THE EFFECT OF SOLVENT COMPOSITION ON THE KINETICS OF DEACYLATION OF LECITHIN

BY

JOHAH IDUH ABALAKA

A thesis submitted to the Postgraduate School Ahmadu Bello University, in partial fulfillment of the requirements for the degree of;

Master of Science in Pharmaceutics
Department of Pharmaceutics and Pharmaceutical
Microbiology Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria

February, 1985

APPROVAL

This thesis submitted by Jonah Iduh ABALAKA meets the regulations governing the award of the degree of Master of Science (Pharmaceutics) of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, and it is approved as a consequence of its contribution to the development of Science.

Internal Examiners: (i) Dr. D. K. Agrawal (B.Pharm., D.Sc. nat (Zurich)
Reader,
Department of Pharmaceutics and
Pharmaceutical Microbiology,
Ahmadu Bello University, Zaria.

(ii) Dr. J. Y. Olayemi (B.Sc., M.Sc., Ph.D.),
Reader, in Chemistry,
Faculty of Science,
Ahmadu Bello University, Zaria.

External Examiner: S. P. Agarwal
Dr. S. P. Agrawal (M.Pharm., Ph.D.),
Reader in Pharmaceutics,
Faculty of Pharmaceutical Sciences,
University of Nigeria, Nsukka.

Dean of Post-Graduate School Professor G. E. Osuide,
(B.Pharm., Ph.D., PSAN, FAS, FPSN.)
Dean, Post-Graduate School,
Ahmadu Bello University, Zaria.

D E C L A R A T I O N

I hereby declare that this thesis has been written by me and that it is record of my research work done under the supervision of Drs. F. B. Banks, D. K. Agrawal and J. Y. Olayemi. It has not been presented in any publication for a higher degree. All quotations have been indicated and sources of information specifically acknowledged by means of references.

[signature]
Abalaka, J. I.

29-3-1985
Date

DEDICATION

In gratitude to God for HIS abundant Love and Care in the LORD JESUS CHRIST.

ACKNOWLEDGEMENTS

I am greatly indebted to my two supervisors, the Head of Department of Pharmaceutics, Dr. D. K. Agrawal and Dr. J. Y. Olayemi of the Department of Chemistry.

In addition I owe a lot of thanks to Dr. F. B. Banks College of the Bahamas, Nasau who initially started with the supervision of the work but had to leave later. I am also highly grateful to Dr. R. D. Gupta of the Katsina College of Arts, Science and Technology, Zaria and Dr. P. F. Olurinola of the Department of Pharmaceutics and Pharmaceutical Microbiology, A.B.U., Zaria for their patience in going through the work and for many helpful suggestions they gave.

I owe a lot of thanks to Dr. M. Shok former Head of Department of Pharmacognosy for helping me with the supply of some chemicals. In addition, I am grateful to Mr. C. Atayi of the School of Basic Studies for his assistance. Mr. M. Stastny has been very helpful with the supply and procurement of chemicals and equipments. I thank Mr. Paul Ekeyi of Biochemistry Department for the supply of a crude sample of lecithin. Mr. P. Katugwa was very helpful in the preparation of some of the equipments used in this work. I am grateful to Mr. Boniface Ochefu for typing this work.

Finally, I thank Dr. O. C. Wambebe, Dean of the Faculty of Pharmaceutical Sciences for his encouragement.

ABSTRACT

A back titration technique has been used to follow up the effect of change in the composition of the propan-1-ol/ water solvent on the kinetics of the deacylation of egg yolk lecithin under second order conditions in alkaline media.

The lecithin was employed at a concentration of 1.5×10^{-2} molar and the alkali concentration was double that of the lipid. The reaction was investigated in concentrations of propan-1-ol ranging from 10 % v/v to 97 % v/v in water.

The work involved the withdrawal of aliquots of 1 cm^3 from the reaction solvent which was maintained at a temperature of 30^0 C under nitrogen reflux and magnetic stiringr Withdrawn aliquots were titrated to phenolphthalein end point using 3×10^{-3} molar hydrochloric acid and a 10 cm^3 burette. readable to 0.02 cm^3. From the titre values obtained estimations of the rate constants for the deacylation process in the various compositions of the propan-1-ol in v/ater solvents employed were obtained.

Association of lecithin in the various solvent systems was examined spoctrophotometrically between 255 and 295 nm using a Pye Unicam SP 30 digital, spectrophotometer using phenolplrthaleln as a probe. The lecithin existed as monomers in 83.33 % v/v propan-1-ol in water while various molecular regates were formed in the other solvent systems.

ABSTRACT (Contd')

The rate constants for the deacylation of lecithin obtained ranged from 2.86×10^{-2} dm mole^{-1} s^{-1} to 86.36×10^{-2} dm^{3} mole^{-1} s^{-1} in the various solvent systems employed. However values of the rate constants for the deacylation of lysolecithin were much smaller, ranging from 2.04×10^{-2} dm^{3} mole^{-1} s^{-1} to 1.09×10^{-3} dm^{3} mole^{-1} s^{-1}

TABLE OF CONTENTS

TABLE OF CONTENTS (Contd')

LIST OF FIGURES

Page

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION TO LIPIDS

Lipids are a class of organic compounds which are very widely distributed in living forms. All plants and animals contain some form of lipid and they constitute a very important storage form of energy in these organisms. They also play an important role along with certain proteins as structural elements of cells.

Though no detailed classification of lipids available is universally acceptable, the following classification by Strickland (1963) has been adopted. It should however be noted that it is necessary to impose the added restriction of Bloor (1943) that lipids must, with a few exceptions, be soluble in the so called fat solvents (e.g. ether, chloroform, benzene etc.).

According to this classification lipids are grouped as indicated below -

A) SIMPLE LIPIDS:-

These are mainly esters of fatty acids and alcohols and include -

(a) glycerol esters (mono, di and tri-glycerides)

(b) cholesterol esters

(c) higher alcohol esters

(d) glycerol ethers

(e) hydrocarbons.

3) PHOSPHOGLYCERIDES:-

These are esters of phosphate and the free α-hydroxyl of α, β-diglyceride or α-(vinyl alkane ether)-β-monoglyceride and include -

(a) phosphate mono esters (phosphatidic acid esters)

(b) phosphate diesters containing

(i) choline. These include lecithin, lysolecithin and choline plasmalogen

(ii) Ethanolamine. These include phosphatidyl ethanolamine, ethanolamine plasmalogen and kephalin B.

(iii) Serine. These include phosphatidyl serine and serine plasmalogen.

(iv) Glycerol. These include phosphatidyl glycerol and cardiolipin.

(v) Inositol. These include phosphatidyl inositol, di and tri phosphoinositides.

(c) phosphate triesters which include complexes of phosphatidyl ethanolamine and phosphatidyl serine.

C) SPHINGOLIPIDS:-

These are sphingosine derivatives containing the N-fatty acyl group and include -

(a) sphingomyelin

(b) cerebrosides

(c) sulphatides

(d) gangliosides (mucolipids and strandin)

D) DERIVED LIPIDS:-

These include -

(a) fatty acids

(b) cholesterol

(c) higher alcohols and

(d) sphingosine.

E) COMPLEX LIPIDS:-

These include -

(a) Lipoproteins

(b) proteolipids and

(c) phosphatidopeptides.

Of the lipids the phosphoglycerides, particularly phosphatidyl choline, exhibit a significant presence in cell membranes.

Cell membranes can be viewed either in terms of their anatomy, as revealed by the electron microscope and other techniques, or in terms of their physiology and function or in terms of their chemistry.

Most lipids of the cell membrane are based on the glycerol molecule

```
  H
  |
H-C-OH
  |
H-C-OH = glycerol
  |
H-C-OH
  |
  H
```

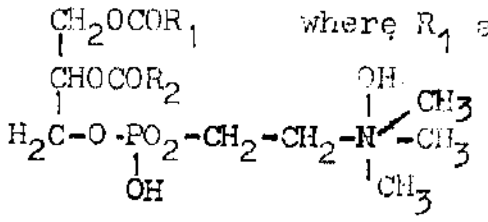
Membrane lipids are usually esterified at the one and two positions by long chain fatty acids. The third position is occupied by a polar group.

Of recent a lot of interest has been generated on the question of membrane structure. It has become necessary to investigate the behaviour of some amphiphiles present in the cell membrane in various solvents especially in water. Of the amphiphiles lecithin is the most employed probably because of its significant presence in actual membranes and it's abundant supply in crude form from egg yolk and other sources.

1.2 INTRODUCTION TO LECITHIN

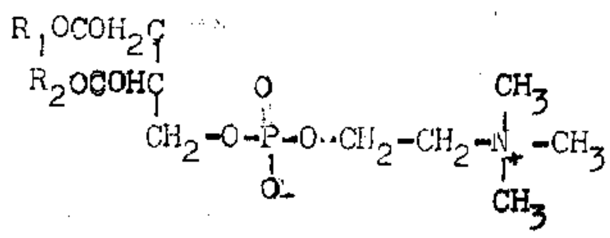
Phosphatidyl choline (lecithin) is the diacyl compound of glycerylphosphoryl choline. It shows a wide distribution in living things and in particular is an integral and major constituent of the phospholipids which are profoundly responsible for the semipermeable properties of the cell, nuclear and mitochondrial membranes.

Phosphatidyl choline on complete hydrolysis yields two molecules of fatty acids, a molecule of glycerol, a molecule of phosphoric acid and a molecule of choline. This led Diakanov (1867, 1868 a, b) and Stretcher (1868) to propose the following structure for lecithin.



where R_1 and R_2 are alkyl radicals.

Jukes (1934) however proposed a zwitterionic structure for lecithin which has been supported by potentiometric titration evidence (Jukes, 1934, Fischgold and Chains, 1935) and by the observed deilectric activity of this lipid in water (Furth, 1923) and ethanol (Kuhn, '<u>et al</u>', 1935).

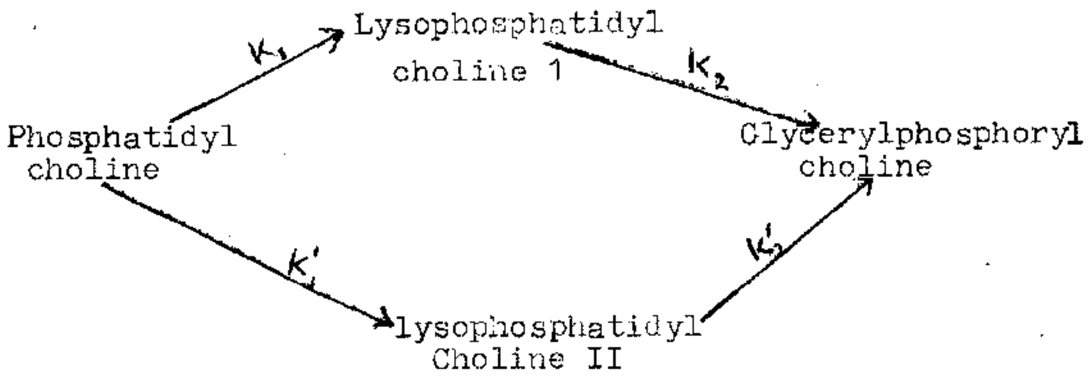


where R_1 is saturated and R_2 is usually unsaturated (Hanahan, 1952, 1954). This statement is however incompatible with the observation that the saturated fatty acids constitute only 35 % of the fatty acids in lecithin (Elworthy and Saunders, 1956). Perhaps the authors intended that the percentage so indicated should be taken on a weight basis and not on a molar basis. It is however interesting to note that the fatty acid composition of the natural lecithins vary since, for instance, both of the alkyl groups in the lecithins occuring in the lungs, are fully saturated.

1:3 REVIEW OF PREVIOUS WORK ON THE DEACYLATION OF LECITHIN.

Though extensive work has been done to investigate the solvolysis of the phosphate ester group in lecithin (Bailly and Gaume, 1926, 1934, 1935 etc., Baer and Kates, 1948 and other workers) research on the deacylation of lecithin has received far less attention. This is probably due to the fact that the process involved is analogous to that of carboxylic ester hydrolysis for which the kinetics are fairly well understood.

Being a diacyl compound the study of the kinetics of the deacylation of lecithin presents challenging problems. The acyl groups are removed in stages leading to appropriate rate constants. Also there is facile acyl migration which complicates the entire problem. There is in addition the relatively insignificant but complicating fact of the migration of the phosphate to the 2-position in the 1-acyl intermediate (Bailly and Gaume, 1934). Neglecting this last point such a process can be represented by constants as indicated below:



The first workers to investigate the kinetics of the process of deacylation are Baer and Kates (1950). Their work involved the titrimetric assay of released fatty acids from synthetic dipalmitoyl-sn-glycerol-3-phosphorylcholine under supposed pseudo first order conditions at 37°C. The solvent employed consists of 8 % v/v carbon tetrachloride, 10 % v/v water and 82 % v/v methanol. Their procedure involved the withdrawal of aliquots from the reaction mixture which was 0.2M in sodium hydroxide and 0.0204M in lipid concentration. This corresponds to a change of over 20 % in the alkali concentration over the entire course of reaction. This large percentage change far exceeds the 10 % limit recommended by Garrett (1967) for compliance with pseudo first order conditions. In view of this, Baer and Kates' conditions can not be said to be first order. Withdrawn aliquots were mixed with a solution of sulphuric acid and then extracted twice with ether. The ether extract was dried and then used for titration. The treatment with sulphuric acid introduces a source of error since the acid can and does act as a deacylating agent. This would lead to titre values higher than expected and hence lead to overestimated values of the rate constant. However this would be opposite to the effect caused by that due to the changing concentration of the alkali. Baer and Kates (1950) treated their data as if the process of deacylation was one step. This can only be true if the rate of removal of the first fatty acid is far greater or far less than that for the removal of the second acyl residue. Under that condition one of the steps becomes rate limiting. Even then the process being consecutive the far portions of the reaction can not be masked off. They obtained pseudo first order rate constants of $2.8 \times 10^{-4}\ s^{-1}$ for deacylation and $1.9 \times 10^{-5} s^{-1}$ for decholination.

Marrinetti (1962) studied the methanolysis of phosphatidylcholine obtained from egg yolk using sodium methoxide. The solvent system employed was 67 % v/v chloroform and 33 % v/v methanol. The mixture was 0.17 molar (1.7×10^{-1}M) in methoxide and 6.4×10^{-3} molar in the lipid hence ensuring pseudo first order conditions. At room temperature, the reaction was too fast to be conveniently followed.

At $0°C$ however, it was slow enough to enable the use of a paper chromatographic procedure to quantitate the lecithin, the lysocompounds, the fatty acids and the glycerylphosphoryl choline. Employing Marrinetti's results and the activation energy for the saponification of various fatty-1-monogycerides as published by Jellinek and Gordon (1951), a pseudo first order rate constant of $2.4 \times 10^{-2} s^{-1}$ may be calculated at $37°C$. This constant (2.4×10^{-2} s^{-1}) refers to that for the removal of the first fatty acid residue from lecithin under pseudo first order conditions. In addition Marrinetti observed a preponderance of saturated fatty acids early in the process. This would imply a preference of methoxide ions for attack at the 1-position of lecithin at which most of the saturated fatty acids of lecithin are esterified.

Fujiwara et al (1967) proceeding with Marrinetti's work investigated the deacylation of egg yolk phosphatidyl choline at a concentration of $3.2 \times 10^{-3}M$. They employed various alkoxides in the corresponding alcohols at a concentration of $6.4 \times 10^{-3}M$. In addition Fujiwara et al employed laurylamine of no specified concentration but the amine was used as a methanolic solution. All the reactions were carried out at 0°C except those for the t-butoxide and laurylamine which were at 40°C and 37°C respectively. The intermediate lysophosphatidyly choline was separated out using preparative thin layer chromatography. Based on a gas liquid chromatographic analysis of both the phosphatidyl choline and the lysophosphatidyl choline, Fujiwara et al observed that n-propoxide attacked the 2-position preferentially. Since the 2-position contains the unsaturated fatty acid residues, this would imply a higher rate constant for the deacylation of the unsaturated residues by n-propoxide and n-butoxide as compared to the saturated fatty acid residues. Though no kinetic data is available from the work of Fujiwara et al, it is obvious that laurylamine and t-butoxide must be relatively poorer deacylating agents since these compounds had to be employed at 37°C and 40°C respectively instead of the 0°C for the other alkoxides.

