THE STABILITY AND FORMULATION OF TOPICAL ANALGESICS

By QUAMRUN NAHAR MASUDA

A thesis submitted for the degree of
Doctor of Philosophy

in the Department Of
PHARMACEUTICAL SCIENCES

University Of ASTON IN BIRMINGHAM

February, 1985.

THE UNIVERSITY OF ASTON IN BIRMINGHAM

SUMMARY

THE STABILITY AND FORMULATION OF TOPICAL ANALGESICS

By QUAMRUN NAHAR MASUDA

A thesis submitted for the degree of Doctor of Philosophy 1985.

High-performance liquid chromatographic methods are developed for the simultaneous determination of various salicylates, their p-hydroxy isomers and nicotinic acid esters. The method is sensitive enough to detect trace amounts ($\sim \mu M/L$) of the product generated from cross reactivity between the drugs and the vehicle. The developed method also allows analysis of various topical products containing salicylate and nicotinate esters in their formulations. Applying this method, the degradation profiles of salicylates, nicotinates, p-hydroxy benzoate, o-methoxy benzoate and aspirin prodrugs in alkaline media are determined. The profile for alkyl salicylate degradation is found to be first order (A$\rightarrow$ B) when the alcoholic radical is similar to that of the ester. In alcohol having a radical different from that of the ester function, the degradation is found to proceed through competitive transesterification and hydrolysis. The intermediates are identified following synthesis and isolation. The rate and extent of transesterification depends on the proportion of alcohol present in the system. Equations are presented to model the time profiles of reactant and product concentration. The reactions are base-catalysed and the predominant pathway involves a concerted solvent attack upon the salicylate anion. Competitive hydrolysis of both ester components also follows this mechanism at moderate pH values but rates increase under strongly alkaline conditions as direct hydroxide attack becomes significant. In contrast, transesterification is independent of base concentration once full ionization is accomplished. The competitive hydrolysis is modelled using equations involving the dielectric constant of the medium. A range of other esters are also shown to undergo base-catalysed transesterification. In non-alcoholic solution phenyl salicylate undergoes a concentration-dependent oligomerisation which yields salsalate among the products. Competitive transesterification and hydrolysis also occur in products for topical use which have vehicles based upon alcohol, glycol or glycol polymers. Such reactions may compromise stability assessments, pharmaceutical integrity and delivery profiles.

KEY WORDS:

Transesterification
Salicylates and nicotinates
Topical formulations
Aspirin prodrugs
High-performance liquid chromatography
Stability

Acknowledgements

I am provided here with an opportunity to convey my thanks to all the people who helped me in preparing this thesis.

I am particularly grateful to my supervisor, Dr.W.J.Irwin, for his guidance, valuable advice, active support and encouragement throughout the duration of this work.

I would like to thank Professors M.F.G.Stevens and M.R.W.Brown for providing laboratory and library facilities and Professor Li Wan Po for his advice and help.

I am grateful to the Commonwealth Commission in the United Kingdom for awarding me a postgraduate research scholarship. I am thankful to the University of Dhaka, Bangladesh for granting me study leave to carry out my research in the United kingdom.

My thanks are also extended to the fellow students, the staff of the computer centre and the technical staff of the Department of Pharmacy,University of Aston for their cooperation.

To Shams and Nasheed

Contents Page No.

Contents

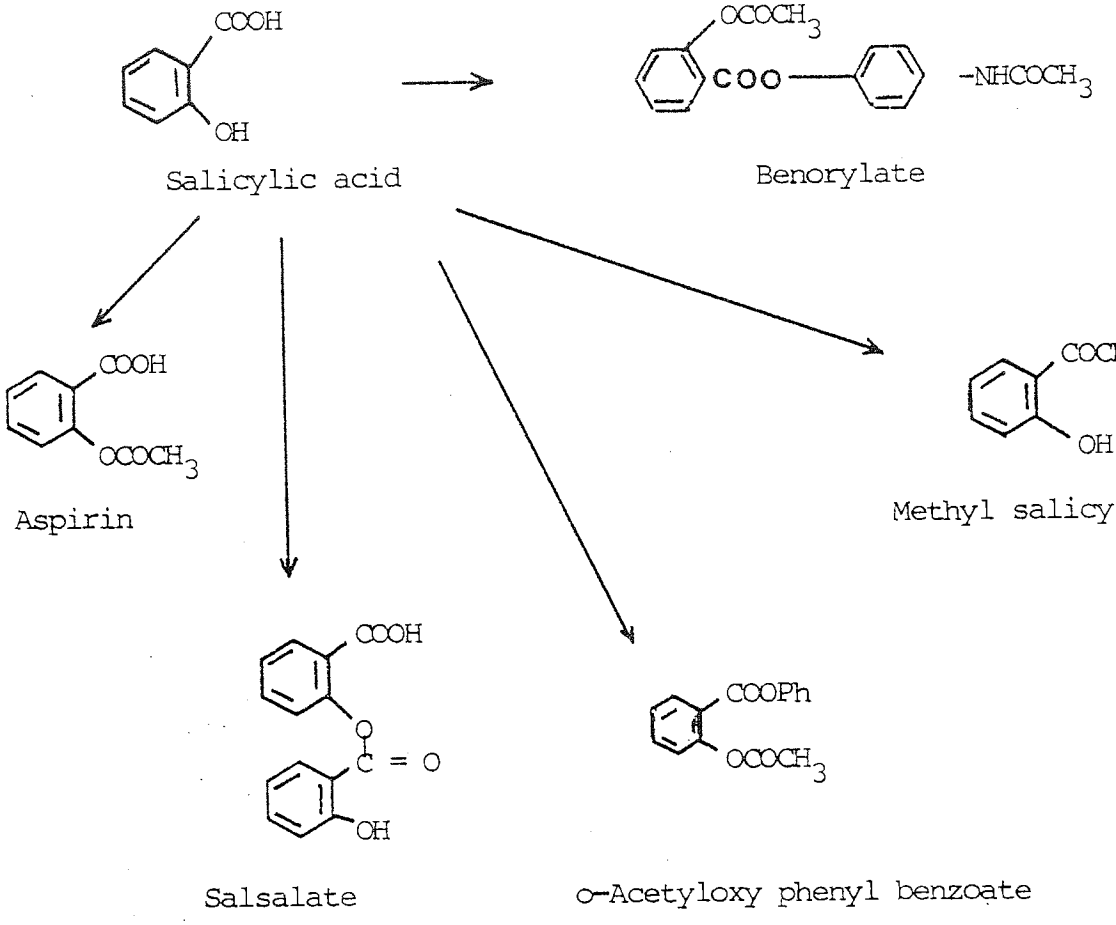
Contents Page No.

Contents Page No.

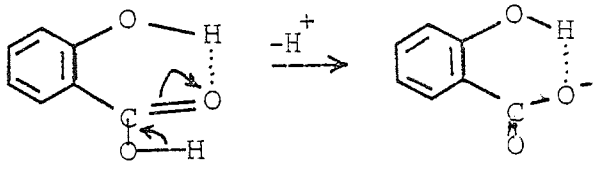
Contents

Page No.

CHAPTER 1 INTRODUCTION



Scheme 1.1. Formation of salicylates from salicylic acid

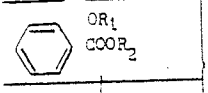
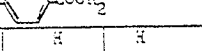


Scheme 1.2. Intramolecular hydrogen bond in salicylic acid

1.1. CHEMICAL STRUCTURES AND SOME PHYSICAL PROPERTIES OF SALICYLATES AND NICOTINATES

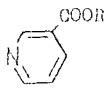
Salicylates are derivatives of 2-hydroxy benzoic acid (salicylic acid) (1). Derivatisation of this bifunctional compound may involve the carboxylic acid group to provide salicylate esters, amides and metallic salts, or the phenolic group to give aspirin (1). Additionally, both functional groups may be involved to yield compounds such as o-acetyloxy phenyl benzoate and Benorylate. The dimer salicylsalicylic acid (salsalate) may also be obtained (2). The pathways for the derivatisation of salicylic acid to salicylates are shown in Scheme.1.1. The ortho-substitution pattern of salicylates allows the formation of intramolecular hydrogen bonds, an effect which influences physical properties such as solubility and ionization when compared to the meta- and para- isomers. Intramolecular hydrogen bonding stabilises the anionic charge of the salicylic acid anion and facilitates the ionization process, enhancing carboxylic acid strength (Scheme.1.2). In contrast, the phenolic ionization requires the rupture of the intramolecular hydrogen bond and ensures a considerable weakening in acidity. The meta- and para- isomers, are devoid of this steric effect. The measured pKa values of these isomeric compounds together with their solubility are presented in Table 1.1. The table also contains structures of some salicylate esters which are frequently used in topical formulations.

Table 1.1. Structural Formulae of Salicylates and Some of Their Isomers Together with Corresponding Physical constants.

Compound	Structural Formulae Salicylates  R_1	R_2	m.wt.	b.p. °C	Solubility in water	pKa	Partion coefficient	Ref.
Salicylic acid	H	H	138.12	bp_{20} 211	1:460	2.97 13.82	octanol/water 2.26	3 4
Methyl salicylate	H	Me	152.14	220 - 224	1:1500	10.51	olive oil/H_2O 343	7
Ethyl salicylate	H	Et	166.17	231 - 234	1:3448	10.50	olive oil/H_2O 1170	7 30
Progyl salicylate	H	Pr	180.20	236 - 238	1:11111	—	olive oil/H_2O 2550	7 30
Butyl salicylate	H	Bu	194.22	237 - 240	-	—	—	30
Phenyl salicylate	H	Ph	214.21	bp_{12} 173	1:8670	—	—	19
Glycol salicylate	H	CH_2CH_2OH	182.20	240 - 242	1:80	—	7.7	7
Aspirin	COMe	H	180.15		1:300	3.49	—	7
Parabens R_1O  $COOR_2$								
p-OH benzoic acid	H	H	138.12	—	1:125	4.16 9.46	octanol/water 1.58	193
Methyl paraben	H	Me	152.14	270	1:400	8.5	—	6

Nicotinates are esters of pyridine 3-carboxylic acid (nicotinic acid). The nitrogen in the pyridine nucleus gives these esters their basic properties. Structural formulae and some physical properties of some of the topically used nicotinates are recorded in Table 1.2.

Table 1.2. Structure of Nicotinates and Their Corresponding Physical Constants



P.C. is partition coefficient
D.C. is diffusion coefficient

Nicotinate	R	UV absorption maxima			b.p.°C/mm	pKa	Solubility in water	P.C.	D.C. $\times 10^{-9}$ m^2/S	Ref.
		C mM/L	λ_{max} nm	ϵ						
Methyl	CH_3	0.43	262.5	2650	85.7/5	3.13	143:100	IPM/H_2O 37°C, 0.39	D_{IPM} 0.51 D_W 0.12	8,9,10,11 12
ethyl	C_2H_5				84.5/5	—	5:100			9,
butyl	C_4H_9	0.44	262.5	2550	120/2	—	0.253:100	IPM/H_2O, 37° 2.6×10^{-2}	D_{IPM} 0.43 D_W 0.99	9,12
hexyl	C_6H_{13}	—	—	—	147 / 2	—	0.46 : 100	IPM/H_2O 1.4×10^{-2}	D_{IPM} 0.4 D_W .92	9,12

1.2. SALICYLATES AND NICOTINATES IN TOPICAL PREPARATIONS

Topical preparations formulated with salicylates and nicotinates are usually administered to exert a local action. Although some salicylates have been used to provide protection in sunscreen preparations, the common application is in the treatment of pain. They are used as topical analgesics to relieve the effects of localised tissue damage caused by muscular strain and joint pain from minor sprains. Additionally, low back pain from poor posture and obesity and post-exercise pain are also frequently treated with these preparations.

The mode of action of topical analgesics has been reviewed (13). Salicylates, on application to the skin, perhaps act as a counter stimulus which competes with pain stimuli in obtaining access to the central nervous system. As a result, the T-cell activity in the spinal cord may decrease and this in turn may close the gate to the incoming pain stimuli. Additionally, massage, which normally follows application of these products, may help dilute and disperse tissue mediators of pain such as prostaglandins and bradykinin. Aspirin may produce analgesia by modifying the cause of pain at the site of origin. This drug is normally used in relieving inflammatory pain. Acute inflammation usually starts with local cell destruction, leading to an accumulation of endogenous pain mediators such as histamine, bradykinin, 5-hydroxy tryptamine and prostaglandins. Aspirin inhibits the synthesis of prostaglandins and prevents the sensitisation of the pain receptors to other pain mediators(14-15).

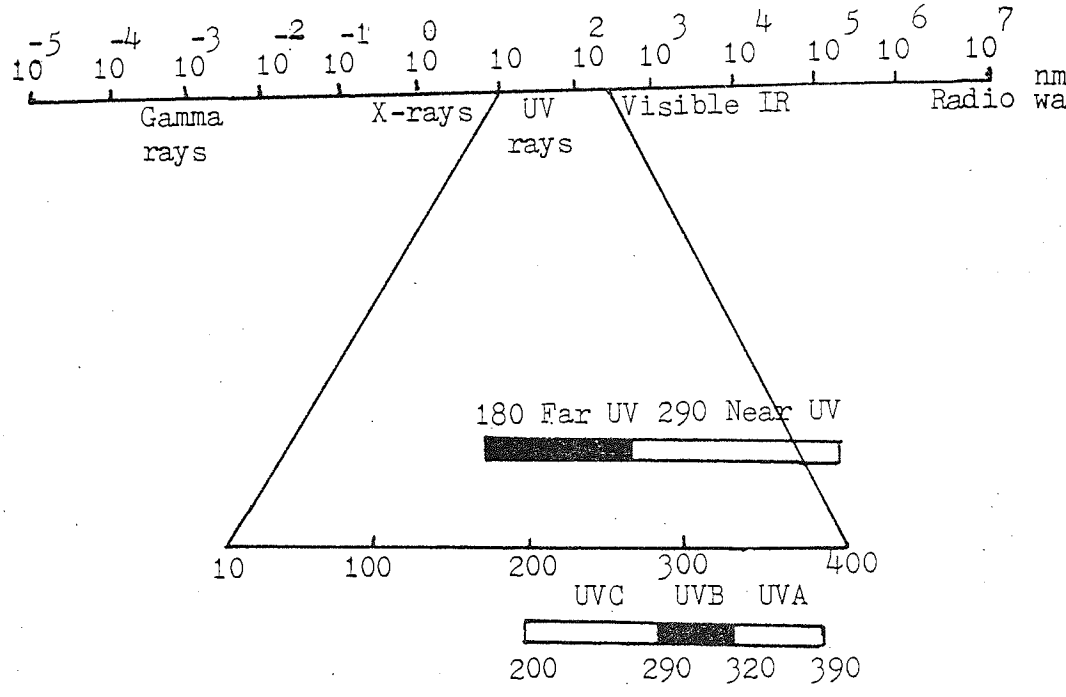


Fig. 1.1. The Electromagnetic Radiation Spectrum Which Induces t Skin Sensitization.

(From'Non-prescription Drugs' (17), P 39).

Salicylic acid is normally used as a keratolytic agent in curing corns. Blank (16) demonstrated that the keratolytic effect of salicylic acid is primarily related to the hydration of the cornified tissue. While comparing the role of vehicle in controlling the keratolytic effect of salicylic acid, he found that no keratolysis developed in a dry excised callus suspended in petrolatum containing salicylic acid. In contrast, when the same preparation was applied to the living cornified skin, the vehicle formed an occlusive covering over the skin permitting water to accumulate in the stratum corneum from the deeper layers. Leaching of salicylic acid reduced the pH of the accumulated water which was better absorbed by the cornified epithelium. The macerated cornified tissue was then removed easily by mechanical means.

When exposed to certain wavelengths of electromagnetic radiation, human skin undergoes photochemical reactions leading to suntan, sunburn, artificial ageing and perhaps even carcinoma. These changes are initiated by a relatively narrow range of the spectrum, within the Ultraviolet region. Due to its biological importance, this region is further subdivided into UV-A, UV-B and UV-C, as shown in Fig.1.1.(17). The highly energetic UV-C region is generally filtered out by the atmosphere. Sunburn is caused by an overexposure of UV-B radiation and is characterised by the development of erythema followed by blisters. In severe cases itching, pain and disturbances such as dizziness, nausea and vomiting may occur. Suntan develops

from a series of biochemical changes of the melanocytes in the epidermis leading to the formation, homogenous distribution and intensification of the colour pigment melanin. UV-A radiation also induces slow tanning but largely without erythema and sunburn. Sunscreen preparations, thus may shield the skin from UV radiation by absorbing the UV-B region but may allow a suntan to develop by being transparent to the longer wavelength radiation. Salicylates such as benzyl (18), phenyl (19), menthyl (20), 2-ethyl hexyl or octyl (17) are formulated as sunscreen preparations. Severe dermatitis is reported to occur following the application of sunscreen preparations containing benzyl and menthyl salicylates (21-22).

Nicotinates produce erythema after topical application by dilatation of cutaneous blood vessels (23). This involves an increase in blood-flow and skin temperature which may exert some counter-irritant effect and a speed up in the removal of pain mediators.

Table.1.3. Topical Formulations With Salicylates And Nicotinates

Product	Manufacturer	Composition
Algipan balm	Wyeth Lab.	1% methyl nicotinate + 1% glycol salicylate
Algi spray	Kirby Warrick Pharm.Ltd.	5% diethylamine salicylate +1% methyl nicotinate + 5% glycol salicylate
Balmosa cream	Pharmax Ltd.	4% methyl salicylate
Bayolin ointment	Bayer UK. Ltd.	10% glycol sal.+2.5%benzyl nicotinate
Bengue's balsam	Bengue & Co Ltd.	20%methyl salicylate
Boots pain relieving balm	Boots Co Ltd	1% nicotinate + 7.5% glycol salicylate
Radian α spirit	Radiol Chemicals Ltd.	21.3% methyl salicylate
Ralgex stick	Eucryl Ltd.	3.01% glycol sal.+ 3.01% Et.sal.+0.6% Me salicylate
Ralgex spray	Eucryl Ltd.	4.8% glycol sal.+4.8% Et.sal.+0.96% Me.sal. + 1.6% methyl nicotinate
SUNSCREEN PREPERATIONS		
Coppertone sun tan lotion	Plough Ltd.	8% menthyl salicylate
Salol Cream Aqueous	Martindale	phenyl salicylate 10%
Ambre Solaire suntan oil.	Golden Ltd.	benzyl salicylate 4.7%

1.3. COMMERCIALLY AVAILABLE SALICYLATES AND NICOTINATES

Some products for topical use which include salicylates and nicotinates in their formulations are shown in Table 1.3.(13). As shown in Table 1.3., these preparations either contain a single drug (Coppertone suntan lotion) or a combination of several drugs(Algipan balm). Though the active ingredients may provide the same spectrum of effectiveness, they are distinguished by their various physical forms. Selection of a particular form is often limited to the consumers' personal preference, unless recommended by a physician. Since the concentration of active ingredients in these preparations varies within the range of 2% to 25%, their irritant potential is expected to be different.

Although generalised toxicity following topical application of these preparations is rare, localised toxicity is not uncommon, especially when applied to inflamed or abraded tissue or to the mucous membrane. However, systemic toxicity may develop because of liberal application to larger areas of the body(24). Though these products may provide invaluable efficacy in relieving an acute condition, their role in progressive degenerative processes may be limited to symptomatic relief. Under these circumstances, topical preparations should be coupled with products for systemic use.

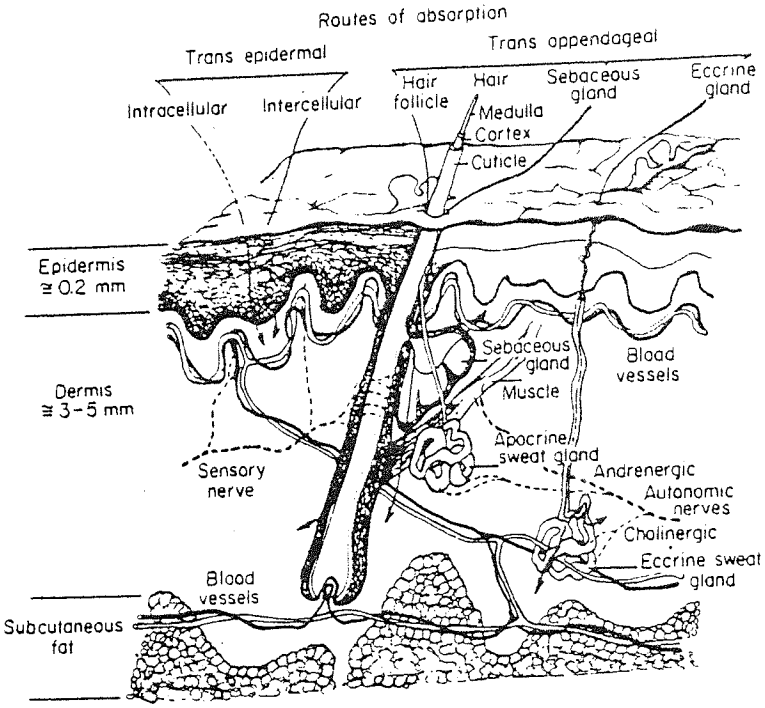


Fig. 1.2. The Three Layers of Skin

(From Drug Design Vol. IV, (5) p 94).

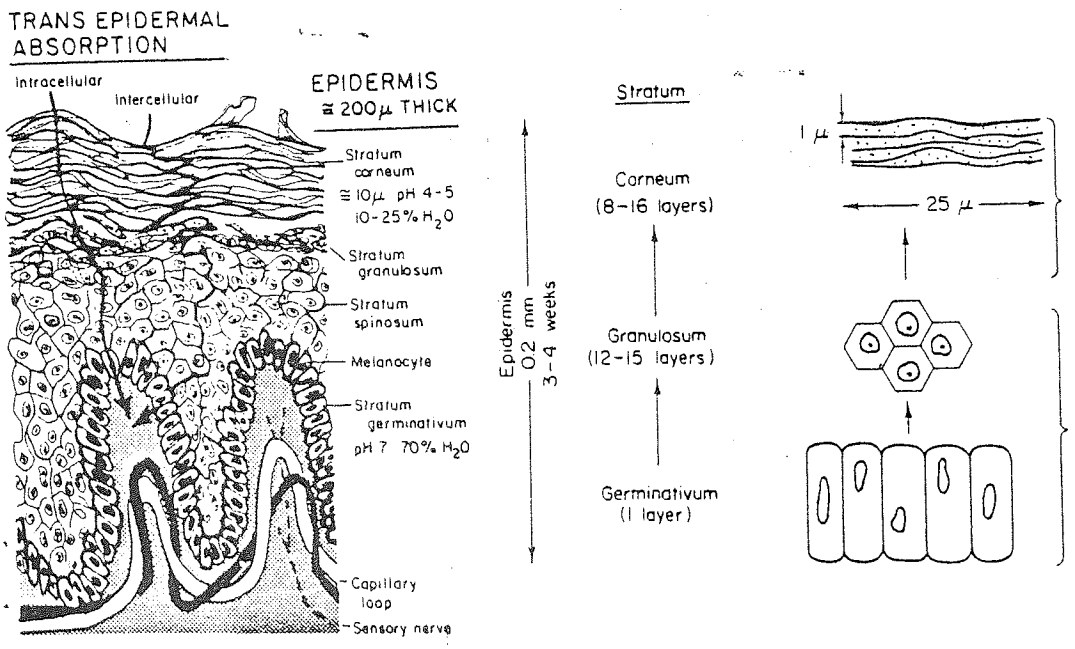


Fig. 1.3. a: The Epidermis (5). , b:Cell Changes in the Epidermis

(From Drug Design, Vol.IV, P 95-96)

1.4. PERCUTANEOUS ABSORPTION :SKIN AS A PERMEABILITY BARRIER

Skin is characterised by three distinct layers, epidermis, dermis and subcutaneous fat, as shown in Fig.1.2.(5). The functional differences of these layers are summarised in Table 1.4.(5). Of the three layers, the outermost layer -epidermis plays the primary role in maintaining the dynamic equilibrium of the skin. The epidermis is conventionally subdivided into stratum germinativum, stratum spinosum, stratum granulosum and stratum corneum. Fig.1.3. displays these layers and their respective cell changes. The germinative basal layer of epidermis undergoes a series of biochemical, physiological and morphological changes in the intracellular materials as well as the intercellular environment. Eventually nonnucleated dead cells migrate towards the uppermost section of the epidermis and intermesh to produce very cohesive laminae which are known as the stratum corneum. The stratum corneum is continuously replenished by the slow upward migration of inner layers and provides the main barrier to the absorption of topically applied substances. An overview of the chemical nature of the stratum corneum is given in Table 1.5.

Characteristic

ad cells (nucle
Keratin
Steral esters
10-20% H_2O
pH 4.2-5.6

ving cells (nucl
Protein
Sterols
Fatty acids
70% H_2O
pH 7.2

s (5).

Table 1.4. The Composition and Function of The Three Layers Of Skin (5)

FEATURE	LAYERS		
	Epidermis	Dermis	Subcutaneous fat
Function	Barrier	Supportive	Insulation
Major Component	Keratin	Collagen	Fat
Thickness	0.2 mm	3 - 5 mm	Variable
pH	4.2 - 6.5	7.1-7.3	—
Water content	10 - 25%	60 - 70%	—
Blood vessels	None	Many	Some
Secretory glands	—	Sweat, sebum	—

Table 1.5. Composition of Stratum Corneum (25)

Tissue component	Gross Chemical Characterization	Percent
Cell membranes	Lipids and nonfibrous proteins	5
Intercellular material	Lipids and nonfibrous proteins	10
Cell Contents	Lipid α-Protein β-Protein Nonfibrous protein	85

The conversion of aqueous epidermal cells into dried, compact, keratin-containing stratum corneum is the crucial event in the continuously developing epidermis that largely determines the low permeability of the skin.

Drugs may be absorbed through the skin by diffusion through the stratum corneum or alternatively by passage through hair follicles, sebaceous glands and sweat glands. The stratum corneum in the epidermis is the principal route of percutaneous absorption, despite the fact that it provides the greatest barrier towards penetration. Absorption through the stratum corneum generally takes place slowly and is characterised by a 'lag period'. During this lag period, the drug achieves equilibrium levels in the epidermis. The continuity of the stratum corneum is interrupted by the hair follicles and their associated paths of sebum secretion and sweat gland pores, which comprise approximately 1/1000 of the entire skin area (26). While the sweat glands give access to hydrophilic substances, the pilosebaceous apparatus allows the entry of lipid-soluble molecules including the macro molecules. These allow the transient permeation of the drug to the epidermal tissues. Eventually the appendageal penetration and diffusion through the stratum corneum merge to form a steady-state diffusion as the drug substances are generally taken up by the lymphatic and blood vessels or are deposited into the deeper layer of the subcutaneous fat. At the steady-state, the transepidermal route (stratum corneum) becomes the dominant pathway, though the total accumulation of the drug in the epidermis is the sum of the fluxes from each of the parallel pathways as

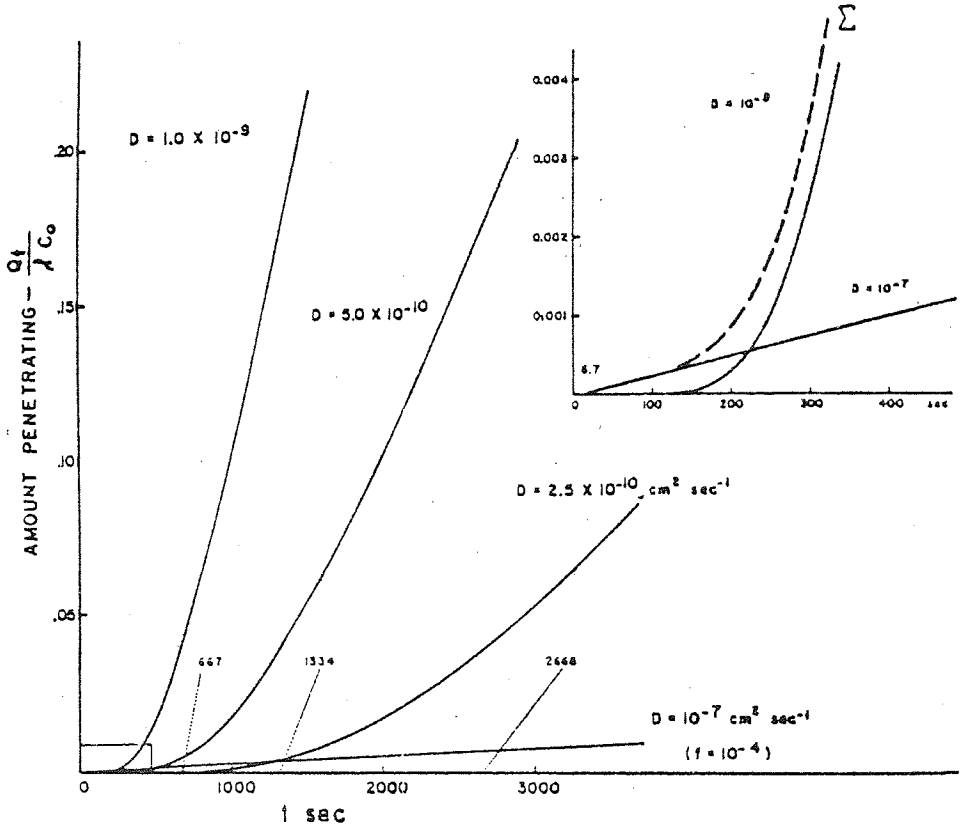


Fig. 1.4. Transient and bulk diffusion leading to steady-state flux of water and similar low molecular weight nonelectrolytes through a membrane. Shunt diffusion through appendageal pathway is shown by the insert. D represents diffusion constant through various pathwa (From J.Physiol.Rev.,1971,51,702-747)

shown in Fig.1.4. Guy and Fleming (27) demonstrated the penetration of methyl and ethyl nicotinates using Millipore filters impregnated with DL-β-γ-dipalmitoyl α-phosphatidylcholine as the phospholipid barrier. The typical lag-times for these drugs as determined by the authors are presented in Fig.1.5.

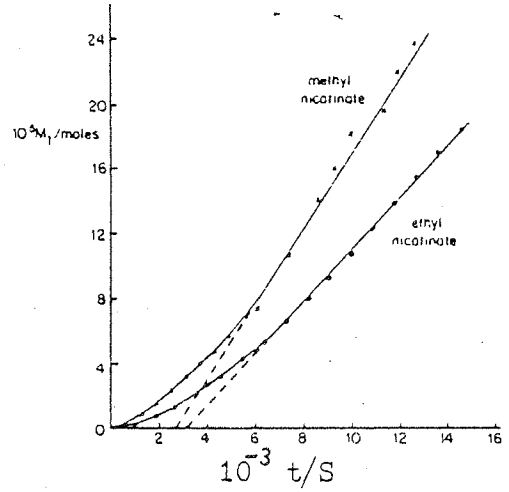


Fig. 1.5. Typical Lag Time for Methyl and Ethyl Nicotinates Crossing the Phospholipid Barrier.

Methyl Nicotinate Lag Time in Sec. 2402 $\pm$ 95
Ethyl Nicotinate 2997 $\pm$ 98

(From J.Colloid. Interface. Sci., 1981,83(1), 130-137)

Table 1.6. Regional Variation in Water Permeability at 37°C (25).

Skin Region	Steady-state flux mg cm^{-2} hr^{-1}	Membrane thickness cm x 10^{-4}	Average membrane diffusion coeff. 10^{-10} cm^2 sec^{-1}	Lag time for diffusion min.
Abdomen	0.34	15.00	6.00	11
Forearm	0.31	16.00	5.90	12
Back	0.29	10.50	3.50	9
Forehead	0.85	13.00	12.90	4
Scrotum	1.70	5.00	7.40	1
Back of hand	0.56	49.00	32.30	22
Palm	1.14	400.00	535.00	83
Plantar(sole)	3.90	600.00	930.00	106

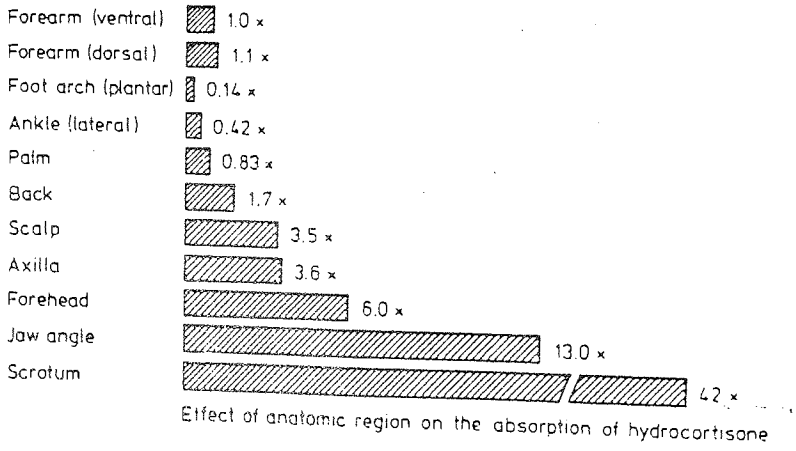


Fig. 1.6. Regional Variations in the Percutaneous Absorption of Hydrocortisone in Man After a Dose of 4 $\mu g/cm^2$ Was Applied to Each Anatomical Site (28).

(From Dermal and Transdermal Absorption, P 56).

1.5. FACTORS AFFECTING PERCUTANEOUS ABSORPTION

1.5.1. REGIONAL VARIATION

The thickness of the stratum corneum varies at various locations of the body causing a variation in the rate and extent of penetration through these regions. A typical example is shown in Table 1.6.(25). Scalla and Schaefer (28) applied a dose of 4 µg/sq.cm of hydrocortisone to various anatomical sites in man and measured percutaneous absorption following urinary recovery of hydrocortisone and its metabolites. Results are displayed in Fig.1.6. Penetration through various anatomical sites are normalised in terms of the penetration through the forearm, which is approximately 1% of the applied dose.

In areas of damp skin such as in the axillary and genital regions, penetration occurs more quickly.

Table 1.7. Penetration of Clocortolone Pivalate Cream 0.1% Through Normal and Inflamed Human Skin (26)

Skin concentration	Inflamed skin ug/ml at 5 hrs.	Normal Skin ug/ml at 5 hrs
Stratum Corneum	—	58.60
Epidermis	6.95	0.039
Dermis	4.50	0.004
Ratio in epidermis		178:1
Ratio in dermis		1125:1

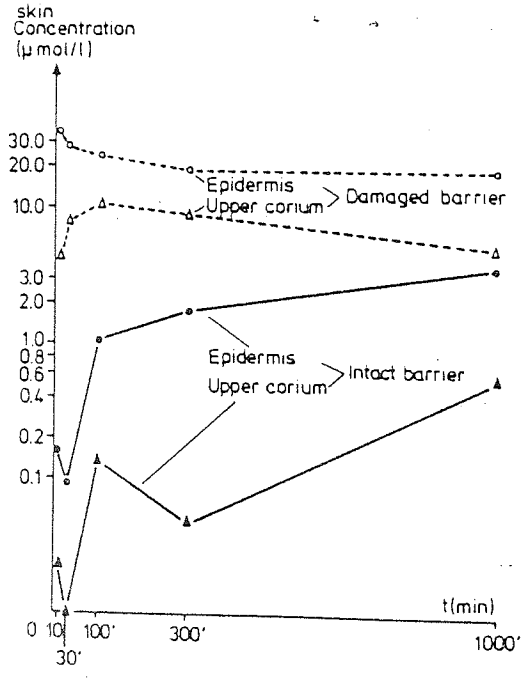


Fig. 1.7. Mean Concentration of Budesonide in the Epidermis With Intact (● ▲) and Stripped Skin (○ △) (28).

(From Dermal and Transdermal Absorption , P 51).

1.5.2. CONDITION OF THE SKIN

Though the stratum corneum is the main barrier against percutaneous absorption in normal healthy skin, its effectiveness varies in diseased skin and in various experimentally induced conditions. When the stratum corneum is removed by stripping, penetration of drugs through the remaining layers of the skin increases because of the lower diffusional resistance offered by these layers. Studies on isolated tissue reveal that the diffusional resistance of the stratum corneum to water is approximately 1000 times the resistance of the dermis (25). The cutaneous penetration of Budesonide through intact and stripped skin(28) is shown in Fig.1.7. This reveals that the diffusional resistance in stripped skin is much reduced. However, in cutaneous disorders, the thickness of the stratum corneum is not necessarily the limiting factor in percutaneous absorption. It has been demonstrated by Scalla and Schaefer (28) that in acute eczema both the influx and the resulting concentration of desoxymethasone in the skin may be increased more than 10 fold, despite the presence of a thicker stratum corneum, compared to the normal condition. This is because of the partial destruction of the cohesiveness of the stratum corneum, largely due to the presence of nucleated parakeratotic cells in this layer. Mechanical injury, cuts, chaps, burns etc. may destroy the barrier property of the stratum corneum and depending upon the severity of the trauma, a remarkable increase in influx may result. A comparative study showing the cutaneous penetration of Clocortolone Pivalate Cream through normal and inflamed skin (26) is presented in Table 1.7.

The data reveal that selective penetration in skin lesions results in higher concentration of the drug compared to the surrounding healthy tissues.

The age of the subject plays an important role in controlling cutaneous absorption of the drug. For example, due to the incomplete development of the skin structure, percutaneous absorption increases in neonates and infants. Scalla et al (29) demonstrated that the removal of the stratum corneum in premature human skin (4 year old children) increases penetration of linoleic acid only slightly compared to the intact skin. This minor increase in penetration in infants reflects an inferior barrier function in the young. In elderly people, barrier function of the stratum corneum may decrease due to actinic atrophy. However, the residence time of the drug within the skin may be increased due to reduced cutaneous blood flow.

Because of the direct exposure of the skin to environmental factors such as contact with water or organic solvents, defatting by soap and UV irradiation from occupational exposure, the stratum corneum may change its barrier properties leading to a change in percutaneous absorption. Occlusive dressing makes the skin damp by preventing perspiration and, moreover, may increase the cutaneous temperature of the occluded area. Scalla and Schaefer (28) demonstrated a 1Ø fold increase in penetration of hydrocortisone over a period of about 4 days in occluded skin compared to the unmodified skin.

Table 1.8. Permeability of Salicylates Through Normal human Skin. Effect of Hydration on Permeability (7).

Experimental Excretion Rates and Other Physical Constants	Salicylates		
	Glycol	Methyl	Ethyl
Hydrous System Rate(moles/100 cm^2/hr)	11.7	8.60	2.0
Anhydrous System Rate(mol/100cm^2/hr)	1.30	2.70	1.5
Rate Hydrous System / Rate Anhydrous System	9.00	3.20	2.0
Water Solubility (%)	1.27	0.08	0.03
Distribution Coefficient (olive oil/water)	7.7	343	1170

Hydration can, thus, alter the barrier properties of the stratum corneum and cause an increase in the permeability of drugs, although the effect becomes more prominent in hydrophilic drugs. A hydration-induced permeability pattern of methyl, ethyl and glycol salicylate through normal human skin has been reported by Wurster and Kramer (7), the findings are summarised in Table 1.8. The ratio of the hydrous to anhydrous rate varies according to the lipophilicity of the homologue.

Brown and Scott (30) organised a similar study in which in vivo penetration of methyl, ethyl, n-propyl, n-butyl and n-amyl salicylates have been measured. The skin surface was flushed with warm water prior to the application of the drug. The authors presented the data in relation to the physical properties of the cutaneous absorption of methyl salicylate normalised to unity. These values are summarised in Table 1.9. The extent of the increase of penetration depends on the partition coefficients of the homologues.

Some drugs in compound preparations may alter the barrier function of the stratum corneum either by producing vasodilatation or by vasoconstriction. Nicotinates have a vasodilatory effect. They are co-formulated with salicylates and may enhance their absorption. Gunther(26) found that topical application of benzyl nicotinate increases vessel permeability and blood flow through the skin. As a result electrolytes and substances of high molecular weight permeate the benzyl nicotinate treated skin at double the normal rate of influx.

Table 1.9. Effect of Temperature on Absorption of Salicylates. Values Are Estimated Considering Methyl Salicylate as Unity (30).

Salicylates	Absorption	Olive oil/water partition coefficient at 20 °C		Viscosity at 25 °C *	Surface tension **		Vapour pressure mm Hg 25°C	
		Absolute	Normalised	Normalised	Absolute	Normalised	Absolute	Normalised
methyl	1.00	343	1	1	44.7	1	0.37	1
ethyl	0.34	1170	3.41	1.004	41.4	0.927	0.20	0.534
n-propyl	0.20	2550	7.43	1.172	38.9	0.872	0.17	0.455
n-butyl	0.26	-	-	1.481	39.37	0.882	0.07	0.197
n-amyl	0.15	-	-	1.701	37.58	0.084	0.04	0.110

* time of outflow,(S), 25°C.
** dynes cm^{-2}, 25°C.

1.5.3. FREQUENCY OF REPEATED APPLICATION, AREA OF APPLICATION AND DEPOT EFFECT

The repeated application of a topical preparation to any particular site does not appreciably alter the penetration kinetics. Scalla and Schaefer (28) monitored the *in vitro* investigations of skin concentrations of linoleic acid in the single skin layers after 1000 min. application period, in which 1,2,3,4 and 8 applications of the substance within the period were compared. The difference in concentrations was insignificant. However, Roberts et al (31) demonstrated that the effect is concentration dependent, using salicylic acid as the model drug. They observed that when 1% salicylic acid was repeatedly applied, penetration remained constant. When the concentration was increased to 5% and 10%, an increase of the flux was observed followed by a decrease. Eaglestein et al (32) found that the clinical effectiveness of Triamcinolone was the same whether the ointment was applied 3 times or 6 times daily.

The stratum corneum also acts as a reservoir, with substances accumulating on and within the stratum corneum. Under approximate steady-state conditions, the reservoir always remains almost full. It has been demonstrated (33) that the stratum corneum serves as a reservoir for steriods and may retain them for about two weeks, if applied under occlusion for a few hours. This depot effect does not exist in stripped skin.

One of the main causes of the formation of a steroid reservoir in the epidermis is the slow removal rate of the drug by the capillary network because of its vasoconstrictior property. The partition coefficients of steroids also regulate their residence time in the stratum corneum.

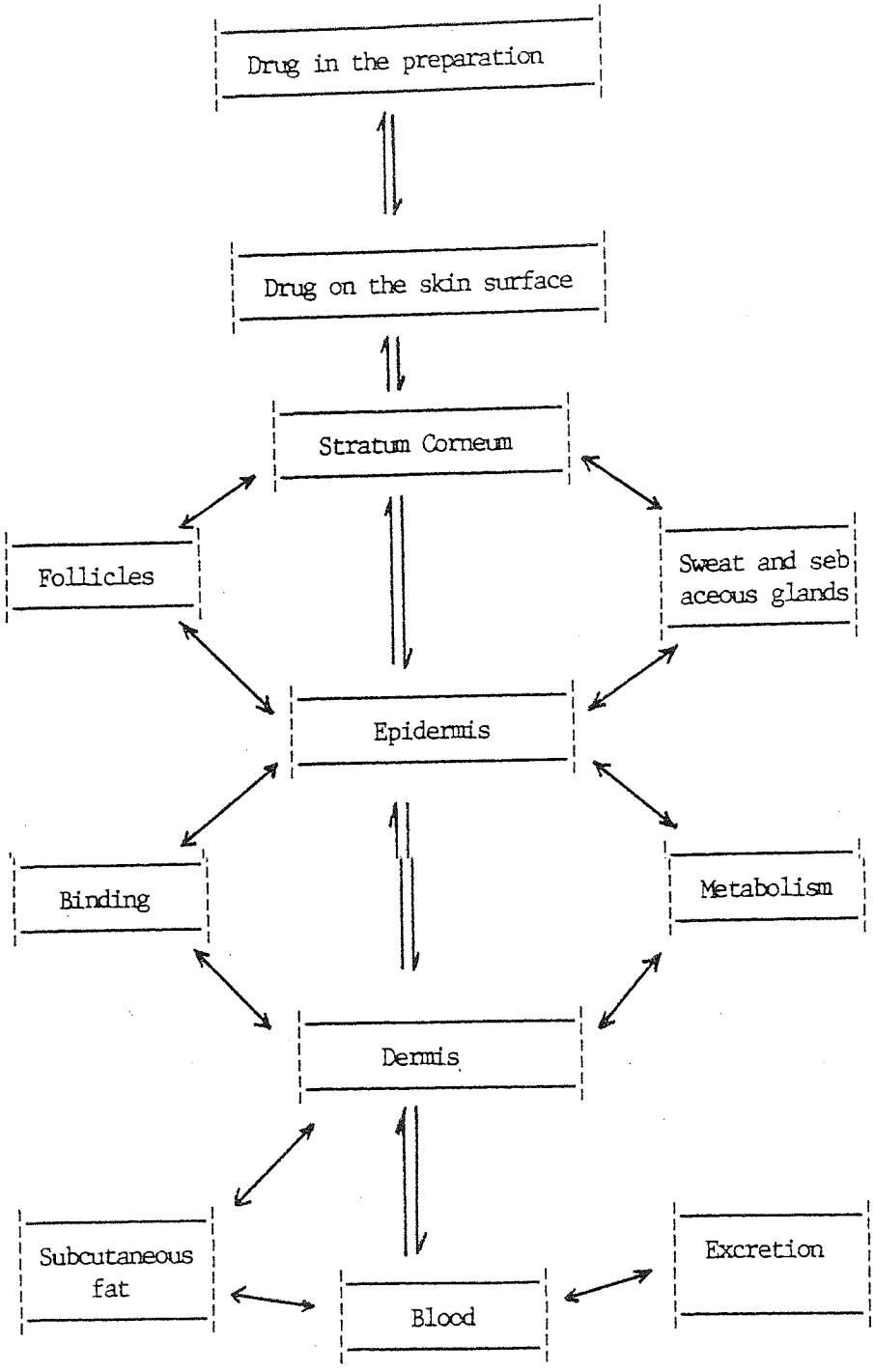


Fig.1.8. Multicompartment Cutaneous Penetration Model

1.6. OPTIMISATION OF PERCUTANEOUS ABSORPTION BY FORMULATION

The drug, its formulation and the skin represent a functional unit which controls the penetration kinetics of topically applied drugs. To reach the target tissue, the drug may have to penetrate and permeate through the multicompartment skin structure, as displayed in Fig.1.8. The parameters which may influence drug delivery are as follows.

1.6.1. SOLUBILITY OF THE DRUG

The solubility of the drug may determine its concentration in the preparation and regulate its release rate. To improve solubility and to ensure better delivery, a binary solvent system is often recommended in which water, the primary solvent is used with various combinations of an adjuvant such as propylene glycol, dimethyl sulphoxide, dimethyl formamide or tetrahydrofurfuryl alcohol.

In a mixed solvent system, drug solubility usually increases with increasing level of adjuvant. This phenomenon may be expressed by a general equation (34-35).

$$\log S_f = \log S_w + af \quad \text{.....equn.1.1.}$$

Where, S_f = solubility of the drug in the binary aqueous system

S_w = solubility of drug in water

f = volume fraction of the non-aqueous adjuvant

a = a constant which depends on the polarity of the solute and the adjuvant.

A plot of f <u>versus</u> log S_f forms a straight line, which gives a useful estimation of solubility, on extrapolation.

The pH of the solvent system may also alter the solubility of the drug. This may be modelled by the equation :

$$pH = pKa + \log (S-S_\emptyset)/S_\emptyset \quad \text{.....equn.1.2.}$$

Where, S = total solubility of the acidic drug

$S_\emptyset$ = intrinsic solubility of the unionised drug.

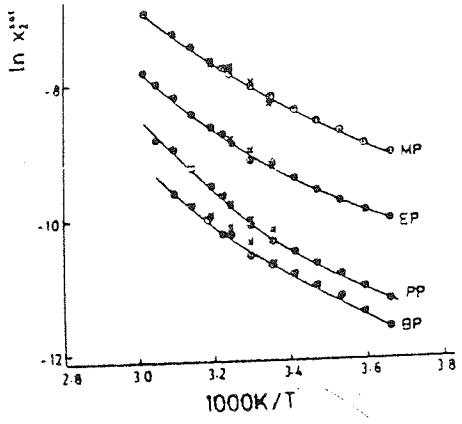


Fig. 1.9. Effect of Temperature on Solubility of Parabens.
MP = methyl paraben ; EP = ethyl paraben; PP = propyl paraben and BP = butyl paraben.
Absolute temperature range 278 - 333°K (38)
Solvemt : Distilled water.

(From Int. J.Pharmaceut., 1984,18, 25-38)

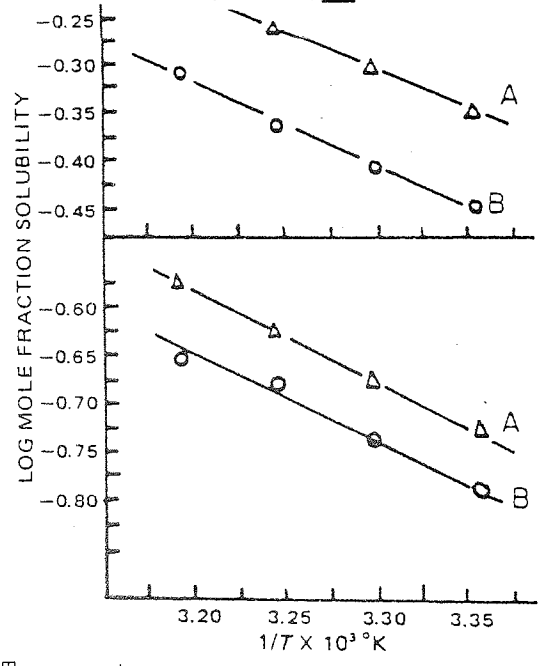


Fig. 1.10. Effect of Temperature on Solubility Profile of Parabens.
Lower figures = ethyl parabens, upper figures = butyl paraben
ideal curves = A from heat of fusion data; actual curve fr
experimental data = B (39). Solvent : aqueous alcohol
(From J.Pharm Sci. 1977, 66, 42-48)

Alkyl salicylates having pKa values of about 10.5 (87), are highly soluble at alkaline pH in their ionic form. At pH value below the pKa, the soluble ionic form is largely converted to its unionized form and precipitates from the solution.

The solubility of the drug also changes with temperature(36), and may be expressed by the equation:

$$\ln x = (-\Delta H_F / R)\ (T_m - T)/T_m T \ldots\ldots \text{equn.1.3.}$$

Where, x = mole fraction solubility

ΔH_F = heat of fusion of the solute

T_m = melting temperature of the solute

T = absolute temperature

R = universal gas constant ($8.3143 J\ mol^{-1} K^{-1}$)

A plot of ln x against 1/T gives a straight line, whose slope is $-\Delta H_F/R$. However, the linearity depends upon the nature of the compound as well as the temperature range. For example, a ln x <u>versus</u> 1/T plot of theophylline, over the temperature range 288^{O} to $350^{O}K$ (15^{O}-$77^{O}C$) is linear (37). A similar plot with parabens over the temperature range 278^{O}-$333^{O}K$(5^{O} -$60^{O}C$) is non-linear. The solubility curve obtained from this data, as shown in Fig.1.9., may be represented by two intersecting straight lines, whose point of

intersection is a function of temperature range (38). A narrow temperature range 298°-313°K (25°-40°C) in another set of data with the same drugs gives an excellent straight line, which is presented in Fig.1.1Ø.(39).

It should, however, be noted that a similar temperature range extracted from Fig. 1.9. Would also show good linearity.

Table 1.10. Effect of Absolute Temperature on the Diffusion Coefficient of Methyl Nicotinate in Water, Aqueous Urea Solution and in Isopropyl Myristate (41)

nicotinates	Temp. ^{o}K	Diffusion Coefficient 10^{-9} $m^2 Sec.^{-1}$		
		water	6 M urea	isopropyl myristate
methyl nicotinte	298	0.88	-	0.41
	303	1.13	-	0.45
	310	1.20	-	0.51
methyl nicotinate	293	-	0.56	0.37
	298	-	0.63	0.41
	303	-	0.81	0.45
	310	-	0.86	0.51

1.6.2. DIFFUSION COEFFICIENT OF THE DRUG

The diffusion coefficient of a drug is an index of the resistance exerted by its environment to the movement of the drug molecule. In topical therapy, diffusion of the drug occurs not only through the vehicle but also through the cutaneous tissue. These processes are respectively described by Higuchi(40) as diffusional resistance of the vehicle (D_V) and diffusional resistance of the stratum corneum (D_S). The mathematical expression used to define this process(40) is :

$$D_V = KT / 6 \; r \eta \quad \text{.....equn.1.4.}$$

Where, D_V = diffusion coefficient of the drug in the vehicle

K = Boltzmann constant ($1.38054 \times 10^{-23} J K^{-1}$)

r = hydrodynamic radius of the diffusing drug

η = viscosity of the vehicle

This expression indicates that D_v is directly proportional to the absolute temperature. However, the skin temperature varies only over a narrow range of temperature under normal conditions and hence the temperature dependency of the release rate is of little importance. The in vitro effect of temperature on the diffusion coefficients of methyl nicotinate in water, 6M urea in water and in isopropyl

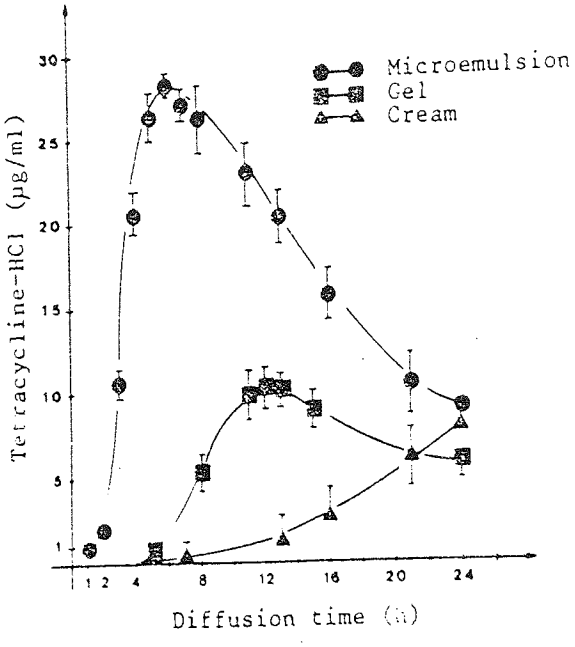


Fig. 1.11. Effect of Viscosity on the Release of 1% Tetracycline -HCl (42).

(From Dermal and Transdermal Absorption, p 73-89).

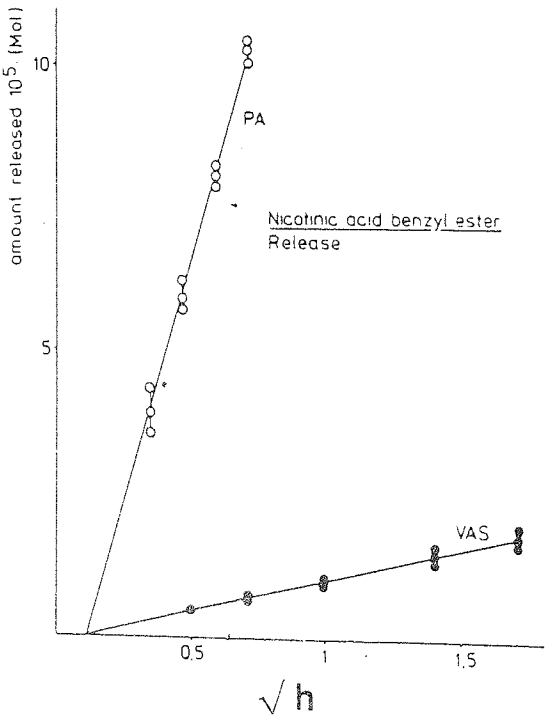


Fig. 1.12. Invitro Release of Benzyl Nicotinate from Poly Acrylate Gel and Vaseline.

C_o = 1%(dissolved); area of barrier = 15.9 cm^2, 32 ± 1° without membrane.

(From Pharm. Ind. 1981,43,1123-1133)

myristate are shown in Table 1.1Ø. In all cases diffusion coefficient increases with increasing temperature (41).

The equation also reveals that D_V decreases with increasing mean radius. However,to achieve a significant change in the mean radius, a gross change in molecular weight is essential. This is why in a homologous series, the slight change in molecular weight of the consecutive members does not alter greatly the diffusion coefficient.

Equation 1.4. also shows that D_V decreases with increasing viscosity of the vehicle. A number of workers have confirmed this effect in vitro. Diffusional resistance to tetracycline -HCl in cream, gel and micro emulsion is displayed in Fig.1.11. (42). Release from the micro emulsion is much faster than from the other two systems. Another example in Fig. 1.12.(43) shows that the diffusional resistance to the release of benzyl nicotinate is considerably higher in vaseline than in polyacrylate gel.

The diffusion coefficient of the stratum corneum may alter the penetration kinetics of the drug which may be expressed by the following equation:

$$dQ/dt = C_V.D_S.K.A/h \quad \text{.....equn.1.5.}$$

te Gel

± 1° C

Where, dQ/dt = steady-state flux of the drug through the skin barrier

C_V = concentration of the diffusing drug in vehicle

D_S = diffusion coefficient of the drug in the stratum corneum

K = partition coefficient of the drug between the skin and the vehicle

A = area of the skin through which the drug penetrates

h = thickness of the stratum corneum

This equation, satisfies only an ideal system , where there is no vehicle or time - induced change in the cutaneous tissue, and when there is no shunt diffusion. Under normal conditions, however,the vehicle may alter the diffusional resistance of the drug in the stratum corneum by changing the degree of hydration and other barrier characteristics, as mentioned in Section 1.5.2.

1.6.3. CONTROLLED RELEASE OF DRUG FROM VARIOUS PREPARATIONS

The physical form of the preparations may control the rate of release of the drug. When a single drug is incorporated in true solution and is uniformly distributed throughout the vehicle, release of the drug is estimated from equation 1.6.(44)

$$Q = h\, C_{\emptyset}\, \{1-8/\pi^2 \sum_{m=\emptyset}^{\infty} 1/(2m+1)^2 \exp[-D_v(2m+1)^2\pi^2 t/4h]\}^2 \quad \text{equn.1.6.}$$

Where, Q = quantity of the drug released to the skin surface per unit area of application

h = thickness of the vehicle layer

C_o = initial concentration of the drug in the vehicle

D_v = diffusion coefficient of the drug in the vehicle

t = time after application

m = integer with values from $\emptyset$-∞

In contrast, from a suspension, in which the drug is uniformly dispersed, the release profile is expressed by the following equation:

$$Q = \sqrt{D_v t}\, (2A - C_s)\, C_s \quad \text{.....equn.1.7.}$$

Where, Q = quantity of the drug released to the skin surface per unit area of application

D_v = diffusion coefficient of the drug in the

C_s = solubility of the drug in the vehicle vehicle

t = time after application

A = concentration of the drug (in units/cm^3)

The permeability of the isolated human epidermis and the whole skin to nicotinic acid, methyl and butyl nicotinate and their ^{14}C-labelled compounds was tested by dissolving them in water, olive oil, ethanol and propylene glycol (45). Skin preimmersion in water increases the permeability of the nicotinates only from olive oil. Permeability from the aqueous vehicle remains unaffected. Another comparative study regarding in vivo penetration of nicotinates also reveals that water is the most suitable vehicle from which methyl and butyl nicotinates penetrate at a faster rate than that from olive oil and propylene glycol (46). The presence of inert excipients in the preparation, such as starch (2-7% wt), calamine (5-10% wt) and zinc oxide (5-10% wt), in methyl nicotinate aqueous and oily cream did not alter the hydration state of the skin, and consequently, the diffusion coefficient of the drug in the stratum corneum remained unchanged (47). The in vitro release rate of methyl salicylate from cocoa butter containing various initial concentrations of the drug was monitored and it was found that the

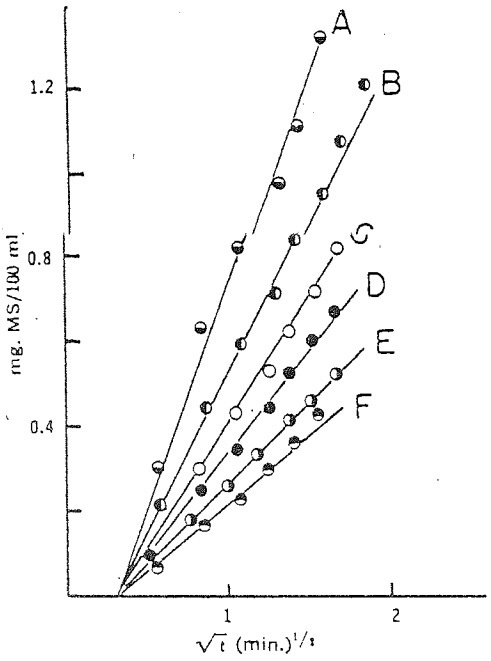


Fig. 1.13. Effect of Initial Concentration on the Release of Methyl Salicylate from Cocoa Butter Ointment (48).

A = 161.8 , B = 124.8 , C = 96.1 , D = 84.6 , E = 71.4 , F = 61.7 (mg/g. cocoa butter).

(From J.Pharm. Sci. 1969, 58 980-982).

rate increases with increasing concentration, as displayed in Fig. 1.13.(48). A similar experiment with methyl, ethyl, butyl, hexyl and benzyl nicotinates supported this finding(49).

1.6.4. PARTITION COEFFICIENT OF THE DRUG

An important criterion of percutaneous absorption of the drug is its relative affinity for the vehicle and for the stratum corneum, in other words, the partition coefficient of the drug between the skin surface and the donor vehicle. To mimic the lipoidal cutaneous tissue, various organic solvents such as isopropyl myristate, octanol, olive oil and benzene are used as the recipient and partition coefficients are determined between the receiving solvent and a binary donor solvent. The relationship between the equilibrium distribution of the drug between an immiscible organic solvent and a binary donor solvent is expressed(35) by the following equation:

$$\text{Log } (P.C.)_f = a \text{ Log}(P.C.)_w + bf \quad \text{.....equn.1.8.}$$

Where, $(P.C.)_f$ = partition coefficient of the drug between an immiscible solvent and a donor solvent with a volume fraction f of a given adjuvant

$(P.C.)_w$ = partition coefficient between the immiscible solvent and water

a and b = constant

A low partition value corresponds to a high degree of interaction with the vehicle and a tendency for the substance to remain in the vehicle. A high partition value, on the other hand, indicates a limited affinity for the vehicle and a quicker rate of release from

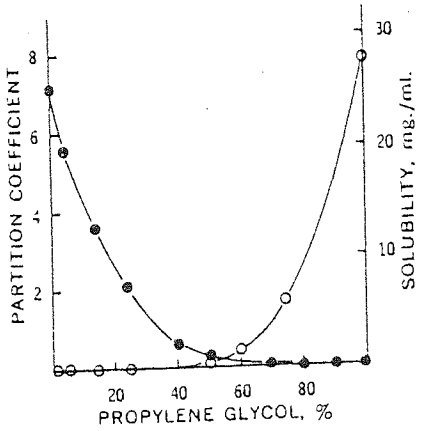


Fig. 1 14. Effect of Proportion of Adjuvant in the Solvent on the Solubility and Partition Coefficient (Isopropyl Myristate/ Propylene Glycol-Water) of Fluocinolone Acetonide (50).

(From J.Pharm. Sci. 1971. 60, 1175-1179)

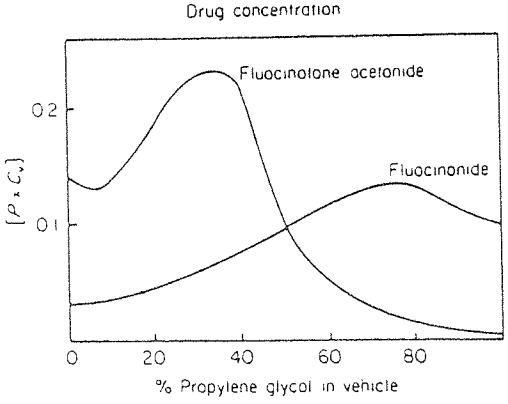


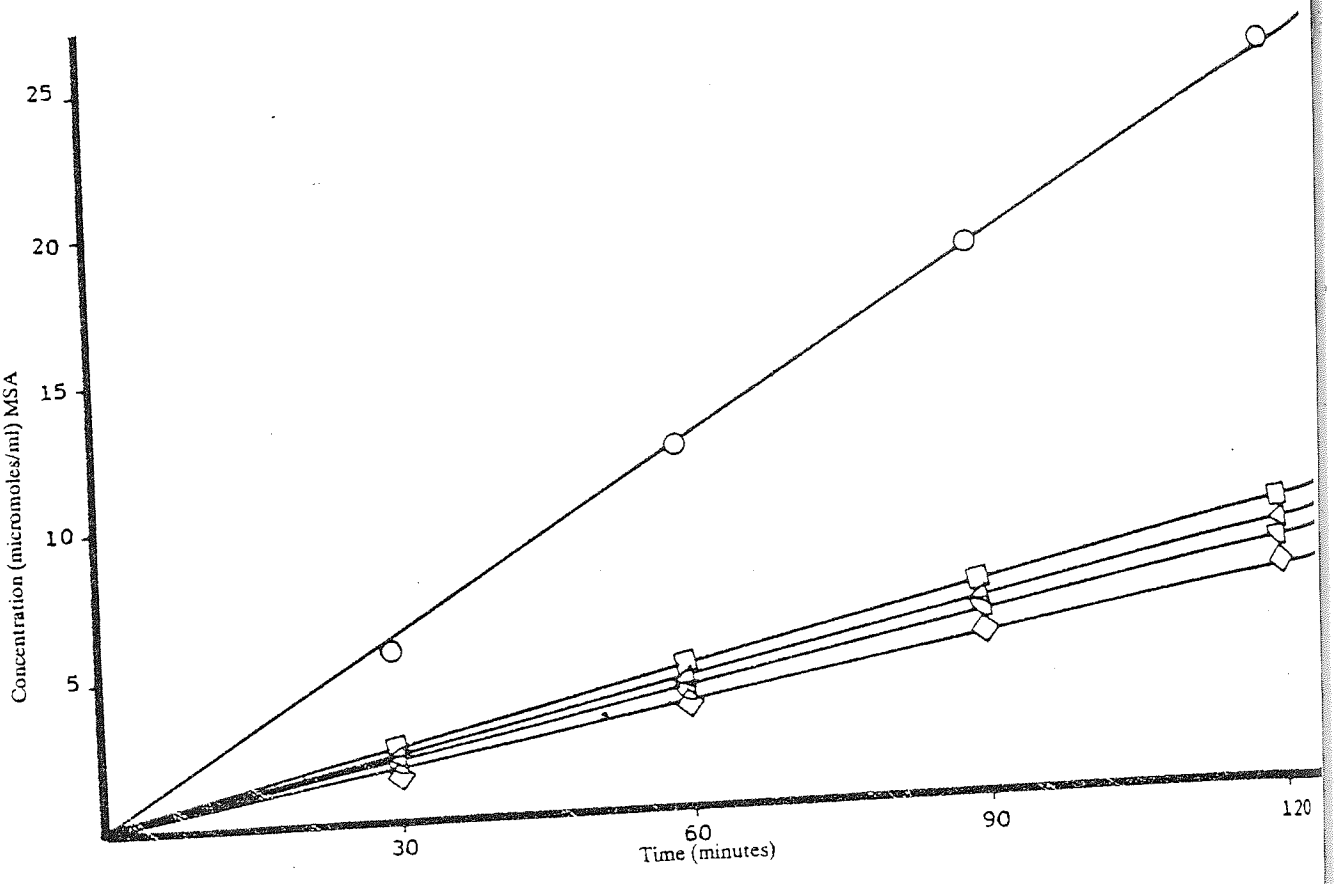
Fig. 1.15. Effect of the % Adjuvant on KC_V of Fluocinolone Acetonide ; Donor Solvent = Propylene Glycol-Water; Organic Solvent = Isopropyl Myristate (50).

(From J.Pharm. Sci., 1971, 60, 1175-1179).

the vehicle. The increase in the proportion of the cosolvent in the donor vehicle increases the drug solubility and decreases its partition coefficient. The mutual dependency is shown in Fig.1.14.

Equation.1.5. reveals that in a donor vehicle, the product of solubility and the partition coefficient is a direct function of the steady-state flux. Several sets of experimental data show that the C_v. K versus %cosolvent plot passes through a maximum (Fig. 1.15.). The crucial solvent concentration, which guarantees maximum delivery is the maximum of the curve.

The partition coefficient also influences the release of the drug from a semisolid preparation. A comparative study of the release of methyl salicylate from various ointment bases (51) shows that a hydrophilic base is most efficient in releasing this drug. The findings are shown in Fig. 1.16.



Key: ○ hydrophilic ointment, ◇ lanolin, ▽ aquaphor, ◁ white petrolatum, □ unibase.

Fig. 1.16. Release of Methyl Salicylate from Ointment Bases (51).

(From Pharm. Acta Helv. 1977, 52(10), 236-238).

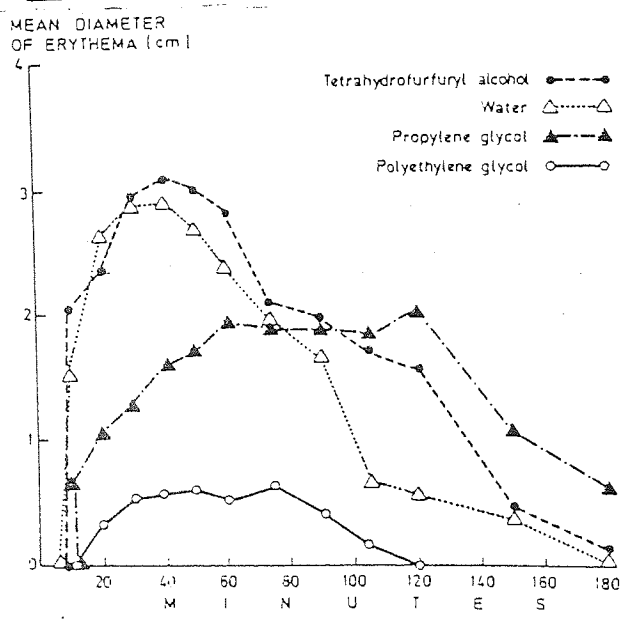


Fig. 1.17. Effect of Vehicle on the Penetration of 0.5% Methyl Nicotinate as Measured by the Mean Diameter of Erythema (Ambient Temperature 23° C. (52).

(From Brit. J. Dermatol. 1969, 81, 202-206).

1.6.5. PENETRATION ENHANCERS

A considerable number of substances can alter the diffusional resistance of the skin and thereby enhance penetration of the drug. Examples are urea, dimethyl sulphoxide, dimethyl formamide, propylene glycol, glycerol, various medium chain organic solvents, and surface active agents. These compounds can form loose bonds with the keratin structure and even lead to a change in the physical state of water in the skin. This may provide a channel for the drug molecule to diffuse through the cutaneous tissue.

