the reaction medium.

6.3.2 EFFECT OF THE STIRRING SPEED ON THE RELEASE OF PROPRANOLOL FROM ITS RESINATE

The effect of the stirring speed on the release profile of propranolol HCl from its resinate in simulated gastric juice, pH 1.6, at 37°C has been studied (Fig 6.5) and it was found that at slow speeds (100 and 150 rpm) the drug-resinate particles were not efficiently stirred and the release of the drug was retarded. This may be due to a hydrodynamic interface between the resinate particles and solvent which provides a diffusional barrier between the dissolution medium and the drug-resin complex particles. At higher speeds (200 to 300 rpm) the release of the drug was independent of the stirring speed due to the more efficient stirring removing this diffusional barrier. To monitor fomulation and system variables rather than diffusion controlled dissolution profiles, a stirring speed of 300 rpm was chosen for subsequent experiments.

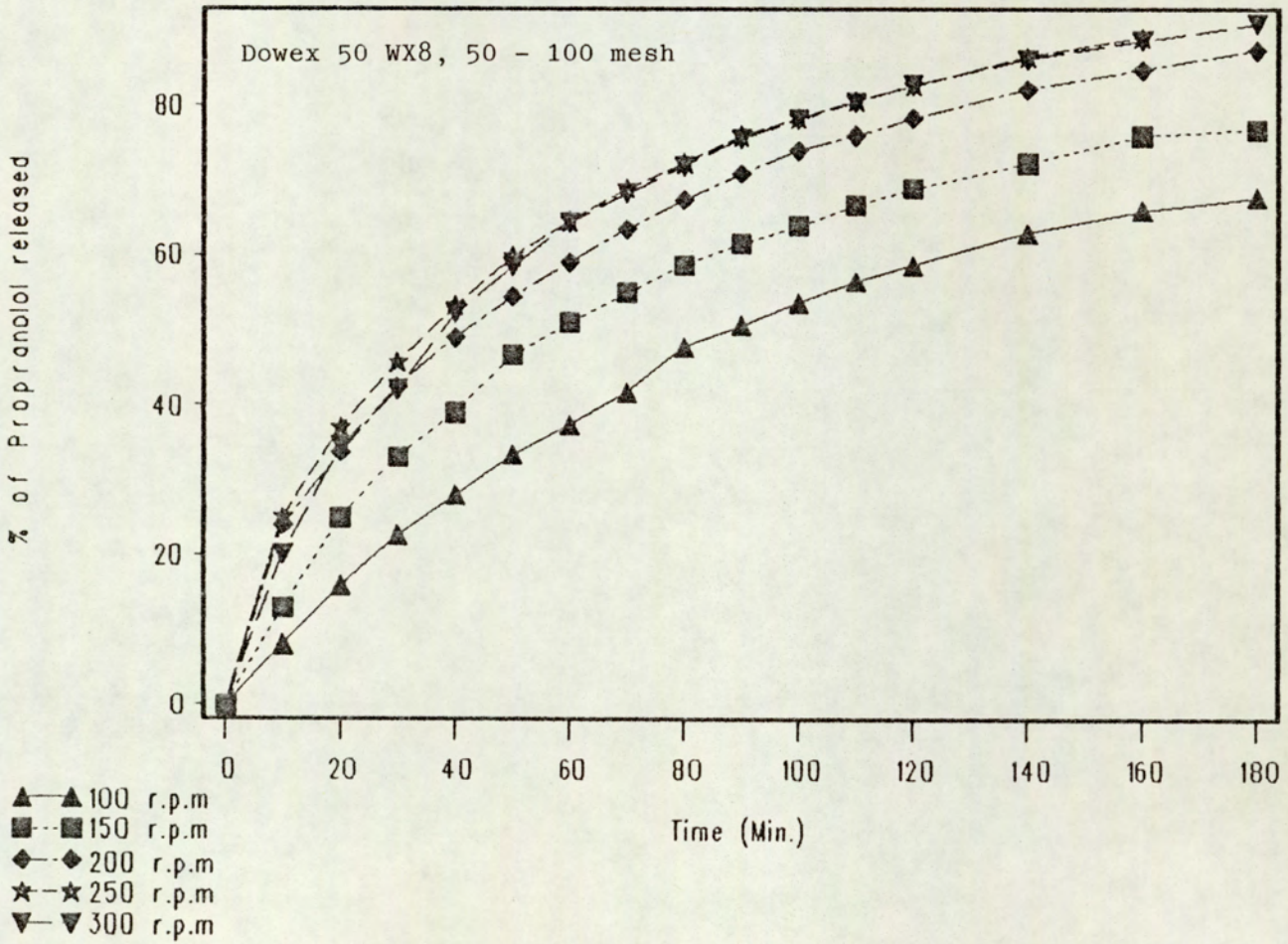


Fig 6.5 Effect of the stirring speed on the release profile of propranolol from its resinite in simulated gastric juice pH 1.6 at 37°C.

6.3.3. EFFECT OF THE RESIN CROSS-LINKING ON THE LOADING AND RELEASE OF O-PIVALOYL PROPRANOLOL FROM ITS RESINATES

The cross-linking degree of the resin has shown an effect on both loading and release from its Dowex resinate (100 - 200 mesh) in simulated gastric juice pH 1.6 at 37°C. Table 6.2 shows the effect of the cross-linking on the percentage of drug content in the resinate.

Ion-Exchange Resin				
Resin	% of Cross-Linking	Particle Size (Mesh)	% Moisture Content	% Content of O-Pivaloyl-Propranolol in the Drug Resin Complex
Dowex 50W	1	100 - 200	80	32.7
Dowex 50W	2	100 - 200	79	29.7
Dowex 50W	4	100 - 200	65	25.6
Dowex 50W	8	100 - 200	56	16.2
Dowex 50W	12	100 - 200	44	10.2

Table 6.2 Effect of the Cross-Linking Degree of the Resin on the % Content of the Drug in the Resinate

Increasing the degree of cross-linking reveals a reduction in both the loading and the release of the drug from the resin-complex (Fig.6.6) . The degree of cross-linkage determines the apparent porosity of the resin which may be defined as the ratio of the volume of interstices of the material to the volume of its mass, thus, the results appear to be consistant with this definition, in which , as the porosity decreases with increasing cross-linkage the capacity of the resin for the drug diminishes and the release rate is also reduced. This may be due to the entrapment of the drug molecules within the pores which provide a greater diffusional resistance. The amount of propranolol HCl loaded onto the

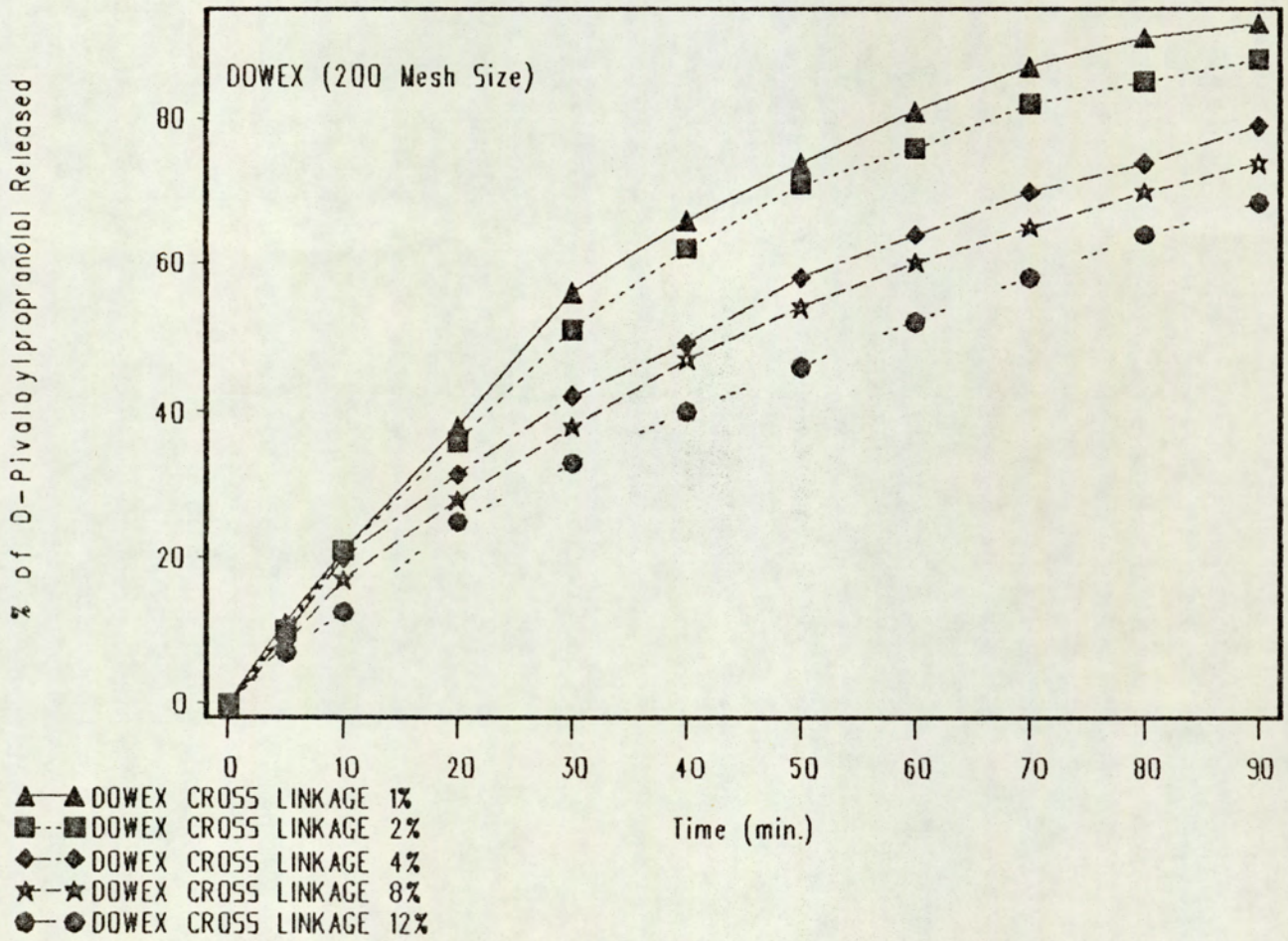


Fig 6.6 Effect of the resin cross-linking degree on the release profile of O-pivaloylpropranolol in simulated gastric juice pH 1.6 at 37°C.

Dowex resin (100 - 200 mesh) with different degrees of cross-linkage was lower than that quoted by Gyselinck et al 1981 (188) Table 1.13 . The low drug loading may reflect a low selectivity coefficient of this batch of resin for propranolol or else it may be due to the presence of DMF in the reaction medium which may compete with the adsorption of propranolol on the cationic resin.

6.3.4 EFFECT OF THE RESIN PARTICLE SIZE ON THE LOADING AND RELEASE OF O-PIVALOYL PROPRANOLOL

One of the major factors influencing the release characteristics of a drug from its resinate is the resin particle size (Fig 6.7). Table 6.3 shows the effect of using a Dowex resin of 8% cross-linking agent with different particle sizes (100 - 200 mesh and 200 - 400 mesh) on the amount of O-pivaloylpropranolol loaded. The O-pivaloylpranolol content in the resinate made with smaller particle size was close to that made with the larger one. These results are in favourable agreement with the finding of Gyselinck and co-workers 1981 (188) who claimed that the change in the particle size of the resin has no effect on the amount of drug bound to the ion-exchanger.

Resin Particle Size (Mesh)	% of O-Pivaloylpropranolol Content in the Resinate
100 - 200	16.2
200 - 400	18.7

Table 6.3 Effect of the Resin Particle Size on the Drug Content in the Resinate

Fig 6.7 shows that the release rate of O-pivaloylpropranolol from the resinate made with the smaller particle size resin in simulated gastric juice, pH 1.6, at 37^{o}C was faster than the larger particle size where the percentages of the drug released in 90 minutes were 96% and

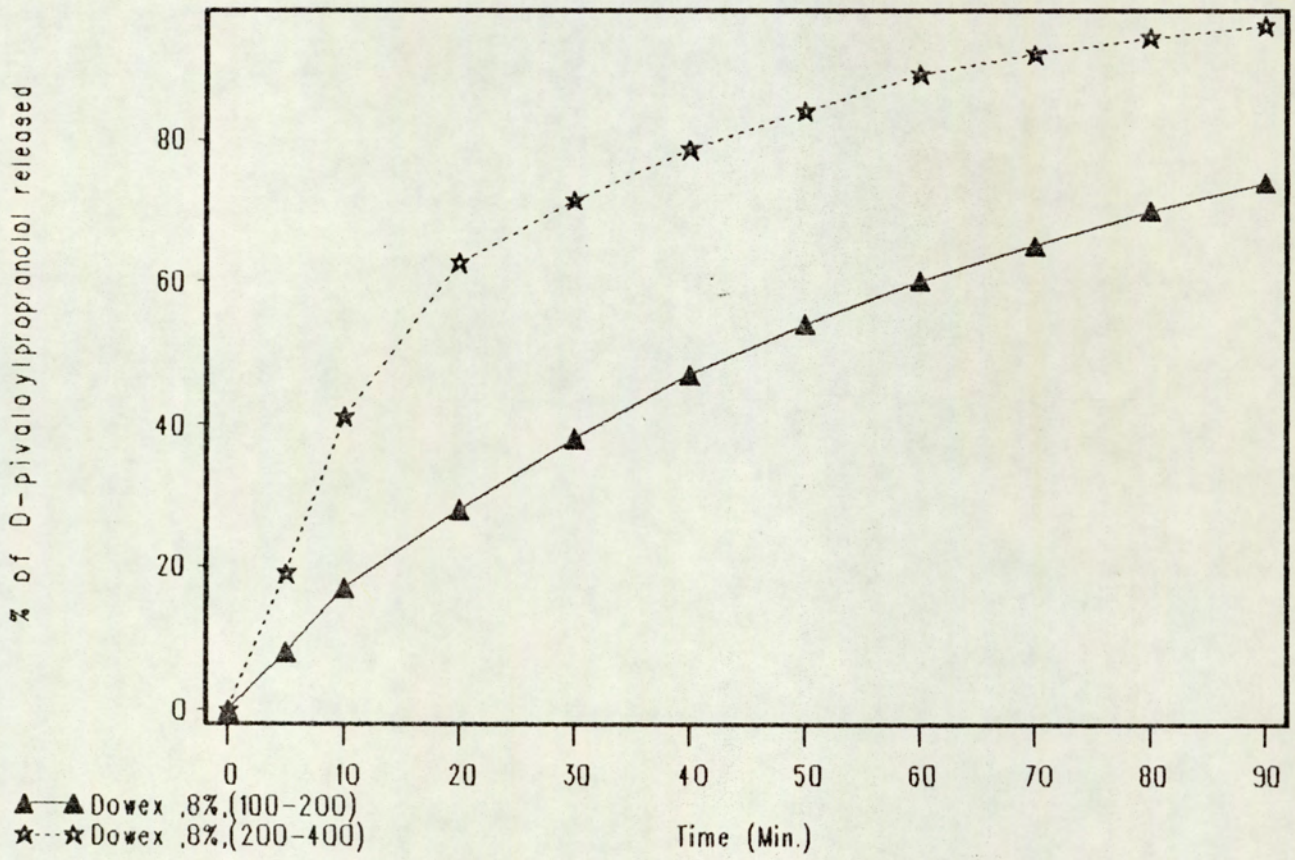


Fig 6.7 Effect of the resin particle size on the release of O-pivaloylpropranolol in simulated gastric juice pH 1.6 at 37°C.

74% respectively. These effects may be attributed to reduced particle size confirming a decrease in the path length available for diffusion of the drug.

6.3.5. EFFECT OF THE DISSOLUTION MEDIUM pH ON THE RELEASE OF O-PIVALOYLPROPRANOLOL FROM STRONG AND WEAK CATIONIC RESINS

The quantity of O-pivaloylpropranolol loaded onto the strongly cationic resin (Dowex 50WX8, 200-400 mesh) and onto the weakly cationic resin (Amberlite IR, 200 - 400 mesh) was 18.7% and 29.9% respectively. The difference in the loading of O-pivaloylpropranolol on both resins may be due to the difference in the selectivity coefficient of both resins for propranolol.

Fig 6.8 a & b shows the comparative release of O-pivaloylpropranolol from the drug-resin complexes made with both resins in simulated gastric juice, pH 1.6, and in phosphate-citrate buffer solution pH 7.4, 0.1M ionic strength at 37°C , where the release of the drug was not greatly affected by the pH of the dissolution medium, but some retardation is observed at the higher value.

This is where reduced protonation may limit the solubility of the ester and this together with small ionic strength of variability may cause the two profiles to deviate.

6.3.6 EFFECT OF THE IONIC STRENGTH OF THE DISSOLUTION MEDIUM ON THE RELEASE OF O-PIVALOYLPROPRANOLOL

The release of O-pivaloylpropranolol from its resinate (made with Amberlite IR, 200 - 400 mesh) in phosphate - citrate (McIlvaine) buffer solution, pH 3, with different ionic strengths (1, 0.5 and 0.077) at 37°C are summarized in Table 6.4, which shows that a decrease in the ionic strength of the buffer delays the release of the drug from its resinate.