Dawson (1954) reported the assay of various phosphoglycerides and other phospholipids present in biological samples. The method used was based on mild alkaline hydrolysis and subsequent separation by paper chromatography of the components of the hydrolysate. This was then followed by the determination of the various products from the chromatogram. By piecing the results of such estimations together, Dawson (1954) could tell what concentration of which lipid he had in his starting material or sample. In continuation of this work, Dawson (1960) simplified the earlier procedure to enable quantitative recoveries of the products and also to enable the assay of the alkaline stable phospholipids. This involved subjecting the sample to mild acid hydrolysis. The treatment with mild acids leads to a break down of the alkaline stable plasmalogens and appropriate long chain aldehydes and phosphodiesters are formed. The solvent employed consisted of 8.7 % v/v carbon tetrachloride, 9.3 % v/v water and 82 % v/v ethanol. The mixture was 2.7×10^{-2} molar in sodium hydroxide. These conditions in contravention of Garrett's rule (Garrett 1967) do not lead to pseudo first order reactions. Though Dawson's solvent appears to be rather less polar than that of Baer and Kates (1950), his reaction is considerably faster with a pseudo first order rate constant of $2 \times 10^{-2}\ s^{-1}$ at 37°C.

Dawson (1966) reported the hydrolysis of lecithin films by phospholipase-A. Phospholipase-A is specific in causing deacylation only at the 1-position of 2-sn-phosphatidyl choline. He observed that when the lecithin was converted to lysolecithin, it was lost from the surface into the underlying aqueous phase where it was rapidly degraded further. Based on this fact, Hauser and Dawson (1967) investigated the kinetics of the deacylation process employing yeast lecithin in the form of surface films on water. The lecithin used was labelled with phosphorus-32. Radioactivity was measurable from the film. On conversion of the lecithin to lysolecithin, an amount of radioactivity corresponding to the quantity of lysolecithin formed was gained by the aqueous phase. In the aqueous phase the lysocompound was degraded further to glyceryl phosphorylcholine (Dawson 1966). Hence this method could be employed to follow up the kinetics of the process.

Under acidic conditions (1.5M HCl corresponding to a pH of -0.18), no loss in radioactivity was observed from the surface films at temperatures below 50°C. This corresponds to a failure of the acid as a deacylating agent. These results were explained as due to the relatively low hydrogen ion concentration at the film surface. Under such highly acidic conditions, the phosphate oxide group gets protonated and the lecithin molecule has a net positive charge from the quartenary ammonium group.

hence hydrogen ions are repelled from the film whose pH becomes significantly less than that of the bulk phase as a result.

If complete protonation of the phosphate ester group is assumed, the surface pH is given by

$$pH_s = pH_b + \frac{\varepsilon \Psi}{2.3kT}$$

where K = Boltzman constant

ε = electronic charge

T = absolute temperature

pH_s = interfacial pH

pH_b = bulk pH and

Ψ = interfacial potential.

The assumption of complete protonation of the phosphate oxide group is consistent with the pKa value of 3 for this group reported by Banks (1978).

The interfacial potential is derivable from the Guoy equation (Davies and Rideal, 1963) and this leads to pH_s of 2.3. The assumption that the phosphate group is protonated at this pH value is consistent with the work of Bangham and Dawson (1959) who reported a sharp change in the surface potential of lecithin below pH 3. The lack of hydrolysis in acid can also be attributed to the poor efficiency of acids generally for hydrolytic processes.

In alkaline sodium hydroxide solution of strength 0.1 - 0.6N having a pH of 13 - 13.78, a slow loss of radioactivity to the bulk phase was observed. The rate of such loss was found to vary linearly with the bulk hydroxyl ion concentration in consistence with the equation for reactions in surface monolayers (Davies and Rideal, 1963).

An activation energy of 14.25 Kcal/mole was calculated for the above saponification. Substitution of sodium hydroxide by either potassium hydroxide or lithium hydroxide produced a slight but virtually negligible decrease in rate. This was interpreted as meaning that neither the ionic diameter nor the hydration of the ion associated with the hydroxide appear to have any significant effect on rate.

In addition, Hauser and Dawson (1967) noted that when 10 - 20 % methanol was added to the solvent system (i.e. to the water) an increase of upto 30 % in reaction rate was obtained. Addition of ethanol to the tune of 10 % of the solvent system caused an increase but not quite as much as that caused by an equal amount of methanol. A still lesser effect was observed with octadecanol. The increasing rate observed with the alcohols was attributed to the presence of their alkoxides in equilibrium with hydroxyl ions (Banks, 1978). It was also felt that the structural organization of the film was such that it presented a barrier to anions but that such a barrier was less effective for the alkoxides than for the hydroxyl ion. This was attributed to the greater lipophilicity of the alkoxides compared to the hydroxide ion. If this be correct, then an increasing potentiatry effect on reaction rate should be expected with increasing chain length for the alcohol. The fact that this wasn't quite so has been attributed to the following equilibrium by Murto (1962).

$EtOH + \bar{O}H \dashrightarrow H_2O + EtO^- \quad K = 0.66$ at $25^\circ C$

$MeOH + \bar{O}H \dashrightarrow MeO^- + H_2O \quad K = 4.5$ at $25^\circ C$

The lack of reaction in acid has been attributed to a surface positive charge (Hauser and Dawson, 1967). If this is so the incorporation of an anionic surfactant in the surface film should cause a marked increase is rate in acidic media. On the other hand, the addition of cationic surfactant should cause a decrease in rate in acid. In alkaline media effects opposite to those indicated above are to be expected as the positive charge on the nitrogen should be rendered 'neutral' by the atmosphere of hydroxyl and alkoxide ions. This has infact been found to be the case by these same workers. On the introduction of cetyl trimethylammonium bromide a rate enhancement was observed while that of phosphatidic acid caused a siginficant decrease in rate. It was assumed that on the basis of this and other works (Phillips et al , 1972; Hauser et al , 1976) that the film was oriented in such a way that any ions approaching it would first encounter a region of high positive charge and then a region of high negative charge. The rate constants obtained are $3.7 \times 10^{-4}\ s^{-1}$ for the pseudo first order constant and $2.2 \times 10^{-3} dm^3 mole^{-1} s^{-1}$ for the apparent second order constant.

It is interesting to note that Alexander and Rideal (1937) working on the saponification of surface films of esters of long chain fatty acids and alcohols have reported a pseudo first order rate constant of $5.0 \times 10^{-4}\ s^{-1}$ corresponding to an apparent second order rate constant of $5.0 \times 10^{-4} dm^3 mole^{-1} s^{-1}$. They employed a hydrolytic solvent which was 1 molar in the alkali.

Marriott (1969) has investigated by titrimetry the release of fatty acids from egg yolk phosphatidyl choline and lysophosphatidyl choline prepared by the action of phospholipase A. He used a solvent that was 1.1×10^{-3}M and 0.8×10^{-3}M in lecithin and lysolecithin respectively and 0.18 molar in the alkali. The solvents employed showed a concentration range of 70 - 92 % v/v in methanol, 4 - 26 % v/v in water and 4 % v/v in chloroform.

It is worth noting that in view of the considerable variation in the water content, a large variation in the dielectric constants of the various hydrolytic solvents in the range 20 - 40 is expected in Marriott's reaction systems. He reported rate constants in the range of $2.04 - 27.34 \times 10^{-3}$ min^{-1}. The hydrolysing agents employed were potassium hydroxide, lithium hydroxide, sodium hydroxide tetramethylammonium hydroxide and tetraethylammonium hydroxide. He observed overall third order kinetics with all the hydroxides except for lithium hydroxide for which overall second order kinetics were observed. Marriott explained his results by postulating that lecithin and lysolecithin in alkaline solution bind lithium ions much more strongly than other cations. He also claimed that only a cation/phospholipid complex could be attacked by the alkalis to release fatty acids. This covertly assumes, as a consequence, that the rate constant for the formation of the cation/phospholipid complex is of the same order of magnitude as that for the deacylation of the complex so formed except for the lithium hydroxide. In addition it is implied that the rate constant for the formation of the lithium/phospho lipid complex is far in excess of that for any of the other alkali ions. Marriott was able to assume this because he thought that the phospholipids were negatively charged in his reaction system. The mixture used for his study was 0.17 molar in hydroxide hence giving a pH of about 13.2. Under these conditions, a net negative charge can be developed by the lecithin only either by co-ordinate bonding of hydroxyl ions to the quarternary ammonium group or by demethylation. It is known that pure lecithin exists zwitterionically between pH 3 and 13 (Banks, 1978). Lecithin contains no labile protons and to assume that the quarternary ammonium group has its charge neutralized by co-ordinate bonding to a hydroxyl ion in the largely methanolic solvent seems odd. In fact in pure water, the lithium hydroxide solution of highest concentration employed will have a pH of 13.6 if complete ionization is assumed. However the solvent system used in the case of lithium hydroxide contained only 9 % water and 87 % methanol, the balance being of chloroform. Bearing in mind that any ions formed need to be hydrated, it seems unlikely that the pH of the solutions in the case of lithium hydroxide would be significantly higher than 13 realising that lithium hydroxide is in any case only about 50 % ionized in pure water (Mellor, 1961). Hence one can not escape the conclusion that the phospholipid molecules existed as zwitterions in Marriott's reaction system.

As for the demethylation, such a process could occur to give methanol. However N-dealkylation reactions involving the quarternary ammonium group require relatively drastic conditions (Tewari et al, 1976). In deed it is hard to see what kind of specific interaction would lead to the loss of the positive charge in view of the fact that it is not counterbalanced by direct bonding to an oppositely charged ion and in view of the stabilizing effect of the phosphate group. Consequently one must conclude that the lipid has a net zero charge within the pH range of interest. Under this condition it becomes difficult to imagine what kind of specific cation/phospholipid interaction Marriott had in mind.

Marriott (1969), like Baer and Kates (1950), treated the kinetic data in such a way as to imply that the process was one step. It becomes particularly difficult to justify this assumption in view of the fact that the $t_{\frac{1}{2}}$ values obtained for the lecithin experiments are very nearly equal to those obtained for the lysolecithin runs (the lysolecithin used was the 2-acyl derivative). This argument might be countered by saying that though lysolecithin is an intermediate in the hydrolysis of lecithin, the compound in the lysolecithin runs is specifically the 2-acyl derivative rather than the mixture of the 1 and 2-acyl compounds as exists in the lecithin runs. This would imply that the rate of removal of the 2-acyl group is much higher than that for the 1-position. The predominantly 1-acyl intermediate from the lecithin then undergoes facile acyl migration to give the 2-acyl derivative which is then hydrolysed at the recorded compartable rate. For this to happen, the rate of acyl migration from position 1 to 2 must far exceed that for deacylation. All these possibilities breakdown in the light of Fujiwara's work (Fujiwara _et al_, 1967). Fujiwara and coworkers reported a preference of methoxide ions for the 1-position.

Banks (1978, 1983) reported a method of quantitating lecithin, lysolecithin, glyceryl phosphoryl choline and fattyacids. The method is based on the linear relationship between the log of spot loading and the peak area. The weight of substance was found by in situ reflectance densitometry of the chromatographic plates after these had been rendered chromophoric by charring in an oven at 210^{o}C for one hour after spraying them with a 20 % aqueous solution of ammonium sulphate. Banks (1978) employed synthetic rac-1, 2-diacylglycerol-3-phosphoryl choline as substrate. The lysocompound was prepared by the action of phospholipase A_2 on egg lecithin. The solvent mixture used was 50 % v/v propan-1-ol in water. The mixture was between 6 x 10^{-4}M and 8 x 10^{-4}M in alkali. In the most dilute alkali solution, a change in base concentration of no more than 6 % was obtained on complete deacylation hence ensuring pseudo first order conditions. He employed the hydroxides of potassium, sodium and lithium for his kinetic investigation. Banks (1978) observed no significant differences in the rates of attack of the three hydroxides on the two lipids in contravention of Marriott's results. However, he [illegible] obtained a pseudo first order rate constant of $2.7 \times 10^{-2}\ s^{-1}$ and $3.0 \times 10^{-2}\ s^{-1}$ for the deacylation of lecithin and lysolecithin respectively. He further obtained a positive dependence of reaction rate constant on base concentration for the lecithin but an opposite effect was noted for the lysolecithin.

1:4 EFFECT OF SOLVENT COMPOSITION ON MICELLIZATION AND RATE:

Banks (1978) explained the various results so far obtained on the basis of the presence or absence of micelles. In his opinion, those reaction systems in which rate constants of the order of $10^{-2}\ s^{-1}$ were obtained contained monomers of the lipid in solution while those of the order of $10^{-4}\ s^{-1}$ contained a micellar dispersion of the lecithin in solution. Since Hauser and Dawson (1967) worked on surface monolayers, it was felt that Baer and Kates (1948), Marriott (1969) and Alexander and Rideal (1937) all worked on solutions containing similar forms of the lipid since the values of their rate constants agree. It is known that Alexander and Rideal (1937) and Hauser and Dawson (1967) worked on surface monolayers. Surface monolayers, structure wise, exhibit some similarity to micelles. The effect observed by

Marriott (1969) on employing lithium hydroxide is hence attributable to a micellar catalytic effect of the type recently reviewed by Fendler (1976). On this basis, it can be said that Jellinek and Gordon (1951), Marrinetti (1962) Dawson (1966) and Banks (1978) all worked on monomers of the lipid in solution.

It is noteworthy that the only results in the unimolecular dispersion group obtained from natural lecithin are those of Marrinetti (1962) and Dawson (1966). Marrinetti employed a solvent system that is 33 % v/v in methanol and 67 % v/v in chloroform. His conditions were therefore non-aqueous. The ion exchange method employed by Perrin and Saunders (1960) for the depigmentation of commercial egg yolk lecithin preparations would tend to indicate, in view of its fast speed and effectiveness, that lecithin forms unimolecular dispersions or small aggregates in equilibrium with monomers in methanol. This is further indicated by the observation of Hauser and Dawson (1967) that molecules of lecithin in a surface film have a very small equilibration constant with those in the underlying medium and vice versa. Since micelles are similar to surface films, an opposite effect, in terms of flow speed and depigmentation efficiency, would have been expected in the presence of large micelles from the work of Perrin and Saunders (1960).

Elworthy and McIntosh (1964) have investigated the effect of solvent dielectric constant on the association of egg lecithin molecules by using various solvent compositions. In benzene for instance, lecithin exists as inverted micelles (Elworthy 1959 a,b,). Elworthy (1960) later demonstrated the solubilization of certain dyes in the micelle interior. Elworthy and McIntosh (1964) have reported that in 93.4 % v/v ethanol in water, lecithin forms a true solution. In 80 % v/v ethanol in water, the lipid forms aggregates containing three molecules each. In 93 % v/v methanol in water, aggregates formed contain four lecithin molecules each. In 70 % v/v ethanol in water, micelles containing twenty-seven monomers each are formed while in 84 % v/v methanol in water, the micelles formed contain ninety monomers per aggregate. In 79.7 % v/v methanol in benzene, egg lecithin forms micelles containing eighty molecules but synthetic lecithin exists as monomers or dimers. However in absolute ethanol and methanol, the synthetic compound forms a true solution. Moreover Banks (1978) reported that synthetic lecithin and the 2-acyl lysolecithin obtained from egg yolk exist as monomers in his 50 % v/v propan-1-ol in water solvent. He went on to indicate the possible presence of pentameric aggregates in his reaction system on the basis of his gel permeation experiments. It consequently becomes obvious that natural lecithin behaves very differently from the synthetic compound as far as association or aggregation is concerned - the former being more susceptible to form micelles in both polar and non-polar solvents. Where association is observed with the synthetic compound, it usually contains far fewer lipid molecules than the natural would contain under similar circumstances.

Dawson (1954) employed a solvent consisting of 82 % v/v ethanol, 8.7 % v/v carbon tetrachloride and 9.8 % v/v water. If he had employed 90 % v/v ethanol and 10 % v/v water, the possibility exists that he would have been working on unimolecular dispersions of the lecithin or small inverted micelles of the lipid.

Furthermore, referring to the work of Marrinetti (1962), one may conclude that micelles were probably present. It is known that lecithin forms micelles in benzene (Marrinetti 1964). In chloroform which is dielectrically similar to benzene, a similar effect is expected. Since it is also known that in 79.7 % v/v methanol in benzene, lecithin forms micelles with an aggregation number of 12, one would expect that in Marrinetti's solvent consisting of 67 % v/v chloroform and 33 % v/v methanol, inverted micelles of aggregation number higher than twelve would be formed since this solvent is less polar than the 79.7 % methanol in benzene solvent. This conclusion is consistent with that of Perrin and Saunders (1960) who found that in a resin column, concentrations of lecithin higher than 2.5 % w/v in methanol lead to a nullification of the ion exchange process. This seems to imply a significant association of the lipid above such a concentration in methanol. Both Marrinetti (1962) and Dawson (1966) have employed the model for a two step reaction and have come up with apparent second order rate constants of about $10^{-1} dm^3 mole^{-1} s^{-1}$. This result makes it hard to explain Marrinetti's results along with those of Baer and Kates (1950). Baer and Kates assumed in effect that these reactions were one step and they employed a solvent not much different from that of Marrinetti but they obtained a pseudo first order constant of $2.8 \times 10^{-4}\ s^{-1}$ and an apparent second order rate constant of $1.9 \times 10^{-3}\ dm^3 mole^{-1} s^{-1}$.