The penetration enhancing capacity of four vehicles through human skin in vivo is shown in Fig.1.17.(52). Methyl nicotinate is the penetrant and its erythemal effect is used as an index of the effect of the vehicles. With water and furfuryl alcohol the erythema developed to a greater extent and more rapidly compared to that with propylene glycol. But the propylene glycol-induced erythema persisted longer. Several authors have proposed mechanisms to explain the enhancing properties of dimethyl sulphoxide (53-56). These include reversible interaction with keratin, the possibility of its interaction with another diffusing solute and its inhibition of the redox polymerisation of hyaluronic acid. The influence of pH on the enhancing characteristics of dimethyl sulphoxide through rabbit skin in vivo was investigated (57), using salicylic acid as the penetrant. Within the pH range 2.97-10.78, the blood levels of salicylic acid were higher at the lowest and the

Table 1.11. Effect of pH on The Dimethyl Sulphoxide-Induced Blood Level of Salicylic Acid (57).

Type of ointment / pH	Salicylic acid Blood Level (mg %)				
	2.97	4.48	6.80	9.23	10.78
without dimethyl sulphoxide	5.57	2.47	2.58	5.49	7.03
with dimethyl sulphoxide	11.54	6.88	7.56	9.74	13.78

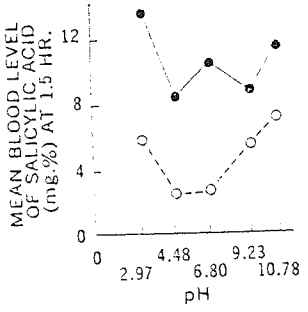


Fig. 1.18. Mean Blood Levels of Salicylic Acid as a Function of pH at 1.5 hr. After Application of Ointment.

DMSO added = ●
No DMSO = O

(From J.Pharm, Sci., 1970, 59(11), 1616-1620)

highest pH values than at the intermediate levels, both with and without dimethyl sulphoxide. This is shown in Table 1.11., with the profile displayed in Fig. 1.18. The highest blood levels were achieved at highest pH without dimethyl sulphoxide and at lowest pH with this enhancer. This shows that the effect of dimethyl sulphoxide is more pronounced with the unionised molecule than with the ion.

Azone^{R}(1-dodecylazacycloheptan-2-one) acts as an enhancer, allowing penetration of about 100 fold of the control values(58). Penetration of hydrophilic drugs are more dramatically enhanced by these enhancers compared to the hydrophilic drugs.

Surface active agents also increase penetration but at the cost of the integrity of the skin structure. These agents interact with the biological membranes by either rupturing them, replacing certain phospholipids present in the lipid micelles, or inducing configurational changes in these micelles (59). However, there is evidence (60) that some nonionic surfactants alter penetration kinetics without causing a significant change in skin properties.

Table 1.12. Cell Types in Various Layers of The Skin (71).

Skin layer	Cell types
Stratum corneum	bacterial flora keratinocytes epidermal cells in different states of differentiation
Epidermis	melanocytes Langerhans cells Merkel cells sebaceous glands sweat glands fibroblasts
Dermis	blood vessels nerves and sense organs histocytes mast cells muscle cells
Subcutaneous fat	fat cells

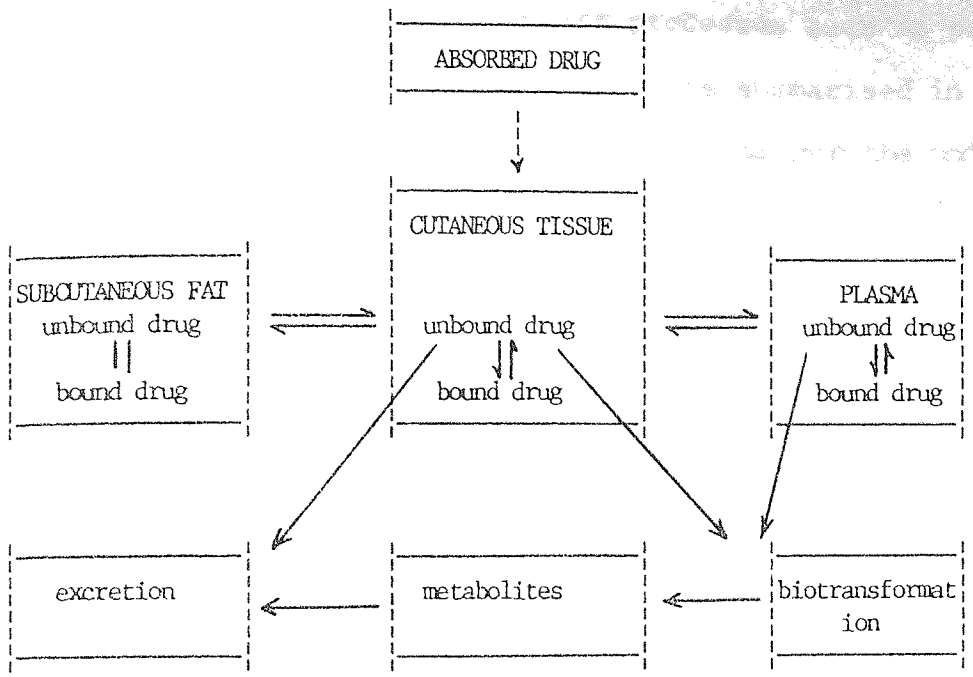


Fig. 1.19.a. Fate of the drug after absorption

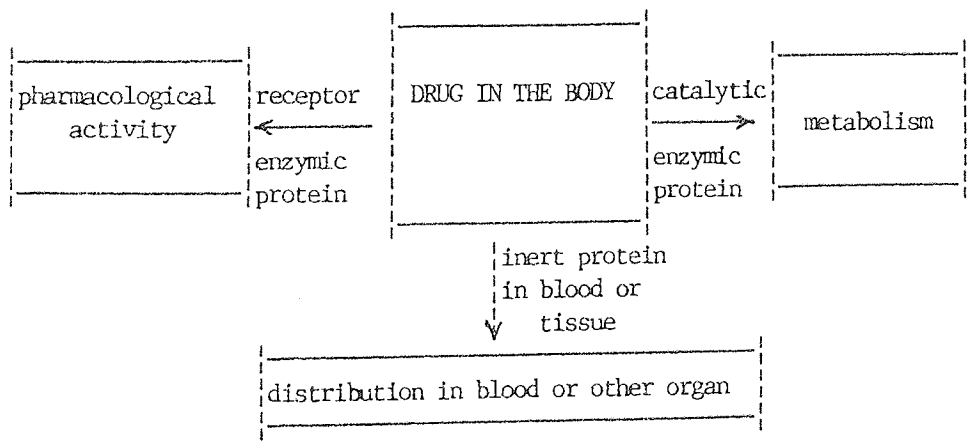


Fig. 1.19.b Possible salicylate-protein interactions

1.7. FATE OF THE DRUG AFTER ABSORPTION

The accumulation of the drug in the target tissue is governed by simultaneous interacting processes such as protein binding and excretion. These processes are summarised in a theoretical model (Fig.1.19). The drug, after entering into the body may interact with the biological proteins in the extracellular fluid, in cell membranes and within the cells. The relative extent of binding may be described by the following equation :

$$\log C_p = \log a + b \log C_f \ldots\ldots\ldots \quad \text{equn.1.9.}$$

Where, C_p = concentration of protein-bound drug

C_f = concentration of free drug

a and b = constants.

Besides drug concentration, the affinity of the drug molecule for the pharmacological site and the capacity of the binding sites also control the degree of binding. The binding capacity and binding site of salicylate has been studied by several authors (61-62). The authors (63-70) have demonstrated that a lysyl ϵ-amino group is the major binding site for salicylates in plasma protein. The unbound drug in the cutaneous tissue remains in dynamic equilibrium with that in the other compartments.

Table 1.13. Physiological Data of Weight and Blood Supply of Skin and liver (71).

Physiological data	Skin	Liver
Total weight	4-5 kg; epidermis 200g	1.6 kg
Blood flow/100 gm tissue	2.5 ml/min.	100 ml/min.
Total blood flow	100-125 ml/min.	1600 ml/min

Table 1.14 Biotransformation Reactions By Human Skin (71).

PHASE I REACTIONS	
OXIDATION	ENZYMES INVOLVED
aliphatic C - atom alicyclic C - atom	mixed function oxidase
aromatic rings	hydroxylase
alcohols	hydroxysteroiddehydrogenases
deamination	monoamineoxidase
dealkylation	deethylase demethylase
REDUCTION	
carboxyl groups C=C - double bonds	ketoreductase 5 α-reductase
HYDROLYSIS	
ester bonds epoxides	esterases epoxidehydratase
PHASE II REACTIONS	
glucuronide formation sulphate formation glutathione conjugation	UDPG-transferase sulpho-transferase glutathione-S-transferase

The various compartments of the skin contain different specialised cells(Tab.1.12) which can contribute to drug metabolism. After topical application, each molecule of the drug which becomes systemically available, must pass through the metabolic barrier of the skin and hence cutaneous metabolism is controlled by the penetration kinetics. However after systemic application, only a small portion of the total dose reaches the skin and is controlled by the distribution volume and cutaneous blood flow. Thus, the gradient is reciprocal to each other, as shown in Fig.1.20. However, the contribution of the skin to the total metabolic clearance, even in systemic therapy is not insignificant. Tauber (71) estimated that the contribution of the skin in epoxihydrase activity is 0.5%, considering the relative physiological data of the skin and the liver (Tab. 1.13). The skin is capable of inducing chemical modification (phase I) and conjugation (phase II) reactions. A summary of these reactions together with the enzymes involved is presented in Table 1.14.

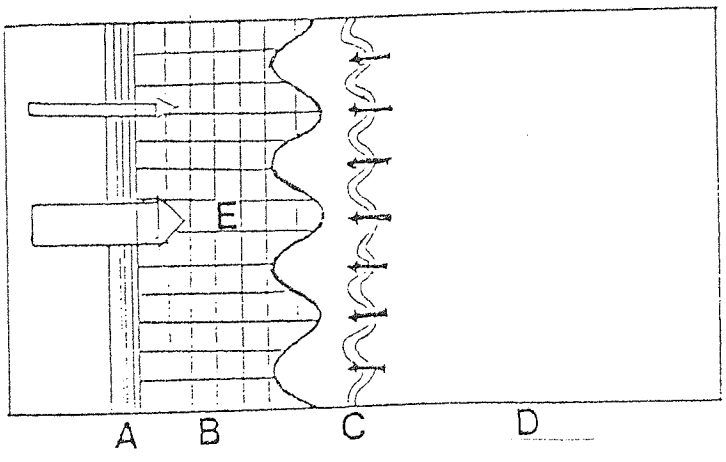


Fig. 1.20. Skin as a metabolic barrier

⟹ Topical

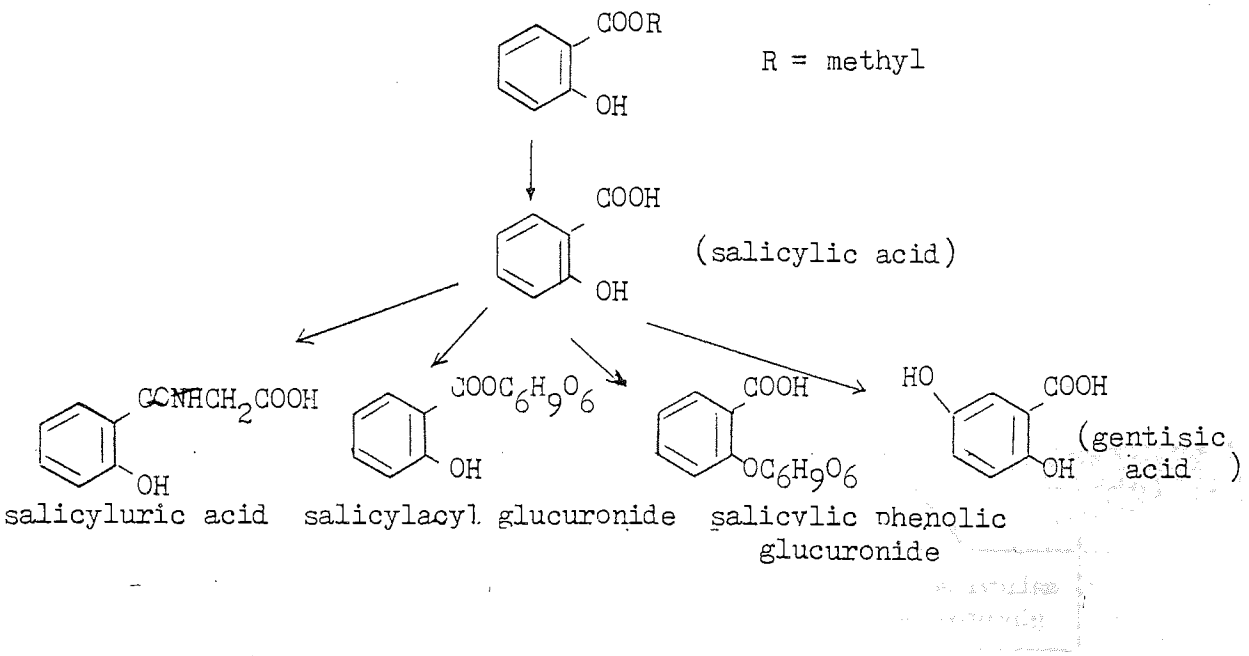
⟵ Systemic

A = Stratum Corneum
B = Epidermis
C = Blood Vessels
D = Dermis
E = Epidermis

Table 1.15. Inhibition of Enzymes by n-Alkyl Salicylates (10^{-3} M). Values are % Inhibition (73).

Salicylates	% inhibition			
	Glyoxalase	Xanthene oxidase	D-Amino acid oxidase	Carbonic anhydrase
ethyl	7	17	11	18
propyl	14	10	-	24
butyl	23	0	11	40
pentyl	54	13	46	49
hexyl	57	8	71	57
heptyl	73	12	100	63
octyl	78	42	100	73
nonyl	85	84	100	90
decyl	80	51	100	52
dodecyl	19	10	92	62
tetradecyl	0	12	96	36
hexadecyl	0	10	86	-
octadecyl	0	-	83	-
myristyl	14	0	53	58

The biotransformation studies of salicylates in man were carried out by Davison et al (72). The major metabolites are displayed in Scheme.1.3.



Scheme 1.3. Biotransformation of salicylate in man

Salicylate esters inhibit a variety of enzymes such as glucose-6-phosphate dehydrogenase, glyoxalase, xanthine oxidase, carbonic anhydrase and D-amino oxidase. The enzyme inhibitory potency of various n-alkyl salicylates is shown in Table 1.15. The inhibitory effect reaches a maximum at the n-nonyl derivative (73).

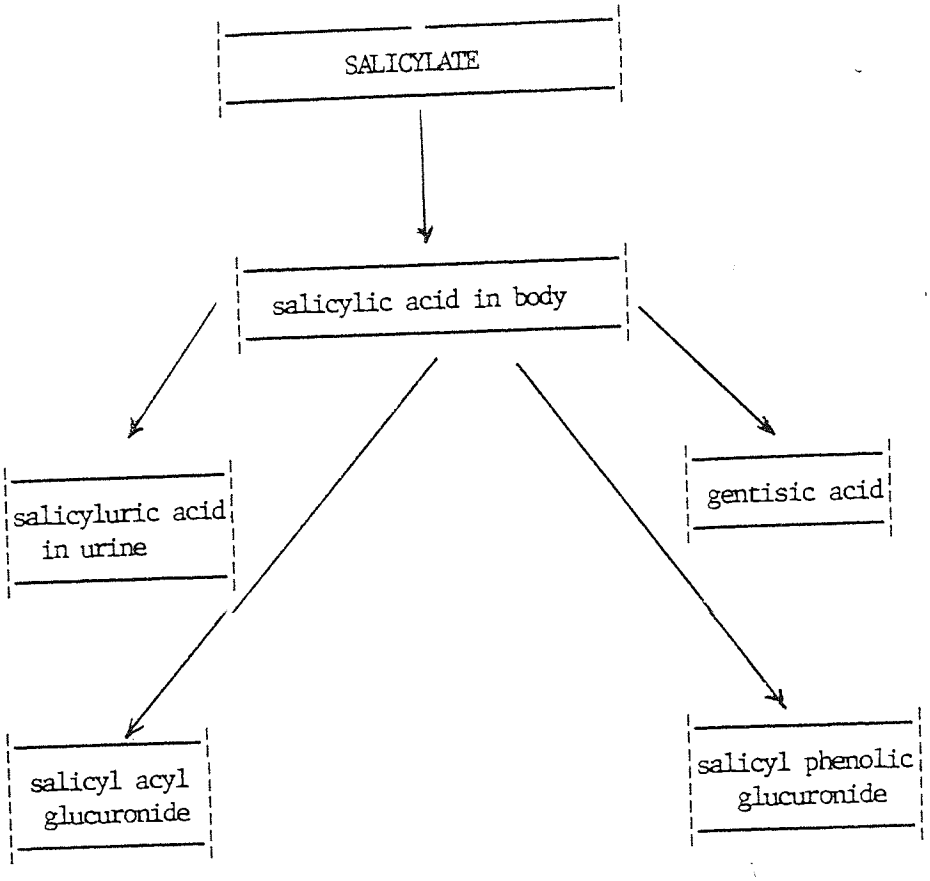
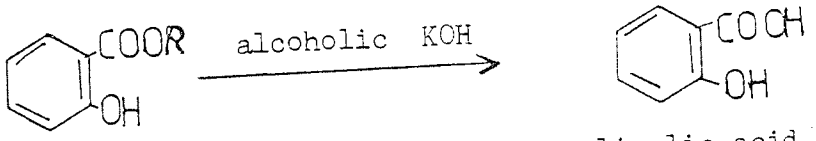


Fig.1.21. Pharmacokinetic model for elimination of salicylate from human.

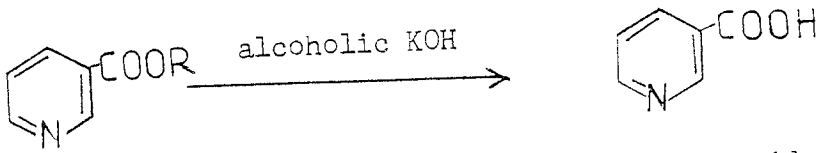
The fate of salicylates such as the methyl, ethyl and glycol esters, following percutaneous absorption, has been studied by several investigators(74-75). The respective elimination rates of these esters were identical to that of salicylic acid. The kinetic models have rate constants for the elimination of the major metabolites of salicylates in man is shown in Fig.1.21. All processes, except the formation of salicyluric acid follows apparent first order kinetics (76-77). At moderate therapeutic body concentrations, salicyluric acid synthesis follows apparent zero-order kinetics.



salicylate

salicylic acid

R = alkyl or aryl group



nicotinate

nicotinic acid

Scheme 1.4. Saponification of salicylates and nicotinates

1.8. ANALYTICAL METHODS FOR THE ESTIMATION OF SALICYLATES AND NICOTINATES

Most of the commercial topical preparations are present in multidrug combinations with mono-, di- or tri-hydric alcohols as solvents, humectants and absorption enhancers. Because of this complexity simultaneous determination of all components is advisable in routine quality control analysis. In kinetic analyses, drug determination becomes more complicated due to the presence of degradation products. Biological samples, too, introduce further problems due to metabolites, endogenous compounds and the low concentrations encountered.

1.8.1. TITRIMETRY AND GRAVIMETRY

Though the modern trend in analysis is to employ rapid and accurate methods of assay, until the mid-fifties salicylates and nicotinates were frequently assayed by the fundamental laboratory methods such as gravimetry and titrimetry. Even in the current British Pharmacopeia, these methods have been described for the estimation of salicylates and nicotinates. Such methods involve a sample preparation step in which the salicylates and nicotinates are hydrolysed to the respective acids using alcoholic alkali.(78-82).

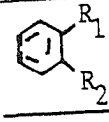
When present in a compound pharmaceutical preparation, these esters are determined by non-aqueous titration. This method has an extra source of error - the extraction of the liberated acid by the organic solvent. This method has its inherent drawbacks such as its

nonspecificity. Besides, in the saponification step the solvent may extract excipients from the product which may be weighed as salicylic acid. Moreover, salicylic acid may sublime causing inaccuracies. These methods are also slow and samples equivalent to 50mg of the active ingredients are required. However, these are very simple methods and the apparatus required is readily available.

1.8.2. COLORIMETRY

Another parallel approach to gravimetry or titrimetry in the quantitative determination of these esters is the application of colorimetry. In common with the previous method, salicylates are hydrolysed to salicylic acid by alcoholic potash. The liberated acid is treated with ferric nitrate to give violet colour to the solution. The intensity of the colour is measured at 520-530 nm and concentrations are determined by calibration with a series of standard solutions (83-85). The intensity of the colour is very sensitive to the pH of the solvent. This test is best performed in acidic solution (0.001M - 0.002M of mineral acid).

Table 1.16. Ultraviolet Absorption Chracteristics of Salicylic Acid and Methyl Salicylate (94).



R_1	R_2	pH	2nd primary band		1st primary band		Secondary band	
			nm	ϵ X 10^3	nm	ϵ X10^3	nm	ϵ X 10^3
OH	COOH	3.00	202.5	36.00	237	9.0	302.5	3.6
OH	COO^-	9.0	—	-	230.5	7.2	296.0	3.5
O^-	COO^-	11.0 (5N NaOH)	-	-	242.0	6.9	306.0	3.4
OH	$COOCH_3$	0.1N HCl	209.5	19.8	252.5	10.9	324.0	3.3
O^-	$COOCH_3$	0.1 N NaOH	225.5	20.2	256.0	5.7	359	5.25

1.8.3. ULTRAVIOLET SPECTROSCOPY

This method allows rapid determination of the drugs (86-93). However, in predicting stability and cross reactivity the reliability of this method is often doubtful despite its merits of rapidity and simplicity. This is because of the presence of identical chromophores in various reactants and products. The ultra violet absorption characteristics of salicylic acid and methyl salicylate are recorded in Table 1.16.(94), and those of nicotinates in Table 1.2.(13).

1.8.4. FLUORIMETRY

Salicylates have intrinsic fluorescence which may be intensified by the addition of alkali and by the choice of a solvent (95-1Ø3). Formation of a fluorescent aluminium complex may increase the sensitivity even more. The method is nonspecific and does not discriminate between closely related metabolites or degradation products. To minimise the background absorption from endogeneous materials, lengthy clean-up procedures are necessary. Besides, the technique has the major drawbacks of cross-quenching from non-fluorescent impurities and self-quenching with increasing concentration of the drug.

1.8.5. CHROMATOGRAPHIC METHODS

To meet the ever increasing demand of simultaneous analysis in the determination of combined drug products, in evaluating reaction pathways and in elucidating drug-drug or drug-vehicle interactions in dosage forms, various chromatographic techniques have been successfully implemented in the quantitative estimation of salicylates. In adsorption chromatography, various components in the mixture are eluted in an order depending upon their affinity for the adsorbent. Similarly, in partition processes the order of elution depends upon their relative stationary phase/mobile phase partition coefficients. These processes are exploited in thin-layer, gas and high-performance liquid chromatography.

1.8.5.1. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography is complementary to other chromatographic methods. A few systems have been reported for salicylates (104-106) and nicotinates (107-108). This method is inexpensive compared to other chromatographic methods and also has the advantage that several assays can be performed simultaneously.

1.8.5.2. GAS CHROMATOGRAPHY

Its selectivity and sensitivity makes gas chromatography valuable for the analysis of multicomponent mixtures. However, despite the specificity and sensitivity, the

Table 1.17. High-Performance Liquid Chromatographic Determination of Salicylates

Solute components	Column	Eluent	nm	Flow rate ml/min	Sample type	Ref.
paracetamol, caffeine, aspirin, phenacetin, sali. acid	prepacked, Whatman Partisil 25cm X 4.6 mm	CH_3CN:AcOH:H_2O 25:5:70	275	1	hydro-alcoholic	114
aspirin, salicylic acid, salsalate, aspirin anhydride	Lichrosorb RP18 25cm X4.6 mm	AcOH:MeOH:H_2O 12.5:537:450	254	1.6	extract from tablet	115
aspirin, gentisic acid, salicyluric acid	prepacked Zorbex 25cm X 6.2 mm	BuOH:Na_2SO_4: AcOH 2:10:5:83	313	3	biological	116
acetaminophen, aspirin, caffeine, codeine, phenacetin salicyl amide	C_{18} bonded 30cm X 4mm	19%MeOH: .1M KH_2PO_4:	285	2	commercial prpn.	117
aspirin, caffeine, phenacetin	Bondapack C_{18} *	MeOH:H_2O:50:50 .01M TBA cation	254	*	aq sol	118
methyl salicylate euginol, thymol	Hypersil SAS *	MeOH:H_2O:50:50	260	2	aq sol	119
salicyl amide, salicylate	C_{18} *	MeOH:Acetate buffer:28:72	*	1	aq sol	120
aspirin, salicylic acid	Lichrosorb RP18 *	MeOH:H_3PO_4:H_2O 55:0.05:45	235	1.5	biolog	121

* no information is available

elevated operating temperatures may occasionally cause thermal degradation of the drugs. Moreover, polar and non volatile compounds are retained on the column or else non-ideal processes result in the tailing of the peaks. Thus it is often necessary to derivatise the samples to increase their volatility and to improve their chromatographic behaviour. In pharmacokinetic studies, the samples in the body fluids frequently need considerable clean up such as solvent extraction to prevent interference from endogenous substances. Al-Khamis et al (109) has analysed methyl salicylate by gas chromatography in the evaluation of the release profile of the drug from various polyethylene glycol bases. The same drug in Wintergreen rubbing alcohol was quantified by Van Atta and Van Atta (110). A mixture of methyl salicylate and menthol in a topical analgesic formulation has also been quantified by gas chromatography. The experimental conditions used were also suitable for the separation of a mixture of salicylic acid, methyl salicylate, ethyl salicylate and menthol in solution (111). Additionally, salicylic acid and aspirin have been derivatised to various esters before gas chromatographic analysis to reduce tailing of these polar acids (112-113).

1.8.5.3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The technique of high-performance liquid chromatography has made a significant impact on analysis in pharmaceutical, clinical and research applications and complements gas chromatography in being useful for both polar and thermally sensitive molecules. This is

Table 1.18. Relative Merits and Demerits of the Physicochemical Methods Used in the Estimation of Salicylates and Nicotinates

METHOD	SAMPLE PREPARATION	APPLICABILITY	SIMPLICITY ease of operation	SENSITIVITY limit of detection	SPEED
Gravimetry	Saponification and drying is essential	restricted	tedious	50 mg/100ml	time consuming
Aqueous titrimetry	Saponification	restricted	tedious	50 mg/100ml	2-5 hours
Non-aqueous titrimetry	Saponification foll-owed by solvent extraction	restricted	tedious	50 mg/100ml	2-5 hours
Colorimetry	Saponification followed by complexation	restricted	tedious	0.8 mg/100 ml	2-5 mins.
Fluorimetry	Clean up is necessary	restricted	easy	100 mg	2-5 hrs
Thin layer Chromatography	minimal clean up	very wide.	easy	20 ng	slow 30 mins. to few hours
Gas Chromatography	Considerable clean up	wide	easy	1 ng- 50 ng	moderate 10 - 30 mins.
High Performance Liquid Chromatography	minimal clean up	wide	easy	1 ng - 1 ug	fast 2 - 20 mins.

perhaps the method of choice for the analysis of pharmaceutical formulations which may contain several drugs and excipients.

Though several HPLC determinations have been reported for aspirin and salicylic acid in various dosage forms as recorded in Table 1.17, Ross and Stewart (119) have pioneered the separation of salicylate esters by this technique. A mixture of eugenol, thymol and methyl salicylate was gradually eluted by a methanol-water (50%,v/v) mobile phase. Another system has been demonstrated by Saotome et al(122), where twelve salicylate esters from a twenty four component mixture were separated over a period of sixty four minutes. Lake et al(123) and Hasegawa et al(124) analysed methyl salicylate by HPLC using methanol-water-acetic acid as the mobile phase.

The relative merits of the available analytical techniques for the determination of salicylates and nicotinates are summarised in Table 1.18.

CHAPTER 2 DEVELOPMENT OF AN HPLC METHOD FOR THE ANALYSIS OF SALICYLATES AND NICOTINATES

2.1. INSTRUMENTATION

All HPLC analyses were carried out using a 10 cm X 4.6 mm Shandon column which was repacked at intervals with Hypersil -ODS (5 μm). A Rheodyne 7120 injection valve with a 20 μl sample loop was used.

Flow rates were maintained using an Altex 100A dual-reciprocating, constant flow solvent- metering pump. Peaks were detected at a wavelength of 235 nm within the attenuation range of 0.02 - 0.64 AUFS, using a Pye LC3 variable wavelength ultraviolet monitor, equipped with an 8 μl flow cell.

^{1}H nmr spectra were determined in neat or in deuteriochloform solution with tetramethylsilane as internal standard using a Varian EM360A spectrometer operating at 60 MH_{z}.

Mass spectra were determined by the direct insertion technique using a Micromass spectrometer operated with an accelerating voltage of 4KV, a trap current of 100 μA and a source temperature of 250°C.

Ultraviolet spectra were determined using Beckman Acta V Spectrophotometer.

A Radiometer PHM 64 Research pH meter with 3 decimal place display of pH was used in measuring the pH.

2.2. REAGENTS AND CHEMICALS

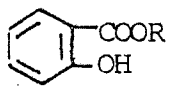
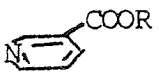
Methyl, ethyl, n-propyl and glycol salicylates were obtained from Graesser Chemicals Ltd.,U.K. Salicylic acid , p-hydroxy benzoic acid, phenyl salicylate, ethyl, n-propyl and n-butyl p-hydroxy benzoates were from Sigma Chemicals. Butyl salicylate, o-acetyloxy phenyl benzoate, o-acetyl salicyloyl ethyl carbonate, methyl and ethyl o-anisates were synthesised in the laboratory. All HPLC solvents were from Fisons Ltd.,in the purest form available. All other chemicals used were either analytical reagent quality or laboratory reagent quality and were obtained from BDH, Fisons or Sigma Chemicals.

All experiments were performed with double distilled water.

2.3. DEVELOPMENT OF AN HPLC SYSTEM FOR ROUTINE ANALYSIS OF SALICYLATES AND NICOTINATES

The aim of an HPLC analysis is the separation of eluted solutes in the shortest time. Resolution of the solute components occurs by the relative movement of the chromatographic zones through the column. This differential migration may be assumed to occur when the solute molecules are selectively soluble in the mobile phase. Hence the composition of the mobile phase and the column packing are the main controlling factors of the differential mobility of the solutes. Optimisation of the mobile phase composition was sought through the evaluation of a series of solvents in terms of retention time, column capacity factor and resolution.

Table 2.1. Molecular Weight of Investigated Drugs

Compound	Structure  R =	mol.wt.
Salicylic Acid	H	138.12
Methyl Salicylate	CH_3	152.14
Ethyl Salicylate	C_2H_5	166.17
n-Propyl salicylate	C_3H_7	180.2
n-Butyl salicylate	C_4H_9	194.22
		
Methyl Nicotinate	CH_3	137.13
Ethyl Nicotinate	C_2H_5	151.15

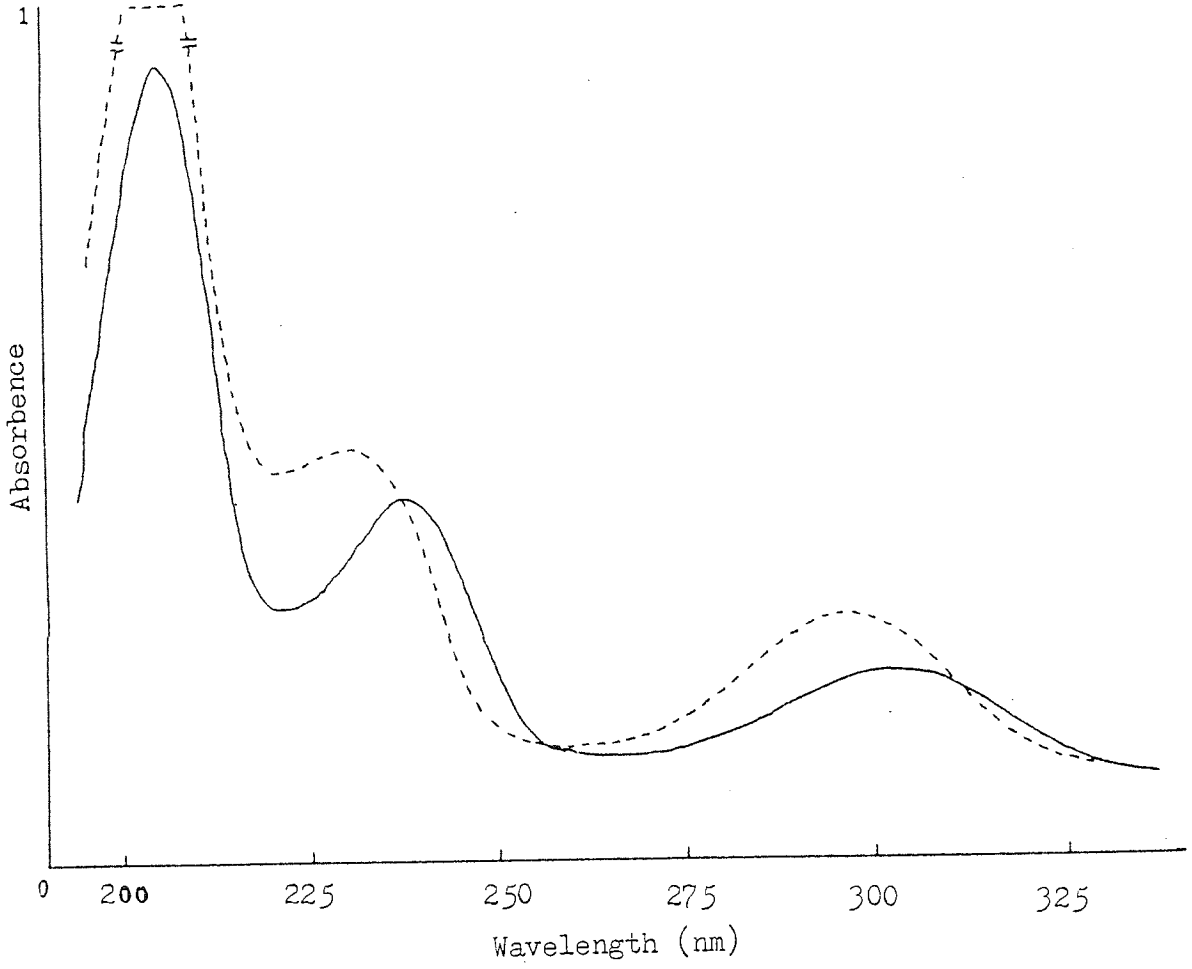


Fig.2.1. Ultra Violet Spectra

Solutes : Salicylic Acid (- - - - - -)
: Butyl Salicylates (——————)

Concentration: 0.05 mM in 50% acetonitrile in water

Reference : 50% acetonitrile in water

2.3.1. ULTRAVIOLET ABSORPTION OF SALICYLATES

Salicylic acid, methyl salicylate, ethyl salicylate, n-propyl salicylate and n-butyl salicylate (0.1 m mol.each; mol. weight in Table 2.1.) were dissolved separately in acetonitrile to provide stock solutions (100 ml, 1 mM). The solutions of salicylic acid and butyl salicylate (5 ml, 1 mM each) were separately diluted with aqueous acetonitrile (50% v/v, 95 ml) to provide samples (0.05 mM, 100 ml) for ultraviolet scan. The absorption spectra over the wavelength range 190 - 450 nm against aqueous acetonitrile (50% v/v) in the reference cell are shown in Fig. 2.1. The absorption characteristics are recorded in Tab.2.2.

Table 2.2. Absorption Data For Salicylic Acid and Butyl Salicylate

Analyte	λ max (nm)	
	Peak 1	Peak 2
Salicylic Acid	294 - 298	229 - 233
Butyl Salicylate	297 - 307	237 - 238
	$\epsilon_{308=4000}$	$\epsilon_{235=8000}$

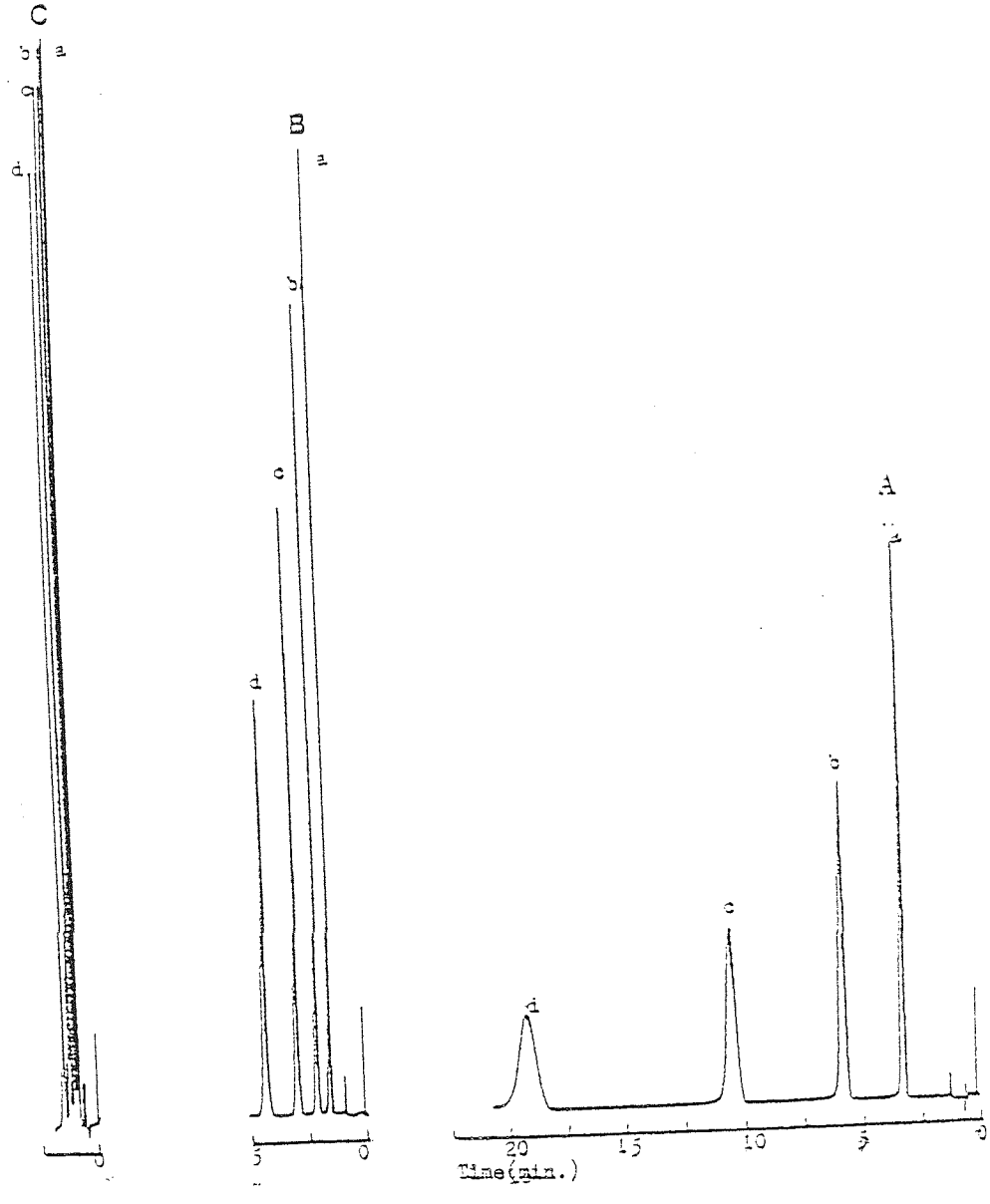


Fig. 2.2. Chromatograms Showing the Effect of the Concentration of Acetonitrile in the Mobile Phase on the Elution of Salicylates :
column 10cm X 4.6mm, Hypersil-ODS
flow rate : 1 ml/min.
detection : 0.64 AUFS ; 235 nm
Solute conc. : 1 mM in acetonitrile-water (50% v/v)

% CH_3CN in mobile phase	Peak	Salicylate
A = 40	a	methyl
B = 55	b	ethyl
C = 75	c	n-propyl
	d	n-butyl

2.3.2 OPTIMISATION OF RESOLUTION AND SPEED OF ANALYSIS : MOBILE PHASE COMPOSITION AND SOLUTE RETENTION

A series of mobile phases were prepared over an acetonitrile concentration range of 40 - 75% in distilled water with the pH adjusted to pH 2 using orthophosphoric acid (0.2%). A four component mixture of methyl, ethyl, propyl and butyl salicylate (1 mM each) was analysed after equilibration of the system with various mobile phases. The chromatograms are shown in Fig.2.2. Retention times were plotted against the proportion of acetonitrile in the mobile phase and are displayed in Fig. 2.3. The retention characteristics of the solutes in various mobile phases are recorded in Tab. 2.3.

The column capacity factor (k'), is expressed as:

$$k' = \frac{t_r - t_o}{t_o} \qquad \text{.....equn.2.1.}$$

where, t_r is the retention time of the solute

t_o is the elution time of non retained solute

It was measured for the four solutes and is displayed as a function of mobile phase composition in Fig. 2.4.

The height equivalent to a theoretical plate (H) and the theoretical plate number (N) are the HPLC parameters which define the band dispersion and are defined as :

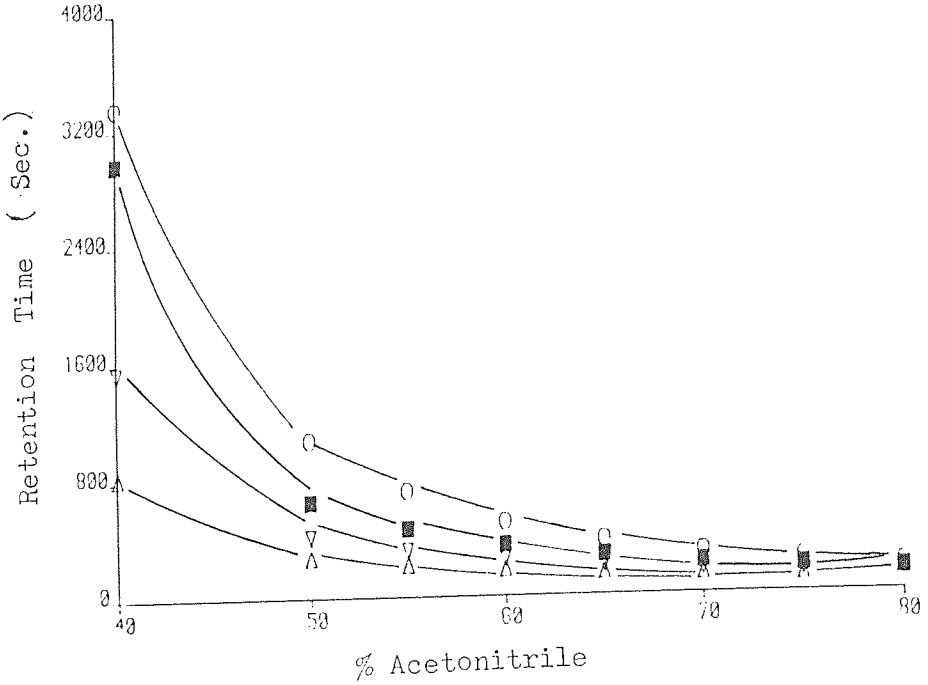


Fig. 2.3. Effect of Proportion of Acetonitrile in Mobile Phase on the Retention Time :

Symbols	Δ	∇	■	○
Salicylates	Methyl	Ethyl	Propyl	Butyl

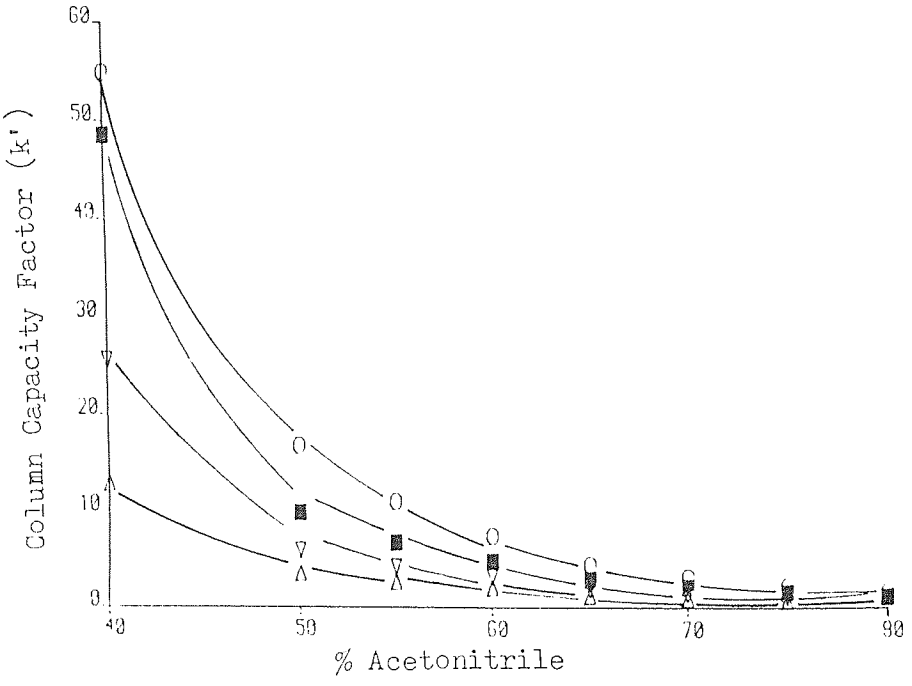


Fig. 2.4. Effect of Proportion of Acetonitrile in Mobile Phase on the Column Capacity Factors :

Symbols	Δ	∇	■	○
Salicylates	Methyl	Ethyl	Propyl	Butyl

$$N = 16 \left(t_r / w_t \right)^2 \text{.....equn.2.2.}$$

or, $$N = 5.54 \left(t_r / w_{1/2} \right)^2 \text{.....equn.2.3.}$$

where, t_r = retention time in time unit (sec.or min.)

w_t = base width of the peak in time unit

$w_{1/2}$ = width of the peak at half of the height

and $$H = L/N \text{ .....equn.2.4.}$$

where L is the column length.

The H and N values, as measured from the chromatograms are recorded in Tab. 2.3

Table 2.3. Chromatographic Parameters Measured For The Chromatograms In Various Mobile Phase Compositions

Mathematical Parameters	%acetonitrile	Salicylates			
		Methyl	Ethyl	n-propyl	n-butyl
Retention time (sec.) t_1	40	828	1542	2970	
	45	282	432	678	1110
	50	270	414	648	1062
	55	216	312	462	714
	60	180	250	345	500
	65	152	202	270	374
	70	137	170	217	278
	75	124	156	185	236
Column Capacity factor k'	40	12.80	25.600	48.500	
	45	3.70	7.100	10.200	17.500
	50	3.50	5.900	9.800	16.700
	55	2.60	4.200	6.700	10.900
	60	2.00	3.170	4.800	7.300
	65	1.17	1.890	2.900	4.300
	70	0.96	1.430	2.100	3.100
	75	0.699	1.370	1.500	2.300
Theoretical plate N	50	324	529	952	1958
	55	432	882	1171	1156
	60	576	625	940	1600
	65	546	637	1139	1726
	70	620	634	837	1287
	75	390	882	871	1547
Height eqivalent H (μm)	50	308.64	189.04	105.02	51.07
	55	236.31	113.26	85.40	86.60
	60	176.61	160.00	106.33	62.50
	65	182.87	156.85	87.79	57.91
	70	161.17	157.66	119.45	77.70
	75	256.11	113.26	114.75	64.64

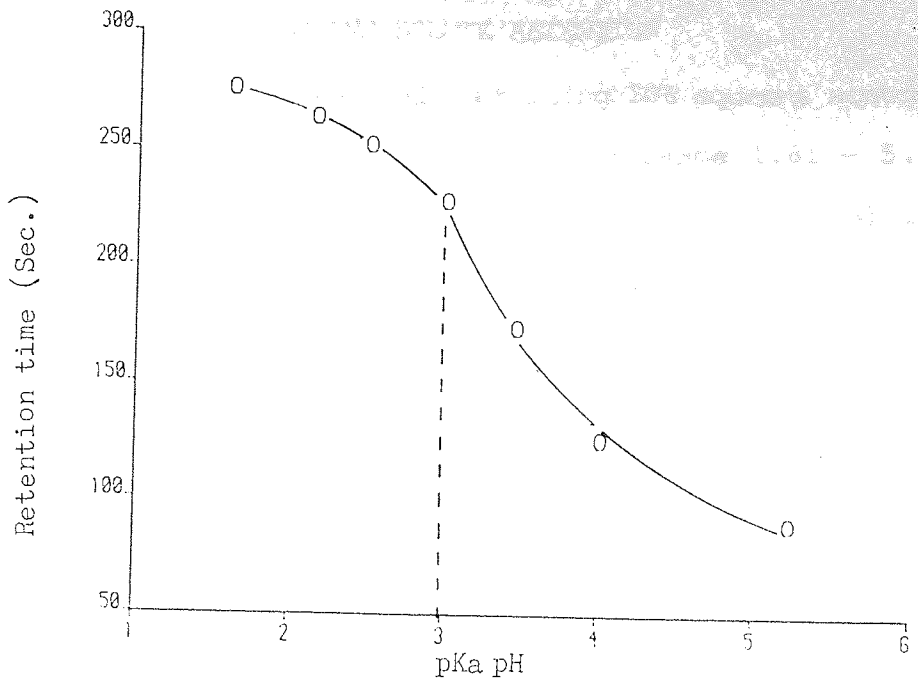


Fig. 2.5. Effect of Eluent pH on the Retention Time of Salicylic Acid :

Eluent : 30% CH_3CN in aq.phosphoric acid
Flow rate : 1 ml/min.

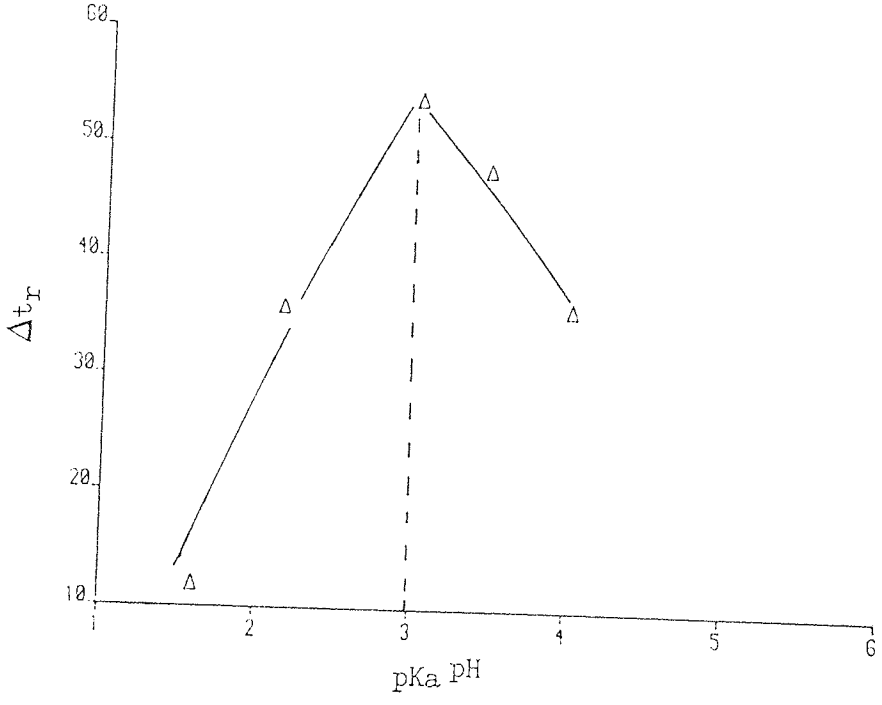


Fig. 2.6. Δt_r Vs. pH Plot for the Elution of Salicylic Acid

2.3.3 MOBILE PHASE pH AND SOLUTE RETENTION

Mobile phases were prepared containing 30% aqueous acetonitrile and the pH values were adjusted over the range 1.61 - 5.23; using orthophosphoric acid. Salicylic acid solution (1 mM) was analysed after equilibration of the HPLC system with the mobile phases. The retention time plot as a function of mobile phase pH is shown in Fig. 2.5. The plot is characterised by a sigmoidal appearance. Within the pH range 1.61 - 2.5, the retention time is almost independent of pH. With further increase in pH the elution becomes more rapid and finally the acid is washed off the column almost with the solvent front. This data gives an estimation of the pKa of salicylic acid in 30% acetonitrile, when $\triangle t_r$ (difference in retention time of salicylic acid at two successive pH values) is plotted as a function of pH, as shown in Fig.2.6. From this study the estimated value for the pKa is 3.0. At this pH value the peak width and the height equivalent pass through a maximum. Due to the high pKa values of the salicylate esters (10.5), the mobile phase pH, which should not exceed ~7.4 in reversed phase HPLC, virtually does not affect their retention.

Considerable difficulty was encountered during the development of a separation of methyl and ethyl nicotinate due to the tailing of the peaks causing poor resolution. A series of mobile phases was prepared containing 40% acetonitrile in water, and pH values were adjusted within the range 1.8 - 6.8 using orthophosphoric acid, dilute ammonia solution and tetramethyl ammonium hydroxide. The chromatograms produced in various mobile phases are displayed in

Fig. 2.7. Effect of Solvent Composition on the Chromatography of Nicotinates.

Flow rate : 1 ml/min.

Solute conc. : 1 mM each in dist. water

Detection : 0.32 AUFS , at 235 nm.

Peaks : a = methyl nicotinate, b= ethyl nicotinate

Column : 10 cm X 4.6 mm, Hypersil- ODS

Composition of Mobile Phases :

Chromatograms :

A = CH_3CN : H_2O:H_3PO_4(88%) :: 40:60:0.2; pH 1.8

B = CH_3CN :H_2O:H_3PO_4(44%):NH_3 solution(10%) ::40:60:0.01:0.1(v/v/v); pH 6.8

C = CH_3CN :H_2O:H_3PO_4:TMAH(12% w/v)::40:60:1:0.5(v/v/v/w);pH 1.8

D = CH_3CN :H_2O:H_3PO_4:TMAH::40:60:0.1:0.05(v/v/v/w); pH 3.0

E = CH_3CN :H_2O:H_3PO_4:TMAH::40:60:0.05:0.5(v/v/v/w); pH 6.8

TMAH = Tetramethyl ammonium hydroxide

Fig. 2.7. Adjustment of the pH with phosphoric acid does not make a significant change to the chromatography. Dramatic improvement occured, however, when the pH was adjusted to pH 6 with dilute phosphoric acid and dilute ammonia solution. However, with tetramethyl ammonium hydroxide in the mobile phase, it was possible to achieve good chromatography and resolution even at lower pH values. The surface silanol groups which remain unbounded are likely to interact with the basic nicotinates and this is a possible reason for peak broadening and tailing. Probably the dramatic change in chromatography, as observed with the tetramethyl ammonium hydroxide in the mobile phase, is due to the blocking of the unreacted surface silanol groups of the support.

This study enables the selection of the optimum composition of the mobile phase in which all components could be analysed within 2 - 5 minnutes. A mobile phase containing 3Ø - 45% of acetonitrile is suitable for the analysis of methyl salicylate together with the degradation product - salicylic acid. For the higher homologues the retention times are rather high. Although adjacent peaks are well resolved, they are very broad and not suitable for routine analyses. In contrast, with concentrations of 6Ø - 75% acetonitrile in the mobile phase, the lower homologues - methyl and ethyl salicylates overlap.This mobile phase range may be useful for the analysis of longer chain salicylate esters but for the current analysis, concentrations of 5Ø - 55% acetonitrile in the mobile phase were found to be the best.At this level the chromatograms are satisfactory with respect to retention time, peak width and resolution.

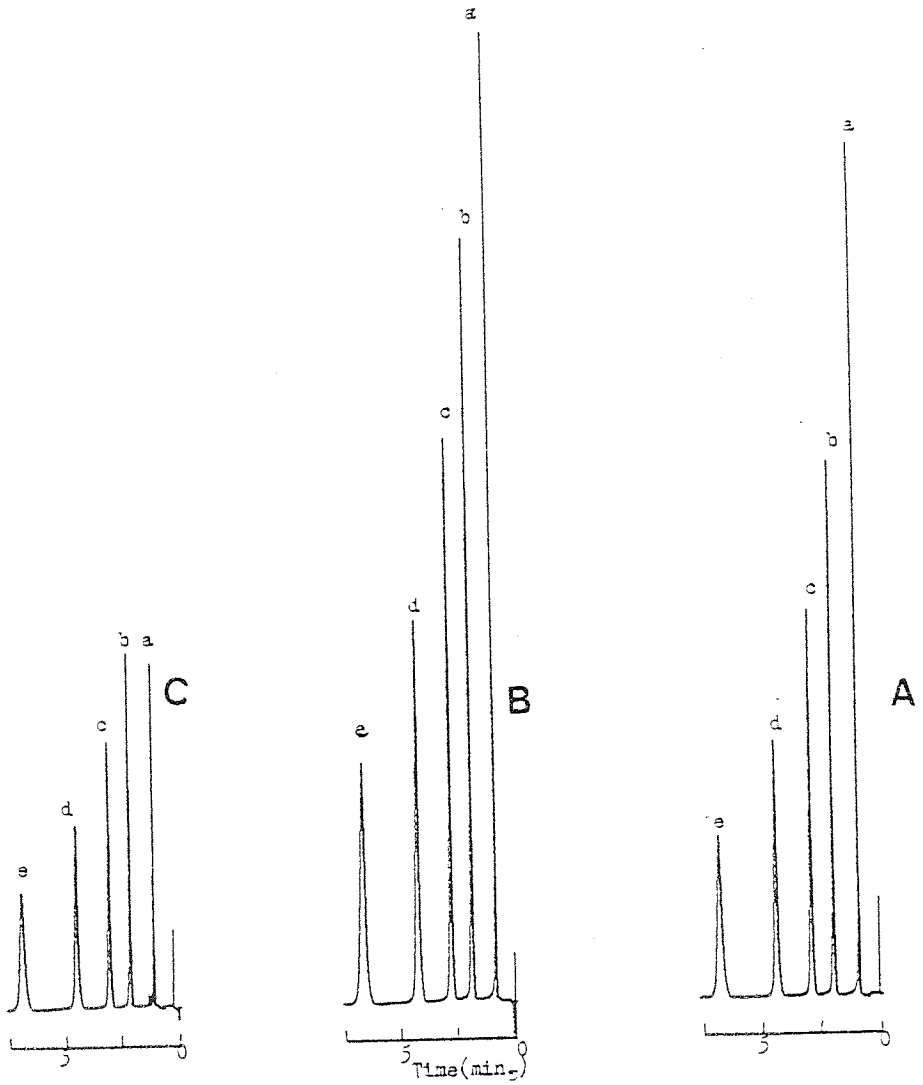


Fig. 2.8. Comparative Chromatograms Showing the Effect of Analytical Wavelength on the Peak Height of Salicylates.

Mobile phase : CH_3CN : Orthophosphoric acid (88%)
: Water::50:0.02:49.8,v/v, pH ~ 2

Flow rate : 1 ml/min.
Column : 10 cm X 4.6 mm, Hypersil - ODS
Detection : 0.64 AUFS

SA : Salicylic Acid

Chromatograms :

Wave length nm	Peaks , Salicylates				
	a	b	c	d	e
A = 225 B = 235 C = 245	SA	Me	Et	Pr	Bu

2.4. SEARCH FOR AN ANALYTICAL WAVELENGTH

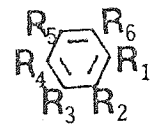
A salicylate mixture (1 mM each of salicylic acid, methyl, ethyl, n-propyl and n-butyl salicylate in 50% acetonitrile - water) was prepared. The solutes were eluted with a mobile phase containing 50% acetonitrile in water at pH 2.0, using a range of detection from wavelengths 225 - 245 nm. Wavelengths were incremented by 2 nm with a detector sensitivity of 0.64 AUFS. The chromatograms are shown in Fig. 2.8. The relative peak heights were measured and are presented in Table 2.4. These peak height data are plotted against wavelength and are shown in Fig.2.9.

Table 2.4. Effect Of Analytical Wavelength On Peak Height.

Wavelength (nm)	Peak height (mm), Salicylate				
	Salicylic acid	Methyl	Ethyl	Propyl	Butyl
225	123	82	66	50	36
227	128	87	71	54	38
229	132	95	78	59	42
231	133	101	83	64	46
233	132	112	92	70	51
235	127	124	102	78	56
237	114	133	109	84	60
239	90	138	114	87	63
241		139	114	87	63
243		134	111	86	62
245		118	98	76	56

The study shows that among the five solutes, butyl salicylate is the least polar and hence is eluted as a broad peak compared to the

Table 2.5. Retention Time and Relative Peak Height of Some Phenolic Compounds. 10 mg/250 ml Aqueous Acetonitrile

% CH_3CN in mobile Phase		Chemical structure R_1	R_2	R_3	R_4	R_5	R_6	t_r min.	pk.ht mm
30% CH_3CN	p-amino benzoic acid	COOH	H	H	NH_2	H	H	1.6	41
	p-OH-benzoic acid	COOH	H	H	OH	H	H	1.6	139
	m-OH-benzoic acid	COOH	H	OH	H	H	H	1.7	123
	Vanillin	H	H	CHO	H	MeO	OH	2.2	90
	Phenacetin	OH	H	H	NHAC	H	H	3.4	123
	Salicylic Acid	COOH	OH	H	H	H	H	4.4	47
40% CH_3CN	Methyl paraben	$COOCH_3$	H	H	OH	H	H	2.2	88
	4-Cl-m-Cresol	OH	H	CH_3	Cl	H	H	2.2	2
	2,4,6,tri-Cl Phenol	OH	Cl	H	Cl	H	Cl	2.3	13
	Ethyl paraben	COOEt	H	H	OH	H	H	3.1	61
	Propyl Paraben	COOPr	H	H	OH	H	H	4.8	35
	Butyl Paraben	COOBu	H	H	OH	H	H	8.4	19
60% CH_3CN	n-Pr 3,4,5-tri-OH benzoate	COOPr	H	OH	OH	OH	H	1.2	72
	3,5,di-OH-toluene (orcinol)	Me	H	OH	H	OH	H	1.2	62
	m-toluic acid	COOH	H	Me	H	H	H	1.6	121
	4-Cl-3,5-Xylenol	OH	H	Me	Cl	Me	H	2.5	8
	Thymol	H	i-Pr	OH	Me	H	H	3.1	5

equimolar concentration of the lower homologues and salicylic acid. The peak height of butyl salicylate maximises at 239 -241 nm but that of salicylic acid is reduced significantly at those wavelengths. Preference was given to 235 nm. This is the point at which the methyl salicylate and salicylic acid curves intersect and this wavelength was chosen as the analytical wavelength for subsequent analyses.

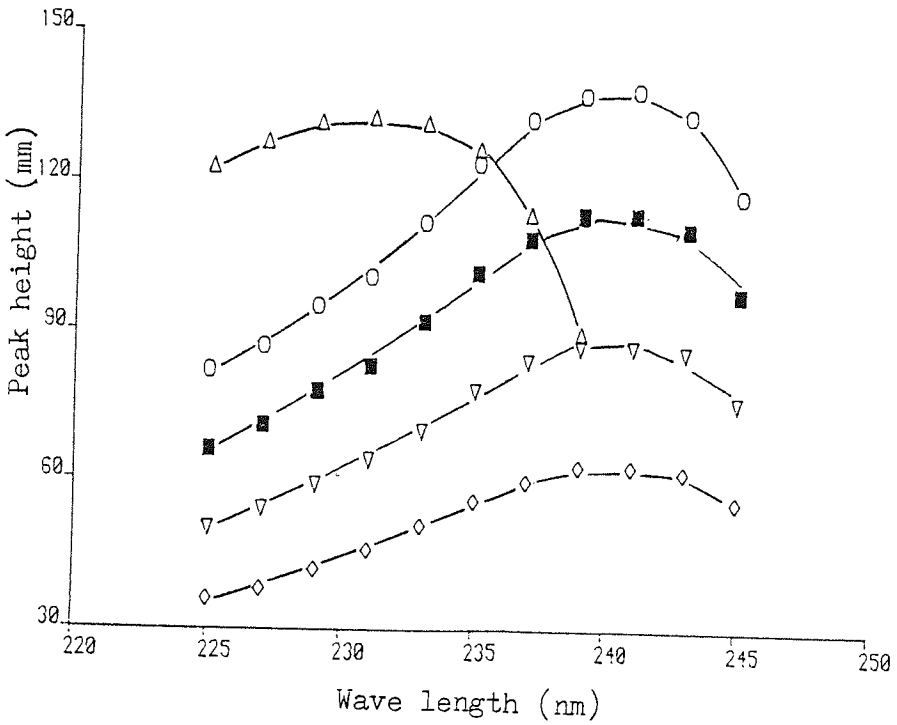


Fig. 2.9. Effect of Wavelength on Peak Heights ; conc. of the Solution, 1 mM each in 50% CH_3CN - H_2O :

Symbol	Δ	○	■	▽	◊
Salicylate	SA	Me	Et	Pr	Bu

SA = Salicylic Acid

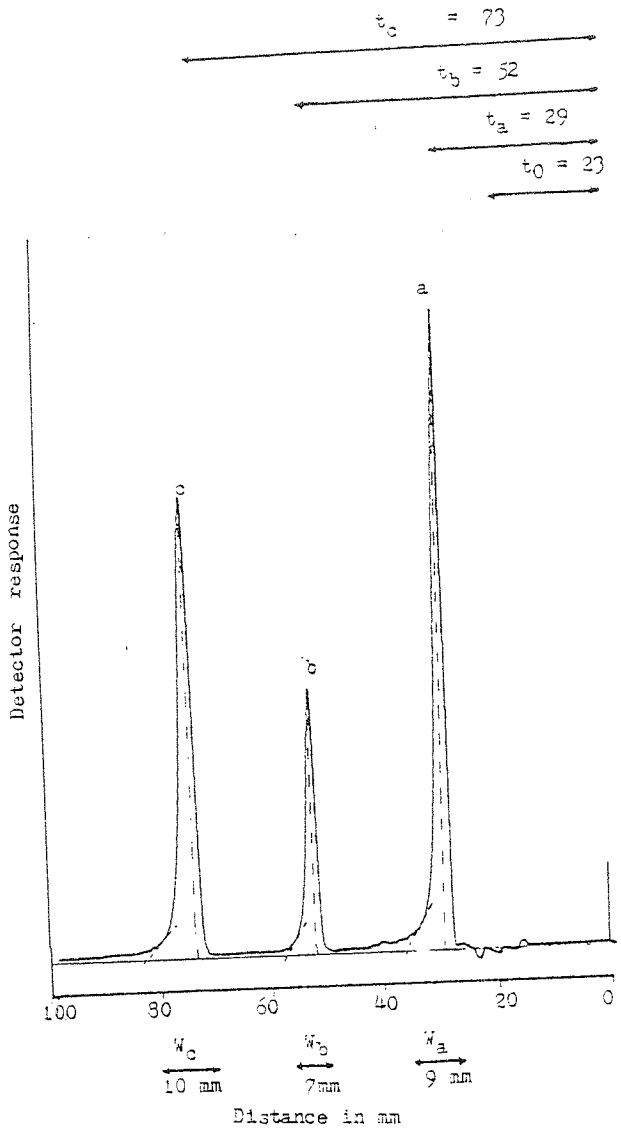


Fig. 2.10. Measurement of Resolution , R_S ,

Peak	Compounds	$R_S = \frac{2(t_{ii} - t_i)}{w_{ii} + w_i}$	R_S
b-a	Propyl paraben salicylic acid	$\frac{2(52 - 29)\text{mm}}{(7 + 9)\text{ mm}}$	2.88
c-b	methyl salicylate propyl paraben	$\frac{2(73 - 52)\text{mm}}{(10+7)\text{mm}}$	2.47

Column : 10 cm X 4.6 mm i.d.; packing: 5 μm Hypersil ODS

Eluent : CH_3CN:88% orthophosphoric acid:H_2O(52: 0.2:47.8, v/v/v)

Detection : UV, at 235 nm, 0.64 AUFS

Chart speed : 20 mm/min.

Flow rate : 1 ml/min.

2.5. QUANTITATIVE ANALYSIS

The analytes may be quantified assuming that the peak heights are proportional to the solute concentration. Although the loop valve injector provides a reproducible method for introducing sample onto the column, this technique is susceptible to errors introduced by the fluctuations in column performance resulting in variations in peak height. Quantification may be improved by incorporating into the sample a known concentration of a pure stable material, which may act as an internal standard. The ratio of the peak heights (analyte/internal standard) would then be directly proportional to the concentration of the analyte.

A series of phenol derivatives were dissolved in acetonitrile - water (30% v/v, 10 mg /250 ml). The structures of these compounds are recorded in Table 2.5. Depending on the polarity of the compound, they were analysed individually by three preselected mobile phases containing 30%, 40% and 60% acetonitrile. The respective retention times and the relative peak heights are shown in Table 2.5.

The preliminary search showed that propyl p-hydroxy benzoate is a satisfactory internal standard for the salicylic acid and methyl salicylate combination. A mobile phase containing 52% acetonitrile was found to be satisfactory and the three component mixture (1 mM each of salicylic acid and methyl salicylate and 0.5 mM propyl p - hydroxy benzoate) was eluted with the mobile phase. Resolution between the adjacent peaks was measured and are recorded in Fig. 2.10.

To minimise the overall error introduced by the difference in peak height between the reactant and the product at the beginning and at the end of the experiments, the peak height of the internal standard was maintained at about half of that of the reactant. A test chromatogram is shown in Fig. 2.11.

Table 2.6. Various Components and their Concentration In Calibration Solutions; Stock Solutions Are 1 mM Each; SA = Salicylic Acid, MS = Methyl Salicylate; ES = Ethyl Salicylate

Analyte in sample solution	% analyte			volume dispensed (ml)		
	Salicylic Acid			S.Acid	CH_3CN-water(10%v/v)	
Salicylic Acid	100			10	0	
	80			8	2	
	60			6	4	
	40			4	6	
	20			2	8	
methyl salicylate + salicylic acid	MS	SA		MS	SA	
	100	0		10	0	
	80	20		8	2	
	60	40		6	4	
	40	60		4	6	
	20	80		2	8	
	0	100		0	10	
Methyl salicylate + ethyl salicylate + salicylic acid	MS	ES	SA	MS	ES	SA
	100	0	0	10	0	0
	80	10	10	8	1	1
	60	20	20	6	2	2
	40	30	30	4	3	3
	20	40	40	2	4	4
	10	60	0	1	6	0
	0	80	0	0	8	0
	0	100	0	0	10	0
	0	0	60	0	0	0
	0	0	80	0	0	6

2.6. CALIBRATION OF INSTRUMENTAL RESPONSE

Stock solutions of salicylic acid, methyl and ethyl salicylates (10 mM) were diluted separately to 1 mM (10 ml to 100 ml) with acetonitrile - water (50% v/v , 90 ml) and were considered as 100% of the analyte. These solutions were rediluted with the solvent either independently or in combination to produce calibration solutions. In multi - component solutions care was taken not to exceed the final 1 mM concentration level for the additive concentration values. The combinations in the various calibration solutions are recorded in Table 2.6. Prior to injection of the solution onto the column, 2 ml of the internal standard (5 mg propyl paraben per 100 ml of 1% acetonitrile in water) were thoroughly mixed with 2 ml of the calibration solution.