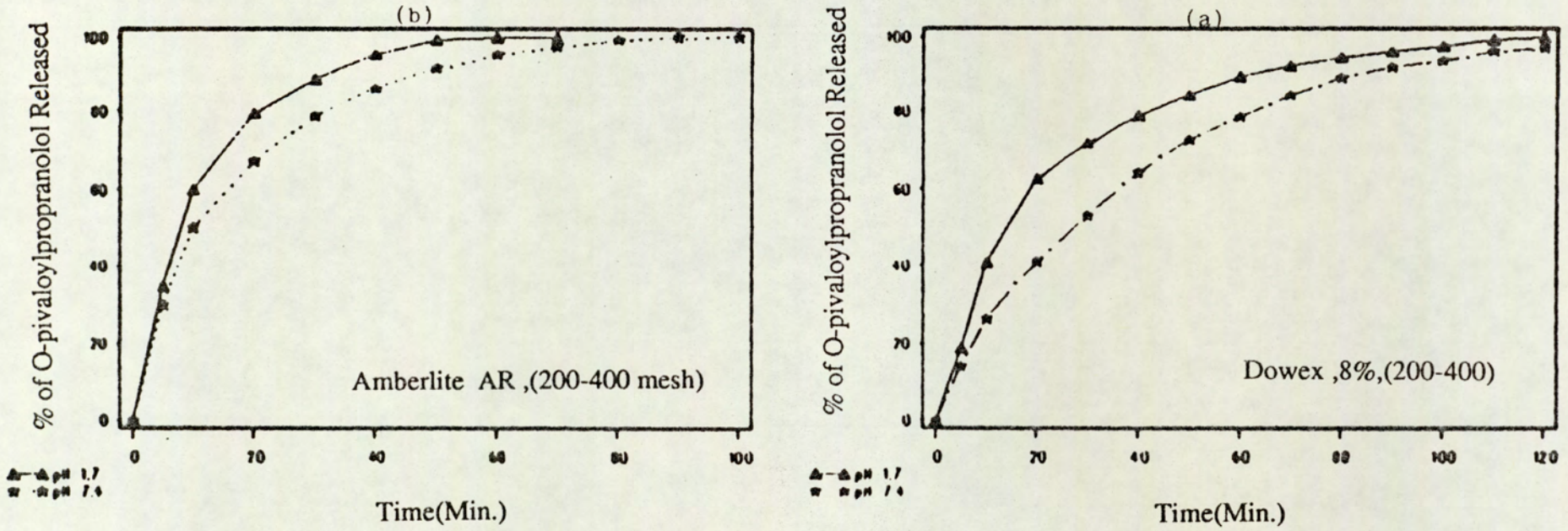


Fig 6.8 Effect of the dissolution medium pH on the release of O-pivaloylpropranolol.

This effect confirms that the release of drug from its resinate depends upon the influx of competitive ion from solution which may be controlled by the ionic strength of the dissolution medium *[Mehta 1986 (299)]*

Time (min).	% of O-pivaloylpropranolol Released		
	Ionic Strength (M) 1	0.5	0.0771
10	55	40	39
20	66	58	54
40	76	69	66
60	84	72	70
90	91	79	74

Table 6.4 Effect of the ionic strength of the dissolution medium on the Release of O-Pivaloylpropranolol from its Amberlite Resinate in a Buffer Solution pH 3 at 37°C.

6.3.7 EFFECT OF TREATMENT OF THE DRUG-RESIN COMPLEX WITH P.E.G. 4000 ON THE RELEASE OF O-PIVALOYLPROPRANOLOL

As shown previously the drug-resin complex rapidly releases the drug in simulated gastric juice pH 1.6, at 37°C, e.g. O-pivaloylpropranolol resinate formed with Dowex 50 WX2, 100 - 200 mesh, released 88% of the drug in 90 minutes in simulated gastric juice , pH 1.6 , at 37°C.

Treatment of this batch of drug-resin complex with an agent such as P.E.G. 4000 showed a slight retardation in the release rate (Fig. 6.9). It has been reported by several authors [*Koff 1964 (199), Amsel 1981 (192) and Raghunathan et al 1981 (198)*] that the treatment of the drug-resin complexes with P.E.G. 4000 helps the resin particles to retain their geometry during dissolution and enable the effective application of a diffusional barrier

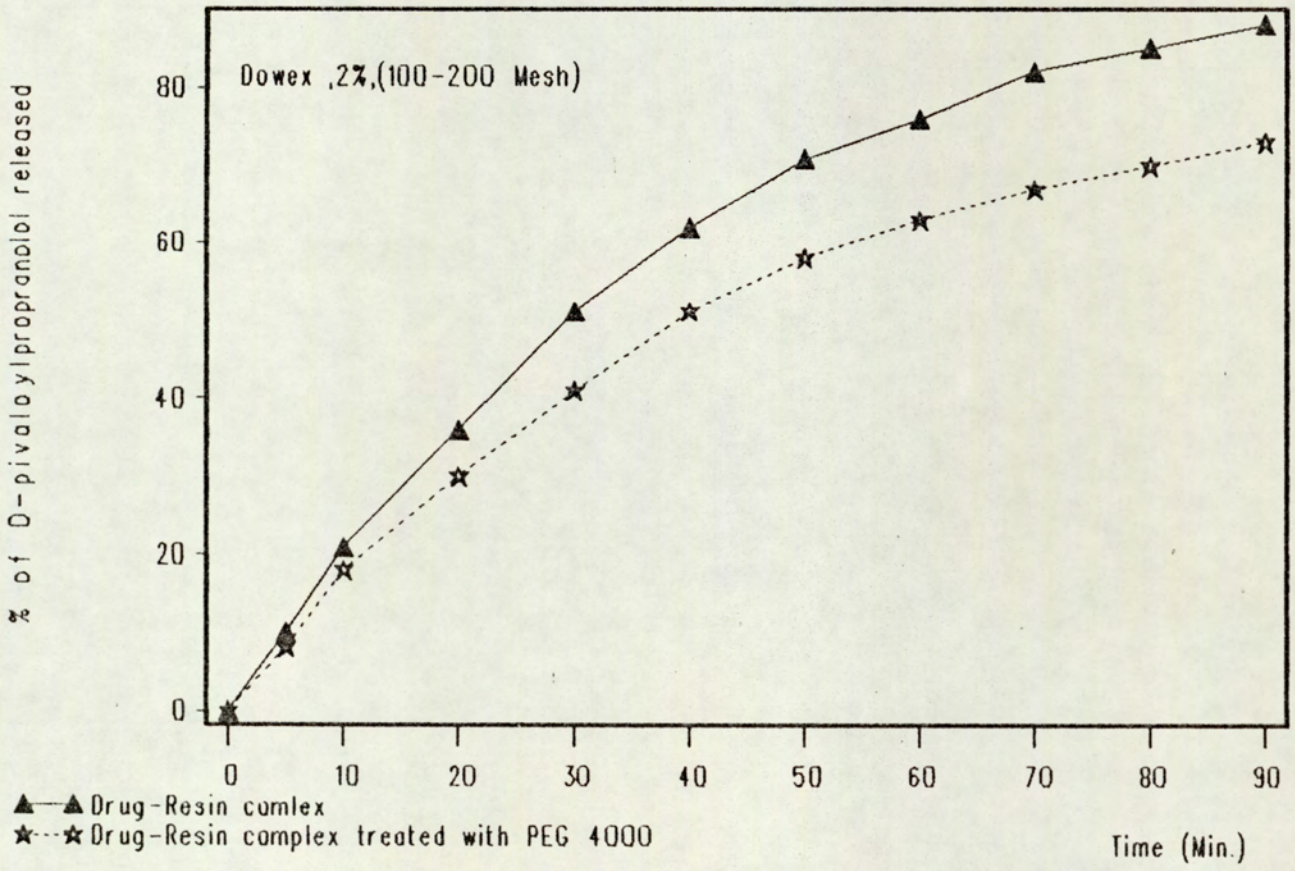


Fig 6.9 Effect of treatment of the drug-resin complex with P.E.G. 4000 on the release rate.

coating to such particles. Direct coating of the drug-resin complex beads was ineffective due to the rapid peeling of the coat as the coated beads swelled and fractured the coat when brought into contact with water or biological fluids.

6.3.8 EFFECT OF TEMPERATURE ON THE QUANTITY OF DRUG BOUND TO THE RESINS AND ITS RELEASE FROM THE RESINATES

The effect of temperature on the amount of propranolol bound to the resin (Dowex 50 WX8, 50 - 100 mesh) and on the release of the drug from the resinate in simulated gastric fluid pH 1.6 at 37°C is summarized in Table 6.5.

Temperature	% of Propranolol in the Resinate	% of Propranolol Released in 5 hours
Room Temp (22°C)	20.00	89.2
90°C	34.60	77.0
Reflux	50.77	61.5

Table 6.5 Effect of Temperature on the amount of O-pivaloylpropranolol bound to the Resin and on the Dissolution of the Drug from its Resinate

From Table 6.5, it is apparent that increasing the temperature results in a higher drug content in the resinate. This may be due to the swelling of the resin particles , due to thermal and hydration effects, to its maximum volume providing access to normally shielded centres of ionic activity. As the temperature drops the resin particles shrink and trap the drug within its structure resulting in an increased load and reduced release rate. The comparative release profiles of propranolol from drug-resin complexes prepared at different temperatures are

shown in Fig 6.10. It has been noted experimentally that all the drug loaded came out into solution at equilibrium.

6.3.9 LOADING AND RELEASE OF O-ACYL PROPRANOLOL-RESINATES

An investigation has been carried out to study the effect of increasing the lipophilicity of a drug on the amount bound to the resin and the release of such drugs from the formed resinate.

Increasing the lipophilicity (logP) by increasing the number of carbon atoms in the ester side chain of O-acyl propranolols from acetyl to hexanoyl has been shown to reduce the actual percentage loading of the drug in the resinate (Table 6.6 and Fig 6.11b).

Propranolol Esters	Log P	Structure Volume (A)	% of Drug Content in the Resinate
Acetyl	4.505	503.662	17.50
Propionyl	4.991	528.711	14.50
Butyryl	5.560	571.192	12.00
Valeryl	6.300	618.462	10.10
Hexanoyl	6.850	667.824	8.89

Table 6.6 Loading of O-acyl propranolols on Ion-exchange Resin (Dowex 50WX8, 50 - 100 mesh) at Room Temperature (22°C).

The release of the esters from their resinates in simulated gastric juice, pH 1.6, at 37°C shows a slight prolongation as the lipophilicity increases (Fig 6.12). This may be due to decreased diffusional mobility but earlier results (Section 6-3-5) suggest that a decrease in the aqueous solubility may also be a controlling factor. The decrease in the drug content of the drug-resin complex may be explained by an increase in the structure volume of the ester

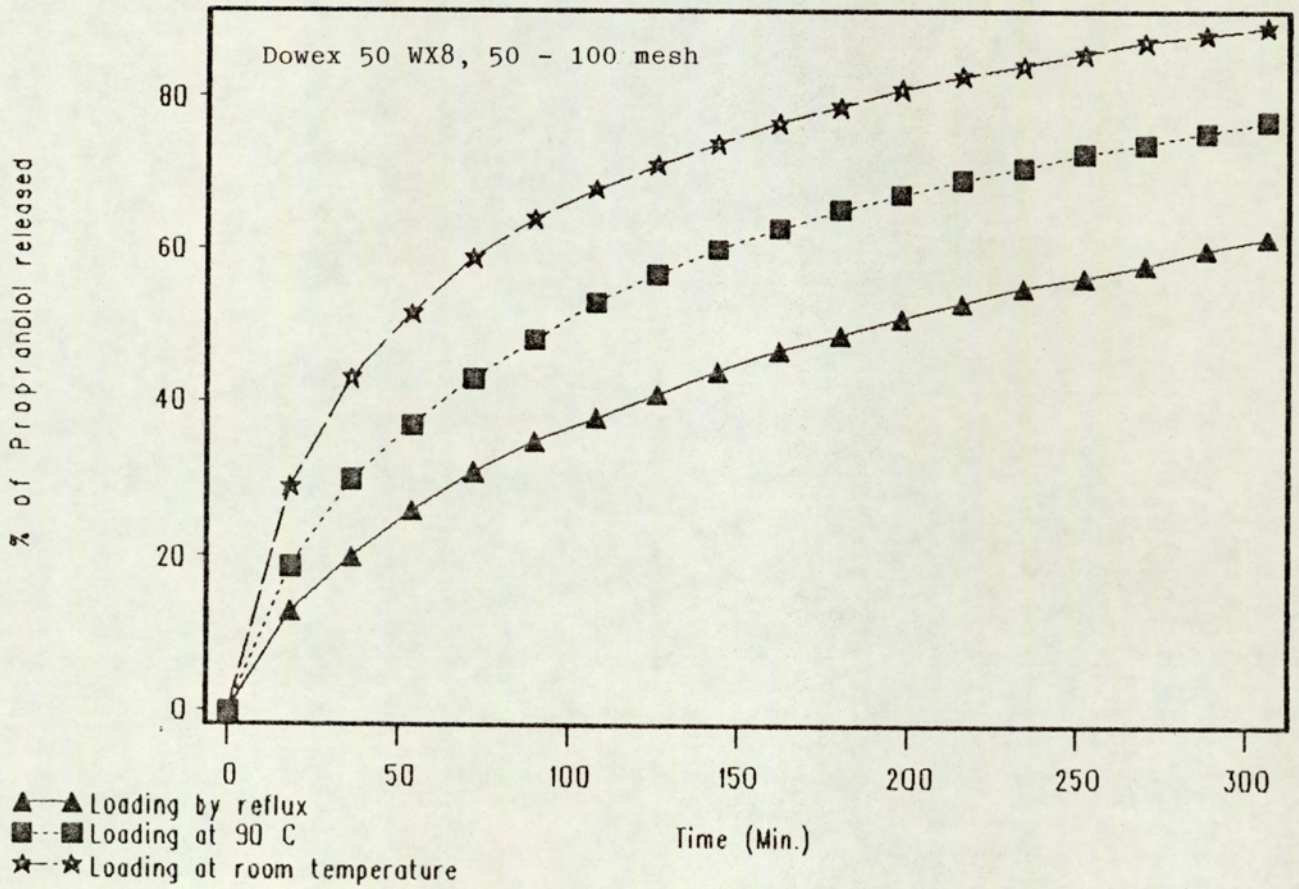


Fig 6.10 Effect of the loading temperature on the release rate of propranolol in simulated gastric juice pH 1.6 at 37°C.

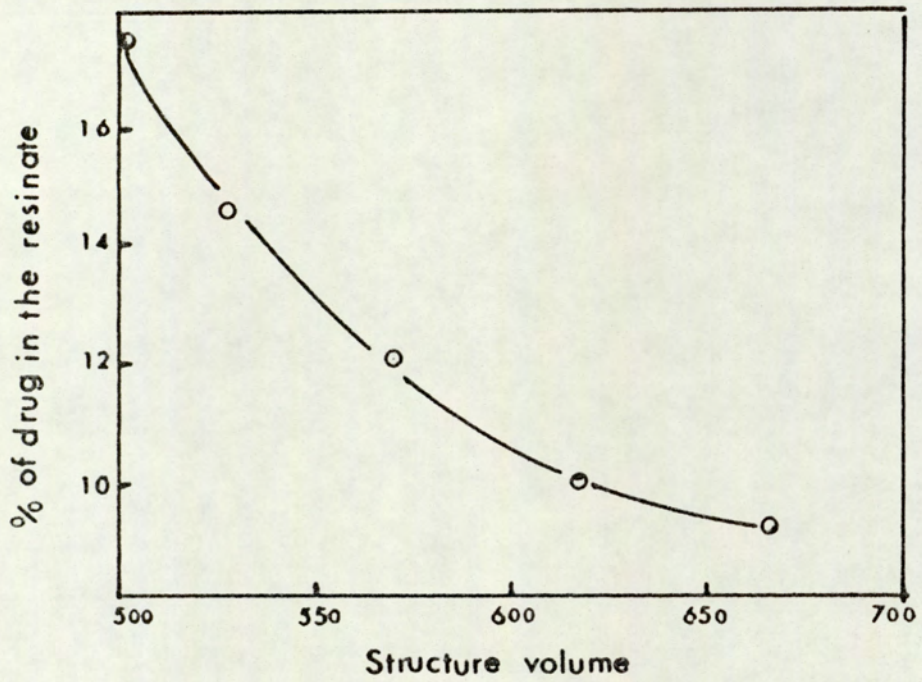


Fig 6.11(a) Effect of the structure volume on the drug content in the resinate

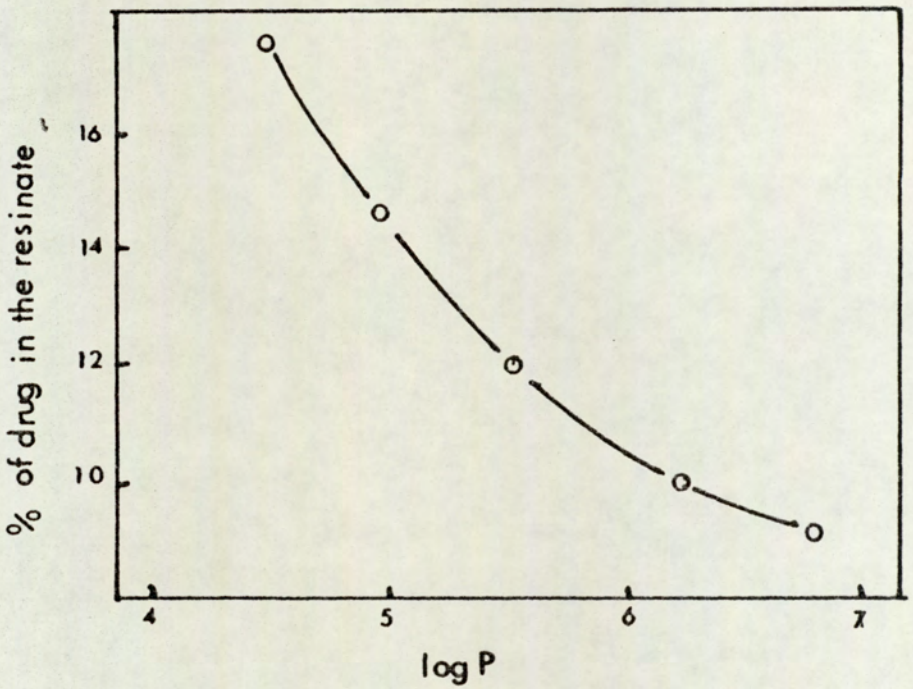


Fig 6.11(b) Effect of lipophilicity on the drug content in the resinate

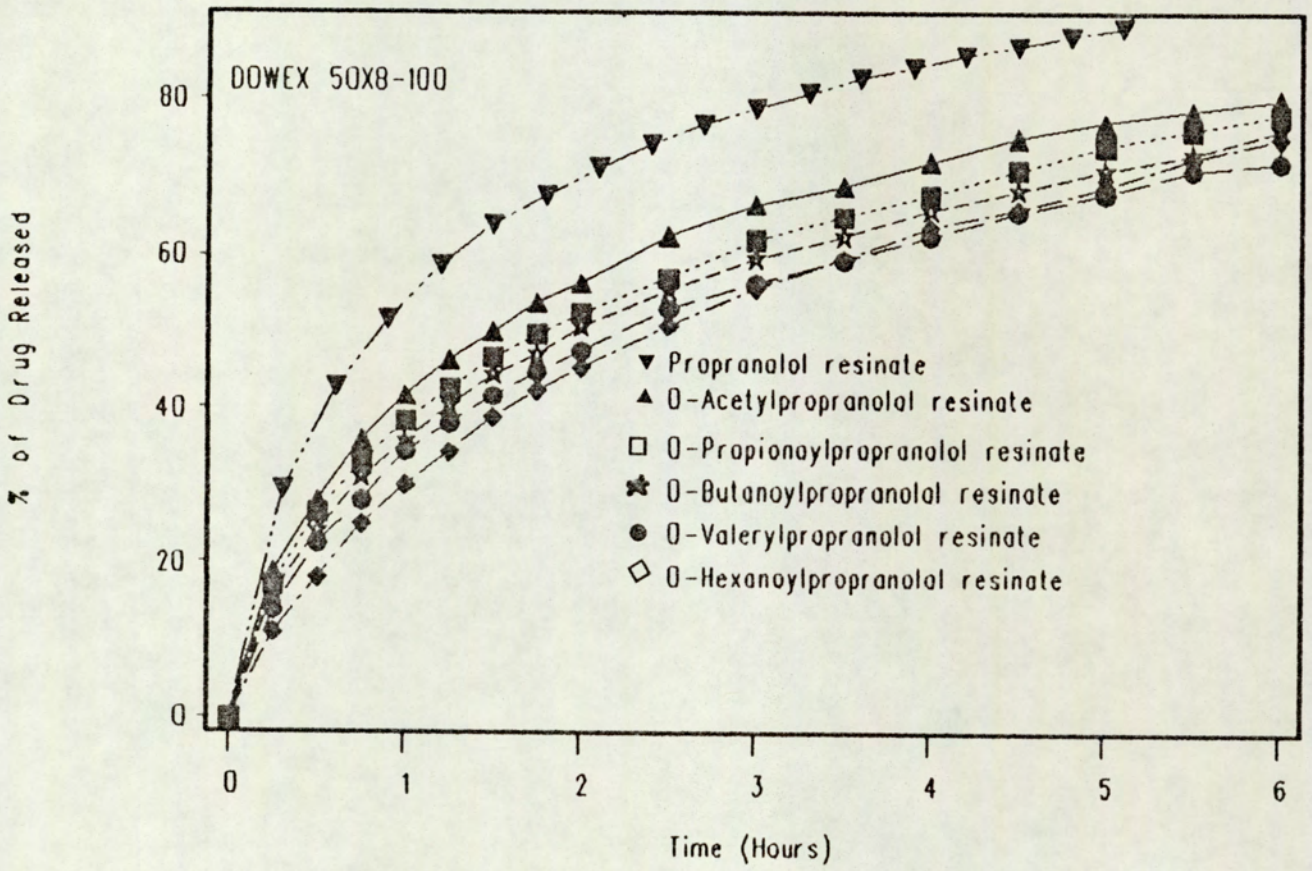


Fig 6.12 Effect of Ester structure on the release rate

molecules binding to the resin. Effect of increasing of the ester structure volumes on the loading onto the resin are shown in Table 6.6 and Fig 6.11a.