With reactions of this nature, it is easily possible to have a micellar catalytic effect in the first part of the reaction and then proceed to an inhibitory effect or vice versa. In the alternative the reaction could start in a solution and terminate in another phase. Consequently, it is sometimes possible to have a very slow second phase of the reaction. Since Baer and Kates (1950) and Marriott (1969) assumed that the reaction was one step, it is difficult to draw any rigid conclusions since various possibilities exist. Banks (1978) concluded that Alexander and Rideal (1937), Baer and Kates (1950), Hauser and Dawson (1967) and Marriott (1969) all worked on micellar systems. However if synthetic lecithin exists as monomers in methanol and is as water tolerant as to exist as monomers in 50 % v/v propan-1-ol in water, it becomes difficult to explain why it should undergo association to form micelles in systems in between these.

Since Marriott (1969) worked on natural lecithin as did Hauser and Dawson (1967) one must infer that they worked on micellar systems. Elworthy and McIntosh (1964) have reported an aggregation number of sixty-eight for a similar solvent mixture, disregarding the small percentage of chloroform.

Banks (1978) also worked on a system that most likely contained the lipid as monomers but the acyl residues of the lipid he used are not indicated. From the behaviour of natural lecithin visavis the synthetic, one sees what difference a change in these residues can make to the physicochemical properties of the lipid. Hence it must be that Baer and Kates were working on some form of associated lecithin.

The impression develops as one reviews these works that apart from solvent effect, differences in the anions employed as the hydrolysing agent are significant. Using sodium methoxide, Marrinetti (1962) reports a preference of methoxide ion for attack at the 1-position. This is consistent with his observation of a higher rate of release of palmitic acid methyl ester in earlier aliquots compared to later aliquots. Banks in addition has reported a preference of n-propoxide for the 2-position in the dipalmitoyl compound which is consistent with the results of Fujiwara *et al* (1967). Fujiwara however observed no positional specificity on employing laurylamine in methanol and the reported preference for the 2-position can only be attributed to the alkoxide employed.

In view of the greater nucleophilicity of the higher alkoxides compared to the methoxide and hydroxide derivatives, one should expect a rate difference with change in the alkoxide. This seems to have been born out by Banks (1978) who reported a higher rate of attack for the 2-position.

The present work is intended to reconcile these two groups of results by investigating the kinetics of the hydrolysis in varying solvent compositions. Also in view of the fact that virtually all the past works in this field had been done under pseudo first order conditions, with the accompanying fact that much of the information becomes masked, the present work has been done under second order conditions.

In view of the many rate constants involved, as earlier indicated, and the fact that the rate of acyl migration in the 1 or 2-acyl intermediate is most probably less than the rate of deacylation of the intermediate (Banks 1978), a simplified model has been employed. This has become necessary since in view of the above factors and the fact that the intermediate lysolecithins can not be easily separated out and analysed for fatty acid composition, no independent rate constants for deacylation at the 1 and 2 positions are determinable. As a consequence the two step consecutive model represented below has been employed.

Lecithin $\xrightarrow{K_1}$ Lysolecithin $\xrightarrow{K_2}$ Glycernyphosphoryl choline

(both the 1 and 2 derivatives)

The scheme assumes that the intermediate lysolecithins behave identically with regard to their kinetics.

CHAPTER TWO

MATERIALS AND METHOD

2.1 INTRODUCTION

Prior to the work of Banks (1978) no in depth study had been done on the kinetics of the solvolysis of these lipids. It appears that previous workers only gave an average of the first and second stage constants. It is possible to calculate a rate constant for the first stage of the reaction from the work of Marrinetti (1962). The design of Marrinetti's work indicates that a calculation of the second stage constant from his data was possible but this was however not done. The analysis of the experimental data to yield a rate constant for such consecutive second order processes becomes very involving and a computer programme may be necessary (Banks, 1978). Using such a programme, Banks succeeded in his estimation of the constants for the two stages of the process - deacylation from lecithin and lysolecithin respectively. His constant shows considerable agreement with that of other workers in the field especially with those of Marrinetti (1962) and Dawson (1966).

Since it was not easy to obtain lysolecithin for the present work and the fact that even the purification of the commercial egg yolk lecithin is very involving, this work was restricted to the estimation of the rate constant for the deacylation of lecithin. Where possible the rate constant for the deacylation of lysolecithin has been estimated. In view of the fact that most works except that of Marrinetti had been done under pseudo first order conditions, it was decided that the present study be carried out under second order conditions.

2.1.1 CHOICE OF TECHNIQUE FOR FOLLOWING UP THE REACTION

There are a wide variety of techniques available for following up the progress of hydrolytic reactions. The most convenient methods usually depend on a quantitation of the hydroxyl or hydrogen ion concentration as the reaction progresses. Other methods include in situ densitometry from chromatographic plates; (Banks, 1983) radioactivity measurements from labelled lipids, (Hauser and Dawson, 1967) and recovery from paper chromatograms (Dawson 1954). In addition spectrophotometry can be employed for the estimation of lecithin on the basis of an absorption maximum it shows at 235 nm (Banks, 1978). Since lecithin is an unsaturated compound, each molecule containing on the average two to three double bonds, it is reasonable to expect it to absorb light in the ultra violet region. However since the released fatty acids and some of the lysolecithins would show more or less absorption around the same peak, a determination of lecithin by spectroscopy would be useless for following up the kinetics of the process.

In view of the requirement for equipment, solvents and the length of time required for chromatographic procedures, a simple back titration technique was decided upon.

.2 CHOICE OF SOLVENT SYSTEM

Much work had been done on the deacylation of phospholipids employing various alcohols and their water mixtures. Most of the works had however been done in methanol or methanolic mixtures with other solvents. From the work of Elworthy and McIntosh (1964), it is obvious that a solution containing monomers of the lipid is not easily obtainable in methanol. These same workers reported a solution of egg lecithin containing monomers in 93.4 % v/v ethanol in water solvent system. Banks (1978) has indicated that lysolecithin obtained by the action of phospholipase A_2 on egg yolk lecithin and synthetic lecithin most probably existed as monomers in his 50 % v/v propan-1-ol in water solvent system. It therefore appears that there is a tendency to form a solution containing monomers of lecithin in ethanol and propan-1-ol. However, higher alcohols have the disadvantage of exhibiting relatively high viscosities and as a consequence thorough mixing of the reaction system, especially at the initial stages would be hampered.

Consequently mixtures of n-propanol and water were chosen as the hydrolytic solvents. The weight of opinion from the previous works is that lecithin forms a solution of monomers in n-propanol. As the water content of the propan-1-ol solvent is varied, one would expect a change in the solution form of the lecithin and this in turn should be evidenced by change in the kinetics of the reaction.

In addition, propan-1-ol/water solvent systems, like most hydro alcoholic solvent systems, have the advantage that they have been quite extensively investigated (Gerrard and Macklen, 1959; Franks and Ives, 1966; Charlot and Tremillion, 1969 and Levitt and Levitt, 1971). In addition the organic and inorganic salts involved in the hydrolysis exhibit a reasonably high solubility in hydropropanolic solvents and the lipid itself is highly soluble in n-propanol.

2.2 MATERIALS

2.2.1 LECITHIN

Two samples of egg lecithin were purchased from the Sigma+ and BDH* companies. However these samples were impure and had to be purified. Also in view of the expected degradation of lecithin both during transportation and storage due to the high thermolability of this lipid, the actual purity of the commercial samples was much less than that indicated by the manufacturers.

For the purification process, a column chromatographic procedure was employed using alumina. However, commercial egg lecithin contains some pigments that are not resolved from the lecithin fraction on column chromatography. Consequently the initial stage of the purification process involved depigmentation of the phosphatide sample using ion exchange resins. Perrin and Saunders (1960) have reported that the pigments are very rapidly removed on a column of Dowex 1 x 4 resin. Since this resin was not available, it's equivalent, amberlite IRA 401 Cl^- (BDH catalogue 1981), was used in its place after conversion to the bicarbonate form. The conversion of the amberlite to the bicarbonate form

---

+ Sigma Chemical Company, P.O.Box 14508, Saint Louis Missouri 63178, USA.

* BDH Chemicals Ltd., Poole England.

was achieved by running 200 cm^3 of a concentrated solution of sodium bicarbonate down a column containing 20 g of the resin. The rate of flow was kept at a fast drip. The column was then washed many times over with distilled water until the eluate was no longer alkaline as indicated by use of a pH indicator paper. The water was then washed off the column using 100 cm^3 of redistilled methanol. A column of methanol about an inch in length was left above the ion exchange resin column.

1 g of the crude egg lecithin was weighed out in a small beaker. Using three 10 cm^3 portions of acetone, the lipid was defatted. The remainder of the lipid was dissolved in 40 cm^3 of methanol to give a 2.5 % w/v solution. This solution was then run down the ion exchange column at a fast rate since depigmentation was very rapid. It was impossible to regenerate the resin contrary to the observation of Perrin and Saunders (1960) with Dowex 1 x 4 resin.

The effluate collected from the column was evaporated to dryness using a rotary evapourator connected to a high powered vacuum pump. This ensured that the temperature did not exceed 0°C for at least 90 % of the evaporation time. This low temperature was necessary because of the instability of lecithin and particularly because of it's susceptibility to autoxidation.

The lipid extract so obtained was dissolved in 20cm^3 of chloroform to give a solution approximately 5 % w/v in the crude lipid. Then according to the method of Singleton et al (1965), 25 g of alumina was weighed out and made into a slurry using 28 cm^3 of chloroform. The slurry was carefully poured into the column. The column was washed using a further 12cm^3 of chloroform. The chloroform was run off the column to about 1 cm. off the alumina surface. The egg phosphatides were poured into the column and the beaker rinsed into the column using 20 cm^3 of chloroform. The chloroform was allowed to run off the column to 1 cm above the surface of alumina. The column was then eluted using 50cm^3 of a solvent system made up of chloroform: methanol, 9:1. Fractions were collected from the chromatographic column and their purity tested using TLC plates of silica gel G. It was usually convenient to collect fractions of about 6cm^3 each. From the TLC plates, fractions containing only pure lecithin were combined and evaporated to dryness on a rotary evaporator under high vacuum. The dry lipid was immediately taken up in propan-1-ol and stored as such. At loadings of over 200 μg the lecithin travelled as a single spot on thin layer chromatographic plates of silica gel G and it remained thus for over three months.

The concentration of the propanolic lecithin solution was determined by evaporating a known volume of the solution to dryness in a previously weighed bottle. The bottle and content were weighed after the drying and the weight of the lecithin found by difference. To confirm the accuracy of this procedure the lecithin stock solution was also determined by the method of saponification. To 1 cm^3 of 1 m. sodium hydroxide in a conical flask 3cm^3 of degassed redistilled propan-1-ol and 1cm^3 of the propanolic solution of lecithin were added and stoppered. After a period of eight hours, the entire content of the conical flask was titrated to phenolphthalein end point using 5×10^{-2} m. hydrochloric acid. These two estimation procedures were in agreement when the molecular weight of 800 as indicated by Elworthy and Saunders (1956) was utilised.

The initial test runs were done using the sample obtained from the Sigma Company but for the experimental runs the sample from BDH laboratories loadings of over 200 μg the lecithin travelled as a single spot on thin layer chromatographic plates of silica gel G and it remained thus for over three months.

The concentration of the propanolic lecithin solution was determined by evaporating a known volume of the solution to dryness in a previously weighed bottle. The bottle and content were weighed after the drying and the weight of the lecithin found by difference. To confirm the accuracy of this procedure the lecithin stock solution was also determined by the method of saponification. To 1 cm^3 of 1 m. sodium hydroxide in a conical flask $3cm^3$ of degassed redistilled propan-1-ol and $1cm^3$ of the propanolic solution of lecithin were added and stoppered. After a period of eight hours, the entire content of the conical flask was titrated to phenolphthalein end point using 5×10^{-2} m. hydrochloric acid. These two estimation procedures were in agreement when the molecular weight of 800 as indicated by Elworthy and Saunders (1956) was utilised.

The initial test runs were done using the sample obtained from the Sigma Company but for the experimental runs the sample from BDH laboratories was employed. The two lipids behaved similarly with respect to their kinetics but the BDH product appeared more susceptible to autoxidation.

Typically the propanolic stock solution contained 3×10^{-4} moles of the lecithin per cm^3 and hence 1 cm^3 of the stock solution was enough for a kinetic run.

2.2.2 PROPAN-1-OL

BDH Laboratory reagent grade. It was redistilled at 97°C on a fractionating column and degassed by exposure to vacuum prior to use. It was stored in glass bottles.

2.2.3 CHLOROFORM

BDH Analar grade. It was first dried by shaking with calcium chloride and then redistilled at a temperature of 58°C – 62°C and stored in glass containers prior to use.

2.2.4 METHANOL

BDH Analar grade. It was redistilled at 64°C – 66°C on a fractionating column and stored in glass containers prior to use.

2.2.5 ETHANOL

+Shermond Company Absolute grade. It was used as obtained after degassing by exposure to a high vacuum.

2.2.6 ACETONE

BDH analar grade. It was used as obtained.

2.2.7 WATER

This was distilled from an all glass still and stored in glass containers prior to use. Just before use it was degassed by boiling and allowed to cool.

2.2.8 HYDROCHLORIC ACID

BDH laboratory reagent grade. Using a small beaker a quantity of the concentrated acid was weighed out. The quantity was used to prepare a solution 0.125 molar in strength. This secondary stock was standardized using standard sodium hydroxide. From the stock so prepared, a dilution of strength 3×10^{-3}M was prepared and this was employed for the titration.

+Shermond Company, Sussex, England.

2.2.9 SODIUM HYDROXIDE

Volucon ampoules of this alkali were obtained from the*May and Baker Company. A volucon ampoule was used to prepare a litre volume of a stock of sodium hydroxide of strength 1 molar. The stock so prepared was standardized using a solution of succinic acid of strength 5 x 10^{-2} molar using both phenolphthalein and methyl red indicators. The standardized sodium hydroxide solution was stored in a tightly sealed volumetric flask.

2.2.10 SUCCINIC ACID

BDH analytical reagent. It was used as obtained.

2.2.11 ALUMINA

BDH laboratory reagents aluminium oxide for chromatographic adsorption analysis. It was labelled as of Brockman activity II. It was used as obtained though in view of the slow rate of flow of solvent experienced with early runs, later samples had to be sieved. The sieves were of sizes 150 µm, 125 µm and 106 µm. The fractions of sizes greater than or equal to 125 µm were combined and used for chromatography.

*May and Baker Ltd., Dagenham, England.

2.2.12 SILICA GEL G

BDH laboratory reagents grade. It was labelled as intended for chromatographic adsorption analysis and has a mesh size distribution of 60 - 120. It was used as obtained.

2.2.13 AMBERLITE IRA 401 (Cl^-) RESIN

This was BDH laboratory reagent grade. It had to be converted to the bicarbonate form prior to use as described earlier.

2.2.14 VACUUM PUMP

This was model EDM2 Edwards High Vacuum Pump. It was indicated as able to produce vacuums of a few millimeters of mercury.

2.2.15 SPECTROPHOTOMETER

This was a pye Unicam SP30 digital spectrophotometer reading upto 0.001.

2.3 METHOD

2.3.1 HYDROLYTIC EXPERIMENTS

In this series of experiments no allowance was made for the difference in the drainage properties of propan-1-ol and water since the experimentally tested delivered volumes by pipette were at least

99.9 % of those indicated on the pipettes. This error of 0.1 % v/v is acceptable when compared with the error limit of 0.1 % indicated on the pipettes.

A 1 cm^3 standard bulb pipette was used for measuring aliquots of 1 cm^3 from the stock solution of lecithin of strength 3 x 10^{-1}M. Into a flat bottomed test-tube (prepared locally) were measured the required volume of propan-1-ol, 1 cm^3 of the propanolic solution of lecithin and the required volume of degassed distilled water. Enough of an aqueous solution of sodium hydroxide of appropriate strength was added both to a total volume of 20 cm^3 and a hydroxide ion concentration of 3 x 10^{-2} molar.