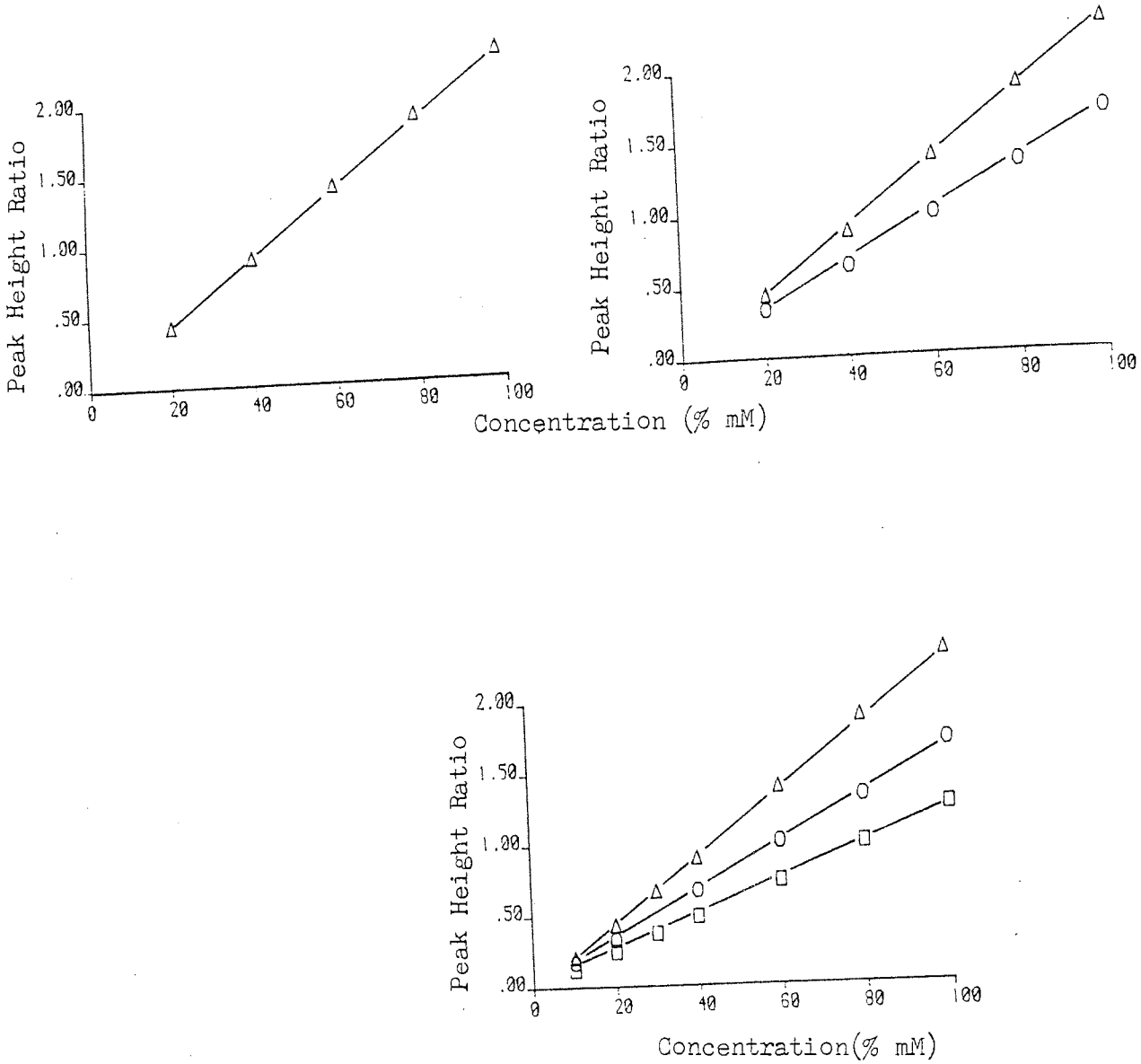


Fig. 2.12. Calibration Curve for Salicylic Acid, Methyl and Ethyl Salicylate in Single and Multicomponent Solutions.

Symbols	Species	Components
Δ	Salicylic Acid	A Single Component
O	Methyl Salicylate	B Double Component
□	Ethyl Salicylate	C Triple Component

The ratios of the peak heights (sample peak height/ internal standard peak height) were determined. The calibration curves were constructed by plotting the peak height ratios against concentration and are shown in Fig.2.12. The statistical parameters of the lines were calculated by the linear regression analysis of the peak height ratios against concentration and are recorded in Table 2.7.

Within the calibration concentration range column performance was reproducible with a linear relationship between the peak height ratio and concentration of the analytes.

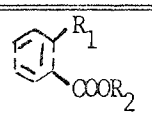
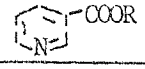
Table 2.7. Statistical Parameters For The Calibration Curves.

Component in solution	Statistical parameters		
	r	s	i
Salicylic Acid	Ø.9999	Ø.Ø236	Ø.Ø387
Salicylic Acid Methyl Salicylate	0.9996 Ø.9995	Ø.Ø235 Ø.Ø164	-Ø.Ø372 -Ø.Ø193
Salicylic Acid Methyl Salicylate Ethyl Salicylate	Ø.9999 Ø.9996 Ø.9999	Ø.Ø235 Ø.Ø166 Ø.Ø123	-Ø.Ø34Ø -Ø.ØØØ7 -Ø.ØØ45

where, r = correlation coefficient of the regression line
s = slope of the regression line
i = intercept of the regression line.

CHAPTER 3 EXPERIMENTAL DETAILS

Table 3.1. Some Physical Properties of Investigated Drugs.

Compound			m.wt.	Solubility in water	Reference
	R_1	R_2	SALICYLATES		
Salicylic acid	H	H	138.12	1:460	3
Me	H	CH_3	152.14	1:1500	88
Et	H	CH_2CH_3	166.17	1:3448	30
n-Pr	H	$(CH_2)_2CH_3$	180.20	1:11111	30
n-Bu	H	$(CH_2)_3CH_3$	194.22	-	-
Ph	H	C_6H_5	214.21	1:6670	19
Aspirin	CH_3COO	H	180.15	1:300	6
Me o-acetoxy	CH_3COO	CH_3	194.00	very sparingly	193
o-Acetyloxy phenyl benzoate	CH_3COO	C_6H_5	256.25	insoluble	193
o-Acetoxysal-icyloyl ethyl carbonate	CH_3COO	$C-O-C_2H_5$	252.00	-	-
	R		NICOTINATES		
Me	CH_3		137.13	soluble	8
Et	CH_2CH_3		151.15	soluble	8

3.1. DEGRADATION OF SALICYLATES IN AQUEOUS SODIUM HYDROXIDE

Salicylic acid (27.6 mg), methyl salicylate (30.4 mg) and ethyl salicylate (33.2 mg) were dissolved separately in distilled water to provide stock solutions (100 ml, 2mM each). Sodium hydroxide stock solution (0.1 M) was prepared by dilution of volumetric solutions (50 ml, 1 M) with distilled water to a final volume of 500 ml. Reactions were initiated by mixing the preheated stock solution (37^{o}C) of the ester (50 ml) with distilled water (40 ml) followed by preheated sodium hydroxide stock solution (10 ml, 0.1 M). Hydrolysis of propyl salicylate in aqueous alkali was carried out by introducing the ester (36 mg) directly into the reaction vessel and adding preheated sodium hydroxide solution (0.01 M, 200 ml). For dissolution of the drug, the reaction mixture was vigorously shaken for few seconds. Aliquots (3 ml) of the samples were withdrawn at various intervals over a period of 2-4 hours depending upon the rate of the reaction. The samples after withdrawal were delivered into chilled tubes kept in a salt ice bath (at~-15^{o}C). Aliquots (2 ml) of the withdrawn samples were pipetted into an acidic solution of internal standard (2 ml, 7 mg % propyl paraben in 10 mM HCl) and stored in the ice bath until required for analysis. The HPLC system for analysis is recorded in Tab. 3.11.

Standard solutions (1mM) of salicylic acid, methyl, ethyl and propyl salicylates were prepared separately in aqueous acetonitrile (10% v/v). Calibration curves were constructed for each ester together with salicylic acid as the degradation product. Standard solutions

Table 3.1. (Contd.)

Compound	Structure	m.wt.	Solubility in water	Reference
HO⟨⟩COOR	R	PARABENS		
p-OH benzoic acid	H	138.12	1:125	3 193
Me	CH_3	152.14	1:400	6
Et	CH_2CH_3	151.00	1:1400	193
n-Pr	$(CH_2)_2CH_3$	166.17	1:2000	193
n-Bu	$(CH_2)_3CH_3$	194.22	1:6500	193

Table 3.2 Composition of Two Component Calibration Solutions.

% ester	% acid	1mM ester (ml)	1 mM acid (ml)	conc.of ester (mM)	conc.of acid (mM)
100	-	10	-	1.0	-
80	20	8	2	0.8	0.2
60	40	6	4	0.6	0.4
40	60	4	6	0.4	0.6
20	80	2	8	0.2	0.8
-	100	-	10	-	1.0

in definite proportions were mixed with each other to prepare calibration solutions within the concentration range Ø.2mM - 1mM (2Ø% - 1ØØ%) for each component. A typical combination is shown in Tab.3.2.

3.2. DEGRADATION OF SALICYLATES IN ALKALINE HYDRO-ALCOHOLIC MEDIA WHERE THE ALKYL GROUP IN ESTER IS IDENTICAL TO THAT OF THE ALCOHOL

Methyl salicylate (152.1 mg), ethyl salicylate (166.1 mg), and propyl salicylate (18Ø.2 mg) were dissolved separately in the corresponding alcohol to prepare stock solutions (1ØØ ml, 1Ø mM). 138.1 mg quantities of salicylic acid were dissolved separately in methanol, ethanol, and n-propanol for stock solution (1ØØ ml, 1Ø mM) in each case. Aliquots (9Ø ml) of alkaline hydro-alcoholic media to provide alcohol concentrations in the range 1Ø - 9Ø% (v/v) in a final volume of 1ØØ ml were prepared by dispensing sodium hydroxide solution (1Ø ml, Ø.1 M), the corresponding alcohol (Ø - 8Ø ml) and distilled water (8Ø - Ø ml) into the reaction vessels. The reaction media were thermally equilibrated at 37°C in a thermostated water bath. Reactions were initiated adding 1Ø ml aliquots of the stock solution of the corresponding ester to each of the reaction vessels. The contents were thoroughly mixed by vigorous shaking for a few seconds. To prevent evaporation, the reaction vessels were sealed with Quick Fit adaptors accommodating syringe needles for sampling.

Table 3.3. Composition of The Reaction Media (100%=90ml) to Investigate The Effect of Solvent Dielectric Constant on The Degradation of Salicylates

reactant and reaction medium	% alcohol in a medium	alcohol required (ml) (10% contribution from the reactant)	0.1 M NaOH (ml)	reactant in alcohol(ml) (10mM)	dist. water(ml) to 90 ml
methyl salicylate in methanol water	10	-	10	10	80
	20	10	10	10	70
	30	20	10	10	60
	40	30	10	10	50
	50	40	10	10	40
	60	50	10	10	30
	70	60	10	10	20
	80	70	10	10	10
	90	80	10	10	-

The initial concentration of the reactant was 1 mM and that of the sodium hydroxide was 10 mM. Aliquots(3 ml) of the sample were withdrawn immediately and at various intervals over a period of 2 - 12 hours depending on the rate of the reaction. Degradation processes were instantly quenched as described in Section 3.1.2. A typical composition of reaction mixture is shown in Table 3.3. Samples were analysed by HPLC as shown in Tab.3.11.

3.2.1. EFFECT OF INITIAL CONCENTRATION OF THE REACTANT ON DEGRADATION RATE

10 ml aliquots of methyl salicylate solution in methanol (10 mM and 5 mM) were added to 90 ml portions of preheated reaction media containing sodium hydroxide (10 ml, 0.1 M) and distilled water (80 ml). Reactions were carried out at 37^{o}C, as described in section 3.2.

3.2.2. EFFECT OF TEMPERATURE ON DEGRADATION OF METHYL SALICYLATE

3.2.3.1. NON - ISOTHERMAL DEGRADATION

The reaction medium(90ml), consisting of methanol (40 ml), distilled water (40 ml) and sodium hydroxide (0.1 M, 10 ml) were thermally equilibrated at 25^{o}C in thermostated water bath, the heater was capable of increasing the temperature to 80^{o}C in 2 hours. The reaction vessel was equipped with a magnetic stirrer for continuous stirring, a syringe needle for sampling and a mercury thermometer (graduated to 0.1^{o}C) for recording temperature. Stock solution of

Table 3.4. Experiments to Investigate the Transesterification of Salicylates.

Purpose	Reactant and Reaction medium	initial Conc.(mM)	Reaction parameter	
			variable	range
Evaluation of the effect of dielectric constant of the solvent on the specific rate constants	methyl salicylate in alkaline (10 mM NaOH) ethanol at 37°C.	1	alcohol conc.	10 - 90%
	ethyl salicylate in alkaline (10 mM NaOH) methanol at 37°C.	1	alcohol conc.	10 - 90%
	propyl salicylate in alkaline (10 mM NaOH) methanol at 37°C	1	alcohol conc.	10 - 90%
	propyl salicylate in alkaline (10 mM NaOH) ethanol, at 37°C.	1	alcohol conc.	10 - 90%
	methyl salicylate in alkaline (100mM NaOH) propanol(40/60,v/v) at 37°C.	0.13	—	—
Dependency of the kinetic model on the reaction medium	methyl salicylate in 50% equimolar methanol -ethanol in 10mM sodium hydroxide at37°C.	1	—	—
	ethyl salicylate in 50% equimolar methanol -ethanol in 10mM sodium hydroxide at37°C.	1	—	—
Dependency of the specific rate constants on the concentration of the alkali	methyl salicylate in alkaline(variable)50% equimolar methanol/ ethanol at 37°C.	1	[NaOH] μ = 2 M	0.01-0.7M

methyl salicylate (1Ø ml, 1Ø mM) was delivered to the reaction vessel and immediately the water bath heater was turned on. Aliquots of the sample (3ml) were withdrawn and stored in a salt ice bath after 1:1 dilution (2 ml to 2 ml) with the acidic solution of the internal standard (7 mg propyl paraben/1ØØ ml of Ø.ØlM HCl). Samples were withdrawn at intervals of 2-5 minutes over a period of about 2 hours. HPLC system for analysis is shown in Tab. 3.11.

3.2.3.2. ISOTHERMAL DEGRADATION

Hydrolyses of methyl salicylate in aqueous methanol (50% v/v) were carried out under various isothermal conditions. Reaction solutions and the samples were prepared as described for the non-isothermal experiment (Section 3.2.3.1.).

3.3. DEGRADATION OF SALICYLATES IN ALKALINE HYDRO-ALCOHOLIC MEDIA WHERE THE ESTER GROUP DIFFERS FROM THAT OF THE ALCOHOL

3.3.1. TRANSESTERIFICATION OF SALICYLATES (METHYL, ETHYL AND PROPYL)

As described previously (Section 3.2.1.) the reactions were initiated by adding the stock solution of the ester (1Ø ml, 1Ø mM) to the preheated reaction medium (9Ø ml). The initial concentration of the reactant was 1 mM in 1Ø mM sodium hydroxide solution (unless otherwise stated). At appropriate time intervals, samples were withdrawn, treated with acidic internal standard (2 ml to 2ml; as shown in Tab.3.11) and stored in a salt ice bath until analysed.

Table 3.4 (Contd.)

Purpose	Reactant and Reaction medium	initial Conc.(mM)	Reaction parameter	
			variable	range
Specific rate constants in buffered solution at controlled pH	methyl salicylate in equimolar methanol ethanol in Britton-Robinson buffer, 50	1	pH	12.246
Specific rate constant in variable ionic strength	methyl salicylate in buffered equimolar methanol:ethanol pH 8.36, at 37°C.	1	ionic strength	0.0993-1 M
Specific rate constants in variable salt concentration	methyl salicylate in buffered equimolar methanol:ethanol pH 8.36, at 50°C.	1	salt conc.	100% -25%

Table 3.5 Composition of Calibration Solution Containing The Reactant (Such as Methyl Salicylate), an Intermediate (Such as Ethyl Salicylate) and the Product (Salicylic Acid).

Reactant and reaction medium	%reactant	% intermediate	% product
methyl salicylate in ethanol 100% reactant = 1 mM.	100	-	-
	80	10	10
	60	20	20
	40	30	30
	20	40	40
	10	60	-
	-	80	-
	-	100	-
	-	-	60
	-	-	80
	-	-	100

A series of similar experiments were carried out and are summarised in Table 3.4.

The three component standard solutions containing a reactant (methyl,ethyl or propyl salicylate, depending upon the experiment), an intermediate ester (depending upon the alcohol in the reaction medium) and the product (salicylic acid) at the maximum concentration of 1mM, were prepared according to Table 3.5.

All standard solutions were prepared in distilled water containing an identical concentration of the corresponding alcohol to that of the analytical solution.

3.3.2. PREPARATION OF EQUIMOLAR METHANOL-ETHANOL MIXTURE

Methanol (64Ø gm, 2Ø mole) was thoroughly mixed with ethanol (92Øgm, 2Ø mole) to provide 1,56Ø gm of equimolar mixture of methanol and ethanol.

3.3.3. PREPARATION OF BUFFER SOLUTIONS

Britton - Robinsons' buffer solutions were prepared over the pH range 1.83 - 11.98 (μ =Ø.5M) (125) using the table in Appendix 2. The appropriate buffer solution (5Ø ml) was mixed with the equimolar methanol-ethanol mixture (4Ø ml) and the stock solution of the ester in equimolar alcohol (1Ø ml, 1Ø mM) to provide the experimental solution (1ØØ ml). The concentration of the buffer in those

Table 3.6. Ionic Strength of Diluted and Undiluted Buffer and of The Individual Components Calculated by IONSTREN - A BASIC Program .

pH	Buffer Composition, M/L							Ionic Strength(M/L)			
	Normal buffer concentration										
				NaOH							
	AcOH	H_3BO_3	H_3PO_4	AcOH	H_3BO_3	H_3PO_4	Σ	AcOH	H_3BO_3	H_3PO_4	Σ
8.350	-	-	0.025	-	-	0.047	-	-	-	0.071	-
8.351	0.025	-	-	0.025	-	-	-	.025	-	-	-
8.355	-	0.025	-	-	0.003	-	.075	=	0.004	=	.099
Diluted Buffer; dilution factor 4.											
8.348	-	-	0.006	-	-	0.012	-	-	-	0.018	-
8.349	0.006	-	-	0.006	-	-	-	.006	-	-	-
8.349	-	0.006	-	-	0.009	-	.019	-	.0009	-	.025

* Σ= total concentration

solutions was 50% of the original composition. The pH values of the experimental solutions were measured at the beginning and at the end of each run. The reactions were carried out at 50°C.

3.3.4. TRANSESTERIFICATION AT CONTROLLED pH AND VARIABLE IONIC STRENGTH

Stock solutions of buffer were prepared by dissolving double the quantities of buffer salt, sufficient to produce 2 litres of double strength buffer of pH 8.36, using the Britton-Robinson table composition (125) in Appendix 2. Dilution (1:1) of this stock solution with distilled water will produce normal strength buffer of ionic strength 0.0993 M. A set of three experimental buffer solutions were prepared by diluting aliquots of the buffer stock solution (50 ml) and appropriate quantities of potassium chloride (Appendix 2) with distilled water to provide 0.0993 M, 0.5 M and 1 M ionic strength in a final 100 ml solution. Solutions for transesterification studies were prepared by diluting aliquots of the corresponding buffer solution (50 ml) with equimolar alcohol (40 ml) followed by the solution of the ester (10 ml, 10mM). The reaction temperature was maintained at 50°C.

3.3.5. TRANSESTERIFICATION AT CONTROLLED pH AND VARIABLE CONCENTRATIONS OF BUFFER SALT

A BASIC computer program IONSTREN (ionic strength) was used to calculate the ionic strength of the diluted Britton-Robinson buffer (pH 8.36) and the individual components are recorded in Table 3.6.

Normal strength buffer solution was prepared by dilution (1:1) of the buffer stock solution. Diluted buffer solution was prepared by diluting 25 ml of the normal strength buffer, containing Ø.5557gm KCl, required to adjust the ionic strength to Ø.Ø993 M, with distilled water. Solutions for transesterification studies were prepared using aliquots of buffer solution (5Ø ml), equimolar alcohol (4Ø ml) and stock solution of ester in equimolar alcohol (1Ø ml, 1ØmM). Reactions were carried out at 5Ø^{O}C and the samples were analysed by HPLC as recorded in Table 3.11

3.3.6. EFFECT OF STRUCTURE ON TRANSESTERIFICATION

3.3.6.1. TRANSESTERIFICATION OF METHYL p -HYDROXY BENZOATE

Transesterification was carried out with a solution containing methyl p-hydroxy benzoate (1 mM) in alkaline (Ø.5M NaOH) equimolar methanol-ethanol (5Ø% v/v) at 8Ø^{O}C. Experimental details are described in Section 3.3.1.

3.3.6.2. TRANSESTERIFICATION OF METHYL o-ANISATE

A solution of methyl o-anisate (1 mM, 1ØØml) was prepared from a stock solution of the ester (1Ø mM, 1Ø ml; dissolving 41.5 mg ester in 25 ml equimolar methanol-ethanol mixture) in Ø.5 M sodium hydroxide. The reaction was carried out at 3Ø^{O}C. The esters - methyl and ethyl o-anisates were synthesised from o-anisic acid according to the usual method of esterification (126).

3.3.7. TRANSESTERFICATION OF PHENYL SALICYLATE

3.3.7.1. EFFECT OF ETHANOL CONCENTRATION ON TRANSESTERIFICATION ØF PHENYL SALICYLATE

A stock solution of phenyl salicylate (1.24 mM,1ØØ ml) was prepared by dissolving the ester (26.5 mg) in ethanol . Reaction mixtures were prepared as described in Section 3.3.1 by diluting the stock solution (1Ø ml) with alkaline (Ø.Ø5 M) ethanol to provide 1ØØ ml solution (1Ø - 9Ø% ethanol in the final solution). Reactions were carried out at 35^{O}C.

3.3.7.2. EFFECT OF TEMPERATURE ON TRANSESTERIFICATION OF PHENYL SALICYLATE

A stock solution of phenyl salicylate (3.1 mM, 25ml) was prepared by dissolving the ester (16.6 mg) in ethanol. The stock solution (2ml) was diluted with alkali (48 ml, Ø.Ø5M) to provide the reaction mixture (5Ø ml). Reactions were carried out at various temperature (25^{O} - 55^{O}C) in increments of 5^{O}-$1Ø^{O}$C.

3.3.7.3. EFFECT OF ALKALI CONCENTRATION ON TRANSESTERIFICATION OF PHENYL SALICYLATE

Aliquots of stock solution of the ester (2 ml, 3.1 mM) were delivered to the reaction vessels containing various concentrations of alkali (48 ml, Ø.Ø1-Ø.7M; μ=2M) to prepare reaction mixtures (5Ø ml). Reactions were carried out at 35^{O}C as described in Section 3.3.1. Samples were analysed by HPLC.

3.3.7.4. EFFECT OF INITIAL CONCENTRATION OF PHENYL SALICYLATE ON TRANSESTERIFICATION

Reaction mixtures were prepared by diluting the stock solution of phenyl salicylate (3.1 mM, 2ml in ethanol) with alkaline-ethanol (Ø.Ø5M NaOH ,8ml ethanol) to provide 5Ø ml of Ø.125 mM ester solution in 2Ø% ethanol. Another solution (Ø.62mM) was prepared by diluting 1Ø ml of the stock solution with alkali (Ø.Ø5 M, 4Ø ml).

3.3.8. TRANSESTERIFICATION OF NICOTINATES

3.3.8.1. TRANSESTERIFICATION OF METHYL NICOTINATE IN ETHANOL CATALYSED BY SODIUM HYDROXIDE

Methyl nicotinate stock solutions (2 ml, 5Ø mM) were delivered to the preheated solvents (1ØØ ml) containing alkali (Ø.Ø1 M, Ø.ØØ1 M and Ø.ØØØ5 M NaOH, 5Ø ml) and ethanol (5Ø ml). The reactions were carried out at 37^{O}C, as described earlier, (Section 3.3.1) and the samples were analysed by HPLC, Table 3.11.

3.3.8.2. TRANSESTERIFICATION OF METHYL NICOTINATE IN BUFFERED EQUIMOLAR METHANOL - ETHANOL MIXTURE

Methyl nicotinate (342.5 mg) was dissolved in distilled water to prepare stock solution (5Ø mM,5Ø ml). Double strength buffer solutions were prepared in the pH range 1.81 - 11.2 (125) such that a 1:1 dilution gave the published compositions with an ionic strength of Ø.5 M. The appropriate buffer solution (5Ø ml) and an equimolar mixed alcohol (methanol: ethanol, 32:46 w/w, 5Ø ml) were mixed together and equilibrated at 5Ø^{O}C. A freshly prepared stock

solution of methyl nicotinate in water (5Ø mM, 2 ml) was added to each reaction vessel and the temperature was maintained at 5Ø°C. Concentrations of the reactant (methyl nicotinate), the intermediate (ethyl nicotinate) and the product (nicotinic acid) were determined immediately and at suitable time intervals by HPLC; Aliquots (2ml) of the sample were withdrawn and quenched by addition to the cooled internal standard solution (2ml) consisting of ethyl 4-hydroxybenzoate in Ø.Ø1 M HCl (7.5 mg %). Sample (2Ø µl) were injected onto the column and concentrations were calculated from a calibration curve covering the concentration range Ø.Ø2-Ø.2 mM for the ethyl ester and Ø.1 - 1 mM for other compounds.

3.3.8.3. TRANSESTERIFICATION OF METHYL NICOTINATE IN ETHANOL - CATALYSED BY 4-DIMETHYLAMINOPYRIDINE

Experimental solutions were prepared with methyl nicotinate (1 mM) containing 4-dimethylaminopyridine (Ø.Ø1 and Ø.Ø2 M) in aqueous ethanol (5Ø%, v/v ,1ØØ ml). Reactions were carried out at 37°C and analysed by HPLC after neutralisation with acidic (equivalent quantities of HCl) internal standard (ethyl paraben, Table 3.11).

3.3.9. TRANSESTERIFICATION BETWEEN SALICYLATE AND NICOTINATE

Methyl nicotinate (1%) and ethyl salicylate (1%) were prepared in acetonitrile (1ØØml) containing alkali (NaOH,1M,few drops). The solution was stored at 37°C. Another solution was prepared by

dissolving nicotinate (5.5 gm) and methyl salicylate (2.7 gm) in acetonitrile (50ml). The solution was diluted to 100 ml with sodium hydroxide (50 ml, 0.001M) solution to give a final pH of 10.57. The solutions were kept in water bath at 37°C.

The solution (1 ml) was diluted (200 ml) with acetonitrile-water (50% v/v) and was analysed by HPLC, Table 3.11.

A third solution was prepared containing methyl nicotinate (5 mM) and ethyl salicylate (2 mM) in acetonitrile (10%) in sodium hydroxide (0.01 M and 0.001 M). Reactions were carried out at various temperatures (20 - 37°C).

3.4. TOPICAL SALICYLATES AND NICOTINATES

3.4.1. ETHYL SALICYLATE GEL IN PROPYLENE GLYCOL

Ethyl salicylate (1 gm) propylene glycol (40 gm) and distilled water (5-7 ml), (Table 3.7) were weighed directly into a wide jar. The contents were mixed thoroughly to a clear solution and equilibrated at 37°C. Carbopol 934 (1 gm) was added to the jar in divided portions while stirring the contents with a magnetic stirrer. Stirring was continued for about 4-6 hours to provide a uniform thick gelling medium. Sodium hydroxide (2 M, 1-3 ml) was pipetted dropwise into the gelling medium and was stirred gently using a glass rod. The gels were stored at 37°C for testing stability. The pH of the gel was recorded by directly immersing an electrode in the

Table 3.7. Topical Salicylates and Nicotinates in Gels.

drug (gm)	adjunct (gm)	geling agent carbopol 934 (gm)	NaOH		dist.water freshly boiled and cooled (ml)	pH	
			Conc.(M)	vol (ml)		direct	diluted gel (10% in water
ETHYL SALICYLATE GEL IN PROPYLENE GLYCOL							
1	40	40	1	2	7	5.4	-
1	40	1	2	2	6	5.5	-
1	40	1	2	3	5	5.6	-
METHYL SALICYLATE GEL IN ETHYLENE GLYCOL							
0.25	40	1	2	1	8	5.4	-
0.25	40	1	2	2	7	5.28	-
0.25	40	1	2	3	6	5.45	-
0.25	40	1	1	8	8	6.66	-
0.25	40	1	5	2	8	8.06	-
METHYL SALICYLATE GEL IN PROPYLENE GLYCOL							
0.25	40	1	5	0.5	8.25	5.92	5.49
0.25	40	1	10	0.5	18.25	6.5	6.14
0.25	40	1	10	1.0	22.50	7.22	7.03
METHYL SALICYLATE AND ETHYL NICOTINATE IN ETHYLENE GLYCOL							
salicylate (gm)	nicotinate (gm)	adjunct (gm)					
1.35	2.25	40.5	1	5	1	4.13	6.55	5.86
1.35	2.25	40.0	1	5	1.5	4.13	7.86	7.41

gel for about 5 minutes. Alternatively, the gel (1 gm) was dissolved in distilled water (10 ml) and the pH of this dilution was taken.

For analysis, the gel (1 gm) was weighed into a wide vial, it was dispersed in acetonitrile (5 ml, Whirlimixer) and was transferred into glass centrifuge tube. Potassium chloride (300 mg) was added into the tube which was then centrifuged (2 minutes). The supernatant solution (2 ml) was diluted with acetonitrile (5 ml). The solution was further diluted (2 ml to 5 ml) with distilled water and was analysed by HPLC, Table 3.11.

The same procedure was followed in the preparation and analysis of other gels, recorded in Table 3.7.

3.4.2. SALOL AQUEOUS CREAM

Table 3.8. was used in preparing salol aqueous cream. Stearic acid (16 gm) was liquefied at 60^{o}C and wool fat (2 gm) was added to the liquefied fat. Phenyl salicylate (10 gm) was added to the melted fat.

The aqueous phase was prepared by dissolving triethanolamine (2 gm) in freshly boiled and cooled distilled water (65 ml). The aqueous phase was equilibrated at 60^{o}C.

Table 3.8 Composition of Salol Aqueous Cream (19)

Component	quantity in gm
Phenyl salicylate	10
Stearic acid	16
Wool fat	2
Triethanolamine	2
Propylene glycol	5
Distilled water freshly boiled and cooled	65

Propylene glycol (5 ml) was added to the fatty mixture and the aqueous phase was mixed thoroughly into the fatty mixture by trituration.

The cream was stored at 37°C for stability testing. The pH of the cream was recorded as described earlier (Section 3.4.1.) as in the case of gels. The cream was extracted in a manner analogous to that used for the gels and analysed by HPLC.

3.4.3. PREPARATION OF TOPICAL ANTIINFLAMMATORY PRODUCT

The components in Table 3.9 were dissolved in ethanol to prepare a topical antiinflammatory product(127). The solution was stored at 37°C. For analysis, the solution (1 ml) was diluted to 250 ml with acetonitrile-water (50% v/v) and was analysed by HPLC; Table 3.11.

3.4.4. PREPARATION OF METHYL SALICYLATE LINIMENT

Methyl salicylate liniment (82) was prepared using the composition in Table 3.10. Methyl salicylate (2.4 ml) was dissolved in iso-propyl alcohol (50 ml) and the solution was diluted with distilled water (22.4 ml). Soft soap BP (0.2gm) was added to the preparation. The pH of the liniment was recorded by direct immersion of the electrode. The liniment was stored at 37°C. For analysis, the liniment (1 ml) was diluted with acetonitrile-water (50% v/v, 250 ml) and was analysed by HPLC, Table 3.11.

3.4.5. ANALYSIS OF SURGICAL SPIRIT (BP)

Surgical spirit (BP)(128) was diluted with acetonitrile-water (50% v/v; 1 ml to 500 ml) and was analysed by HPLC, Table 3.11.

3.5. SYNTHESIS AND DEGRADATION OF ASPIRIN PRODRUGS

3.5.1. SYNTHESIS OF o-ACETYLOXY PHENYL BENZOATE

o-Acetyloxy phenyl benzoate was prepared according to the method described for the synthesis of phenyl acetate (129). Sodium hydroxide (60 gm, 1.5 mole) was dissolved in distilled water (100 ml). Phenyl salicylate (21 gm, 0.1 mole) was triturated with the alkali solution. Care was taken not to form solid lumps of the sodium salt of phenyl salicylate. The alkaline mixture was poured into crushed ice (~ 500 gm) and was stirred to mix completely. Acetic anhydride (128 gm, 1.25 mole) was quickly added to the chilled mixture and was vigorously stirred for a few secs. The o-acetyloxy phenyl benzoate was precipitated as a white paste which was collected by filtration and was washed several times with distilled water and crushed ice. The precipitate was dried in a vacuum desiccator for enough time. The crude product was dissolved in ether (50 ml) and pure ester was precipitated from the ethereal solution by addition of hexane.

3.5.2. SYNTHESIS OF o-ACETYL SALICYLOYL ETHYL CARBONATE

This prodrug was synthesised following the method described in the patent(130). Aspirin (9 gm, Ø.Ø5 mole) was dissolved in tetrahydrofuran (5Ø ml) and was cooled to 15°C in asalt ice bath. Triethylamine (5.Ø5 gm) was added to the reaction vessel. Ethyl chloroformate (5.43 gm) solution in tetrahydrofuran (2Ø ml) was added dropwise to the reaction vessel and the mixture was brought back to the room temperature. Agitation was carried out for 3 hours. The crude reaction product was filtered and the solvent (THF) was evaporated under vacuum (4 mm Hg). o-Acetoxy salicyloyl ethyl carbonate was collected as a colourless oily liquid and was stored in 1 ml ampoules.

3.5.3. DEGRADATION OF o-ACETYLOXY PHENYL BENZOATE

o-Acetyloxy phenyl benzoate (16 mg, Ø.Ø625 m moles) was dissolved in acetonitrile (25 ml) to provide a stock solution (2.5 mM) which was stored in an ice bath. Experimental solutions were prepared adding the stock solution (4 ml) to preheated (5Ø°C) normal strength Britton-Robinson buffer (1ØØ ml, μ =Ø.5M) within the pH range 1.8 - 11.2. Samples were withdrawn immediately and at various intervals over a period of 8 hours depending on the speed of the reaction in alkaline media. Samples from the acidic solutions were withdrawn less frequently because of their stability. The withdrawn samples (2 ml) were delivered to chilled acidic internal standard solution (2 ml, 1.5 mg% propyl paraben in Ø.ØØ5 M HCl) which reduced the pH of

Table 3.9. Composition of Topical Antiinflammatory Product (127)

Component	weight (gm)
Phenyl salicylate	5
Eugenol	0.25
Methyl salicylate	3
Propylene glycol	12
Ethanol	80

Table 3.10. Composition of Methyl Salicylate liniment (82)

Component	Concentration (ml)
Methyl salicylate	2.4
Isopropyl alcohol	50.0
Soft soap BP, instead of green soap concentrate	0.2 gm
Distilled water	22.4

the sample to 2. The collected samples were frozen and stored at -20°C until required for analysis.

3.5.4. IDENTIFICATION OF SALICYL SALICYLATE AS A DEGRADATION PRODUCT OF o-ACETYLOXY PHENYL BENZOATE

A solution of o-acetyloxy phenyl benzoate (1 ml, 10 mM) in acetonitrile was added to preheated (37°C) alkaline (0.01 M NaOH) acetonitrile (50% v/v, 50 ml), and the reaction was carried out at 37°C. The process was repeated in borate buffer (pH 7.55) with various initial concentrations of phenyl salicylate (0.1, 0.2 and 0.4 mM). Control experiments were carried out in alkaline (0.01 M NaOH) acetonitrile (50% v/v) containing phenyl salicylate, salicylic acid, aspirin and o-acetyloxy phenyl benzoate (0.2 mM each).

3.5.5. DEGRADATION OF BENORYLATE

Benorylate was precipitated from Tridol suspension. The dried solid was dissolved in buffered acetonitrile(10% v/v, pH10.14, μ=0.5M). Degradation was carried out at 45°C.

3.6. DETERMINATION OF pKa OF METHYL SALICYLATE IN AQUEOUS METHANOL(50% V/V), AQUEOUS ACETONITRILE (30% V/V) AND IN EQUIMOLAR METHANOL-ETHANOL MIXTURE IN WATER (50% V/V)

76 mg quantities of methyl salicylate were dissolved in aliquots (50 ml) of the solvents. Temperature was maintained at 37°C using a

Table 3.11 HPLC Conditions for the Analyses of Drugs Listed in Table 3.1. SA=salicylic acid, MS=methyl salicylate, ES=ethyl salicylate, PS=propyl salicylate, BS=butyl salicylate, PhS=phenyl salicylate

Solute Components	mobile phase	internal standard	approx.elution time(min)
MS + SA MS + ES + SA ES + SA	$CH_3CN:H_3PO_4:H_2O$ 52:0.2:47.8 pH 1.8–2	5–7 mg% propyl paraben in 0.01 M HCl	5 – 7
PS, when SA was washed off the column with the solvent front	$CH_3CN:H_3PO_4:NH_3:H_2O$ 60:0.01:0.1:39.9 pH 6.8	5 mg% thymol in 0.01 M HCl	5
PS + MS + SA PS + ES + SA	$CH_3CN:H_3PO_4:H_2O$ 54:0.2:45.8, pH 2	7 mg% propyl paraben or 5 mg% Cl-xylenol in 0.01 M HCl	6 – 8
PhS + ES + SA PhS + ES	$CH_3CN:H_3PO_4:H_2O$ 56:0.2:39.5, pH 2 $CH_3CN:H_3PO_4:NH_3:H_2O$ 60:0.01:0.1:39.9 pH 6.8	2 mg% butyl paraben	10 – 12 9 – 10
Et o-anisate, Me o-anisate & anisic acid in 0.5 M NaOH solution	$CH_3CN:H_3PO_4:H_2O$ 50:0.2:49.8 pH 1.8 – 2	9 mg/100ml butyl paraben in 0.5 M HCl	5
ethyl paraben, methyl paraben & p-OH-benzoic acid in 0.5 M NaOH	$CH_3CN:H_3PO_4:H_2O$ 35:0.2:39.8 pH 2	8 mg /100 ml propyl paraben in 0.5 M HCl	6
methyl, ethyl, propyl & butyl salicylate in distilled water	$CH_3CN:H_3PO_4:H_2O$ 60:0.2:39.8 pH 2	5 mg/100 ml ml propyl paraben in distilled water	6
Salol Aqueous Cream	$CH_3CN:H_3PO_4:H_2O$ 45:0.4:54.6 pH 1.7	no internal std.	17 min.
	$CH_3CN:H_3PO_4:H_2O$ 45:0.4:54.6 pH 2	no int.std.	15 min.for degradation products

jacketed vessel and allowing warm water through the jacket from a recirculating water bath. The solution was titrated with freshly prepared sodium hydroxide solution (Ø.5M in freshly boiled and cooled distilled water). pH was recorded after each addition of sodium hydroxide from a microburette.

3.7. HPLC SYSTEMS FOR THE ANALYSIS OF INVESTIGATED DRUGS

Analytical solutions containing alkali (sodium hydroxide) in hydro alcoholic media (1Ø-9Ø% alcohol) were analysed after neutralisation with an equivalent quantity of hydrochloric acid containing internal standard (2 ml to 2 ml).

Buffered solutions were diluted (2 ml to 4 ml prior to injection to prevent blockage of the column packing material by the buffer salts. The diluent was the internal standard solution in distilled water.

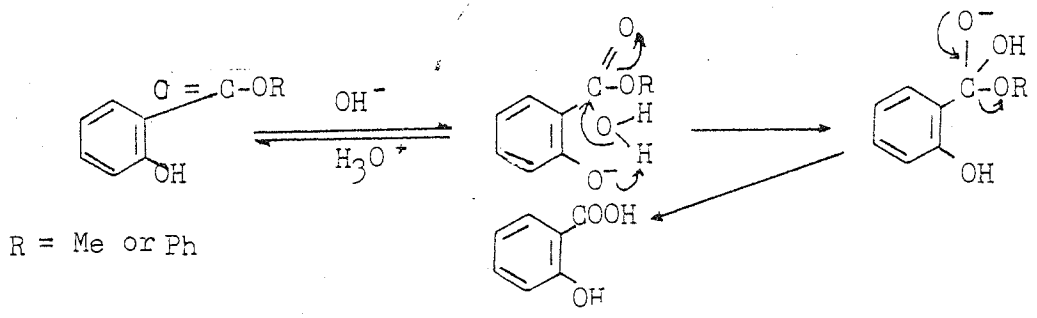
To prevent tailing in samples containing potassium chloride either the first analyte (salicylic acid) was washed off the column with the solvent front, using a mobile phase of pH 6.9 or the samples were diluted (2 ml to 5 ml) with distilled water containing the internal standard. Care was taken to maintain identical solvent composition for both the analytical and the calibration solvents.

Various compositions of the mobile phases which were used for the analyses of the experimental solutions are recorded in Table 3.11.

Table 3.11. (Contd.)

Solute Components	mobile phase	internal standard	approx.elution time(min)
salicylate gels	$CH_3CN:H_3PO_4:H_2O$ 45:0.04:54.6 pH 2	no int. std.	8 min.
	$CH_3CN:H_3PO_4:H_2O$ 25:0.1:74.9 pH 2		15 min.
methyl and ethyl nicotinates	$CH_3CN:H_3PO_4:NH_3:H_2O$ 40:0.0001:0.0025:60 pH 7	5 mg% ethyl paraben	4 min.
phenol, salicylic acid salicyl salicylic acid & phenyl salicylate	$CH_3CN:H_3PO_4:H_2O$ 54:1:45 pH 1.88	no int. std.	12 min.
o-acetyl phenyl benzoate+phenyl salicylate + aspirin + salicylic acid + phenol	$CH_3CN:H_3PO_4:H_2O$ 45:0.2:54.8, pH 1.8	propyl paraben 2 mg%	18
degradation products of Benorylate: p-acetamidophenyl salicylate+ Benorylate	$CH_3CN:H_3PO_4:NH_3:H_2O$ 30:0.005:0.5:70 pH 6.8	Phenacetin	6
aspirin, paracetamol and salicylic acid	$MeOH:H_3PO_4:H_2O$ 30:1:69; pH 1.6	phenacetin	16
o-acetoxysalicyloyl ethyl carbonate +ethyl carbonate salicylate + aspirin+salicylic acid	$CH_3CN:H_3PO_4:H_2O$ 45:0.5:54.5	no int. std.	8 - 9

CHAPTER 4 HYDROLYSIS OF ALKYL SALICYLATES IN AQUEOUS OR IN HYDROALCOHOLIC SOLUTION



R = Me or Ph

Scheme 4.1. Solvent attack on ionised ester

4.1. INTRODUCTION

The degradation of salicylates in aqueous solvents has been investigated by several researchers. Emphasis has been given to the determination of order and the mechanism of the degradation process (87,90,131). The temperature dependence and the solvent effect have also been subjects of interest to a number of investigators (87-89,131). The catalytic effect of acids and bases in addition to the effect of buffer salts have also been given due consideration (90). The investigators have agreed that the hydrolysis follows pseudo first order kinetics and have assumed two kinetically active forms of the ester. These are the ionized ester which may react with the solvent, as shown in Scheme 4.1., and the unionized ester which may react with hydroxide ion.

The dielectric constant of the solvent may influence the rate of reaction changing the stability of the intermediate tetrahedral anion in Scheme 4.1. This has been investigated (88-89). The ester-alcohol combinations described in the literature are variable and include methyl salicylate in n-propanol (88,132) and phenyl salicylate in ethanol (89-90,133). The degradation rates have been assessed by ultraviolet spectroscopy. This technique lacks specificity and is discussed (134) as a potential source of error.

4.2. ESTIMATION OF RATE OF DEGRADATION AND OTHER PARAMETERS

The degradation of salicylate esters in aqueous solutions or in hydroalcoholic solutions when the alcohol is the same as that of ehe ester function, follows first order kinetics such that :

$$A \xrightarrow{k} C \quad \text{.....equn.4.1.}$$

where, A represents the ester,

C represents salicylic acid and

k is the degradation rate constant.

The residual concentration of the ester A_t at any time t is dependent upon the initial concentration $A_\emptyset$ and is given by :

$$A_t = A_\emptyset \, .e^{-kt}$$

or, $$\ln A_t = \ln A_\emptyset - kt \text{.....equn.4.2.}$$

The Arrhenius parameters were determined from the Arrhenius equation:

$$\ln k = \ln A - E/RT \text{.....equn.4.3.}$$

where, k is the rate constant at temperature T

E is the energy of activation

R is the universal gas constant (8.3143 J $mol^{-1}K^{-1}$)

T is the temperature in degrees Kelvin

A is the frequency factor.

The least squares analysis required by equn.4.2. and 4.3. were performed automatically by a BASIC computer package WJIRATE.

The half life $t_{1/2}$ of the hydrolytic process was determined by :

$$t_{1/2} = \ln (2) / k = 0.693/k \quad \text{.....equn.4.4.}$$

The dielectric constants of the solvent compositions were calculated from :

$$\varepsilon_{\text{solvent}} = x\,\varepsilon_x + (1-x)\,\varepsilon_y \quad \text{.....equn.4.5}$$

where, x = mole fraction of the solvent component

ε_x and ε_y = dielectric constant of the pure solvents(136).

For example, the dielectric constant of aqueous methanol (90% v/v) was calculated as follows :

$$x = \frac{(90 \times 0.8435) / 32}{(90 \times 0.8435)/32 + (10 \times 1.0135)/18} = 0.8082$$

ε methanol-water = (0.8082 X 33.7) + (1- 0.8082) X 80

(90% v/v) = 42.5811.

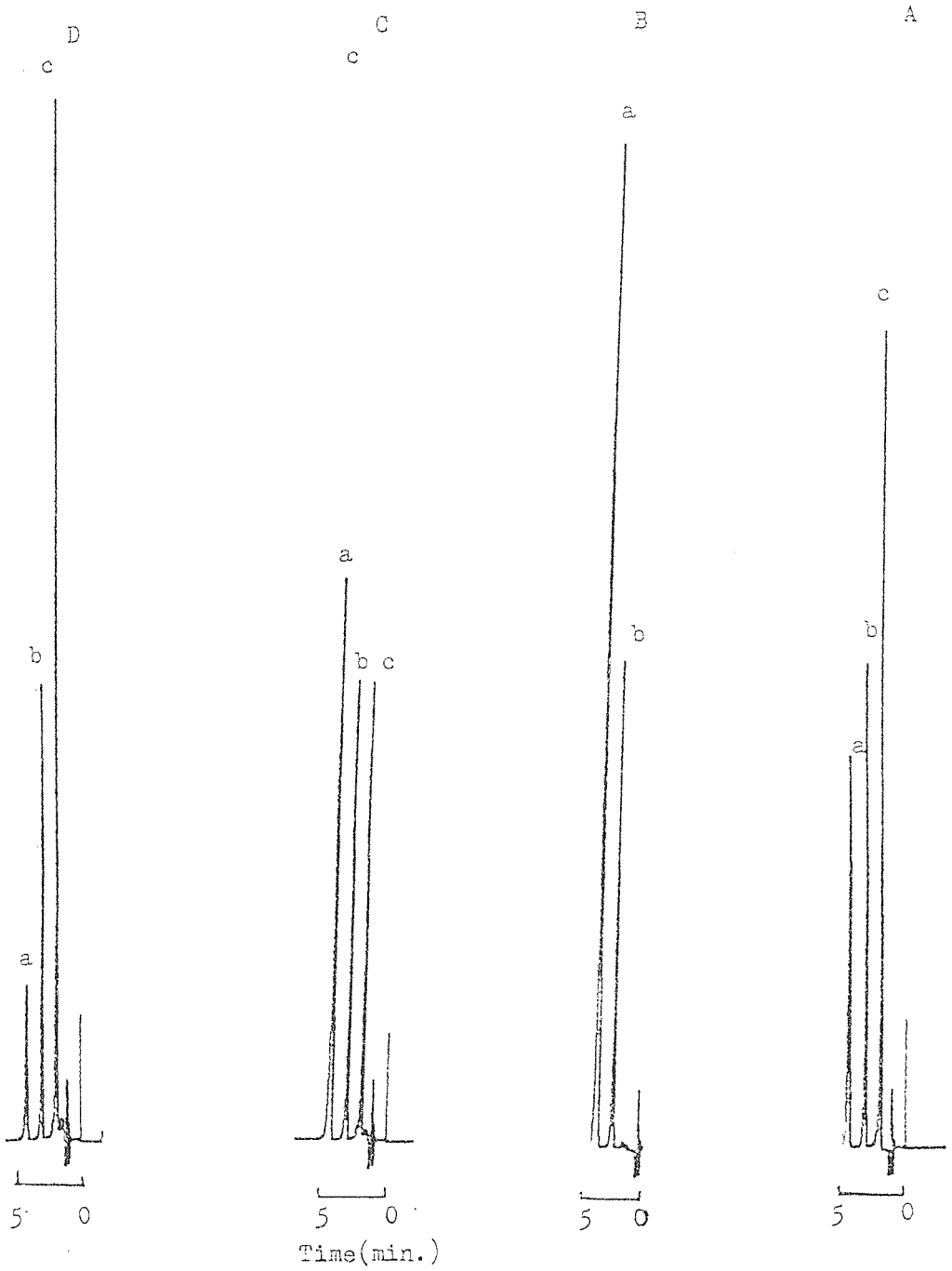


Fig. 4.1. High Performance Liquid Chromatograms Showing the Degradation of Methyl Salicylate (1 mM) in Methanol/H_2O (10% v/v); Temp. 37° C., NaOH = 0.01M; Detection : 0.64 AUFS at 235 nm; Solvent : CH_3CN : H_2O : H_3PO_4 :: 52 : 47.8 : 0.2, pH 1.8

Chromatogram	A	B	C	D
	'Std. Containing 60%c + 40% a	t = 0	t = 30min.	t= 120 min.

a	b	c
Methyl Salicylate	Propyl Paraben 5mg% int. Std.	Salicylic acid

4.3. RESULTS AND DISCUSSION

4.3.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

A series of high performance liquid chromatograms, showing the course of the reaction of methyl salicylate (1 mM) in alkaline (10 mM NaOH) methanol-water (10% v/v), together with a standard chromatogram is shown in Fig.4.1. The chromatograms reveal that under the experimental conditions, methyl salicylate gradually breaks down forming salicylic acid. It is also apparent in the figure that the column performance remains the same for both the experimental and the standard samples.

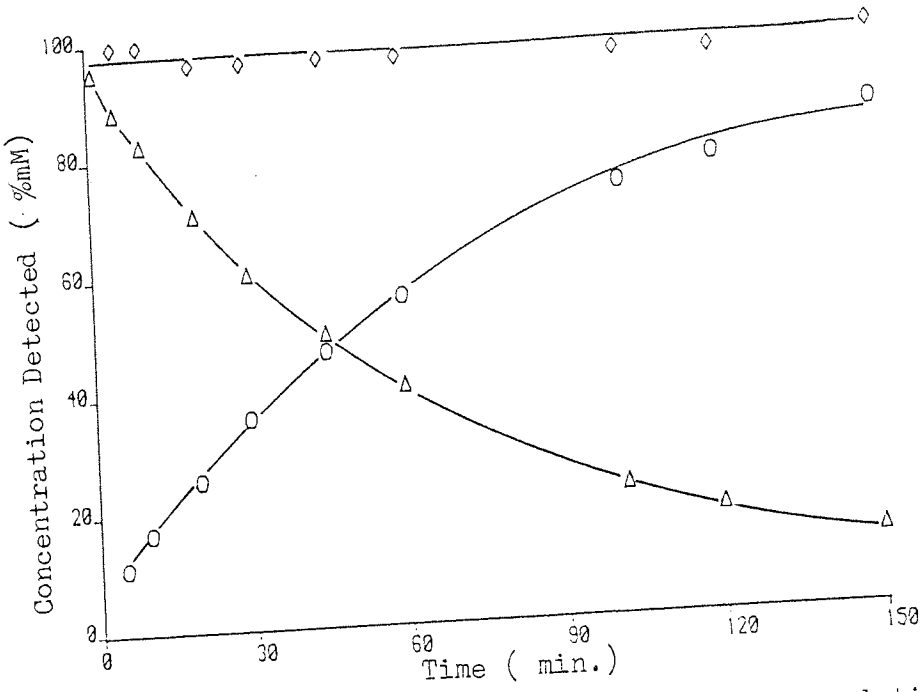


Fig. 4.2. Concentration-Time Profiles Showing the Degradation of Methyl Salicylate (1 mM) in Methanol-Water (10% v/v) at 37°C., With 10 mM NaOH.

Symbols	Δ	○	◇
Salicylates	Methyl	Salicylic Acid	Mass Balance

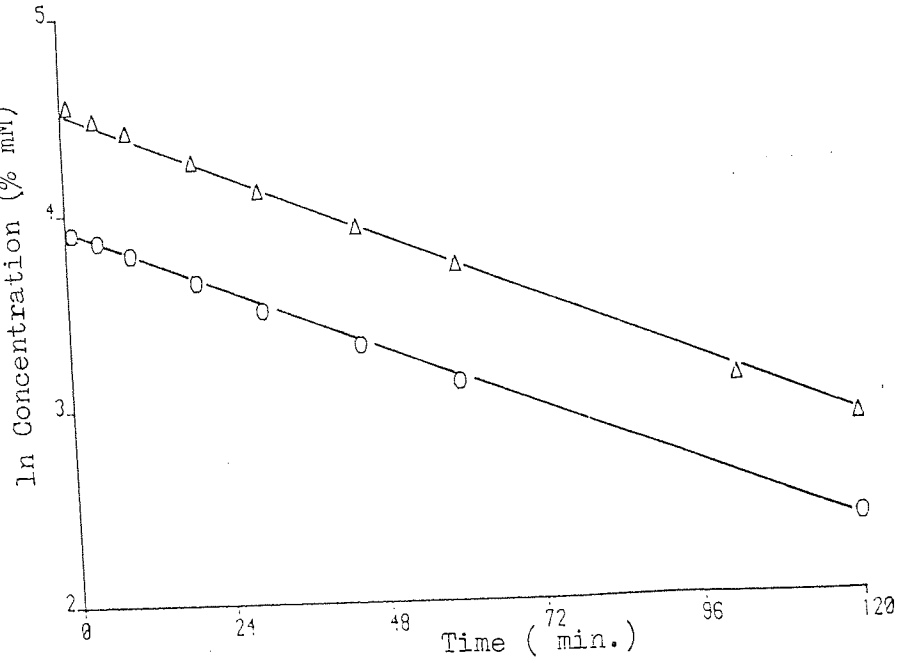


Fig. 4.3. First Order Plot Showing the Effect of Initial Concentration on the Degradation of Methyl Salicylate in Methanol-Water (10% v/v); NaOH = 0.01M; Temp. 37°C.

Symbols	Δ	○
Initial Conc. mM	1	0.5

4.3.2. CONCENTRATION - TIME PROFILES AND ORDER OF THE REACTION

The time - courses reflecting the degradation of methyl salicylate (1 mM) in alkaline (10 mM NaOH) aqueous methanol (10% v/v) at 37°C and the subsequent formation of salicylic acid are shown in Fig.4.2. At any time, the mass balance of the residual methyl salicylate and the generated salicylic acid corresponds to the initial methyl salicylate concentration, as shown in Fig.4.2. Degradation of this ester was also conducted under the same experimental conditions but with 0.5 mM initial concentration. Semilogarithmic plots of these degradation profiles were found to fit a first order model (equn.4.2. Section 4.2), as shown in Fig.4.3. The parallel lines signify equivalent rate constants for the degradation. The measured rate constants and the half-lives are in close agreement with each other, as shown in Table 4.1. These results suggest that the hydrolysis rate is independent of the initial concentration of the ester and confirms the first order degradation under these conditions.

Table 4.1 Effect of Initial Concentration of Methyl Salicylate On the Degradation Rate Constants NaOH=10 mM; temp.37°C; methanol Conc. 10% v/v.

initial Conc.(mM)	$k\ min^{-1} \times 10^{-3}$	$t_{1/2}$(min)	r
0.5	13.70	9.495	0.999
1.0	13.60	9.425	0.999

The rate constants equivalent to the absolute slopes of lines in Fig. 4.4. were determined by linear regression analysis and are recorded in Table 4.2. The rate constants of formation of salicylic acid were also measured and are similarly recorded.

Table 4.2 Effect of Concentration of Methanol (Ø-9Ø% v/v) on the Alkaline (1Ø mM NaOH) Hydrolysis of Methyl Salicylate (1 mM) at 37°C.

methanol in water(% v/v)	dielectric constant	rate constants for hydrolysis of methyl salicylate $k\ min^{-1} X 1Ø^{3}$	rate const.for formation of salicylic acid $k\ min^{-1} X\ 1Ø^{3}$
Ø	8Ø.ØØ	14.66	14.29
1Ø	77.71	12.65	13.4Ø
2Ø	75.15	11.17	11.38
3Ø	72.26	9.34	9.24
4Ø	68.99	7.57	7.22
5Ø	65.24	6.39	
6Ø	6Ø.9Ø	4.33	3.98
7Ø	55.83	3.Ø1	2.86
8Ø	49.82	1.67	1.68
9Ø	42.58	Ø.6Ø	Ø.62

The rate constants estimated in this way are in close agreement. As the concentration of methanol was increased in the reaction medium, a significant reduction of the rate of degradation of the ester was observed. Amis (137) in his study, explained that the solvent may alter the rate without influencing the mechanism by changing the

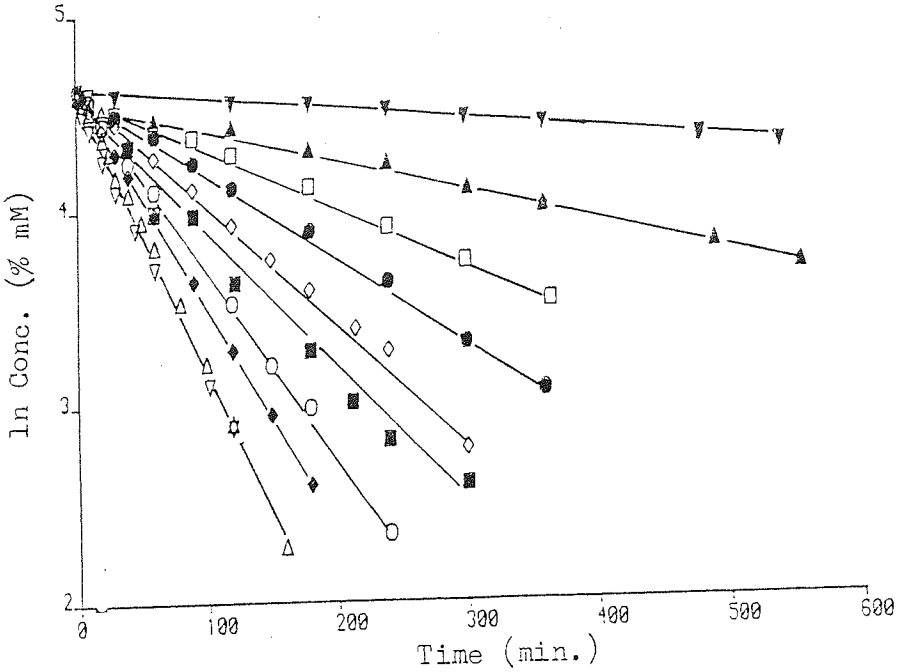


Fig. 4.4. First Order Plots of the Degradation of Methyl Salicylate (1mM) in Various Proportions of Methanol-Water. Temp. 37°C; NaOH = 10mM.

Symbols	▽	△	♦	○	■	◇	●	□	▲	▼
Methanol % v/v	0	10	20	30	40	50	60	70	80	90

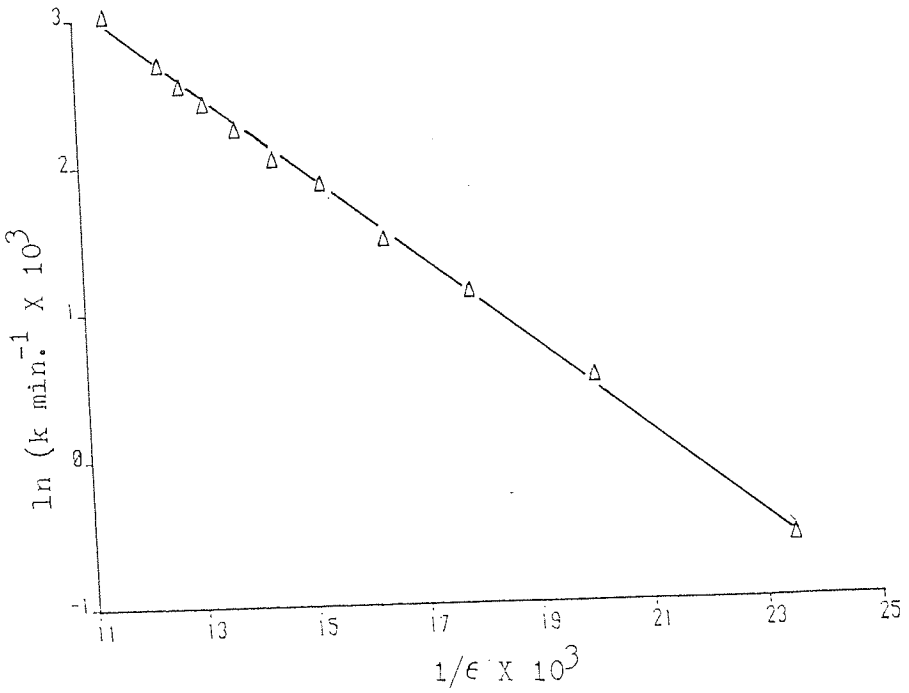


Fig. 4.5. Plots Showing Dependence of Rate Constant on Dielectric Constant of the Mixed Solvent.
Reactant : Methyl Salicylate (1mM) and NaOH = 10mM
Solvent : Methanol in Water 0-90% v/v; Temp. 37° C.

force between the reacting particles, and hence altering the stability of the transition state. He has explained this phenomenon by the effect of dielectric constant of the mixed solvent system on electrostatic forces among reacting particles. The change in specific rate constant for a reaction with changing dielectric constant of the solvent at constant temperature is expressed quantitatively(134-135) by the equation:

$$\ln k = \ln k_{\varepsilon=\infty} - \frac{Z_A Z_B N e^2}{RTr\varepsilon} \quad \text{.....equn.4.6.}$$

where, k = the specific rate constant

$k_{\varepsilon=\infty}$ = rate constant in a medium of infinite D.C.

Z_A, Z_B = electrical charges on the reacting species

N = Avogadro's number ($6.02 \times 10^{23} mol^{-1}$)

e = electrostatic charge

r = interionic distance within the activated complex

R = universal gas constant

T = temperature in degrees Kelvin.

ε = dielectric constant of the medium

Plot of $1/\varepsilon$ Vs. ln k for methyl salicylate degradation in methanol - water is shown in Fig.4.5. These rate constants follow the relationship in equation 4.6. closely and the least squares analysis gives :

$$\ln k = -0.06946\ (+\ 0.0534) - \frac{285.9\ (+\ 3.3)}{\varepsilon};\ r=-0.9995;\ n=10$$

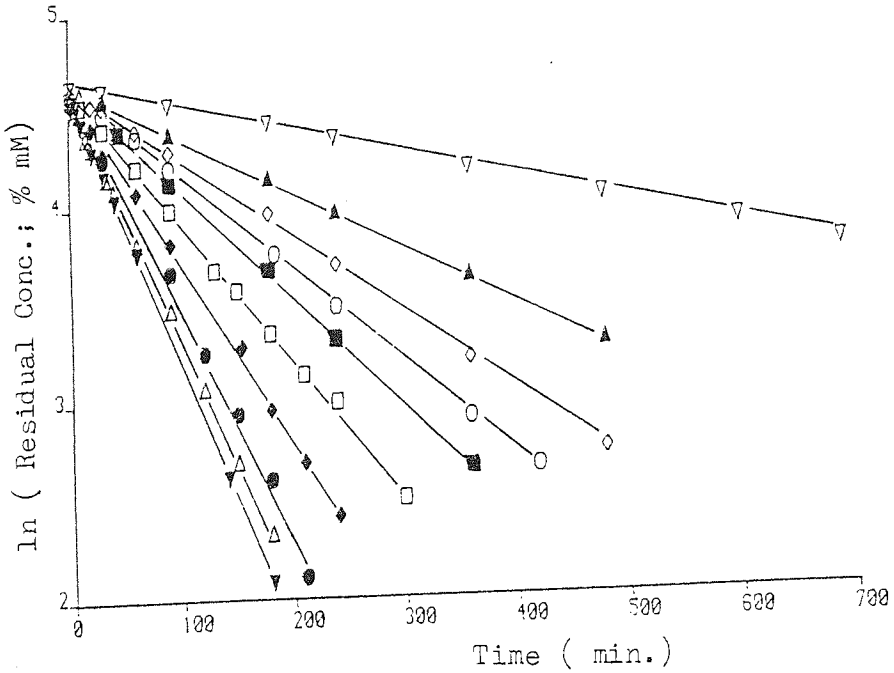


Fig. 4.6. First Order Plots for the Degradation of Ethyl Salicylate (1 mM) in Various Proportions of Ethanol-Water; Temp. 37° C., NaOH = 10 mM.

Symbols	▼	△	●	♦	□	■	○	◇	▲	▽
Ethanol % v/v	0	10	20	30	40	50	60	70	80	90

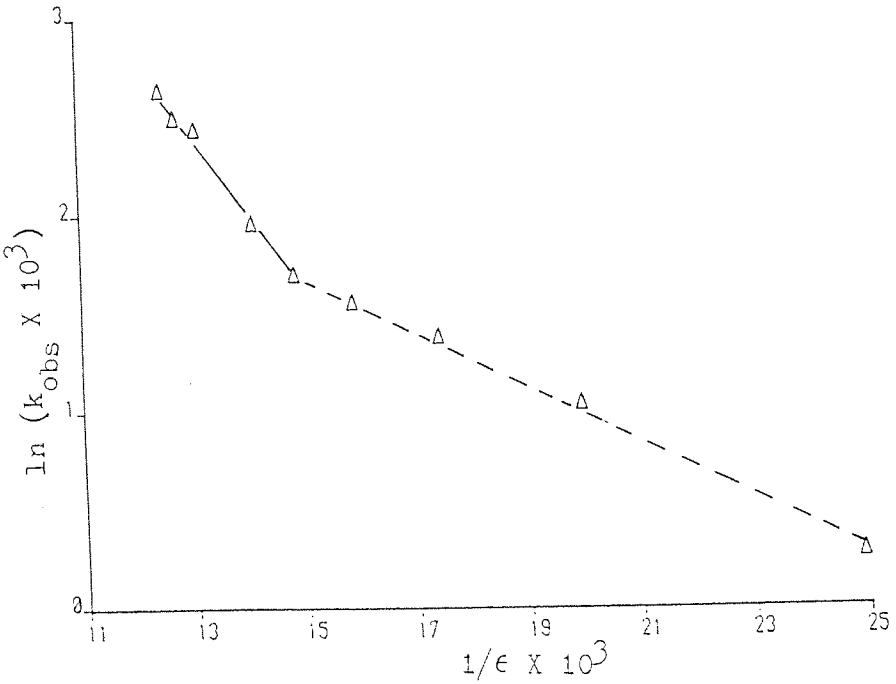
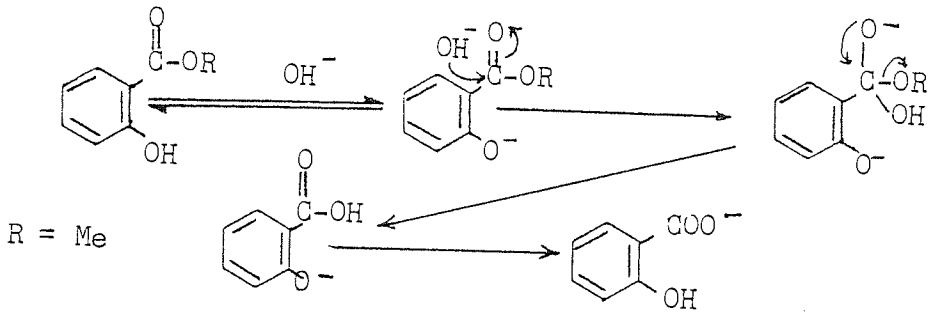


Fig.4.7. Effect of dielectric constant on the degradation of ethyl salicylate in ethanol - water. Initial conc. 1 mM, Temp. 37°C, NaOH 10 mM.

providing a limiting value for $k_{\epsilon=\infty}$ of Ø.4993 min^{-1}, which is the notional rate constant in a medium of infinite dielectric constant. The negative slope indicates that the ions in the transition state have like charges and signifies that the reaction involves an attack of hydroxide ion upon the salicylate anion (134), as shown in Scheme 4.2.



Scheme 4.2. Hydroxide attack on salicylate anion

Ethyl salicylate behaves in a manner similar to methyl salicylate. The observed rate constants for the degradation of this ester in various proportions of ethanol in water, measured from the slopes of the regression lines in Fig.4.6. are recorded in Table 4.3.

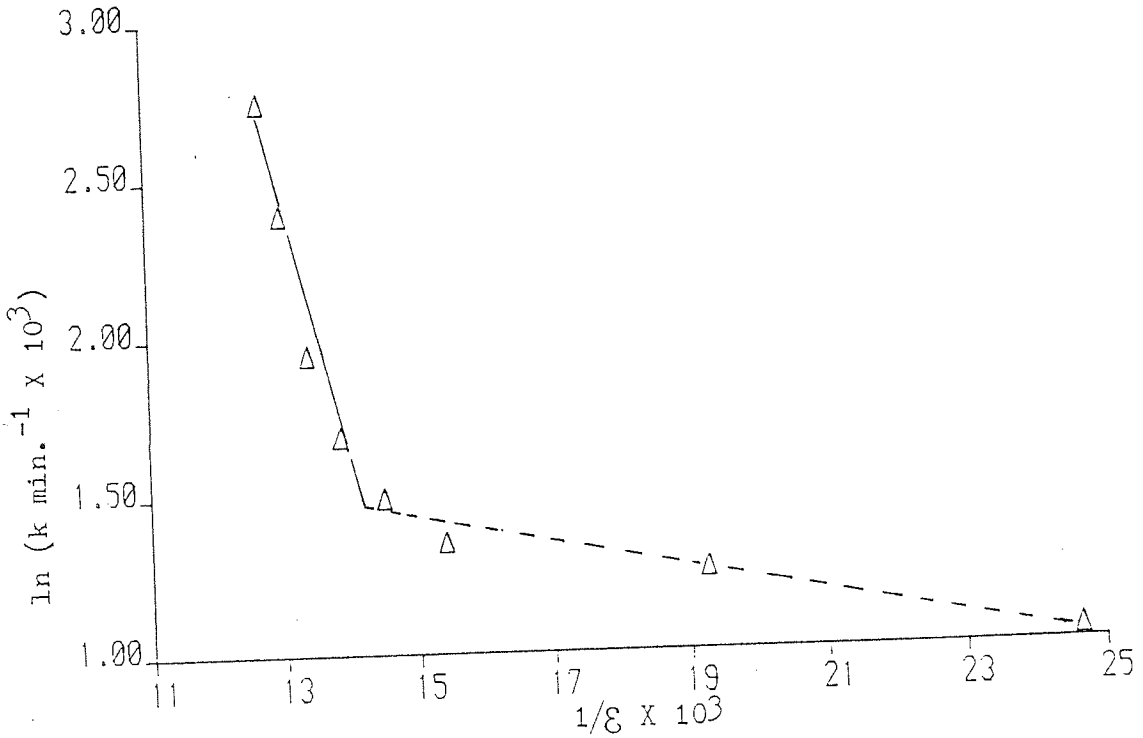


Fig. 4.8. Effect of dielectric constant on the degradation of propyl salicylate in ethanol-water. Initial conc. 1 mM, NaOH 10 mM, Temp. 37°C.