The structure volumes of O-acylpropranolols were determined using a computer program named CHEM-X , which runs on the VAX mainframe computer system and supporting Tektronix graphics terminals. The use of such a program enables modelling of large or small structures which can be quickly and accurately assembled for display on a screen either from crystallographic coordinates or built from individual atoms or fragments. Model techniques such as surface mapping, docking or tracing the locii of important atoms and energy and geometrical calculations for such atoms may also be employed. Colour may be used to identify various atoms and/or groups. Figures 6.13 and 6.14 show examples of the application of this program for modelling the structure of O-hexanoylpropranolol in a form of a solid space filling model and a surface map for the determination of the structure volume.

6.3.10 CONTROLLED RELEASE OF O-PIVALOYLPROPRANOLOL-RESIN COMPLEX ENCAPSULATED BY GELATIN-ACACIA

To provide further control of the release profile a diffusional barrier coating for the drug-resin complex was investigated. This was felt to be particularly advantageous for those resinates prepared with fine particle size resins.

Encapsulation of O-pivaloylpropranolol resin (Dowex 50WX2, 100 - 200 mesh) complex with gelatin-acacia (core:wall, 1:6) was shown to delay the drug release from the coated resinate in simulated gastric juice, pH 1.6, at 37^{o}C by about 3 times compared to release from the uncoated resinate (Fig 6.15). The major disadvantage of this process is that the drug content in the microencapsulated drug-resin complex was much reduced; from 33.5% in the uncoated resinates to 5.5% in the coated ones. This makes this process of little application for drugs with a high therapeutic dose.

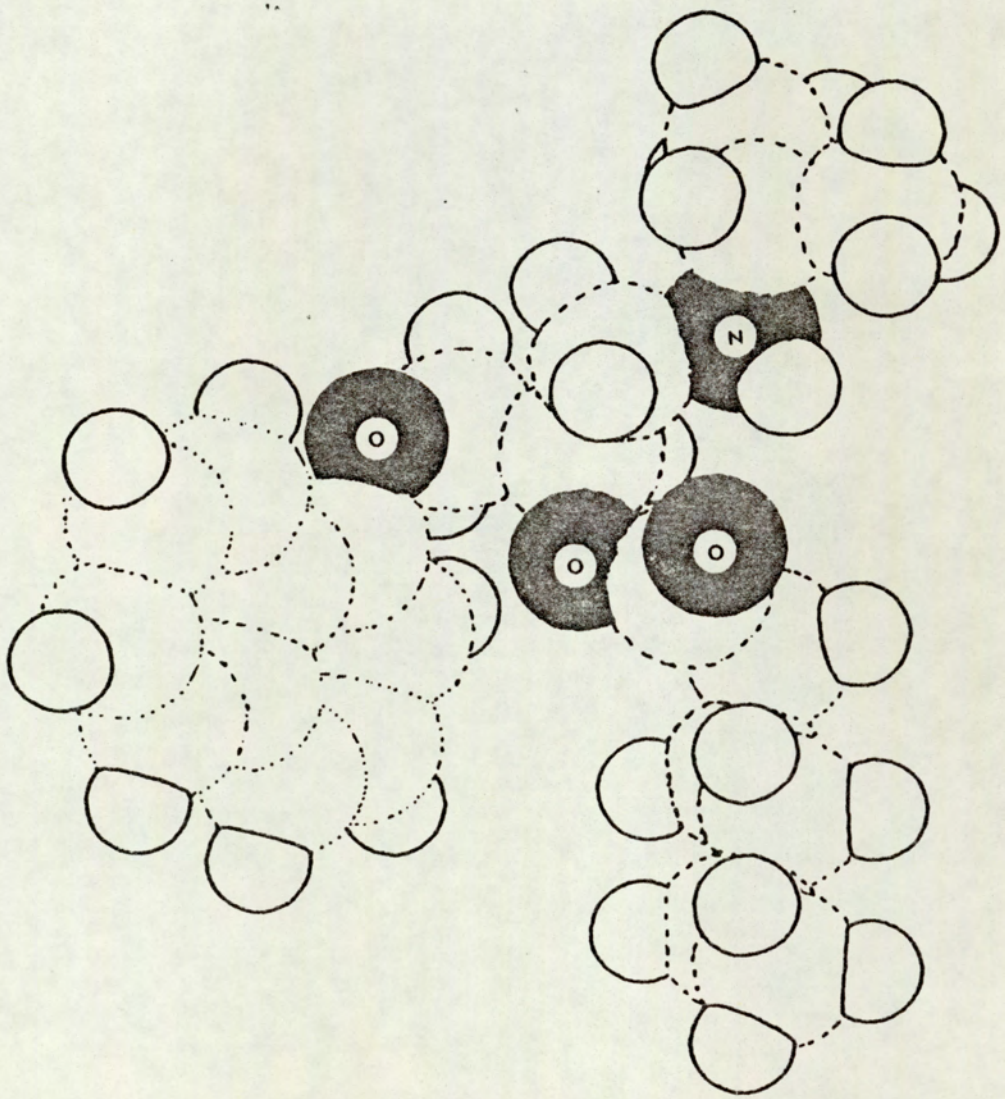


Fig 6.13 Space-filling model of O-hexanoylpropranolol using the CHEM-X program.

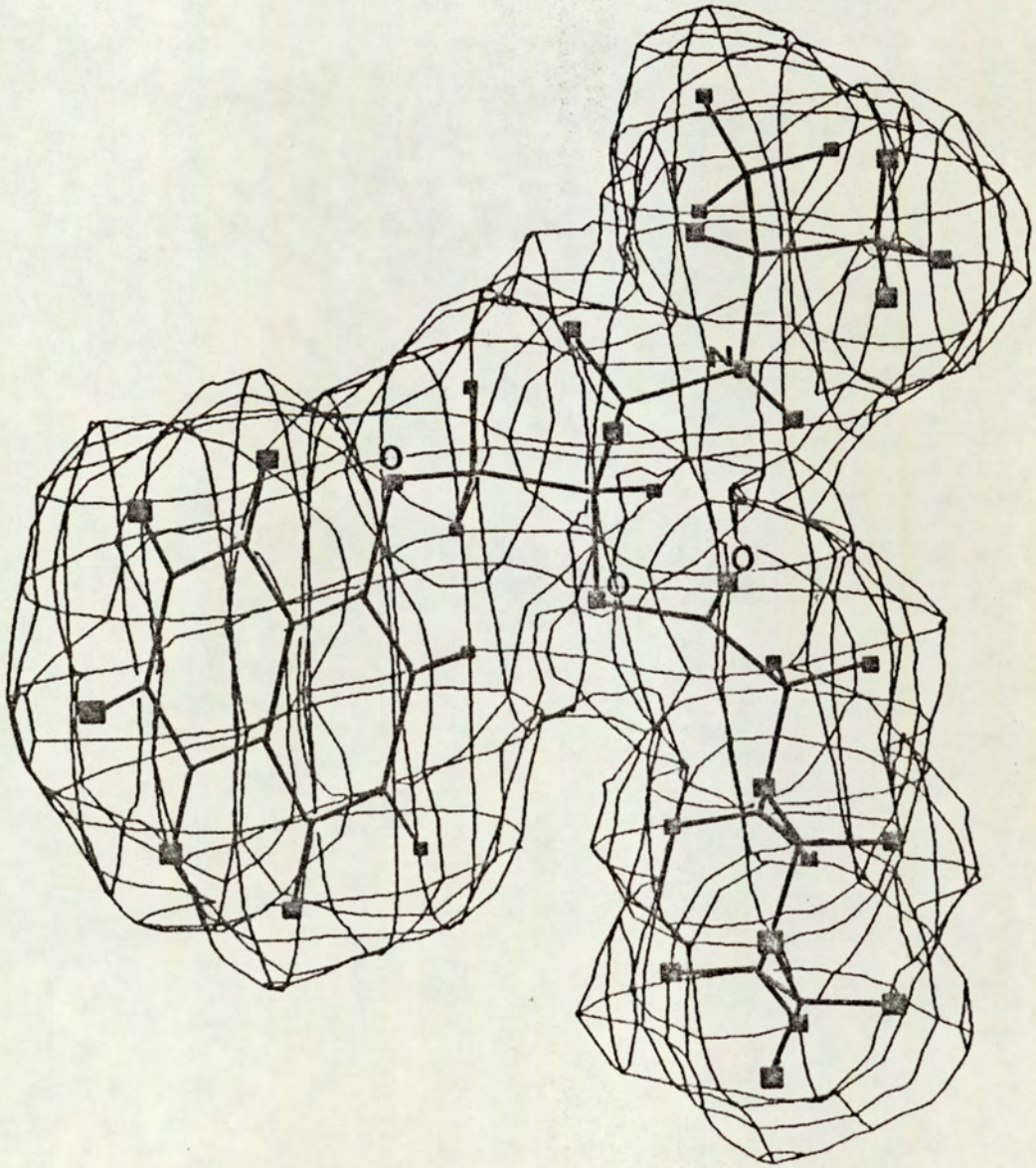


Fig 6.14 Surface map model of O-hexanoylpropranolol using the CHEM-X program.

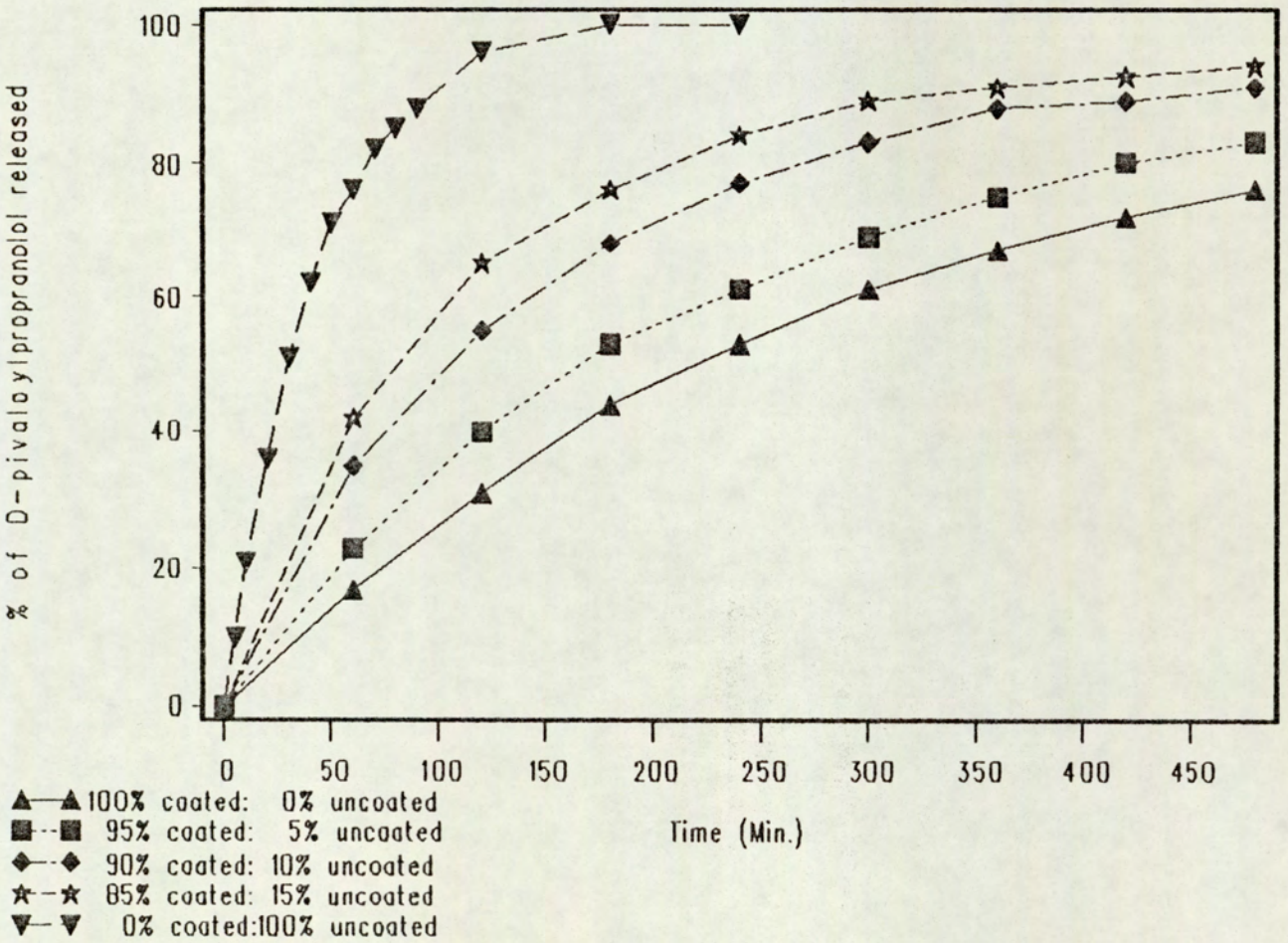


Fig 6.15 Effect on the release rate of mixing the coated and uncoated O-pivaloylpropranolol resinate (Dowex 50 WX2, 100 - 200 mesh).

In order to control the drug content and the release of the drug from the final preparation, mixtures of coated and uncoated drug-resin complexes were blended together with various ratios (coated:uncoated, 100:0 ; 95:5 ; 90:10 ; 85:15 and 0:100) and dissolution in simulated gastric juice, pH 1.6, at 37^{o}C was monitored.

Table 6.7 shows the effect of the biphasic control system (coated and uncoated drug-resin complex mixture) on the drug content in the final preparation and on the release characteristics from this preparation, which are graphically presented in Fig 6.15.These results were in agreement with that of the Pennwalt biphasic-control drug delivery system [Pennwalt Prescription Products 1980 (291)].

The more rapid drug release from the uncoated particles provides an initial increase in availability while the coated particles provided a more sustained profile.

Ratio of Coated to Uncoated Resinates	Amount of O-pivaloylpropranalol in 1g mixture (mg)	% of O-Pivaloylpropranolol Released in 2 Hours
100 : 0	55	31
95 : 5	69	40
90 : 10	83	55
85 : 15	97	65
0 : 100	337	100

Table 6.7 Effect of Biphasic System on the Drug Content and Drug Release

6.3.11 EFFECT OF THE CORE TO WALL RATIO ON THE RELEASE OF DRUGS FROM THEIR MICROENCAPSULATED RESINATES

In order for a drug to be displaced from the ion-exchange resin, ions from the gastro-intestinal fluids must first defuse through the outer coating. The diffusion of drug through the semipermeable coating becomes the rate limiting step in drug release.

By varying the thickness of the coating, the rate of drug release can be predetermined and controlled. During passage through the gastro-intestinal tract, ions present in the gastro-intestinal fluids displace drug from the ion-exchange resin matrix. Since the concentration of ions within these fluids remains constant, interpatient variation in rates of drug release are expected to be small. Drug-resin complexes were prepared for propranolol and O-pivaloylpropranolol using an irregularly shaped, fine particle (100 - 500 mesh) pharmaceutical grade resin (IRP69) which was encapsulated by gelation-acacia with various core to wall ratios.

Formulation	Recovery (% Encapsulated)		Drug Content in 1g Preparation (mg)		% of Drug Released in One Hour	
	O-PP.	P.	O-PP.	P.	O-PP.	P.
Uncoated Resinate	-	-	395.3	434.0	78.8	100
Core:Wall 1:4	78	-	97.8	-	56.6	-
Core:Wall 1:5	87	-	90.4	-	43.4	-
Core:Wall 1:6	93	96	70.8	73.0	28.2	85.9
Core:Wall 1:12	-	91.8	-	43.0	-	71.6
Double Coated Resinates	91	90.2	34.5	40.5	23.0	58.3

Abbreviations O-PP. = O-pivaloylpropranolol , and P. = Propranolol

Table 6.8 Effect of Core to Wall Ratio and Double Coating of the Microencapsulated resinate on the % of Capsules Recovered, Drug Loading and Drug Release

Table 6.8 shows that an increase in the proportion of the coating material decreases the amount of drug content and prolongs the drug release profile (Fig 6.16 a & b). The prolonged-release characteristic of the drugs was attributed to the thickness of the coating layer which controls the drug diffusion rate.