As an example, for the 50 % v/v propan-1-ol/water solvent system, into the clean dry testtube was pipetted 9 cm^3 of redistilled degassed propan-1-ol. To this was added 1 cm^3 of the propanolic solution o of the lecithin and 9 cm^3 of distilled degassed water. The mixture was corked by means of a rubber bung and put on a water bath at 30°C. Propan-1-ol/water saturated nitrogen gas was admitted into the test tube via a delivery tube. This was to flush carbon dioxide out of the reaction system. The tube and its contents were then allowed to equilibrate with the water bath. Stirring was ensured by means of a magnetic stirrer whose setting had been standardized. After equilibration, 1 cm^3 of an aqueous solution of sodium hydroxide of strength 6×10^{-1} molar was added to the mixture over a period of time not exceeding 7 seconds and the clock started half way through the addition. Aliquots of 1 cm^3 were withdrawn from the reaction container at the earliest possible time after thorough mixing had set in. The 1 cm^3 aliquot was immediately mixed with 7.5 cm^3 of degassed ethanol. The dilution factor of 8.5 meant a very significant decrease in the rate of reaction leading to a decrease of 72.25 times in reaction rate. Hence the time of addition of the ethanol was taken in practice as the time of withdrawal of the aliquot. To this mixture was added 2 drops of phenolphthalein indicator. The mixture was then titrated to a colourless endpoint using 3×10^{-3}M hydrochloric acid. A 10 cm^3 burrette readable to 0.02 cm^3 was employed through out the work. Further aliquots were subsequently withdrawn and titrated at convenient time intervals. Two other runs were carried out.

From the titre values obtained, a graph of the concentration of the hydroxide against time was plotted (Fig. 1). From the graphs, using readings in the $t_{10\%}$ region, where it can be assumed that no significant conversion of the intermediate lysolecithin to glycerylphosphorylcholine had occurred, a graph of log of the ratio of the hydroxide to the lecithin was plotted against time. From the slope of such a graph, the rate constant for the removal of the first acyl residue from lecithin was calculated.

Using other compositions of the propan-1-ol water solvent (table 2:1) other sets of titrations were carried out.

Table 2.1 Percentage of propan-1-ol in the various solvents employed

97	86.67	81.67	60	30
90	85	80	50	20
88.33	83.33	75	40	10

In some cases, the graph of the concentration of hydroxide ion against time obtained could not be used to obtain accurate readings in the $t_{10\%}$ region due to a high rate of reaction. In such cases, it became necessary to prepare magnifications of the original graphs in the area around the $t_{10\%}$ region.

2.4 SPECTROPHOTOMETRIC EXPERIMENTS

From the kinetic experiments varying rates were obtained. Since constant concentrations of the hydroxide and lecithin were used through out, and in view of the amphiphilic nature of lecithin, it was felt that any changes in the kinetics of the reaction in terms of reaction rate and order should be attributable to changes in the solution form of the lipid. Hence it became necessary to investigate the association form of the lecithin.

In view of the amphiphilic nature of the lecithin, the most likely cause of change in kinetic behaviour is change in the solution form of the lipid leading to the formation of micelles and various lipid aggregates.

Osipov (1962) has shown that some compounds are solubilized by micelles. Other workers (Elworthy, 1960; Martin et al', 1975 and Rawlins, 1979) have also given evidence of micellar solubilization of dyes. Since phenolphthalein was used through out the kinetic study as an indicator, it was decided to use it as a probe into the nature of the lipid aggregates that were formed. Phenolphtalein also has been reported as insoluble in water but it shows fair solubility in ethanol and ether (Martindale, 1977). The formation of micelles would lead to selective partitioning of the phenolphthalein between the micellar phase and the solvent phase.

Solutions of lecithin in the various solvent compositions employed were prepared at a concentration of lecithin equivalent to those used in the hydrolytic experiments. However, sodium hydroxide was left out in view of its hydrolysing property.

Blank and test solutions were prepared in exactly the same way. However since the phenolphthalein was being used as a probe, it was unnecessary to incorporate it in the blank. The phenolphthalein was employed as a propanolic solution and its final concentration in the experiments was 20 $\mu g/cm^3$. The absorption spectrum was determined. From the spectrum, graphs of absorbances against the wavelength for the various solvent compositions employed were prepared.

It was impossible to work on propan-1-ol/water systems with propan-1-ol content higher than 97 % v/v due to the insolubility of the alkali.

CHAPTER THREE

RESULTS

.1 HYDROLYTIC EXPERIMENTS

From the titre values obtained in the kinetic experiments, values of the concentration of sodium hydroxide against time were obtained (see the appendix). Figure 1 is a graph of the concentration of the alkali against time for selected solvent compositions (97, 88.33, 85, 81.67, 60 and 10 % v/v propan-1-ol in water).

For second order reactions of the type being investigated,

$$K = \frac{2.303}{t(a-b)} \log \frac{b(a-x)}{a(b-x)} \quad . \; . \; . \; . \; . \; 3.1$$

where K = the second order rate constant for the deacylation of lecithin.

t = time after the start of the reaction

a = initial concentration of the alkali

b = " " " " lipid

x = change in the concentration of the alkali.

Hence a-x gives the concentration of the alkali remaining at any particular point in time and b-x similarly corresponds to the concentration of the lipid remaining at time t.

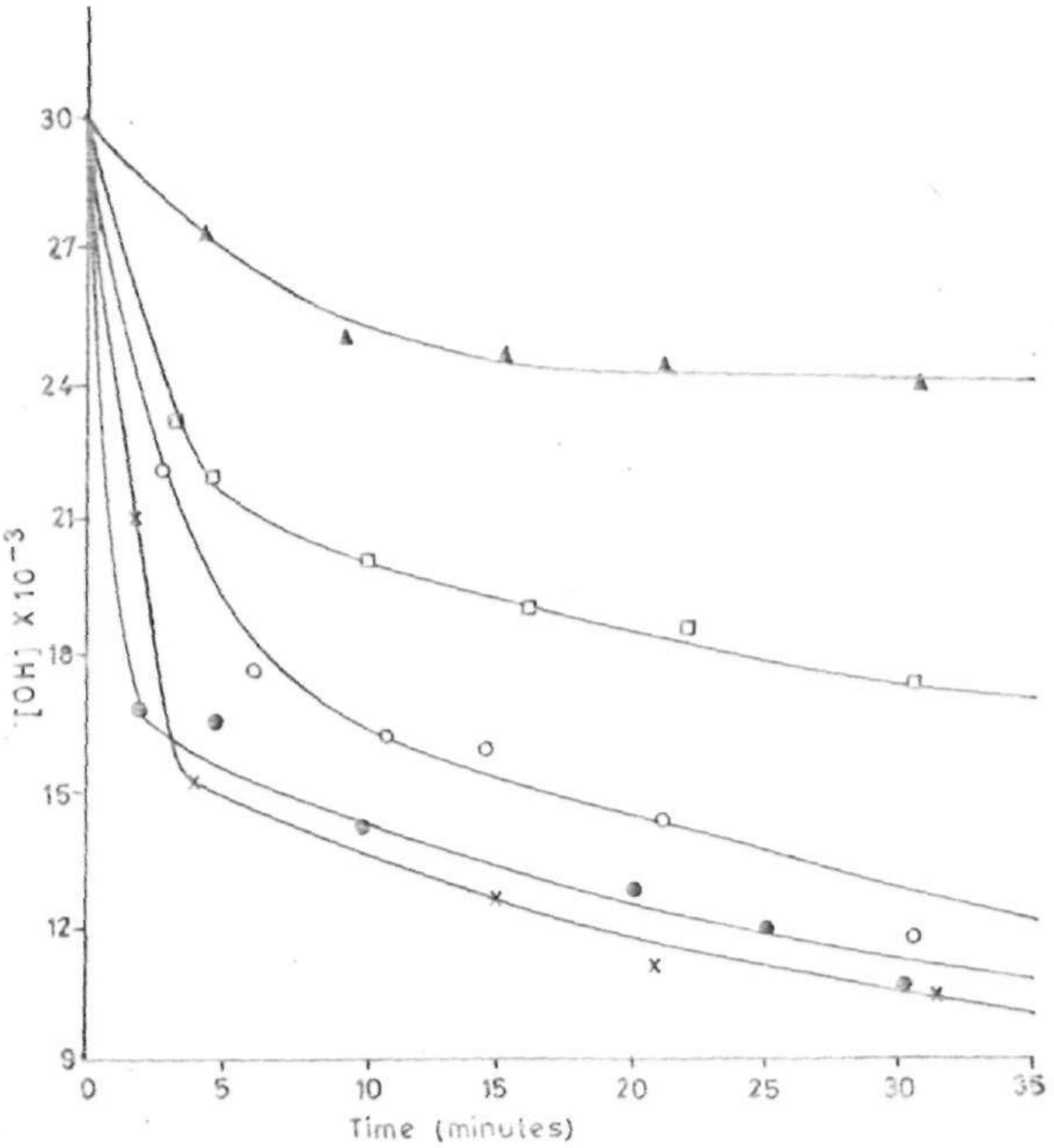


Fig. 1 A plot of the concentration of sodium hydroxide against time for various compositions of the propan-1-ol/water solvent

□ = 97% $\frac{V}{V}$ propan-1-ol, ○ = 88·33% $\frac{V}{V}$ propan-1-ol
● = 85% $\frac{V}{V}$ propan-1-ol x = 81·67% $\frac{V}{V}$ propan-1-ol
▲ 10% $\frac{V}{V}$ propan-1-ol

From figure 1, it is obvious that the reaction is very fast initially but shows a slow second stage. For instance, the solvent composition at which the reaction is fastest is 85 % v/v propan-1-ol and the $t_{\frac{1}{2}}$ value for the first phase of the reaction in this solvent is five minutes. However in view of the slow rate observed in the second phase of the reaction, it can be assumed that no significant proportion of the intermediate lysolecithin formed is converted to glycerylphosphoryl choline within the $t_{10\,\%}$ region. Hence within the $t_{10\,\%}$ region, the concentration of the lecithin can be approximated, with reasonable accuracy, to half that of the alkali. As the reaction progressed beyond this region, it became impossible to estimate the concentration of the lecithin and hence readings outside the $t_{10\,\%}$ region have not been used for the estimation of the first stage rate constant.

Equation 1 rearranges to

$$\log \frac{a-x}{b-x} = \frac{a-b}{2.303} kt - \log 0.5 \quad \ldots 3-2$$

Graphs of $\log \frac{a-x}{b-x}$ against time were plotted. For solvent compositions 97 %, 85 % and 60 % v/v propan-1-ol in water, the plots are shown in figures 2, 3 and 4 respectively.

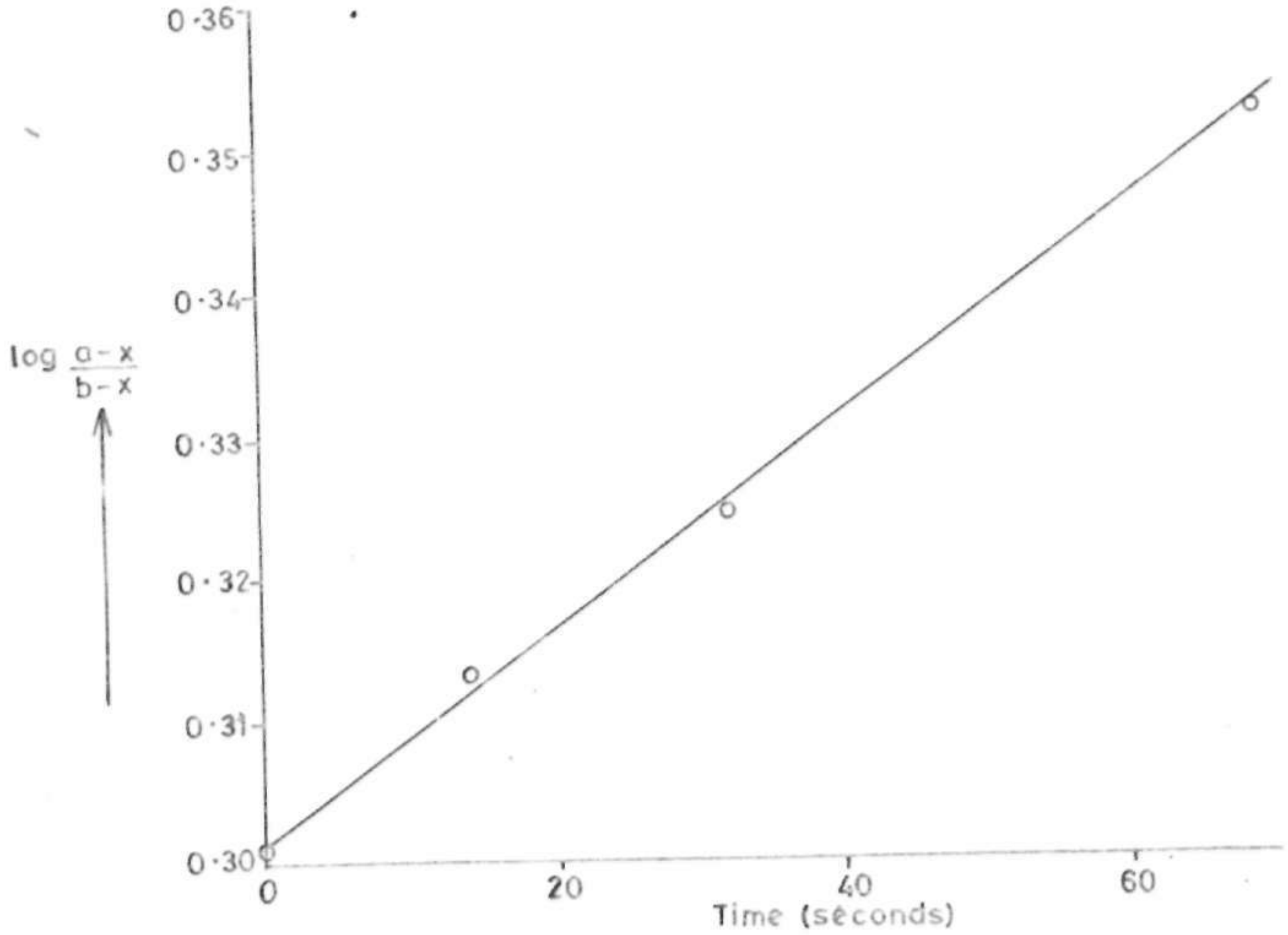


Fig. 2 Log $\frac{a-x}{b-x}$ versus time plot for 97% $\frac{v}{v}$ propan-1-ol in water solvent

Equation of straight line is $\log \frac{a-x}{b-x} = 7\cdot353 \times 10^{-4}t + \cdot 301$

Correlation coefficient = 0·9735

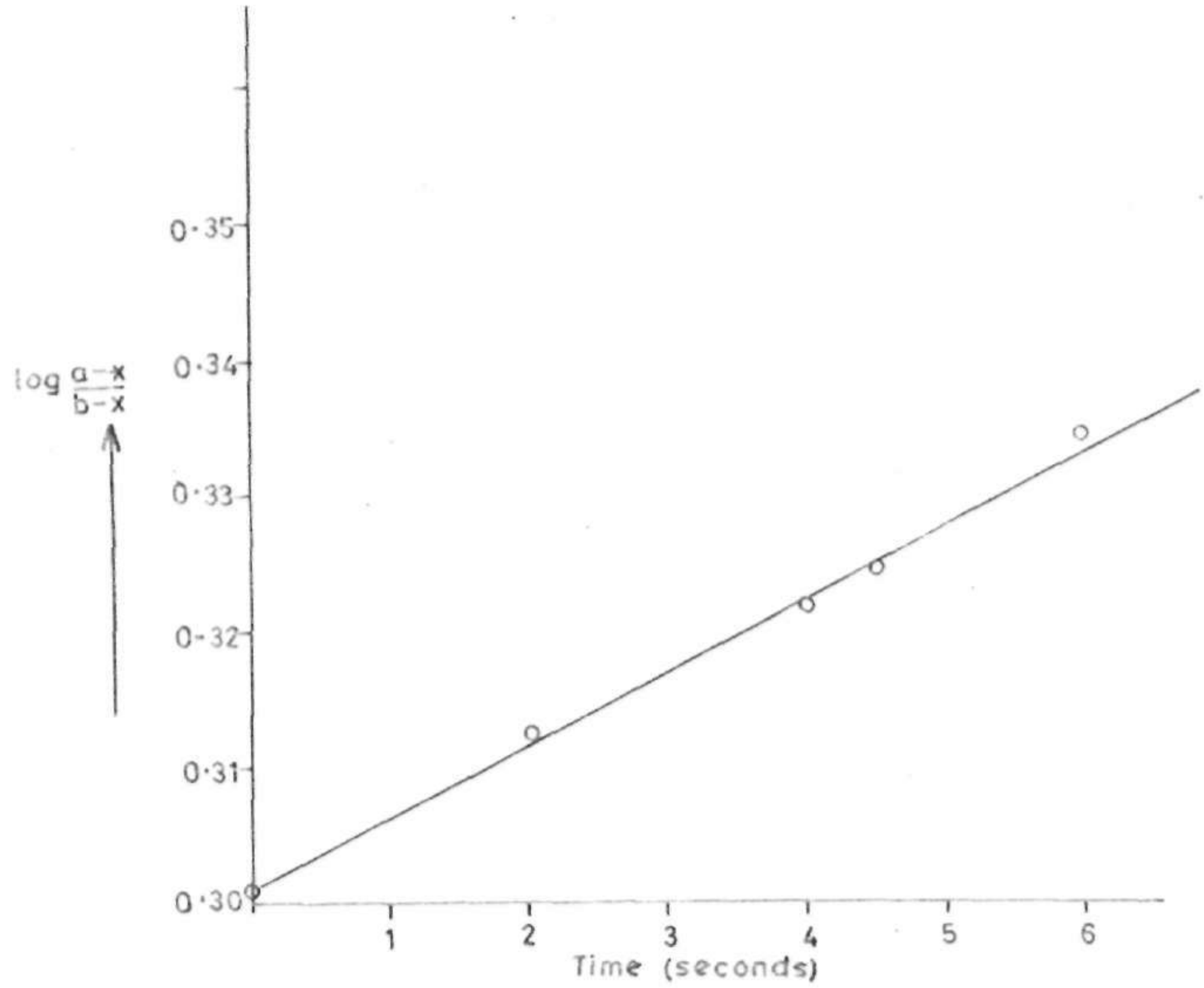


Fig. 3 Log $\frac{a-x}{b-x}$ versus time plot for 85% $\frac{v}{v}$ propan-1-ol solvent.