Table 4.3. Effect of Ethanol Concentration (Ø–90% v/v) on the Alkaline (1Ø mM NaOH) Hydrolysis of Ethyl Salicylate (1 Mm) at 37°C.

Conc.of ethanol (% v/v)	dielectric constant	rate const.for hydrolysis of ethyl salicylate $k\ min.^{-1} \times 1Ø^{3}$	rate const.of formation of salicylic acid $k\ min.^{-1} \times 1Ø^{3}$
Ø	8Ø.ØØ	13.99	14.31
1Ø	78.35	12.12	11.25
2Ø	76.13	11.44	1Ø.13
4Ø	7Ø.79	7.Ø8	7.33
5Ø	67.26	5.44	6.32
6Ø	62.9Ø	4.72	5.1Ø
7Ø	57.36	3.95	3.65
8Ø	5Ø.Ø9	2.81	2.48
9Ø	4Ø.15	1.32	1.Ø6

The $1/\varepsilon$ against the ln k plot is biphasic as shown in Fig.4.7. The linear sections intersect at 50% (v/v) alcohol level of mole fraction Ø.2346. The coefficients of the regression lines are :

low alcohol concentration $\ln k = Ø.7524 - \frac{4Ø1.8}{\varepsilon}$; r=-Ø.996, n=5

high alcohol concentration $\ln k = -3.1Ø17 - \frac{14Ø.754}{\varepsilon}$; r=-Ø.999; n=5

The rate constants for the hydrolysis of propyl salicylate in various proportions of n-propanol in water were also measured and are recorded in Table 4.4. The $1/\varepsilon$ against ln k plot is again biphasic (Fig.4.8) with the point of intersection of the two linear sections being the 50% (v/v) propanol concentration (mole fraction Ø.189). The coefficients of the lines are :

low alcohol concentration $\ln k = 5.558 - \frac{773.6}{\varepsilon}$; r=Ø.976; n=5

high alcohol concentration $\ln k = -4.883 - \frac{4Ø.Ø7}{\varepsilon}$; r =-Ø.974; n = 4

Amis et al (138) plotted log k Vs. $1/\varepsilon$ for the reaction between negative divalent tetrabromophenol sulfonaphthalein ions and negative univalent hydroxide ions in ethanol-water solvent system at 25°C. The line was straight with negative slope, down to a dielectric constant of around 65 (which is equivalent to the ethanol concentration of about 50% v/v). The deviation of the ln k Vs. $1/\varepsilon$ plots from linearity in lower dielectric constant values of the mixed solvent has been explained in several studies (139-143) in terms of preferential adsorption of water on the reactant ions. It has been suggested that upto 30 or 40% by weight of the organic

Table 4.4. Effect of Propanol Concentration (10–90% v/v) on the Alkaline (10 mM NaOH) Hydrolysis of n-Propyl Salicylate (1 mM) at 37°C.

Conc.Of propanol % v/v	dielectric constant	rate const.of hydrolysis $k\ min.^{-1} \times 10^{3}$
10	78.53	15.52
20	76.79	10.89
30	74.71	7.00
40	72.17	5.41
50	68.99	4.45
60	64.92	3.86
80	51.91	3.51
90	40.58	2.83

component in the mixed solvent system (ethanol-water) the reactant ions cling rather exclusively to the more polar component. This relative affinity for water exist until the latter is replaced by the larger molar volume of the organic component. As the dielectric constant of the mixed solvent decreases to lower values ($\sim$ 50% or less), the rates are less dependent upon this parameter than is predicted by equation 4.6.

Table 4.5 Rate Constants for the Degradation of Methyl Salicylate in Methanol-Water (50% v/v) Obtained from Non-Isothermal Experiment Using NONISO.

temp. °C	experimental rate const. k $min.^{-1}$ X 10^{3}	calculated rate const. k $min.^{-1}$ X 10^{3}
38.20	7.423	7.148
41.00	10.152	9.612
42.00	11.462	10.671
43.00	12.785	11.893
44.20	14.158	13.398
45.80	15.616	15.779
47.00	17.192	17.819
51.00	24.096	26.551
52.20	26.585	29.869
53.20	29.352	32.926
56.00	39.555	43.118
56.80	43.658	46.533
57.80	48.143	51.158
58.40	53.022	54.135
59.40	58.310	59.461
60.40	67.032	65.275
61.20	73.385	70.303
62.80	80.175	81.467
63.40	87.406	86.065
64.20	95.078	92.574
65.00	103.190	99.540
65.80	111.739	109.994
66.40	120.718	112.925

4.3.3. TEMPERATURE DEPENDENCE OF HYDROLYSIS OF METHYL SALICYLATE IN METHANOL - WATER (50% V/V)

A recently developed BASIC computer program -NONISO (144) is reported to be applicable in establishing the full temperature-stability profile from a single non-isothermal experiment. This method was applied to determine the temperature dependence of methyl salicylate in aqueous methanol (50% v/v). The rate constants at various experimental temperatures were determined fitting the time - temperature - concentration data to the program NONISO and are recorded in Table 4.5. To check the validity of the method, hydrolysis of methyl salicylate was conducted at various isothermal conditions. The first order plots of these concentration - time profiles are shown in Fig.4.9. and the respective rate constants are given in Table 4.6. The 1/T against ln k plots for the isothermal and the non-isothermal results are shown in Fig.4.10. together with the Arrhenius parameters in Table 4.7.

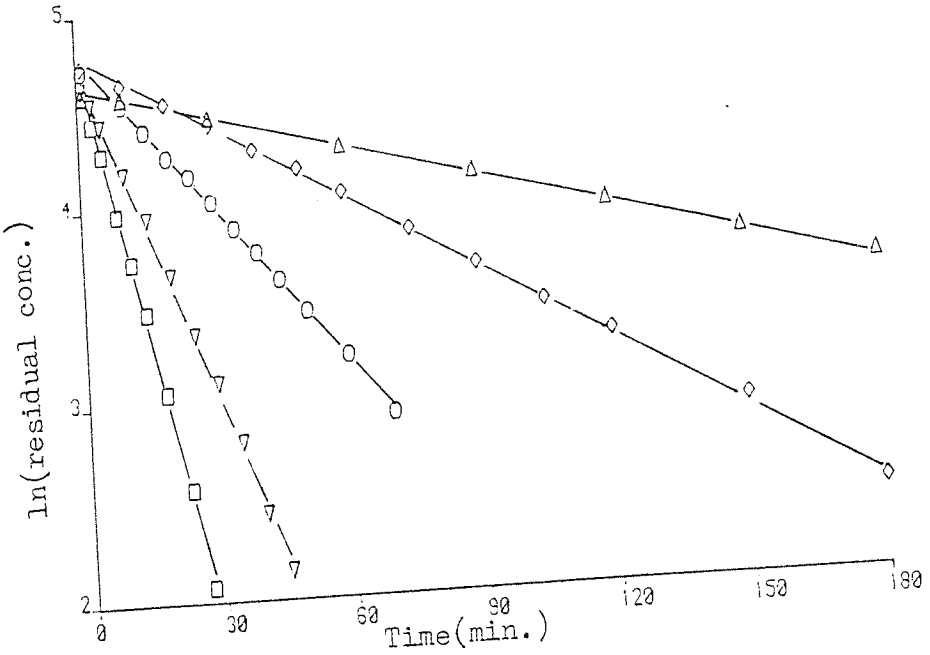


Fig. 4.9. First Order Plot of the Residual Concentration of Methyl Salicylate Showing The Dependence of Hydrolysis on Temperature. Initial Ester Conc. 1mM, NaOH = 0.01M.

Symbol	△	◊	○	▽	□
Temp. °C.	37	43	51	58.4	65

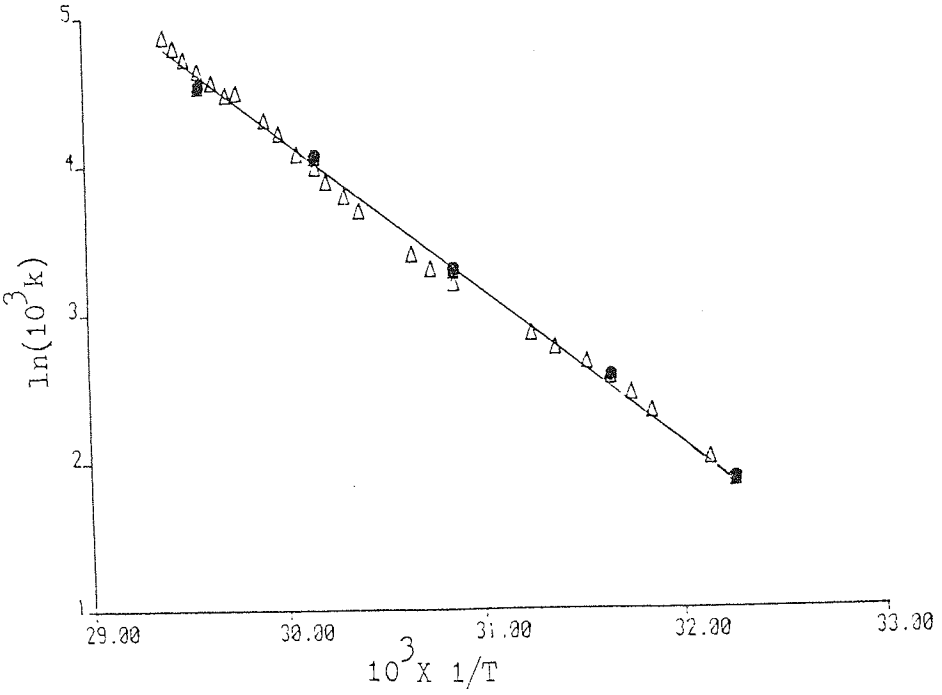


Fig. 4.10 Arrhenius Plots for Methyl Salicylate (1mM) in Methanol - Water (50% v/v); The Lines Represent the Theoretical Rate Constants Calculated from the Non-Isothermal Experiment Using 'NONISO'

Symbols	●	Isothermal Rate Constants
	△	Rate Constant from a Non-Isothermal Experiment.

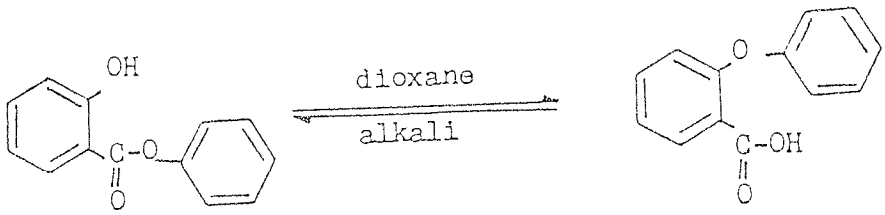
Table 4.6. Effect of Temperature on the Alkaline (10 mM NaOH) Hydrolysis of Methyl Salicylate (1 mM) in Methanol-water (50% v/v).

Temp. ^{o}C	rate constant of hydrolysis, $k\ min^{-1} \times 10^{3}$
37	6.386
43	12.770
51	26.20
58.4	56.90
65.0	92.40

Table 4.7. Arrhenius Parameters Measured from Isothermal and Nonisothermal Degradation of Methyl Salicylate in Methanol-Water (50% v/v).

Parameters	values	
	Isothermal	Nonisothermal
E_{act} ($KJ\ mol^{-1}$)	85.36	83.72
A min^{-1}	1.464 X 10^{12}	0.801 X 10^{12}
r	0.998	-0.999

CHAPTER 5 TRANSESTERIFICATION OF SALICYLATES



Scheme 5.1. Smiles' rearrangement, showing the intramolecular rearrangement of phenyl salicylate to o-carboxy diphenyl ether in alkaline dioxane

5.1. INTRODUCTION

The degradation profiles of methyl, ethyl and propyl salicylates in the corresponding hydroalcoholic medium, discussed in Chapter 4 reveal that the cleavage of the ester function may occur via solvent attack on the ionized reactant, forming a tetrahedral intermediate. In a hydroalcoholic medium, both the aqueous and alcoholic components are capable of attack on the reactant ester by providing H^+OH^- or H^+OR^-, though the more polarised component predominates at the reacting site. The rate studies in Chapter 4 were undertaken with alcohols in which the radicals were identical to those of the leaving ester functions. Consequently the question of the participation of this species in the reaction process remained unsolved. Taft and coworkers (145) reported that in alkaline methanol transesterification occurs in esters of substituted benzoic acid. Subsequently they found that in alkaline aqueous methanol the esters degrade via transesterification and hydrolysis through a similar mechanism. Scowen et al (146) detected transesterification in the methanolysis of p-nitro phenyl acetate in alkaline medium forming methyl acetate and p-nitro phenol. Fersht and Kirby (147) detected the formation of the methyl ester of aspirin in methanol. After quantification they found that about 12% of the initial concentration of aspirin was converted to the methyl ester. Bernard and Smiles (148) reported that in alkaline dioxane aryl salicylate undergoes intramolecular rearrangement to form the o-carboxy diphenyl ether, as shown in Scheme 5.1. Bender et al (91) stated that in ethanol such rearrangement does not occur even in Ø.1 M

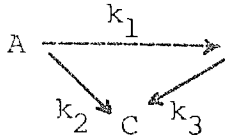
sodium hydroxide and used 32.8% ethanol in studying hydrolysis of p-nitro phenyl salicylate in phosphate buffer over the pH range 6.98-8.Ø at 25°C.

The aforementioned reactions have been reported to occur under conditions of high temperature and pH. The work to be described in this chapter reveals that the alcohol may not be an inert additive but may directly participate in the reaction with the formation of an intermediate ester. The purpose was to investigate the effect of the hydroalcoholic medium in which the alcohol differs from the ester function, and to study the possible decomposition of salicylates through a simultaneous-parallel model via competitive transesterification. Such effects are contrary to those reported by previous authors whose analytical methodology was incapable of revealing the formation of intermediate products.

5.2. KINETIC MODEL

5.2.1. IRREVERSIBLE TRANSESTERIFICATION

When the alcohol in the reaction medium does not correspond to that of the ester function (A), the reaction may proceed via transesterification to produce a second ester (B) and both esters undergo hydrolysis to salicylic acid (C). This transesterification may be presented by equation 5.1.



.....equn. 5.1.

Where,

A = the parent ester

B = the intermediate ester

C = salicylic acid

k_1 = rate constant of transesterification

k_2 = rate constant of hydrolysis from the parent ester.

k_3 = rate constant of hydrolysis from the intermediate ester.

The rate of change of concentration of species A,B and C are given by :

$$dA/dt = -A\,(k_1 + k_2) \quad \text{.....equn.5.2.}$$

$$dB/dt = k_1A - k_3B \quad \text{.....equn.5.3.}$$

$$dC/dt = k_2A + k_3B \quad \text{.....equn.5.4.}$$

Integration of these equations between time zero and the current time t enables expressions for instantaneous concentrations of each species to be obtained. Thus :

$$A_t = A_{\emptyset} \cdot e^{-(k_1 + k_2).t} \quad \text{.....equn.5.5.}$$

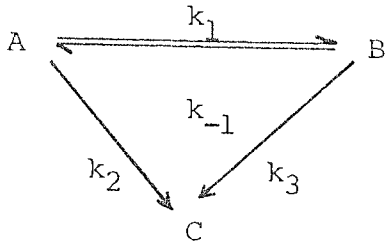
$$B_t = A_{\emptyset} \cdot k_1 \cdot \frac{e^{-k_3 t} - e^{-(k_1+k_2).t}}{(k_1 + k_2 - k_3)} \quad \text{.....equn.5.6.}$$

$$C_t = A_{\emptyset} \cdot \left[1 - \frac{k_1 e^{-k_3 t} + (k_2 - k_3) e^{-(k_1+k_2)t}}{(k_1 + k_2 - k_3)}\right] \quad \text{...equn.5.7.}$$

Derivation of these equations is presented in Appendix 3.

5.2.2. REVERSIBLE TRANSESTERIFICATION

When the transesterification reaction is reversible, the kinetic model is modified to



.....equn.5.8.

The rates of change in concentration of species A,B and C are now given by :

$$dA/dt = k_{-1}B - A\,(k_1 + k_2) \quad \text{.....equn.5.9}$$

$$dB/dt = k_1A - B\,(k_{-1} + k_3) \quad \text{.....equn.5.10.}$$

$$dC/dt = k_2A + k_3B \quad \text{.....equn.5.11.}$$

Intregration of these expressions from time zero to time t allows the concentration of each species at time t to be determined :

$$A_t = A_\emptyset.\left[\frac{(k_{-1} + k_3 - \gamma_1).e^{-\gamma_1 t} - (k_{-1}+k_3-\gamma_2).e^{-\gamma_2 t}}{(\gamma_2 - \gamma_1)}\right] \quad \text{eqn.5.12}$$

$$B_t = A_\emptyset.k_1\left[\frac{e^{-\gamma_1 t} - e^{-\gamma_2 t}}{(\gamma_2 - \gamma_1)}\right] \quad \text{.....equn.5.13.}$$

$$C_t = A_{\emptyset} \cdot \left[1 - \frac{(k_1+k_{-1}+k_3-\gamma_1)e^{-\gamma_1 t} - (k_1+k_{-1}+k_3-\gamma_2).e^{-\gamma_2 t}}{(\gamma_2 - \gamma_1)} \right] \text{eq5.14}$$

Where γ_1 and γ_2 are quadratic root functions of the various rate constants and are given by :

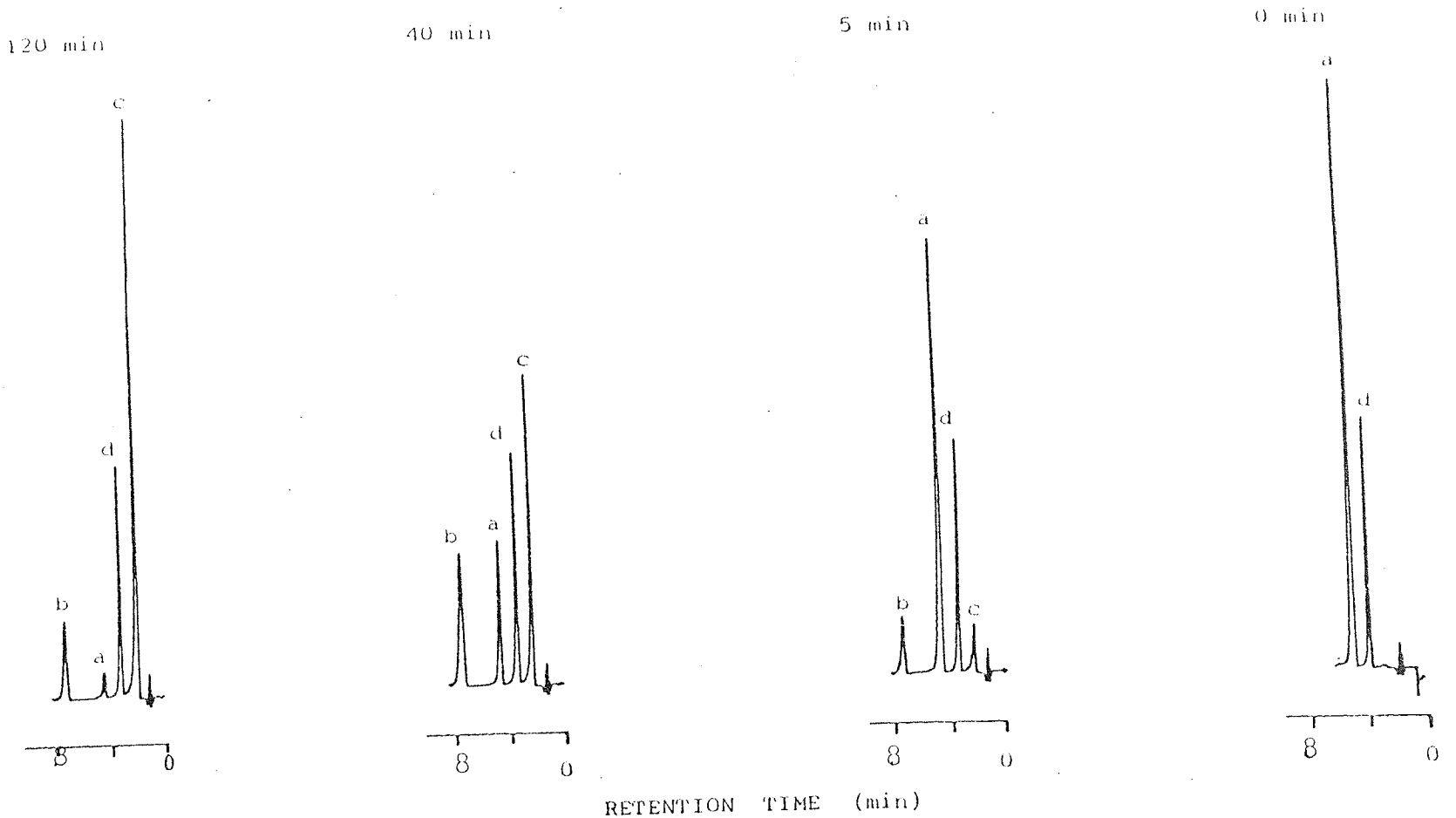
$$\gamma_1 = \frac{\sqrt{(k_1+k_{-1}+k_2+k_3)+(k_1+k_{-1}+k_2+k_3)^2-4(k_{-1}k_2+k_1k_3+k_2k_3)}}{2} \quad \text{equn.5.15}$$

$$\gamma_2 = \frac{\sqrt{(k_1+k_{-1}+k_2+k_3) - (k_1+k_{-1}+k_2+k_3)^2 - 4(k_{-1}k_2+k_1k_3+k_2k_3)}}{2} \quad \text{equn.5.16}$$

Fig. 5.1. High Performance Liquid Chromatogram of methyl salicylate (1mM) degradation in alkaline aqueous ethanol, 20% v/v, 37°C, NaOH 10mM.

peaks	salicylates
a	methyl
b	ethyl
c	salicylic acid
d	internal standerd, propyl paraben

solvent : $CH_3CN:H_2O:H_3PO_4$ :: 52 : 47.9 : .1
detection : 235 nm , 0.64 AUFS
flow rate : 1 ml / min,
Column : 10 cm X 4.6 mm, Hypersil-ODS



5.3. RESULTS AND DISCUSSION

5.3.1. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatograms following the degradation of methyl salicylate (1 mM) in alkaline ethanol(20% v/v,Ø.Ø1 M NaOH) at 37°C are displayed in Fig. 5.1. The initial trace (t = Ø) indicates the presence of methyl salicylate and the internal standard. After 5 mins. traces of salicylic acid together with a large fourth peak are also apparent. The retention time of the fourth peak is identical to that of ethyl salicylate, and suggests that a transesterification process is favoured under these conditions. The identity of this component was confirmed by its isolation from a solution of methyl salicylate (Ø.Ø7 M) in absolute ethanol containing sodium hydroxide (Ø.ØØ2 M) stored at 37°C for 5 days. The product displayed identical chromatographic characteristics to the transesterified material, as shown in Fig. 5.2. and was shown to be ethyl salicylate by ^{1}H nmr and mass spectral analysis, as displayed in Fig. 5.3.and 5.4. together with the mass spectral data in Tab.5.1.

Table 5.1. Mass Spectral Data for Ethyl Salicylate

M/Z	166	149	141	139	138	133	122	121	12Ø	94	93
RI(%)	76.36	1Ø.39	1Ø.39	1Ø.13	34.55	3.39	29.Ø9	76.36	77.27	2Ø.91	38.18

M/Z	92	81	79	78	77	76	71	66	65	64	63
RI(%)	76.36	12.73	8.Ø5	6.75	23.64	11.82	1Ø.91	15.45	9Ø.91	1ØØ	52.73

M/Z	53	51	5Ø	4Ø	39	32	3Ø	28
RI(%)	27.27	17.27	13.63	8.83	37.27	7.Ø1	24.54	83.64

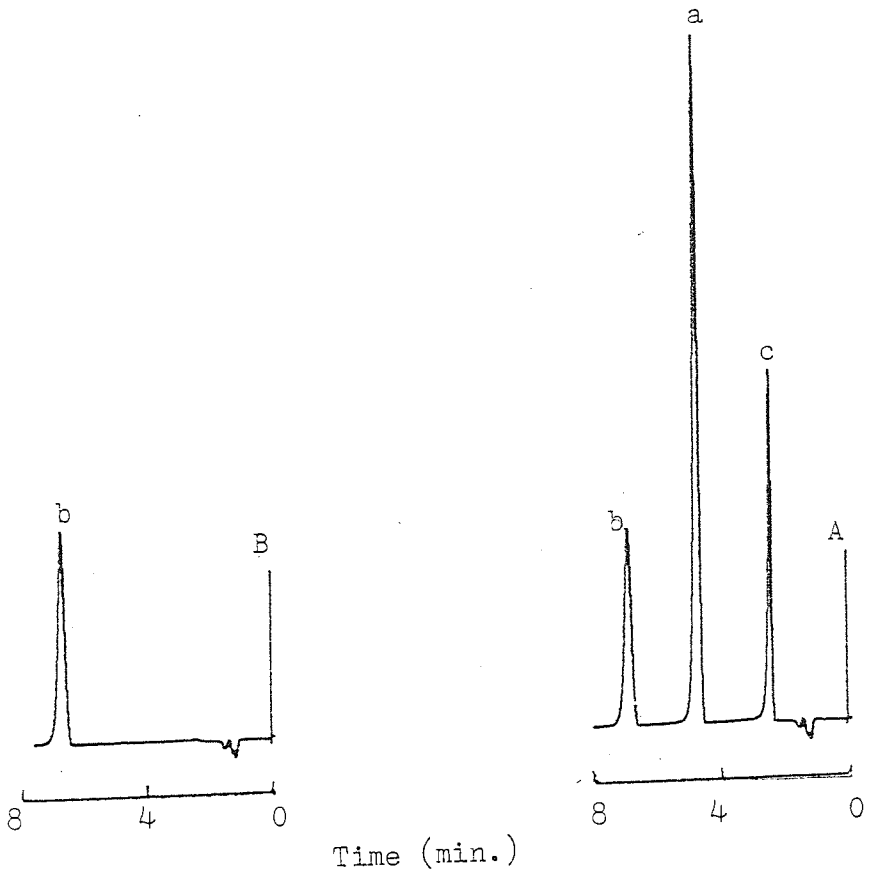


Fig. 5.2. A. Chromatograms of the Experimental Sample Containing Degradation Products of Methyl Salicylate in 20% v/v Aqueous Ethanol.

B. Chromatogram, When the New Component in A is Isolated by Synthesis and Distillation.

Peaks	a	b	c
Salicylates	Methyl	Ethyl	Salicylic acid

Column : 10 cm X 4.6 mm, Hypersil-ODS.
Solvent: CH_3CN:H_3PO_4:H_2O :: 52:0.1:48
Flow rate : 1 ml/min.
Detection : 235 nm, 0.32 AUFS

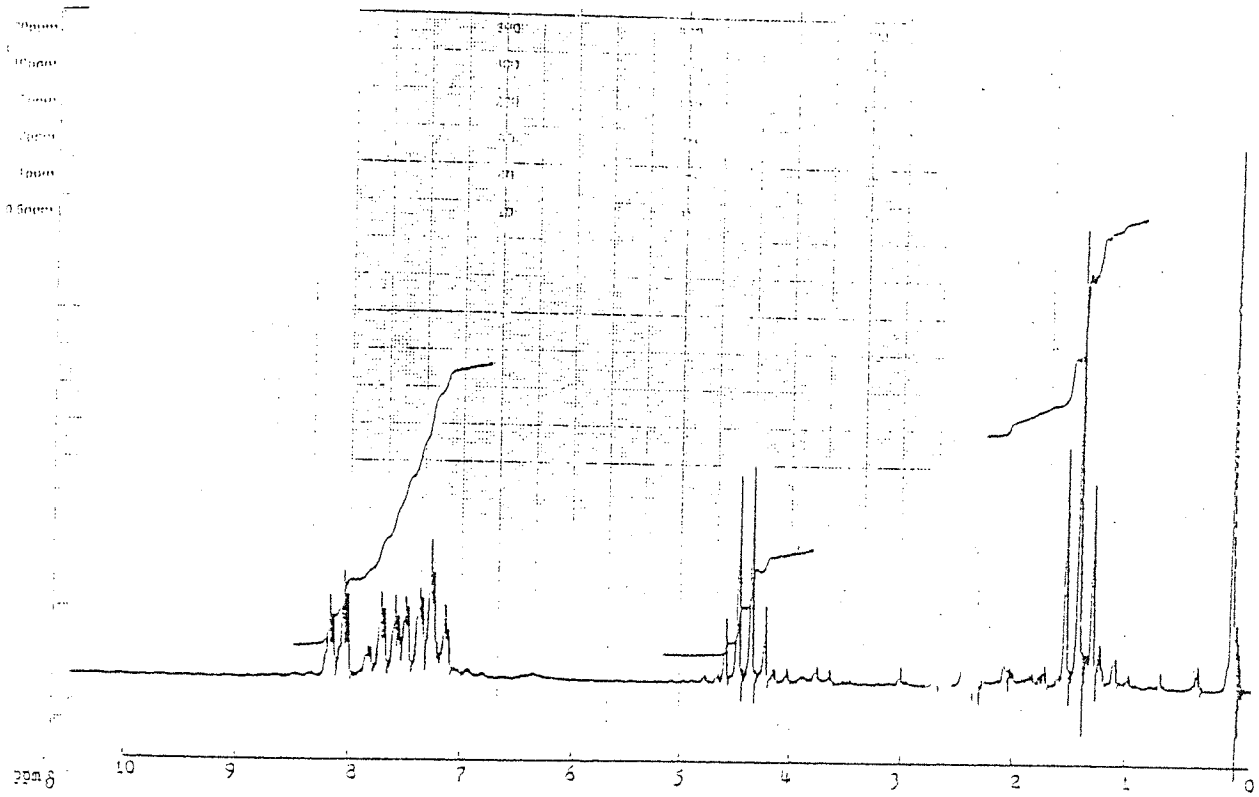
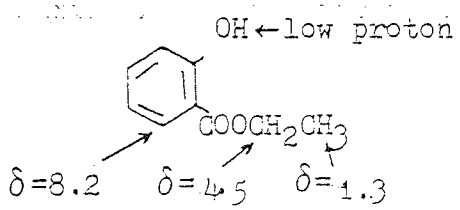


Fig. 5.3. ^{1}HNMR Spectrum of Ethyl Salicylate



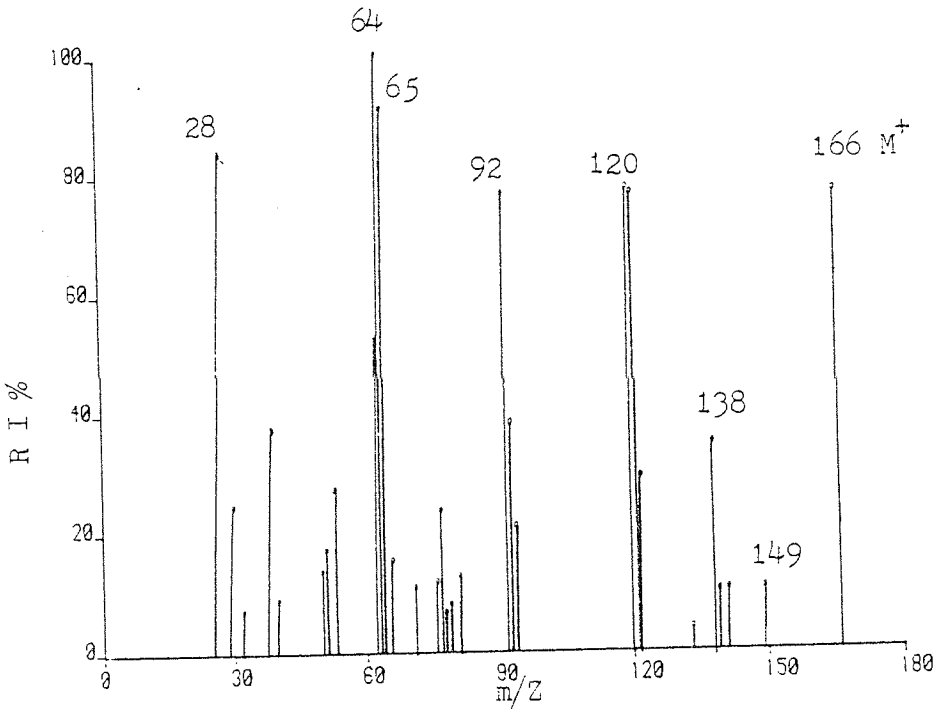
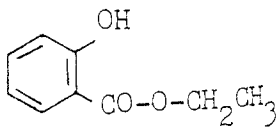


Fig. 5.4. Mass spectrum of ethyl salicylate



5.3.2. CONCENTRATION TIME PROFILE

The reaction profiles showing the fate of the reactant (methyl salicylate), the intermediate esters (ethyl salicylate) and the product (salicylic acid) are displayed in Fig.5.5. At any instant the sum of the molar concentrations of methyl salicylate, ethyl salicylate and salicylic acid maintain mass balance equivalent to the initial concentration of methyl salicylate. It is apparent in the profiles that under the experimental conditions of alkaline ethanol (20% v/v, 0.01 M NaOH, 37^{o}C), a maximum level of ethyl salicylate is formed after 40 minutes and corresponds to 45% of this product. Control experiments show that under these conditions salicylic acid is not esterified, indicating that ethyl salicylate must arise by transesterification of methyl salicylate in competition with the hydrolytic process. The profiles also reveal that both esters suffer depletion by hydrolysis to salicylic acid.

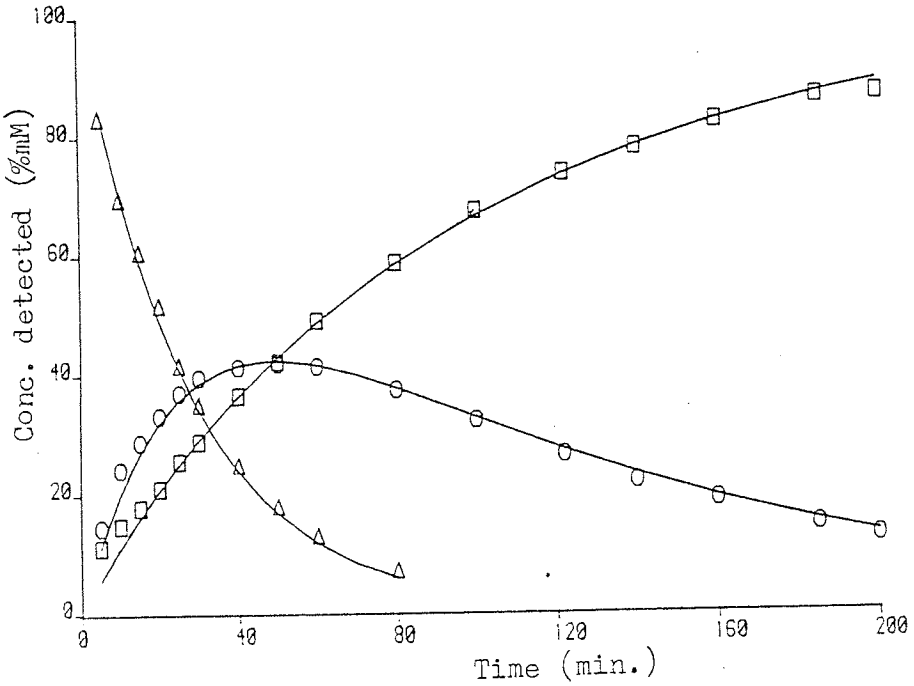
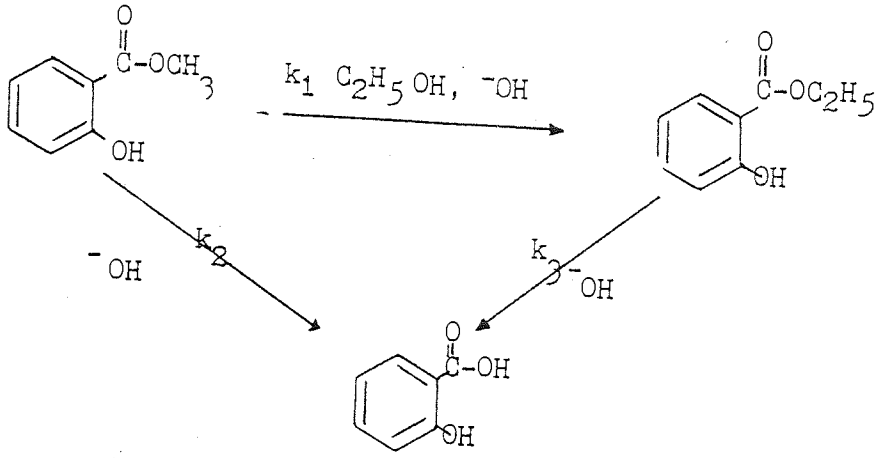
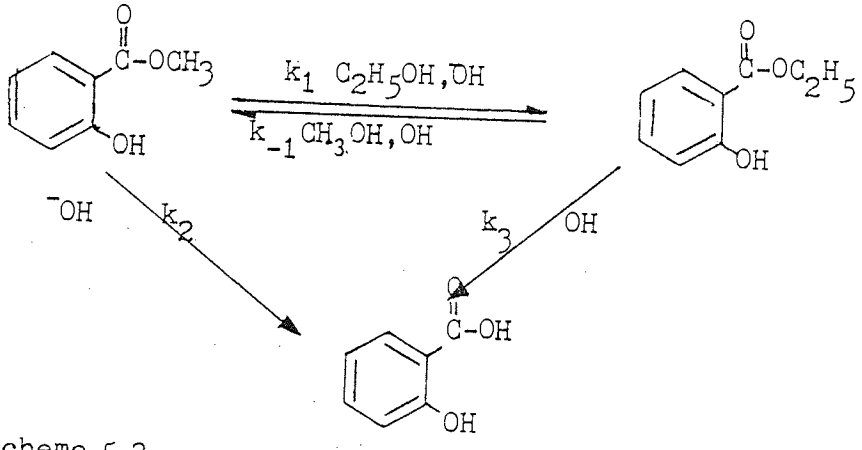


Fig. 5.5. The Time - Course for the Degradation of Methyl Salicylate (1 mM) in Alkaline (0.01M NaOH) Ethanol-Water (20% v/v) at 37°C.

Symbols	Δ	○	□
Salicylate	Methyl	Ethyl	Salicylic acid



Scheme 5.2.



Scheme 5.3.

Models showing possible routes of transesterification and hydrolysis of methyl salicylate in aqueous ethanol

5.3.3. ESTIMATION OF VARIOUS RATE CONSTANTS

The disappearance of methyl salicylate is presented as a semi-logarithmic plot in Fig.5.6. The excellent linearity confirms the overall first-order degradation expected by equn. 5.5. The logarithmic form of which is :

$$\ln . A_t = \ln . A_\emptyset - (k_1+k_2).t \text{ ....equn.5.17.}$$

The measured slopes thus yield the sum of the hydrolysis and transesterification rate constants for the disappearance of methyl salicylate. The competitive transesterification and hydrolytic process may be represented by the mechanistic model as sketched in Scheme 5.2. and Scheme 5.3. In justifying the applicability of these models, methyl salicylate in 20% aqueous-ethanol, in which all rate processes reasonably compete with each other were considered.The time - courses of the reactants and the products were fitted to equations 5.5,5.6 and 5.7 using the FORTRAN version of NONLIN . The primary estimates for the specific rate constants were chosen from the rate constants of the overall disappearance. The final estimates, as obtained from NONLIN are recorded in Table.5.2. Specific rate constants were also estimated by fitting the time - course to the reversible kinetic model (equn.5.8, Scheme 5.3.), using equations 5.12; 5.13 and 5.14. The comparison of the summation of the rate constants of the hydrolysis and transesterification reveal that the irreversible model in Scheme 5.2.(equn.5.2.) gives a close approximation to the overall disappearance process. The reverse rate constant of transesterification is very slow compared

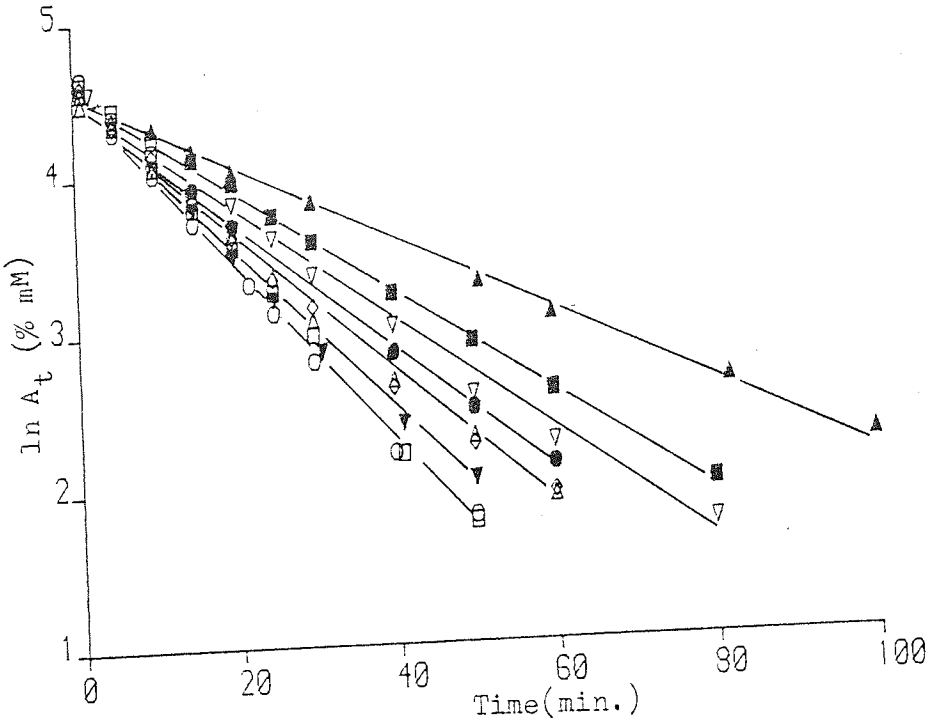


Fig. 5.6. First Order Plots Showing the Overall Degradation Methyl Salicylate in Various Concentrations of Aqueous Ethanol , A_0= 1mM, temp. 37°C., NaOH = 10 mM

Symbol	▲	■	▽	●	◊	Δ	□	○	▼
%EtOH	10	20	30	40	50	60	70	80	90

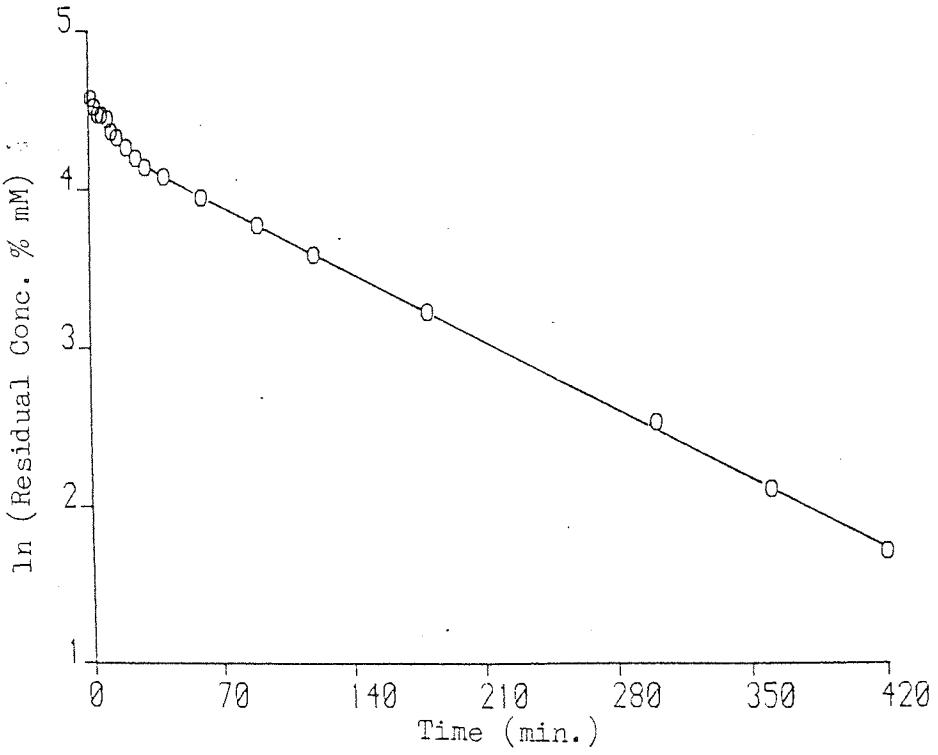
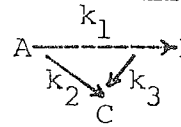
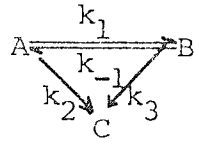


Fig. 5.7. First Order Plots Showing the Overall Degradation of Methyl Salicylate in Aqueous Equimolar Methanol:Ethanol (50% v/v), temp. 37° C., A_0 = 1 mM, NaOH = 10 mM

to the forward rate constant of the transesterification. The ratio of the forward to the reverse rate constant of transesterification of methyl salicylate in 20% aqueous ethanol at 37°C is 7.61.

Table 5.2 Specific Rate Constants for the Degradation of Methyl Salicylate in Aqueous Methanol-Water (50% v/v) Measured by NONLIN Considering the Possibilities of Both Mechanistic Models.

System	Kinetic Model	$k\ min^{-1}.X\ 10^3$ specific rate constant				r			k_1+k_2 (overall) $k\ min^{-1}X10^3$
		k_1	k_2	k_3		r_1	r_2	r_3	
methyl salicylate in 20% aq-methanol		24.5	11.7	9.89		.999	.998	.999	33.12
methyl salicylate in 20% aq-ethanol		k_1	k_2	k_3	k_{-1}				
		26.1	12.4	8.4	3.4	.999	.995	.999	

The formation of the methyl ester from the degradation of the ethyl ester in alkaline aqueous methanol indicates the reversible nature of the reaction. However, the reversible model is not appropriate here because of very low concentration of second alcohol present in the system. When further studies were conducted with an increase in the concentration of the competing alcohol the reversed reaction became significant. In order to provide a high

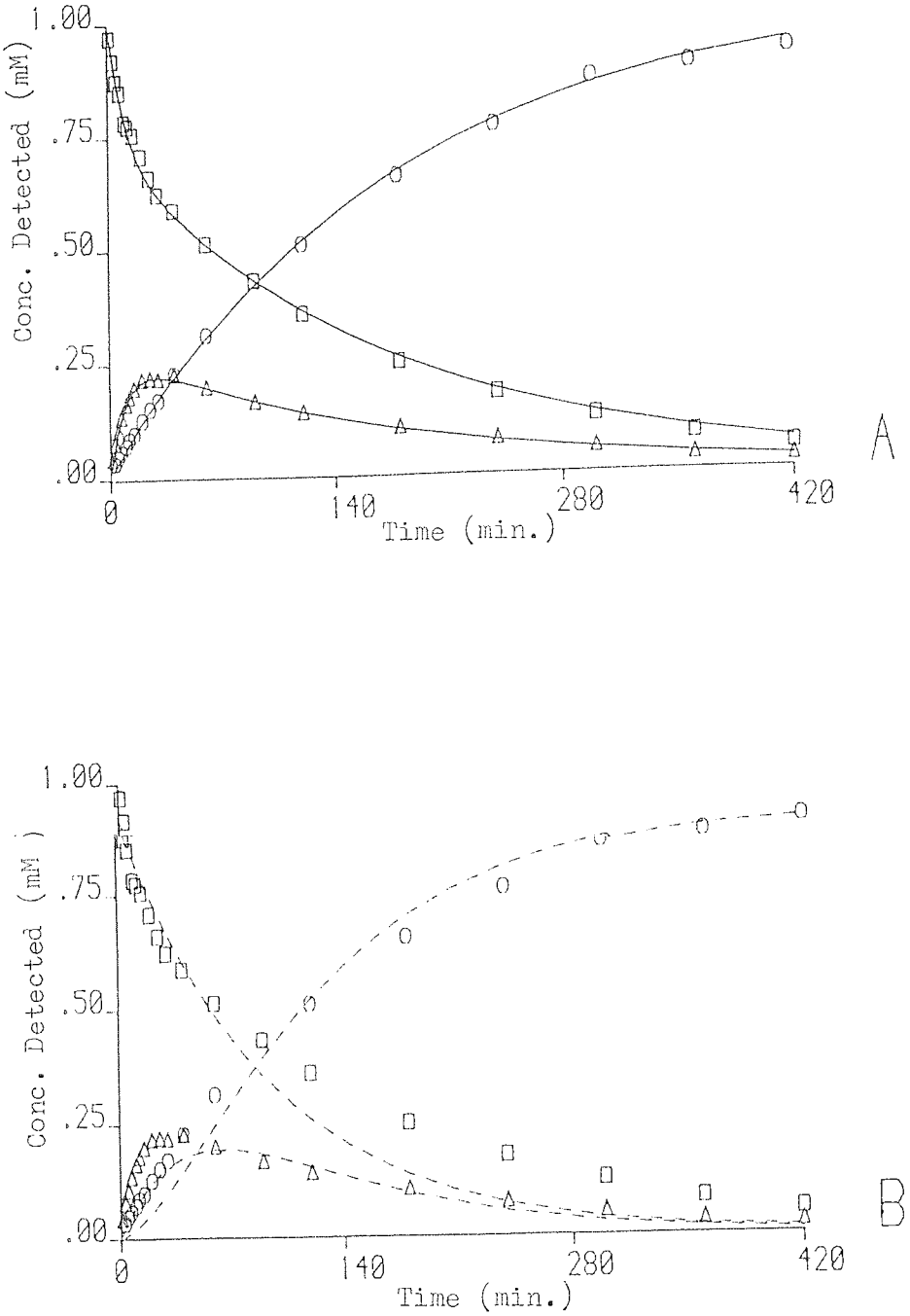


Fig. 5.8. Concentration-Time Profiles Showing the Degradation of Methyl Salicylate(1mM) in Alkaline(0.01M NaOH) Equimolar Methanol:Ethanol(32:46 w/w) in Water(50% v/v) at 37° C.,

Symbols □ △ ○

Salicylate Methyl Ethyl Salicylic acid

Continuous Lines in Fig. 5.8.A. Represent Reversible Kinetic Model (equn. 5.8.) and the Broken Lines in Fig. 5.8.B. Represent Irreversible Kinetic Model (equn. 5.1.)

concentration of alcohol for both the forward and reversed transesterification, equimolar alcohol consisting of methanol and ethanol (32 gm : 46 gm) in alkaline medium (50% v/v,0.01 M NaOH) was used. The overall disappearance process is displayed in Fig.5.7. by a first - order plot. The plot is nonlinear because of the reversibility of the reaction. The time courses for the reactants and the products were fitted to both of the kinetic models in Scheme 5.2.and Scheme 5.3. and are displayed in Fig.5.8. The degree of fit of the experimental values to those calculated from the reversible model is very good with the correlation coefficients 0.999, 0.998 and 1 for the three functions - the methyl ester, ethyl ester and salicylic acid respectively. The correspondence between theoretical and measured data is much poorer in the irreversible model with the corresponding correlation coefficients 0.992, 0.838 and 0.997. The specific rate constants under these conditions were determined and are presented in Table 5.3. This result suggests that in the former studies, the reverse rate was very slow because this process was dependent on the amount of alcohol released by the original reactant ester. The comparative studies in Tab.5.3. shows that when both alcohol functions becomes available in excess quantities, compared to the salicylate anion, the forward and reversed rate of transesterification significantly compete with each other. However, the value of the ratio between the two depends upon the relative reactivity of the two alcohols.

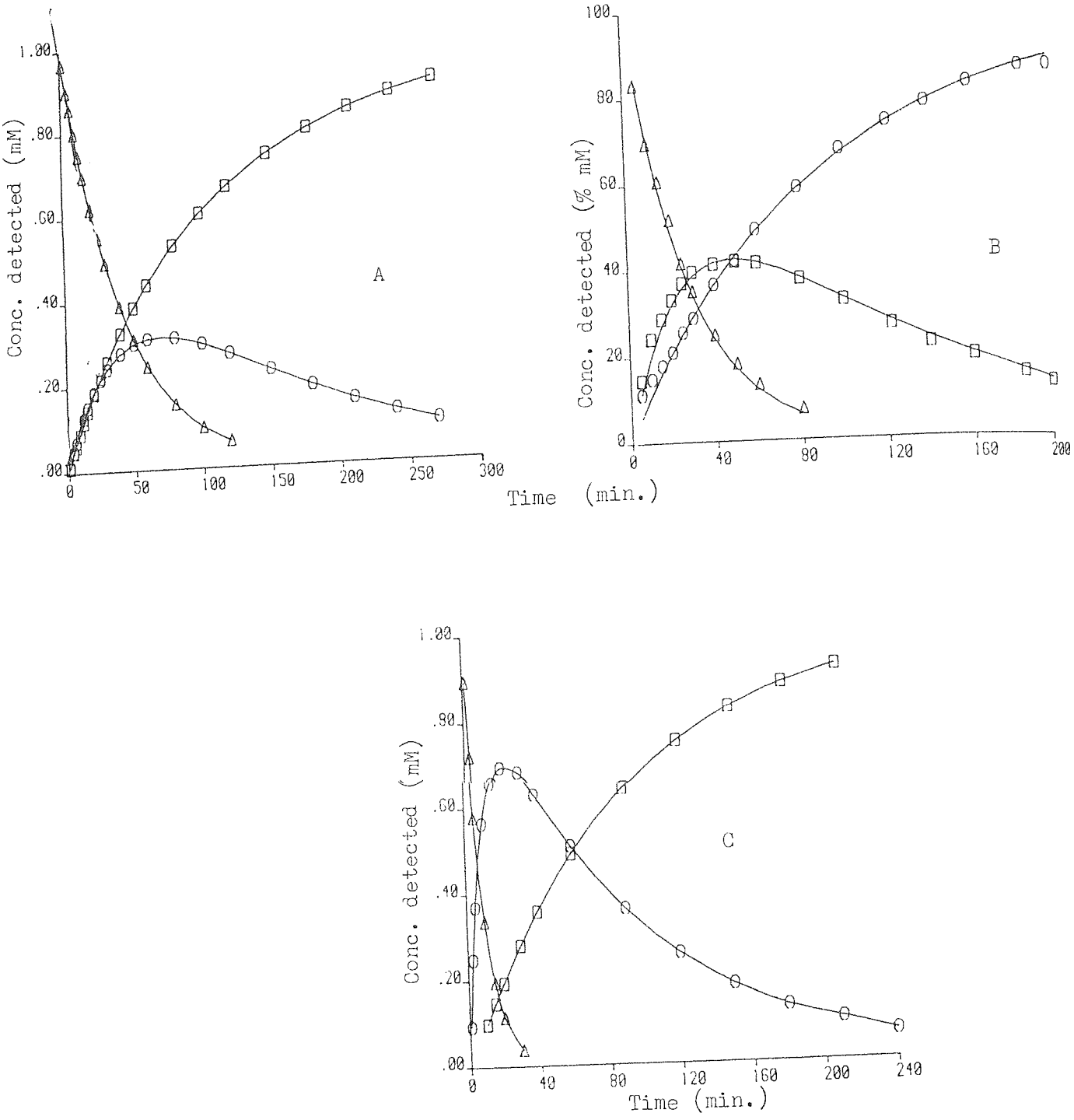


Fig. 5.9. Concentration Time-Profile Showing Transesterification of Salicylates in Alkaline Hydro-Alcoholic Medium.

Profiles	Reactant and Medium	Symbols	Time - Course
A	Propyl Ester in 20% EtOH	△	Propyl ester
		○	Ethyl ester
		□	Salicylic acid
B	Methyl Ester in 20 EtOH	△	Methyl ester
		□	Ethyl ester
		○	Salicylic acid
C	Propyl Ester in 20% MeOH	△	Propyl ester
		○	Methyl ester
		□	Salicylic acid

Table 5.3.A. Specific Rate Constants For The Degradation of Methyl Salicylate in Alkaline equimolar Methanol:Ethanol(20% v/v) at 37°C, NaOH = 10 mM

kinetic model	Specific rate constants $k\ min.^{-1} \times 10^3$				Correlation coefficient(r)		
	k_1	k_2	k_3		r_1	r_2	r_3
$A \xrightarrow{k_1} B$; k_2 C k_3	9.1	1.6	22.00		.992	.838	.997
	k_1	k_2	k_3	k_{-1}			
$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$; k C k	26	7.2	2	71	.999	.998	.997

Table 5.3.B. Comparative Studies Showing the Effect of the Concentration Of Available alkoxy Function on Forward and Reversed Rate of transesterification, A_0 = 1 mM; temp.= 37°C, NaOH = 10 mM .

reactant and reaction medium	Specific rate constants $k\ min.^{-1} \times 10^3$		ratio k_1 / k_{-1}
	forward transesterification	reversed transesterification	
methyl salicylate in 50% ethanol	43.9	2.52	17.42
methyl salicylate in 50% equimolar methanol:ethanol	26.0	71.86	0.362
ethyl salicylate in 50% methanol	131.31	1.80	72.95
ethyl salicylate in 50% equimolar methanol:ethanol	54.4	20.10	2.706

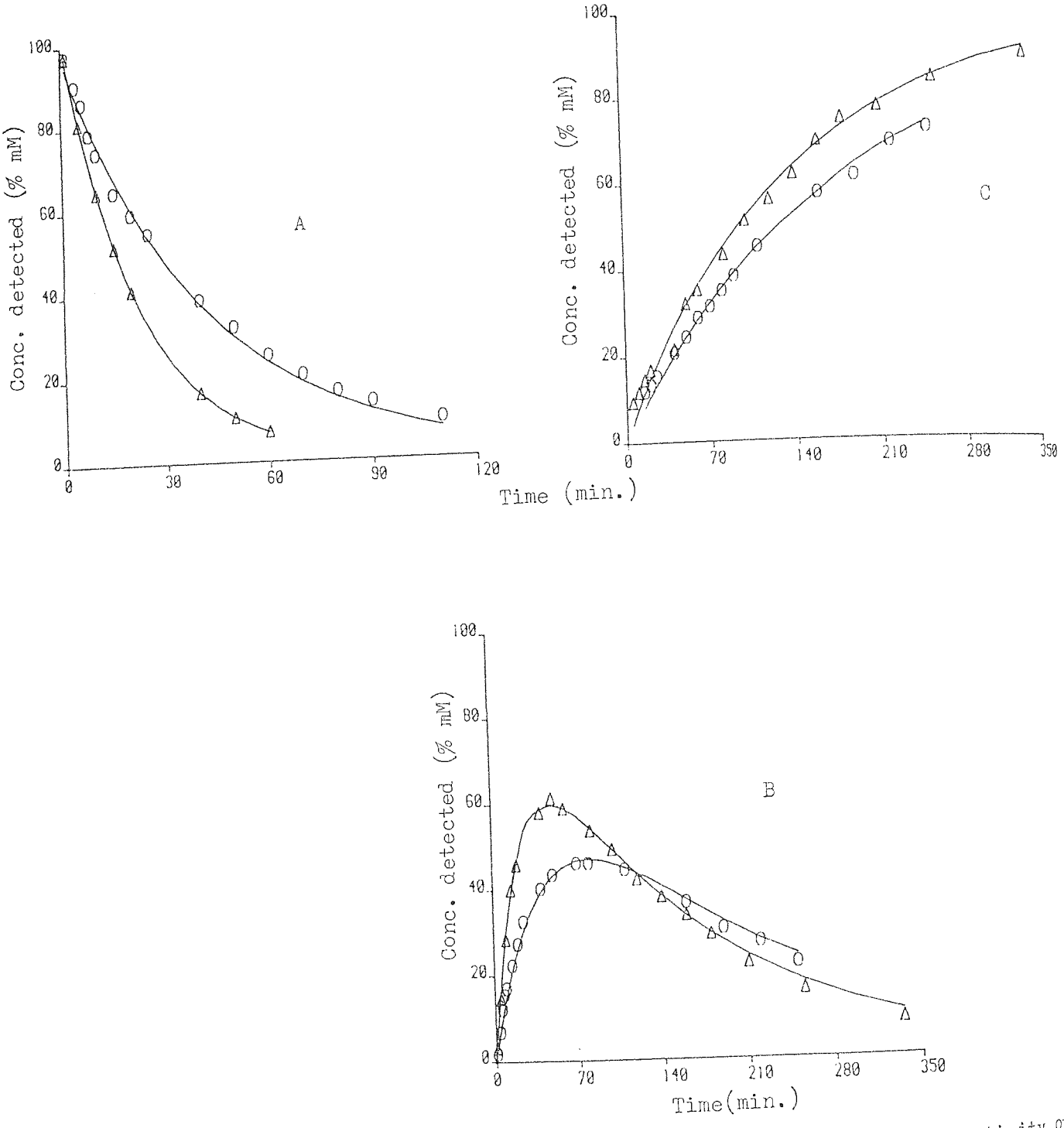


Fig. 5.10. Concentration-Time Profiles Showing the Relative Reactivity of Esters in Aqueous Ethanol(40% v/v)

Symbols	Esters	Profiles	Processes
○	Propyl	A	Disappearance of the parent ester
		B	Formation and disappearance of the intermediate ester
Δ	Methyl	C	Formation of Salicylic acid

5.3.4. DEPENDENCE OF THE SPECIFIC RATE CONSTANTS ON THE CONCENTRATION OF ALCOHOL IN THE REACTION MEDIA

Transesterification appears to be a general reaction of salicylates in alkaline hydroalcoholic media as shown in the concentration-time profile in Fig.5.9. Typical reaction profiles of methyl and n-propyl esters in 40% aqueous-ethanol (in Fig.5.10.) show that the methyl ester is more reactive than the propyl ester. The respective $t_{1/2}$ and maximum level of transesterification is shown in Table 5.4. This difference in reactivity of the leaving ester function may be explained in terms of bulk effect and steric inhibition to solvent attack (149-150). n-Propyl as the leaving group is relatively bulkier compared to methyl. This bulky substituent may also hinder the orientation of solvent molecules round the reaction site in the transition state compared to the ground state.

Table 5.4. Relative Reactivity of Methyl and Propyl Salicylate in 40% Aqueous-Ethanol at 37°C. NaOH = 0.01 M, A_0 = 1 mM

ester	$t_{1/2}$ (min.)	max.level of transesterified ethyl ester	Specific rate constants k min^{-1} X 10^3			Correlation coefficient		
			k_1	k_2	k_3	r_1	r_2	r_3
methyl	15	59% after 60 min.	36.98	8.38	6.68	.997	.999	.996
propyl	30	46% after 60 min.	17.80	5.84	5.57	.999	.998	.998

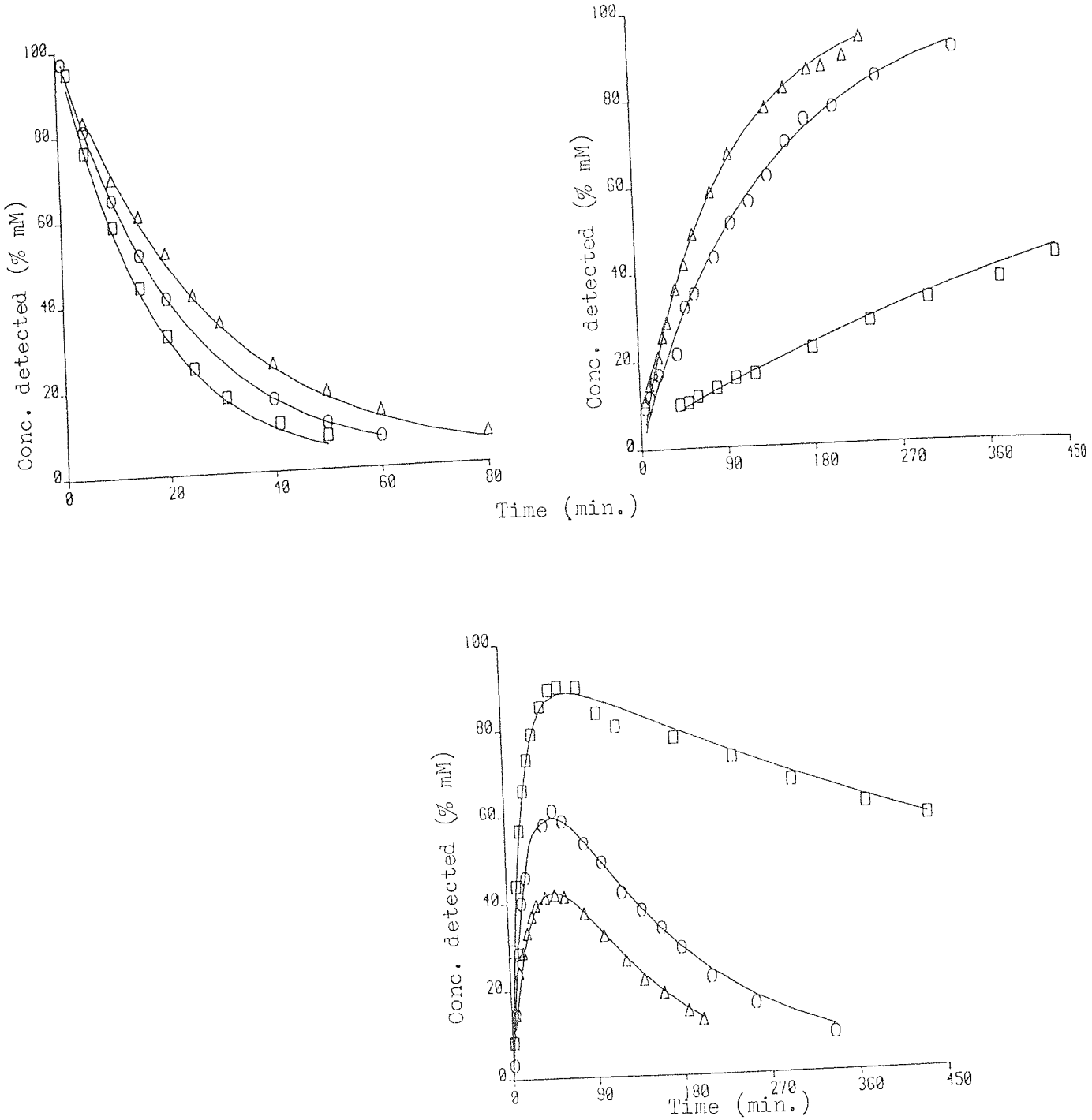


Fig. 5.11. Concentration-Time Profiles Showing the Effect of Ethanol Concentration on the Degradation of Methyl Salicylate.

Symbols	%(v/v) EtOH	Profiles	Processes
Δ	20	A	Disappearance of the parent esters
○	40	B	Formation and disappearance of intermediate ester
□	90	C	Formation of salicylic acid

The concentration of an alcohol influences the degradation profiles in various ways. While transesterification increases with increasing alcohol concentration, hydrolysis decreases significantly, as displayed by typical concentration-time profiles for the degradation of methyl salicylate in 20-90% aqueous-ethanol in Fig.5.11. The transesterification process cannot be modelled by equation.4.6., discussed in section.4.3.2., as shown in Fig. 5.12. as both dielectric constant and reactant concentration vary simultaneously. Initially the rate increases slowly with increasing concentration of alcohol upto 40% (v/v) of alcohol, after which the process becomes almost independent of alcohol concentration. This effect appeared in all systems studied.

The reaction profiles in Fig.5.13. show that an increase in the chain length of the alcohol decreases the reactivity of a particular ester, as demontrated by the degradation of n-propyl salicylate in 40% aqueous-methanol and 40% aqueous ethanol. The mechanistic model in Section 4.3.3.,Scheme 4.2. suggests that the reaction takes place by attack of the solvent molecules on the ionized ester. As the dielectric constant of the solvent changes (80 for water, 33.7 for methanol, 25.7 for ethanol and 21.8 for propanol) its ionizing capacity decreases and hence the ability to stabilise charged intermediate is reduced. Selective solvation on the other hand tends to increase the rate constants. As the medium changes from aqueous-methanol to aqueous-propanol, selective solvation becomes more prominant, as found in Chapter 4. A comparison of the rate constants is summarised in Table 5.5.