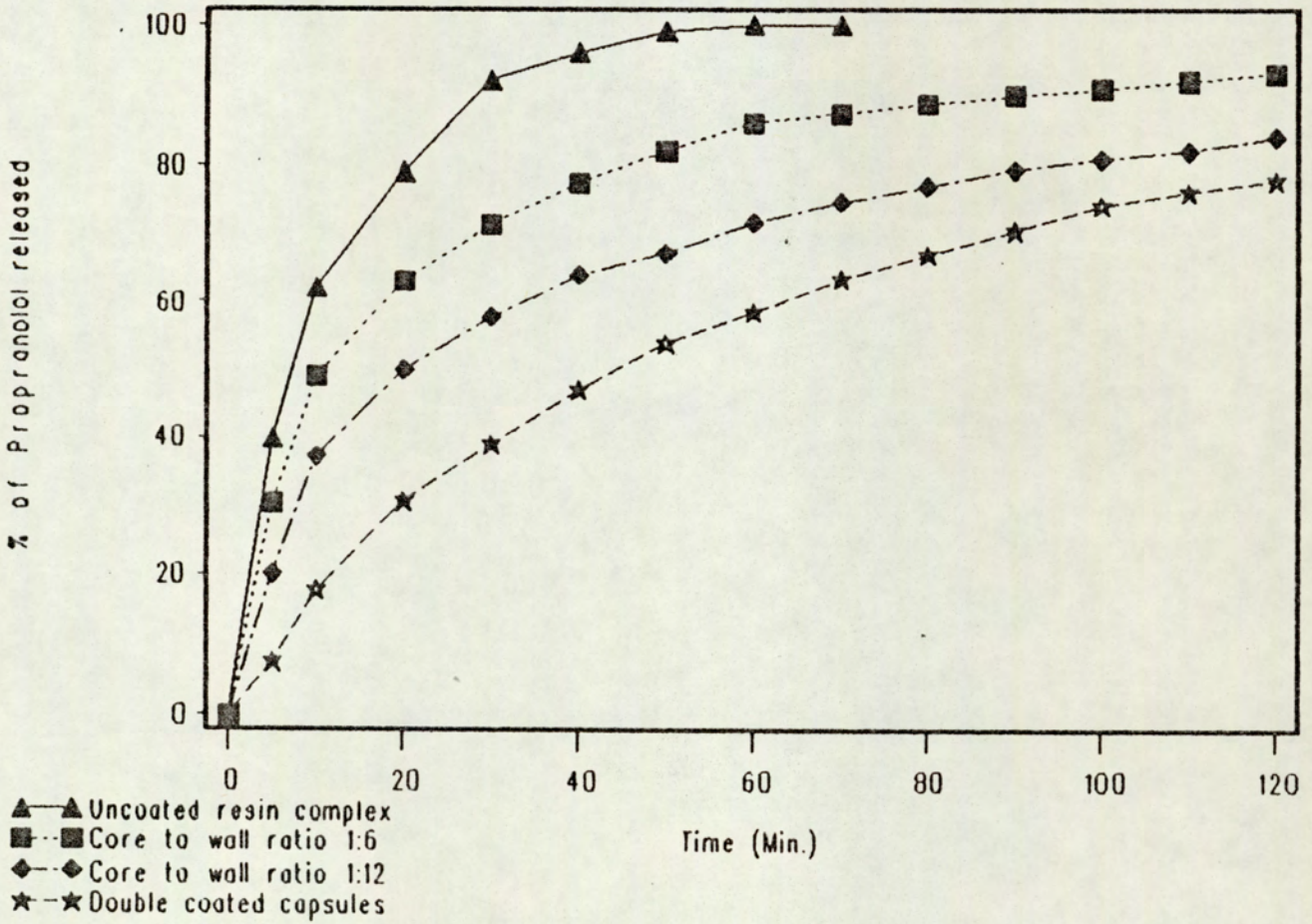


Fig 6.16 (a) Release profile of propranolol from pharmaceutical grade resin Fine particle size (1RP69) encapsulated with different ratios of gelatin-acacia.

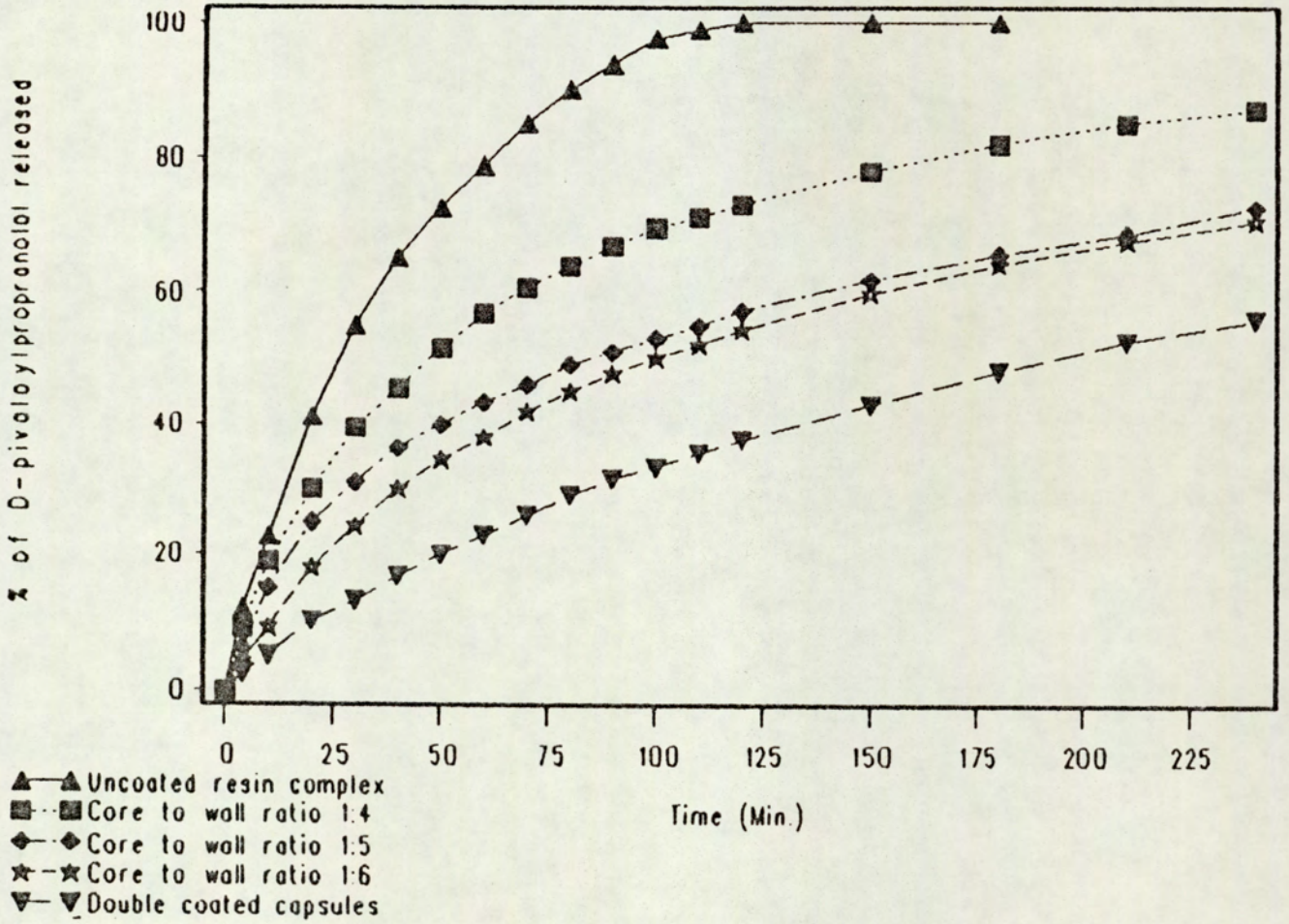


Fig 6.16 (b) Release profile of O-pivaloylpropranolol from pharmaceutical grade resin (Fine particle size) encapsulated with different ratios of gelatin-acacia.

Tables 6.9 a and 6.9b show the sieve analysis of the prepared microcapsules for propranolol and O-pivaloylpropranolol resinates respectively, using a nest of B.S. 1796 sieves by manual shaking. The fraction 53 - 90 μm from the single coated resinates and the fraction 125 - 150 μm from the double coated ones were used for the in-vitro-dissolution tests.

The microscopic examination of the drug-resin complex and the microcapsules were carried out after staining the particles with Sudan III. Figures 6.17 a and b show the microscopic appearance of the pharmaceutical grade resin (IRP69) - drug complex before and after trituration with glycerol prior to encapsulation.

The resinate particles triturated with glycerol are shown to be broken up into microparticles which facilitates encapsulation. The microscopic appearance of the microencapsulated drug-resin complex before hardening with formaldehyde (Fig 6 - 18) was translucent, smooth and round while after hardening, (Fig 6.19) the translucency was lost due to dehydration and the resin particles cannot be seen.

The appearance of these microcapsules (after dissolution in simulated gastric juice pH 1.6) under the microscope (Fig 6.20) shows that the translucency is much greater than before hardening due to the rehydration of the capsules during dissolution. The shape of the microcapsules is still intact. The microscopic appearance for the double coated drug-resin complex is shown in Fig 6.21.

6.3.12 STABILITY OF THE UNCOATED AND COATED DRUG RESIN COMPLEXES

Dissolution studies have been carried out in cooled, boiled double-distilled water on the uncoated drug-resin complex as well as the coated ones. No significant release of drug was noted from either system.

Particle Size µm	Fraction Retained		
	Core to Wall Ratio		Double Coated Capsules
	1 : 6	1 : 12	
> 150	0.3	0.5	3
125 - 150	7.6	0.6	77
90 - 125	27.9	14.3	22
53 - 90	56.9*	72.0	-
38 - 53	6.5	11.3	-
< 38	0.8	1.3	-

* This fraction was used for preparing the double coated micro-capsules

Table 6.9a Sieve analysis of the microencapsulated propranolol-resin (IRP69) complexes.

Particle Size µm	Fraction Retained			
	Core to Wall Ratio			Double Coated Capsules
	1 : 6	1 : 12	1 : 6	
> 150	1.9	0.6	0.2	3.0
125 - 150	6.0	4.7	0.7	67.0
90 - 125	32.9	25.0	21.5	30.0
53 - 90	50.2	61.0	65.2*	-
38 - 53	5.3	6.7	11.2	-
< 38	3.7	2	0.8	-

* This fraction was used for preparing the double coated micro-capsules

Table 6.9b Sieve analysis of the microencapsulated O-pivaloylpropranolol-resin (IRP69) complexes.

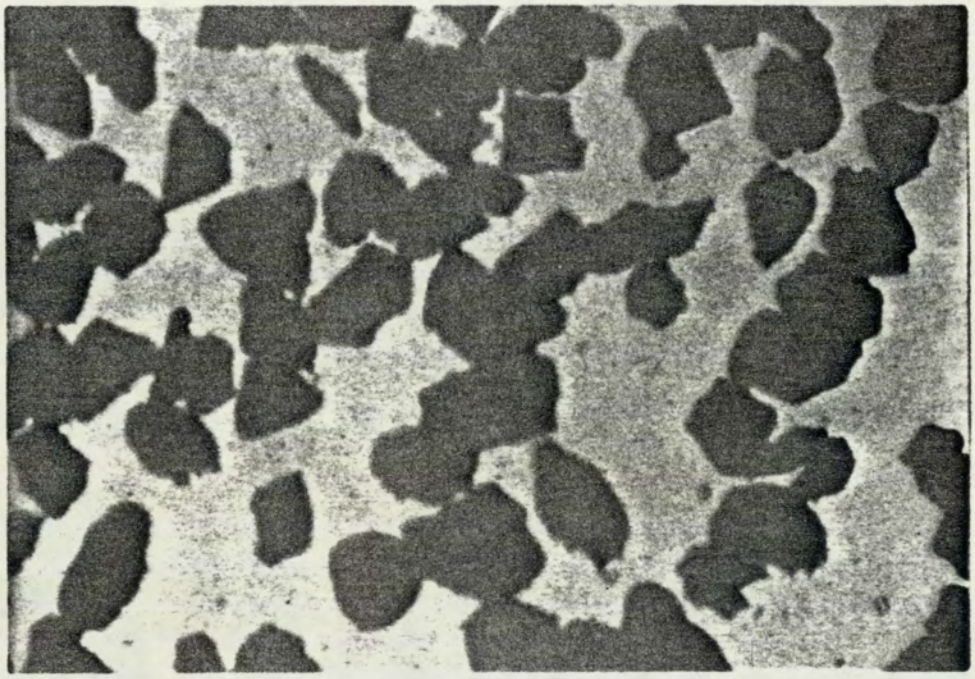


Fig 6.17.a Propranolol resin IRP69) complex before encapsulation (X400)

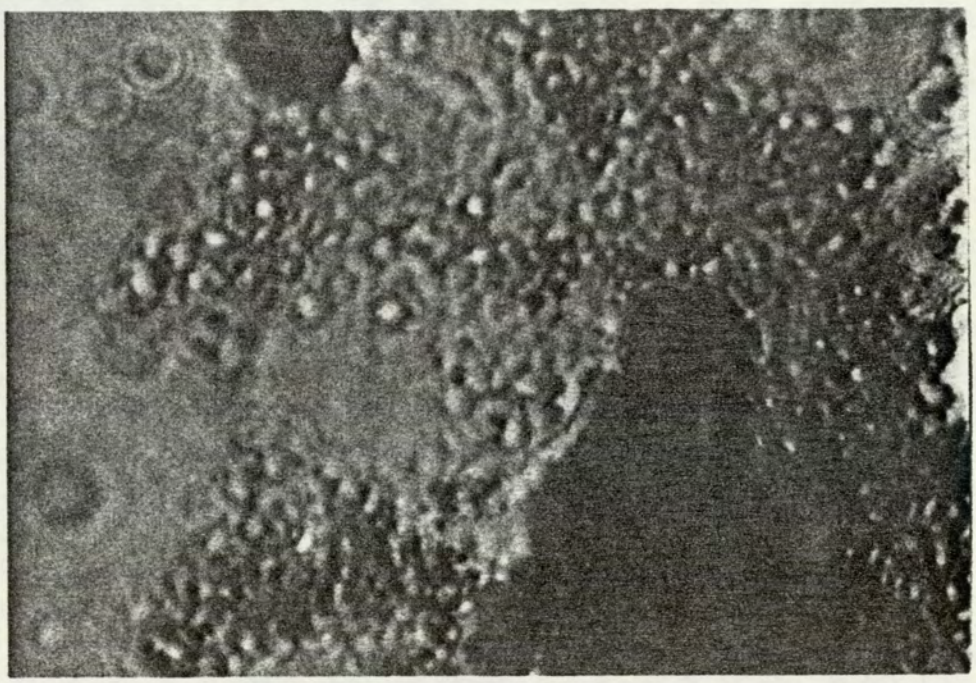


Fig 6.17.b Propranolol resin (IRP69) complex particle during trituration with glycerol prior to encapsulation (X800)

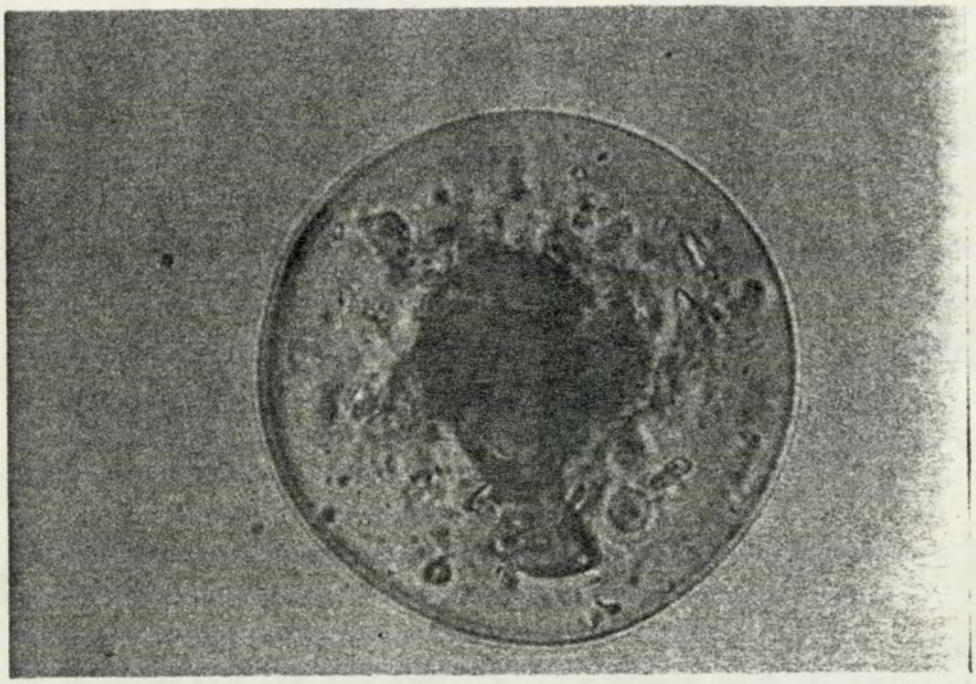


Fig 6.18 Microencapsulated propranolol resin (IRP69) complex before hardening (X400)