Equation of straight line is $\log \frac{a-x}{b-x} = 5\cdot625 \times 10^{-3} t + \cdot301$

correlation coefficient = 0·9128

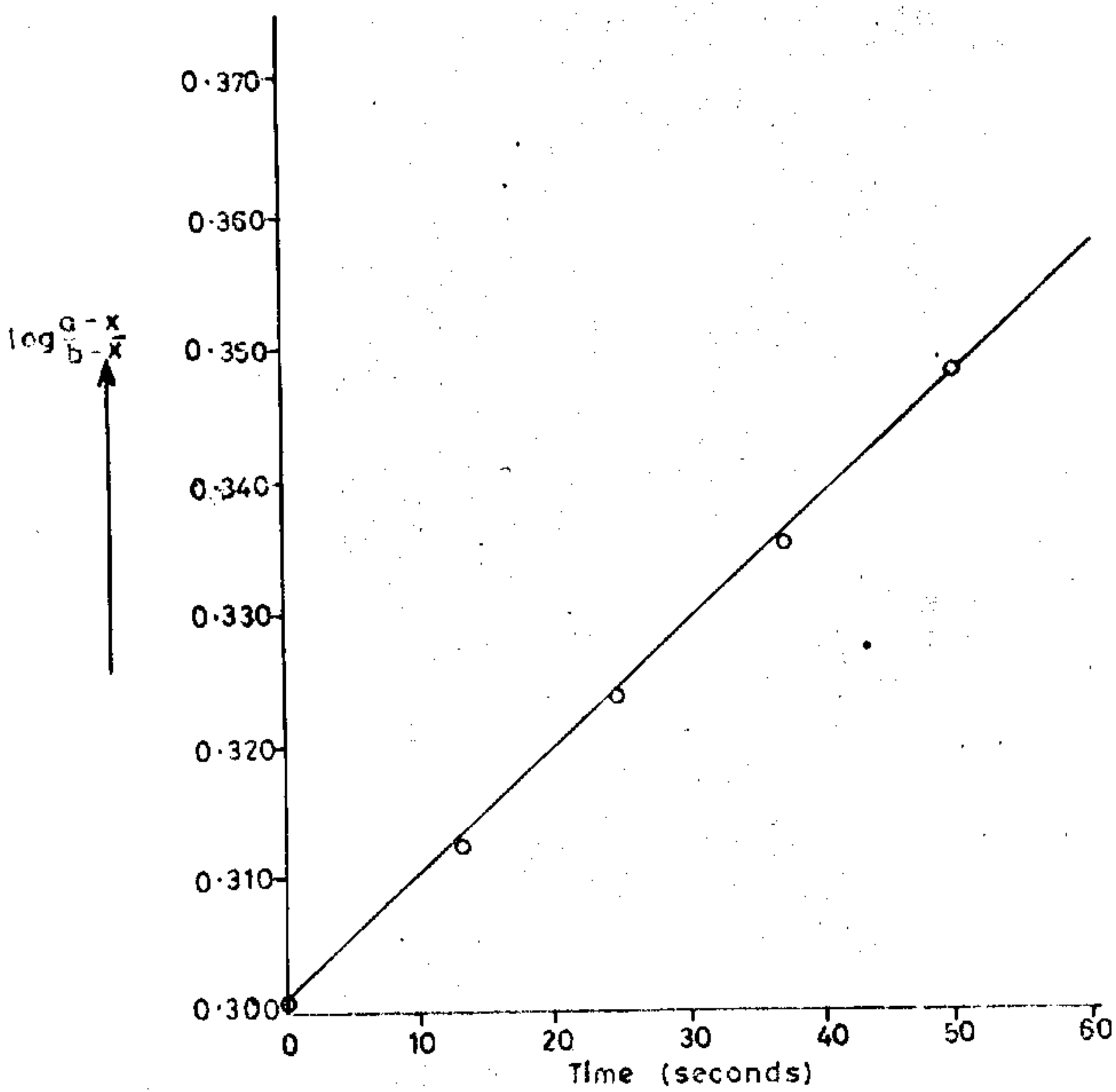


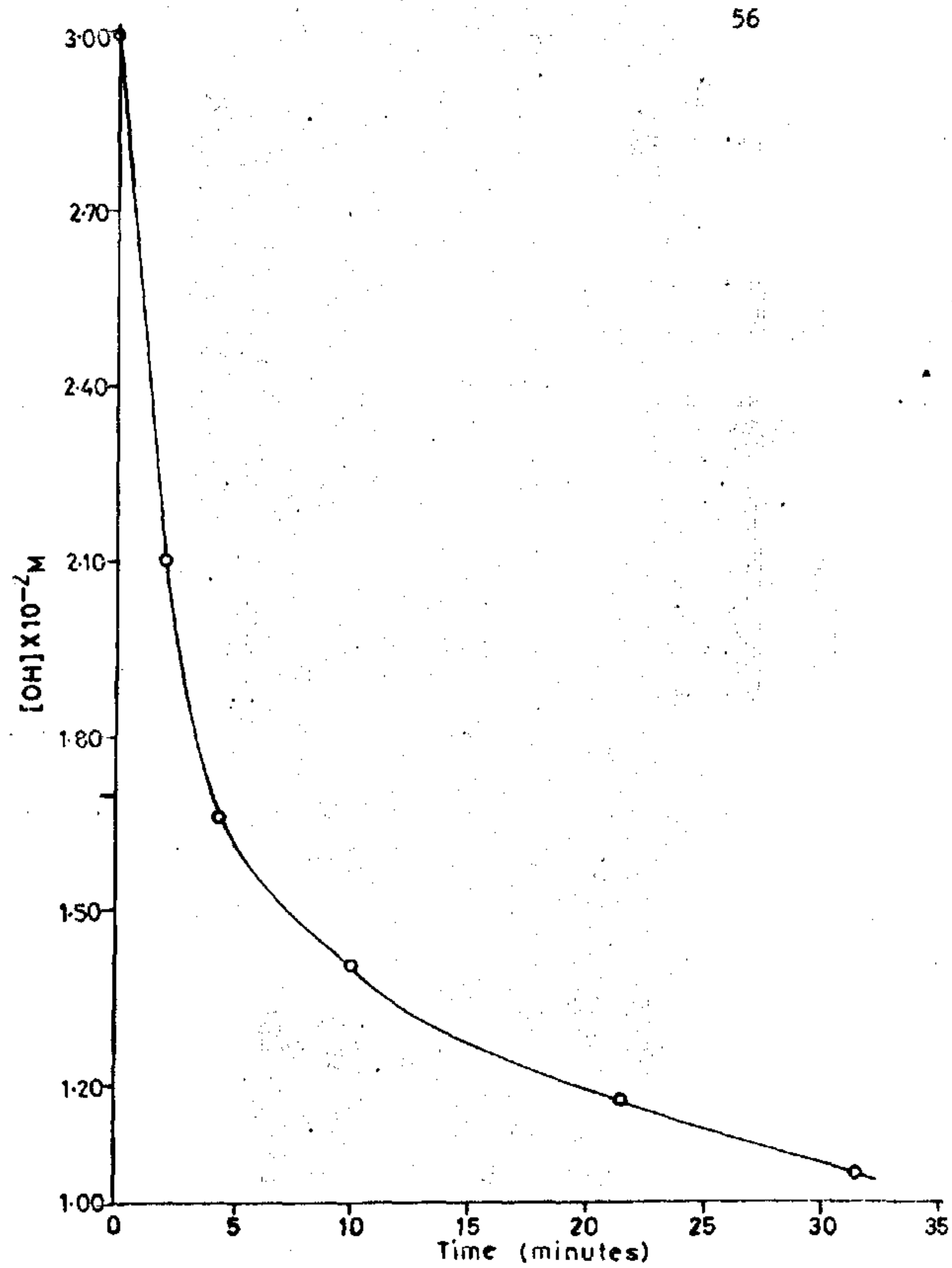
Fig. 4 Log $\frac{a-x}{b-x}$ versus time plot for 60% $\frac{v}{v}$ propan-1-ol solvent.

Equation of straight line is $\log \frac{a-x}{b-x} = 9.327 \times 10^{-4} t + \cdot 301$

Correlation coefficient = 0·990

Where it was impossible to obtain readings in the $t_{10\,\%}$ region from the experimentally obtained results presented graphically, magnifications of the concentration of sodium hydroxide versus time plot were made in the $t_{10\,\%}$ region so that accurate readings in that region could be obtained. For instance, Fig. 5 shows a plot of hydroxyl ion concentration against time in minutes for 81.67 % propan-1-ol in water solvent. However readings in the $t_{10\,\%}$ region of this graph can't be taken accurately and as a consequence readings in the $t_{40\,\%}$ region have been reproduced on an enlarged scale (Fig. 6). Fig. 7 shows a similar enlargement of readings in the $t_{10\,\%}$ portion based on Fig. 6.

Using this method the rate constant was similarly determined for every solvent composition employed. Table 3:1 shows the rate constants obtained in the indicated solvent compositions.



Fig·5 A graph of hydroxyl ion concentration versus time for the 81·67% $\frac{V}{V}$ propan-1-ol in water solvent

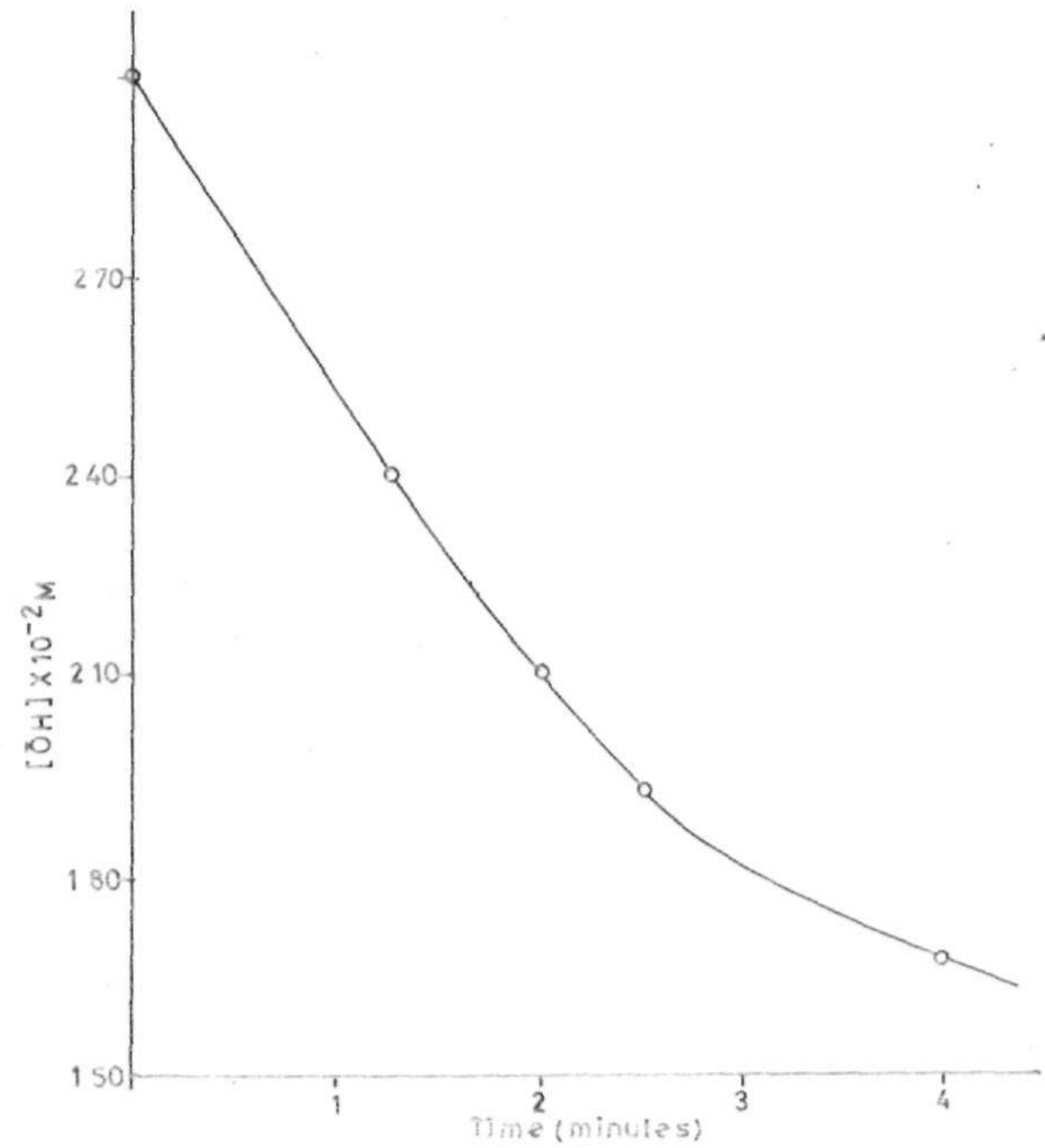


Fig. 6 A graph of hydroxyl ion concention against time for the 81·67% $\frac{V}{V}$ propan-1-ol in water solvent (magnification of the t_{40}% region)

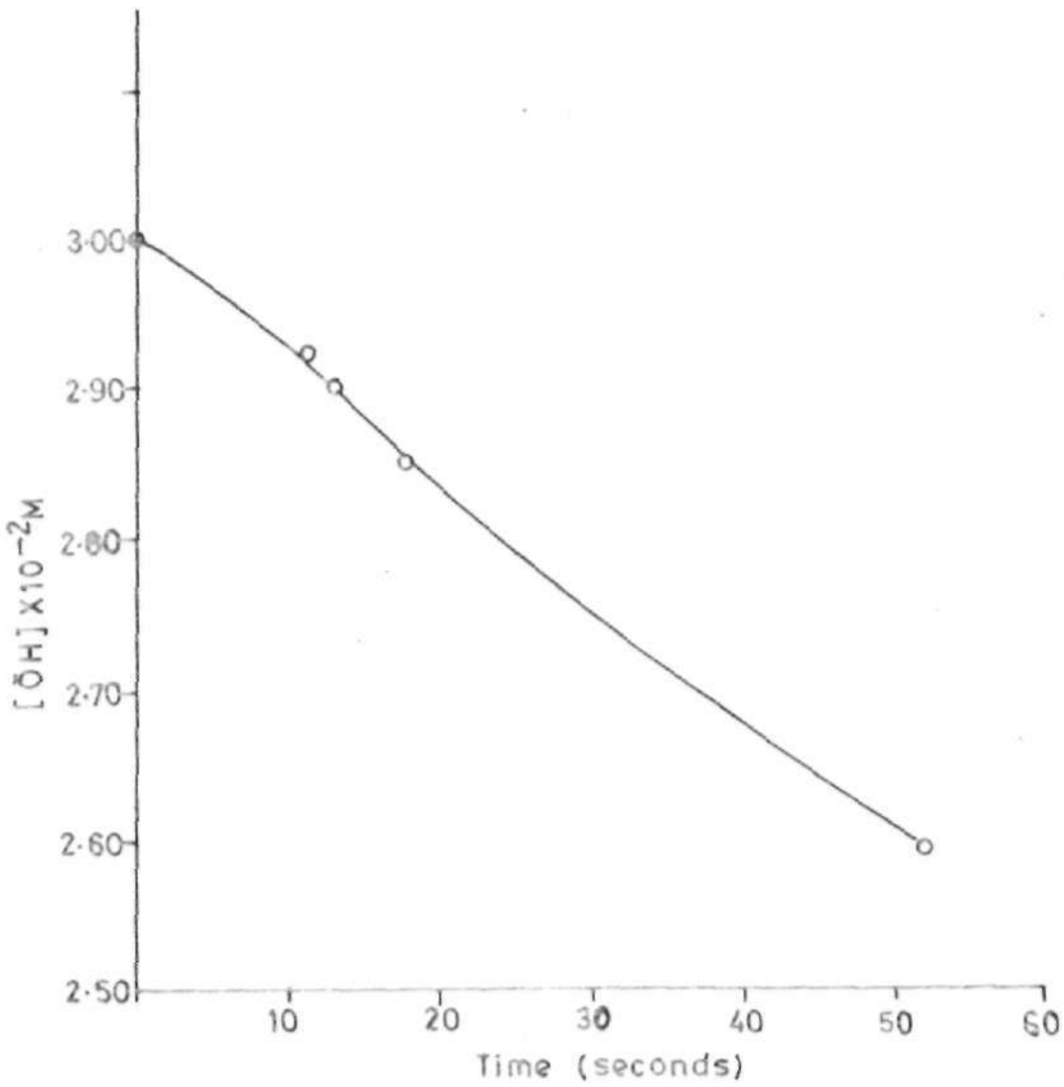


Fig. 7 A graph of hydroxyl ion concetration against time for the 81·67 % $\frac{v}{v}$ propan-1-ol in water solvent (magnification of the ^{t}10% region

Table 3.1 Second order rate constants for the deacylation of lecithin, K_1, in the different solvent systems.