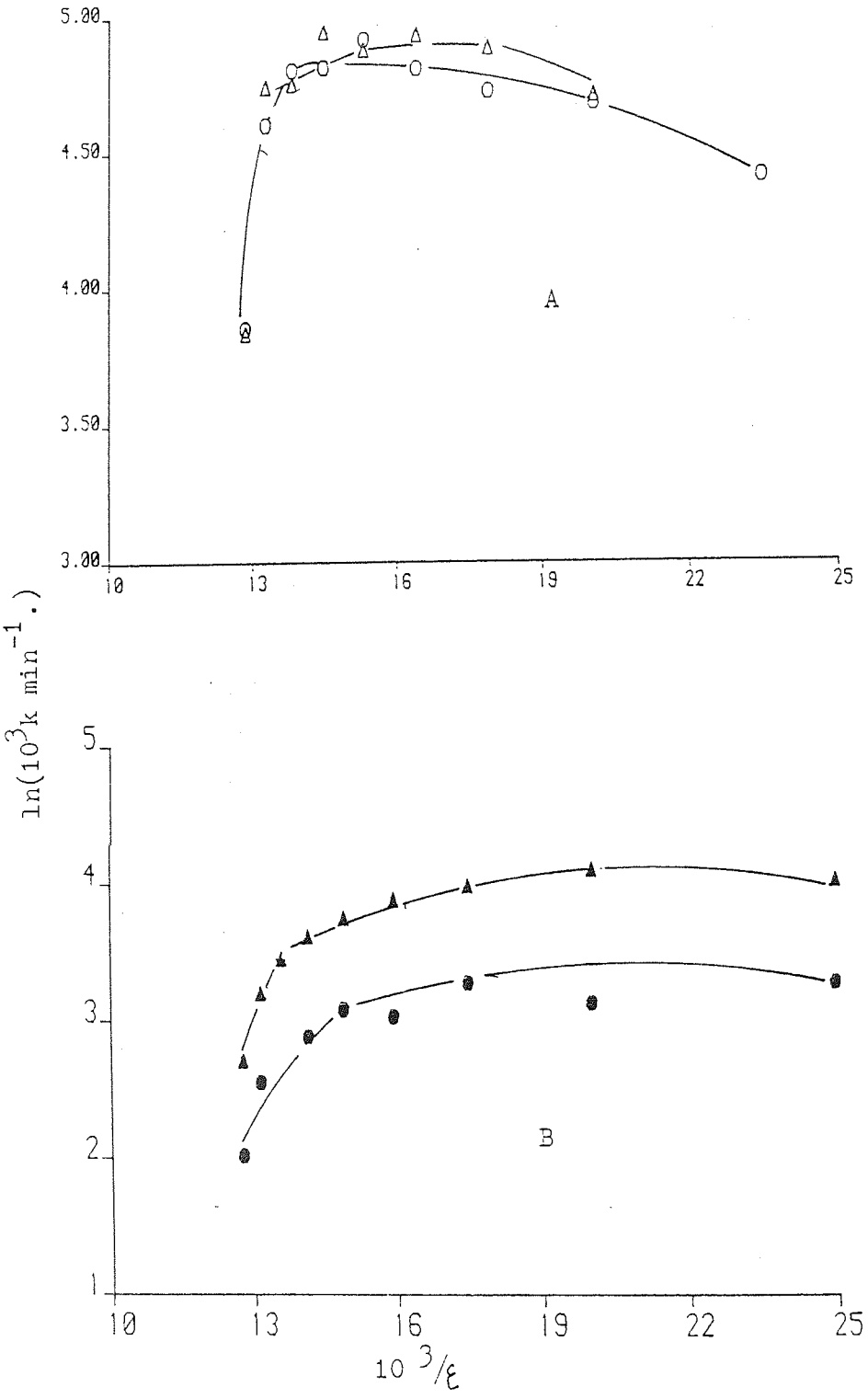


Fig. 5.12. Effect of solvent dielectric constant on transesterification of salicylates; A = methanol-water, B = ethanol-water

Symbol	Ester	Medium
Δ	ethyl	methanol
○	propyl	methanol
▲	methyl	ethanol
●	propyl	ethanol

Table 5.5. Specific Rate Constants for Degradation of Various Salicylates In Hydro-Alcoholic Medium, Using Kinetic Model A $\xrightarrow{k_1}$ B, A $\xrightarrow{k_2}$ C, A $\xrightarrow{k_3}$ C

% alcohol / % methanol (v/v)	Specific rate constants $k\ min^{-1} \times 10^3$			correlation coefficient		
	k_1	k_2	k_3	r_1	r_2	r_3
	n-propyl salicylate in aqueous-methanol					
10	47.1	11.40	13.20	0.999	1.0	0.999
20	99.1	9.6	11.50	0.999	0.999	0.999
30	121.0	8.12	9.83	0.994	0.995	0.999
40	122.0	7.43	7.08	0.999	0.997	1.000
50	135.0	5.89	5.81	0.999	0.998	0.999
60	121.0	3.53	3.96	0.996	0.993	0.994
70	111.0	0.215	2.72	0.999	0.997	0.999
80	106.0	4.46	1.37	0.998	0.997	0.997
90	81.0	0.013	0.59	0.999	0.993	1.000
% ethanol	n-propyl salicylate in aqueous-ethanol					
10	7.51	13.60	9.43	1.000	0.965	0.998
20	12.80	10.50	7.89	0.999	0.983	0.999
30	32.20	13.60	8.03	0.989	0.954	0.997
40	17.80	5.86	5.57	0.999	0.996	0.999
50	21.70	6.53	4.78	0.977	0.990	0.999
60	20.60	5.25	3.89	0.998	0.997	0.998
70	26.20	3.17	3.49	0.991	0.997	0.998
80	22.60	2.61	2.50	0.997	0.998	0.998
90	26.20	2.35	0.93	0.999	0.998	0.997

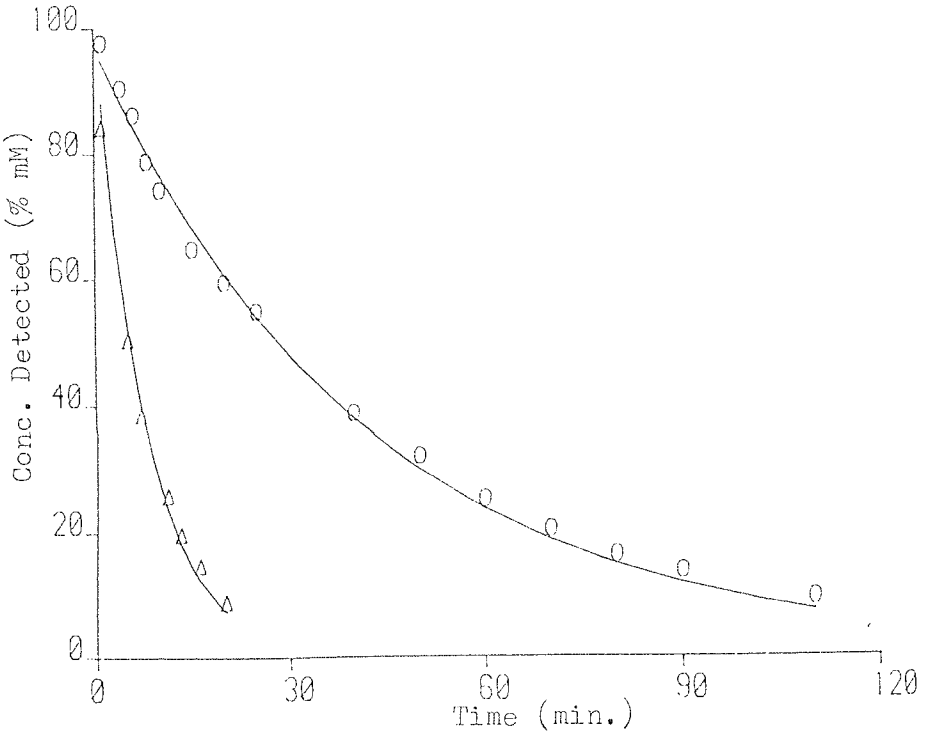
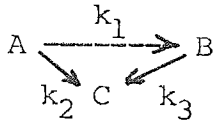


Fig. 5.13. Degradation of Propyl Salicylate in Alkaline Hydroalcoholic Media.

Symbol	Δ	O
Reaction medium	40% MeOH	40% EtOH

Table 5.5. (Contd.) Specific Rate Constants for Degradation of Various Salicylates in Hydro-Alcoholic Medium,

Using Kinetic Model



% alcohol	Specific rate constants $k\ min.^{-1} \times 10^{3}$			Correlation Coefficient		
% ethanol (v/v)	k_1	k_2	k_3	r_1	r_2	r_3
	methyl salicylate in aqueous - ethanol					
10	15.00	13.10	12.50	0.999	0.999	0.999
20	24.50	11.70	9.89	0.998	0.998	0.999
30	31.50	10.80	8.14	0.997	0.997	0.998
40	37.00	8.37	6.69	0.998	0.997	0.998
50	42.20	7.60	4.30	0.999	0.999	0.999
60	48.1	7.48	4.30	1.0	0.999	0.998
70	53.10	5.87	3.52	0.998	0.999	0.999
80	59.60	4.45	2.06	0.999	0.997	0.999
90	55.10	3.56	1.20	0.999	0.997	0.999
% methanol	ethyl salicylate in aqueous-methanol					
10	46.30	10.90	13.00	0.999	0.997	0.999
20	114.0	10.10	11.20	0.998	0.998	0.999
30	115.0	10.80	8.61	0.999	0.999	0.999
40	139.0	8.86	6.92	0.998	0.998	0.999
50	130.0	8.79	5.20	0.999	0.999	0.999
60	137.0	7.73	3.84	0.999	0.998	0.999
70	130.0	4.43	2.80	0.999	0.998	0.999
80	109	6.77	1.24	0.999	0.998	0.999
90	135.0	2.23	0.497	0.999	0.999	0.999

Both hydrolytic rate constants (k_2 and k_3) are reduced as the concentration of alcohol increases. The $1/\varepsilon$ against ln k plots, according to equation.4.6. for various hydrolytic processes are shown in Fig.5.14. Hydrolysis of various esters in methanol closely follows the equation. As the chain length in alcohol increases, this line deviates from linearity forming a shallow curve which may be represented by two intersecting straight lines with point of intersection near 50% of alcohol concentration, as observed in previous case. The coefficients, as calculated from the least squares linear regression of the data, shown in Fig. 5.14 are summarised in Tab. 5.6.

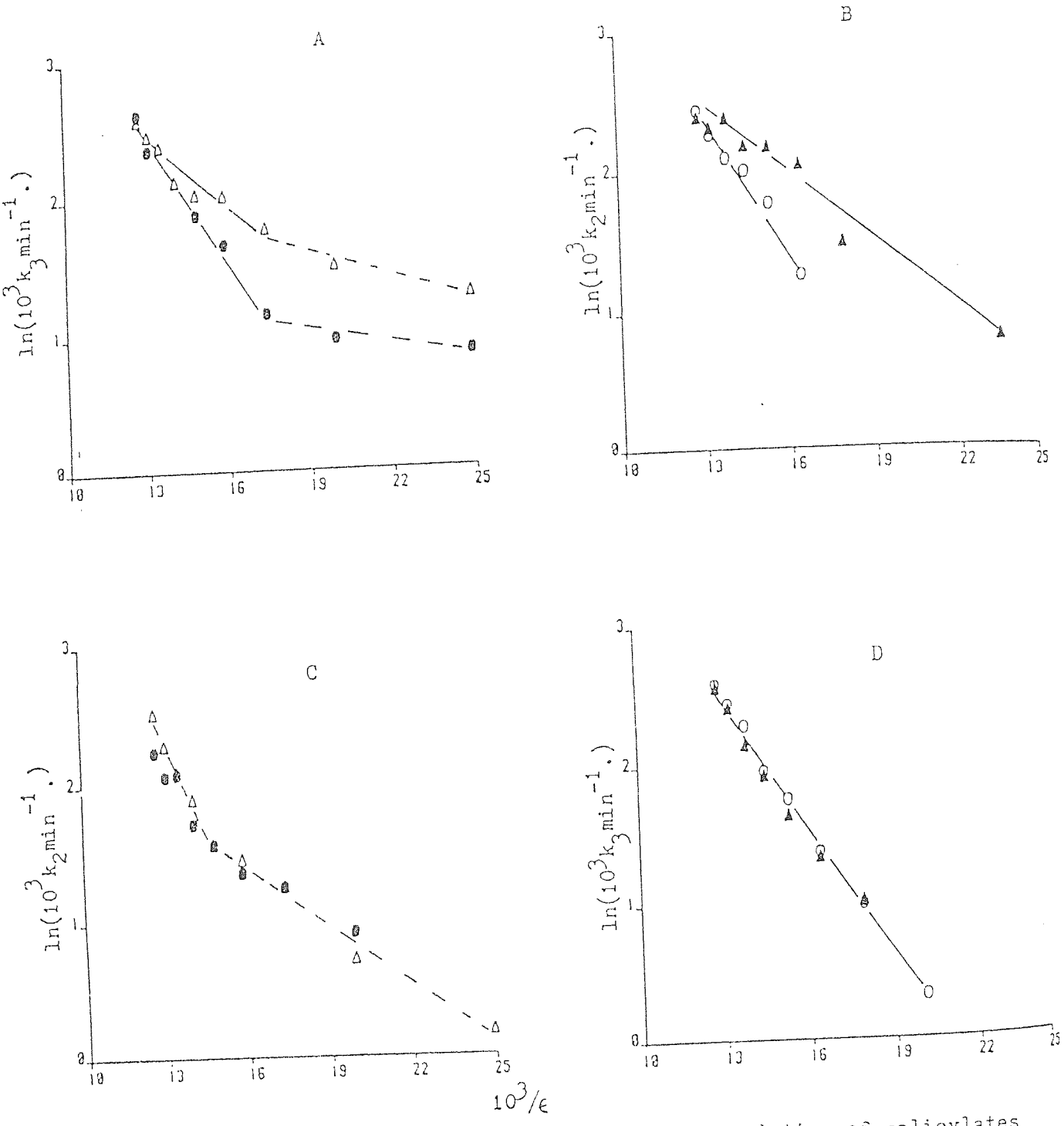


Fig. 5.14. Effect of solvent dielectric constant on degradation of salicylates

Rate processes and medium			Symbols	Component
A	k_3	in ethanol	Δ	Methyl ester
B	k_2	in methanol	▲	Ethyl ester
C	k_2	in ethanol	●	propyl ester
D	k_3	in methanol	○	propyl ester

Table 5.6. Coefficients Of Regression Lines Generated From Data held In Fig.5.14.

Reaction medium	Reactant	Source	Coefficients of regression line	n	r
aqueous methanol	methyl salicylate	from ethyl salicylate	$\ln k_3 = -0.4469 - 308.5413/\varepsilon$	7	0.992
	methyl salicylate	from propyl salicylate	$\ln k_3 = -0.6287 - 292.4781/\varepsilon$	8	0.996
	ethyl salicylate	ethyl salicylate	$\ln k_2 = -3.6818 - 67.6177/\varepsilon$	7	0.944
	propyl	propyl	$\ln k_2 = -0.5187 - 307.3059/\varepsilon$	6	0.986
aqueous ethanol	ethyl salicylate	from methyl salicylate	low alcohol : $\ln k_3 = 1.1668 - 437.981/\varepsilon$	5	0.996
			high alcohol : $\ln k_3 = -3.1976 - 143.5016/\varepsilon$	5	0.992
	ethyl salicylate	from propyl salicylate	low alcohol : $\ln k_3 = -2.1806 - 206.558/\varepsilon$	5	0.927
			high alcohol : $\ln k_3 = -2.4685 - 180.1492/\varepsilon$	3	0.996
	methyl salicylate	from methyl salicylate	low alcohol : $\ln k_2 = -0.7634 - 289.040/\varepsilon$	5	0.902
			high alcohol $\ln k_2 = -2.7650 - 162.4291/\varepsilon$	3	0.924
	propyl salicylate	propyl salicylate	low alcohol : $\ln k_2 = -0.8838 - 271.0243/\varepsilon$	5	0.932
			high alcohol : $\ln k_2 = -3.731 - 78.7994/\varepsilon$	4	0.961

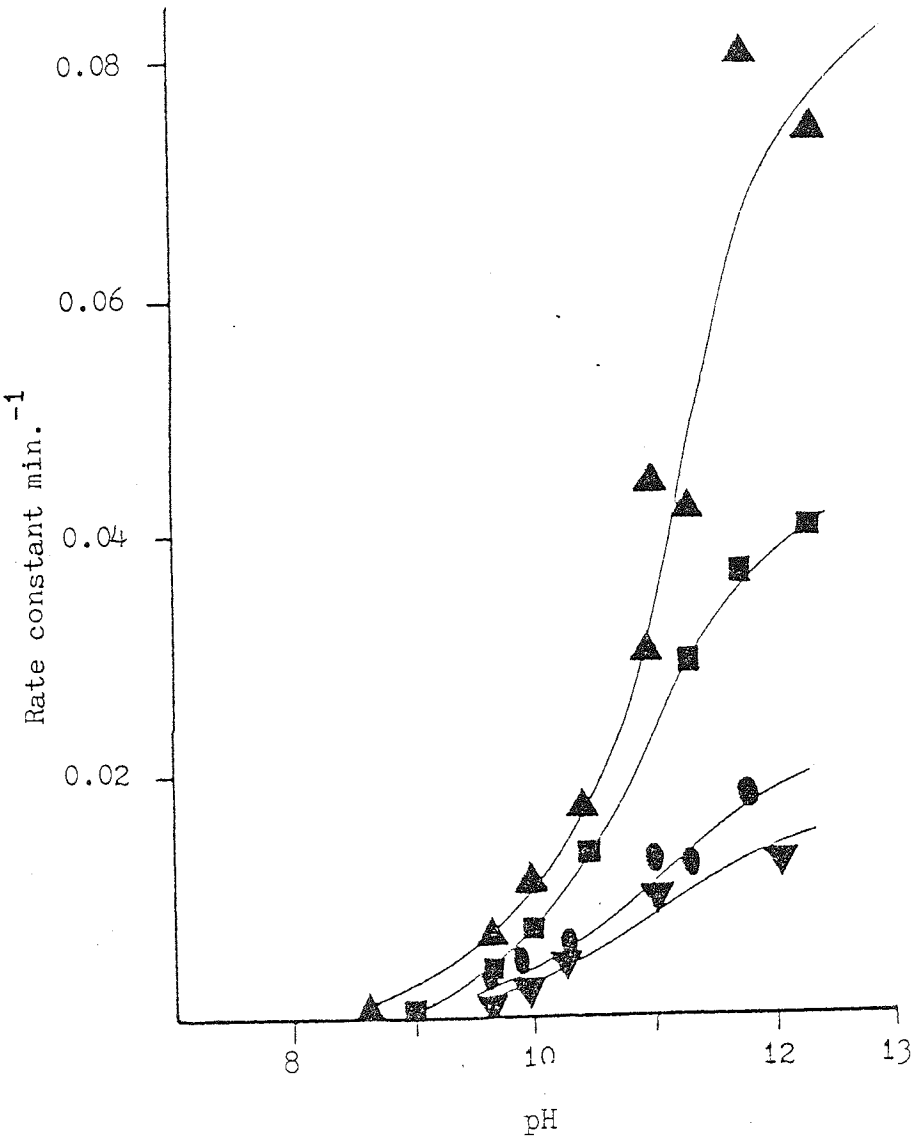


Fig. 5.15. Effect of pH on the transesterification and hydrolysis of methyl salicylate in 50% methanol:ethanol

Symbols	Specific rates
■	transesterification, forward
●	hydrolysis from methyl salicylate
▼	hydrolysis from ethyl salicylate
▲	transesterification, reversed

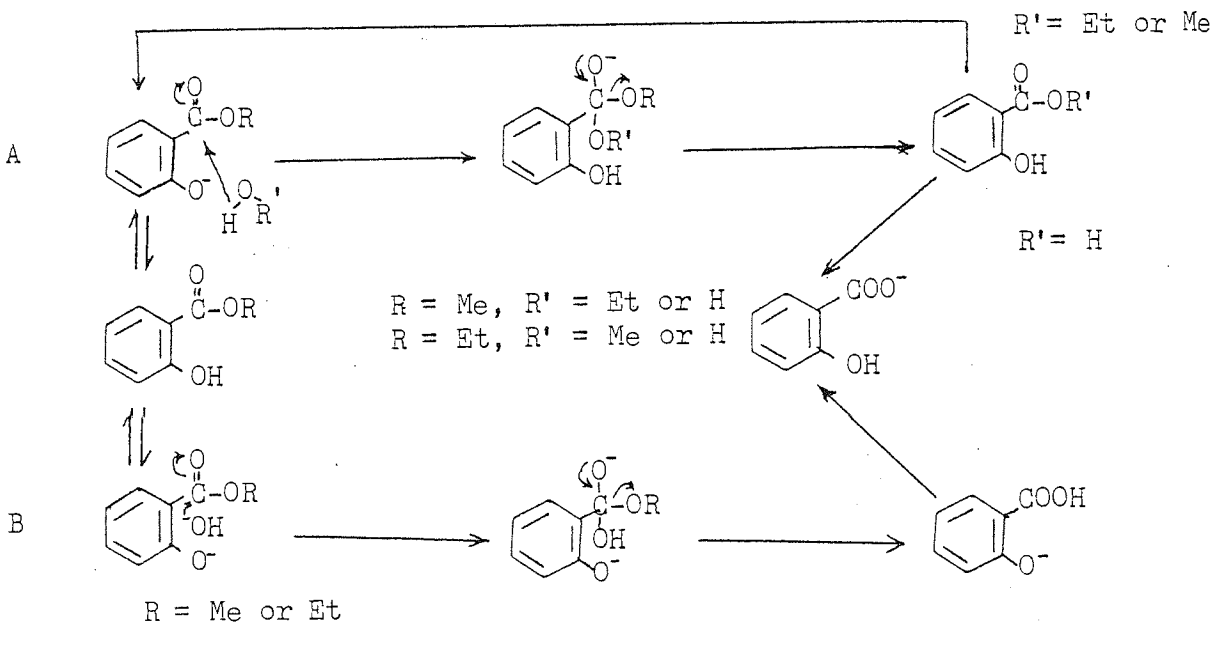
5.3.5. MECHANISM OF TRANSESTERIFICATION AND HYDROLYSIS OF SALICYLATES

The specific reaction rates, measured for the degradation of methyl salicylate in aqueous-equimolar methanol:ethanol (32:46 w/w; 50% v/v) in Britton-Robinson buffer (pH 7-12) are recorded in Tab 5.7. and the profiles are displayed in Fig. 5.15.

Table 5.7. Effect Of pH On the Degradation of Methyl Salicylate in Britton-Robinson Buffered Equimolar Methanol:Ethanol (50% v/v) at 50°C; μ = 0.5 M

pH	%[A^-]	Specific rate constants $min.^{-1} \times 10^3$				Correlation coefficients		
		k_1	k_2	k_3	k_{-1}	r_1	r_2	r_3
7.325	0.00843	0.0112	0.0053	0.0032	0.012	.996	0.989	0.991
8.595	0.0988	0.42	0.19	0.0002	0.51	.989	0.983	0.990
9.70	1.956	4.55	1.97	0.26	6.63	.998	0.992	0.999
9.923	3.23	4.10	1.38	0.91	5.17	.999	0.996	0.997
10.033	4.12	8.76	3.14	2.01	11.20	1.00	0.993	0.998
10.363	8.41	13.11	5.32	2.87	18.75	.998	0.996	0.997
10.982	27.64	30.70	12.70	10.00	45.80	.999	.985	1.000
11.309	44.78	29.20	12.40	13.70	42.50	.999	.991	0.999
11.723	67.78	37.40	17.00	10.60	79.60	.999	.980	0.995
12.246	87.523	40.48	12.72	36.65	73.57	.999	.981	0.999

Each curve in Fig.5.15. describing an individual rate constant is sigmoid with little degradation appearing below pH 8. Above this



Scheme 5.4. Mechanism of salicylate degradation in alkaline hydroalcoholic medium,
A = Weakly alkaline pathway
B = Strongly alkaline pathway.

value the rate of reaction increases rapidly upto pH 12. The pKa of methyl salicylate in 25.26% methanol-water at 30°C is reported as 10.5 (87). In 50% (v/v) aqueous equimolar methanol:ethanol at 37°C the apparent pKa was found to be 11.4 (data are recorded in appendix 4). Above a pH value of 8, the ionization of salicylate increases rapidly. This corresponds to the increase in the degradation rate of the ester which suggests that a possible major mechanistic pathway involves the reaction of the salicylate anion with the solvent. This reaction follows the weakly alkaline pathway shown in Scheme 5.4. In this pathway, the ionized ester forms a tetrahedral intermediate with the solvent molecule in the transition state. Eventually the original ester function is expelled by stabilisation of distributed charge. Capon et al (90) and Bender et al (91,151) proposed a similar mechanism in support of intramolecular catalysis by the phenolic group in the hydrolysis of phenyl salicylate and p-nitrophenyl 5-nitrosalicylate. To prove this hypothesis Capon et al studied the solvent deuterium - isotope effect and found a ratio for $k_1(H_2O)/k_1(D_2O)$ of 1.7 confirming this proposal. The weakly alkaline reaction pathway in Scheme 5.4. is confirmed by the linearity of the plots of anion percentage $[A^-]$ from salicylate against the observed rate constnat (k') which closely follows the relationship (134):

$$k' = k's + k''s\,[A^-] \quad \text{.....equn.5.18.}$$

where, k's = specific rate constant for attack of hydroxide or alkoxide ion on the salicylate molecule

k''s = specific rate constant for attack of solvent on

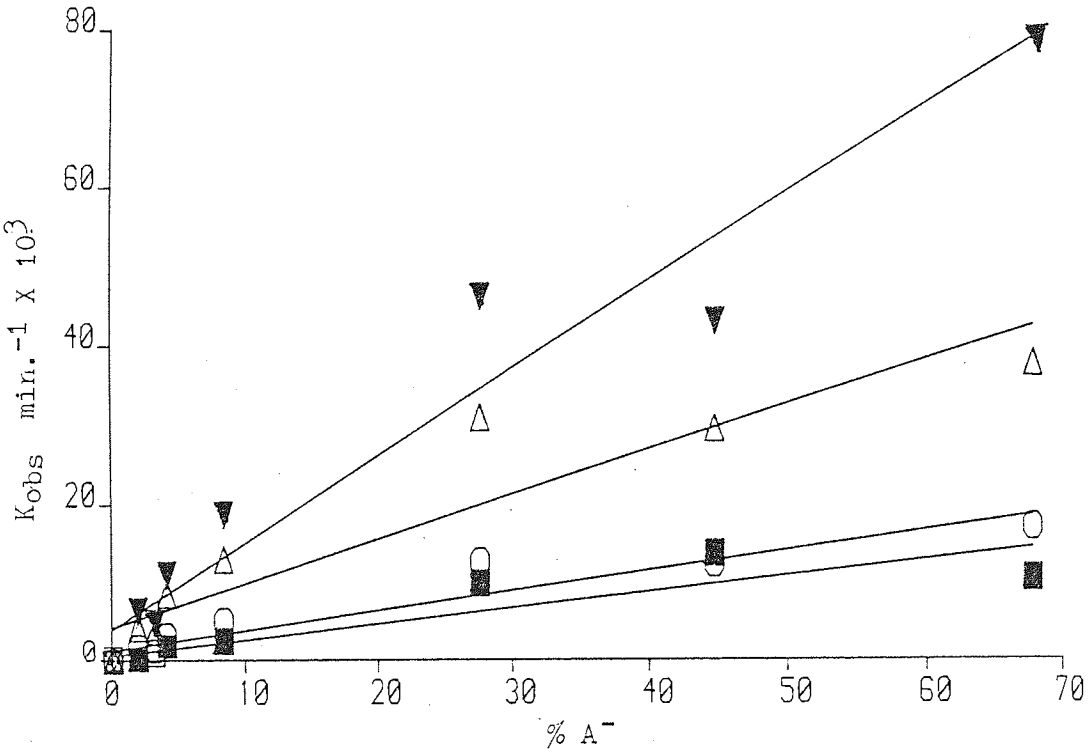


Fig. 5.16. K_{obs} Versus % of Salicylate Anion Plots for the Degradation of Methyl Salicylate in Equimolar Ethanol: Methanol in Britton-Robinson Buffer, Temperature 50°C, Initial Concentration 1mM, = 0.5M.

Symbol	Δ	O	■	▼
Specific Rate Constants	k_1	k_2	k_3	k_{-1}

the salicylate anion

$[A^-]$ = concentration of ionized salicylate ester

The coefficients of the lines are :

$$k_1' = 0.00449 + 0.000556\ [A^-] \quad (n = 9,\ r = 0.937)$$
$$k_{-1}' = 0.00423 + 0.00109\ [A^-] \quad (n = 9,\ r = 0.973)$$
$$k_2' = 0.00166 + 0.000250\ [A^-] \quad (n = 9,\ r = 0.954)$$
$$k_3' = 0.00098 + 0.000200\ [A^-] \quad (n = 9,\ r = 0.896)$$

Plots are presented in Fig. 5.16

As the hydroxyl ion concentration increases further, the conversion of salicylate ester to the anion becomes complete and the direct attack by hydroxide becomes a significant degradation pathway. This is represented by the strongly alkaline pathway in Scheme.5.4. In this instance, the observed rate constant (k) becomes dependent upon both solvent (k') and hydroxyl (k'') rate processes (134) which is represented by equation 5.19.

$$k = k' + k''[OH^-] \quad \text{.....equn.5.19.}$$

Where k = the observed rate constant

k' = specific rate constants for attack of solvent

k'' = specific rate constant for attack of hydroxyl ion

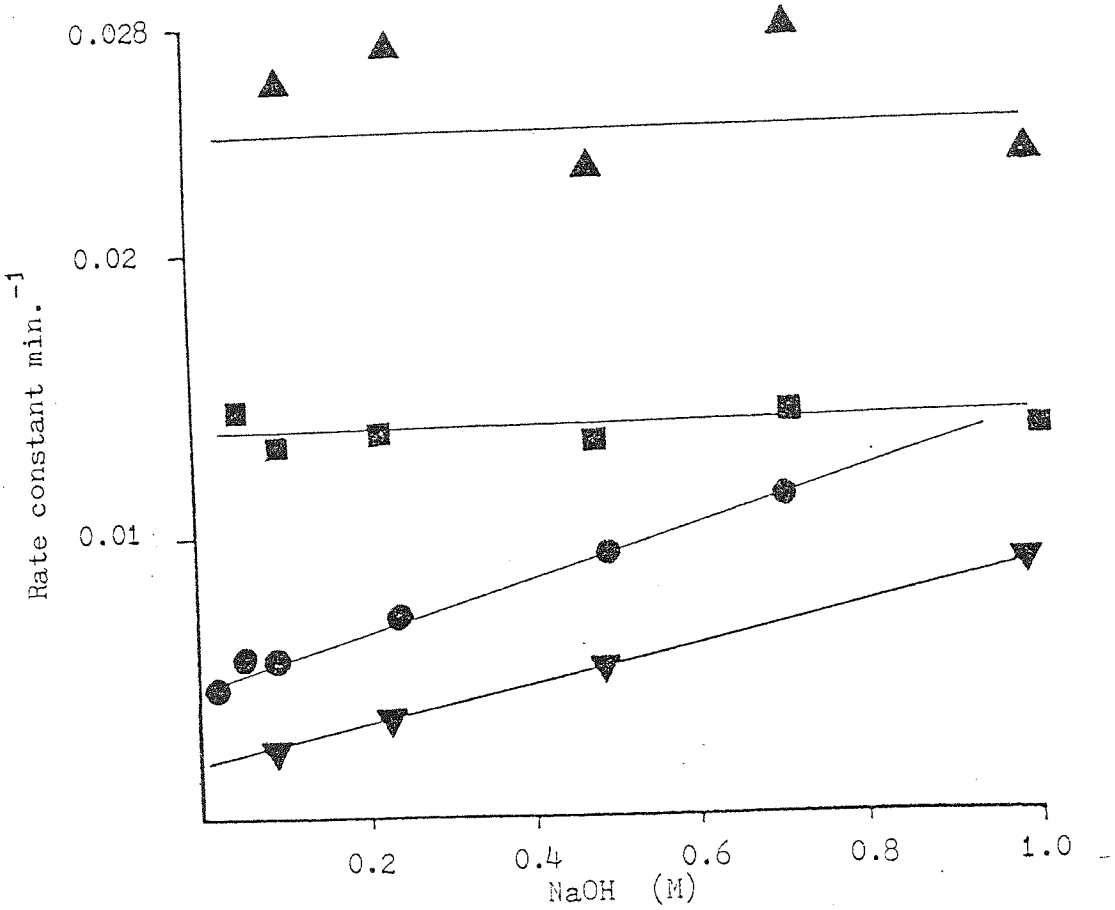


Fig. 5.17 Effect of Hydroxide concentration on the transesterification and hydrolysis of methyl salicylate in 50% aqueous methanol:ethanol

Symbols	specific rate constants
■	transesterification, forward
●	hydrolysis from methyl salicylate
▼	hydrolysis from ethyl salicylate
▲	transesterification, reversed

The degradation of methyl salicylate with Ø.Ø1-1 M sodium hydroxide in aqueous equimolar methanol:ethanol follows this Scheme closely. The specific rate constants are recorded in Tab.5.8.

Table 5.8. Effect of Concentration of Sodium Hydroxide on the Specific Rate Constants of Degradation of Methyl Salicylate (1 mM) in 5Ø% Equimolar Methanol : Ethanol, temp = 37°C;

[NaOH] (M)	Specific rate constants $k\ min^{-1} X\ 10^3$				correlation coefficient r		
	k_1	k_2	k_3	k_{-1}	r_1	r_2	r_3
Ø.Ø1	15.4Ø	4.666	3.337	32.4Ø	Ø.997	Ø.989	Ø.999
Ø.Ø5	15.1Ø	5.633	1.983	33.9Ø	Ø.998	Ø.989	Ø.999
Ø.1Ø	12.52	5.817	2.611	25.9Ø	Ø.996	Ø.994	Ø.998
Ø.25	13.66	6.647	3.735	25.9Ø	Ø.996	Ø.944	Ø.999
Ø.5Ø	12.8Ø	9.Ø49	4.7Ø9	22.ØØ	Ø.999	Ø.948	Ø.998
Ø.7Ø	14.2Ø	11.2Ø	3.1Ø	28.ØØ	Ø.998	Ø.977	Ø.998
1.ØØ	13.2Ø	11.4Ø	9.135	22.5Ø	1.ØØØ	Ø.988	Ø.999

Linear regression of the hydroxyl ion concentration against each observed rate constant is displayed in Fig.5.17. The figure reveals that once complete ionization of salicylate ester is achieved, the transesterification process (both forward and reversed) becomes independent of the concentration of the hydroxyl ion. In contrast, decomposition by hydrolysis proceeds through both concerted solvent attack and by direct hydroxide involvement.

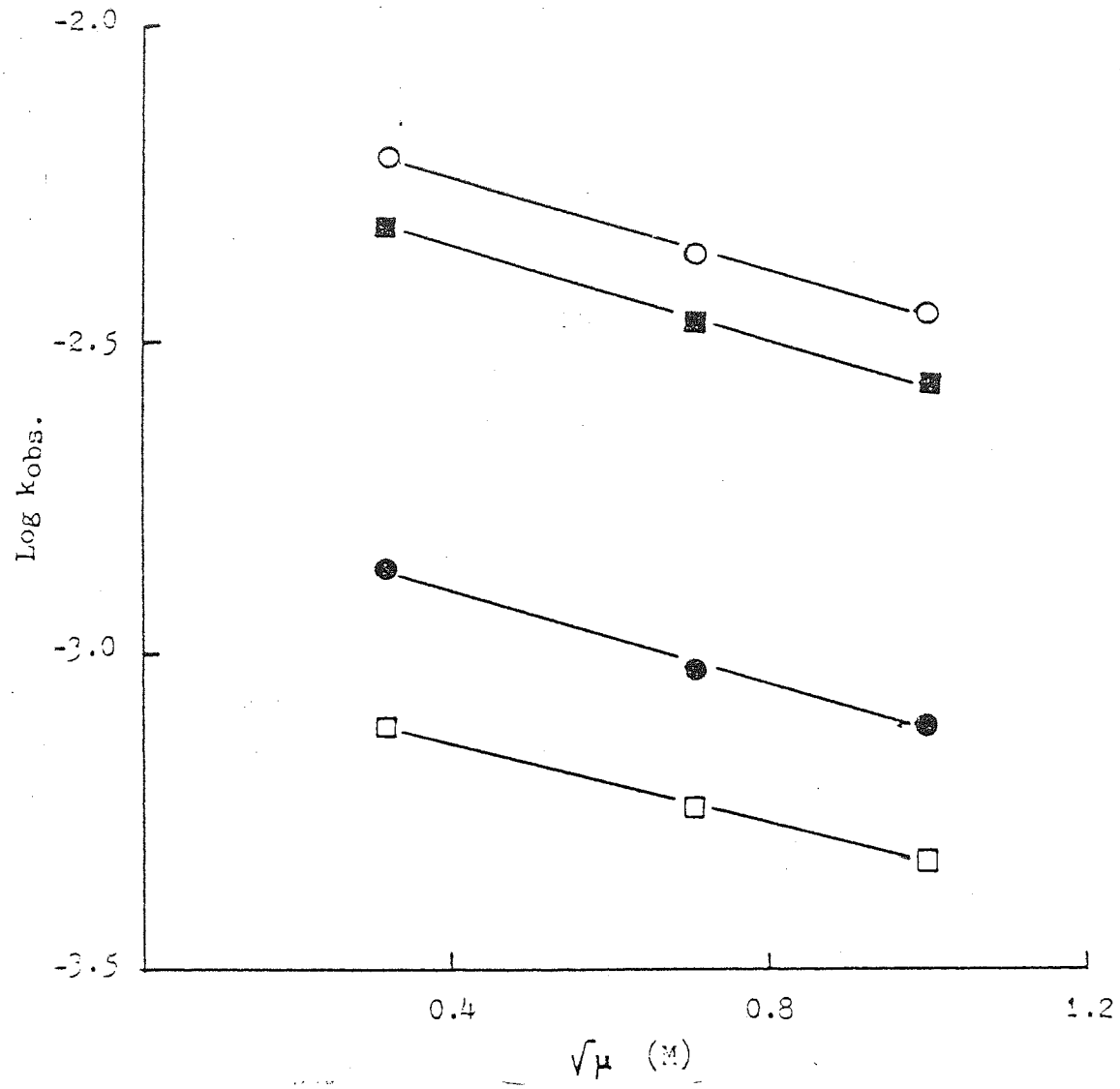


Fig. 5.18. Log k_{obs} vs $\sqrt{\mu}$ plot for degradation of methyl salicylate in equimolar methanol:ethanol pH 8.35; Temp. 50°C.; Initial conc. 1 mM;

Symbols :
- □ k_1 transesterification, forward
- ○ k_2 hydrolysis from methyl ester
- □ k_3 hydrolysis from ethyl ester
- ○ k_{-1} transesterification, reversed

The coefficients of the regression lines in Fig.5.17., according to equation 5.19. are :

$$k_2 = 0.00509 + 0.00718\ [OH^-] \quad ; \quad (r = 0.973,\ n = 7)$$

$$k_3 = 0.002187 + 0.00653\ [OH^-] \quad ; \quad (r = 0.964,\ n = 6)$$

The observed rate constants in Tab.5.9. at various ionic strength of Britton Robinson buffer (pH 8.35) in 50% (v/v) aqueous-equimolar methanol:ethanol for the degradation of methyl salicylate at 50°C were fitted to the equation 5.20.

$$\log k = \log k_{\mu=0} + 1.02\ Z_A Z_B \sqrt{\mu} \quad \text{.....equn.5.20.}$$

Where, $k_{\mu=0}$ = rate constant in an infinitely dilute solution

$Z_A\ Z_B$ = charge on the reactants A and B.

The regression lines are presented in Fig.5.18 together with the coefficients of regression in Tab. 5.10. A negative effect on the rate is observed when the ionic strength is increased. However, this effect corresponds to a slight decrease in pH as shown in Tab.5.9.

Table 5.9 Effect of Ionic Strength on the Specific Rate Constants of Degradation of Methyl Salicylate in 50% (v/v) Aqueous Equimolar Methanol:Ethanol (32:46 w/w) at 50°C, A_0 = 1 mM

Ionic strength μ (M)	pH of the medium	Specific rate constant $k\ min^{-1} \times 10^3$				Correlation coeffecient (r)		
		k_1	k_2	k_3	k_{-1}	r_1	r_2	r_3
1.00	9.787	3.411	1.153	0.755	4.360	0.997	0.996	0.997
0.50	9.862	4.104	1.376	0.914	5.165	0.999	0.996	0.998
0.0993	9.915	5.592	1.950	1.165	7.113	0.999	0.996	0.998

Table 5.1Ø Coefficients Of Regression Lines held in Fig. 5.18

Specific process	Coefficient of regression line	n	r
transesterifcation of methyl salicylate	$\log k_1 = -6.844 - 3.172\ \mu$	3	Ø.998
reversed rate of transesterification	$\log k_{-1} = -6.495 - 3.165\ \mu$	3	Ø.996
hydrolysis of methyl sal	$\log k_2 = -7.669 - 2.941\ \mu$	3	Ø.996
hydrolysis of ethyl sal	$\log k_3 = -1Ø.413 - 3.658\ \mu$	3	Ø.9999

Similarly, a slight decrease in specific rate constants was observed with dilution of the buffer salt. Tab.5.11. reveals that this effect corresponds to the decrease in pH resulting from dilution.

Table 5.11 Degradation of Methyl Salicylate in Britton-Robinson Buffer Effect Of Concentration Of Buffer Salt; μ = Ø.Ø993; Solvent Methanol:Ethanol 5Ø%v/v
Temperature 5Ø°C; Initial Concentration of Methyl Salicylate 1 mM.

Concentration of buffer salt	pH of the solvent	Rate constants, $min^{-1} X\ 1Ø^3$						
		k_1	k_2	k_3	K_{-1}	r_1	r_2	r_3
1ØØ%	9.83	4.5Ø94	1.541	Ø.1579	6.8Ø42	.998	.999	.997
25%	9.73	3.755	1.221	Ø.1Ø73	6.3Ø73	.998	.996	.997

CHAPTER 6 TRANSESTERIFICATION OF PHENYL SALICYLATE

6.1 INTRODUCTION

During the course of the work described in chapter 4 and 5, a publication appeared which described the stability of phenyl salicylate in highly alkaline medium with ethanol as a co-solvent (89). Ultraviolet spectroscopic method was used to monitor the degradation profiles following disappearance of phenyl salicylate at 340 nm. A few more reports were found (90- 92) on the stability of phenyl salicylate and substituted phenyl salicylate in which ethanol was used as a co-solvent to overcome the solubility problem. Bender et al (91) used 32.8% (w/w) ethanol in phosphate buffer within the pH range 6.8 - 8 at 25^{o}C. It has been shown in chapter 5 of this thesis that in weakly alkaline medium the transesterification process may become the major route of degradation. Since the work of Bender et al was conducted at 25^{o}C, it may be assumed that the reaction conditions were favourable towards transesterification instead of hydrolysis. Capon and Ghosh (90) conducted the hydrolysis of phenyl salicylate, p-nitro phenyl salicylate, salicyl salicylate and other structurally related compounds in 0.1% ethanolic solution within the pH range 6.58 -11.5 at 59.2^{o}C. Since the temperature was very high and the level of ethanol in the reaction medium was low, only trace amount of ethyl salicylate may be expected to be formed during the course of the reaction. However, these authors failed to detect the transesterification process possibly due to the non-specificity of ultraviolet spectroscopic method which they used for analysis. Recently Khan et al (89) have used phenyl salicylate as the test ester in modelling the effect of alkalinity, dielectric constant and temperature on salicylate ester hydrolysis. Although

only 4% ethanol was used as co-solvent to ensure complete solubility of the ester in monitoring the effect of alkali and temperature, a wider range (5-95% ethanol) was used in determining the effect of solvent dielectric constant. According to the report of Capon and Ghosh(90), the rate constant of hydrolysis of phenyl salicylate at pH 11.5, temperature 59.2°C is 7.12 X 10^{-3} $Sec.^{-1}$ Table 4.2. of this thesis shows that the rate of hydrolysis of methyl salicylate in 50% aqueous methanol at 58.4°C, pH 12.0 is 0.95 X 10^{-3} Sec^{-1}. This suggests that phenyl salicylate is much more reactive (~8 fold) than methyl salicylate. The present study also evidenced that transesterification is a general phenomenon in alkaline hydroalcoholic medium with salicylate esters having a leaving group different from that of the alcohol group of the medium. Considering a particular condition of degradation of methyl salicylate in aqueous-ethanol (30% v/v, 0.01 M NaOH, 37°C, pH 12.0) it has been found that ethyl salicylate forms as an intermediate, the concentration of which reaches to a maximum in ~40 mins. This is equivalent to ~53% of the initial concentration of methyl salicylate. Due to these facts, it was felt necessary to develop an assay method which would allow simultaneous analysis of all potential reaction components of degraded phenyl salicylate to provide an insight into the true degradation pathway of this compound.

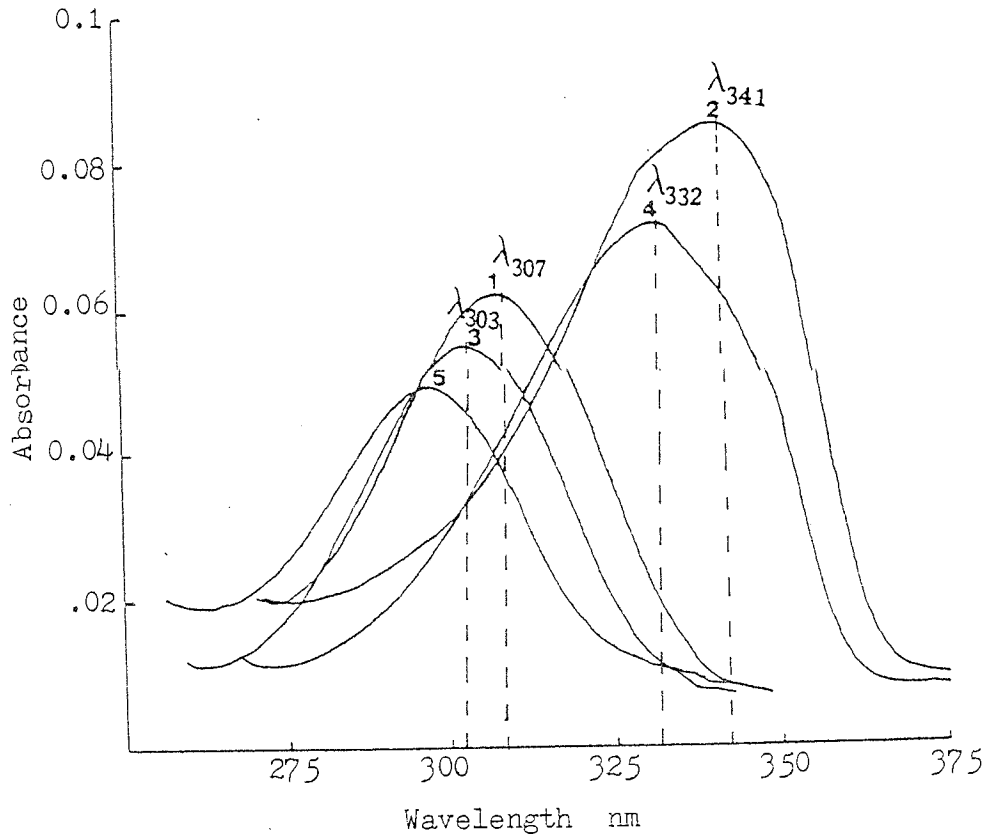


Fig. 6.1. Ultraviolet Spectral Change in Phenyl and Ethyl Salicylate (Conc. 0.1 mM) and Salicylic Acid (Conc. 0.1 mM)

1 = Phenyl Salicylate in 4% Ethanol
2 = Ethyl Salicylate in 4% Ethanol
3 = Phenyl Salicylate in 4% Ethanol in Alkali (.05M NaOH)
4 = Ethyl Salicylate in 4% Ethanol in Alkali (.05M, NaOH)
5 = Salicylic Acid in 4% Ethanol in Alkali (.05M, NaOH)

Condition	λ_{max} nm		ϵ_{max}	
	Et.Ester	Ph.Ester	Et.Ester	Ph. Ester
Neutral Soln.	302-304	306-309	3790.32	4354.88
Alkaline "	332-333	340-342	4600.00	6200.00

6.2. RESULTS AND DISCUSSION

6.2.1. ULTRAVIOLET ABSORPTION SPECTRUM OF PHENYL AND ETHYL SALICYLATE

Ultraviolet absorption spectra of phenyl and ethyl salicylate are presented in Fig.6.1. This compares the absorption of ethyl and phenyl salicylate and shows the spectral changes which occur on the addition of alkali. Salicylic acid has also been included for reference. In neutral solution (4% aqueous-ethanol) both esters give similar absorptions with the λ max being at 302 -304 nm (ε_{max} =3790) for ethyl salicylate and 306 - 309 nm (ε_{max} = 4354) for phenyl salicylate showing a some what higher extinction. Immediately after addition of alkali (0.05 M NaOH in 4% ethanol) the peaks shift to a lower wavelength with the λ max at 332 -333 nm (ε_{max}=4600) for ethyl ester and 340 - 340 nm (ε_{max} =6200) for phenyl ester due to the formation of phenoxide ion. Khan and co-workers (89) followed the gradual decrease in absorbance at 340 nm and interpreted that in terms of simple first order degradation of phenyl salicylate. The chromatographic analysis however, shows the formation of ethyl salicylate which reaches a maximum concentration in 4% ethanol of 26- 37% of the initial concentration of phenyl salicylate. At 340 nm phenyl salicylate ion has the extinction of 6200 and ethyl salicylate exhibits an extinction of 4600 . This significant contribution of ethyl salicylate to the absorbence at 340nm ensures that the degradation profile of phenyl ester cannot be adequately monitored in a system where transesterification is evident.

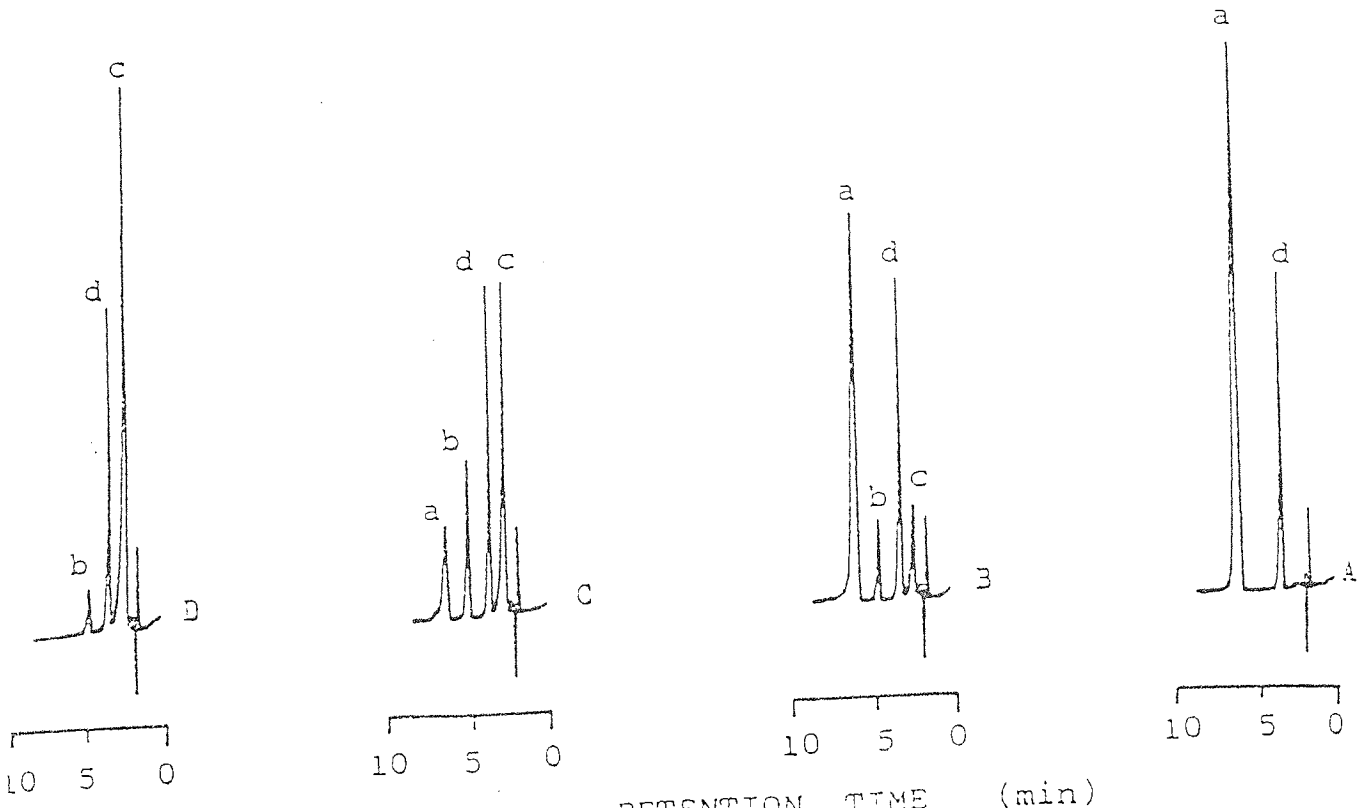


Fig. 6.2. High-Performance Liquid Chromatography of Ph-Salicylate
Initial Conc. = 0.124 mM ; NaOH = 0.05 M ; Temp. 35° C.

Chromatograms	Peaks	Salicylates	HPLC Conditions
A t = 0 min	a	Phenyl	Mobile phase; $CH_3CN:H_2O:H_3PO_4$ 60:39.9:0.2; pH 2.0
B t = 5 min	b	Ethyl	Flow rate : 1 ml/min.
C t = 25 min	c	Salicylic acid	Detection :.08,AUFS,235 nm
D t =120 min	d	Int. Std.Butyl Paraben	Column :10cm X 4.6mm, Hypersil-ODS

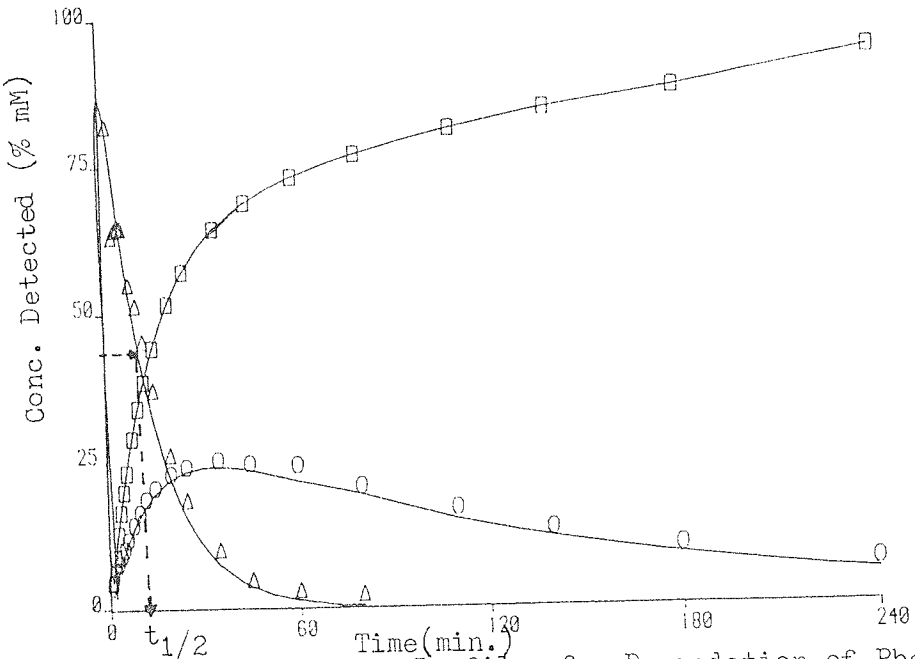


Fig. 6.3. Concentration-Time Profiles for Degradation of Phenyl Salicylate in Aqueous Ethanol, Temp. 35°C., NaOH = 0.05M ; 4% Ethanol

Symbol	Salicylates	Specific Rate Constants ($min.^{-1}$)	
Δ	Phenyl	k_1	0.024
O	Ethyl	k_2	0.0494
□	Salicylic Acid	k_3	0.00787

6.2.2. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND RELATIVE RATE OF DEGRADATION

Chromatograms following degradation of phenyl salicylate in aqueous-ethanol in experimental conditions similar to that used by Khan *et al* (89) (4%v/v in Ø.Ø5M NaOH, 35°C, $A_{Ø}$=Ø.124 mM) are displayed in Fig.6.2. The initial trace (t=Ø) shows only phenyl salicylate and the internal standard. After 5 minutes the chromatograms reveal a significant decrease in the intensity of the shenyl salicylate peak together with the appearance of two new peaks. The early peak corresponds to salicylic acid, and the later one has been identified as ethyl salicylate by retention time and by isolation(134). After 25 minutes very little phenyl ester remains although ethyl salicylate is still a major component. Degradation of ethyl salicylate takes a much longer, as shown in the chromatogram. After 12Ø mins. ethyl salicylate, equivalent to 1Ø% of the initial concentration of phenyl salicyate, is still present. The fate of each component of the reaction mixture in Fig.6.2. is displayed by the concentration-time profiles in Fig. 6.3. These profiles also reveal the rapid disappearance of phenyl salicylate together with the formation of ethyl salicylate, which undergoes slow hydrolysis to salicylic acid. The time-courses were fitted to equations 5.5-5.7. by NONLIN and are displayed by the curves in Fig.6.3. The kinetic model represented by equation 5.1. wa used to measure the specific rate constants which are recorded in Tab.6.1. The half-life for the overall disappearance of phenyl salicylate is determined from the measured rate constants ($k_1 + k_2$) as 9.44 mins. and is indicated in Fig. 6.3. The true half life of hydrolysis was found to be 14.03 mins.

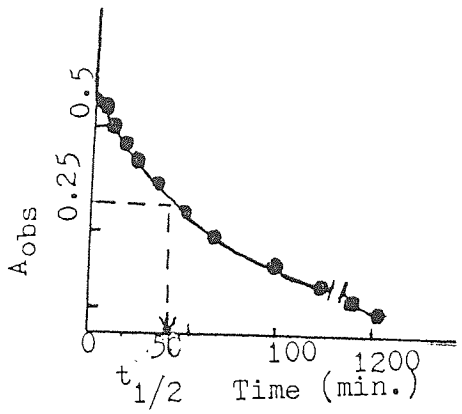


Fig. 6.4. Concentration - Time Profiles Reproduced from Fig. 1. of the Experiment of Khan et al.(89) Showing the Degradation of Phenyl Salicylate.

A_o= 0.124 mM, EtOH Conc.= 4% v/v, NaOH = 0.05 M, Temp. 35° C.

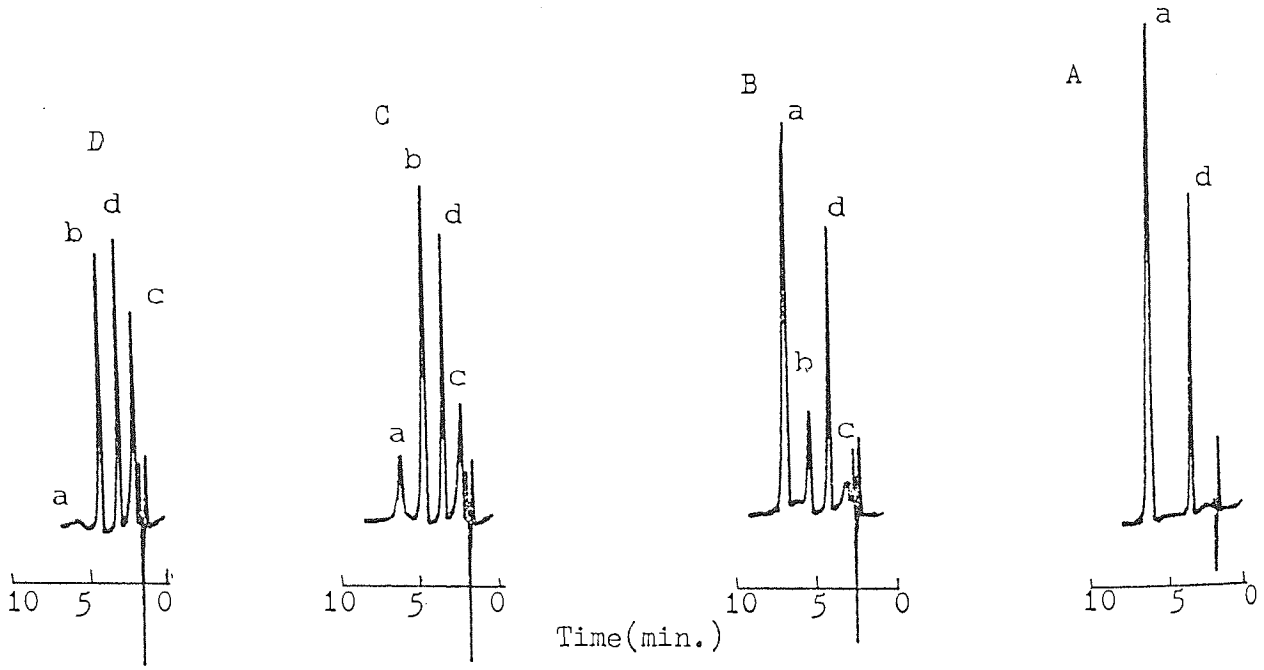


Fig. 6.5. High Performance Liquid Chromatography of Phenyl Salicylate. Initial Conc. = 0.124mM, NaOH = 0.05M, Temp. 35° C., EtOH=20%

Chromatograms	Peaks	Salicylates	HPLC Conditions
A t= 0 min.	a	Phenyl	Mobile Phase: $CH_3CN:H_2O:H_3PO_4$ 60:39:8:0.2, pH 2.0
B t= 3 min	b	Ethyl	
C t=30 min.	c	Salicylic acid	Detection: 0.08AUFS, 235nm
D t=120 min.	d	Int. std.; butyl Paraben	Column : 10 cm X 4.6 mm, Hypersil - ODS.

mins. The same parameter describing the hydrolysis of ethyl salicylate was calculated as 88.1 min. The degradation profile presented by Khan et al(89) is reproduced in Fig.6.4, which shows the half-life of disappearance of of phenyl salicylate is 40 minnutes, (calculated value from their data is 39.8 mins.). It is obvious that the ultraviolet assay procedure underestimates the true hydrolysis rate due to the slower removal of the absorbing transesterified ethyl salicylate. The rate and the extent of the transesterification process, however, depends upon the proportion of ethanol in the solvent. The chromatograms in Fig.6.5. shows that in 20% ethanol phenyl salicylate degrades rapidly essentially via transesterification to yield ethyl salicylate. The fate of these components is modelled by typical NONLIN fit in Fig. 6.6., which shows that the concentration of ethyl salicylate reaches a maximum concentration equivalent to 70% of the initial concentration of phenyl salicylate within 40 mins. The previous investigators assumed that hydrolysis was the only degradation pathway and the reaction followed a simple first-order kinetics. However, HPLC analysis reveals that the non-specificity of the ultraviolet spectroscopic method misled the investigators.

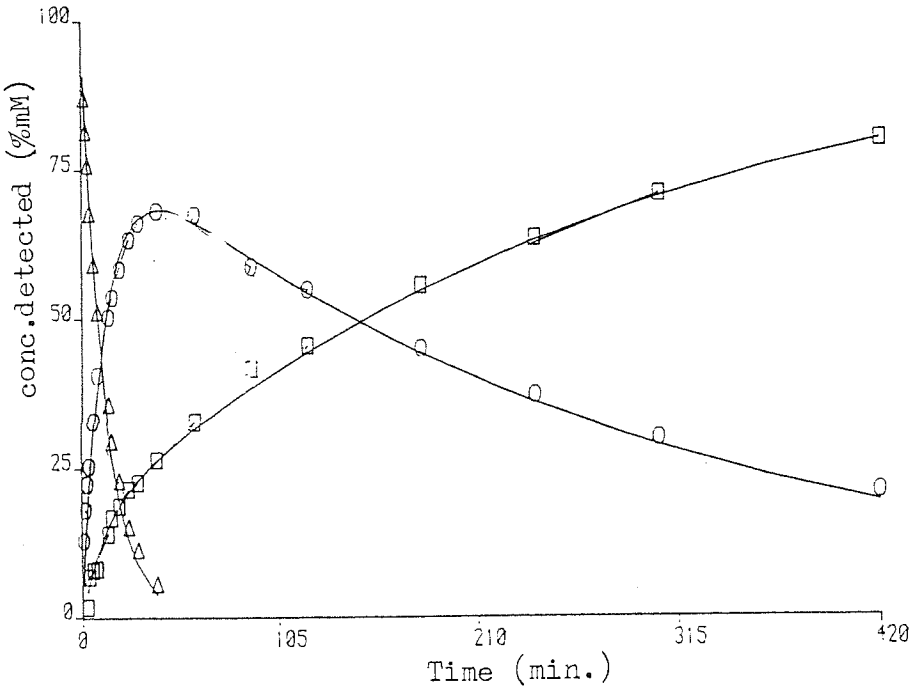
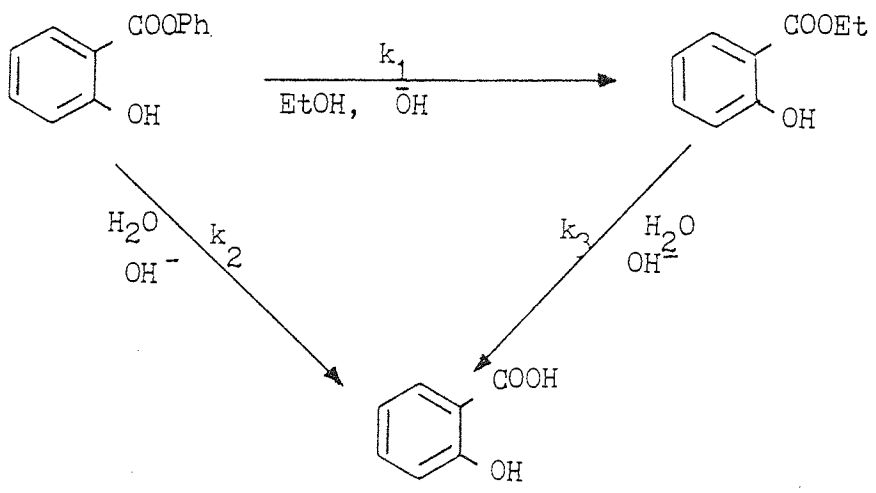


Fig. 6.6. Concentration - Time Profiles Showing the Degradation of Phenyl Salicylate in Aqueous Ethanol, Temp. 35° C., NaOH = .05M, EtOH = 20% v/v

Symbols	Salicylates	Specific Rate Constants k k min.$^{-1}$ X 10^3		
		k_1	k_2	k_3
Δ	Phenyl	12.7	5.6	13.4
○	Ethyl			
□	Salicylic acid			

6.2.3. EFFECT OF CONCENTRATION OF ETHANOL ON SPECIFIC RATE CONSTANT

The control experiment containing phenol and ethyl salicylate in the reaction medium failed to generate phenyl salicylate. This suggests that the transesterification process proceeds through an irreversible kinetic model, represented by equation 5.1. The overall process is displayed by Scheme 6.1. Specific rate constants were estimated fitting the concentration-time profiles to Scheme 6.1. Primary estimates of the specific rate constants were determined from the slopes of the first-order plots describing the overall disappearance ($k_1 + k_2$) of phenyl salicylate, displayed in Fig.6.7. The effect of alcohol concentration on each kinetic process is shown by specific rate constants in Table 6.1.



Scheme 6.1. Transesterification and hydrolysis of phenyl salicylate

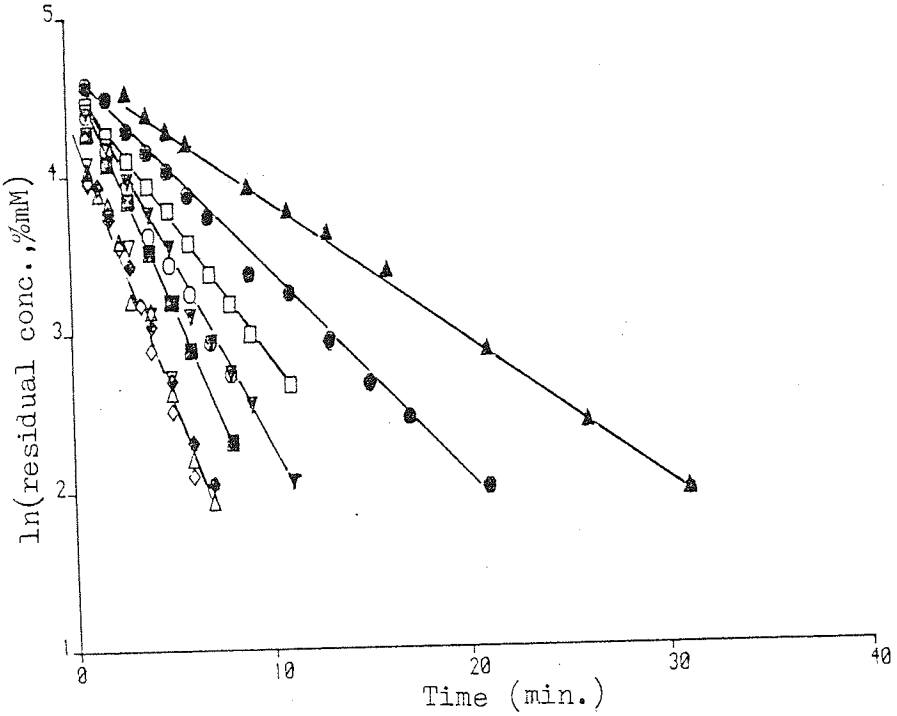


Fig. 6.7. First Order Plots Showing the Effect of Concentration of Ethanol on Degradation of Phenyl Salicylate. Temp. 35° C, NaOH = 0.01M, A_0 = 0.5 mM.

Symbol	▲	●	□	▼	○	■	△	◆	▽	◇
% EtOH	5	10	20	30	40	50	60	70	80	90

Table 6.1. Effect of Ethanol Concentration on the Transesterification and Hydrolysis Rates of Phenyl Salicylate; Temp.=35°C, NaOH=Ø.ØlM Initial Concentration = Ø.5 mM

%EtOH		Rate const.k min^{-1} X 10^3			Correlation coefficient (r)			$(k_1+_3k_2)min^{-1}$ X 10^3 from fig.6.7	r
		k_1	k_2	k_3	r_1	r_2	r_3		
5	79.11	28.3	55.1Ø	21.4Ø	.987	.981	.997	89.5Ø	.999
1Ø	78.35	72.3	6Ø.Ø	15.3Ø	.998	.994	.998	131.3Ø	.998
2Ø	76.13	127.Ø	56.1Ø	13.4Ø	.999	.994	.996	18Ø.6Ø	.999
3Ø	73.69	175.Ø	5Ø.4Ø	11.5Ø	.998	.994	.995	24Ø.ØØ	.997
4Ø	7Ø.79	194.Ø	41.2Ø	8.11	.999	.993	.998	238.7Ø	.999
5Ø	67.26	236.1	39.1Ø	6.Ø6	.995	.996	.996	287.6Ø	.998
7Ø	57.36	368.Ø	36.ØØ	4.29	.989	.992	.991	355.8Ø	.992
8Ø	5Ø.Ø9	39Ø.Ø	13.3Ø	3.Ø4	.99Ø	.979	..997	331.7Ø	.998

The transesterification rate increases dramatically with increasing concentration of ethanol giving an almost linear relationship. The linear least squares regression parameters are.

$$k_1 = 4.731 \times 10^3 \ (EtOH\%) + Ø.Ø1877 \ ; \ r = Ø.994, \ n = 9.$$

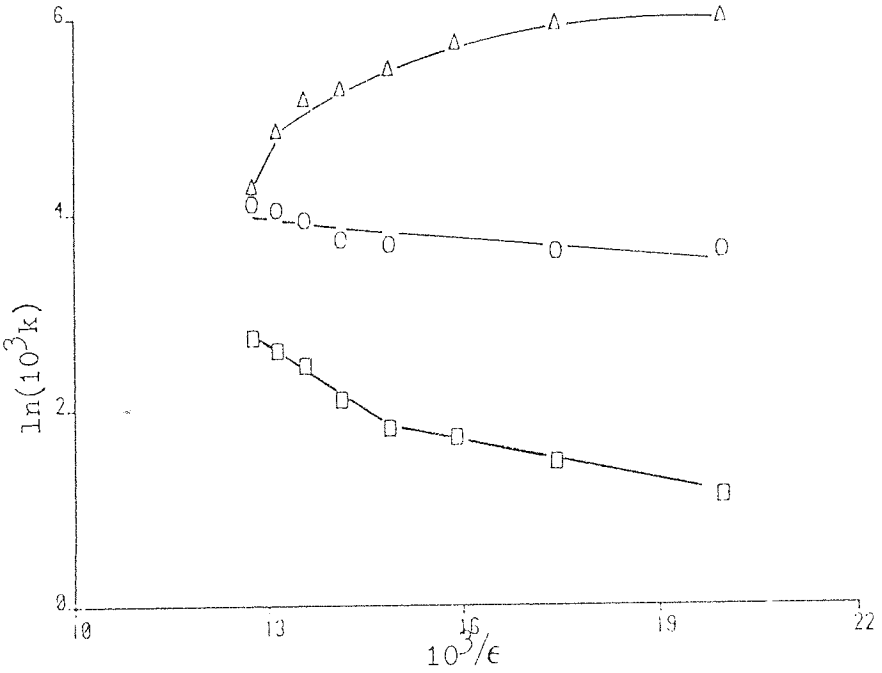


Fig. 6.8. Dependence of the Specific Rate Constants for the Degradation of Phenyl Salicylate in Aqueous Ethanol on Solvent Dielectric Constant.