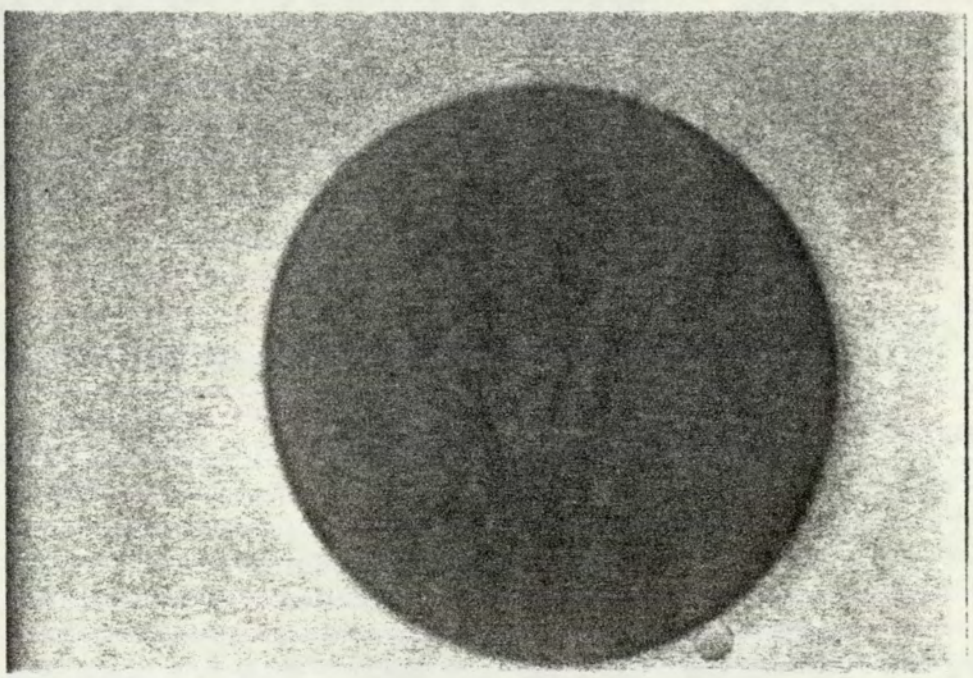


Fig 6.19 Microencapsulated propranolol resin (IRP69) complex after hardening with formaldhyde for 14 hours (X400)

Fig 6.20 Microencapsulated Propranolol resin (IRP69) complex after Dissolution (X400)

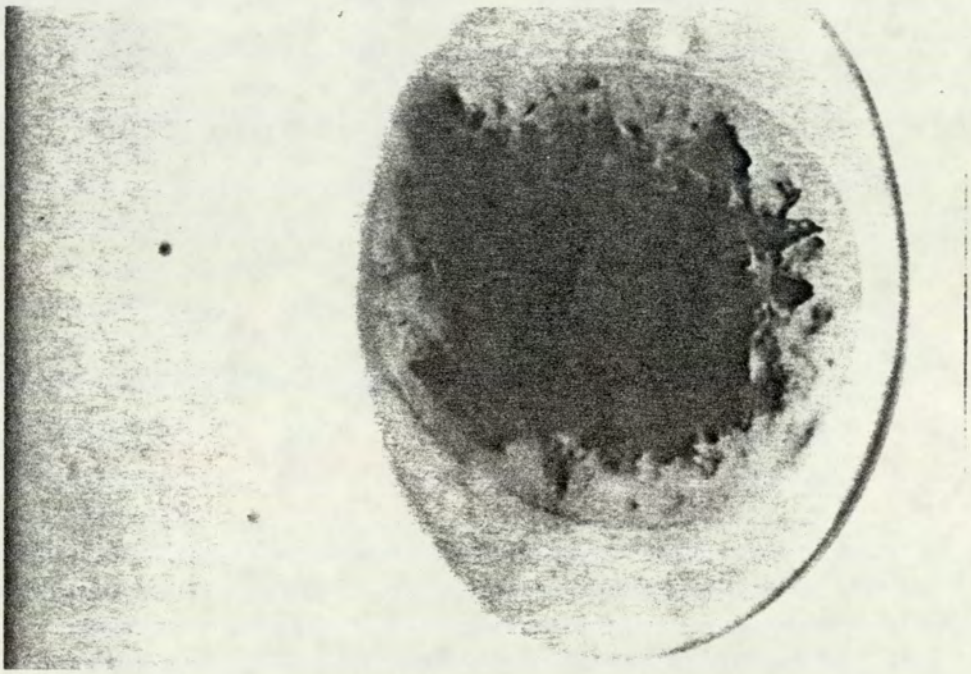


Fig 6.21 Double coated microcapsule containing propranolol resinates (X400)

6.3.13 APPLICATION OF ION-EXCHANGE RESIN SYSTEMS FOR A SUSTAINED-RELEASE FORMULATION OF SOME OTHER DRUGS

Drugs such as nadalol, promazine HCl, doxipen HCl, nortriptyline HCl and amitriptyline HCl have been loaded onto a Dowex resin of 8% cross-linking and particle size of 50 - 100 mesh at room temperature (22°C).

The loading of these drugs is recorded in Table 6.10. The variation of the drug content probably results from differences in the volume structure of the drug molecules and from the orientation in which the drug interacts with the anionic centre within the resin particle.

Compound	% Loading
Nadalol	32.10
Promazine HCl	10.96
Nortriptyline HCl	12.73
Amitriptyline HCl	13.97
Doxipen HCl	16.50

Table 6.10 Loading of Nadolol and some anti-depressant Drugs onto Dowex 50 WX8, 50 - 100 mesh

Fig 6.22 shows comparative release profiles of these compounds in simulated gastric fluid, pH 1.6, at 37°C, the variation of the release rate may be related to their aqueous solubility and hence the more soluble the drug (e.g. nadolol) the faster its release.

Table 6.11 shows comparative loading of amitriptyline in the drug-resin complex prepared at room temperature and by reflux and the percentage of drug released in the same dissolution medium described earlier at 37°C after 5 hours.

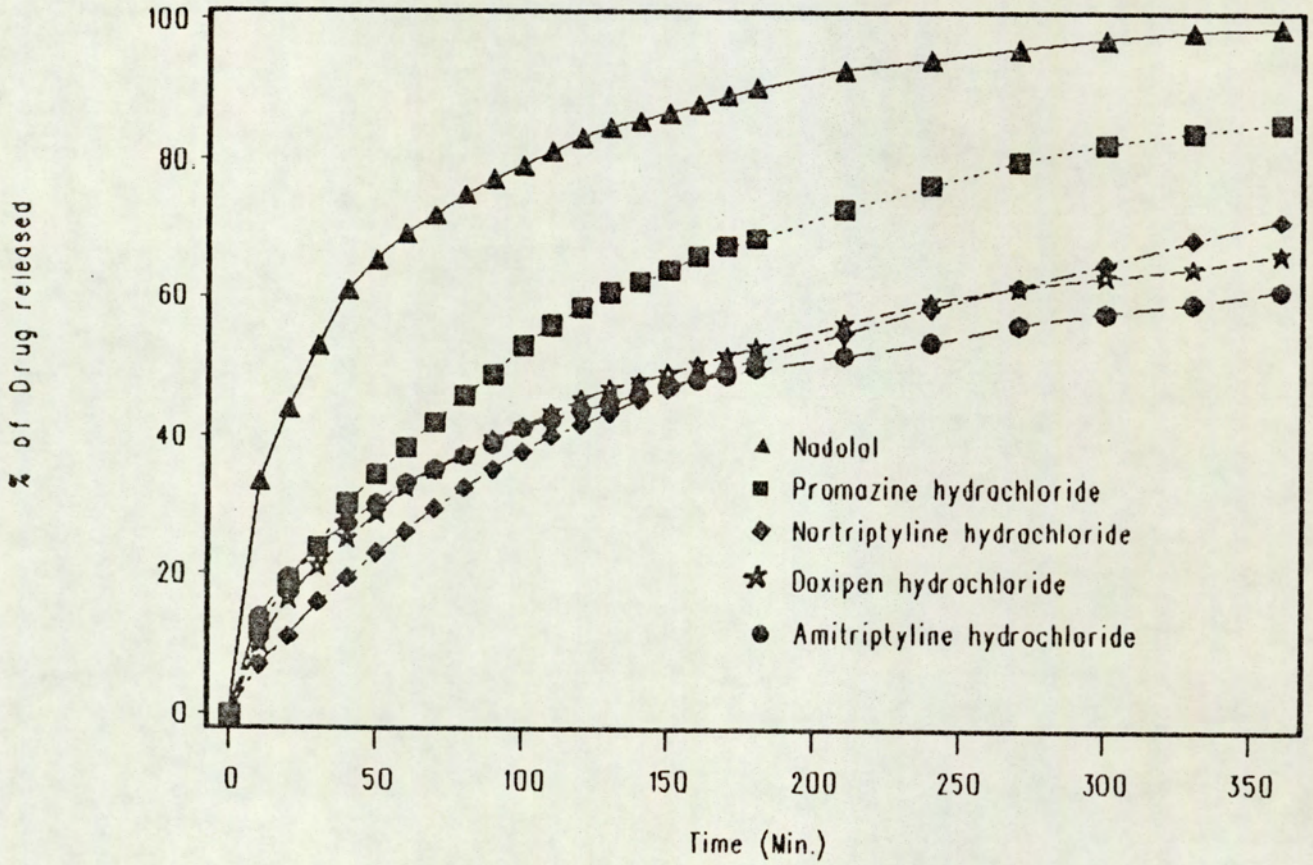


Fig 6.22 The drug release rate from the resinate made with Dowex 50 WX8, 50 - 100 mesh.

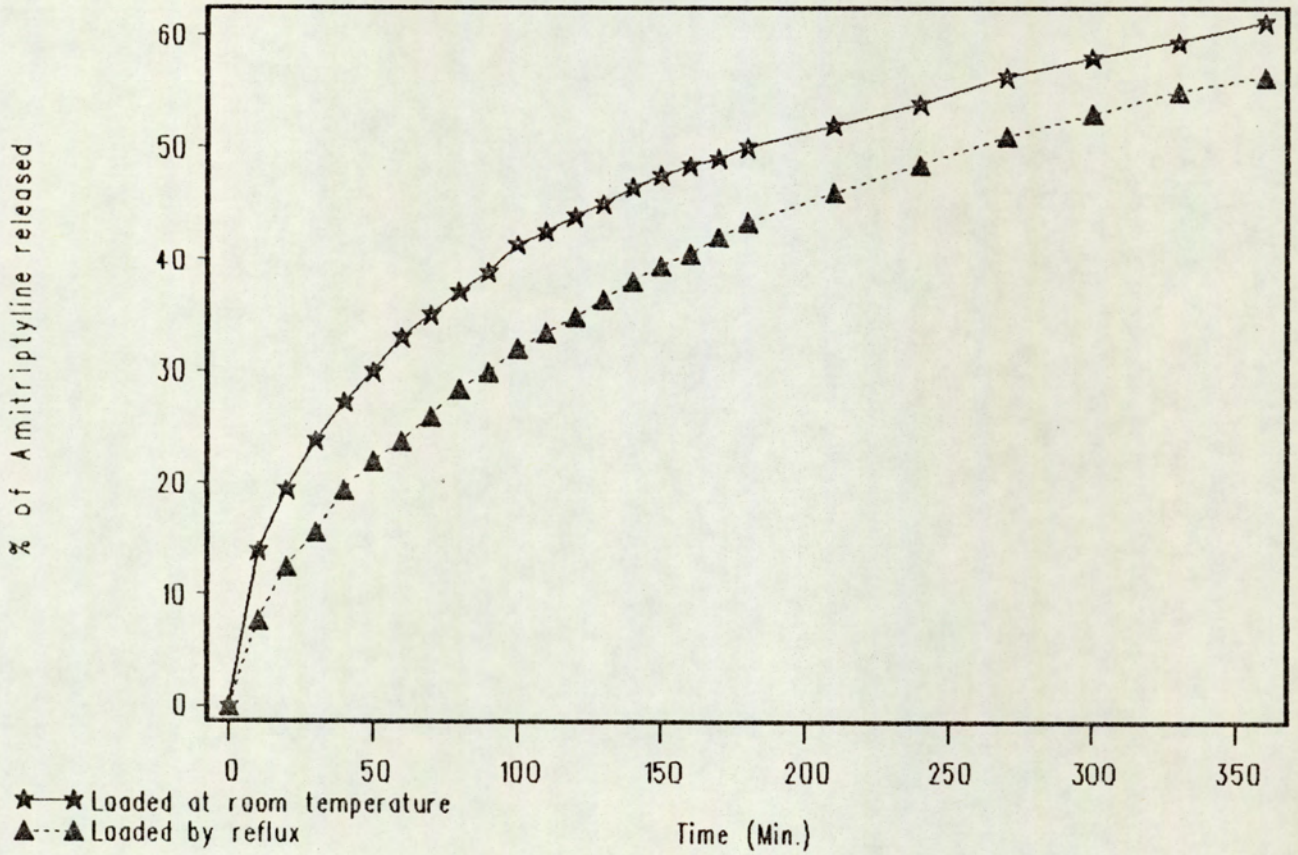


Fig 6.23 Effect of the loading temperature on the release of Amitriptyline HCl is simulated gastric juice pH 1.6 at 37°C.

Temperature	% Loading	% of Amitriptyline Released in 5 Hours
Room Temperature ($22^{o}C$)	13.97	58
Reflux	29.70	53

Table 6.11 The Effect of Temperature on the Content and Release of Amitriptyline from the Resinates

Loading of amitriplyline by reflux has been shown to produce a resinate with high drug content with more than twice of that loaded at room temperature. This is probably due to the same reason discussed earlier in Section 6.3.8, although the effect on the release rate in this instance is less significant (Fig 6.23).

6.4. CONCLUSIONS

The following conclusions can be drawn based upon the in-vitro dissolution tests.

- Despite drying all the drug-resin complexes in the same way, the moisture contents were different. This emphasises the necessity of the determination of the adsorbed water in such resinates.
- The amount of drug bound to the resin increases by decreasing the degree of cross-linking.
- The release rate of the drug from its resinate decreases as the degree of cross-linking and the particle size increase.

Therefore, the release rate can be controlled to some extent by choosing the correct cross-linking degree and the appropriate particle size.

- Loading of drugs onto the ion-exchanger by reflux increases the drug content and reduces the release rate. The major limitation of this process that it can not be used for thermolabile drugs.

- By microencapsulation of the drug resin complex, the release rate may be suppressed to provide usable sustained profiles.
- The release rate can easily be adapted to requirements by either mixing the coated and uncoated drug-resin complexes in the appropriate ratios or by selecting the correct core to wall ratios.
- Micro-encapsulation of the drug resinate with gelatin-acacia yielded a low drug content product which makes this process limited to potent drugs of low therapeutic dose.

CHAPTER SEVEN

BIOAVAILABILITY OF SUSTAINED-RELEASE PROPRANOLOL

7. BIOAVAILABILITY OF SUSTAINED-RELEASE PROPRANOLOL

7.1 INTRODUCTION

Bioavailability has been defined as the measurement of both the relative amount of an administered dose that reaches the general circulation and the rate at which this occurs [*Notari 1971 (216)*].

The absorption rate may be the more critical pharmacokinetic parameter in the totality of drug effect. A drug that enters the circulation very rapidly may induce untoward responses whereas if it is absorbed too slowly, it may not achieve sufficient body levels to produce a desired intensity of pharmacologic response, even if the entire dose is ultimately absorbed. It is equally obvious that the onset of pharmacologic response from a single dose of a drug is directly influenced by the rate of availability.

One of the most difficult problems encountered in developing a pharmacokinetic profile of a drug is the assessment of the rates of bioavailability, which, are always model-dependent and must frequently be attempted with the most shocking paucity of data [*Gibaldi and Perrier 1975 (300)*]. The most satisfactory approach to the quantification of the availability of a drug is to compare plasma levels or urinary excretion data following oral, intramuscular or other administration of a drug in a given dosage form to that following intravenous administration of a solution of the drug. For practical reasons, intravenous administration is often not possible, and in such cases comparisons of the test drug and/or dosage form may be made to a secondary reference standard such as an orally administered solution of a drug or a dosage form which has been accepted as standard. If a secondary reference standard is employed, however, one measures the relative availability of a drug as opposed to the absolute availability which is determined when an intravenous dose is used as the reference standard.

The most frequently used approach to the measurement of availability is to compare the area under the plasma concentration-time curve (AUC) following administration of oral and standard dosage forms. This leads to:-

$$\text{Apparent availability} = \frac{(AUC)\,T}{(AUC)\,s}$$

where the terms (AUC)T and (AUC)s are the areas under the plasma concentration versus time from time zero to infinity for the test and the standard. Two methods for calculating area under a plasma concentration-time curve are the "cut and weigh" method and application of the trapezoidal rule. Simpson's method is also available but this requires a strict protocol for data collection. The trapezoidal rule involves the description of a given plasma concentration-time curve by a function that depicts the curve as a series of straight lines, thereby enabling the area under the curve to be divided into a number of trapezoids. The area of each trapezoid is easily calculated (using equation 7.1) and the sum of all the areas of all the trapezoids yields an estimate of the true area under the curve of interest.

$$AUC = \int_{to}^{tn} \emptyset\,(t)\,dt = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}\,(C_i + C_{i+1}) \qquad 7.1$$

where the term $\int_{to}^{tn} \emptyset\,(t)\,dt$ is equivalent to the estimated area under the curve, C_i is the plasma concentration at time t_i and t = time.

There are a number of circumstances which may arise which make it difficult to determine the availability of a drug following administration of a single dose. The plasma levels of certain drugs may be very low (e.g. propranolol) such that an assay method sensitive enough to measure those levels may not be available. Other drugs have very long half-lives and the determination of the total area under a plasma concentration-time curve requries plasma samples to be obtained for excessively long periods of time.

In this chapter, the bioavailability of an oral single dose sustained-release preparation containing propranolol HCl has been studied using an aqueous propranolol as the reference standard formulation.

7.2 EXPERIMENTAL

7.2.1 FORMULATION

The sustained-release formulation comprises a propranolol-resin complex prepared with a cationic resin (Dowex) of 4% cross-linking agent and 50 - 100 mesh particle size (32% drug content), was suspended in 1% aqueous gum tragacanth with a concentration of 32mg drug-resinate per 100ml to give a final propranolol concentration in the suspension of 1mg/ml. This fomulation is denoted by SRP and is compared with aqueous propranolol (1mg/ml) which is denoted by CP and is used as a reference standard.

In-vitro dissolution for the SRP formulation has indicated that the time taken for 90% of the drug to dissolve in simulated gastric juice, pH 1.6, at 37^{o}C is 3 hours.

7.2.2 METHOD

Five male Wistar rats were used for each formulation (SRP and CP). The rats were fasted overnight and weighed (370g average weight). The dose for each rat was calculated (2mg/kg) and administered orally using a 5ml syringe fitted with a curved animal feeding needle.

About 1.2 ml of blood samples were collected by cardiac puncture using disposable syringes equipped with a 21 gauge, 1.5 inch needle at time interval up to 24 hours. Immediately after collection, blood was transferred into 10ml tubes containing lithium heparin as anticoagulant (Brunswick 10ml lithium heparin polystyrene tubes) and gently agitated. The samples were centrifuged at 2500 rpm for 20 minutes to separate the plasma, a quantity of which (0.5 ml) was then transferred to a centrifuge tube. To the 0.5 ml of the heparinized rat plasma, 100 µl of internal standard solution (desimipramine in acetonitrile 5µg/ml) and 100µl of 1M NaOH was added. This was followed by the addition of 3 ml of ethylacetate (HPLC grade) and the system was vortex-mixed for 30 seconds and then centrifuged for 10 minutes at 2500 rpm. The clear organic layer (2.5ml) was transferred to

a clean glass test-tube for evaporation under a gentle stream of nitrogen at 30°C. The residue was redissolved in 0.5 ml of the mobile phase by placing the test-tube in an ultrasonic water bath for 5 minutes to insure complete solubilization. Samples were analysed by HPLC with fluorometric detection.