Percentage propan-1-ol (% v/v) in water	Rate constant (dm^3 $mole^{-1}$ s^{-1}) x 10^{-1}
97	1.129
90	1.149
88.33	1.414
86.67	3.538
85	8.636
83.33	4.138
81.67	2.022
80	1.445
75	1.433
60	1.432
50	1.036
40	0.965
30	0.602
20	0.287
10	0.286

Fig. 8 shows a graphical presentation of these results in respect of the deacylation of lecithin.

3:2 CALCULATION OF CONSTANT FOR THE DEACYLATION OF LYSOLECITHIN

Estimates of the rate constants for the removal of the first acyl residue (either the saturated or unsaturated acyl residue) from lecithin, K_1, have been made from a log $\frac{a-x}{b-x}$ versus time plot (where a, b and x are as previously defined). Values used

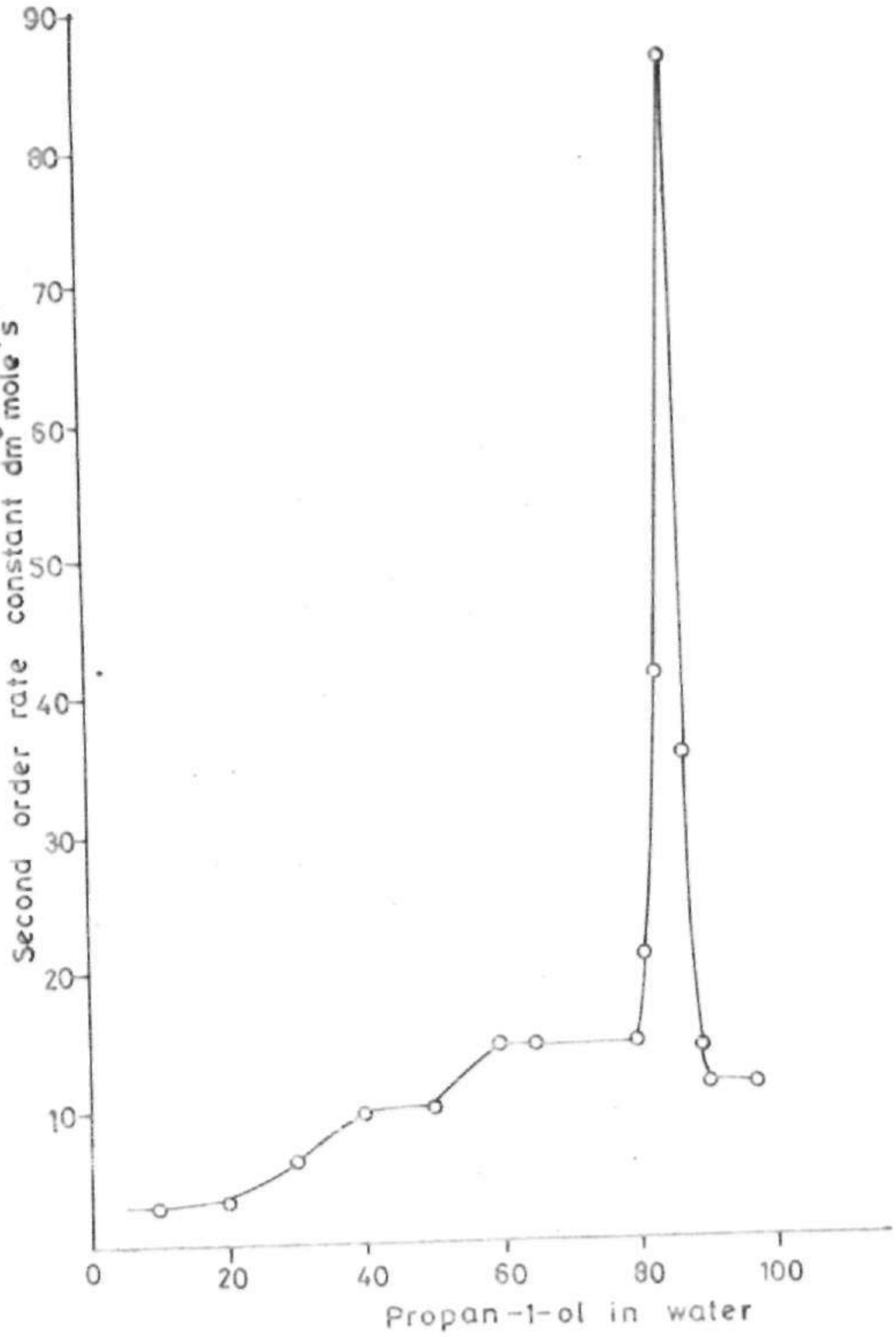
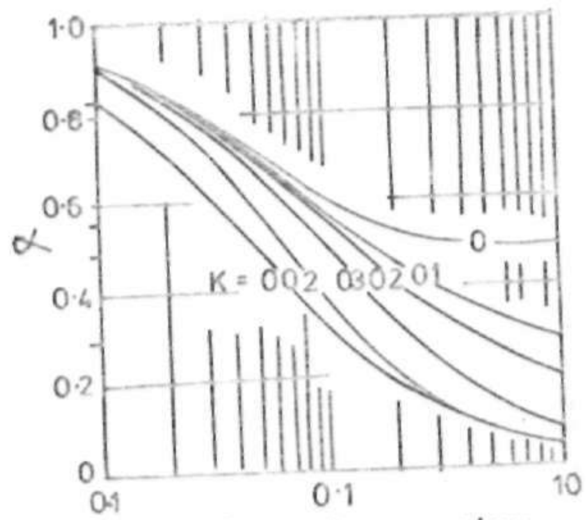


Fig. 8 Rate constant versus % $\frac{V}{V}$ propan-1-ol in water plot

for the plots were restricted to the $t_{10\,\%}$ region to minimize the effect of the deacylation of lysolecithin on the estimated constants. Based on the determined values of K_1, assuming that the reaction rate constant (K_1) remains the same through out the course of reaction, the concentration of lecithin at any point in time can be calculated from the second order equation (3.1 page 49) by substituting for the values of K_1 and t and the a-x value would be as experimentally determined. Relating the experimental values of the hydroxide ion concentration to the calculated values of the lecithin concentration, the lysolecithin concentration at any point in time can be calculated.

However, the analysis of the kinetic data for the estimation of K_2, the rate constant for the deacylation of the lysolecithin becomes very difficult due to two main reasons. One is the second order nature of the runs and the second is the consecutive nature of the deacylation process. In view of the consecutive second order nature of the reactions, a problem arises as to the possible non-compliance of the reaction with this expected condition, especially the second order kinetic behaviour expectation, due to possible changes in the solution form of the lipid.

Frost and Schwemer (1952) have however recommended a procedure for calculating the two rate constants in a consecutive second order reaction. In addition, they have concluded that the kinetic data from a consecutive two step second order process must comply with the shape in figure 9 where α is the ratio of the concentration of the alkali at time t to the initial concentration of the alkali. From fig. 10 it is obvious that the kinetic data obtained in 85 % v/v propan-1-ol in water complies with this condition. From the paper by Frost and Schwemer (1952), the α values were used to approximate the value of a constant K defined such that $K = \frac{k_1}{K_2}$ where K_1 and K_2 are the rate constants for the deacylation of lecithin and lysolecithin respectively. However it was impossible to use the table in the paper by Frost and Schwemer relating α, K, K_1 and K_2 because the value of K obtained from figure 10 and the accompanying kinetic data is far outside the range covered in the aforementioned paper. However the similarity of fig. 10 to fig. 9 shows that the hydrolysis of lecithin under the experimental conditions complies with consecutive second order kinetic behaviour for the 85 % v/v propan-1-ol in water solvent.



Frost and Schwemer (1952)

Fig. 9 Plot of the fraction of hydroxide remaining (α) against the log time parameter T ($T = bk_1t$) for various values of $1/k = k_2/k_1$

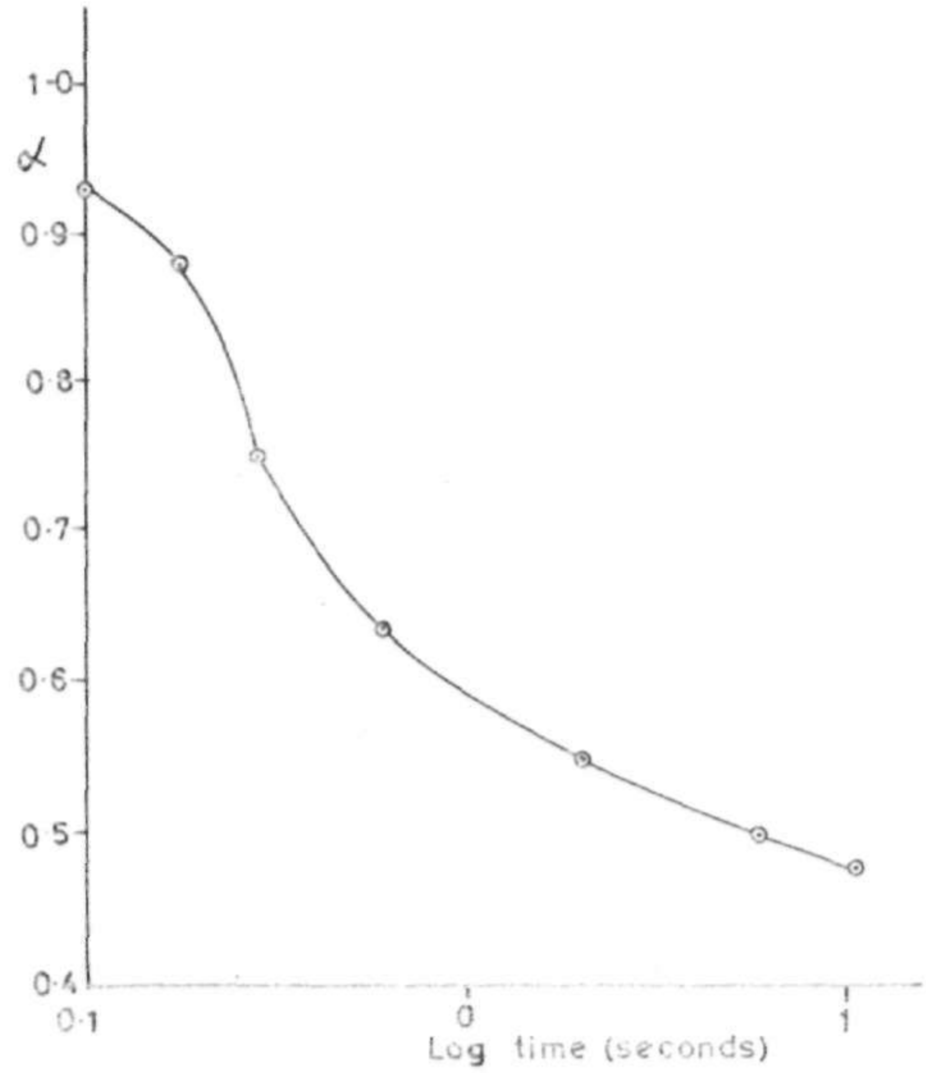


Fig.10 The plot of the fraction of hydroxide remaining (α) against the log time (seconds) parameter for 85% $\frac{v}{v}$ propan-1-ol in water

A different approach has however been employed to calculate K_2. On the basis of the K_1 values as obtained from the earlier plots, consecutive estimates of lecithin, lysolecithin and glycerylphosphorylcholine concentrations were made at suitable time intervals until the calculated lecithin concentration was judged to be practically and kinetically insigificant. From that point on, the lecithin concentration was approximated to zero and the hydroxyl ion and the lysolecithin concentration became equal as a result. Consequently the second order equation applicable when equal concentrations of two reactants are involved in a chemical reaction

$$k = \frac{1}{t} \frac{x}{a(a-x)} \quad \cdot\ \cdot 3.3 \text{ (Osol and Hoover, 1975)}$$

became relevant and was used as a consequence after the lecithin had been used up. In the above equation,

K = constant for the deacylation of lysolecithin

t = time elapsed after lecithin had been used up

a = concentration of the lysolecithin = concentration of the alkali

x = fraction of lysolecithin reacted in time t.

A plot of $\frac{x}{a-x}$ against time should give a straight line graph with a slope of Ka. For examplification, the data for the 85 % v/v propan-1-ol in water solvent run has been employed.

Recalling equation 3 - 1

$$K = \frac{2.303}{t(a-b)} \log \frac{b(a-x)}{a(b-x)}$$

where all the parameters are as previously defined,

five minutes after the start of the experiment,

$$0.864 = \frac{2.303}{300\ (1.5 \times 10^{-2})} \log \frac{a}{2a} \frac{a-x}{b-x}$$

($K = 0.864\ dm^3 mole^{-1} s^{-1}$ $b = \frac{a}{2}$ and t = 300 seconds).

Substituting for a - x from the experimental data, $b - x = 1.552 \times 10^{-4}$ M = Conc. of lecithin. From the observed hydroxyl ion concentration, $a - x = 1.512 \times 10^{-2}$M, hence the concentration of lysolecithin after five minutes = 1.481×10^{-2}M. This approximation was continued until the lecithin concentration became kinetically insigificant.