Symbol	k Specific Rate Constants
Δ	k_1 Transesterification of Phenyl Salicylate
O	k_2 Hydrolysis of Phenyl Salicylate
□	k_3 Hydrolysis of Ethyl Salicylate

In contrast, both hydrolysis rates (from phenyl salicylate and from ethyl salicylate), are suppressed with increasing concentration of alcohol. The rate constants of hydrolysis (k_2 and k_3) follow equation 4.6., which models the variation in the degradation constant with the change in dielectric constants caused by solvent change. The $10^3/\varepsilon$ Vs $\ln(10^3\ k\ min^{-1})$ plots of this data are shown in Fig.6.8. The linear least squares parameters for these specific rate constants of hydrolysis according to equation 4.6.are

Phenyl salicylate hydrolysis: $\ln k_2 = 0.175 - 208/\varepsilon$, r=0.998, n=6

Ethyl salicylate hydrolysis:

low alcohol : $\ln k_3 = 1.642 - 454/\varepsilon$, r=0.996, n=5

high alcohol : $\ln k_3 = -3.019 - 139/\varepsilon$, r=0.997, n=4

The k_3 plot, representing hydrolysis of ethyl salicylate shows a change in slope near 50% ethanol and both lines are reported individually. This effect parallels that observed previously, viz, when the hydrolysis of ethyl salicylate in aqueous ethanol was followed (Section 4.3.2.) and also when the hydrolysis of the transesterified ethyl salicylate from propyl or from methyl ester was monitored in aqueous ethanol (Section 5.3.4.). However, equation 4.6 cannot be used in modelling the effect of dielectric constant on transesterification as the reactant concentration is simultaneously altered.

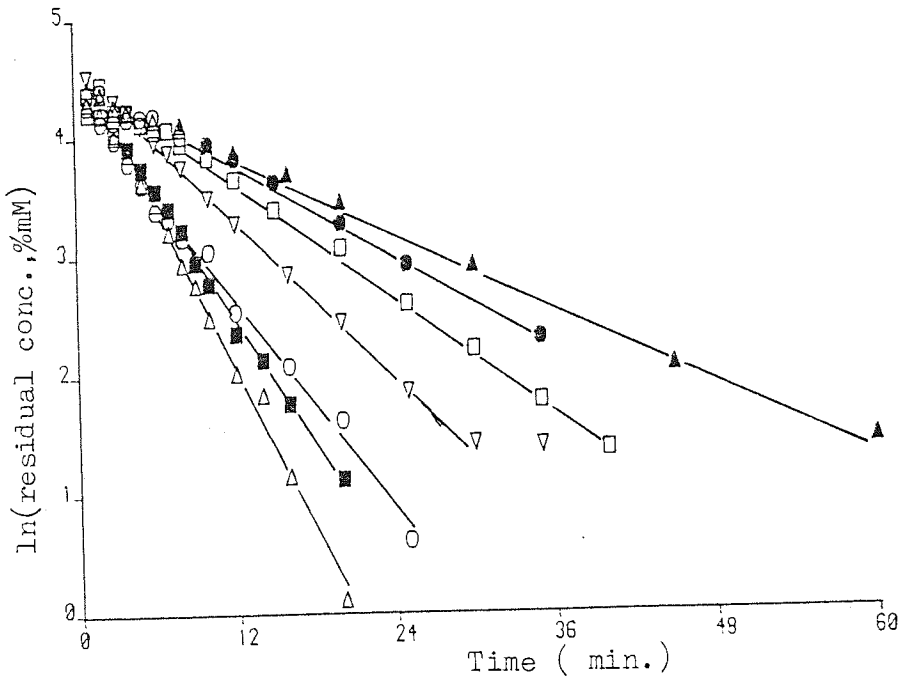
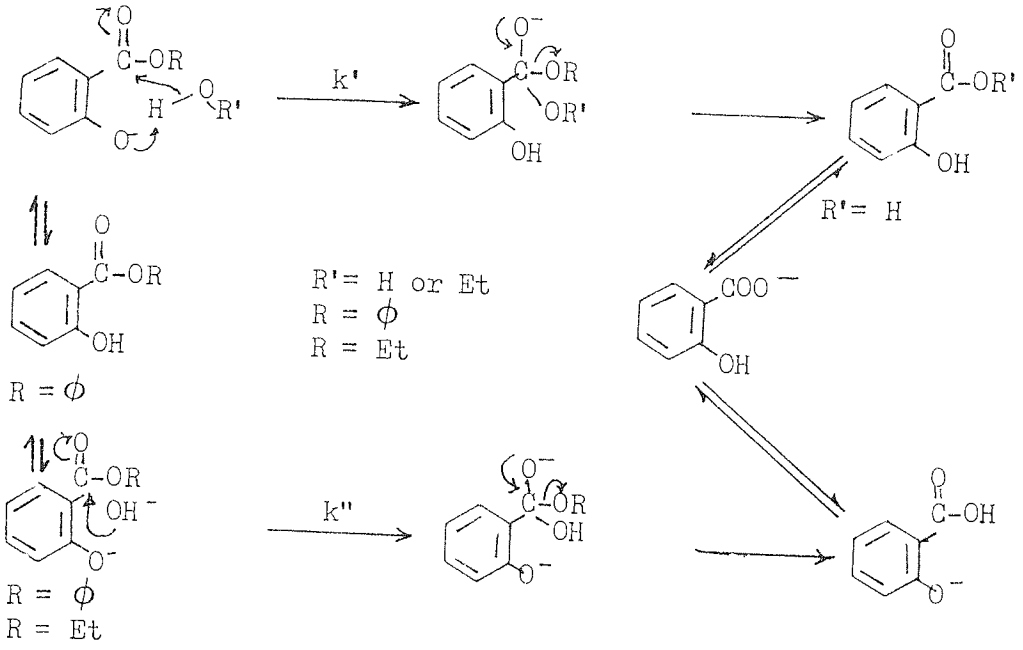


Fig. 6.9. Effect of Concentration of Sodium Hydroxide on the Degradation of Phenyl Salicylate Temp. = 35°C, A_o = 0.124 mM, EtOH = 4% v/v

Symbols	▲	●	□	▽	○	■	△
NaOH , M	0.01	0.05	0.1	0.3	0.5	0.6	0.7



Scheme 6.2. Mechanism of phenyl salicylate degradation in alkaline aqueous ethanol

6.2.4. EFFECT OF ALKALI CONCENTRATION ON SPECIFIC RATE CONSTANTS

First order plots showing the overall disappearance of phenyl salicylate at various concentration of sodium hydroxide are displayed in Fig.6.9. The absolute slopes of these lines (in Tab.6.2.) are used as primary estimates in measuring specific rate constants by NONLIN. The specific rate constants, together with the earlier data (89) are presented in Table 6.3. This covers only part of the range used by Khan et al, due to the very rapid degradation of the phenyl ester at higher alkali concentration.

Table 6.2 Effect of the Concentration of Sodium Hydroxide on the Overall Rate of Disappearance of Phenyl Salicylate, $A_{\emptyset}$ = Ø.124 mM ; Temp.= 35°C ; EtOH = 4% v/v

NaOH M	$(k_1 + k_2)min^{-1} X\ 10^3$	r
Ø.Ø1	51.1	Ø.999
Ø.1Ø	77.8	Ø.997
Ø.3Ø	1ØØ.7	Ø.993
Ø.5Ø	146.5	Ø.997
Ø.6Ø	175.6	Ø.999
Ø.7	222.7	Ø.998

At the concentration of ethanol used (4% v/v), the hydrolysis of phenyl salicylate is much faster than the transesterification

Table 6.3. Effect of Hydroxide ion Concentration on the Degradation of Phenyl Salicylate

Literature Value			Experimentally Observed				
Conditions	$[OH^-]$ M	$10^2 k\ min^{-1}$	Conditions	$[\bar{O}H]$ M	$10^2\ k\ min^{-1}$		
					k_1	k_2	k_3
initial phenyl salicylate = 1.2×10^{-4} M temp.=35°C μ=2M EtOH conc.4%	0.05	1.11+0.18	initial phenyl salicylate = 1.24×10^{-4} M temp.=35°C μ=2M, EtOH conc 4%	0.01	2.46	5.02	0.634
	0.07	1.31+0.10		0.05	2.42	4.94	0.787
	0.10	1.30+0.02		0.10	2.49	5.75	1.060
	0.20	1.50+0.01		0.30	2.77	7.07	1.45
	0.50	2.56+0.02		0.50	3.45	13.3	2.49
	0.70	3.24+0.05		0.60	2.22	15.8	2.78
	*			0.70	2.72	17.0	3.80

*The degradation of phenyl salicylate in alkali concentrations higher than 0.7M NaOH was too fast to measure the specific rate constants.

process, although the formation of ethyl salicylate under these conditions approached 20-30% of the initial concentration of phenyl salicylate. The comparison shows that the previous investigators underestimated the true hydrolysis rate, due to interference by ethyl salicylate. The effect of the concentration of sodium hydroxide on transesterification and hydrolysis is illustrated in Fig.6.10. It has been proposed in Section 5.3.5., that the degradation of salicylates under strongly alkaline conditions takes place by a concerted reaction involving solvent and salicylate anion competing with direct hydroxide attack. A similar mechanism is shown in Scheme 6.2. The observed rate constant k has been shown to depend upon both processes(89):

$$k = \frac{k'k_i' [H_2O][OH^-] + k''k_i' [OH^-]^2}{1 + k_i' [OH^-]} \quad \text{.....equn.6.1.}$$

where $k'_i = \frac{Ka}{Kw}$

For phenols, pKa ~ 9-10, $k_i' [OH^-] >> 1$ at the levels of hydroxide used and equn.6.1.may be simplified to :

$$k = k'[H_2O] + k''[OH^-] \quad \text{.....equn.6.2.}$$

This equation, rather than equn.6.1. used by Khan et al requiring non-linear parameter estimation, may be used to model the variation of the degradation processes with hydroxide concentration on the

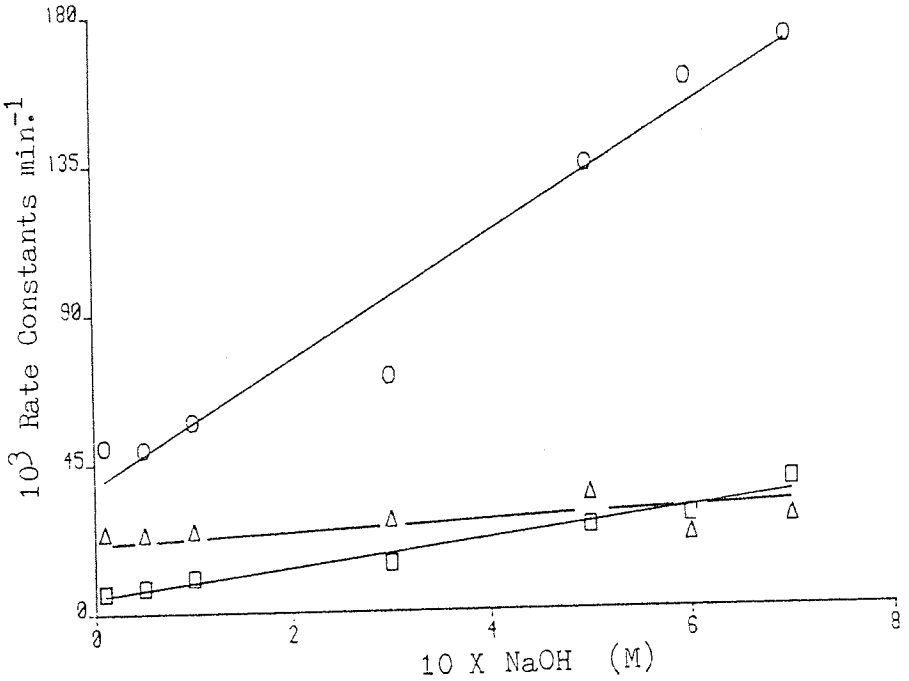


Fig. 6.10. Effect of Sodium Hydroxide Concentration on Phenyl Salicylate Degradation; Initial Concentration of Phenyl Salicylate = 1.24×10^{-4} M; Temp. 35°C; Ethanol in the Reaction Medium = 4%

Symbols	k	Rate process
Δ	k_1	Transesterification Ph-Salicylate
O	k_2	Hydrolysis of Phenyl Salicylate
□	k_3	Hydrolysis of Ethyl Salicylate

specific rate constants. The estimated values for the contribution of each mode of attack are recorded in Table 6.4. These suggest that:

(i)transesterification (k_1) is independent of hydroxide concentration in this region and involves the anchimeric attack of alcohol at the ester function of the salicylate anion.

(ii) the hydrolysis reactions (k_2, k_3) involve anchimeric attack of water and also the direct attack of hydroxide ion at the ester function of the salicylate anion which is presumably responsible for the observed dependence upon dielectric constant.

Phenyl salicylate is significantly less stable than the ethyl ester in both modes of degradation due to the phenoxide ion being an effective leaving group.

Table 6.4. Specific rate Constants for the Decomposition of Phenyl Salicylate in 4% Ethanol Via Solvent and Anion Attack.

Degradation Process	Rate Constants for degradation via			
	Solvent attack k' $min.^{-1}$	Anion Attack k'' $min.^{-1}$	n	r
phenyl salicylate transesterifcation k_1	Ø.Ø251	-	-	-
Phenyl salicylate hydrolysis k_2	Ø.Ø384	Ø.185	7	Ø.976
ethyl salicylate hydrolysis k_3	Ø.ØØ5178	Ø.Ø415	7	Ø.98Ø

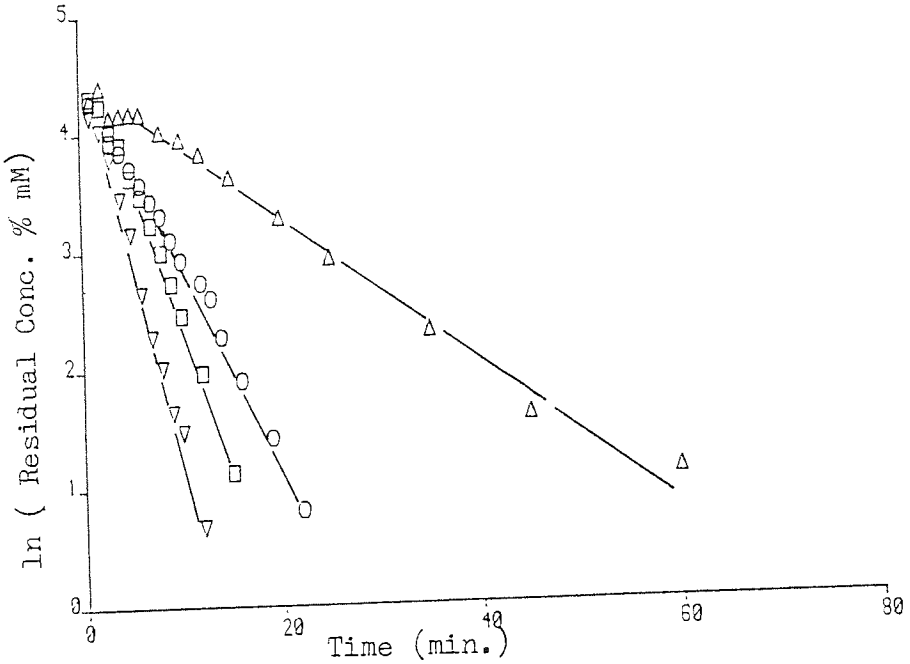


Fig. 6.11. First Order Plots Showing the Effect of Temperature on Degradation of Phenyl Salicylate.

Symbols	Δ	○	□	▽
Temperature °C.	35	45	50	55

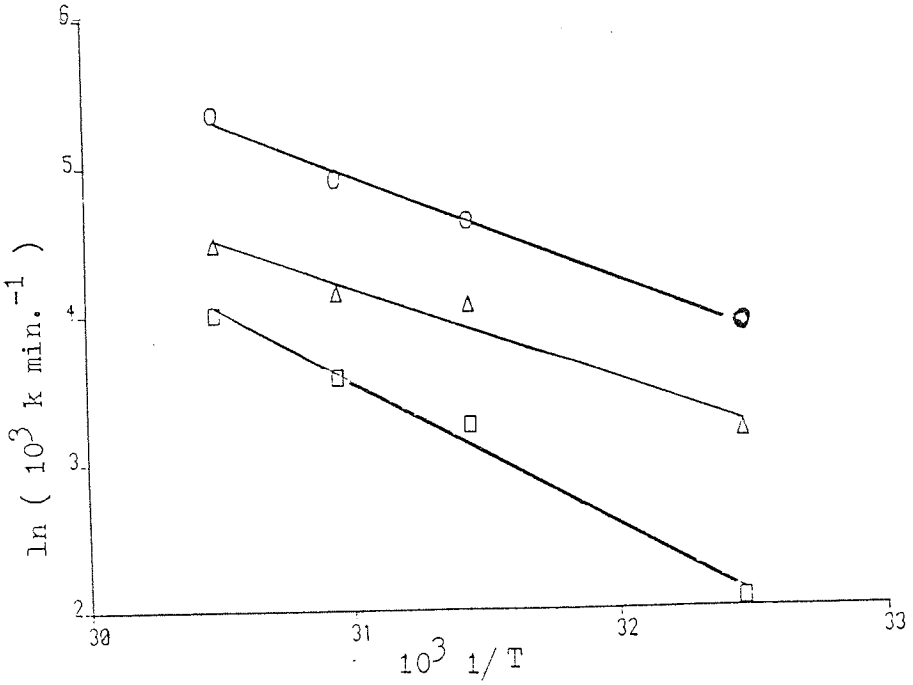


Fig. 6.12. Arrhenius Plots of Degradation of Phenyl Salicylate in 4% Ethanol With 0.05 M NaOH (μ = 2M);

Synbols	k	Rate Processes
Δ	k_1	Transesterification of Phenyl Salicylate
○	k_2	Hydrolysis of Phenyl Salicylate
□	k_3	Hydrolysis of Ethyl Salicylate

6.2.5. EFFECT OF TEMPERATURE ON DEGRADATION OF PHENYL SALICYLATE

The first order plots showing the overall degradation of phenyl salicylate within the temperature range 35^{o}-55^{o}C are displayed in Fig.6.11. The absolute slopes of these lines were used as primary estimates in measuring specific rate constants. The measured values for the specific rate constants are recorded in Tab.6.5.The Arrhenius and thermodynamic parameters are shown in Table 6.6. The Arrhenius plots for the individual rate constants are shown in Fig.6.12. The measured values of E_{act} and A contrast markedly with earlier values of 69.22 KJ mol^{-1} (E_{act}) and 1.55 X 10^{-6} $min.^{-1}$(A)(89). The calculation of the thermodynamic parameters is similarly compromised. These confirm the facile nature of both degradation pathways available to phenyl salicylate compared to ethyl salicylate hydrolysis.

Table 6.5. Rate Constants for Degradation of Phenyl Salicylate in 4% Ethanol With Ø.5M NaOH (μ = 2 M), Effect of Temperature

Temp ^{o}C	Specific rate constants $min.^{-1}$ X 10^{3}			Correlation coefficient			$(k_1 + K_2)$ $min^{-1}X10^{3}$ 1st order plot	r
	k_1	k_2	k_3	r_1	r_2	r_3		
35	24.16	49.39	7.865	Ø.982	Ø.996	Ø.982	5Ø.8Ø	Ø.983
45	57.46	1ØØ.3Ø	25.49	Ø.998	Ø.997	Ø.993	163.2Ø	Ø.994
5Ø	62.54	134.3Ø	35.61	Ø.993	Ø.99Ø	Ø.995	228.2Ø	Ø.995
55	87.36	21Ø.1Ø	54.66	Ø.988	Ø.995	Ø.993	328.9Ø	Ø.996

Table 6.6. Arrhenius Parameters for Degradation of Phenyl Salicylate in 4% Ethanol With 0.05 mM NaOH (μ = 2 M).

Parameter	For Specific Rate Constant		
	k_1	k_2	k_3
E_{act} K J mol^{-1}	52.91	59.66	81.47
A min^{-1}	2.34×10^{7}	6.13×10^{8}	5.17×10^{11}
r	-0.981	-0.997	-0.995
G K J mol^{-1}	85.62	83.78	88.51

CHAPTER 7 TRANSESTERIFICATION OF NICOTINATES AND OTHER ESTERS STRUCTURALLY RELATED TO SALICYLATES; TRANSESTERIFICATION IN FORMULATIONS

7.1. INTRODUCTION

Transesterification has been reported in substituted benzoic acids (145-148). Besides these, intramolecular migration of ester function has also been reported. Examples are the Fries rearrangement (152) of the derivatives of salicylic acid and substituted benzoic acids. The intramolecular migration of the 17-esters of hydrocortisone or betamethasone to the less active isomers (153-154) is also important. These processes frequently involve forcing conditions and the pharmaceutical importance of such reactions under milder conditions of analytical or formulation work have not been stated. During the course of the present study involving transesterification, it has been observed that phenyl or methyl salicylate solution in absolute ethanol undergoes transesterification at ambient temperature. Although the extent of conversion of the methyl ester to the ethyl ester is less than 1% over a period of 48 hrs., interconversion of the phenyl ester to the ethyl ester is very fast. Detectable conversion takes place within an hour and over a week about 25% of the initial concentration is converted to the ethyl ester. The use of hydroalcoholic solutions for physico-chemical and analytical work and the availability of the alcoholic excipients such as propylene glycol and polyethylene glycol leads to the possibility that many transesterification reactions may be detected with sensitive and specific analytical techniques. Lack of specificity in analytical method may result in erroneous stability profiles in preformulation studies. In alkaline 4% ethanol, for example, phenyl salicylate was shown to have a half-life of 9.4 mins. by HPLC analysis (134). This contrasted with

an estimate of 40 mins. based upon UV assay only which could not detect the rapid formation of the more stable ethyl ester[89]. Other solvent-vehicle interactions with medicaments recently reported include the increased melting point of aminophylline suppositories through reactions of ethylene diamine with the fatty base (155) and also the presence of indomethacin and p-chlorobenzoic acid esters of polyethylene glycols in indomethacin suppositories (156-157). A more insidious transesterification is that involving salicylate esters used as topical analgesics (134), where the alcoholic component present as vehicle or as solvent interchanges rapidly with that of the drug to produce a second ester. As the substantivity and percutaneous absorption of salicylates is markedly dependent upon the ester function (7) such transformations may lead to a significant change in the activity profile. The stability profiles of the various salicylates described in previous chapters suggest that these facile reactions threaten the integrity of pharmaceutical formulations and may compromise nonspecific analytical work. The purpose of this study is to present evidence that various mono- or polyhydric alcohols used in pharmaceutical formulations as inert excipients may directly participate in the reactions with the formation of an intermediate ester of altered polarity which may change the integrity of the preparations.

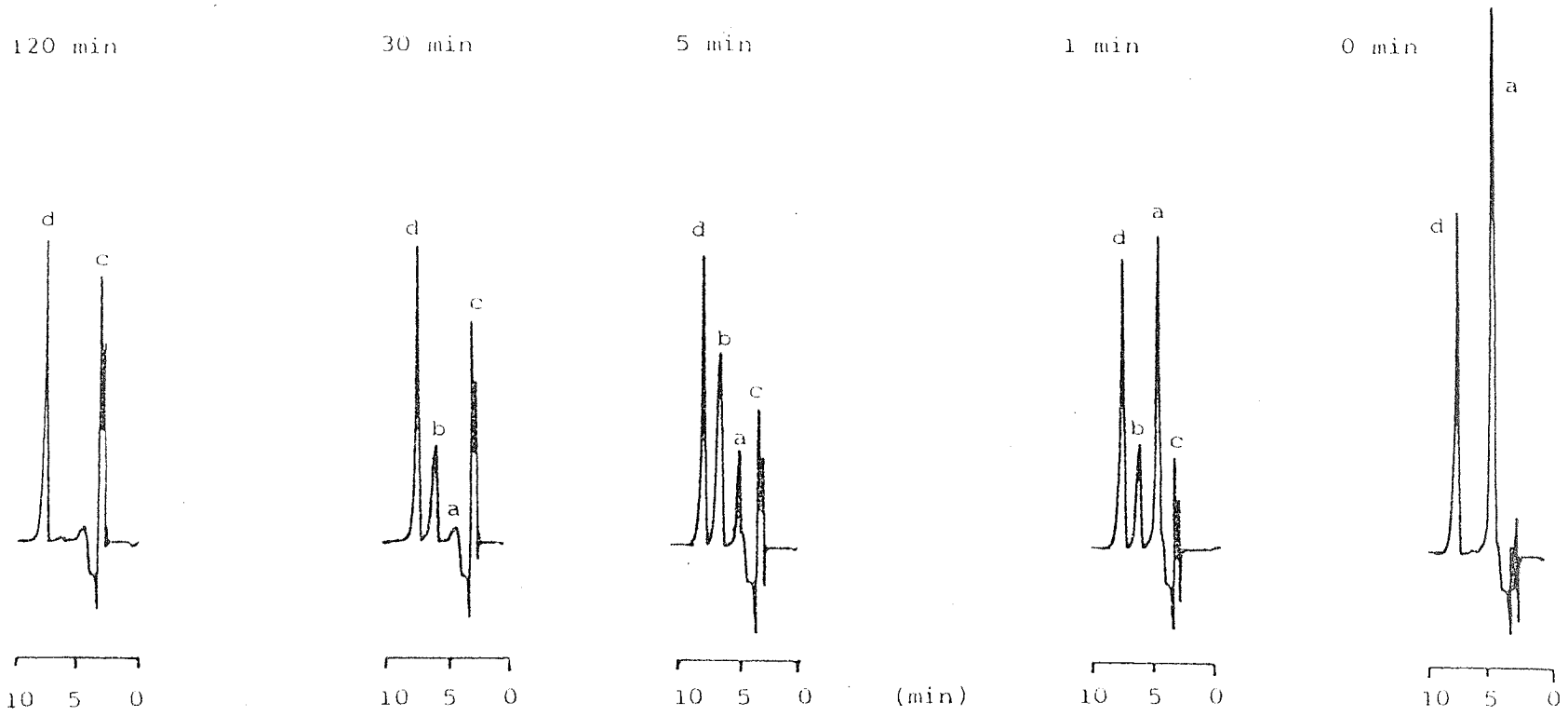


Fig. 7.1. Chromatograms Showing Transesterification of Methyl Nicotinate (1mM) to Ethyl Nicotinate in Alkaline Ethanol (50% v/v; 0.0005M NaOH), Temperature = 37°C.
Column : 10 cm X 4.6 mm, Hypersil-ODS

Peaks	Nicotinates
a	Methyl
b	Ethyl
c	Nicotinic Acid
d	Internal Standard.

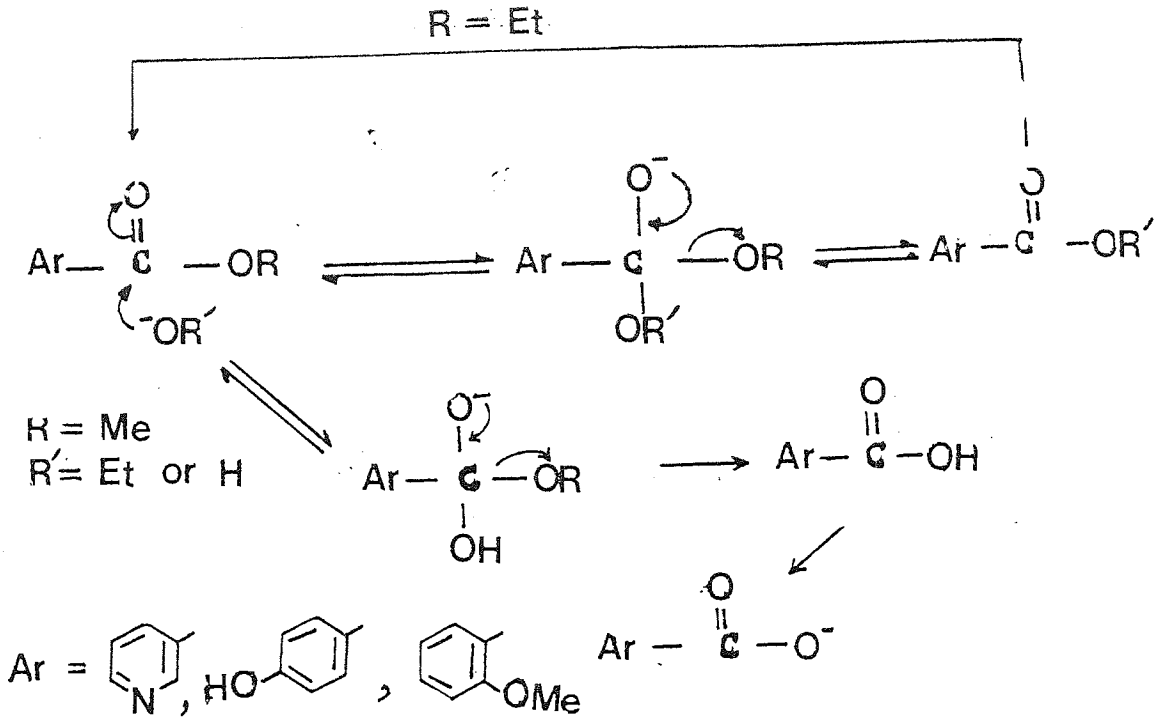
7.2. RESULTS AND DISCUSSION

7.2.1. TRANSESTERIFICATION OF NICOTINATES AND OTHER COMPOUNDS STRUCTURALLY RELATED TO SALICYLATES IN SOLUTIONS

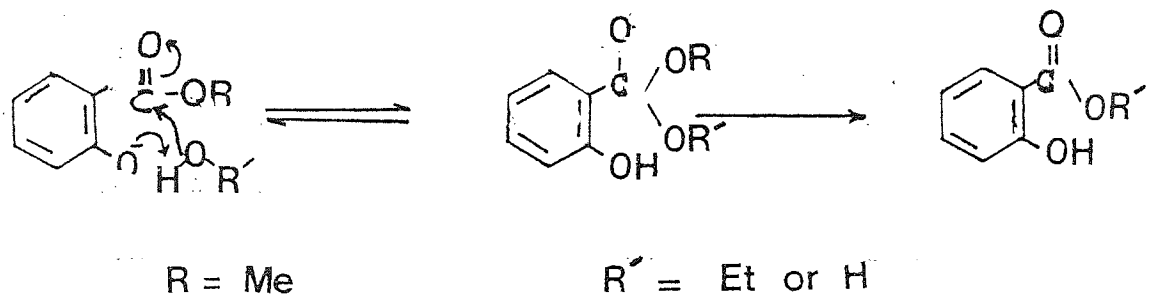
Transesterification of ethyl nicotinate in alkaline aqueous-ethanol (50% v/v, 0.005 M NaOH) at 37°C is displayed by high-performance liquid chromatograms in Fig.7.1. At time zero, methyl nicotinate and the internal standard are the only components present but after 1 min. significant degradation is observed and ethyl nicotinate and nicotinic acids are readily detected. In solutions containing higher amounts of hydroxide methyl nicotinate has totally disappeared within 5 mins. although traces of ethyl nicotinate are detectable for upto 30 mins.(0.001M NaOH) or 10 mins.(0.01M NaOH). These reactions follow the model in equation 5.1. and the specific rate constants obtained by nonlinear regression analyses (158) are:

k_1 = 0.0263 $min.^{-1}$, k_2 = 0.0125 $min.^{-1}$ and k_3 = 0.00143 $min.^{-1}$

When methanol was also included in the solvent in equimolar ratio (methanol:ethanol, 32:46 w/w), in 50% aqueous medium, reversible transesterification, according to Scheme 5.2. was observed. The typical concentration - time profiles together with the theoretical models are presented in Fig.7.2. The specific rate constants within the pH range 8.315 - 11.395 were determined, fitting the concentration-time profiles to the equations 5.12.-5.14. Initial values were determined from the absolute slopes of the first order plots of the time course of disappearance of methyl nicotinate at various pH values.



Scheme 7.1. Base catalysed transesterification and hydrolysis of nicotinates, p-hydroxy benzoates and o-methoxy benzoates



Scheme 7.2. Mechanism of transesterification and hydrolysis of salicylates under weakly alkaline conditions

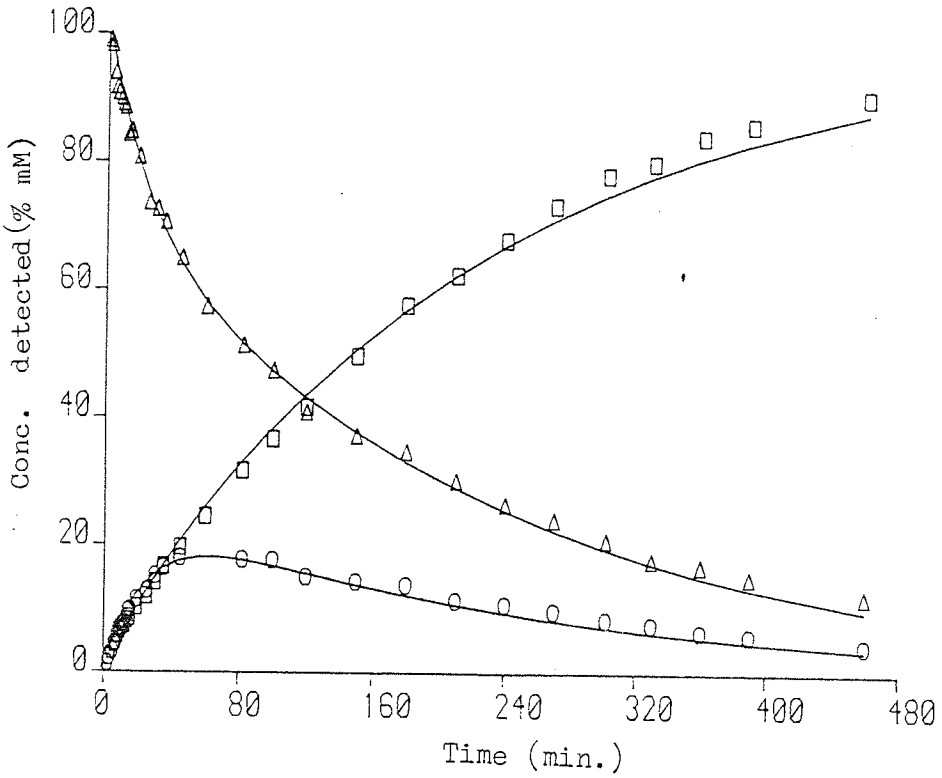


Fig. 7.2. Concentration-Time Profiles Showing the Degradation of Methyl Nicotinate in 50% Equimolar Methanol/ Ethanol. The Lines Represent Theoretical Models According to Equations 5.12-5.14.

Symbols	Δ	○	□
Nicotinates	Methyl	Ethyl	Nicotinic Acid

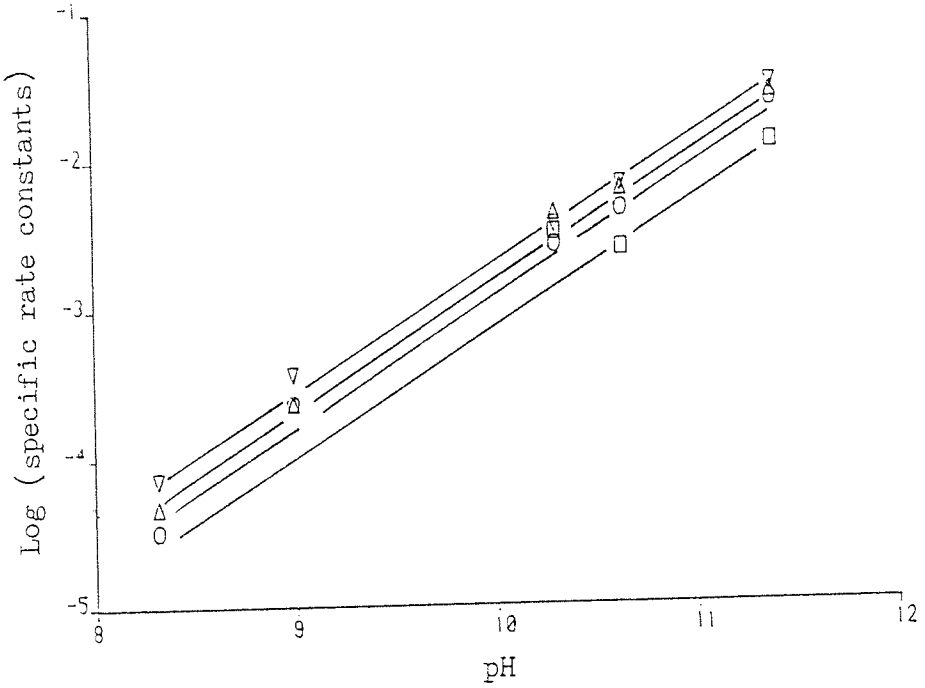


Fig. 7.3. Semi-Logarithmic Plots Showing the Effect of pH on Specific Rate Constants, Using Equation Log $k_{specific}$ = a + bpH

Symbols	Δ	□	○	▽
Rate Constants	k_1	k_2	k_3	k_{-1}
Coefficient of Regression Lines	k_1 = -11.68+0.89pH; r = 0.998; n=5 k_2 = -11.81+0.89pH; r = 0.997; n=5 k_3 = - 8.93+0.64pH; r = 0.901; n=3 k_4 = -10.95+0.83pH; r = 0.999; n=5			

The individual rate constants are recorded in Table 7.1.

Table 7.1. Specific Rate Constants for the Degradation of Methyl Nicotinate in Equimolar Methanol:Ethanol, as a Function of pH.

pH	Specific rate constants k $min.^{-1}$ X 10^{3}							$k_1 + k_2$ from 1st order plots	r
	k_1	k_2	k_3	k_{-1}	r_1	r_2	r_3		
8.315	Ø.047	.Ø323	.ØØ1	.Ø723	Ø.994	Ø.994	Ø.985	Ø.Ø413	-Ø.98Ø
8.995	Ø.231	.23ØØ	.ØØ1	.36ØØ	Ø.994	.988	Ø.995	Ø.1941	-Ø.984
1Ø.31Ø	4.16Ø	2.55Ø	3.Ø8Ø	3.45Ø	Ø.994	Ø.988	Ø.981	5.5685	-Ø.993
1Ø.642	5.95Ø	4.36Ø	2.4ØØ	6.34Ø	Ø.998	Ø.996	Ø.988	8.353	-Ø.993
11.395	26.ØØ	23.ØØ	12.ØØ	29.4Ø	Ø.998	Ø.985	Ø.997	24.ØØ7	-Ø.985

The specific rate constants are plotted as a function of pH in semilogarithmic form, as shown in Fig.7.3. The plots are linear and reveal that under the experimental conditions described, transesterification rates exceeds the hydrolysis rates. Little reaction is observed over the pH range 2.5 - 7. The rate of reactions increase with pH. This suggests that the probable mechanism involves direct attack of hydroxide and alkoxide ions on the ester function, according to Scheme 7.1. This is in contrast to the reactivity of salicylate esters in which a concerted attack of solvent molecules upon the salicylate anion is the main reaction ly alkaline conditions, Scheme 7.2.

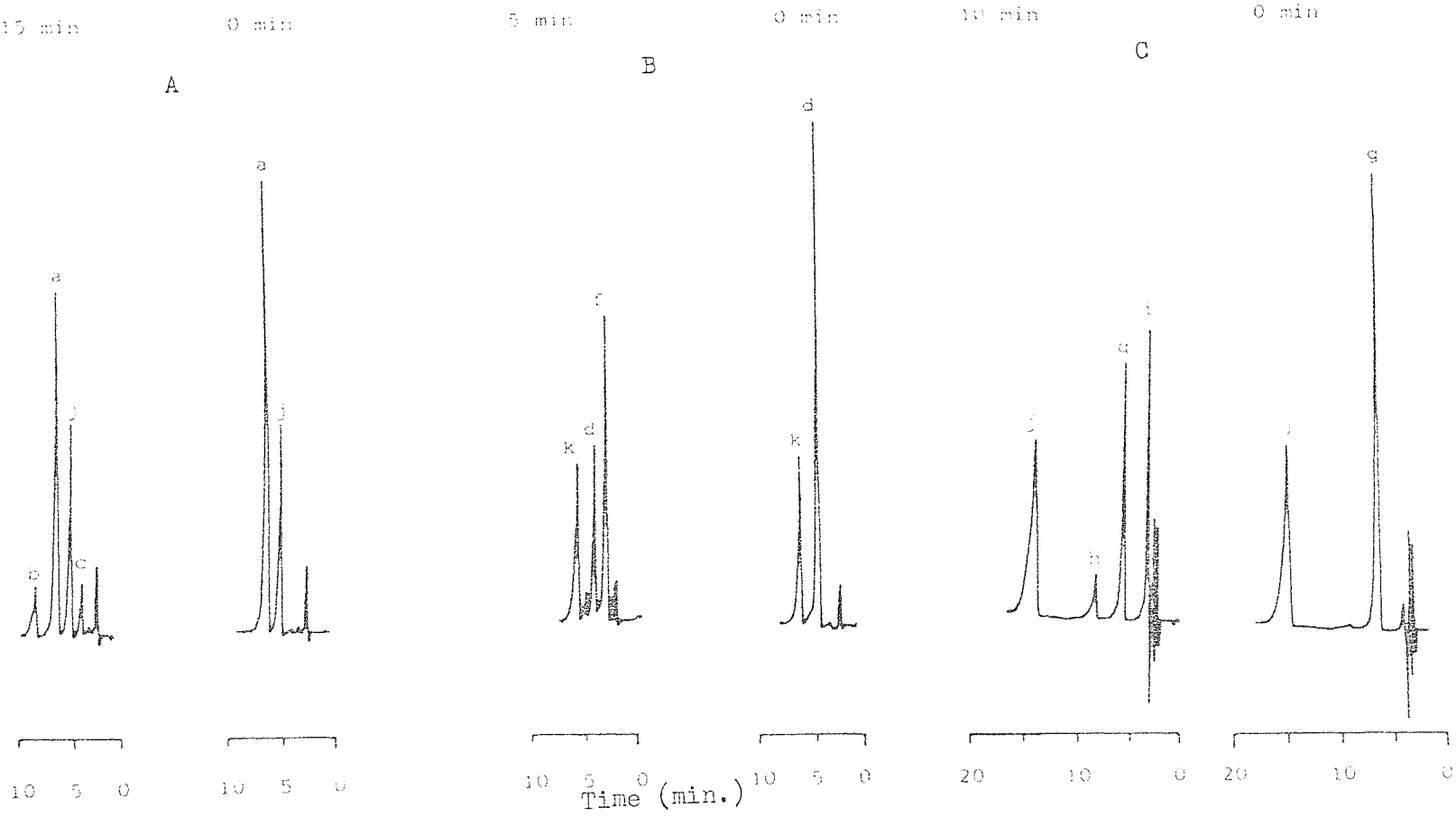


Fig. 7.4. Chromatograms Showing Transesterification and Hydrolysis in Aqueous Equimolar Methanol:Ethanol (50% v/v); NaOH = 0.5M, A_0 = 1 mM. Column : 10 cm X 4.6 mm, Hypersil-ODS.

Chromatograms		Peaks	Components
A	Methyl Salicylate	a	Methyl Salicylate
		b	Ethyl Salicylate
		c	Salicylic Acid
		j	Propyl 4-Hydroxybenzoate
B	Methyl 2-Methoxybenzoate (30° C.)	d	Methyl 2-Methoxybenzoate
		e	Ethyl 2-Methoxybenzoate
		f	2-Methoxybenzoic Acid
		k	Buty 4-Hydroxy benzoate
C	Methyl 4-Hydroxybenzoate (80° C.)	g	Methyl 4-Hydroxybenzoate
		h	Ethyl 4-Hydroxybenzoate
		i	4-Hydroxybenzoic Acid

These reactions are also catalysed by 4-dimethylaminopyridine (159) and the specific rate constants are recorded in Table 7.2.

Table 7.2. Specific Rate Constants of Transesterification and Hydrolysis of Methyl Nicotinate and Methyl Salicylate in 50% Equimolar Methanol:ethanol Catalysed by 4-Dimethylaminopyridine (0.01 M) Temp.= 37°C, A_0 = 1 mM , pH 9.8

Ester	Specific Rate Constants, k min^{-1} X10				Correlation coefficient		
	k_1	k_2	k_3	k_{-1}	r_1	r_2	r_3
Methyl salicylate	2.26	1.26	0.107	2.60	.997	.998	.997
Methyl nicotinate	2.31	0.364	0.100	0.55	.998	.997	.998

Chromatograms illustrating the transesterification of other derivatives of benzoic acid such as methyl 4-hydroxy benzoate and methyl 2-methoxy benzoate under comparable experimental conditions are displayed in Fig.7.4. Each chromatogram shows the presence of transesterified product. Methyl 4-hydroxy benzoate is less reactive than the ortho derivatives and the reaction was forced to completion at elevated temperature (80°C). The concentration-time profiles are shown in Fig. 7.5.

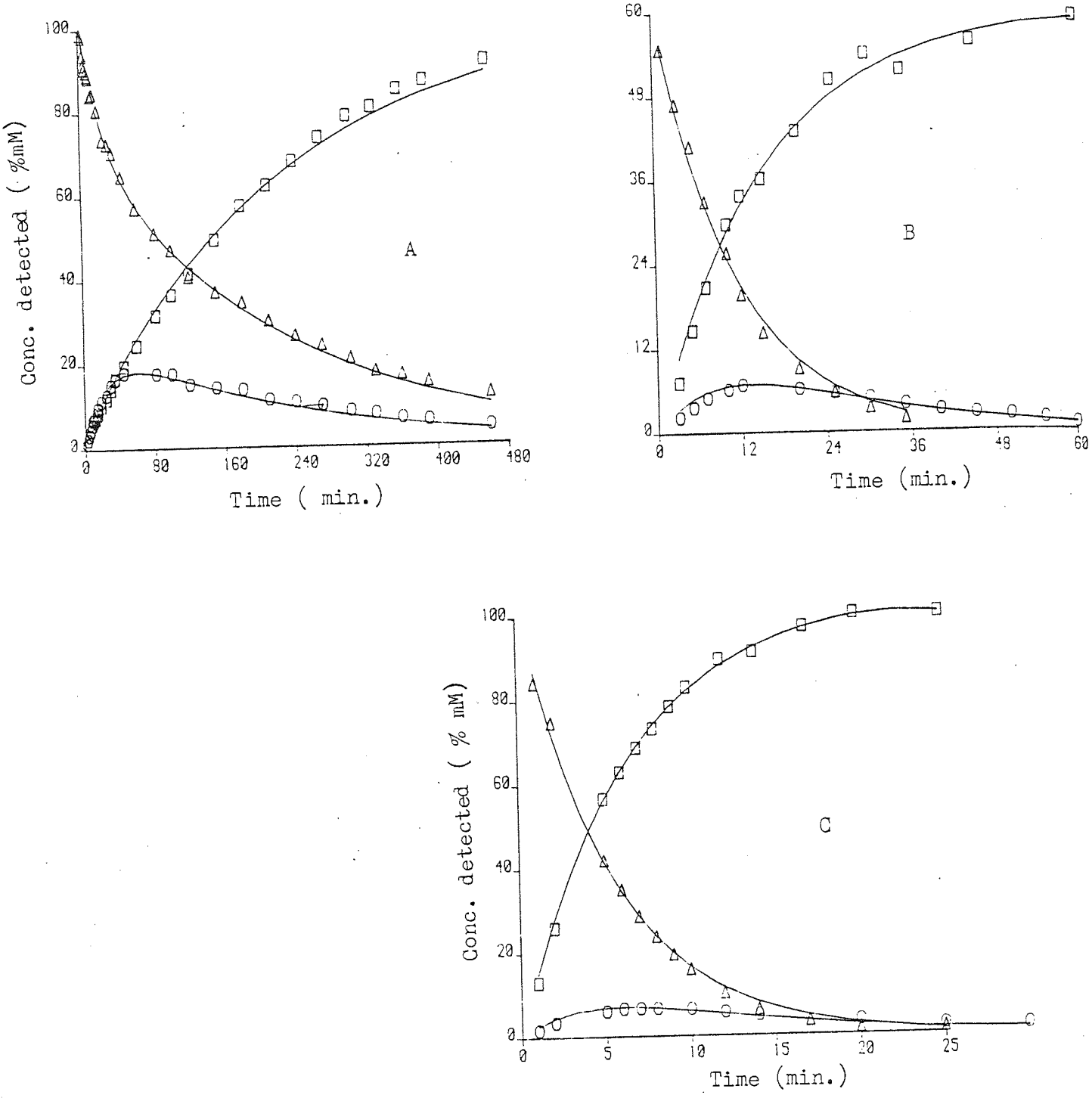


Fig.7.5. Concentration-Time Profiles Showing Transesterification and Hydrolysis in Mixed Alcohol (50% v/v, NaOH 0.5).

Reactants	Symbols	Components
A Methyl Salicylate	△ ○ □	Methyl Salicylate Ethyl Salicylate Salicylic Acid
B Methyl Paraben	△ ○ □	Methyl Paraben Ethyl Paraben p-OH-benzoic Acid
C Methyl o-Anisate	△ ○ □	Methyl o-Anisate Ethyl o-Anisate o-Anisic Acid

The specific rate constants are recorded in Table 7.3.

Table 7.3. Structural Effect On the Specific Rate Constants Of Transesterification and Hydrolysis. NaOH = Ø.5 M, Solvent = Aqueous MeOH/EtOH (50% v/v).

Compound	temp. °C	Specific rate constants $k\ min.^{-1} \times 10^{3}$				Correlation coefficient		
		k_1	k_2	k_3	k_{-1}	r_1	r_2	r_3
methyl salicylate	3Ø	9.46	5.73	.496	29.5Ø	Ø.999	Ø.981	Ø.999
methyl paraben	8Ø	23.5Ø	64.7Ø	62.9Ø	Ø.Ø17	Ø.999	Ø.957	Ø.999
methyl o-anisate	3Ø	29.9Ø	155.ØØ	148.Ø	43.9Ø	Ø.999	Ø.945	1.ØØØ

The comparative study leads to the conclusion that increased resistance to alkaline degradation in their 4-hydroxy derivative is due to ionization of the phenolic residue which reduces the reactivity of the ester function towards nucleophilic attack by hydroxide or alkoxide anion, Scheme 7.1. In salicylate esters this ionization enables a concerted transesterfication-hydrolysis pathway involving solvent moleculeto occur (Scheme 6.2.). In methyl 2-methoxy benzoate, in which no initial ionization is possible, the degradation rate is significantly faster(158).

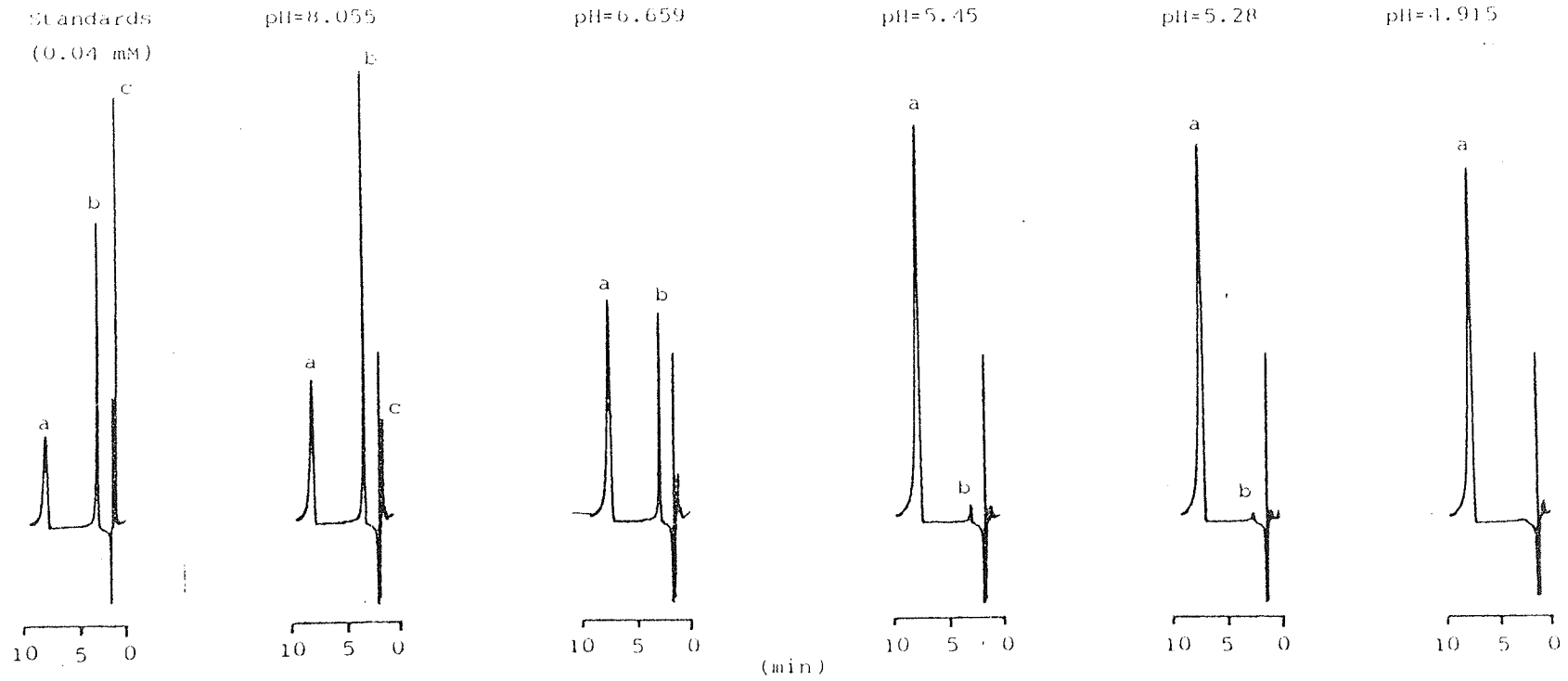


Fig. 7.6. Transesterification of Methyl Salicylate Gel in Ethylene Glycol Showing the Effect of pH. Time = 72 Hours ; Temperature = 37° C.; Column : 10 cm X 4.6 mm, Hypersil-ODS

Peaks	Species
a	Methyl Salicylate
b	Ethylene Glycol Salicylate
c	Salicylic Acid

7.2.2. TRANSESTERIFICATION IN FORMULATIONS

High-performance liquid chromatograms showing the effect of formulation on the transesterification of semiaqueous gels containing methyl salicylate in ethylene glycol are displayed in Fig.7.6. Ethylene glycol was preferred rather than the more usual propylene glycol due to the availability of the potential breakdown product glycol salicylate. The chromatograms show the stability of the gels as a function of pH within the pH range 4.92-8.Ø6, after a storage time of 72 hrs. at 37°C. At pH 4.92 no degradation is evident but at all higher pH degradation is observed. At pH 5.28 and 5.45 only traces of glycol salicylate are apparent but as the medium becomes sufficiently alkaline to produce higher concentrations of salicylate anion the transesterification reaction increases in importance and at pH 8.Ø6 the glycol ester becomes the major component of the formulation. Competitive hydrolysis is also indicated by the appearance of salicylic acid although this is not the major degradation pathway due to the small amount of water in the formulations. However, the gels become more stable when propylene glycol is used and also when an ethyl salicylate-propylene glycol combination is considered. Chromatograms which illustrate the degradation of these gels over a period of 8 months (37°) are displayed in Fig. 7.7.ge period of 8 months at 37°C.

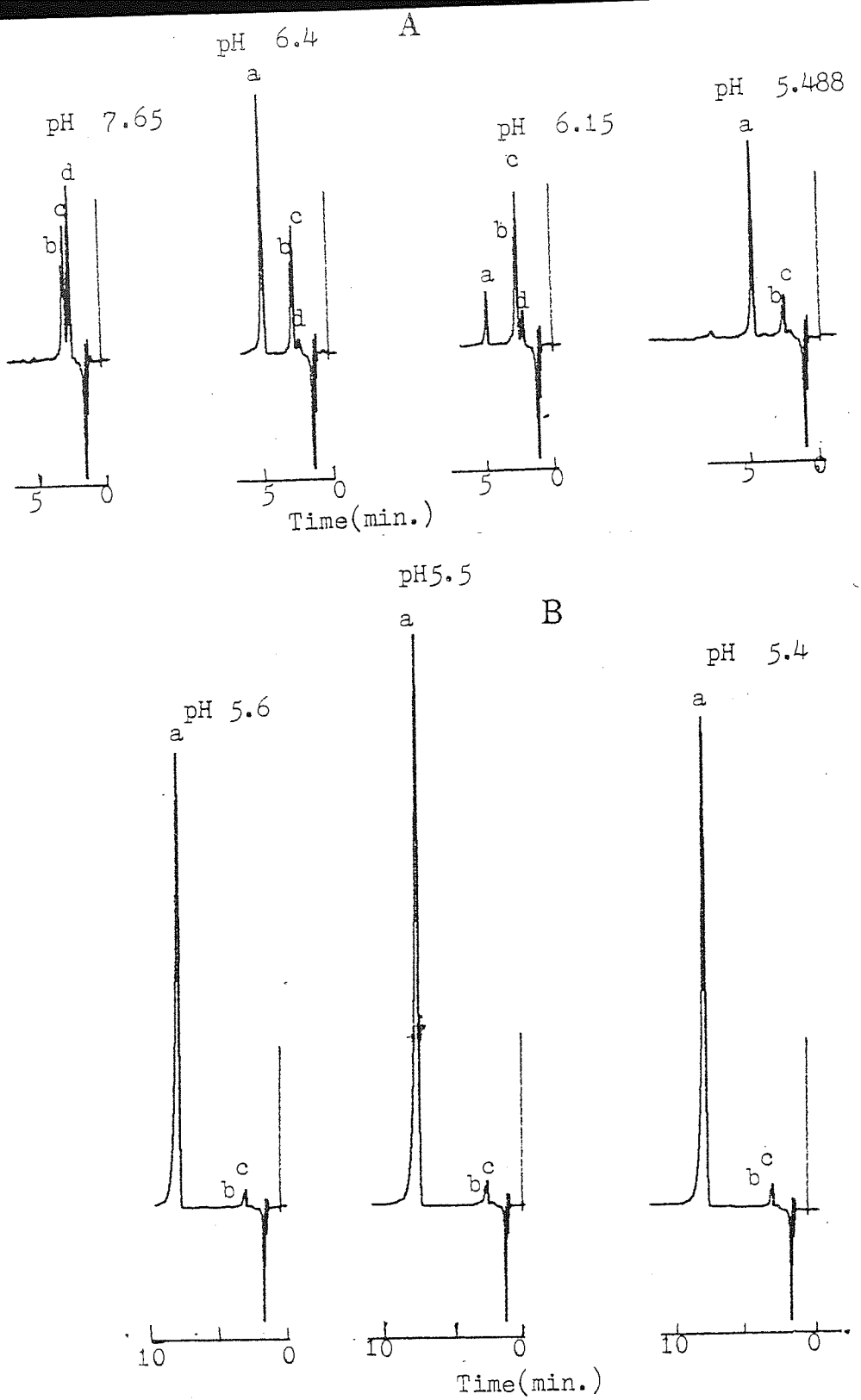
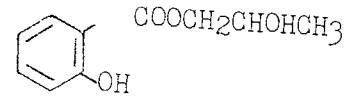


Fig. 7. 7. Transesterification in Salicylate Gels in Propylene Glycol.

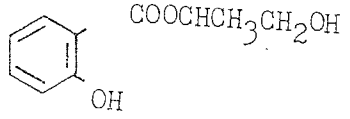
Salicylate Gels		Peaks	Salicylates
A	Methyl	a	Methyl
		b	2'-Hydroxy Propyl
		c	1'-Hydroxymethyl Ethyl
		d	Salicylic Acid
B	Ethyl	a	Ethyl
		b	2'-Hydroxy Propyl
		c	1'-Hydroxymethyl Ethyl
		d	Salicylic Acid

Column : 10 cm x 4.6 mm, Hypersil-ODS.

In propylene glycol, two intermediate esters develop wtich are the hydroxy derivatives of propyl salicylate, shown in Scheme.7.3.



2'-hydroxy propyl salicylate



1'-hydroxymethyl ethyl salicylate

Scheme 7.3. Formation of hydroxy derivatives of propyl salicylate.

Surgical spirit is a product which contains methyl salicylate, diethyl phthalate and castor oil in an ethanolic solution. Transesterification is possible in this formulation with production of ethyl salicylate. Of five samples examined a freshly purchased sample showed only methyl salicylate and diethyl phthalate but older preparations showed traces of ethyl salicylate in solution, as shown in Fig.7.8.

A liniment containing methyl salicylate in isopropanol (82) has been reported, which when reproduced in the laboratory was found to be unstable. Transesterification occurs producing iso-propyl salicylate on storage of the preparation at 37^{o}C for a week. During the course of the present studies, several patent reports appeared which contained various alcohols together with salicylates and nicotinates in their formulations. One has been reported to be a topical antiinflammatory preparation(127) containing phenyl salicylate and methyl salicylate together with ethanol and propylene glycol.

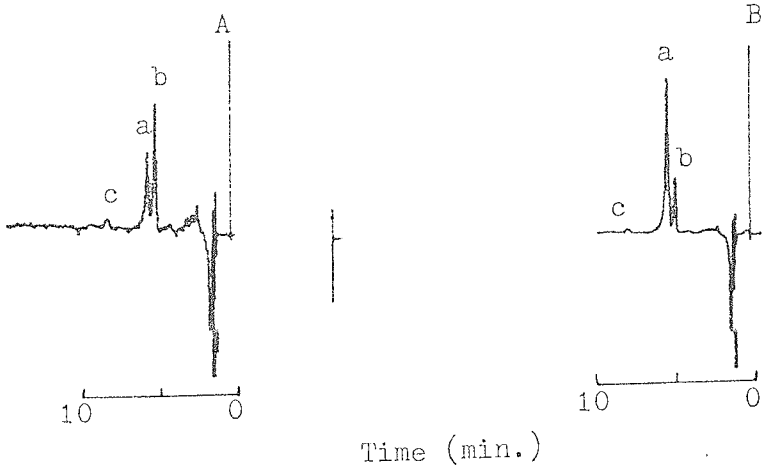


Fig. 7.8. High Performance Liquid Chromatograms of Surgical Spirit.

Peaks	Species	HPLC Conditions
a	Methyl Salicylate	CH_3CN : H_2O : H_3PO_4
b	Ethyl Salicylate	50 : 49.8 : 0.2
c	Diethyl Pthalate	pH 2

Column : 10 cm X 4.6 mm, Hypersil -ODS.

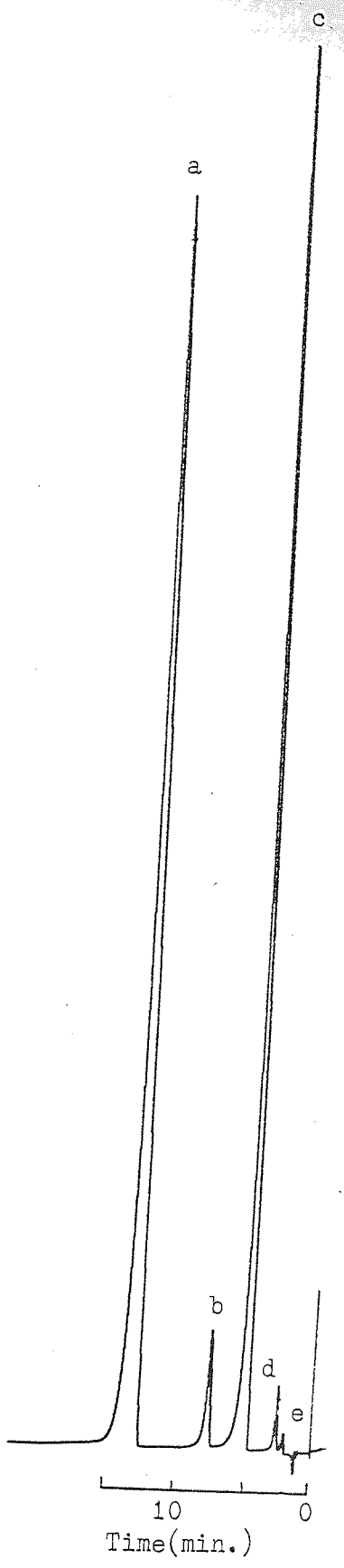
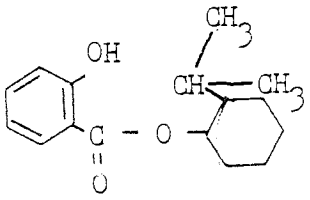


Fig. 7.9. Transesterification in Topical Antiinflammatory Product.

Peaks	Salicylates	HPLC Conditions
a	Phenyl	
b	Ethyl	CH_3CN : H_2O : H_3PO_4
c	Methyl	50: 49.8 : 0.2
d	2' Hydroxy Propyl	
e	1' Hydroxy Methyl Ethyl	pH 2

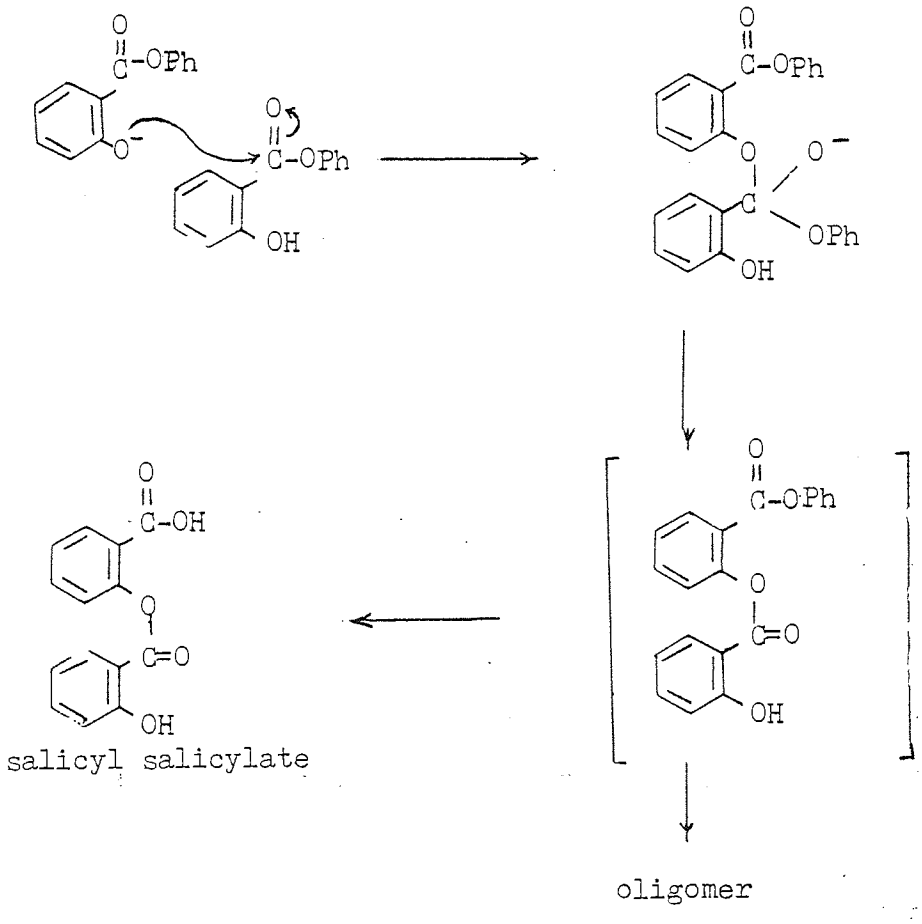
Column : 10 cm X 4.6 mm, Hypersil-ODS



Scheme 7.4. 1-(2-isopropyl)cyclohexyl o-hydroxy benzoate

A high-performance liquid chromatogram of the preparation, on storage at 37°C for 6 weeks is shown in Fig. 7.9. Ethyl salicylate and hydroxy derivatives of propyl salicylate were formed.

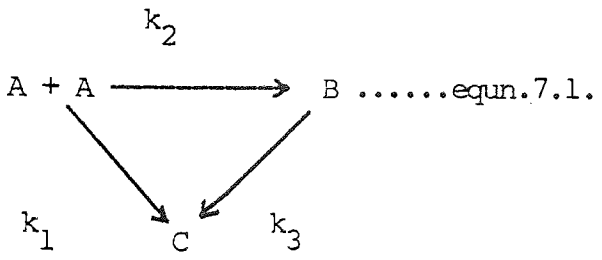
A sunscreen preparation has been reported (160) which contains 1-(2-isopropyl) cyclohexyl o-hydroxy benzoate, in (Scheme.7.4.), in combination with ethanol. From evidence of transesterification in other preparations it may be assumed that the sunscreening property of the preparation may be threatened due to possible transesterification to yield ethyl salicylate. A further preparation has been reported which contains methyl and glycol salicylate in presence of ethanol and ethylene glycol (161). It seems possible that, here too, methyl salicylate may suffer depletion by transesterification to ethyl and glycol salicylate. A sustained-released topical antiinflamatory preparation has been reported which contains methyl salicylate in the presence of ethanol (162), which also may generate ethyl salicylate on storage. A surgical dressing has been formulated (163) which contains methyl salicylate in combination of glycerin , another potential cross reacting agent and may produce transesterified compounds. Methyl nicotinate and glycol salicylate have been formulated together with ethanol and propylene glycol to produce a product for localised slimming (164). This preparation too, may suffer transesterification.