7.2.3 CHROMATOGRAPHIC APPARATUS AND CONDITIONS

The high-performance liquid chromatograph consisted of an Altex 100A double piston constant flow solvent-metering pump, a Rheodyne 7120 injector fitted with a 20µl loop, a Perkin Elmer LS.5 luminescence spectrometer equipped with a 25 µl flow cell operated at 285 nm (excitation) and 405 nm (emission); the slit openings both for excitation and for emission were 15 and 20 nm respectively, with the sensitivity range set at 7.2%, and a J.J chart recorded (J.J Instruments). The column used in this study was 10 cm x 4.6 mm. I.D. Shandon stainless steel column packed with Hypersil-ODS, 5µm. The mobile phase consisted of acetonitrile, diethylamine, orthophosphoric acid (88%) and water (65 : 0.02 : 0.08 : 34.9) pH3 (Table 3.8). This solution was degassed before use in an ultrasonic bath. The mobile phase flow rate was 1 ml/min. The minimum concentration of propranolol that could be quantified was 0.5 ng/ml.

7.2.4 REPRODUCIBILITY STUDIES

Reproducibility studies were performed at two concentrations of propranolol by doing five replicate analyses of plasma samples which had been spiked, such that the concentrations were 50 and 200 ng/ml. At 50 ng/ml level, the coefficient of variation was 8.3% while at the 200 ng/ml level the corresponding value was 5%.

7.2.5 PREPARATION OF THE STANDARD SOLUTIONS

Stock solutions containing 1 mg/ml propranolol HCl and 4-hydroxypropranolol HCl were prepared separately in acetonitrile. From these solutions a stock plasma standard was

made containing propranolol HCl and 4-hydroxypropranolol (10 μg/ml each as the free base). The stock plasma standard was further diluted with drug-free plasma to cover a concentration range of both compounds in plasma of 10 - 200 ng/ml. Solutions were analysed by treatment with the internal standard, extraction and chromatography as described earlier in Section 7.2.2. In order to provide standard curves, the peak height ratio of propranolol and 4-hydroxypropranolol to desimipramine were plotted against the concentration of propranolol and 4-hydroxypropranolol. A linear regression analysis was used to determine the concentration of propranolol and 4-hydroxypropranolol from the calibration curves.

7.3 RESULTS AND DISCUSSION

7.3.1. PLASMA PROPRANOLOL CONCENTRATION

Concentrations of propranolol in heparinized plasma samples were determined by high-performance liquid chromatography using fluorometric detection [excitation and emission wavelength were set at 285 nm and 405 nm respectively, according to the published method [*Albani et al 1982 (133)*]. The typical chromatograms obtained from a plasma blank, propranolol and 4-hydroxypropranolol-supplemented plasma and a plasma sample from a rat treated with drug are shown in Fig 7.1 The chromatographic studies show that the traces of 4-hydroxypropranolol have not been detected from the plasma samples which may be due to failure to liberate this metabolite from conjugates or to the oxidation of 4-hydroxypropranolol during storage of the blood samples after collection.

Mean plasma propranolol concentrations obtained during the single-dose studies of SRP and CP preparations are listed in Table 7.1 and are graphically displayed in Fig 7.2. After administration of an oral single 2mg/kg dose (Table 7.1, Fig 7.2), SRP preparation produced mean plasma levels from 1 to 8 hours that were lower than those of CP preparation.

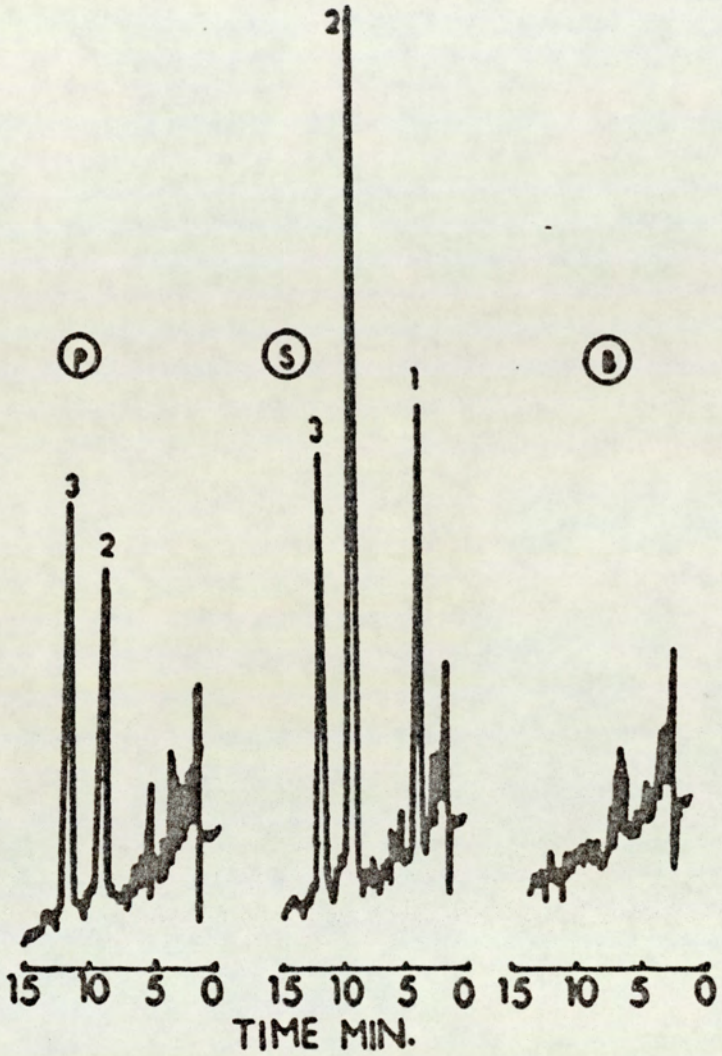


Fig 7.1 High-performance liquid chromatograms of blank plasma {B}, plasma spiked with 4-hydroxypropranolol (1),propranolol (2) and desimipramine (3)[100,100 and 500 ng/ml respectively]{S} ; and a plasma sample collected 5 hours after administration of SRP dose and supplemented with 500 ng desimipramine (Int. Std.){P}.

Column :10 cm x 4.6 mm I.D ,Hypersil-ODS (5µm)
Mobile phase :MeCN : Et_2NH : H_3PO_4 : H_2O
(65 : 0.02 : 0.08 : 34.9 v/v/v/v)pH 3
Flow rate : 1ml/min.
Detection :285 nm (excitation),and 405 nm (emission)
sensitivity : 7.2 %

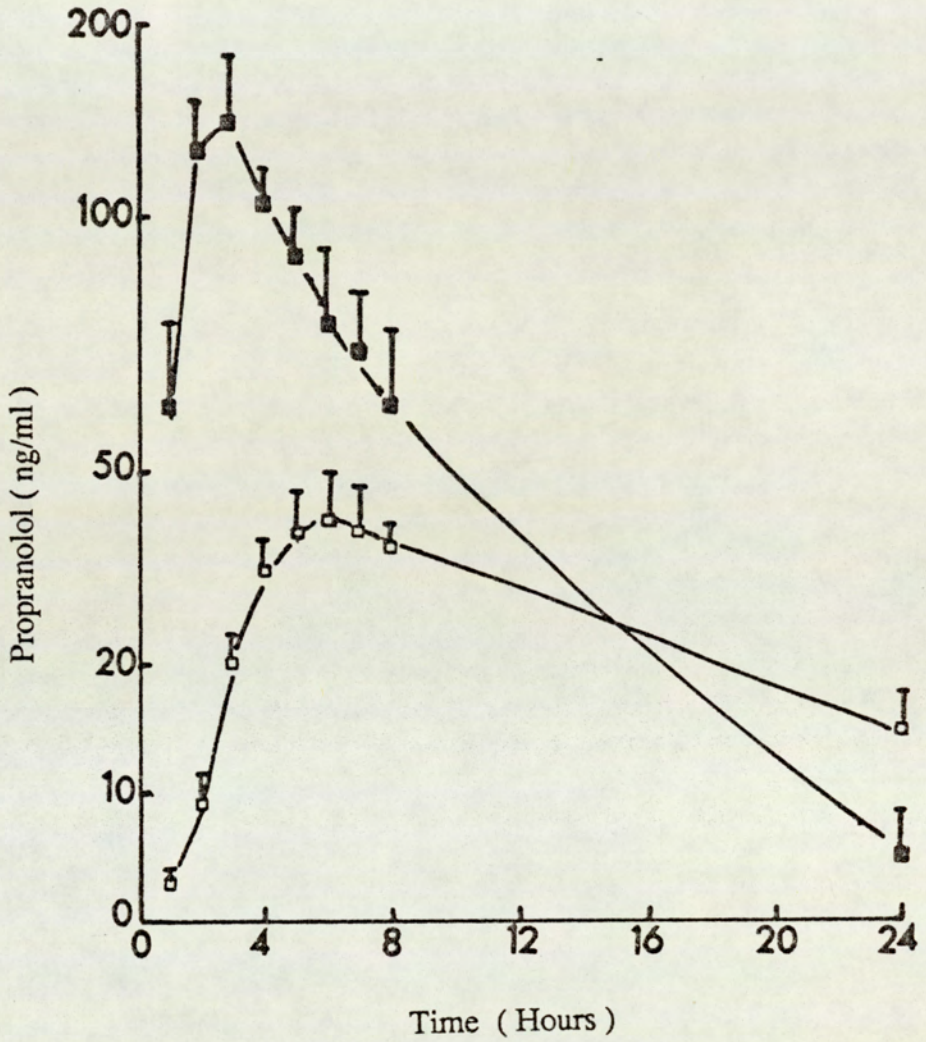


Fig 7.2 Mean ± S.D. mean plasma propranolol concentration following administration of an oral single-dose (2mg/ml) of SRP (- □ -) and CP (- ■ -) preparations.

Time after dosing (hour)	Plasma Propranolol Concentrations (ng/ml)	
	SRP Preparation	CP Preparation
0	-----	--------
1	60 ± 20	2 ± 0.8
2	131 ± 22	9 ± 3.2
3	148 ± 41	21 ± 4
4	107 ± 18	35 ± 5
5	90 ± 15	41 ± 7
6	74 ± 16	43 ± 7
7	69 ± 10	40 ± 8
8	61 ± 12	38 ± 5
24	5 ± 3	15 ± 3

Table 7.1 Summary of the mean plasma propranolol concentration ± S.D, following of an oral single-dose of SRP and CP preparations.

7.3.2 PHARMACOKINETIC COMPARISON

Table 7.2 shows the comparative pharmacokinetic parameters of SRP and CP preparations. Peak plasma concentrations (C_{max}) recorded after administration of the SRP preparation were significantly lower than the CP values, and the times to peak levels (t_{max}) were 3 and 6 ± 1 hours for CP and SRP respectively.

The relative bioavailability of SRP preparation compared to that of the CP formulation was estimated from the area under the plasma concentration-time curves from 0 to 24 hours (0 - 24 AUC). Calculated in this way, the bioavailability was significantly reduced for the sustained release formulation (SRP) in comparison with those achieved by conventional propranolol (CP) in oral single doses. This difference could be due to either incomplete absorption across the gut wall, or to a higher degree of metabolism on the first pass through the liver due to a slower rate of absorption. Paterson et al 1970 (301) have demonstrated in man that absorption across the gut wall with conventional propranolol appears to be complete, but the effect of different rates of gastric emptying must be taken into account.

Parameter	Value	
	SRP Preparation Dose: 2mg/kg	CP Preparation 2mg/kg
C_{max} (ng/ml)	43 ± 7	148 ± 41
t_{max} (hours)	6 ± 1	3 ± 0
0 - 24 AUC ng/ml x h	646 ± 100	1237 ± 287
Elim.$t_{1/2}$ (hours)	12	4.5

Table 7.2 Comparative pharmacokinetic parameters for the administration of an oral single dose (2mg/kg) of propranolol in sustained-release(SRP)and aqueous (CP) preparations

CONCLUSIONS

CONCLUSIONS

The results of the present study reveal that, by esterification of propranolol, lipophilicity was improved by the increase of the true partition coefficient of the drug. As the ester chain length increases, the partition coefficient increased with decreasing aqueous solubility of the free bases.

High-performance liquid chromatography (HPLC) methods were developed for the simultaneous determination of these pro-drugs and their degradation products during kinetic assessments of stability profiles.

The degradation of O-n-acyl propranololsunder alkaline conditions was found to proceed via competing hydrolysis to the required propranolol, and rearrangement to yield the N-acyl derivatives. This is in contrast to the O-pivaloyl derivative which undergoes a simple hydrolysis to propranolol without involvement of competing rearrangement.

The N-acyl derivatives were stable under the conditions of the experiments and reaction did not proceed further. The formation of N-acyl propranolols is pH dependent. The mechanism is not intermolecular and presumably involves the attack by the amino pair of electrons at the acyl carbon atom. Such a reaction should be inhibited under acidic conditions. The hydrolysis rate is also dependent upon pH with maximum stability demonstrated at pH 3 - 3.5.

The transition state for intramolecular acyl rearrangement appears rather bulky, and it might be expected that , as larger substituents are examined, the rearrangement process may becomes less important. The increased bulk of the substitutent also reduces the hydrolysis rate, but overall in buffered-DMF pH 10 at 37^{o}C, the propionyl compound is a significantly better precursor of propranolol than the acetyl derivative; favouring the hydrolysis pathway by almost five-times compared with the shorter chain ester.

Higher chain homologues complement these results and show a somewhat reduced hydrolysis rate coupled with further suppression of the rearrangement reaction.

Maximal effects are observed with the O-hexanoyl derivative and further increase in chain length has little effect upon the pure chemical stability profiles.

In enzymatic systems, using isolated esterases and 90% rabbit serum, the specifity of the enzyme was such that the competing rearrangement reaction was much inhibited. Indeed, with ester homologues larger than O-propionyl, no rearrangement was detected at all.

The generation of propranolol under these conditions was found to occur at a maximum rate with the O-hexanoyl derivative, but all compounds showed acceptable release rate for propranolol.

Experiments in isolated everted sac of rat small intestine confirmed these results and showed that degradation essentially occurred in the serrosal tissue.

Incorporation of these prodrugs into controlled release delivery systems using cation exchange resin for the formulation of liquid dosage forms, shows that, the release rate of the drugs from their resinate decreases as the degree of cross-linking and the particle size increase. Better control of release rates was achievable when the drug resin complex was coated with gelatin-acacia co-acervate (microencapsulation) and the release rate was further reduced when drug-resin complex particles were double coated. The release rate can easily be adapted to requirements by either mixing the coated and uncoated drug-resin complexes in appropriate ratios or by selecting the correct core to wall ratios.

The bioavailability study of the sustained release formulation showed a decrease in the absorption and elimination rates. The availability was found to be lower than that of the standard reference (aqueous propranolol) which may be due to the incomplete absorption and/or to the first-pass effect.

APPENDICES

APPENDIX I LIST OF ABBREVIATIONS

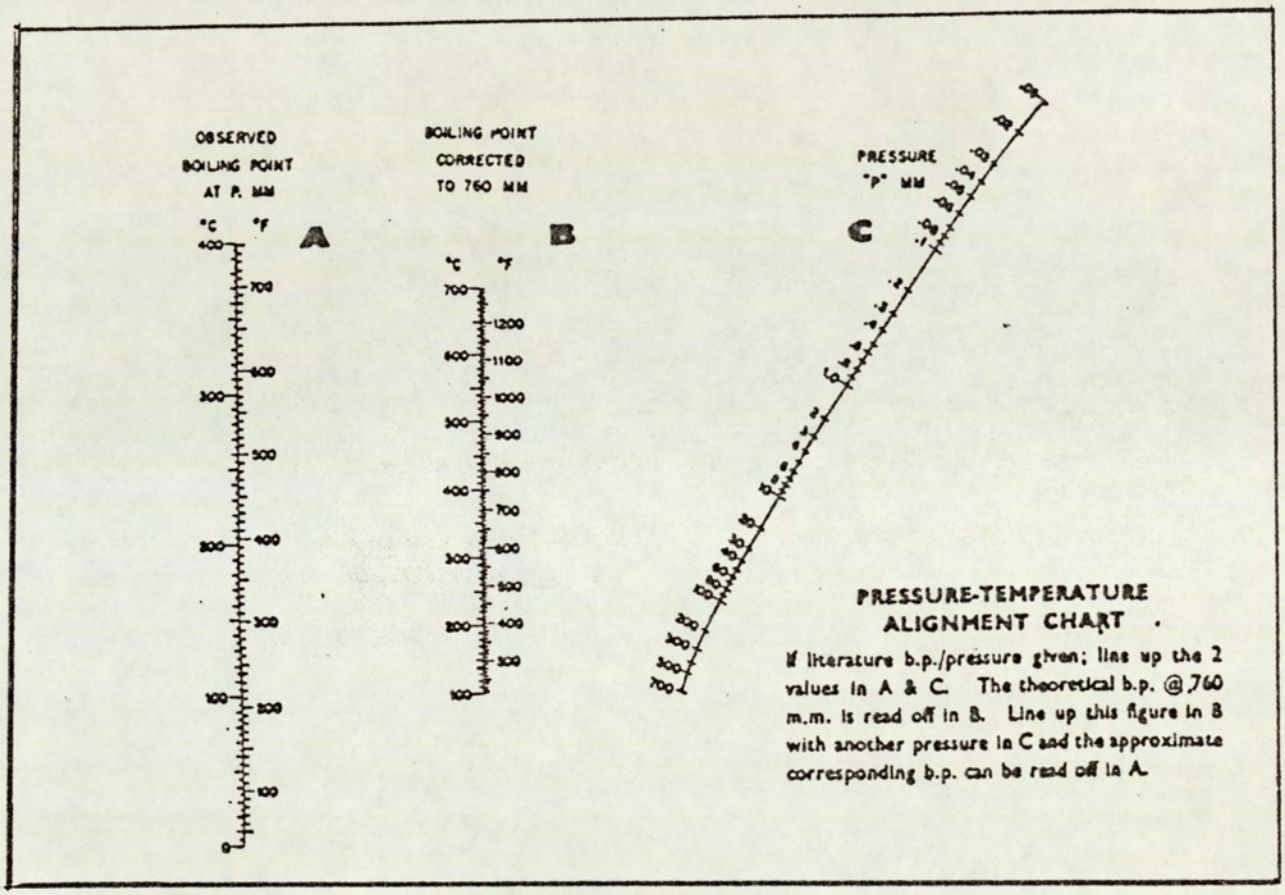
AR	Analytical reagent
AUFS	Absorbance units full scales
BP	British Pharmacopoeia
DMF	Dimethylformamide
DTAB	Dodecyltrimethylammonium bromide
GPR	General purpose reagent
HPLC	High - performance liquid chromatography
Int. Std.	Internal standard
ODS	Octadecylsilane
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
PG	Propylene glycol
SLS	Sodium lauryl sulphate
SLR	Standard laboratory reagent
TAC	Tetramethylammonium chloride
UV	Ultraviolet