The data obtained from this calculation has been used to calculate the second stage constant for the deacylation in the 85 % v/v propan-1-ol in water solvent run (Fig. 11). Similarly figures 12 and 13 represent the data for the 50 % v/v and 20 % v/v propan-1-ol in water solvent runs. Estimates of

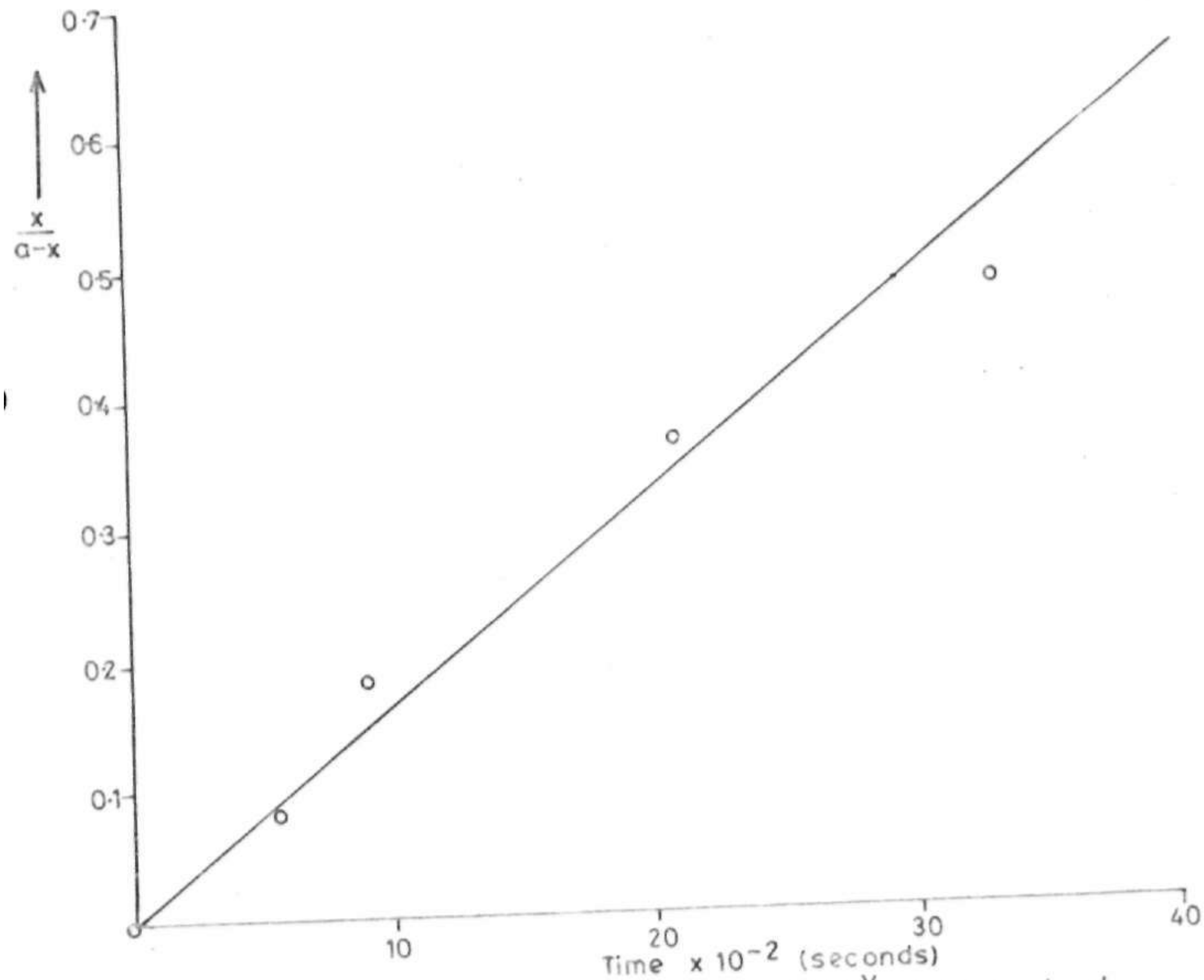


Fig. 11 $\frac{x}{a-x}$ versus time plot for the 85% $\frac{v}{v}$ propan-1-ol in water solvent

Equation is $1{\cdot}125 \times 10^{-4} t = \frac{x}{a-x}$

Correlation coefficient = 0·980

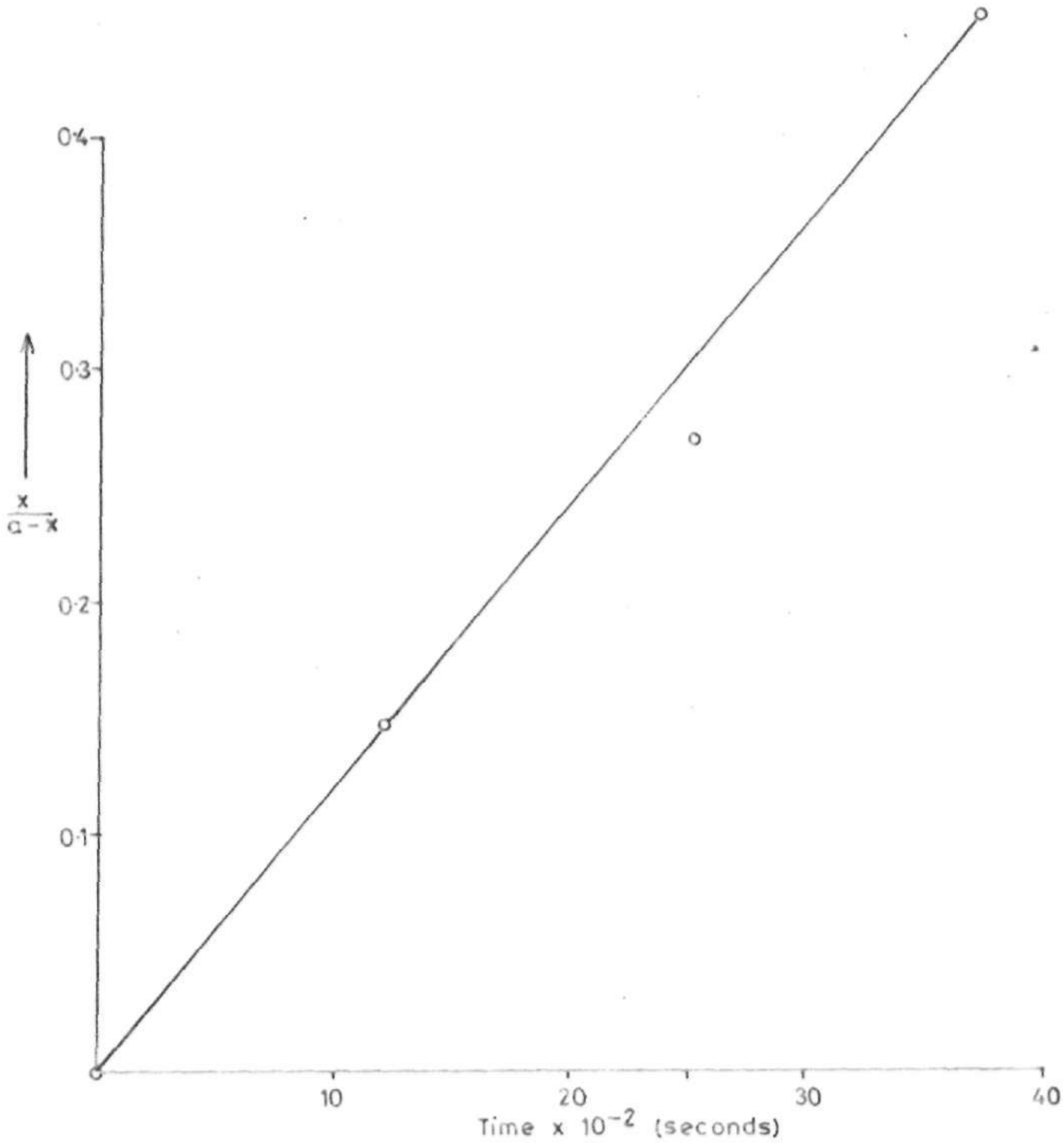


Fig.12: $\frac{x}{a-x}$ versus time plot for 50% $\frac{v}{v}$ propan-1-ol in water solvent

Equation is $1{\cdot}071 \times 10^{-4} t = \frac{x}{a-x}$

Correlation coefficient = 0·997

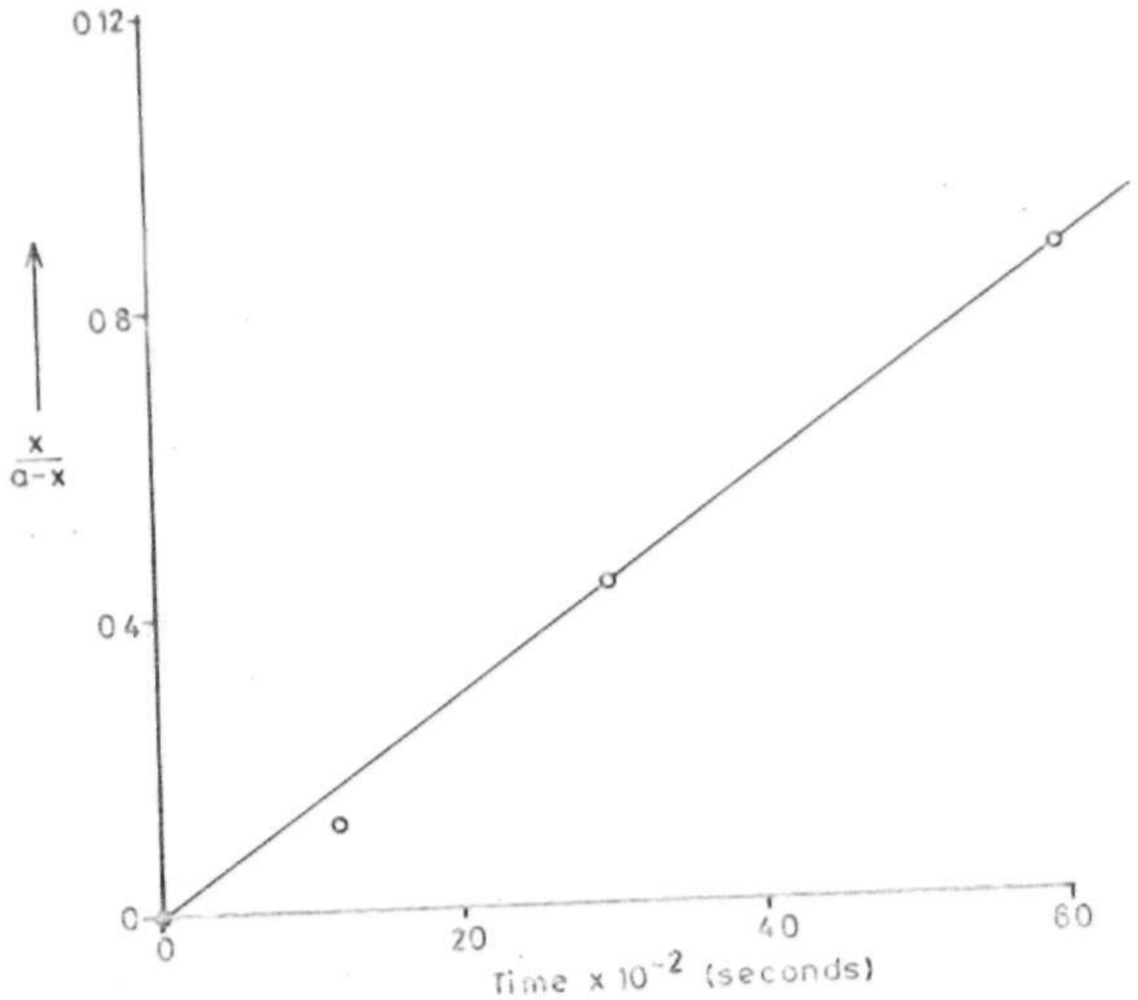


Fig. 13 $\frac{x}{a-x}$ versus time plot for 20% $\frac{v}{v}$ propan-1-ol in water solvent

Equation is $1.471 \times 10^{-5} t = \frac{x}{(a-x)}$

Correlation coefficient = 0.995

K_2 values have been made for 86.67, 83.33, 81.67, 75, 60, 40, 30 and 20 % v/v propan-1-ol in water solvent systems.

Table 3.2 - Second order second stage rate constants

% v/v propan-1-ol	K_2 value ($dm^3 mole^{-1} s^{-1}$)
86.67	3.19×10^{-3}
85	1.09×10^{-2}
83.33	2.98×10^{-3}
81.67	2.04×10^{-2}
75	2.50×10^{-3}
60	1.70×10^{-2}
50	1.19×10^{-2}
40	7.79×10^{-3}
30	1.18×10^{-3}
20	1.09×10^{-3}

It was impossible to obtain these rate constants for 97, 90, 88.33 and 80 % v/v propan-1-ol in water solvent systems. This was due to the failure of the kinetic data obtained to fit into this method of analysis. In these four cases, the concentration of the glyceryl phosphorylcholine obtained did not show a consistent increase with time. In all the four

K_2 values have been made for 86.67, 85, 83.33, 81.67, 75, 60, 50, 40, 30 and 20 % v/v propan-ol in water solvent systems.

Table 3.2 - Second order second stage rate constants for the deacylation of lecithin

% v/v propan-1-ol	K_2 value (dm^3 $mole^{-1}s^{-1}$)
86.67	3.19×10^{-3}
85	1.09×10^{-2}
83.33	2.98×10^{-3}
81.67	2.04×10^{-2}
75	2.50×10^{-3}
60	1.70×10^{-2}
50	1.19×10^{-2}
40	7.79×10^{-3}
30	1.18×10^{-3}
20	1.09×10^{-3}

It was impossible to obtain these rate constants for 97, 90, 88.33 and 80 % v/v propan-1-ol in water solvent systems. This was due to the failure of the kinetic data obtained to fit into this method of analysis. In these four cases, the concentration of the glyceryl phosphorylcholine obtained did not show a consistent increase with time. In all the four cases decreases in the calculated concentrations of glyceryl phosphorylcholine occurred as time progressed. For the 10 % v/v propan-1-ol in water solvent however, enough experimental data was not obtained and so a calculation of the second order second stage constant could not be attempted.

3.3 SPECTROSCOPIC EXPERIMENTS

Fig. 14 shows the graph of the absorbance of a solution of phenolphthalein of strength 20 µg/cm^3 in propan-1-ol against wavelength.

From the absorbance obtained for the solutions containing 20 µg of phenolphthalein per cm^3 and the kinetic concentration of the lecithin, 12 mg per cm^3, two peaks are evident (Fig. 15 and 16) around 258 nm and 290 nm respectively for the various solvents employed. Fig. 15 shows a graph of absorbance against wavelength for the 258 nm region and fig. 16 shows the same plot for the 290 nm region. From these two plots (Figs. 15 and 16), it is obvious that the absorbances change with change in solvent composition. For purposes of comparison Figs. 17 and 18 have been prepared to compare the sets of absorbances for 88.33, 60 and 50 % v/v propan-1-ol in water (Fig. 17) and 83.33, 60 and 40 % v/v propan-1-ol in water

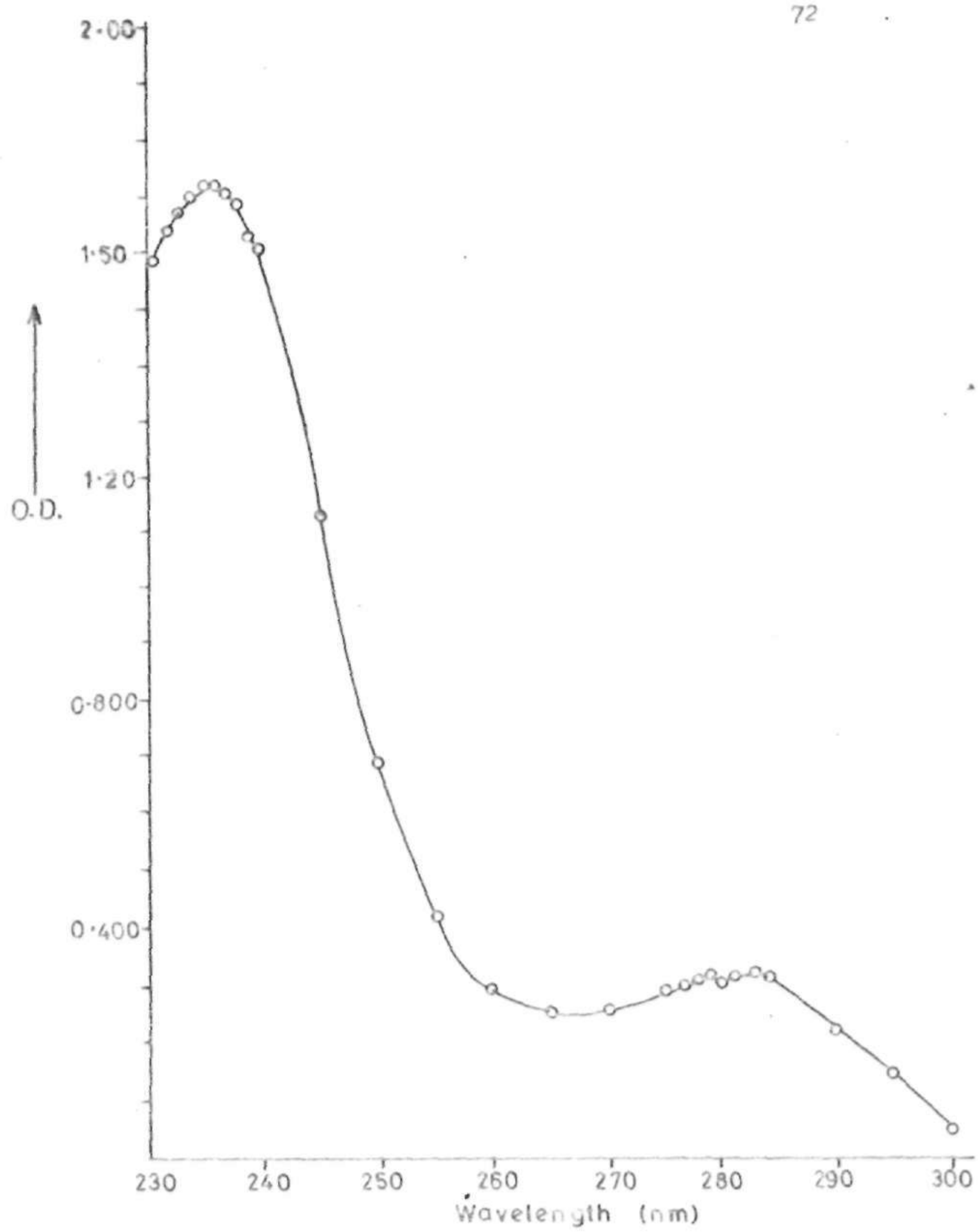


Fig. 14 Absorbance versus wavelength (nm) plots for 20 $\mu g/cm^3$ phenolphthalein in propan-1-ol