Scheme 7.5. Formation of dimer and oligomer from phenyl salicylate

7.2.3. DIMERISATION OF PHENYL SALICYLATE

The transesterification reactions discussed so far have involved reaction with solvent or vehicle. It may be assumed that suppression of unwanted reactions merely involves the selection of an inert vehicle. This is not necessarily true. Phenyl salicylate, for example, undergoes rapid transesterification in alcoholic solution (135) but the degradation of solutions in alkaline acetonitrile is not limited to the expected hydrolysis to salicylic acid and phenol. Chromatograms in Fig.7.1Ø. show that, in addition to the expected products, a Ø.2 mM phenyl salicylate solution yields a fourth peak. This component is concentration dependent. It is not produced in Ø.1 mM phenyl salicylate solution but is enhanced, together with the appearance of a further new peak, in Ø.4 mM solution. This product has been identified as salicyl salicylate (salsalate) produced by dimerisation of phenyl salicylate (Scheme 7.5.), possibly together with trimer. The disappearance of phenyl salicylate under the conditions which yield dimer together with salicylic acid may be described by the kinetic model in equn. 7.1.:



Where, A represents phenyl salicylate

B represents salicyl salicylate

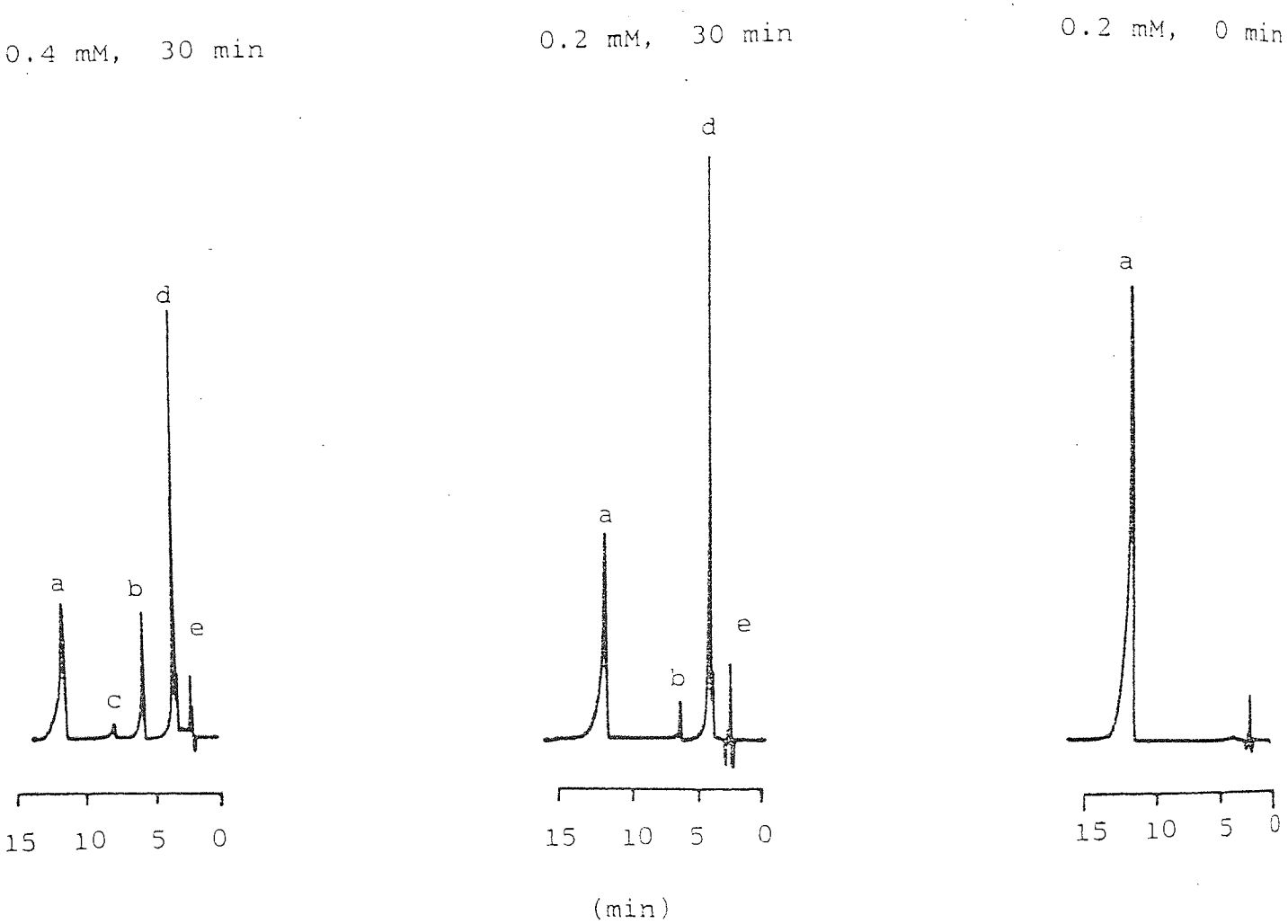


Fig. 7.10. Chromatograms Showing the Concentration Dependent Dimerisation and Oligomerisation of Phenyl Salicylate in 50% Aqueous CH_3CN. Column : 10 cm X 4.6 mm, Hypersil-ODS.

Peaks	Species
a	Phenyl Salicylate
b	Salicyl Salicylate
c	Trimer
d	Salicylic Acid
e	Phenol

C represents salicylic acid, and

k_1 and k_3 are first order hydrolysis rate constants and k_2 is the second order dimerisation rate constant.

the degradation of phenyl salicylate, under the conditions reported here follows competing first order hydrolysis to salicylic acid and second order dimerisation yielding salicyl salicylate (salsalate). the rates of change in concentration of the species A,B and C are given by :

$$dA/dt = -A(k_1+k_2A) \quad \text{.....equn.7.2.}$$

$$dB/dt = k_2A^2-k_3B \quad \text{.....equn. 7.3.}$$

$$dC/dt = k_1A+k_3B \quad \text{.....equn. 7.4.}$$

Integration of equation.7.2. between time zero and the current time t enables an expression for the instantaneous concentration of phenyl salicylate (A_t) to be obtained:

$$A_t = \frac{A_\emptyset k_1 e^{-k_1 t}}{k_1+A_\emptyset k_2-A_\emptyset k_2 e^{-k_1 t}} \quad \text{..... equn.7.5.}$$

where $A_\emptyset$ represents the initial concentration of phenyl salicylate.

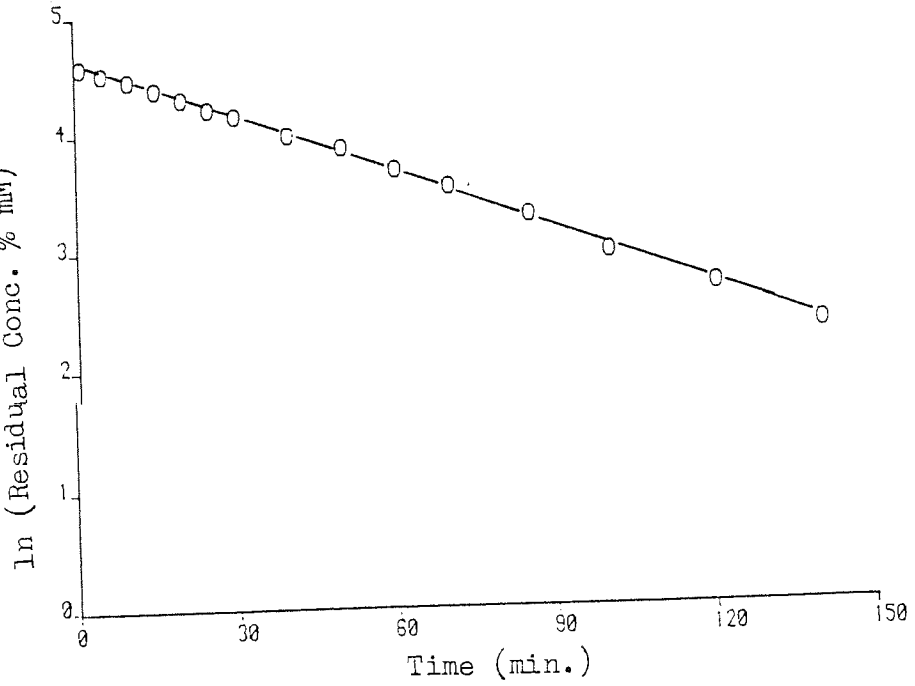


Fig. 7.11. First Order Plot Showing the Overall Degradation of Phenyl Salicylate in Aqueous Acetonitrile (50% v/v)

As the first order rate constant k_1 is usually larger than k_2 and as the difference in magnitude increases equn.7.5. approximates to the first order case. When $k_1 >> A_\emptyset k_2$, the k_2 terms in the denominator may be neglected providing the limiting first order case :

$$A_t = A_\emptyset e^{-k_1 t} \quad \ldots\ldots\ldots \text{ equn. 7.6.}$$

This approximation is also true at very early time points when

$$A_\emptyset k_2 \simeq A_\emptyset k_2 e^{-k_1 t}.$$

The first order plot of the concentration time profile is shown in Fig. 7.11. The rate constant for the hydrolysis of phenyl salicylate obtained from the slope of the regression line in Fig.7.11 is Ø.Ø164 min^{-1}. Non-linear least squares regression of the measured concentration-time profile, using the kinetic model in equn.7.5., provides an estimate for k_1 of Ø.Ø16 min^{-1}. This first order rate constant considerably exceeds that of the second order dimerisation reaction and the discrepancy between the exact approach and the estimate obtained using a simple first-order model is small (Ø.ØØØ4 min^{-1}.).

This type of reaction parallels those of aspirin observed in solid-state degradation (165-169) which yields products with immunogenic properties (17Ø). Were such transformations to occur in topical

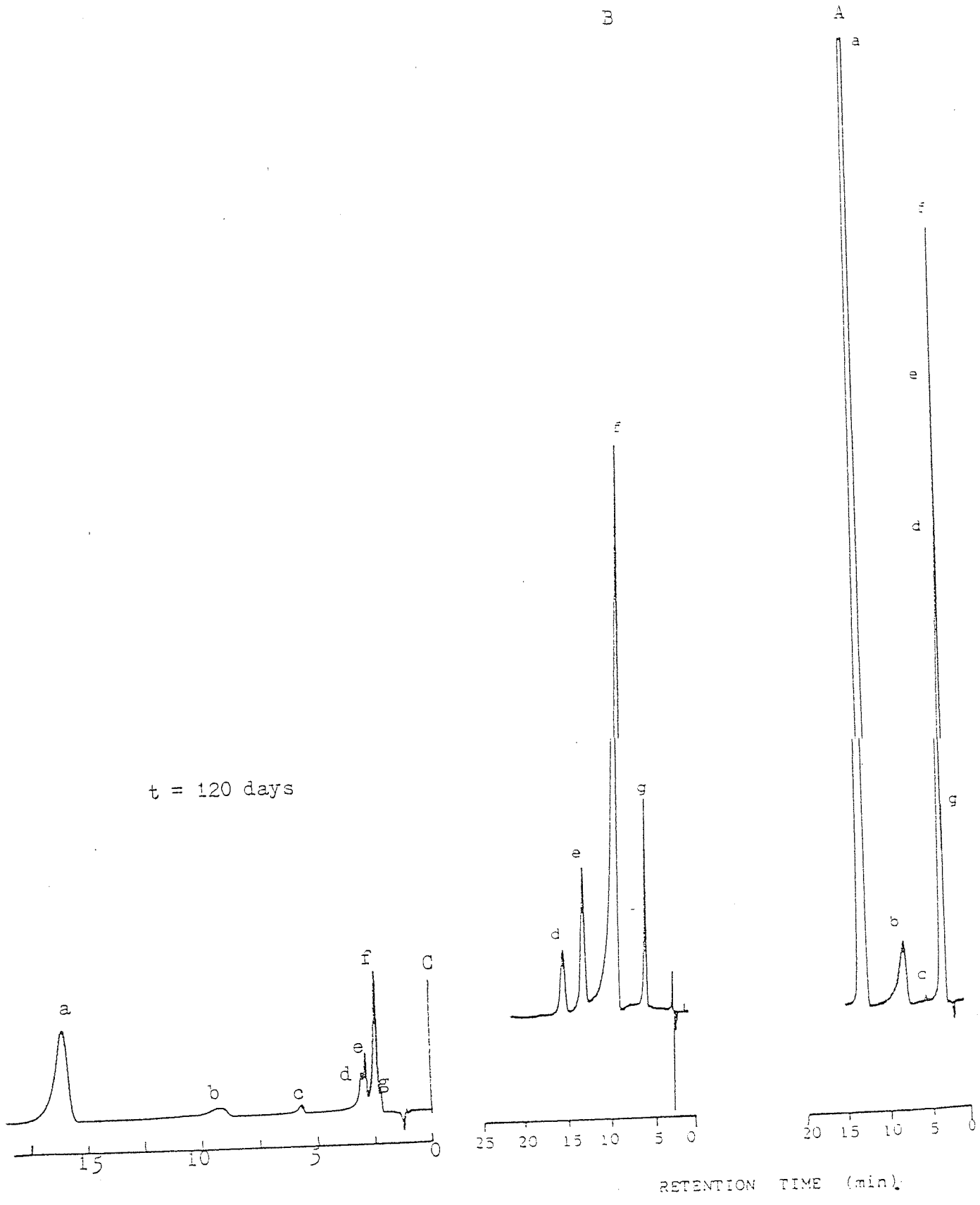
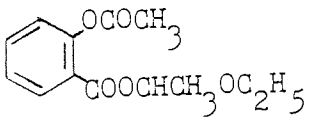


Fig. 7.12. Chromatograms Showing the Degradation of Salol Aqueous Cream on Storage at 37° C. Column : 10 cm X 4.6 mm, Hypersil-ODS.

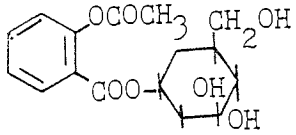
Solvent	Peaks	Components
A $CH_3CN:H_3PO_4:H_2O$:: 50:0.5: 48.5 , pH 2	a	Salol
	b	Triethanolamine -Salicylate Product
	c	Salicyl Salicylate
B $CH_3CN:H_2O:H_3PO_4$::25:74.9: 0.1 , pH 2	d	1' Hydroxymethyl Ethyl Salicylate
	e	2' Hydroxypropyl Salicylate
	f	Salicylic Acid
C $CH_3CN:H_2O:H_3PO_4$::45:54.8: 0.2 , pH 2	g	Phenyl

formulations allergic reactions to the preparation might be expected. Such reports have been made concerning topical phenyl salicylate preparations used as sunscreens (171-172). Salol Aqueous Cream is a product which contains phenyl salicylate in a cream base with 5% propylene glycol. This clearly has some potential for degradation by transesterification or dimerisation although the biphasic formulation may provide a considerable stabilising influence. The chromatograms obtained from this cream after 37 days at 37^{o}C is shown in Fig.7.12. together with a trace after a storage time of 120 days. Considerable degradation is evident with the transesterified propylene glycol products being readily identified. Two new peaks are also observed. Those are triethanolamine salicylate and salicyl salicylate. These products indicate that dimerisation and possible oligomerisation are potential degradation pathways in this formulation and may lead to adverse activity profiles.

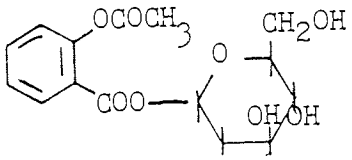
CHAPTER 8 DEGRADATION OF ASPIRIN PRODRUGS IN BRITTON ROBINSON BUFFER



(1'-ethoxy)ethyl 2-acetoxy benzoate

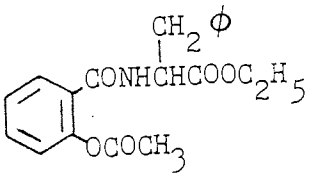


1-o-(2'-acetoxybenzoyl)-2-deoxy α-D- glucopyranose

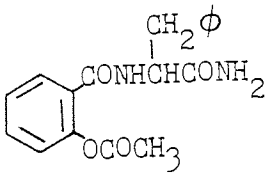


1-o-(2'-acetoxy)benzoyl- α-D -2-deoxyglucopyranose

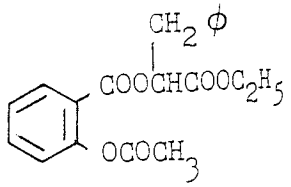
Scheme 8.1. Structures of acylal type aspirin derivatives



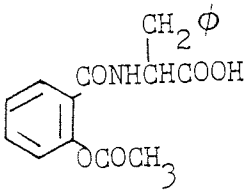
aspirin phenylalanine ethyl ester



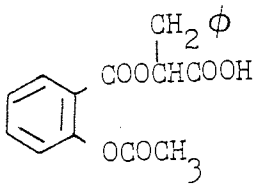
aspirin phenylalanine amide



aspirin phenyllactic ethyl ester



aspirin phenylalanine

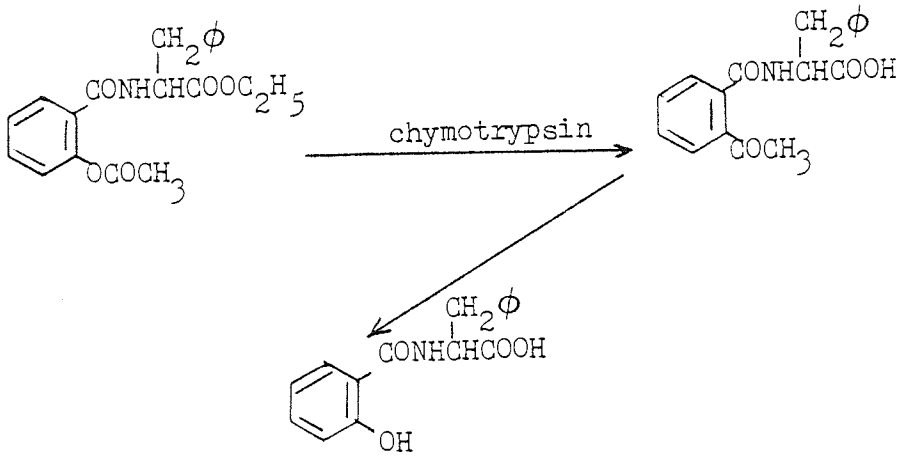


aspirin phenyllactic acid

Scheme 8.2. Phenylalanine type aspirin derivatives

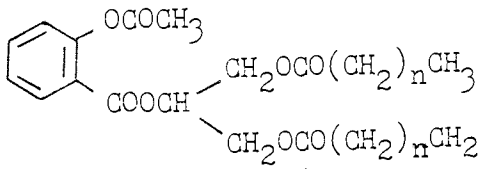
8.1. INTRODUCTION

In drug design the concept of a prodrug has been utilized to improve the physico-chemical properties of the drug for their better availability. Properties such as solubility, lipophilicity, absorption and stability may be modified and optimised. This allows controlled delivery of the drug and may produce better delivery and efficacy. Additionally, a prodrug may also provide better patient acceptance by minimizing taste or odour problems and irritation. The prerequisite for the design of aspirin prodrugs is that the carboxyl ester should cleave at a faster rate than the acetyl groups to release aspirin rather than a salicylate ester. Pierre and Jenks (173) masked the carboxyl group of aspirin by forming the methyl ester. This resulted in an insoluble , nonirritating prodrug which on hydrolysis liberated methyl salicylate instead of aspirin. Hussain et al (174-176) have recently reported the development of various aspirin prodrugs in which the carboxyl group has been esterified with acylal-type compounds,(structures in Scheme.8.1.) Samples were analysed by ultraviolet spectroscopy and it was claimed that these compounds rapidly generate aspirin. The non-specificity of the assay method leads to possible doubt of the correctness of such deductions. Banarjee and Amidon (177-179) prepared phenyl alanine type derivatives, as shown in in Scheme.8.2, and proposed that this ester generated aspirin by activation through hydrolysis involving -chymotrypsin and carboxypetidase-A enzymes. They also used a nonspecific method (pH) to analyse samples for kinetic studies. Although they (177-179) claimed to have detected aspirin using TLC, Hussain and Mulin-eldeen (180) have refuted this

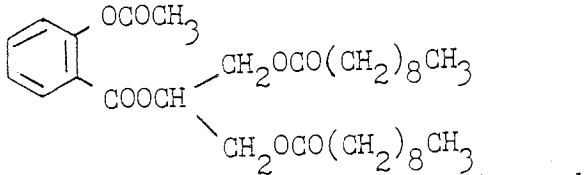


salicyl phenylalanine

Scheme 8.3. Formation of salicyl phenylalanine from aspirin phenyllactic ethyl ester

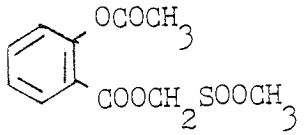


1,3-Bis(alkanoyl)-2-(o-acetylsalicyloyl) glyceride



2- (1,3-di-decanoyloxy)-propyl 2-acetyloxybenzoate

Scheme 8.4. Structures of aspirin triglycerides



methylsulfinylmethyl 2-acetoxybenzoate

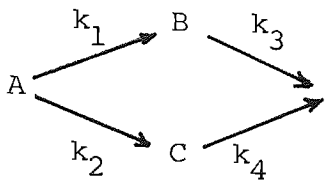
Scheme 8.5. Thioderivative of aspirin

conclusion and have shown by HPLC analysis that these compounds did not liberate aspirin under the specified conditions. Rather it was shown that the prodrug was hydrolysed to aspirin phenylalanine in the presence of chymotrypsin as shown in Scheme 8.3. Aspirin triglycerides, in which aspirin is attached to the second position of glycerol and fatty acids of various chain lengths are attached in position one and three (181-182),(Scheme.8.4.) have also received attention. These drugs have been evaluated by monitoring the salicylate ion blood concentration by fluorimetry in the rat in vivo. This also failed to provide evidence that the generated salicylate ion was from aspirin and from the corresponding salicylate ester or salicylic acid. Levy and Gagliardy (183) and Pffeiffer and Pankey (184) conducted experiments on aspirin anhydrides. However, due to the immunogenic properties, further development on these derivatives has not been encouraged. Interest has also been shown in sulphur derivatives of aspirin (185-186).It has been shown(186) that in vivo studies, with analysis by HPLC, methylsulfinylmethyl 2-acetyloxy benzoate is a true prodrug of aspirin which on hydrolysis on human plasma at 37^{o}C yielded about 90% aspirin. Both the prodrug and aspirin were hydrolysed to salicylic acid. In some patent literature syntheses of various aspirin derivatives have been reported but without adequate information (130,187-190). The p-acetamidophenyl salicylate derivative benorylate has been synthesised as a true aspirin prodrug (191).

The purpose of this study was to investigate the properties of aspirin prodrugs to liberate aspirin under mild conditions, to evaluate the degradation profile of these derivatives and to provide a full kinetic model of the transformations.

8.2. KINETIC MODEL

In aqueous solvent, the degradation of an aspirin prodrug may proceed via a simultaneous parallel model, forming intermediate esters which are aspirin and the corresponding salicylate ester. Thes intermediate esters degrade by hydrolysis, forming salicylic acid. The following kinetic model may be used to represent these processes:



..... equn.8.1.

where, A = the prodrug

B = the corresponding salicylate ester

C = aspirin

D = salicylic acid

k_1= rate constants for the formation of salicylate

k_2= rate constants for the formation of aspirin

k_3= rate constant for the hydrolysis of salicylate ester

K_4= rate constants of hydrolysis of aspirin

The rates of change of the concentrations of species A,B,C and D are given by :

$$dA/dt = -A(k_1+k_2) \quad \text{.....equn.8.2.}$$

$$dB/dt = k_1A-k_3B \quad \text{.....equn.8.3.}$$

$$dC/dt = k_2A-k_4C \quad \text{.....equn.8.4}$$

$$dD/dt = k_3B+k_4C \quad \text{.....equn.8.5}$$

Integration of these equations between time zero and the current time t, provides expressions which give simultaneous determinations of each species.

$$A_t = A_\emptyset e^{-(k_1k_2)t} \quad \text{..equn.8.6}$$

$$B_t = A_\emptyset k_1[\{e^{-k_3t} - e^{-(k_1k_2)t}\} / (k_1 - k_2 - k_3)] \text{..equn.8.7}$$

$$C_t = A_\emptyset k_2[\{e^{-k_4t} - e^{-(k_1+k_2)t}\}/(k_1-k_2-k_3)] \text{ .equn.8.8}$$

$$D_t = A_\emptyset[1-e^{-(k_1+k_2)t} - k_1\{[e^{-k_1t} - e^{-(k_1+k_3)t}]/(k_1+k_2-k_3)\} - k_2\{[e^{-k_4t} - e^{-(k_1+k_2)t}]/(k_1+k_2-k_3)\} \text{ ..equn.8.9}$$

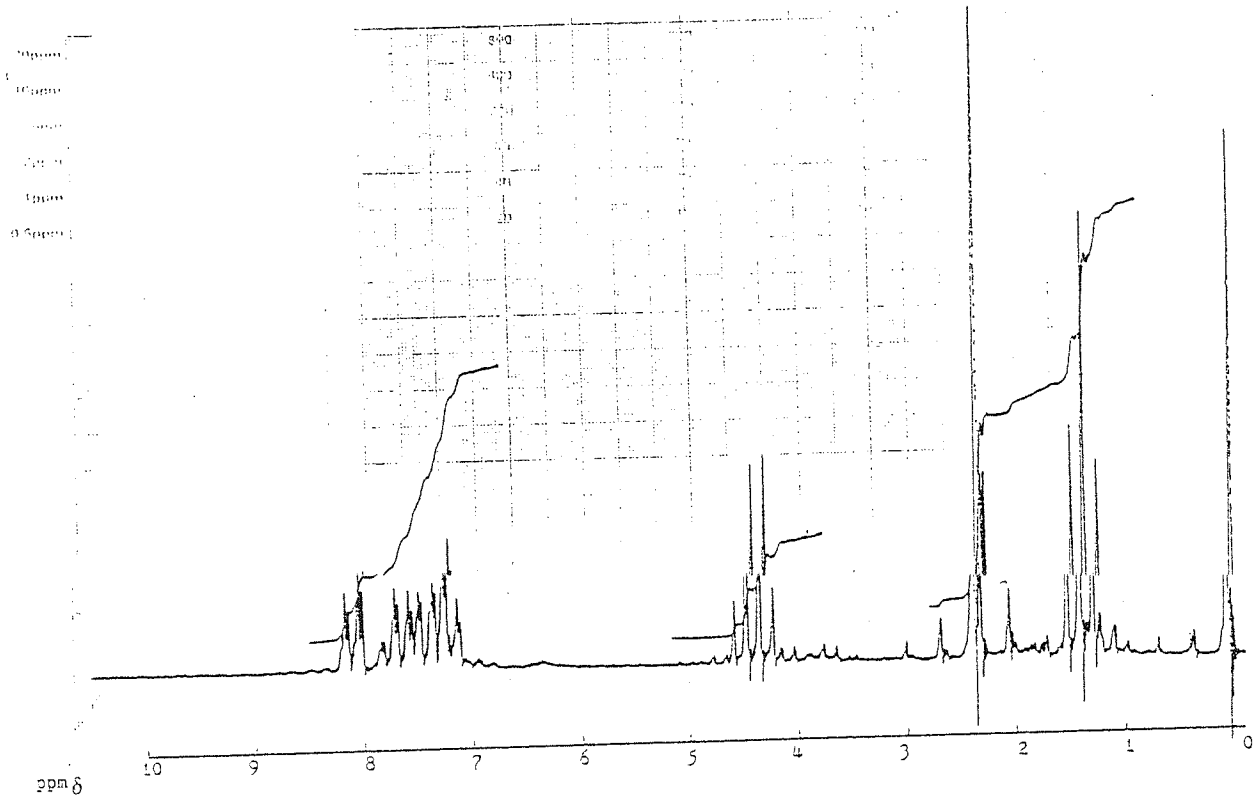
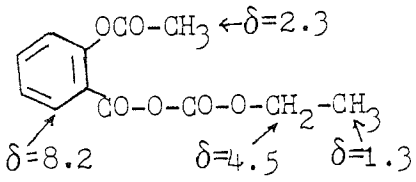


Fig.8.1 ^{1}Hnmr spectrum of o-acetoxy salicyloyl ethyl carbonate



8.3. ^{1}H-NMR, MASS AND HPLC ANALYSIS OF ASPIRIN PRODRUGS

^{1}H-NMR spectrum of the prodrug o-acetyloxy phenyl benzoate is shown in Fig.8.1.A., and that of o-acetyloxy salicyloyl ethyl carbonate in Fig.8.1.B.

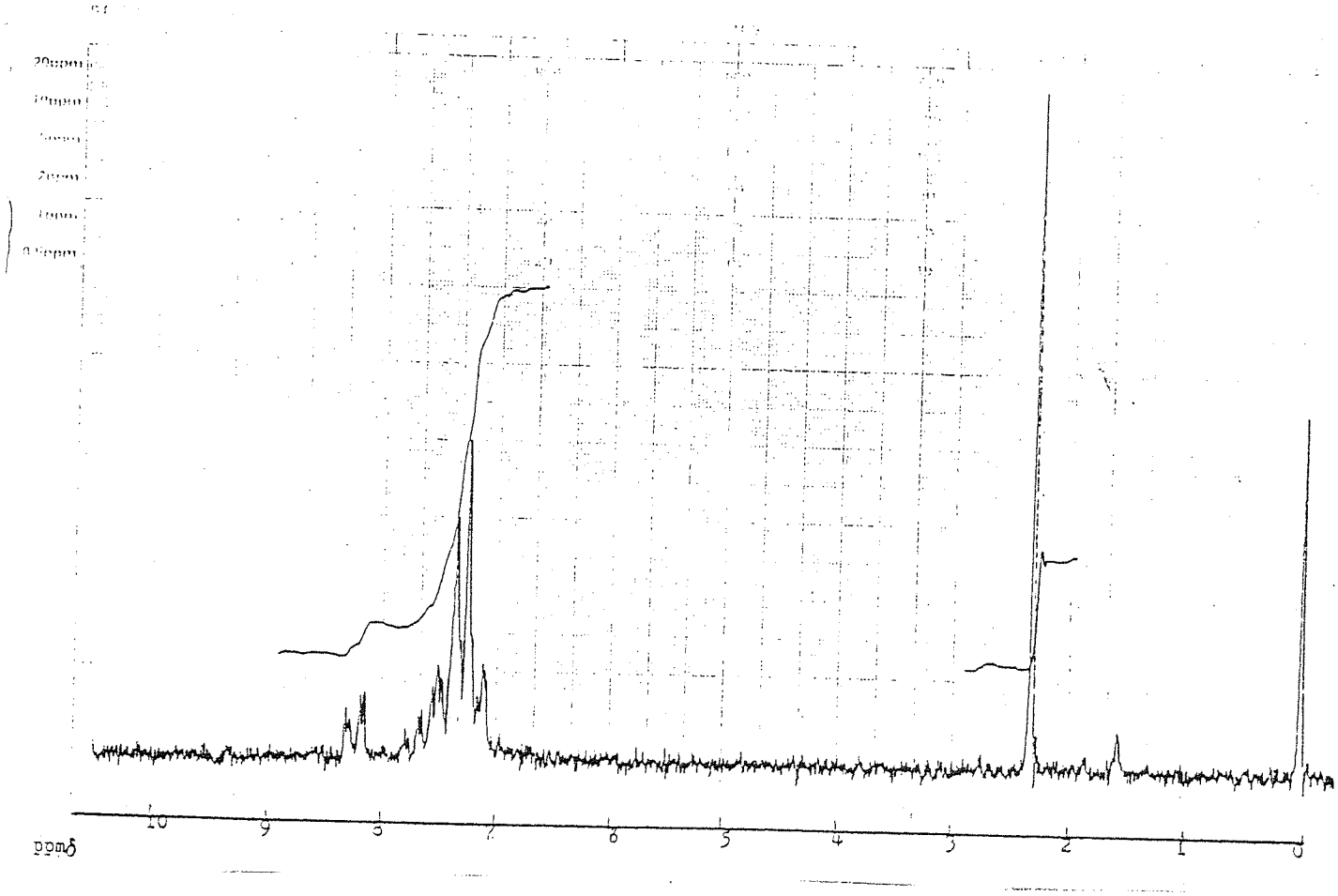
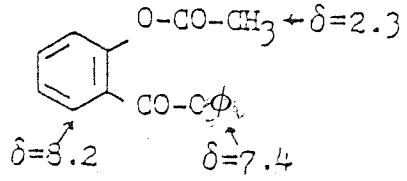


Fig. 8.1.B. ^{1}Hnmr spectrum of o-acetyloxy phenyl benzoate



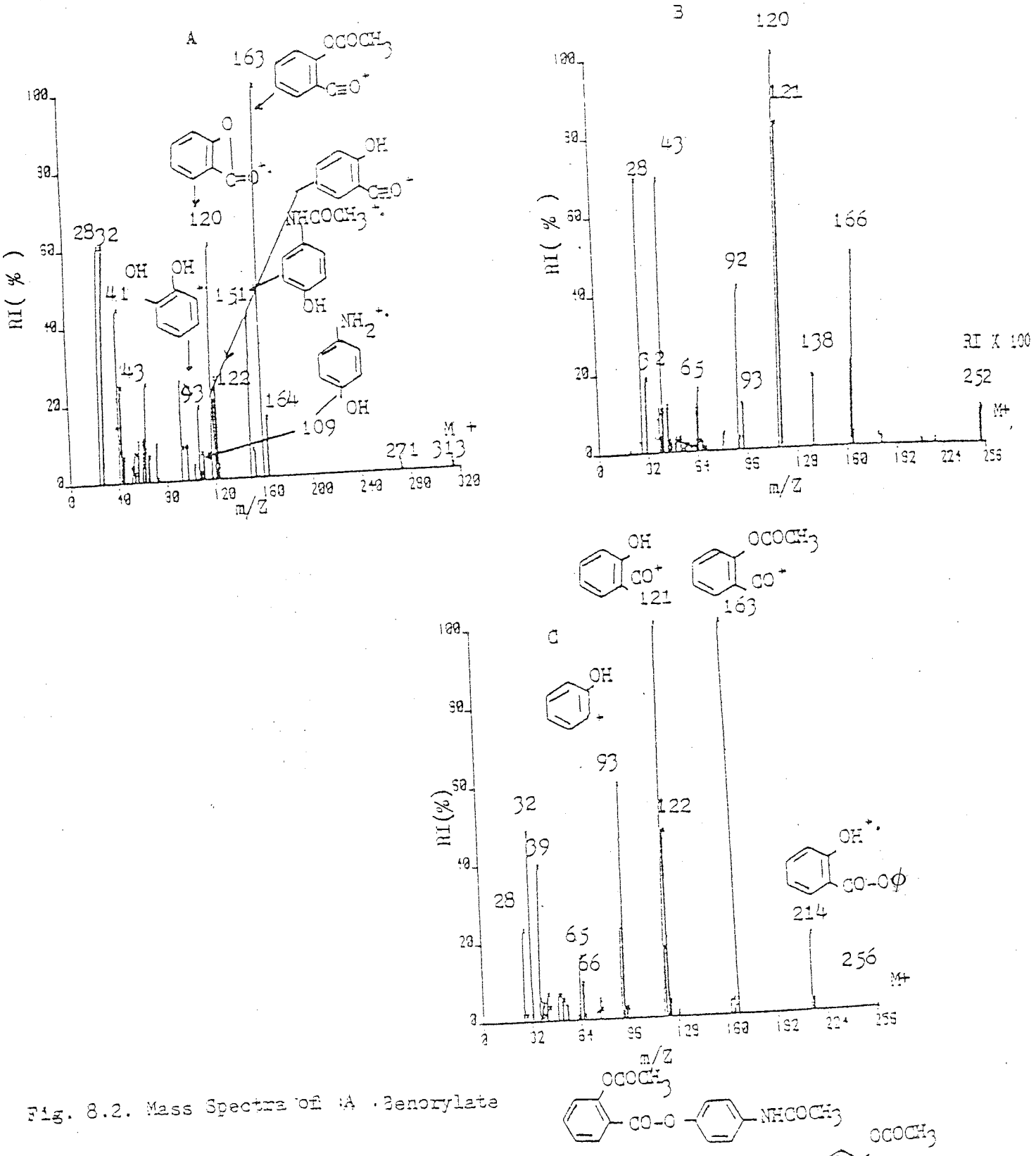


Fig. 8.2. Mass Spectra of A - Benorylate

B. O-Acetoxy Salicyloyl Ethyl Carbonate

C. O-Acetyloxy Phenyl Benzoate

The mass spectra obtained by the fragmentation of o-acetyloxy phenyl benzoate, o-acetyloxy salicyloyl ethyl carbonate and benorylate are displayed in Fig.8.2. The M/Z values against relative intensities of the fragments are given in Tab. 8.1. assigning the most abundant ion in the spectrum to the base peak.

These analyses confirm that the compounds had the expected structures.

Table 8.1.A. Mass Spectrum Data for O-Acetyloxy phenyl benzoate;

m/Z	256	215	214	213	165	164	163	128	123	121	120-
R.I(%)	.194	2.729	19.59	1.267	1.755	3.894	100	1.170	3.802	100	17.54

m/Z	95	94	93	92	91	78	77	76	75	74	67
R.I(%)	2.73	2.632	59.65	23.68	1.560	2.534	4.971	2.241	2.144	1.852	1.072

m/Z	66	65	64	63	62	55	53	52	51	50	44
R.I(%)	9.36	16.08	15.21	3.704	1.17	3.704	5.263	4.386	6.433	6.725	3.411

m/Z	43	42	41	40	39	38	37	32	29	28	27
R.I(%)	6.82	2.242	1.365	4.368	39.47	5.555	1.657	48.25	1.560	23.39	2.339

Table 8.1.B. Mass Spectrum Data for O-Acetyl Salicylol Ethyl Carbonate,

m/z	252	210	208	180	166	164	163	138	121	120	93	92	91
R.I(%)	.095	1.0	.95	2.6	48.7	3.13	21.4	17.95	82.05	100	11.4	41.03	2.85

m/Z	81	80	69	67	66	65	64	63	62	61	60	59	58	57
R.I(%)	4.27	2.56	.85	.52	2.56	15.67	11.4	10.5	3	1.23	.80	1.09	1.7	2.04

m/z	56	55	54	53	52	51	50	47	46	45	43
R.I.(%)	1.37	1.94	0.52	3.7	2.56	2.71	3.41	.90	2.85	11.68	69.23

m/z	42	41	40	39	38	37	32	29	28	26
R.I.(%)	10.68	9.40	3.85	10.83	8.12	3.7	18.80	69.23	69.23	2.56

Table 8.1.C. Mass Spectrum Data for Benorylate

m/z	314	313	272	271	164	163	152	151	123	122	120	110	109
R.I.(%)	.82	2.04	.82	2.24	15	100	7.14	41.43	3.27	25.71	20	6.74	1.84

m/z	108	103	97	94	93	92	91	73	72	66	65	63
R.I.(%)	18.57	3.67	8.78	8.37	25	17.2	2.86	.61	9.39	6.43	25	8.16

m/z	61	57	56	55	54	53	52	51	50	45	44	43
R.I.(%)	10.7	10.2	4.29	7.14	2.24	7.35	4.08	4.08	3.67	6.33	24.29	44.29

m/z	42	41	40	39	32	28
R.I.(%)	7.86	10.71	21.43	14.29	60.71	60.71

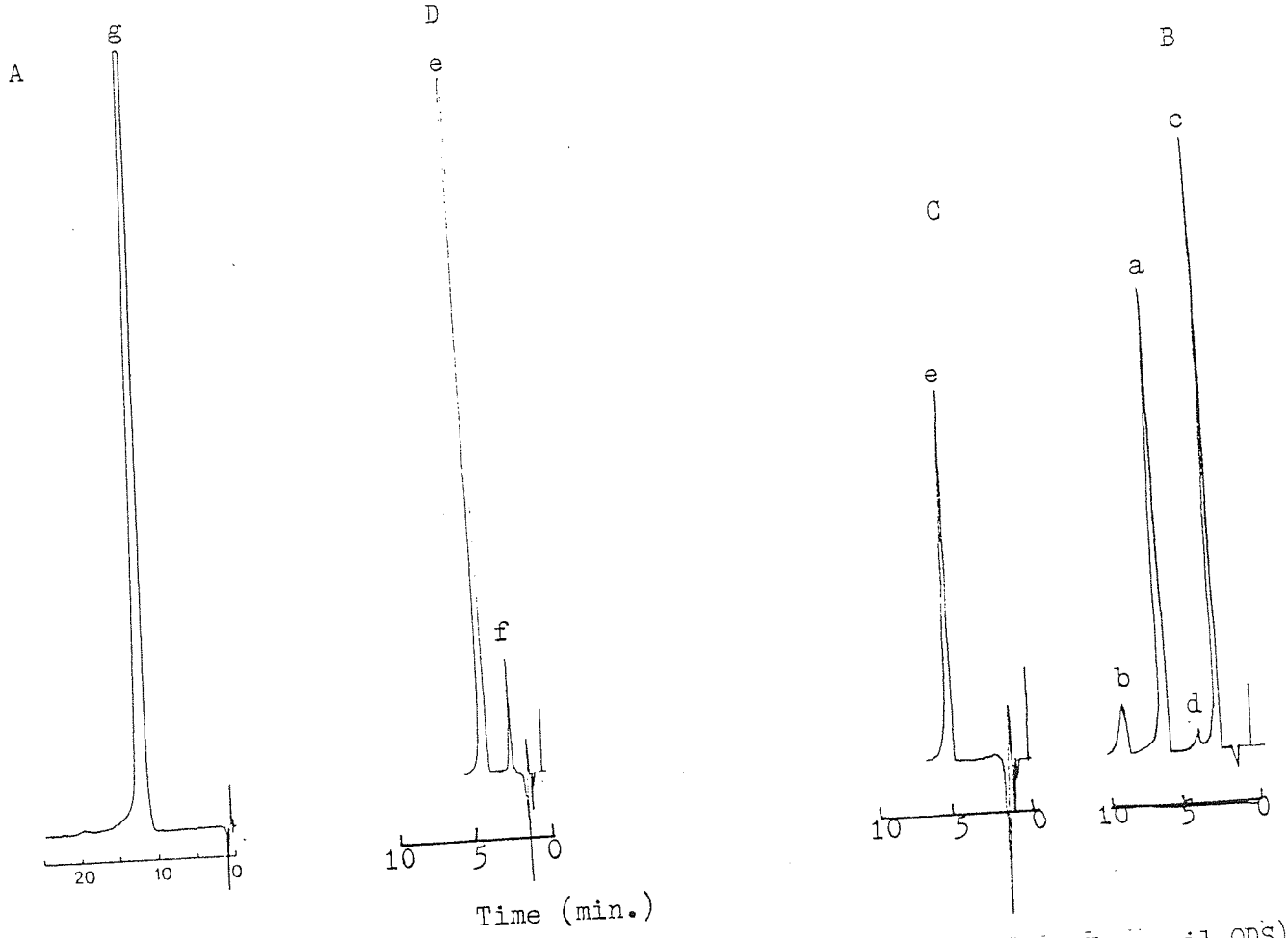


Fig. 8.3. Chromatograms of Aspirin Prodrug (Column : 10 cm X 4.6 mm Hypersil ODS)

Chromatograms		Mobile Phase	Peaks	Components
A	O-Acetyloxy phenyl benzoate	$CH_3CN:H_3PO_4:H_2O$ 45:0.5:54.5 pH 1.8	g	o-Acetyloxy phenyl benzoate
B	o-Acetyl Salicyloyl Ethyl Carbonate	$CH_3CN:H_3PO_4:H_2O$ 45:0.5:54.5 pH 1.8	a b c d	o-Acetyl Salicyloyl Ethyl Carbonate Salicylate (Corresponding) Aspirin Salicylic Acid
C	PPtd. Solid from Tidol. Suspension	$CH_3CN:H_3PO_4:H_2O$ 45:0.5:54.5 pH 1.8	e	Benorylate
D	Diluted Solution	$CH_3CN:H_3PO_4:H_2O$ 45:0.5:54.5 pH 1.8	e f	Benorylate Aspirin

The high-performance liquid chromatograms of these prodrugs are displayed in Fig.8.3. The prodrug o-acetyl salicyloyl ethyl carbonate was very unstable and was sensitive even to trace amounts of water. The HPLC trace showed that the freshly prepared aqueous solution had four peaks, which were identified as aspirin, salicylic acid, the prodrug and salicyloyl ethyl carbonate. The presence of salicylic acid and salicyloyl ethyl carbonate peaks in the HPLC trace and at the same time the absence of aromatic carboxylic acid proton in the ^{1}Hnmr spectrum suggests that the additional peaks appeared due to rapid hydrolysis of the prodrug during analysis.

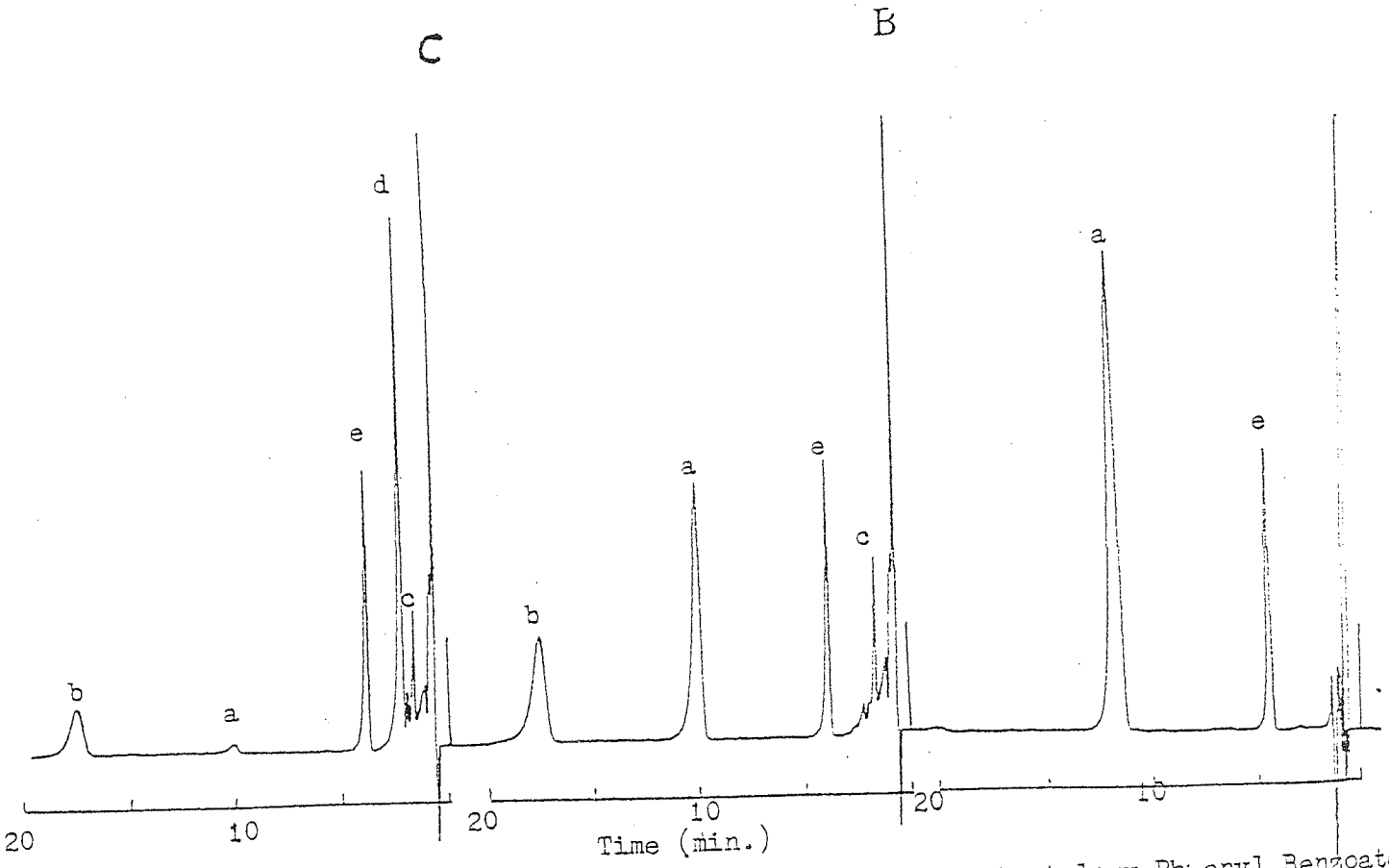
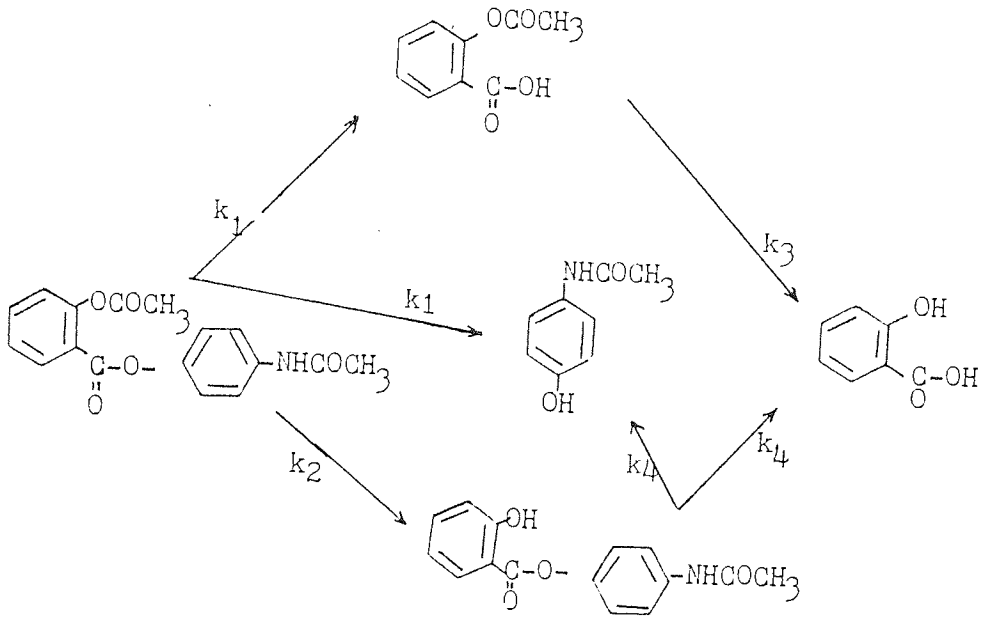


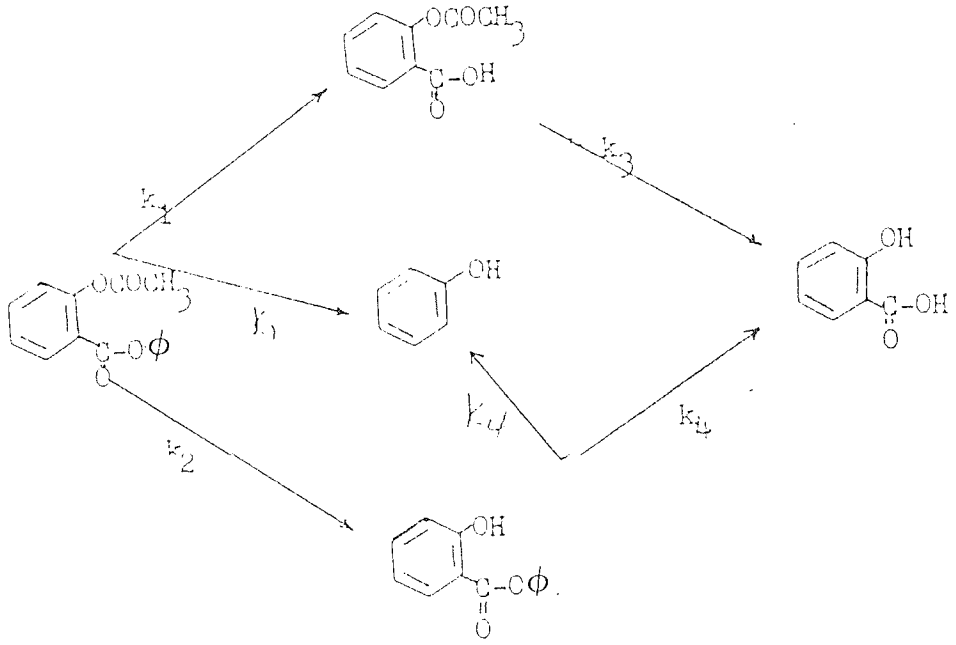
Fig. 8.4. Chromatograms Showing the Degradation of o-Acetyloxy Phenyl Benzoate

Slovent	Chromatogram	Peaks	Components
$CH_3CN:H_3PO_4:H_2O$ 45 : 0.5 : 54.5	A t = 0 min. B t = 3 min. C t = 10 min.	a b c d e	O-Acetyloxy Pheryl Benz Salol Aspirin Salicylic Acid Pripyl Paraben (Int.St

Column : 10 cm X 4.6 mm, Hypersil -ODS



Scheme 8.6. Possible Routes of Degradation of benorylate.



Scheme 8.7. Possible Routes of Degradation of O-Acetyloxy Phenyl Benzoate.

8.4. DEGRADATION PRODUCTS OF o-ACETYLOXY PHENYL BENZOATE AND BENORYLATE ANALYSED BY HPLC

o-Acetyloxy phenyl benzoate following degradation in aqueous solvent, produces aspirin, phenol, salicylic acid and phenyl salicylate (displayed by HPLC chromatograms in Fig. 8.4). Due to the difference in polarity of aspirin and phenyl salicylate, a compromise was made between the resolution of the earlier peaks and column performance of the longer retained peak phenyl salicylate. The degradation profile is shown in Fig.8.5. The elution time of phenyl salicylate was considerably longer (17 minutes) and storage of samples was necessary.The frogen samples at reduced pH maintained their stability. This is shown by the chromatograms in Fig. 8.5. The degradation process is proposed in Scheme 8.7 .

o-Acetyloxy p-acetamido phenyl salicylate (benorylate), following degradation in aqueous solution, produced aspirin, paracetamol, p-acetamidophenyl salicylate and eventually salicylic acid according to Scheme.8.6. Paracetamol, aspirin and salicylic acid together with the internal standard phenacetin were quantified following elution in one mobile phase Fig.8.6.A.). Benorylate and the p-acetamidophenyl salicylate were eluted as broad peaks after 30 mins. and 45 mins. respectively. These two components were analysed using a second mobile phase (8.6.B). The acetonitrile concentration in the second mobile phase was sufficient to elute paracetamol with the solvent front. Moreover, the high pH of the mobile phase (pH 6.8) washed salicylic acid and aspirin off the column, leaving the internal standard phenacetin well resolved. In

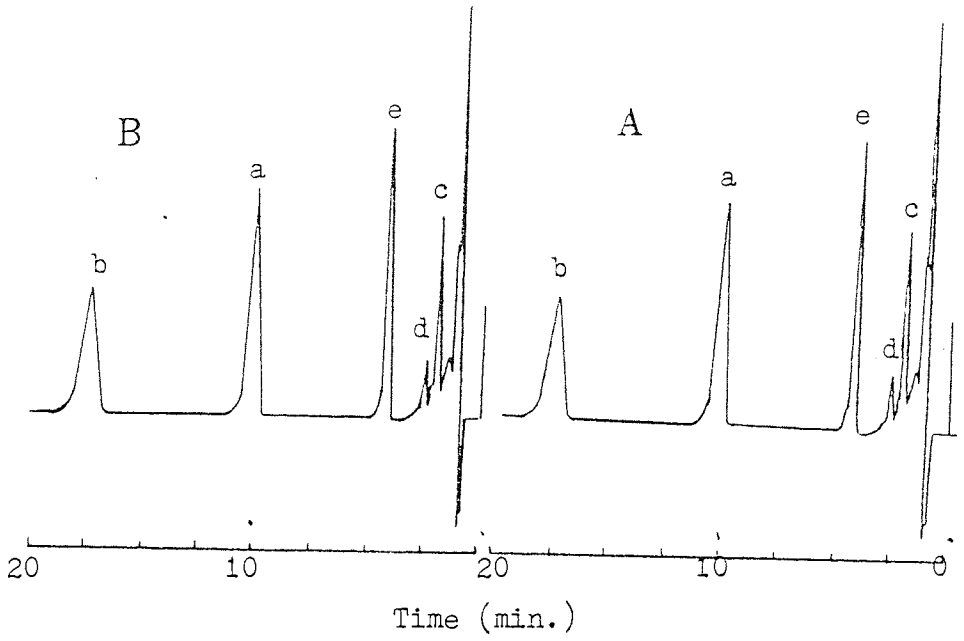


Fig. 8.5. Test Chromatograms Showing the Stability of the Stored Samples Collected During the Degradation Study of o-Acetyloxyphenyl Benzoate.
Conditions : pH of the Samples Reduced to 2 With 0.01M HCl and Solidified.

Solvent	Chromatogram	Peaks	Components
CH_3CN :H_2O:H_3PO_4	A t=0, Immediate Assay	a	0-Acetyloxy Phenyl Benzoate
45:54.5:0.5	B t= 8 Hrs. Stored	b	Salol
pH		c	Aspirin
		d	Int. Std. Propyl Paraben

Column : 10 cm X 4.6 mm, Hypersil - ODS

the second mobile phase, the chromatograms record the gradual time-dependent formation and disappearance of a further compound, which was possibly the corresponding salicylate, p-acetmidophenyl salicylate.

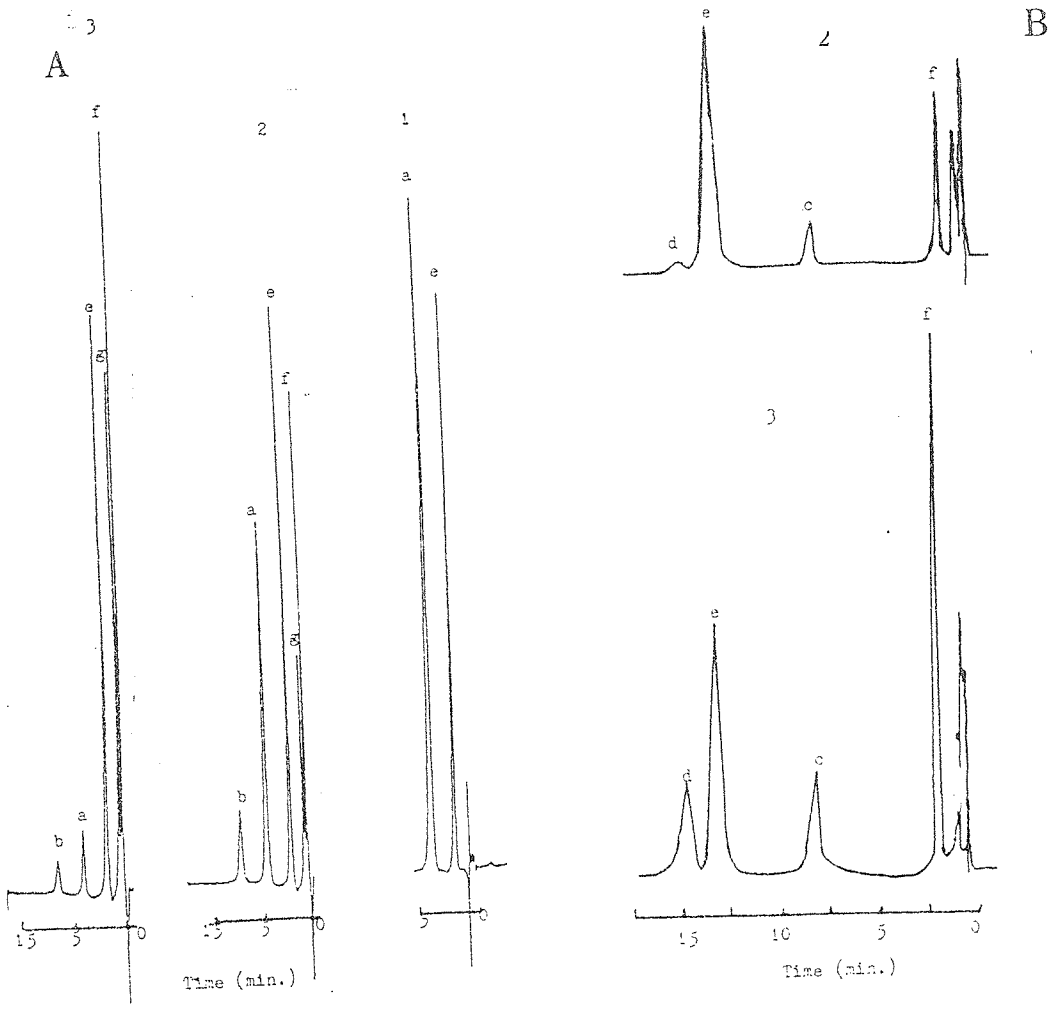


Fig. 8.6. Chromatograms Showing the Fate of Benorylate (0.1mM) in 4% Buffered Acetonitrile (v/v, pH 10.12, = 0.5M) at 45° C.

Solvent	t = min.	Peaks	Components
A $CH_3CN:NH_4OH:H_3PO_4:H_2O$ 30:0.5:0.05:64.45 pH 6.8	1 = 0 2 = 5 3 = 10	a b e f g	Benorylate p-Acetamido Salol Int. Std. Phenacetin Paracetamol Aspirin & Salicylic Acid
B $MeOH:H_3PO_4:H_2O$ 30:1:69, pH 1.8	2 = 5 3 = 10	c d e f	Aspirin Salicylic Acid Phenacetin, Int. Std. Paracetamol

Column : 10 cm X 4.6 mm, Hypersil-ODS

8.5. RESULTS AND DISCUSSION

8.5.1. CONCENTRATION-TIME PROFILES

The time-course, showing the fate of the prodrugs o-acetyloxy phenyl benzoate and o-acetyloxy p-acetamidophenyl salicylate, together with their degradation products are displayed in Fig.8.7.

The effect of pH on the degradation of o-acetyloxy phenyl benzoate is displayed by the degradation-time profiles in Fig.8.8. This shows the fate of salol (Fig.8.8.A) and aspirin (Fig.8.8.B). These plots reveal that the extent of formation of aspirin is almost independent of pH within the experimental pH range (8.2 - 11.25). Under these conditions, aspirin concentration maximises at about 22% of the initial concentration of the prodrug (Ø.1mM). In contrast, the maximum level of formation of salol was controlled by the pH of the medium, reaching a maximum of about 15% at pH 9.7 and 6Ø% at pH 11.25.

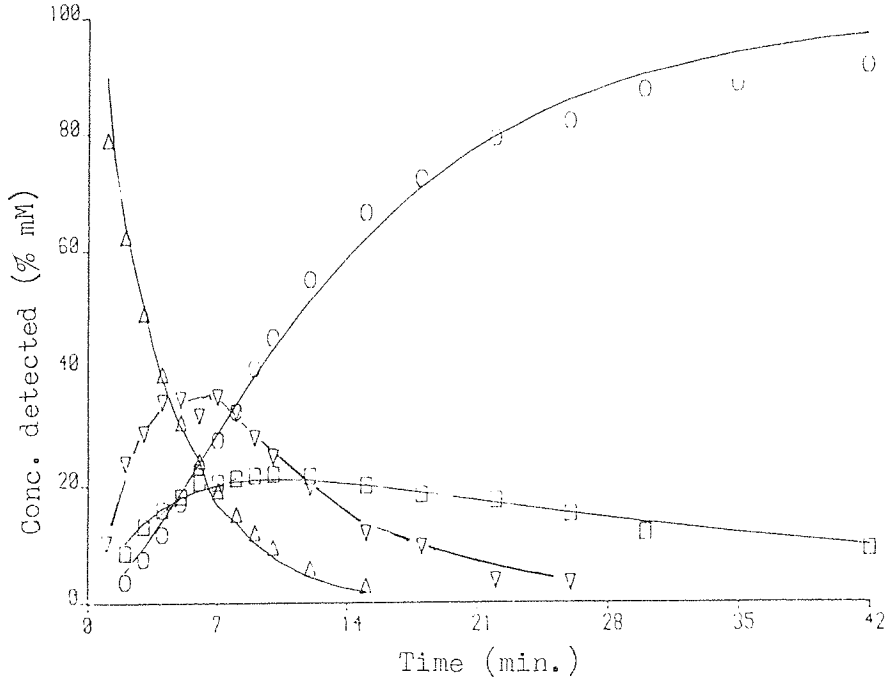


Fig.8.7.A. Concentration - Time Profiles Showing the Degradation of O-Acetyloxy Phenyl Benzoate in Buffered Acetonitrile (4% v/v, Britton-Robinson Buffer).

Symbols	Components	Conditions
△	O-Acetyloxyphenyl Benzoate	pH 10.45
▽	Salol	μ 0.50M
□	Aspirin	Temp. 50°C.
○	Salicylic Acid	A_0 0.1mM

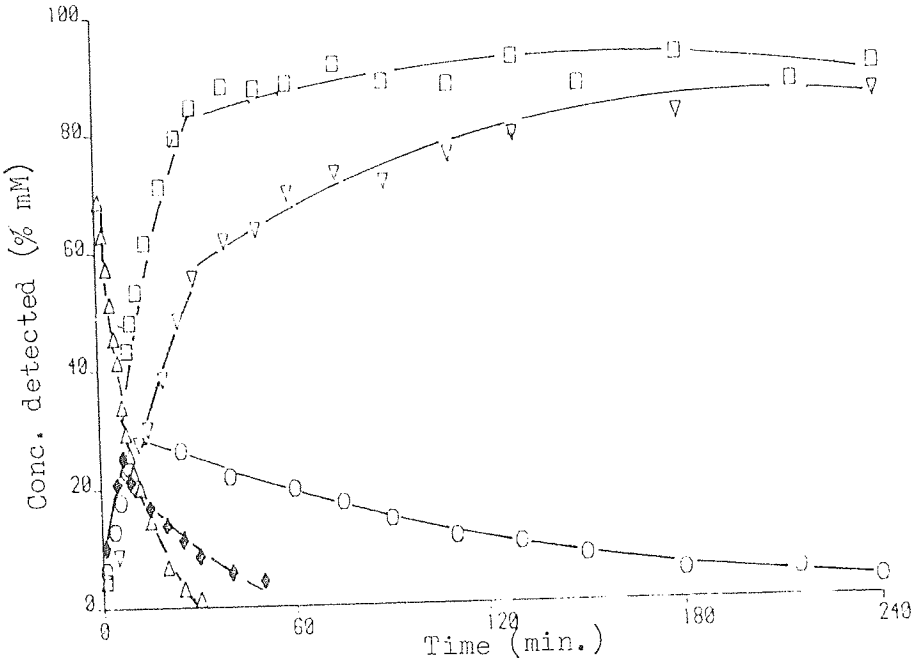


Fig. 8.7.B. The Time-Course for the Degradation of Benorylate in Buffered Acetonitrile (4% v/v, Britton-Robinson Buffer).

Symbols	Components	Conditions
△	Benorylate	pH 10.12
◇	P-Acetamido Salol	μ 0.50M
○	Aspirin	
▽	Salicylic Acid	Temp. 45° C.
□	Paracetamol	A_0 0.1mM

8.5.2. ESTIMATION OF VARIOUS SPECIFIC RATE CONSTANTS

The concentration-time profiles indicate the rapid formation of the intermediate esters and an induction period in the formation of salicylic acid, which emphasises that salicylic acid was formed from the intermediate esters and not directly from the prodrug. Considering this, the simultaneous parallel and consecutive degradation pathway (Scheme.8.6-8.7.) may be proposed for the degradation of o-acetyloxy phenyl benzoate and benorylate. The overall rate of disappearance of o-acetyloxy phenyl benzoate and benorylate were measured from the slopes of the semi-logarithmic plots of the time-courses of these prodrugs. The plots are reasonably linear (Fig.8.9), indicating that the overall degradation follows pseudo first order kinetics. The kinetic model in equation 8.1. has been used to calculate the theoretical concentrations of each component at current time, fitting the experimental concentration-time profiles to equations 8.2.-8.5. by non-linear least squares regression techniques (NONREG, NONLIN). The preliminary estimates were chosen arbitarily so that the sum (k_1+k_2) equalled the slope of the overall first order plots of the prodrugs held in Tab.8.2.

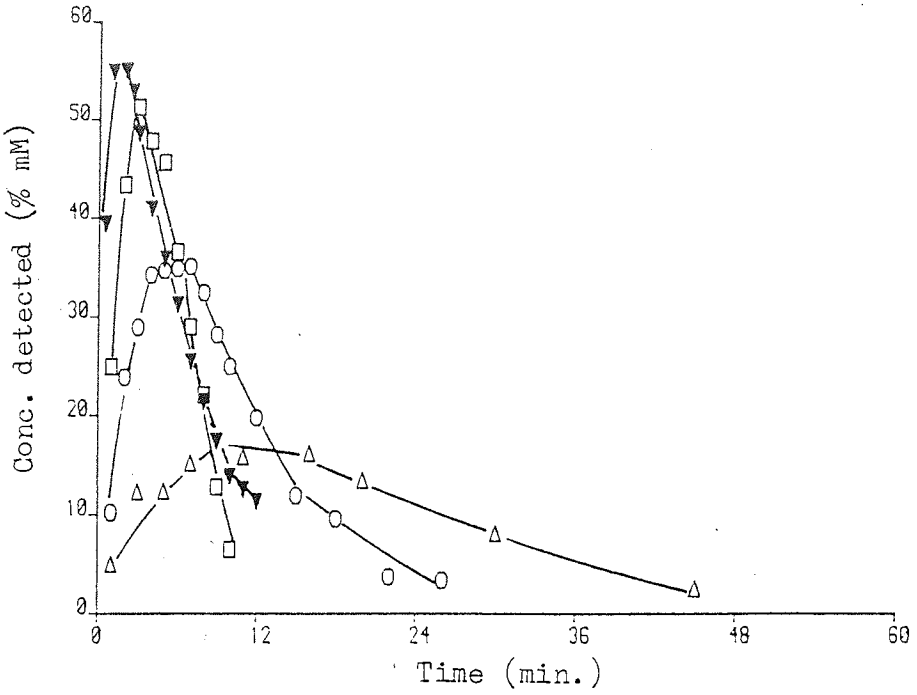


Fig. 8.8.A. pH-dependent Formation of Phenyl Salicylate from o-Acetyloxy Phenyl Benzoate.

Symbols	pH
△	3.70
○	10.45
□	11.08
▼	11.25

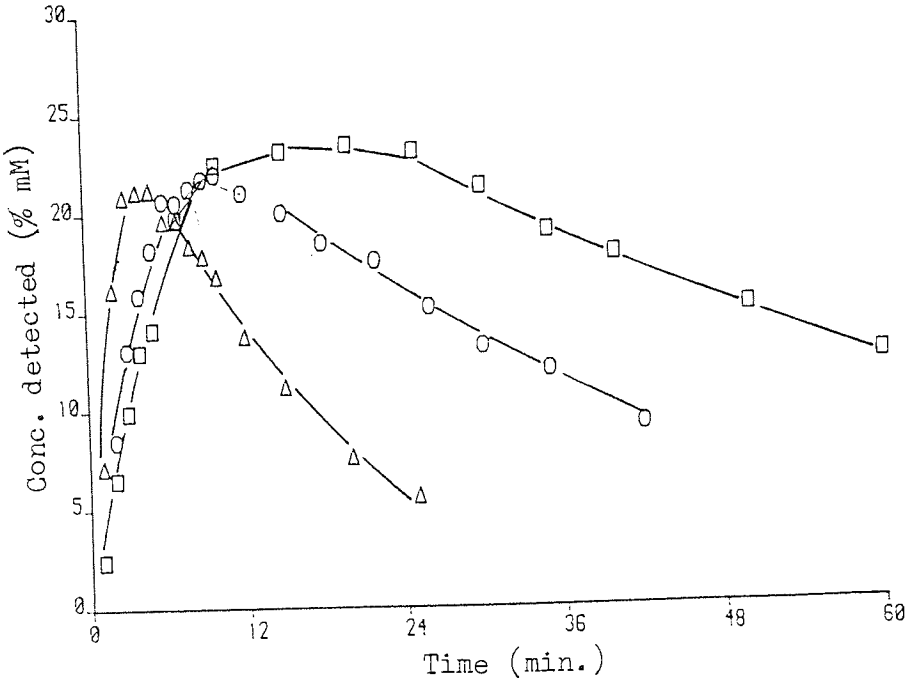


Fig. 8.8.B. pH-dependent Formation of Aspirin from O-Acetyloxyphenyl Benzoate

Symbols	pH
□	10.84
○	10.45
△	10.14

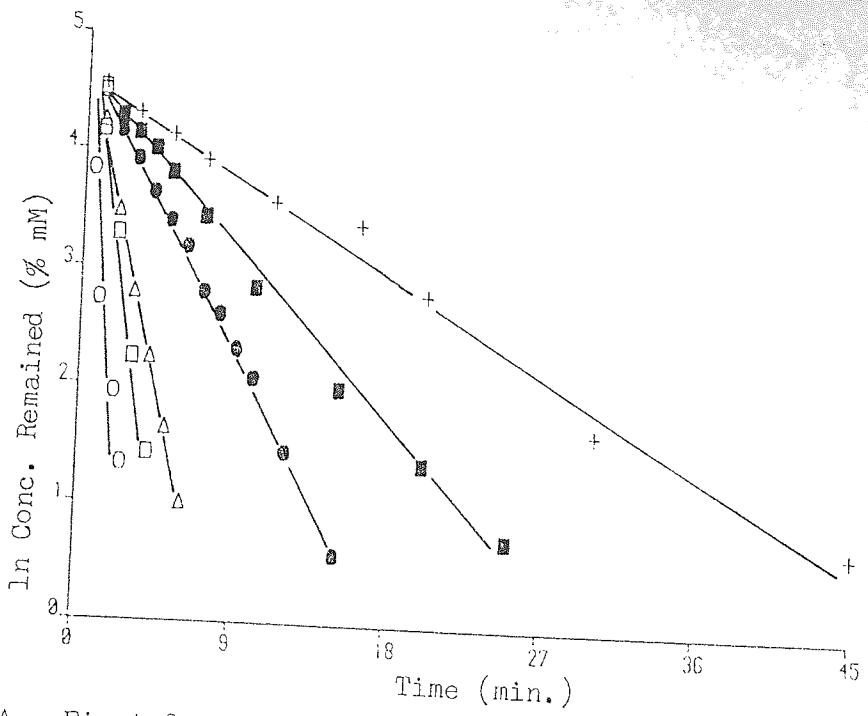


Fig. 8.9.A. First Order Plots, Showing the Effect of pH on the Overall Degradation of o-Acetyloxy Phenyl Benzoate in Britton-Robinson Buffer (μ =0.5M) at 50°C.