APPENDIX II LIST OF CHEMICALS

CHEMICAL	GRADE	SUPPLIER
Acacia	BP.	Macarthys
Acetic acid	AR	Fisons.
Acetonitrile	HPLC	Fisons
Acyl chlorides (from acetyl to decanoyl)	AR	Sigma
Amberlite resins (IRP69)	AR	Rohm &Haas
Amberlite CG50	AR	Matlinckrodt laboratory
Amitriptyline.HCl	AR	Lilly Research Centre
Boric acid	AR	B.D.H
Beta - Blockers (free samples)		I.C.I
Carboxylesterase	120 units/mg	Sigma
Chloroform	GPR	Fisons
Citric acid	SLR	Fisons
Desimipramine	AR	Sigma
Diethylamine	LR	Fisons
Diethylether	GPR	B.D.H
Dimethylformamide	SLR	Fisons
Disodium hydrogen ortho-phosphate	SLR	Fisons
DTAB	AR	Sigma
Dowex resins (different particle size and cross-linking)		Sigma
Doxipen	AR	Pfizer
Ethanol	SLR	Fisons
Ethyl acetate	AR	Fisons
Ethylchloroformate	SLR	Fisons
Formaldehyde 38%	SLR	Fisons
Gelatin	BP	Macarthys
Glucose	AR	B.D.H.
Glycerol	BP	Macarthys
Hydrochloric acid	SLR	Fisons
Hypersil-ODS (5 µm)		Shandon
Karl-Fischer reagent	AR	Fisons
Methanol	AR	Fisons
Methylene chloride	SLR	Fisons
Nortriptyline HCl	AR	Lilly Research Centre
Octanol	Spectral	Fisons

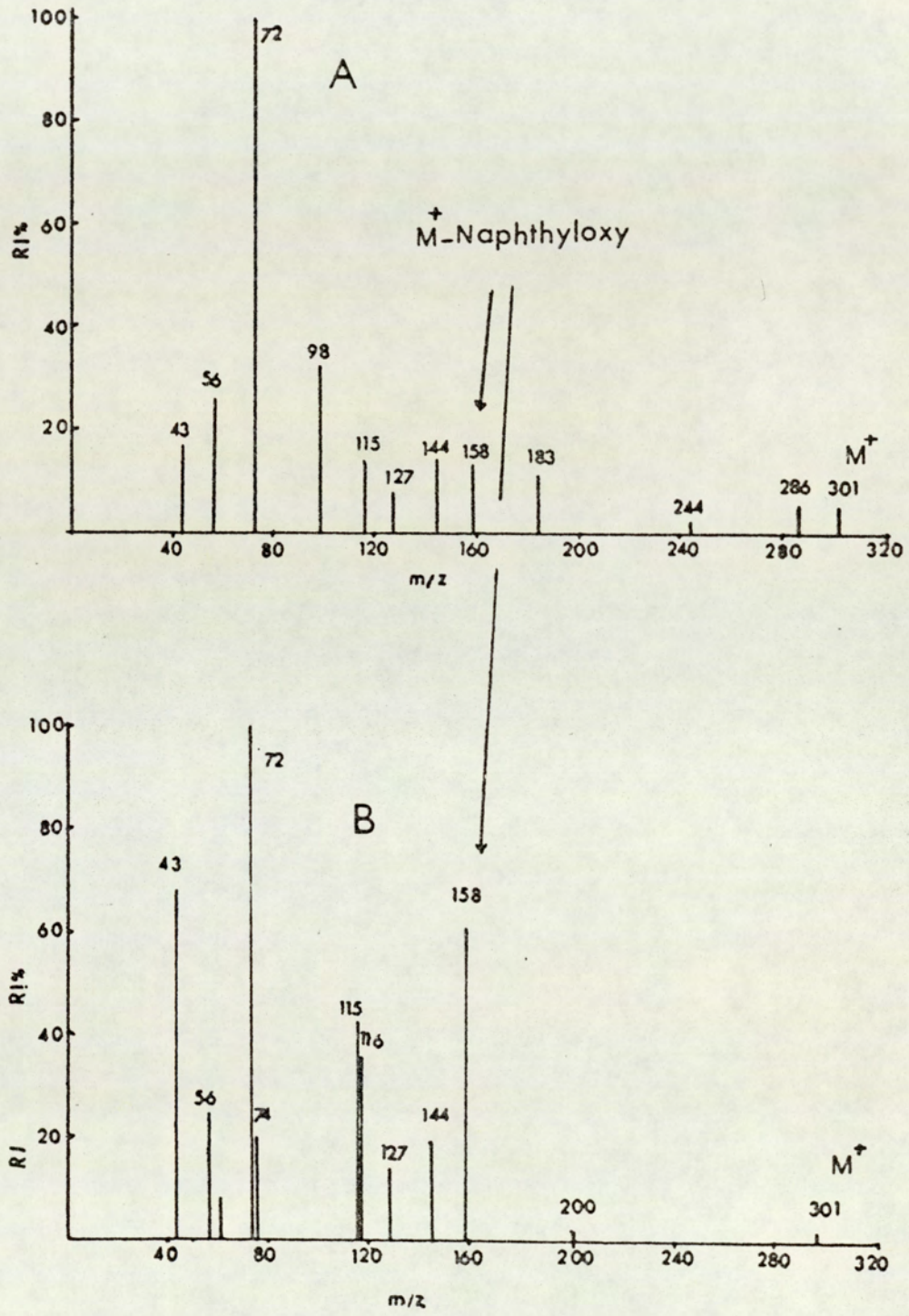
APPENDIX II Cont'd

CHEMICAL	GRADE	SUPPLIER
Parabens (from methyl to butyl ester)		Sigma
Phosphoric acid (88%)	SLR	Fisons
Polyethylene glycol (1000 to 20,000)		B.D.H
Polyvinylpyrrolidone (10,000 to 700,000)		Sigma
Potassium chloride	AR	Fisons
Potassium dihydrogen phosphate	GPR	Hopkins & William
Promazine HCl	AR	Wyeth
Propan -2-ol	AR	Fisons
Propranolol HCl		Sigma
Propylene glycol	GPR	B.D.H
Sodium bicarbonate	AR	B.D.H
Sodium carbonate	GPR	B.D.H
Sodium dihydrogen phosphate	LR	B.D.H
Sodium hexanesulphonate	AR	Fisons
Sodium hydroxide pellet	SLR	Fisons
Sodium hydroxide (volumetric solution)		B.D.H
Sodium lauryl sulphate	LR	Fisons
Sodium sulphate (anhydrous)	GPR	B.D.H
Tetramethylammonium chloride	AR	Aldrich
Tragacanth powder	BP	Thornton & Ross
Triethylamine	SLR	Fisons
Tween 80		Sigma

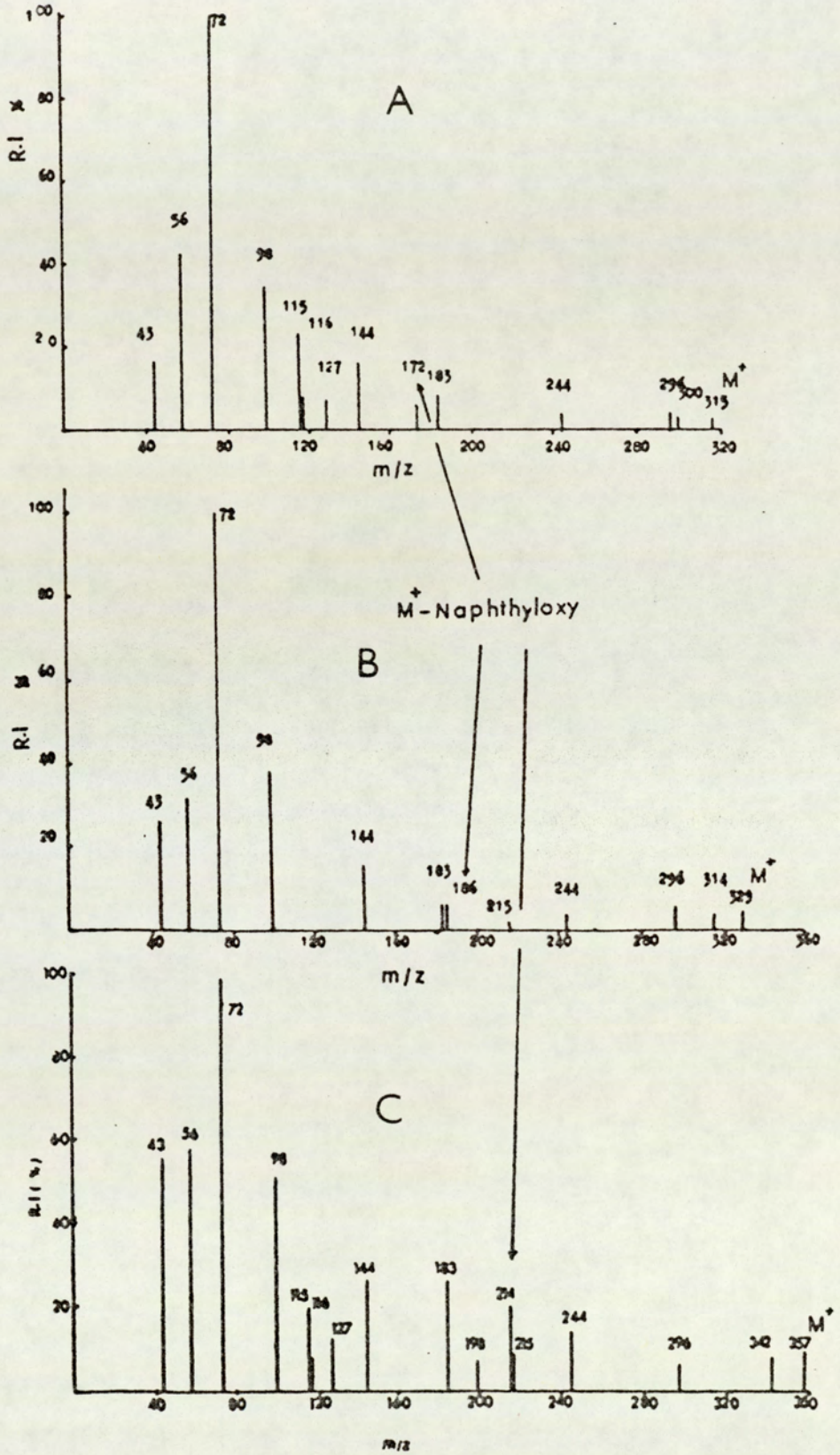


Appendix III Boiling Point-Pressure Nomogram.

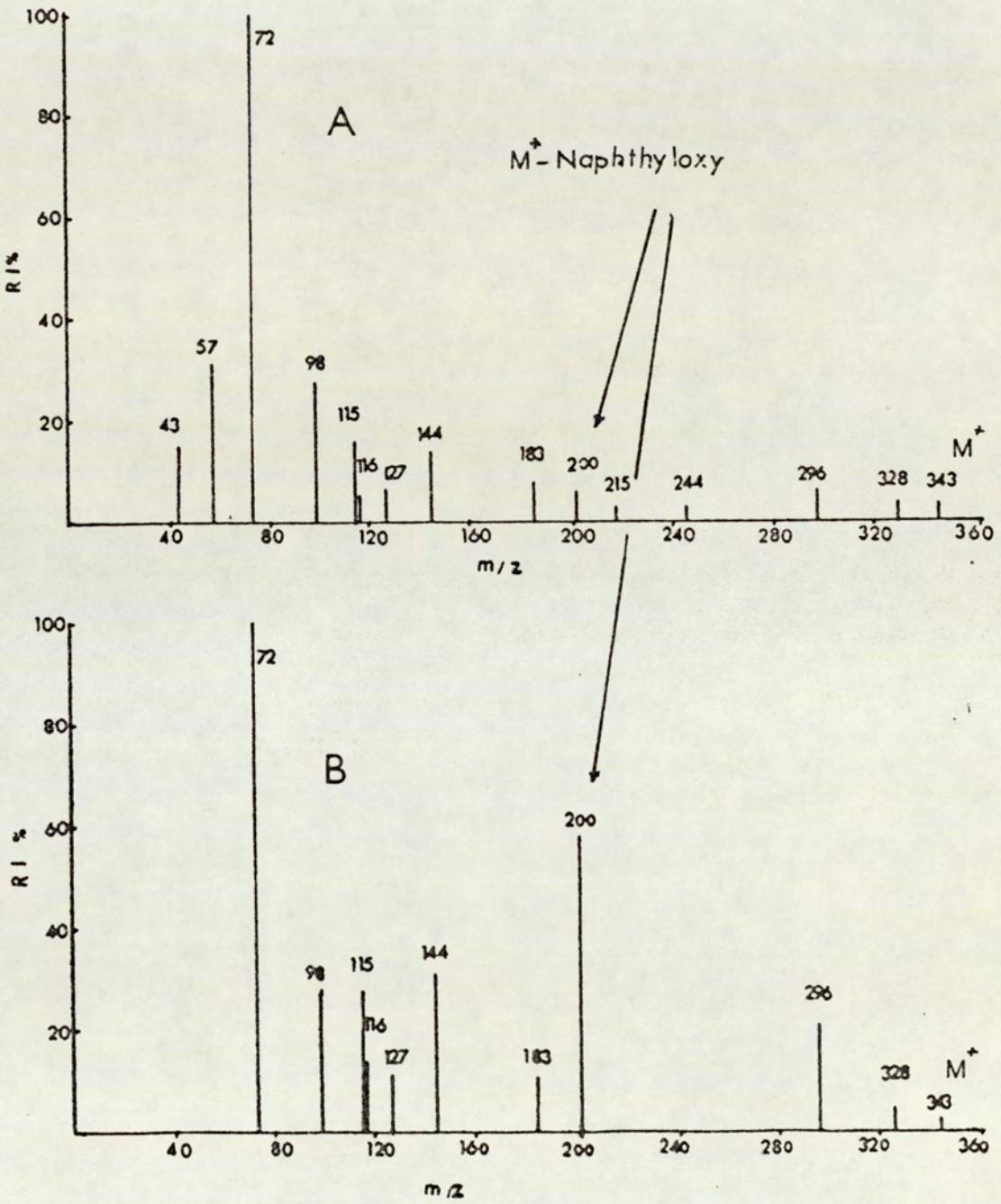
"Reproduced from one supplied by:
Maybridge Chemical Co. Ltd, Tintagel,
Cornwall, England".



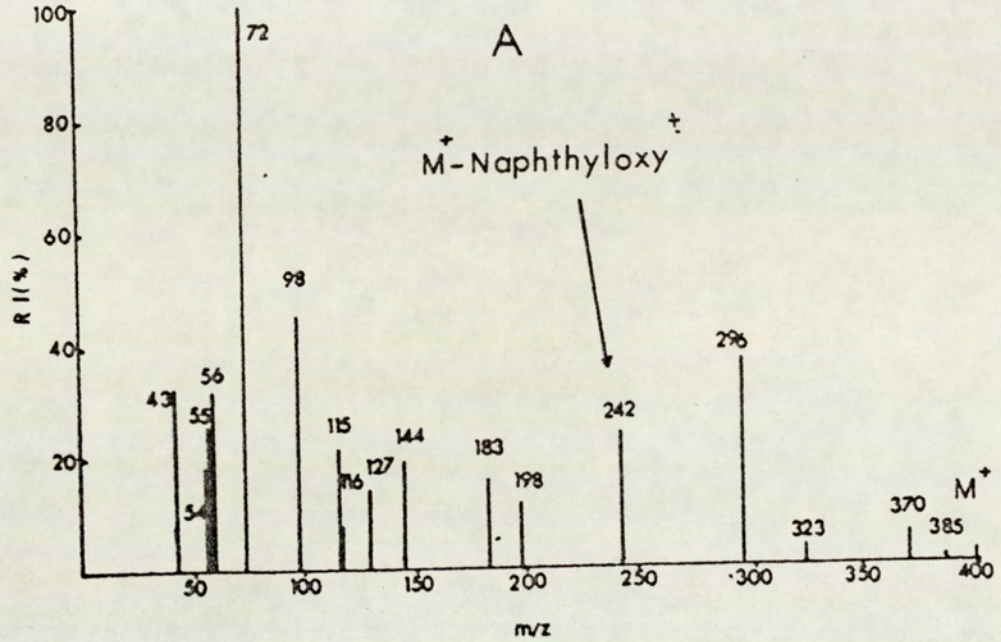
Appendix IV.1 Mass Spectral Fragmentation of O-acetylpropranolol (A), and N-acetylpropranolol (B).

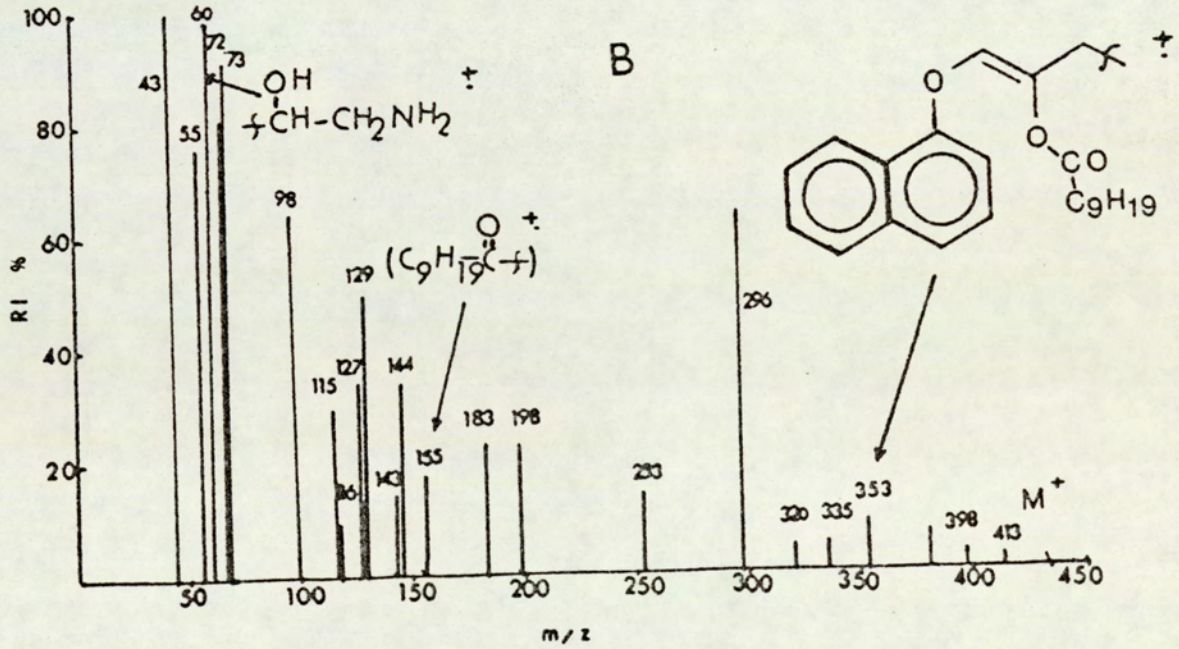


Appendix IV.2 Mass Spectral Fragmentation of O-Propionylpropranolol (A), O-Butarylpropranolol (B), and O-Hexanoyl-Propranolol (C).

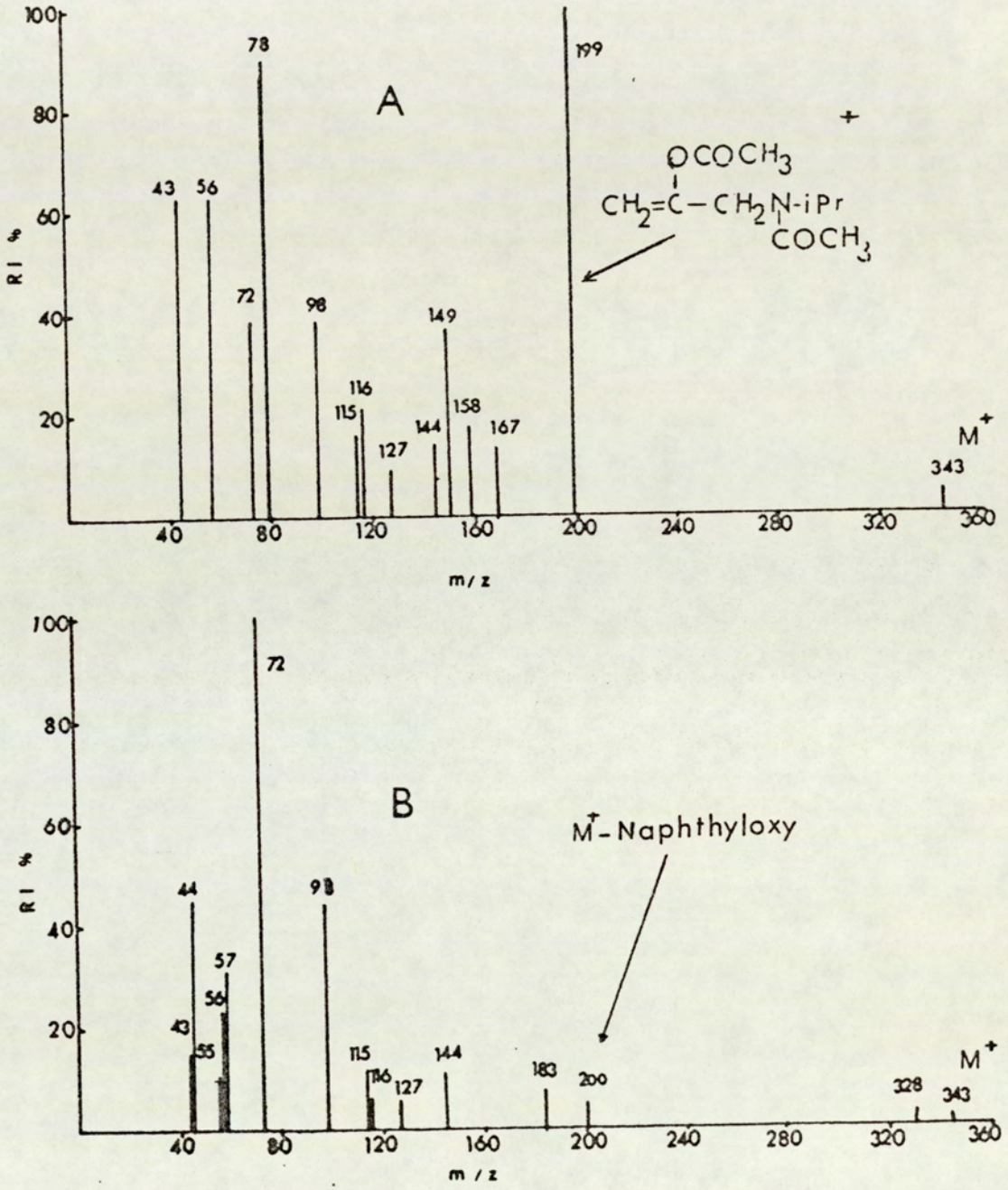


Appendix IV.3 Mass Spectral Fragmentation of O-valerylpropranolol (A), and N-valerylpranolol (B).





Appendix IV.4 Mass Spectral Fragmentation of O-octanoylpropranolol (A) and O-decanoylpropranolol (B).



Appendix IV.5 Mass Spectral Fragmentation of N-O-diacetylpropranolol (A), and O-pivaloylpropranolol (B).

Preparation of Constant Ionic Strength McIlvaine Buffered Solutions *

pH Desired at 25° C.	Composition, G./Liter Solution		Buffer System Ionic Strength, M	G. HCl Added per Liter of Solution to Produce Ionic Strength of	
	Na_2HPO_4. $12H_2O$	$H_3C_6H_5O_7$. H_2O		1.0 M	0.2 M
2.2	1.43	20.6	0.0108	74.8	37.2
2.4	4.44	19.7	0.0245	73.7	35.4
2.6	7.80	18.7	0.0410	71.6	34.2
2.8	11.25	17.7	0.0592	70.2	33.0
3.0	14.7	16.7	0.0771	68.7	31.4
3.2	17.7	15.8	0.0934	67.6	30.2
3.4	20.6	15.0	0.113	66.2	28.9
3.6	23.6	14.2	0.128	64.9	27.6
3.8	25.4	13.6	0.143	64.0	26.7
4.0	27.6	12.9	0.157	62.8	25.8
4.2	29.7	12.3	0.173	61.7	24.6
4.4	31.6	11.7	0.190	60.4	23.1
4.6	33.4	11.2	0.210	58.9	21.6
4.8	35.3	10.7	0.232	57.2	19.9
5.0	36.9	10.3	0.254	55.8	18.3
5.2	38.4	9.75	0.278	53.8	16.6
5.4	40.0	9.29	0.302	52.1	14.8
5.6	41.5	8.72	0.321	50.8	13.3
5.8	42.8	8.22	0.338	49.5	12.2
6.0	43.2	7.74	0.344	48.9	11.8
6.2	47.8	7.12	0.358	47.9	10.6
6.4	49.8	6.47	0.371	46.9	9.63
6.6	52.1	5.72	0.385	45.8	8.66
6.8	54.4	4.78	0.393	44.8	7.93
7.0	58.9	3.70	0.427	42.7	6.44
7.2	62.8	2.74	0.457	40.4	5.10
7.4	65.0	1.91	0.488	38.3	0.488
7.6	67.2	1.23	0.516	36.0	..
7.8	68.8	0.803	0.540	34.3	..
8.0	80.6	0.439	0.550	33.9	..

* Anal. Chem. (1956) , 28(7) 1179-1180

APPENDIX V McILVAIN BUFFER OF KNOWN IONIC STRENGTH

Temperature = 25 ^{o}C

Amount of O-acetylpropranolol added = 10 mg

Molecular weight = 337.8

Molarity of the titrant = 0.1M

Volume of solution in the titration cell = 25 ml

Solvent	pH 0.1M NaOH added (ml)									
	0.02	0.04	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20
0.02M D.T.A.B.	6.40	6.83	7.06	7.23	7.38	7.51	7.63	7.76	7.88	8.00
0.02M S.L.S pH7	8.80	9.35	9.54	9.67	9.81	9.90	10.02	10.10	10.18	10.25
0.02M S.L.S pH > 7	9.35	9.60	9.86	10.00	10.12	10.21	10.30	10.38	10.44	10.495

Appendix VI Data for the determination of pKa of O-acetylpropranolol HCl in presence of cationic and anionic micelles.

Propranolol Esters	pH 0.1M. NaOH added (ml.)									
	0.02	0.04	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20
Acetyl	7.52	7.88	8.10	8.28	8.41*	8.51	8.58	8.65	8.71	8.80
Propionyl	7.60	7.82*	7.89	7.95	8.00	8.06	8.12	8.20	8.29	8.38
Butyryl	7.28*	7.34	7.38	7.42	7.47	7.52	7.58	7.65	7.73	7.83
Valeryl	6.79*	6.83	6.88	6.93	6.97	7.03	7.10	7.17	7.27	7.39
Hexanoyl	6.31*	6.35	6.39	6.44	6.50	6.56	6.63	6.73	6.84	6.99
Pivaloyl	7.24*	7.50	7.82	8.36	8.70	8.94				

* The pH at which the compound started precipitate to

Appendix VII Data for the determination of the basic solubility of propranolol ester hydrochlorides using non-logarithmic titration method for determining pKa using the B_{sol} = 1.

Concentration of the ester = 10 mg/25 ml cooled boiled distilled water
Temperature = 25°C

TABLE 2 (*)

pH	Britton-Robinson buffer *					KCl added per liter of buffer to produce an ionic strength of		
	Composition (g/l)				Ionic strength (M)			
	NaOH	CH_3CO_2H	H_3PO_4	H_3BO_3		0.1 M	0.5 M	1 M
1.81	0.000	2.402	3.920	2.473	0.0134	6.389	36.211	73.489
1.89	0.195	2.343	3.824	2.413	0.0161	6.255	36.077	73.355
1.98	0.381	2.288	3.733	2.355	0.0180	6.113	35.935	73.213
2.09	0.558	2.234	3.647	2.301	0.0200	5.964	35.786	73.064
2.21	0.772	2.184	3.564	2.248	0.0228	5.755	35.577	72.855
2.36	0.889	2.135	3.484	2.198	0.0246	5.621	35.443	72.721
2.56	1.043	2.089	3.409	2.151	0.0273	5.420	35.242	72.520
2.87	1.191	2.044	3.336	2.105	0.0302	5.203	35.025	72.303
3.29	1.333	2.002	3.267	2.061	0.0331	4.987	34.809	72.087
3.78	1.469	1.961	3.200	2.019	0.0360	4.771	34.593	71.971
4.10	1.600	1.922	3.136	1.979	0.0388	4.562	34.384	71.662
4.35	1.725	1.884	3.075	1.940	0.0417	4.346	34.168	71.446
4.56	1.846	1.848	3.015	1.902	0.0445	4.137	33.959	71.237
4.78	1.962	1.813	2.958	1.867	0.0475	3.914	33.736	71.014
5.02	2.074	1.779	2.904	1.832	0.0506	3.683	33.505	70.783
5.33	2.182	1.747	2.851	1.799	0.0539	3.436	33.258	70.536
5.72	2.286	1.716	2.800	1.767	0.0571	3.198	33.020	70.298
6.09	2.386	1.686	2.751	1.736	0.0603	2.959	32.781	70.059
6.37	2.483	1.657	2.703	1.706	0.0636	2.713	32.535	69.813
6.59	2.576	1.628	2.658	1.677	0.0671	2.452	32.274	69.552
6.80	2.667	1.601	2.613	1.649	0.0712	2.147	31.969	69.247
7.00	2.754	1.575	2.570	1.622	0.0758	1.804	31.626	68.904
7.24	2.839	1.550	2.529	1.596	0.0815	1.379	31.201	68.479
7.54	2.921	1.525	2.489	1.570	0.0882	0.879	30.701	67.979
7.96	3.000	1.501	2.450	1.546	0.0952	0.357	30.179	67.457
8.36	3.077	1.478	2.412	1.522	0.0993	0.052	29.874	67.152
8.69	3.152	1.456	2.376	1.499	0.102	—	29.672	66.950
8.95	3.224	1.434	2.340	1.477	0.104	—	29.523	66.801
9.15	3.294	1.413	2.306	1.455	0.106	—	29.374	66.652
9.37	3.362	1.392	2.272	1.434	0.107	—	29.300	66.578
9.62	3.429	1.373	2.240	1.413	0.109	—	29.151	66.429
9.91	3.493	1.353	2.208	1.393	0.110	—	29.076	66.354
10.38	3.556	1.334	2.178	1.374	0.111	—	29.001	66.279
10.88	3.616	1.316	2.148	1.355	0.112	—	28.927	66.205
11.20	3.676	1.298	2.119	1.337	0.114	—	28.778	66.056
11.40	3.733	1.281	2.091	1.319	0.116	—	28.629	65.907
11.58	3.789	1.264	2.063	1.302	0.118	—	28.430	65.758
11.70	3.844	1.248	2.036	1.285	0.121	—	28.256	65.534
11.82	3.897	1.232	2.010	1.268	0.123	—	28.107	65.385
11.92	3.949	1.216	1.985	1.252	0.126	—	27.883	65.161
11.98	4.000	1.201	1.960	1.237	0.128		27.734	65.012

(*) Since pH values depend on ionic strength (*), the actual pH of each solution must be checked experimentally. The values reported are the Britton-Robinson original ones (without potassium chloride).

* Anali di Chemica , 64-1974

APPENDIX VIII BRITTON-ROBINSON BUFFER OF KNOWN IONIC TRENGTH

Physiological salt solutions
Composition in g./litre

	Krebs[1] (1950)	Krebs-Henseleit[2] (1932)	Locke[3] (1901)	Tyrode[4] (1910)	Ringer[5] (1894)	Clark-frog-Ringer[6]
NaCl	5·5	6·87	9·0	8·0	9·0	6·5
KCl	0·35	0·4	0·42	0·2	0·42	0·14
$MgCl_2$ $MgSO_4.7H_2O$	— 0·11	— 0·14	— —	0·1 —	— —	— —
$CaCl_2$	0·28	0·28	0·24	0·2	0·24	0·12
NaH_2PO_4	—	0·14	—	0·05	—	0·01
KH_2PO_4	0·16	—	—	—	—	—
$NaHCO_3$	2·1	2·1	0·15	1·0	0·5	0·2
Glucose	2·0	2·0	1·0	1·0	1·0	2·0
pyruvic acid fumaric acid glutamic acid	0·27 0·62 0·45 } Converted to sodium salts with $NaHCO_3$					

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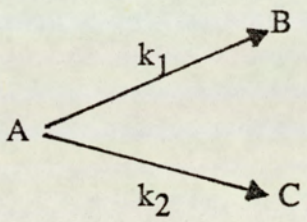
APPENDIX IX. COMPOSITION OF PHYSIOLOGICAL SALT SOLUTIONS

APPENDIX X. DERIVATION OF THE RATE EQUATION FOR THE DEGRADATIONS OF O-ACYL PROPRANOLOLS



$$\frac{dA}{dt} = -(k_1 + k_2)[A]$$

$$\frac{dB}{dt} = k_1[A] \qquad \frac{dC}{dt} = k_2[A]$$

$$1 - \frac{dA}{dt} = -(k_1 + k_2)[A]$$

$$\int_{A_o}^{At} \frac{dA}{A} = -(k_1 + k_2) \int_0^t dt$$

$$\ln At = \ln A_o - (k_1 + k_2)\, t$$

$$-(k_1 + k_2)t$$

and $At = A_0.e$

$$2 - \frac{dB}{dt} = k_1 A \quad , \quad \frac{dC}{dt} = k_2 A$$

$$\frac{dB}{dC} = \frac{k_1}{k_2}$$

$$k_2 \int_o^B dB = k_1 \int_o^C dC$$

$$k_2 B = k_1 C$$

$$A_o = A + B + C$$

$$C = A_o - A - B$$

$$k_2 B = k_1 (A_o - A - B)$$

$$B (k_1 + k_2) = k_1 (A_o - A)$$

$$A_o - A = \frac{B (k_1 + k_2)}{k_1}$$

$$A = A_o - \frac{B (k_1 + k_2)}{k_1}$$

and

$$\frac{dB}{dt} = k_1 A = A_o k_1 - B (k_1 + k_2)$$

$$\int_o^B \frac{dB}{A_o k_1 - B(k_1 + k_2)} = \int_o^t dt$$

$$t = \left[- \frac{1}{(k_1 + k_2)} \ln \{A_o k_1 - B (k_1 + k_2)\} \right]_o^B$$

$$= \left[+ \frac{1}{(k_1 + k_2)} . \ln (A_o k_1) - \frac{1}{(k_1 + k_2)} . \ln \{A_o k_1 - B(k_1 + k_2)\} \right]$$

$$- \frac{1}{(k_1 + k_2)} . \ln \{A_o k_1 - B(k_1 + k_2)\} + \frac{1}{k_1 + k_2} . \ln (A_o k_1) = t$$

$$\frac{1}{(k_1 + k_2)} \left[- \ln \{A_o k_1 - B (k_1 + k_2)\} + \ln A_o k_1 \right] = t$$

$$\ln \left[\frac{A_o k_1 - B (k_1 + k_2)}{A_o k_1} \right] = - (k_1 k_2) t$$

$$\frac{A_o k_1 - B (k_1 + k_2)}{A_o k_1} = e^{- (k_1 + k_2) t}$$

$$A_o k_1 - B(k_1 + k_2) = A_o k_1 \cdot e^{-(k_1 + k_2)t}$$

$$-B(k_1 + k_2) = A_o k_1 \cdot e^{-(k_1 + k_2)t} - A_o k_1$$

$$B(k_1 + k_2) = A_o k_1 - A_o k_1 \cdot e^{-(k_1 + k_2)t}$$

$$B_t = \frac{A_o k_1 - A_o k_1 \cdot e^{-(k_1 + k_2)t}}{(k_1 + k_2)}$$

and similarly for C_t

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