Symbols	pH
+	9.70
■	10.14
●	10.45
Δ	10.84
□	11.08
○	11.25

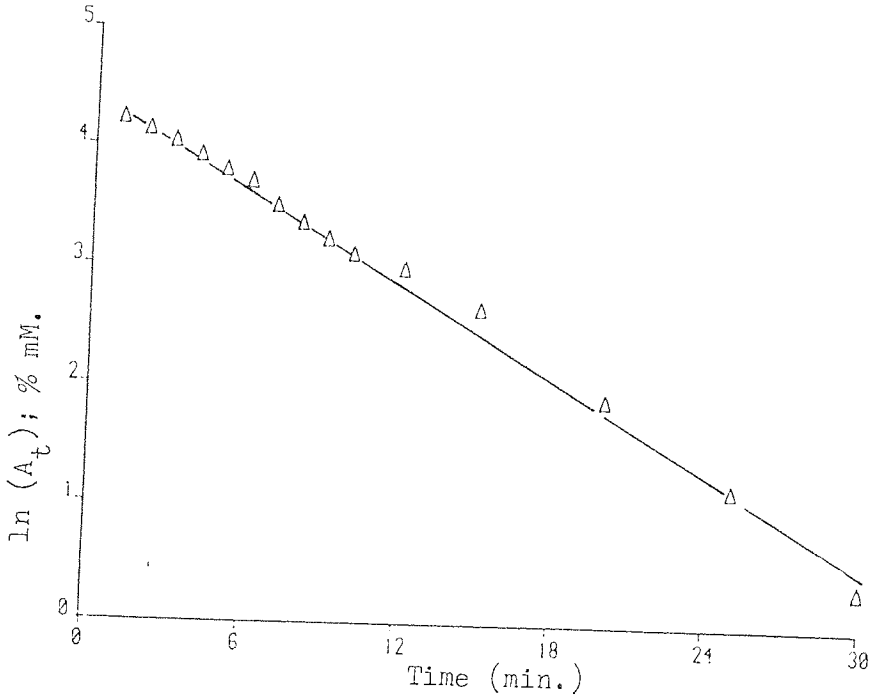


Fig. 8.9.B. First Order Plot Showing the Degradation of Benorylate in Alkaline Medium.

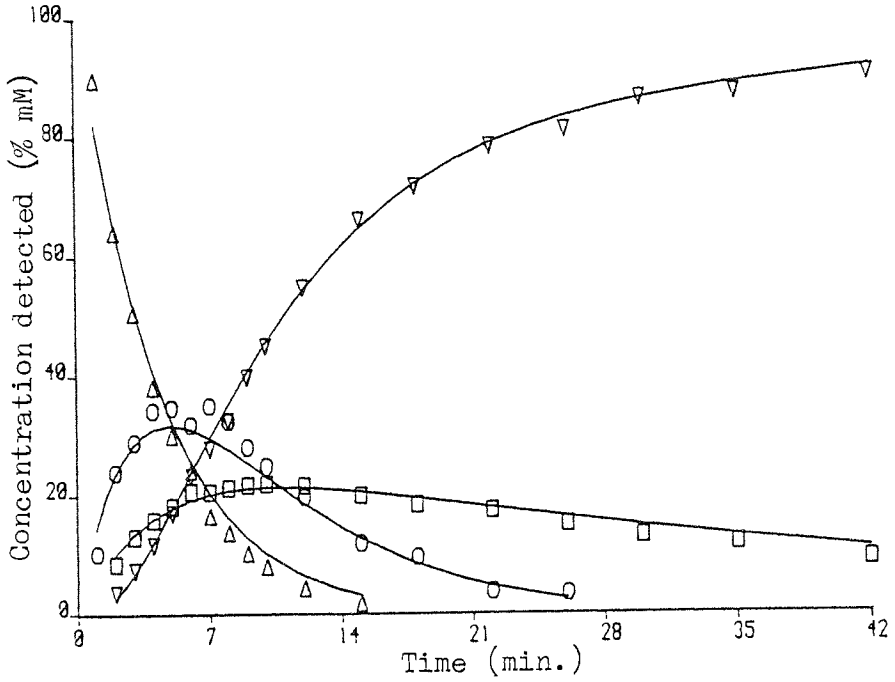


Fig. 8.10. Non-Linear Regression analysis of the Concentration-Time Profiles for Degradation of O-Acetyloxy Phenyl Benzoate at pH 10.45. Lines Represent the Theoretical Model.

Symbols	Species	Lines
Δ	O-Acetyloxy Phenylbenzoate	A_t
O	Salol	B_t
□	Aspirin	C_t
∇	Salicylic Acid	D_t

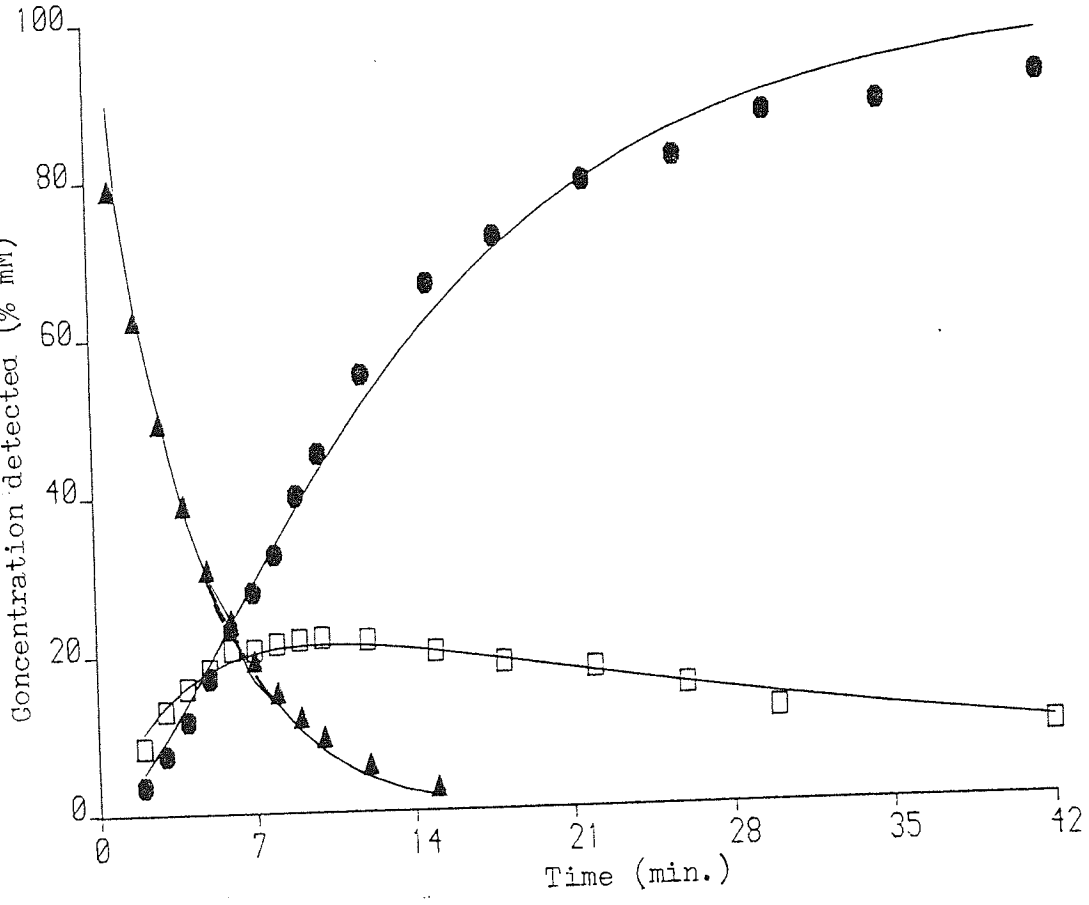


Fig. 8.11. Non-linear Regression Analysis to the Concentration-Time Profiles for the Degradation of O-Acetyloxy Phenyl Benzoate at pH 10.45, omitting Data for Salol. Lines Represent the Theoretical Model.

Symbols	Species	Lines
Δ	O-Acetyloxy Phenyl Benzoate	A_t
□	Aspirin	C_t
○	Salicylic Acid	D_t

Table 8.2. Overall Rate of Degradation of o-Acetyloxy Phenyl Benzoate Using First Order Model; Effect of pH .

pH	Rate constant $min^{-1} \times 10^{3}$ $k_1 + k_2$	r	n
8.20	0.34	0.999	14
9.7	6.50	0.999	10
10.14	17.00	0.998	9
10.45	27.00	0.999	11
10.84	64.00	0.999	6
11.08	93.00	0.999	4
11.25	116.00	0.980	4

Due to very broad [illegible] salol peak, the goodness of fit for this component to the model was poor (Fig.7.10.). To improve the NONLIN fit, a constant value for A_0 was assigned to the program and the concentration-time profiles for prodrug, aspirin and salicylic acid were fitted to equations 8.6.,8.8. and 8.9. with the ommission of the salol data. The comparitive profiles for the theoretical and experimental time-course are displayed in Fig.8.11., together with the parameters in Tab.8.3.

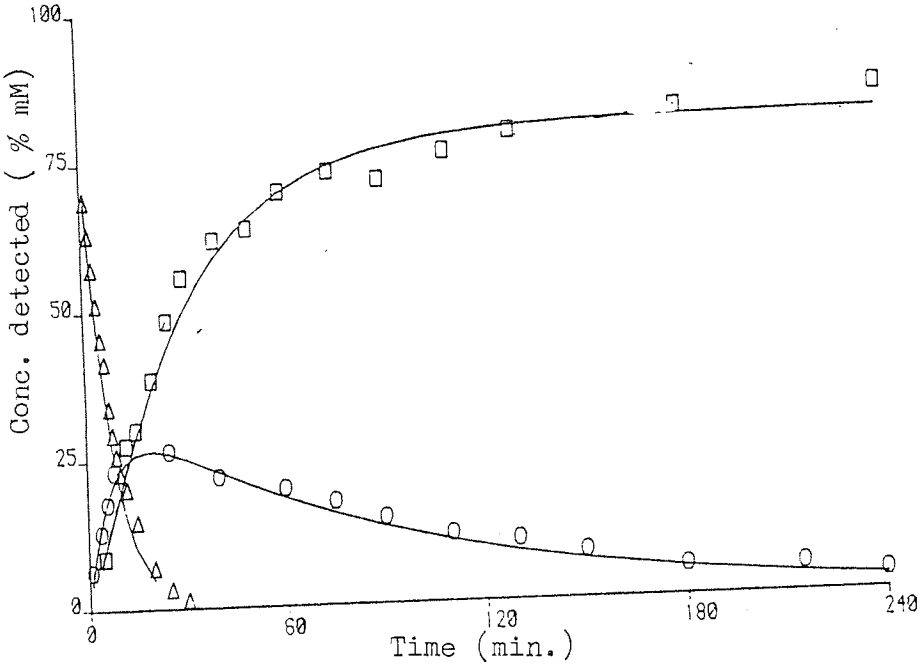


Fig. 8.12. Time Course for the Degradation of Benorylate in Buffered Acetonitrile (4% v/v) in Britton-Robinson Buffer(pH 10.14, μ = 0.5M) at 45°C., Showing the Goodness of NONLIN Fit; Lines are Generated by Equations 8.6, 8.8 and 8.9.

Symbols	Components
△	Benorylate
○	Aspirin
□	Salicylic Acid

Table 8.3. Specific Rate Constants for the Degradation of o-Acetyloxy phenyl benzoate pH 10.45 ; Comparative NONLIN Fit Between Theoritical and Experimental Profiles.

Equations used to generate thoretical profiles	Specific rate constant $k\ min.^{-1} \times 10^3$				Correlation coefficient r			
	k_1	k_2	k_3	k_4	r_1	r_2	r_3	r_4
8.2. - 8.5. A_t, B_t, C_t and D_t	17.0	6.3	17.6	2.49	0.997	0.986	0.989	0.999
8.2, 8.4, and 8.5. A_t, C_t and D_t	16.9	6.81	17.5	3.90	0.998	0.989	0.999	-

Similarly, the specific rate constants for the degradation of benorylate were measured using equations 8.2, 8.4 and 8.5, and are held in Fig.8.12. and in Tab.8.4

Table 8.4. Specific Rate Constants for the Degradation of Benorylate at 45°C in Britton-Robinson Buffer, pH 10.12 , μ= 0.5 M ; Together With the Overall Psendo First Order Degradation rate of the Prodrug

Specific rate constants $k\ min.^{-1} \times 10^3$				Correlation coefficient		
k_1	k_2	K_3	K_4	benorylate	aspirin	salicylic acid
8.62	5.41	7.31	1.29	0.991	0.980	0.998

Overall degradation rate from linear regression analysis			From NONLIN
$k_{obs}\ min.^{-1} \times 10^3$	r	n	
$k_1 + k_2$			$k_1 + k_2$
12.04	-0.997	13	14.03

Table 8.5 Effect Of pH On The Specific Rate Constants For The Degradation Of o-Acetyloxy phenyl benzoate(.1mM) In 4%(v/v) Acetonitrile In Britton Robinson Buffer(μ=.5M) at 50°C.

pH	Specific rate constants k $min.^{-1}$ X 10^{3}						
	k_1	k_2	k_3	k_4	r_1	r_2	r_3
8.2	Ø.151	.156	-	.574	Ø.999	Ø.907	Ø.999
9.7	4.49	1.85	2.78	Ø.89	Ø.999	.989	Ø.998
1Ø.14	12.7	4.39	11.6	1.38	Ø.999	Ø.997	Ø.974
1Ø.45	16.9	6.81Ø	17.5Ø	3.94Ø	Ø.998	Ø.989	Ø.999
1Ø.845	29.3Ø	14.6Ø	33.4Ø	6.86Ø	Ø.984	Ø.986	Ø.993
11.Ø8	31.7	2Ø.1	38.1	11.3	Ø.99Ø	Ø.934	Ø.981
11.25	56.8	34.6	69.8	11.5	Ø.98Ø	Ø.844	Ø.978

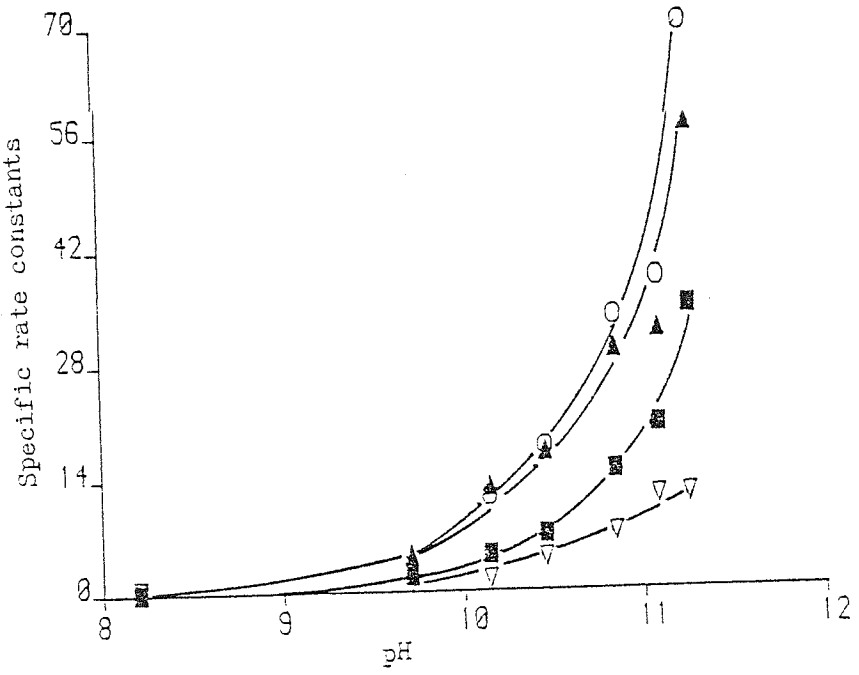


Fig. 8.13. Effect of pH on specific rate constants for the degradation of o-acetyloxy phenyl benzoate in 4% acetonitrile in Britton Robinson buffer(μ = .5M) temp. 50°C, initial conc. .1mM.

Symbols	Specific rate constants
▲	k_1 formation of salol
■	k_2 formation of aspirin
○	k_3 hydrolysis of salol
▽	k_4 hydrolysis of aspirin

8.5.3. EFFECT OF pH ON DEGRADATION OF o-ACETYLOXY PHENYL BENZOATE

The specific rate constants at various pH values within the range 8.2-11.25 were measured as described in section 8.5.2. The pH Vs. specific rate constant plots (using Tab.8.5) are displayed in Fig.8.13.

Regression analysis of log of the specific rate constants against pH represent a linear relationship, shown in Fig.8.14.together with the statistical parameters in Tab.8.6.This type of behaviour indicates that during the course of degradation, the most probable mechanism involves direct attack of hydroxide ion on the ester function.

Table 8.6 Specific Rate Constants for the Degradation of o-Acetyloxy phenyl benzoate, Least Squaes Regression Analysis, Log k v.pH

	Degradation Process	Slope	intercept	r	n
k_1	formation of salol	Ø.8195	-9.4Ø54	Ø.988	7
k_2	formation of aspirin	Ø.7545	-9.Ø178	Ø.999	7
k_3	hydrolysis of salol	Ø.8148	-9.3344	Ø.978	6
k_4	hydrolysis of	Ø.784Ø	-1Ø.6862	Ø.985	6

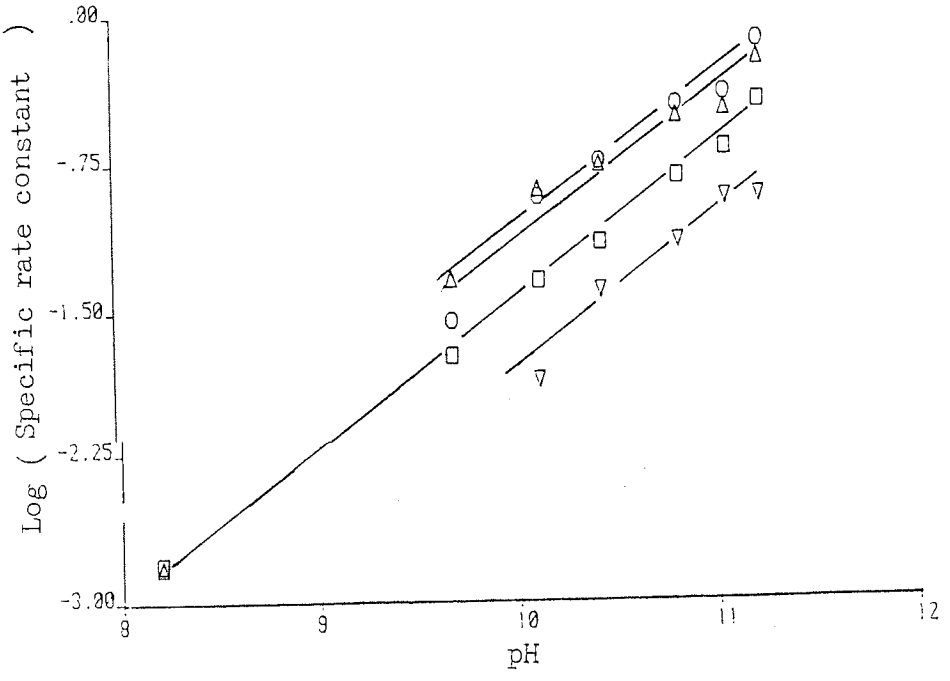


Fig. 8.14. Semi-Logarithmic Plots Showing the Effect of pH on the Specific Rate Constants.

Symbols	Δ	□	○	▽
k	k_1	k_2	k_3	k_4

APPENDICES

APPENDIX 1 LIST OF ABBREVIATIONS

AUFS	Absorbance units full scale
CARBOPOL	Carboxypolymethylene polymers
FBC	Freshly boiled and cooled
GC	Gas chromatography
HPLC	High-performance liquid chromatography
MS	Mass spectrometric
TBA	Tetrabutylammonium
THF	Tetrahydrofuran
UV	Ultraviolet

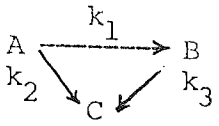
APPENDIX 2 BRITTON-ROBINSON BUFFER OF KNOWN IONIC STRENGTH

pH	Britton-Robinson buffer					KCl added *per* liter of buffer to produce an ionic strength of		
	Composition (g/l)				Ionic strength (*M*)	0.1 *M*	0.5 *M*	1 *M*
	NaOH	CH_3CO_2H	H_3PO_4	H_3BO_3				
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	—	28.480	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

-(From Ann.Clin.(Rome).,1974,64,409-412).

Appendix 3. Derivation of the Rate Equation for Simultaneous Transesterification and Hydrolysis of Salicylates:

The kinetic equations stated in section 5.2.1. may be derived as follows using the Laplace - Canon operator method :-



..... equn.5.1

For non-zero initial concentrations

$$dA/dt = SA - SA_{\emptyset} = -A(k_1 + k_2)$$

$$\frac{A - A_{\emptyset}}{A} = -\frac{(k_1 + k_2)}{S}$$

$$1 - \frac{A_{\emptyset}}{A} = -\frac{(k_1 + k_2)}{S}$$

$$\frac{A_{\emptyset}}{A} = \frac{(k_1 + k_2)}{S} + 1 = \frac{S + k_1 + k_2}{S}$$

$$A = A_{\emptyset}\frac{S}{S + k_1 + k_2} \quad \text{..... equn.A.1}$$

Transforming using $S/(S+a) \longrightarrow e^{-at}$

$$A_t = A_{\emptyset}.e^{-(k_1 + k_2)t} \quad \text{.....equn.5.5}$$

$$dB/dt = SB = k_1A - k_3B \quad \text{.....equn.5.3}$$

$$SB + k_3B = k_1A$$

$$B(S+k_3) = k_1A$$

$$B = \frac{k_1A}{(S+k_3)}$$

Substituting for A, using elqun. A.1.

$$B = \frac{k_1}{(S + k_3)}.A_{\emptyset}.\frac{S}{(S+k_1+k_2)}$$

$$= \frac{A_\emptyset k_1 S}{(S+k_3)(S+k_1+k_2)} \qquad \text{.....equn.A.2}$$

Transforming using $\frac{S}{(S+a_1)(S+a_2)} = \frac{1}{(a_2-a_1)} e^{-a_1 t} + \frac{1}{(a_1-a_2)} e^{-a_2 t}$

$$B_t = A_\emptyset k_1 \left[\frac{e^{-k_3 t}}{(k_1+k_2+k_3)} + \frac{e^{-(k_1+k_2)t}}{(k_3-k_1-k_2)}\right]$$

$$\text{or, } B_t = A_\emptyset k_1 \left[\frac{e^{-k_3 t} - e^{-(k_1+k_2)t}}{k_1+k_2-k_3}\right] \qquad \text{.....equn.5.6.}$$

$$dC/dt = SC = k_2 A + k_3 B \qquad \text{..... equn.5.4.}$$

Substituting for B using equation A.2 and for A using equation A.1

$$SC = \frac{k_2 A_\emptyset S}{(S+k_1+k_2)} + k_3 k_1 A_\emptyset \frac{S}{(S+k_3)(S+k_1+k_2)}$$

$$C = A_\emptyset \left[\frac{k_2}{(S+k_1+k_2)} + \frac{k_1 k_3}{(S+k_3)(S+k_1+k_2)}\right]$$

$$= A_\emptyset \left[\frac{k_2(S+k_3)+k_1 k_3}{(S+k_3)(S+k_1+k_2)}\right]$$

$$= A_\emptyset \left[\frac{k_2 S + k_2 k_3 + k_1 k_3}{(S+k_3)(S+k_1+k_2)}\right]$$

$$= A_\emptyset k_2 \left[\frac{S + k_3 + (k_1 k_3)/k_2}{(S+k_3)(S+k_1+k_2)}\right]$$

Transforming using :

$$\frac{(S+b)}{(S+a_1)(S+a_2)} = \frac{b}{a_1 a_2} - \frac{(b-a_1)}{a_1(a_2-a_1)} e^{-a_1 t} - \frac{(b-a_2)}{a_2(a_1-a_2)} e^{-a_2 t}$$

$$C_t = A_\emptyset k_2 \left[\frac{k_3 + \frac{k_1 k_3}{k_2}}{k_3(k_1 + k_2)} - \frac{\frac{k_1 k_3}{k_2} e^{-k_3 t}}{k_3(k_1 + k_2 - k_3)} - \frac{k + \frac{k_1 k_3}{k_2} - (k_1 + k_2) e^{-(k_1 + k_3)t}}{(k_1 + k_2)(k_3 - k_1 - k_2)} \right]$$

$$\text{or, } C_t = A_\emptyset \left[1 - \frac{k_1 e^{-k_3 t} + (k_2 - k_3) e^{-(k_1 + k_2)t}}{(k_1 + k_2 - k_3)} \right] \quad \text{equn.5.7}$$

APPENDIX 4. DETERMINATION OF pKa OF METHYL SALICYLATE IN EQUIMOLAR METHANOL : ETHANOL (50% V/V).

pKa of methyl salicylate was determined in aqueous equimolar methanol : ethanol (32 : 46 w/w ; 50% v/v) by direct titration with sodium hydroxide (0.5 M) and fitting the data to the program pKa (219) which was the exact form of the Henderson - Hasselbalch equation

$$pKa = pH - \log\left[\frac{[salt] + [H_3O^+] - [OH^-]}{[acid] - [H_3O^+] + [OH^-]}\right]$$

A typical batch of data together with the estimated pKa and statitical parameters are recorded in Table A.4.1.

Table A.4.1. : Data for the determination of pKa of methyl salicylate.

Amount of methyl salicylate used : 76 mg
initial volume of the solvent : 50 ml
molar concentration of methyl salicylate : 10 mM
concentration of sodium hydroxide : 0.5 M
temperature : 37°C.

NaOH added (ml)	.16	.26	.28	.30	.32	.36	.42	.48	.54
pH	10.55	10.81	10.89	10.93	10.95	11.00	11.17	11.24	11.31
estimated pKa	11.4	11.42	11.48	11.49	11.48	11.46	11.41	11.38	11.36

Average pKa :11.4222
95% limit :11.33-11.54
t value :32.73

APPENDIX 5 COPIES OF PUBLISHED PAPERS

International Journal of Pharmaceutics, 21 (1984) 35–50
Elsevier

IJP 00706

Transesterification of salicylate esters used as topical analgesics

W.J. Irwin, Q.N. Masuda and A. Li Wan Po

Pharmaceutics Research Group, Division of Pharmaceutics and Pharmaceutical Chemistry, Department of Pharmacy, University of Aston in Birmingham, Birmingham B4 7ET (U.K.)

(Received February 28th, 1984)
(Modified version received March 14th, 1984)
(Accepted March 30th, 1984)

Aston University

Content has been removed for copyright reasons

Tetrahedron Vol. 40, No. 24, pp. 5217 to 5223, 1984
Printed in the U.S.A.

0040-4020/84 $3.00 + .00

TRANSESTERIFICATION KINETICS OF PHENYL SALICYLATE

W. J. IRWIN,* Q. N. MASUDA and A. LI WAN PO
Pharmaceutics Research Group, Division of Pharmaceutics and Pharmaceutical Chemistry,
Department of Pharmacy, University of Aston in Birmingham, Gosta Green,
Birmingham B4 7ET, England

Aston University

Content has been removed for copyright reasons

REFERENCES

REFERENCES

1. Text Book Of Organic Medicinal and Pharmaceutical Chemistry; Edited by Wilson, C.O.; Gisvolt, O. and Doerge, R.F.; 7th edition, 1977, J.B. Lippincott Company.

2. Li Wan Po,A.; Mroso,P.V. and Irwin,W.J.; " Modelling Decomposition In The Solid State Stability of Salsalate In Suspension In The Presence Of Excipients";
Int. J. Pharmaceut., 1983,16(1),115-23.

3. Albert,A. and Serjeant,P. in " The Determination Of Ionization Constants ", 1971, Chapman and Hall Ltd.

4. Argen,A.; " The Complex Formation Between Iron (III) Ion and Some Phenols. IV. "The Acidity Constant Of The Phenolic Group",
Acta. Chem. Scand., 1955,9,49-56.

5. Katz,M., " Design Of Topical Drug Products : Pharmaceutics ", in Drug Design , Edited by Ariens,E.J., Vol.IV, pg 93-148,Academic Press, 1973.

6. Martin,A.N.; Swarbrick,J.; Cammarata,A. in " Physical Pharmacy ", 1969, pg 194, " Ionic Equilibria "; Lea and Febiger, Philadelphia.

7. Wurster,D.E. and Kramer, S.F.; " Investigation Of Some Factors Influencing Percutaneous Absorption ";
J. Pharm. Sci., 1961,50,288-293.

8. Stefanovic,D.;Gruetzmacher,H.F.,"Mechanism Of Mass Spectrometric Fragmentations.XIII. Ionization Potential Of Substituted Pyridines"
Org.Mass Spectrom.,1974,9,1052-4.Through Chem.Abstr. 82,169790n.

9. Albery,W.J.; Hadgraft,J.; " Percutaneous Absorption :Interfacial Transfer Kinetics",
J. Pharm. Pharmacol., 1979,31,65-68.

10. Guy,R.H.;Aquino,T.R.III; Honda,D.H.; "Kinetics Of Solute Transfer Across Aqueous Phase Liquid Hydrocarbon Interfaces ",
J. Phys. Chem., 1982,86(2),280-283.

11. Huber,W.; Boehme,W. and Laskowski,S.C.; " Water- insoluble Forms Of Thiamine and Niacin ",
J. Amer. Chem. Soc., 1946,68,187-191.

12. Kyte,C.T.; Feffery,G.H. and Vogel,A.I.; " Physical Properties and Chemical Constitution. Part XXVIII. Pyridine Derivatives ",
J.Chem.Soc. B, 1960,4454-4472.

13. Li Wan Po,A.; " Topical Analgesics " in Non - prescription Drugs; 1982, pg 405-407, Blackwell Scientific Publication.

14. Rabinowitz,J.L.; Feldman,E.S.; Weinberger,A. and Schunacher,H.R.;

" Comparative Tissue Absorption Of Oral ^{14}C- Aspirin and Topical Triethanolamine ^{14}C-Salicylate In Human and Kanine Knee Joints" J. Clin. Pharmacol., 1982,22,42-48.

15. Hamberg,M.; " Inhibition Of Prostaglandin Synthesis In Man ", Biochem. Biophys. Res. Comm., 1972,49,720-726.

16. Blank,I.H. in " The Evaluation Of Therapeutic Agents and Cosmetics ", pg 95-101,1964, Edited by Sternberg,T.H. and Newcomer,V.D., McGraw Hill.

17. Li Wan Po,A. " Sunscreen and Suntan Preparations " in Non-prescription Drugs, 1982, pg 39, Blackwell Scientific Publication

18. Rietschel,M.R.L. and Lewis,C.C.W.; " Contact Dermatitis To Homomenthyl Salicylate ", Arch. Dermatol., 1978,114,442-443.

19. Martindale, The Extra Pharmacopeia, 28th edition, p1498.

20. Goswin,W.; Van Han and Wolfgang P.H.; " The Design Of Sunscreen Preparations " in Drug Design , Vol. IV, Edited by Ariens,E.J., 1973, pg 209, Academic Press.

21. Rothenberg,H.W. and Hjorth,N., " Allergy To Perfumes From Toilet Soaps and Detergents In Patients With Dermatitis ", Arch. Dermatol., 1968,97,417-421.

22. Khan,G.; " Intensified Contact Sensitization To Benzyl Salicylate Phototoxic Effect Of Psoralen Therapy ", Arch. Dermatol., 1971,103,497-500.

23. Lippold,B.C., " In Vitro Methods For Simulating The Absorption Of Drugs " in Dermal and Transdermal Absorption , edited by Brandav,R. and Lippold,B.H.,pg 101-115, 1982, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

24. Anderson,J.A.R. and Ead,R.D., " Percutaneous Salicylate Poisoning ", Clin. Exp. Dermatol., 1979,4,349-51.

25. Scheuplein,R.J. and Blank,I.H., " Permeability Of The Skin ", Physiol. Rev., 1971,51(4),702-747.

26. Gunther,S.,"Drug Absorption by Intact And Damaged Skin" in Dermal and Transdermal Absorption, Edited by Brandav,R. and Lippold,B.H., pg 27-40.

27. Guy,R.H.; Fleming,R.; " Transport Across a Phospholipid Barrier" J. Colloid. Interface. Sci., 1981,83(1),130-137.

28. Scalla,W. and Schaefer,H.; " Mechanism Of Penetration Of Drugs

Into The Skin " in Dermal and Transdermal Absorption , Edited by Brandav,R. and Lippold,B.H., pg 41-72, 1982, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

29. Scalla,W.; Gazith,J.; Bauer,E.; Stuck,B. and Schaefer,H.; "Influx Of Essential Fatty Acids (EFA) and Triglycerides Into The Skin In Relation To The Age Of Human Beings ", J. Invest. Dermatol., 1979,72,287-288.

30. Brown,E.W. and Scott,W.O., " The Comparative Absorption Of Certain Salicylate Esters By The Human Skin ", J. Pharmacol. Exp. Theraput., 1934,50,373-385.

31. Roberts,M.S.;Anderson,R.A.;Swarbrick,J and Moore,D.E. " The Percutaneous Absorption of Phenolic Compounds: The Mechanism of diffusion Across the Stratum Corneum", J. Phar. Pharmacol. 1978, 30, 486.

32. Eaglstein,W.H.; Farzad,A. and Capland,L.;"Topical Corticosteroid Therapy: Efficacy of Repeated Application" Arch. Derm., 1974,110,955.

33. Vickerst,C.F.H.,"Existance of Reservoir In The Stratam Corneum" Arch. Derm., 1963,88,20-23.

34. Turi,J.S.; Danielson,D. and Woltersom,J.W., " Effect Of Polyoxypropylene 15 Stearyl Ether and Propylene Glycol On Percutaneous Penetration Rate Of Diflorasone Diacetate ", J. Pharm. Sci., 1979,68(3),275-280.

35. Yalkowsky,S.H. and Flynn,G.L., " Correlation and Prediction Of Mass Transport Across Membranes. II. Influence Of Vehicle Polarity On Flux From Solutions and Suspensions ", J. Pharm. Sci., 1974,63,1276-1280.

36. Hildebrand,J.H. and Scott,R.L., in " The Solubility Of Nonelectrolytes ", pg 28, 1965, Dover Publications, Inc.

37. Fokkens,J.G.; " A Thermodynamic Study Of The Solubility Of Theophylline and Its Hydrate ", Int. J. Pharmaceut. 1983,14,79-93.

38. Grant,D.J.W.; Mehdizadeh,M.; Chow,A.H.L. and Fairbrother,J.E., "Non-linear Van't Hoff Solubility - Temperature Plots and Their Pharmaceutical Interpretation ", Int. J. Pharmaceut., 1984,18,25-38.

39. Alexander,K.S.;Mauger,J.W.;Petersen,H.Jr.and Paruta, "Solubility Profiles and Thermodynamics Of Parabens In Aliphatic Alcohols ", J. Pharm. Sci., 1977,66,42-48.

40. Poulsen,B.J.;"Design Of Topical Drug Products :Biopharmaceutics" in Drug Design, Vol.IV, Edited by Ariens,E.J., 1973, pg 149-192, Academic Press.

41. Guy,R.H.; Aquino,T.R.III and Honda,D.H.; " The Influence Of Urea On The Kinetics Of Interfacial Transfer ", J. Colloid. Interface. Sci., 1982,87(1),107-114.

42. Ziegenmeyer,J., " The Influence Of The Vehicle On The Absorption and Permeation of drugs ", in Dermal and Transdermal Absorption, pg 73-89, 1982, edited by Brandav,R. and Lippold,B.H., Wissenschaftliche Verlasgesellschaft mbH, Stuttgart.

43. Lippold,B.C. and Taubner,A.; " The Effect Of Different Ointment Bases On The Activity Of Dissolved Nicotinic Acid Benzyl Ester ", Pharm. Ind., 1981,43(11),1123-33.

44. Higuchi,T.,"Rate Of Release Of Medicaments From Ointment Bases Containing Drugs In Suspension ", J. Pharm. Sci., 1961,50,874-5.

45. Matsushima,I.and Osamura,H.,"Studies On The Permeability Of Human Skin To Nicotinic Acid, Methyl Nicotinate and Butyl Nicotinate With Emphasis On The Influence Of Liquid Vehicles and That Of Water Immersion ", Nippon Hifuca Gakkai Zasshi,1980,90(8),687-99,(JPN), Through Chem. Abstr. 1981,94,197485g.

46. Henschel,V.and Jaminet,F.,"Percutaneous Absorption Of Nicotinic Acid Esters. Effect Of Excipients ", J. Pharm. Belg., 1972,27(6),743-754 (Fr.), Through Chem. Abstr., 1973,78,119070y.

47. Hazratwala,B.R., " The Percutaneous Absorption Of Methyl Nicotinate From Aqueous and Oily Creams Containing Inert Ingredients ", Proc. Univ. Otago Med. Sch., 1976,54(1),14-15, Through Chem. Abstr. 1976,91,162973a.

48. Weiss,A.L.and Sciarrone,B.J.,"Release Rates Of Salicylates From Cocoa Butter ", J. Pharm. Sci., 1969,58,980-982.

49. Lippold,B.C.,"Biopharmaceutical Viewpoints On The Development and Use Of Ointments", Pharm. Ind., 1976,38(2),201-7,(Ger.), Through Chem. Abstr. 1976,84,155589g.

50. Ostrenga,J.;Steinmetz,C.and Poulsen,B.,"Significance Of Vehicle Composition. I. Relationship Between Topical Vehicle Composition ", J. Pharm. Sci., 1971,60,1175-1179.

51. Plakogianis,F.M.and Yaakob,M.,"Influence Of Ointment Bases On The In Vitro Release Of Methyl Salicylate ", Pharm. Acta Helv., 1977,52(10),236-238.

52. Fountain,R.B.; Baker,J.W. and Sarkany,I., " The Rate Of Absorption and Duration Of Action Of Four Different Solutions Of Methyl Nicotinate ", Brit. J. Dermatol., 1969,81,202-206.

53. Elfbaum,S.C.;Laden,K.,"The Effect Of Dimethyl Sulphoxide On Percutaneous Absorption : A Mechanistic Study, Part III. ",
J. Soc. Cosmet. Chem., 1968,19,841-847.

54. Price,V.H.;Meneffe,E.,"On The Effect Of Dimethyl Sulphoxide On Hair Keratin ",
J. Invest. Dermatol., 1967,49(3),297-301.

55. Morain,W.D.;Replogle,C.A. and Curran,P.F.,"Effect Of Dimethyl Sulphoxide On Permeability and Electrical Properties Of Frog Skin"
J.Pharmacol. Exper. Ther., 1966,154,298-302.

56. Barker,S.A.;Crews,S.J.;Marsters,J.B.and Stacey,M.,"Inhibition Of Hyaluronic Acid Degradation By Dimethyl Sulphoxide" Nature, 1965,207,1388-1389.

57. Marcus,F.;Colaizzi,J.L.; Barry,H.,"pH Effect on Salicylate Absorption From Hydrophilic Ointment ",
J. Pharm. Sci., 1970,59(11),1616-1620.

58. Stoughton,R.B. and McClure,W.O.;"Azone^{R} : A New Non-toxic Enhancer Of Cutaneous Penetration ",
Drug Dev. Ind. Pharm., 1983,9(4),725-744.

59. Mezei,M.and White,G.N.,"Dermatitic Effect Of Non-ionic Surfactants. III. Incorporation Of ^{32}P Into Phospholipids and Acid Soluble Material Of Normal and Surfactant Treated Rabit Skin In Vitro ",
J. Pharm. Sci., 1969,58,1209-1212.

60. Dalvi,U.G.and Zatz,J.L.;" Effect Of Non-ionic Surfactants On Penetration Of Dissolved Benzocaine Through Hairless Mouse Skin",
J. Soc. Cosmet. Chem., 1981,32,87-94.

61. Mereish,K.A.;Rosenburg,H. and Coby,J.; "Glucosylated Albumin and Its Influence On Salicylate Binding ",
J. Pharm. Sci., 1982,71,235-238.

62. Steele,W.H.; Boobis,S.W.; Moore,M.R.; Goldberg,A. and Brodie,M.J., " Protein Binding Of Salicylate In Cutaneous and Hepatic Porphyria"
Eur. J. Clin. Pharmacol., 1978,13,309-313.

63. Wosilait,W.D., "Theoretical Analysis Of Binding Of Salicylate By Human Serum Albumin. The Relationship Between Free and Bound Therapeutic Levels ",
Eur. J. Clin. Pharmacol., 1976,9,285-290.

64. Trnavska,Z. and Trnavsky,K., " Characterization Of Salicylate Binding To Sinovial Fluid and Plasma Protein In Patients With Rheumatoid Arthritis ",
Eur. J. Clin. Pharmacol., 1980,18,403-406.

65. Furst,D.E.; Tozer,T.N. and Melmon,K.L., " Salicylate Clearance, The Resultant Of Protein Binding ",

Clin. Pharmacol. Ther., 1979,26,380-389.

66. Zaroslinski,J.F.; Keresztes-Nagy,S.; Mais,R.F. and Oester,Y.T., " Effect Of Temperature On The Binding Of Salicylate By Human Serum Albumin ",
Biochem. Pharmacol., 1974,23,1767-1776.

67. Skidmore,I.F. and Whitehouse,M.W., " Effect Of Non-steroid Anti-inflammatory Drugs On Aldehyde Binding To Plasma Albumin : A Novel in vitro Assay For Potential Antiinflammatory Activity ",
J. Pharm. Pharmacol., 1965,17,671-673.

68. Whitehouse, M.W. and Skidmore,I.F., " Concerning The Regulation Of Some Diverse Biochemical Reactions, Underlining The Inflammatory Response By Salicylic Acid, Phenyl Butazone and Other Acidic Antiarthritic Drugs ",
J. Pharm. Pharmacol., 1965,17,668-670.

69. Cham,B.E.; Boechner,F.; Imhoff,D.M.; Byrne,G. and Gunsberg,M., " in vivo and in vitro Studies On The Binding Of Salicylate To Human Plasma Protein. Evidence For One Type Of Binding Site ",
J. Pharmacol. Exp. Ther., 1982,220,648-653.

70. Hamzah,R.Y. and Tu,S., " Determination Of The Position Of Monooxygenation In The Formation Of Catecol Catalysed By Salicylate Hydroxylase ",
J. Biol. Chem., 1981,256,6392-4.

71. Tauber,U., 1982, " Metabolism Of Drugs On and In The Skin ", in Dermal and Transdermal Absorption, pg 133-151, Edited by Brandav,R. and Lippold,B.H., Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

72. Davison,C.; Zimmerman,E.F. and Smith,P.K., " On The Metabolism and Toxicity Of Methyl Salicylate ",
J. Pharm. Pharmacol., 1961,132,207-211.

73. McCoubrey,A.; Smith,M.H. and Lane, A.C., " Inhibition Of Enzymes By Alkyl salicylic Acid ",
J.Pharm. Pharmacol.,1970,22,333-7.

74. Levy,G., " Salicylurate Formation - Demonstration Of Michaelis - Menten Kinetics In Man ",
J. Pharm. Sci., 1965,54,496,(Communication To the Editor).

75. Levy,G. and Hollister,L.E., " Variation In Rate Of Salicylate Elimination By Humans ",
Brit. Med. J., 1964,11,286-288.

76. Levy, G., " Pharmacokinetics Of Salicylate Elimination In Man ",
J. Pharm. Sci., 1965,54,959-967.

77. Hollister,L.E. and Levy, G., " Some Aspects Of Salicylate Distribution In Man ",
J. Pharm. Sci., 1965,54,1126-1129.

78. Garret,D.C. in " Drugs and Galenicals, Their Quantitative Analysis ", 1950,pg 214-217, Chapman and Hall Ltd.

79. Wadodker,S.G.; Kasture,A.V. and Mandle,A.M., " Estimation Of Methyl Salicylate In Formulations Containing Oils ", Ind. Drugs, 1978,16,13-14; Through Chem Abstr.,1979,90,110042f.

80. Sherman,E.B. and Zabokritskii,M.R., " Aquametric Microdetermination Of Drugs Containing An Ester Group ", Khim.-Farm. Zh., 1977,11,136-8 (Rus.); Through Chem. Abstr., 1977,86,127390h.

81. Das Gupta,V. and Ho,H.W., " Potassium Hydroxide Solution 10% For The Fast Hydrolysis Of Esters For Analysis ", Amer. J. Hosp. Pharm., 1977,34,653-654.

82. Das Gupta,V., " Quantitative Determination Of Methyl Salicylate In Liniment ", Amer. J. Hosp. Pharm., 1974,31,1001-1002.

83. Pankrtz, R.E. and Bandelin, E.J., " The Colorimetric Determination Of Salicylates ", J. Amer. Pharm. Assoc. (Sci. Ed.), 1952,41,267-270.

84. Trinder,P.,"Rapid Determination Of Salicylate In Biological Fluids" Biochem. J., 1954,301-303.

85. Gordy,R.W.; Graziano,J.H.; Akers,H.A. and Cerami,A. " The Development Of New Iron-chelating Drugs ", J. Pharmacol. Expt. Ther., 1976,196,478-485.

86. Leonard,D. and Vanderbelt,J.M., " The Ultraviolet Absorption Spectra Of Simple Unsaturated Compounds. II. m- and o- Disubstituted Benzene Derivatives ", J. Amer. Chem. Soc., 1949,71,1521-2954.

87. Pal,S.K.; Lahiri,S.C. and Aditya,S., " Spectrophotometric Investigation On The Alkaline Hydrolysis Of Methyl Salicylate ", Z. Phys. Chemie. (Leipzig)., 1974,255,226-237.

88. Mata,F. and Mucientes,A., " Kinetic Study Of The Hydrolysis Of Ortho and Para Methyl Hydroxy Benzoates ", Z. Phys. Chemie. (Leipzig)., 1977,259,881-888.

89. Khan,M.N.; Olagbemiro,T.O. and Umar,U.Z., " The Kinetics and Mechanism Of Hydrolytic Cleavage Of Phenyl Salicylate Under Highly Alkaline Medium ", Tetrahedron., 1983,39,811-814.

90. Capon,B. and Ghosh,B.C., " The Mechanism Of The Hydrolysis Of

Phenyl Salicylate and Catechol Monobenzoate In The Presence and Absence Of Borate ions ",
J. Chem. Soc. B .,1966,472-478.

91. Bender,M.L.; Kezdy,F.J. and Zerner,B, " Intramolecular Catalysis In The Hydrolysis Of p- Nitro Phenyl Salicylates ",
J. Amer. Chem. Soc., 1963, 85, 3017-3024.

92. Khan,M.N., " Structure - Activity Relationship and The Rate Determining Step In The Neucleophilic Cleavage Of Phenyl Salicylate With Primary and Secondary Amines ",
J. Org. Chem., 1983, 48, 2046-2052.

93. Van De Vaart,F.J.; Hulshoff,A. and Indemans,A.W.M., " Analysis Of Creams. I. Quantitative Determination Of Drugs In Creams By UV Spectrophotometry ",
Pharm. Weekbl., Sci. Ed., 1980,2,179-185 (Eng.), Through Chem. Abstr., 1981,94,109424h.

94. Gordy,W., " Spectroscopic Evidence Of Hydrogen Bonds : Effects Of Chelation On The Carbonyl Frequency ",
J. Chem. Phys., 1940,8,516-19.

95. Tsunetoshi,K.; Koji,K. and Toshihiro,I., " Fluorimetric Determination Of Phenol Derivatives . I. Fluorimetric Determination Of Salicylic Acid Esters In A Solution Of N,N- Dimethyl Formamide ",
Bunseki Kagaku., 1971,20,(7),801-6.(JPN)., Through Chem. Abstr. 1972,76,20986y.

96. Kloepffer,W. and Naundorf,G., " Fluorescence Of Methyl Salicylate In Hydrogen Bonding Solvents ",
J. Lumin., 1974,8(6),457-61. Through Chem. Abstr., 1974,81,77108w.

97. Kovi,P.J.; Miller,C.L. and Schulman,S.G., " Biprotonic Versus Intramolecular Phototautomerism Of Salicylic Acid and Some Of Its Methylated Derivatives In The Lowest Excited Singlet State ",
Anal. Chim. Acta, 1972, 61, 7-13.

98. Schachter,D. and Mannis,J.G., " Salicylate and Salicyl Conjugates :Fluorimetric Estimation, Biosynthesis and Renal Excretion In Man ",
J. Clin. Invest., 1958, 37, 800-807.

99. Lange,W.E. and Bell,S.A., " Fluorimetric Determination Of Salicylate In Blood ",
J. Pharm. Sci., 1966,55,386-389.

100 Saltzman,A., " Fluorimetric Method For The Estimation Of Salicylate In Blood ",
J. Biol. Chem., 1948,174,399-404.

101. Harris,P.A. and Riegelman,S., " Acetylsalicylic Acid Hydrolysis In Human Blood and Plasma I. Methodology and in vitro Studies ",
J. Pharm. Sci., 1967,56,713-716.

102. Chirigos,M.A. and Sidney,U., " A Simple Fluorimetric Procedure

For Determining Salicylic Acid In Biological Tissues ",
J. Lab. Clin. Med., 1955,54,769-772.

103. Khabarov,A.A. and Povalyaeva,L.I., " Fluorimetric Determination Of Salicylic Acid Derivatives;
Farmatsiya (Moscow), 1980,29,39-41. Through Int. Pharm. Abstr., 1982,19,3288.

104. Khemani,L. and French,I.W., " Thin-Layer Chromatography Of Aminobenzoates and Salicylates ",
J. Chromatogr., 1969,41,274-275.

105. Grizodub,A.I. and Georgivskii,V.P., " Evaluation Of Chromatographic Separation In A Solvent System Of Benzene-Methanol ",
Zh. Anal. Khim., 1980,35(3),535-8. (Rus.). Through Chem. Abstr., 1980,92,221440v.

106. Van De Vaart,F.J., " Separation Of Some Fatty Ointment Excipients By Gradient Elution HPLC ",
Pharm. Weekbl., 1981,116,1098-1101. Through Chem.Abstr., 1981,94,24633a.

107. Thielemann,H.; Paepke,M., " Thin Layer Chromatographic Estimation and Identification Of 3-Pyridine Carboxylic Acid (Nicotinic Acid), 3-Pyridine Carboxylic Acid Amide (Nicotinamide) and Nicotinic Acid Benzyl Ester On Silufol UV 254 Films ", Sci. Pharm., 1972,40,210

108. Beisenbekov,A.S., " Determination Of Derivatives Of Nicotinic Acid and Isonicotinic Acid By Paper Chromatography ",
Mater. Nauchn. Konf. Alma-At. Gos. Inst., 43rd, 1971,427-30 (Ger.). Through Chem. Abstr., 1975,82,121060w.

109. Al-Khamis,K.; Davis,S.S.; Hadgraft,J. and Mills,S.," The Determination Of Thermodynamic Activity By Gas Chromatography Head Space Analysis and Its Use In Studying Release Rates Of Drugs From Topical Preparations ",
Int. J. Pharmaceut.,1982,10,25-28.

110. Van Atta,R.E. and Van Atta, R.L., "Gas Chromatographic Determination Of Methyl Salicylate In Rubbing Alcohol",
J. Chem. Educ., 1980,57(3),230-1.

111 Walash,M.I. and Hassan,S.N., "A Note On The Identification Of Volatile Oils In Vaginal Powders",
J. Drug Res., 1978,10,85-9. Through Chem. Abstr., 1980,92,11281t.

112. Ali,S.L.,"Simultaneous Determination Of Acetylsalicylic Acid and Salicylic Acid by Gas Liquid Chromatography Using A New Methylation Technique",
Chromatographia, 1973,6,478-480.

113. Ali,S.L.; "A Comparative Study Of The Derivatization Of

Salicylic Acid and Acetyl Salicylic Acid With BSTFA, MSTFA and Methyl Iodide In Presence Of Potassium Carbonate Prior To GLC Determination",
Chromatographia, 1975,8,33-34.

114. Williams,K.J.; Li Wan Po,A. and Irwin,W.J.,"Sample Solvent Induced Peak Broadening In The Reversed Phase High-Performance Liquid Chromatography Of Aspirin and Related Analgesics",
J. Chromatogr .,1980,194,217-223.

115. Reepmeyer,J.C. and Kirchhoefer,R.D.,"Isolation Of Salicylsalicylic Acid, Acetylsalicylic Acid and Acetylsalicyl anhydride From Aspirin Tablets by Extraction and High-Performance Liquid Chromatography",
J. Pharm. Sci., 1979,68,1167-1169.

116. Cham,B.E.;Bochner,F.; Imhoff,D.M.; Johns,D. and Rowland,M., "Simultaneous Liquid Chromatographic Quantitation Of Salicylic Acid, Salicyluric Acid and Gentisic Acid In Urine",
Clin. Chem. ,1980,26,111-114.

117. Das Gupta,V.,"Simultaneous Quantitation Of Acetaminophen, Aspirin, Caffeine, Codeine Phosphate, Phenacetin and Salicyl Amide by High-Performance Liquid Chromatography",
J. Pharm.Sci., 1980, 69,110-112.

118. Stewart,J.T.; Honigberg,I.L. and Coldren,J.W.,"Liquid Chromatography In Pharmaceutical Analysis XI. Determination Of Muscle relaxant Analgesic Mixtures Using Reversed Phase and Ion Pair Techniques",
J.Pharm.Sci.,1979,68,32-36.

119. Ross,M.and Stewart,F.,"Application Of High-Performance Liquid Chromatography To The Analysis Of Volatile Oils",
J.Chromatogr.,1980,160,199-204.

120. Lo,L.A.,and Bye,A.;"Specific And Sensitive Method For The Determination Of Aspirin And Salicylic Acid In Plasma Using Reversed Phase High-Performance Liquid Chromatography",
J.Chromatogr, 1980,181,473-477.

121. Harrison,L.I.; Funk,M.L. and Ober,R.E.,"High-Performance Liquid Chromatographic Determination Of Salicylsalicylic Acid, Aspirin and Salicylic Acid In Human Plasma and Urine",
J. Pharm. Sci., 1980,69,1268-71.

122. Saotome,T.; Ogasawara,Y.; Ishijma,I. and Masuda,N.,"The Use Of Pre-column To Avoid The Interference Of Polyamides In The High-Performance Liquid Chromatographic Analysis Of Salicylates",
Chem. Lett., 1980,pg 245-246.

123 Lake,O.A.; Hulshoff,A.; Van De Vaart,F.J. and Indemans,A.W.M., "Analysis Of Creams IV. Application Of High-Performance Liquid Chromatography. Part I",
Pharm. Weekblad (Sci. Ed.), 1983,5,15-21.

124. Hasegawa,K.; Matsuno,T.; Ikagawa,S. and Hoshida,H., "Determination Of Acid Chloride By HPLC", Yakugaku Zasshi, 1981,101(11),1059-64(JPN), Through Chem. Abstr.,1982,96,96783p.

125. Mongay,C. and Cerda,V.,"Britton Robinson Buffer of Known Ionic Strength", Ann. Clin.(Rome).,1974,64,409-412.

126. Vogel,A.I. in Quantitative Organic Analysis, 2nd edn.,1966,Longman.

127. Kelley,K.L.(Beecham, Inc.),"Topical Antiinflammatory Composition", Can.CA.,1,142,090; Through Chem.Abstr.,1983,98,p204451f.

128. British Pharmaceutical Codex, 1968, p1258.

129. Chattaway,F.D.,"Acetylation in Aqueous Alkaline Solutions", Soc. 1931,2495-2496.

130. Kallianos,A.G.;Mold,G.D.;Simpson,M.I.,"Carbonate Anhydride Esters of Acetyl Salicylic Acid", U.S.Patent, 3,646,201, 29th Feb.1972

131. Pekkarinen,L. and Tommila,E., "The Hydrolysis of Alkyl Salicylates in Alkaline Solutions", Acta Chem. Scand., 1959,13,1019-1030.

132. Khan,M.N. "Kinetic Evidence For The Occurance Of The Oxydianionic Tetrahedral Intermediates In The Hydrolysis Of Methyl Salicylate and Methyl O-methoxybenzoate In Highly Alkaline Medium", J.Org.Chem.,1982,47,3695-3699.

133. Hurst,G.H. and Bender,M.L."Mechanism Of Monofunctional and Bifunctional Catalysis Of An Ester Hydrolysis. The Kinetics Of Methyl,2,6-Dicarboxy Benzoate Hydrolysis" J.Amer.Chem.Soc.,1970,93,704-711.

134. Irwin,W.J.; Masuda,Q.N. and Li Wan Po,A.,"Transesterification Kinetics of Phenyl salicylate"; Tetrahedron, 1984,40(24),5217-5223

135 Irwin,W.J.; Masuda,Q.N.and Li Wan Po,A. "Transesterification Of Salicyate Esters Used As Topical Analgesics", Int.J.Pharm.,1984,21,35-50.

136. Sorby,D.L.;Bitter,R.G. and Webb,J.G.,"Dielectric Constants Of Complex Pharmaceutical Solvent Systems I. Water-Ethanol-Glycerin And Water-Ethanol-Propylene Glycol",J.Pharm.Sci.,1963,52(12),1149-1153.

137. Amis,E.S.;"The Polar Properties Of Solvent And the Conductance of Electrolytes At Infinite Dilutions", J.Phys.Chem.,1956, 60,428-430.

138. Amis,E.S, and LaMer,V.K.;"The Entropies And Energies Of Activation Of Ionic Reactions. The Kinetics Of Alkaline Fading Of Bromo Phenol Blue In Isodielectric Media",

J.Amer.Chem.Soc.,1939,61,905-913.

139. Amis,E.S. in Kinetics of Chemical Change in Solution, Chapter 4, 1949,MacMillan, NY.

140. Minnick,L.J. and Kilpatrick,M.;"Acid-Base Equilibria In Aqueous And Non-Aqueous Solutions
J.Phys.Chem.,1939,43,259-274.

141. Harmed,H.S. and Embree,N.D.;"The Ionization Constant Of Acetic Acid In Methyl Alcohol-Water Mixture From 0-40°",
J.Amer.Chem. Soc.,1935,57,1669-1670.

142. Harned,H.S.and Kazanjia,G.L.;"The Ionization Constant Of Acetic Acid In Dioxane-Water Mixture",
J.Amer.Chem. Soc.,1936,58,1912-1915.

143. Hughes,E.D.;"Mechanism Of Substitution At A Standard Carbon Atom. Part V. Hydrolysis Of tert-Butyl Chloride",
J.Chem. Soc.,1935,255-258.

144. Hempenstall,J.M.; Irwin,W.J.;Li Wan Po,A.and Andrews,A.H. ;"Non-isothermal Kinetics Using A Micro Computer.A Derivative Approach To The Prediction Of The Stability Of Penicillin Formulations";
J.Pharm.Sci.,1983, 72(6), 668-673.

145. Taft,R.W.;Newman,M.S. and Verhoek,F.H.;"Kinetics Of The Base-Catalysed Methanolysis Of Ortho-,Meta-, and P-substituted l-Menthyl Benzoates";
J.Amer. Chem. Soc.,1950,72,4511-4519.

146. Scowen,R.L.and Behn,C.G.;"Nucleophilic Catalysis By Acetate Ion In The Methanolysis Of p-Nitrophenyl Acetate",
J.Amer.Chem.Soc.,1968,90,5839-5844.

147. Fersht,A.R.and Kirby,A.J.;"Intramolecular Nucleophilic Catalysis In the Hydrolysis of Substituted Aspirin Acids";
J.Amer.Chem.Soc.,1968,90,5826-5832.

148. Bernard,T.T.and Smiles,S.;"A Rearrangement Of Aryl Salicylates",
J.Chem. Soc.,1938,141,1897-1900.

149. Bender,M.L.,"Oxygen Exchange As Evidence For The Existance Of An Intermediate Ester In Hydrolysis";
J.Amer.Chem. Soc.,1951,73,1626-1629.

150. Chapman,N.B.;Shorter,J.and Utley,J.H.P.;"The Seperation Of Polar and Steric Effect.Part II.The Basic And the Acidic Hydrolysis Of Substituted Methyl Benzoate";
J.Chem. Soc.,1963,1291-1299.

151. Bender,M.L.,Chow,Y-L and Cholupek,F.;"Intramolecular Catalysis Of Hydrolytic Reactions.II.The Hydrolysis Of Phthalamic Acid";
J.Amer. Chem. Soc.,1958.80,5380-5384.

152. Amin,J.H. and Desai,R.D.,"Fries Reaction. IV. Rearrangement Of The Derivatives Of Salicylic Acid And Substituted Benzoic Acids" J.Sci.Ind.Research.,1954,13B,178-80.

153. Shafik,R.M. and Askar,A.A.A.,"New Biphenyl Derivatives as Potential adrenoceptor blocking agents"

J.Pharm.Sci.1979.72,776-781

154. Li wan Po,A.Irwin,W.J.and Yip,Y.W.;"High-Performance Liquid Chromatographic Assay Of Betamethasone 17-Valerate And Degradation Products.
J.Chromatogr.,1979,176,399-405.

155. Brower,J.F.;Juenje, E.C.;Page,D.P. and Dow,M.L.;"Decomposition Of Aminophilline In Suppository Formulations",
J.Pharm.Sci.,1980,69,942-945.

156. Curran,N.M.; Lovering,E.G.; McErlane,K.M. and Watson,J.R.; "Impurities In Drugs IV Indomethacin",
J.Pharm.Sci.,1980,69,187-189.

157. Ekman,R.;Liponkoski,L. and Kahela,P.;"Formation Of Indomethacin Esters In Polyethylene Glycol Suppositories"
Acta Pharmaceutica Succica,1982,19,241-246.

158. Irwin,W.J.;Masuda,Q.N.and Li Wan Po,A.;"Transesterification : An Analytical And Formulation Problem",
J.Pharm.Biomed.Anal.,(In Press)

159. Höfle,G.;Steglich,W. and Vorbruggen,H."4-Dialkylaminopyridines As Highly Active Acylation Catalysts"
Angew. Chem. Int. Edn.,1978,17,569-588.

160. Roque Margenat,Jose Maria,"Product For Protecting The Skin Against The Sun" Span. Es.506.622(C/BoJ8/10). Through Chem. Abstr.1983,98, p132150g

161. Lion Corp.JPN.Kokai Tokkyo,Koho JP57,185,215[82,185,215],(Cl.A61k9) "Therapeutic Lotions Containing Polar Drugs And Alcohols", Through Chem.Abstr.,1983,98,p78150s.

162. Mitsubishi Chem.Ind.Co.Ltd.,JPN.,Kokai Tokkyo Koho JP.,57,209,215[82,209,215],(Cl.A61k9/08),"Sustained Release Antiinflammatory Pharmaceuticals For Topical Application",Through Chem.Abstr.,1983,98,p95690k.

163. JPN.Kokai Tokkyo Koho JP 58,21,612[83,58,21,612],"Surgical Dressings Containing Crosslinked Poly(Vinylpyrrolidone)", Through Chem.Abstr.,1983,98,p204464n.

164. Champion,J.,"Topical Compositions Containing Vasodilators", Brit.Pat., Through Chem.Abstr.,1979,91,p151543j.

165. Mroso,P.V.;Li Wan Po,A. and Irwin,W.J.,"Solid-state Stability Of

Aspirin In The Presence Of Excipients: Kinetic Interpretation, Modeling And Prediction"
J.Pharm.Sci.,1982,71,1096-1101.

166. Reepmeyer,J.C."Thermal Decomposition Of Aspirin: Formation Of Linear Oligomeric Salicylate Esters"
J.Pharm.Sci.,1983,72,322-3

167. Taguchi,V.Y.;Cotton,M.L.;Yates,C.H. and Millar,J.F."Determination Of Drug Stability In Aspirin Tablet Formulations By High-Pressure Liquid Chromatography"
J.Pharm.Sci.,1981,70,64-67.

168. Kirchhoefer,R.D.;Reepmeyer,J.C. and Juhl,W.E.,"Aspirin - A National Survey III. Determination Of Impurities In Bulk Aspirin and Aspirin Formulations By High Pressure Liquid Chromatography And Spectrophotometry"
J.Pharm.Sci.,1980,69,550-553.

169. Reepmeyer,J.C.and Kirchhoefer,R.D.,"Isolation Of Salicylsalicylic Acid, Acetylsalicylsalicylic Acid And Acetylsalicylic Anhydride From Aspirin Tablets By Extraction And High Pressure Liquid Chromatography"
J.Pharm.Sci.,1979,68,1167-1169.

170. Bundgaard,H. and DeWeck,A.L.,"Role Of Amino Reactive Impurities In Acetylsalicylic Acid Allergy"
Int.Arch.Allergy Appl.Immunol.,1975,49,119-124.

171. Bundgaard,H.,"High Pressure Liquid Chromatographic Determination Of Immunogenic Contaminants Of Acetylsalicylic Acid Preparations"
Arch.Pharm.Chem.Sci.Ed.,1976,41,601-606.

172. Groves,G.A.,"Current Drug Informations. Continuous Photobiology. Part 2."
J.Aust.Pharm.,1975,56,601-606.

173. Pierre,T.St.and Jenks,W.P.;"Intramolecular Catalysis In The Reactions Of Nucleophilic Reagents With Aspirin";
J.Amer.chem. Soc.,1968,90,3817-3827.

174. Hussain,A.A.,Yamasaki,M and Truelove,J.E.;"Kinetics And Hydrolysis Of Acylals Of Aspirin:Hydrolysis Of (1'-Ethoxy) Ethyl 2-Acetoxy Benzoates";
J.Pharm.Sci.,1974,63,627-628.

175. Hussain,A.A., Truelove,J.E,and Kostenbauder,H.;"Kinetics And Mechanism Of Hydrolysis Of 1-(2'-Acetoxybenzoyl)-2-deoxy-a-D-glucopyranose,A Novel Aspirin Prodrug";
J.Pharm.Sci.,1979,68,299-301.

176. Hussain,A.A.,Truelove,J.E.and Kostenbauder,H.B.;"Synthesis Of 1-O-(2'-acetoxy)benzoyl-a-D-2-deoxyglucopyranose,A Novel Aspirin Prodrug";

J.Pharm.Sci.,1980,69,231-232.

177. Banerjee,P.and Amidon,G.L.;"Physico-chemical Property Modification Strategies based On Enzyme Substrate Specificities I.Rationale,Synthesis and Pharmaceutical Properties Of Aspirin Derivatives";
J.Pharm.Sci.,1981,70,1299-1303.

178. Banerjee,P.and Amidon,G.L.;"Physico-chemical Property Modification Strategies Based On Enzyme Substrate Specificities II"
J.Pharm.Sci.,1981,70,1304-1306.

179. Banerjee,P.and Amidon,G.L.;"Physico-chemical Property Modification strategies Based On enzymes substrate specificities III.Carboxypeptidase-A hydrolysis Of Aspirin Prodrug";
J.Pharm.Sci.,1981,70,1307-1309

180. Hussain,A.A.,and Mulin-eldeen,Z.;"Is Aspirinphenylalanine Ethyl Ester a Prodrug for Aspirin";
J.Pharm.Sci.,1983,72,1094-1095.

181. Paris,G.Y.,Garmaise,D.L.and Cimon,D.G.;"Glycerides As Prodrugs.I.Synthesis and Antiinflammatory Activity Of 1,3-Bis(Alkanoyl)-2-(O-Acetyl Salicyloyl) Glycerides (Aspirin Triglycerides)";
J.Med.Chem.,1979,22,683-686.

182. Carter,G.W.;Young,P.R.;Swett,L.R.and Paris,G.Y.;"Pharmacological Studies In The rat with [2-(1,3-di-decanoloxy)-propyl] 2-acetyloxybenzoate(A-45474); An Aspirin Prodrug With Negligible Gastric Irritation"
Agents and Actions, 1983,10,240-245.

183. Levy,G. and Gagliardi,B.A., "Gastrointestinal Absorption Of Aspirin Anhydride",
J.Pharm.Sci.,1963,52,730-732.

184. Pfeiffer,C.D. and Pankey,J.W.;"Determination Of Related Compounds In Aspirin By Liquid Chromatography"
J.Pharm.Sci.,1982,71,511-4.

185. Hardy,P.M.; Rydon,H.N.and Thomson,R.C.,"The Use Of Methylthioethyl Esters For The Protection Of Carboxyl Group In Peptide Synthesis: Removal Through -Methyl Sulphonyl Ester"
Tet.Lett.,1968,20,2525-2526.

186. Duggan,E.D.;Hooke,K.F.;Nole,R.M.;Hucker,H.B.and Arman,G.V.,"Comparative Disposition Of Sulindac And Metabolites In Five Species"
J.Biochem.Pharmacol.,1978,27,2311-2320

187. Borrows,E.T.;Johnson,J.M,;"Antiinflammatory Salicylic Acid Derivatives"

Brit.Pat.,1,220,447, Through Chem. Abstr.,1971,75,p35462c.

188. Wojciech,G.;Maria,G.;Edward,B.;Leonard,M.;Elzbieta,P.;Zdzislaw,W.and Henryk,S.,"Salicylic Acid Derivatives"
Pol.Pat., 114,342, Through Chem. Abstr.,1983,98,p71696r.

189. Kallianos,A.G.;Mold,J.d.and Simpson,M.I.;"Salicylate Compounds In Sustained Released Drugs";
S.African Pat. 7108,141, Through Chem.abstr.,1973,79,p129097x.

190. Driver,G.W.and Paige,M.F.C.,"Ethyl Esters Of Substituted Thiobenzoic Acids"
Brit. Pat.782,909, Through Chem.Abstr.,1958,52,2915c.

191. Croft,D.N.;Cuddigan,J.H.P.and Swettland,C."Gastric Bleeding And Benorylate - A New Aspirin";
Brit. Med.J.,1972,3,545-547.

192. Li Wan Po,A. and Irwin,W.J.;"The Determination of pKa and Partition Data : An Automated Approach";
Laboratory Practice, 1980, January, 21-25.

193. The Merck Index, 10th Edition, 1983, Merck and Co.,Inc.