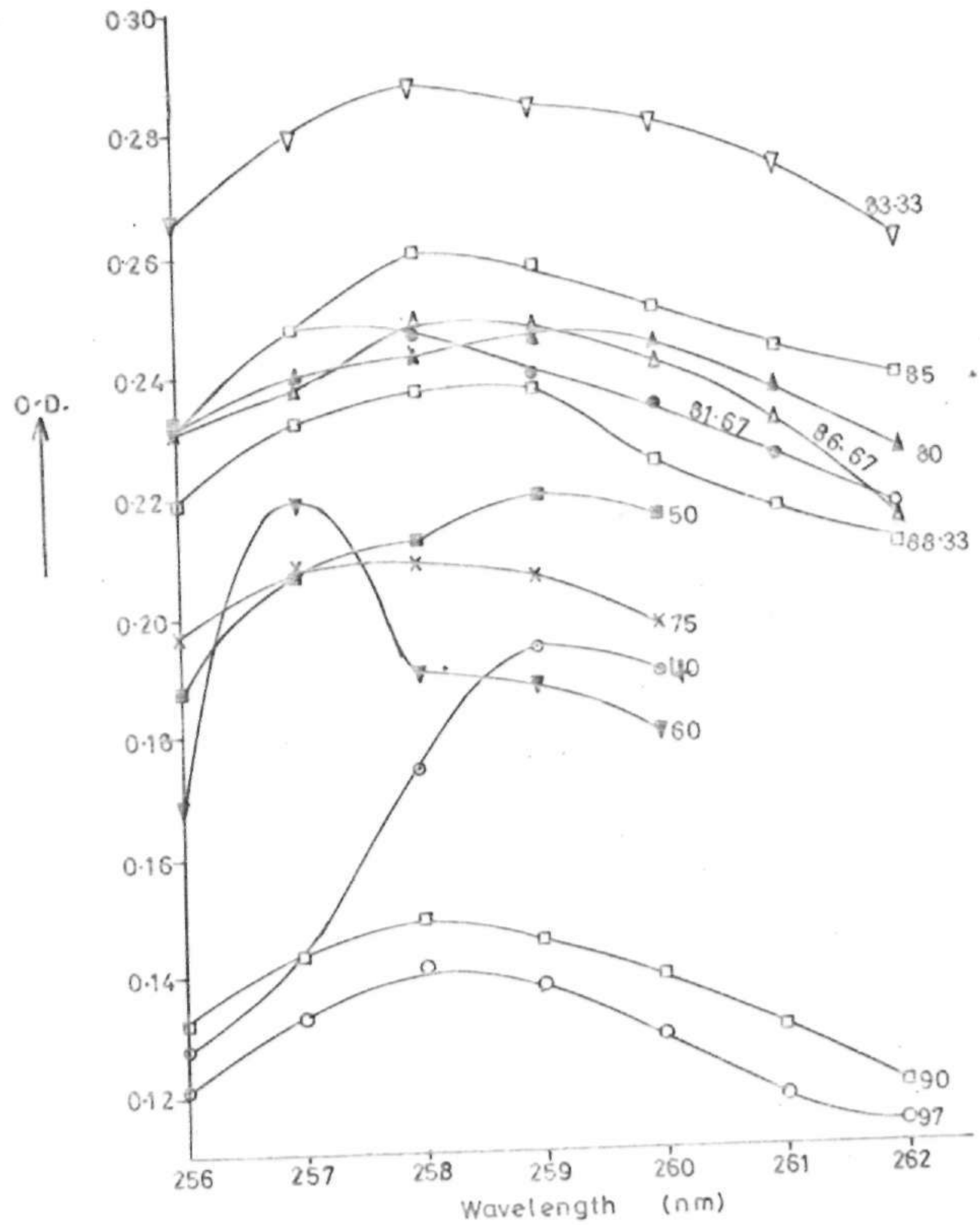


Fig. 15 Absorbance versus wavelength plots for phenolphthalein in the indicated solvents (% propan-1-ol in water) for the 256 to 262 nm region.

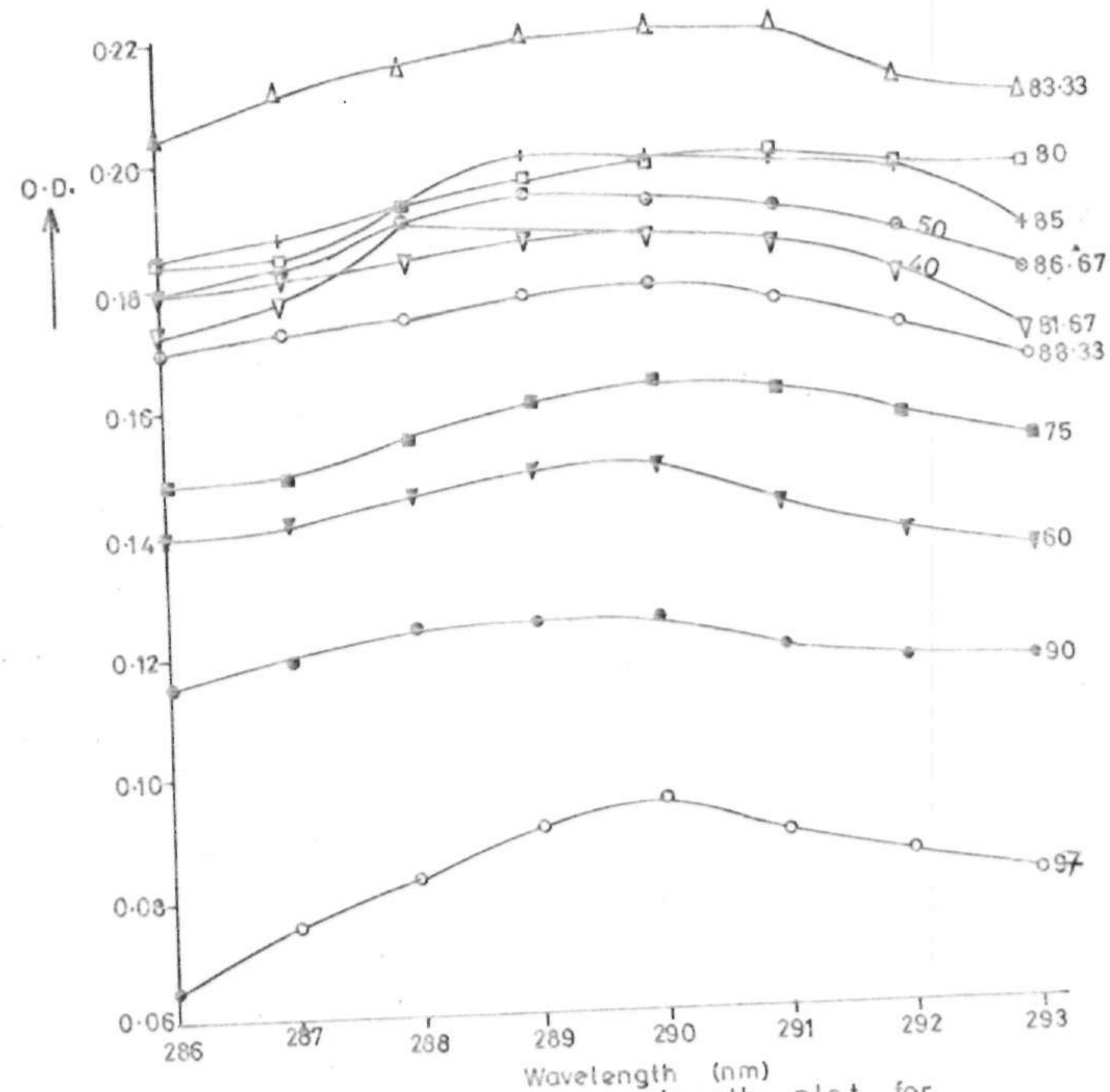


Fig. 16 Absorbance versus wavelength plot for phenolphthalein in the indicated solvent systems (% propan-1-ol in water) in the 286 to 293 nm region

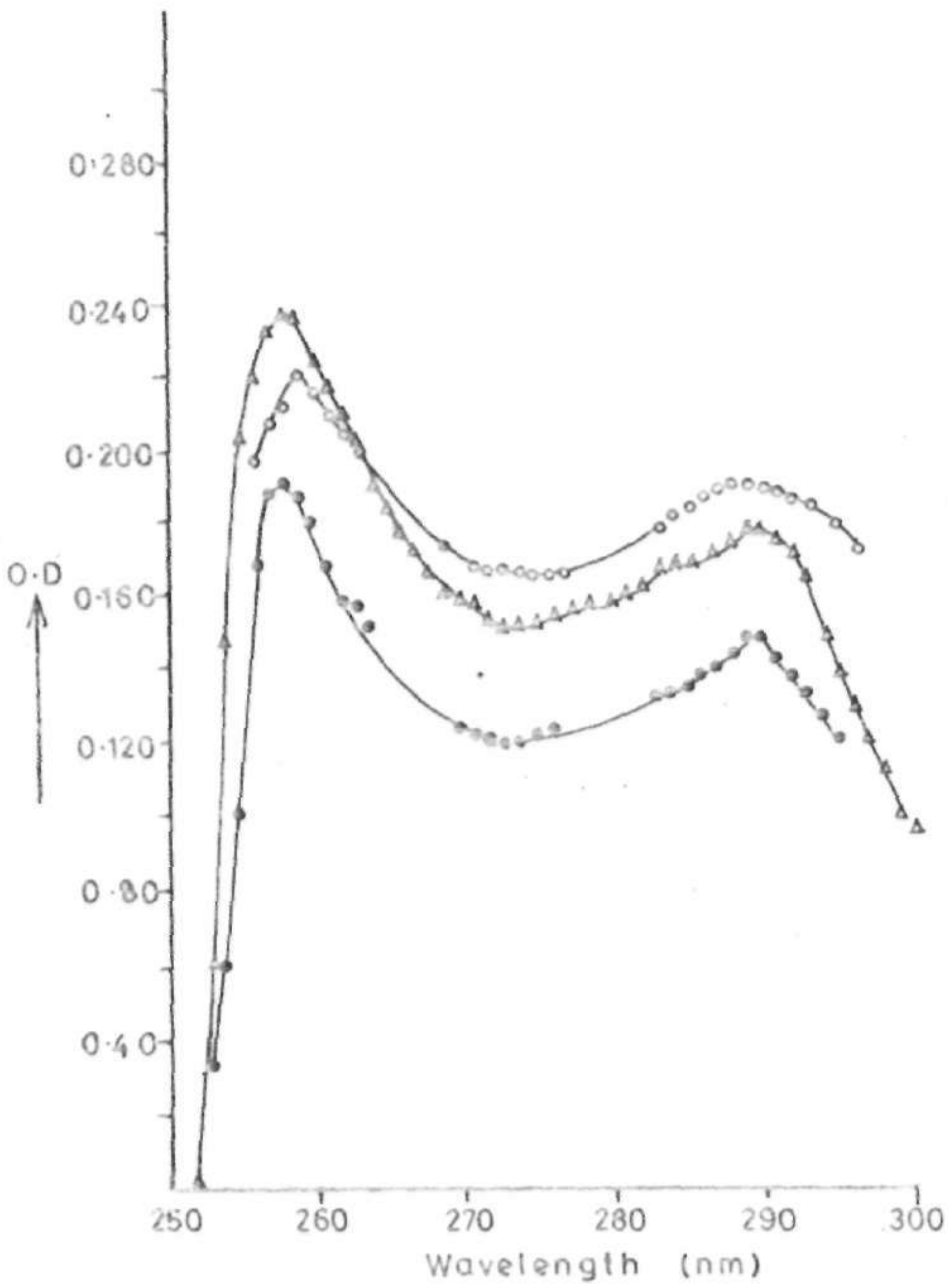


Fig. 17 Absorbance versus wavelength plot for phenolphthalein in the 250-300 nm region for selected solvents (% propan-1-ol in water)

Δ = 88·33%, ◉ = 60%, ○ = 50%

(Fig. 18). Fig. 19 is a graph of absorbance agasint solvent composition in the 288 to 291 nm region and Fig. 20 represents the absorbance versus solvent composition plot for the 257 to 259 nm region.

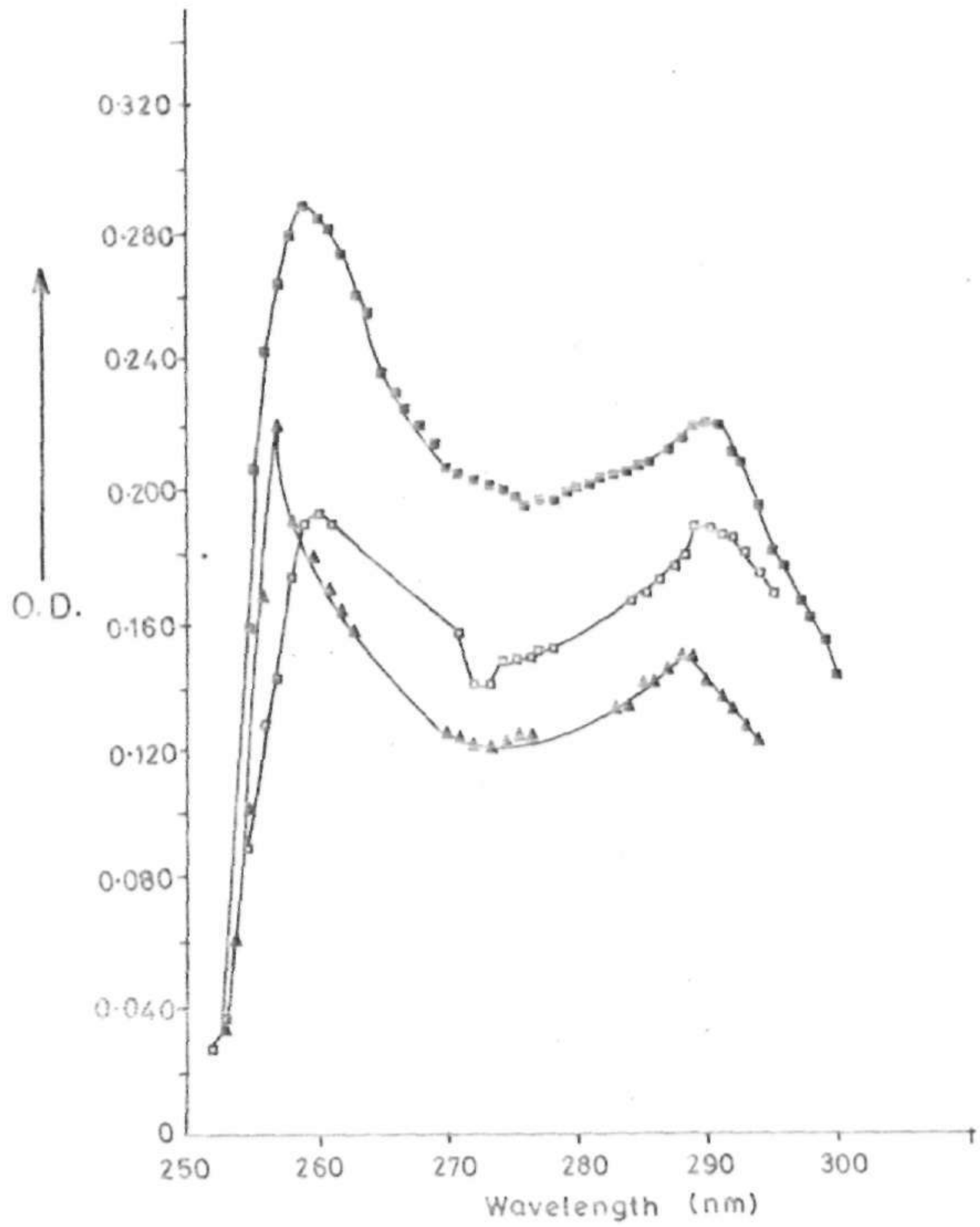


Fig. 18 Absorbance versus wavelength plot for phenolphthalein in selected solvents for the 250-300 nm region

■ = 83⅓%, ▲ = 60% □ = 40%

λmax for 83·33% propan-1-ol = 258 nm, 290 nm
λmax for 60% propan-1-ol = 257 nm, 288, 289 nm
λmax for 40% propan-1-ol = 259 nm, 289 nm

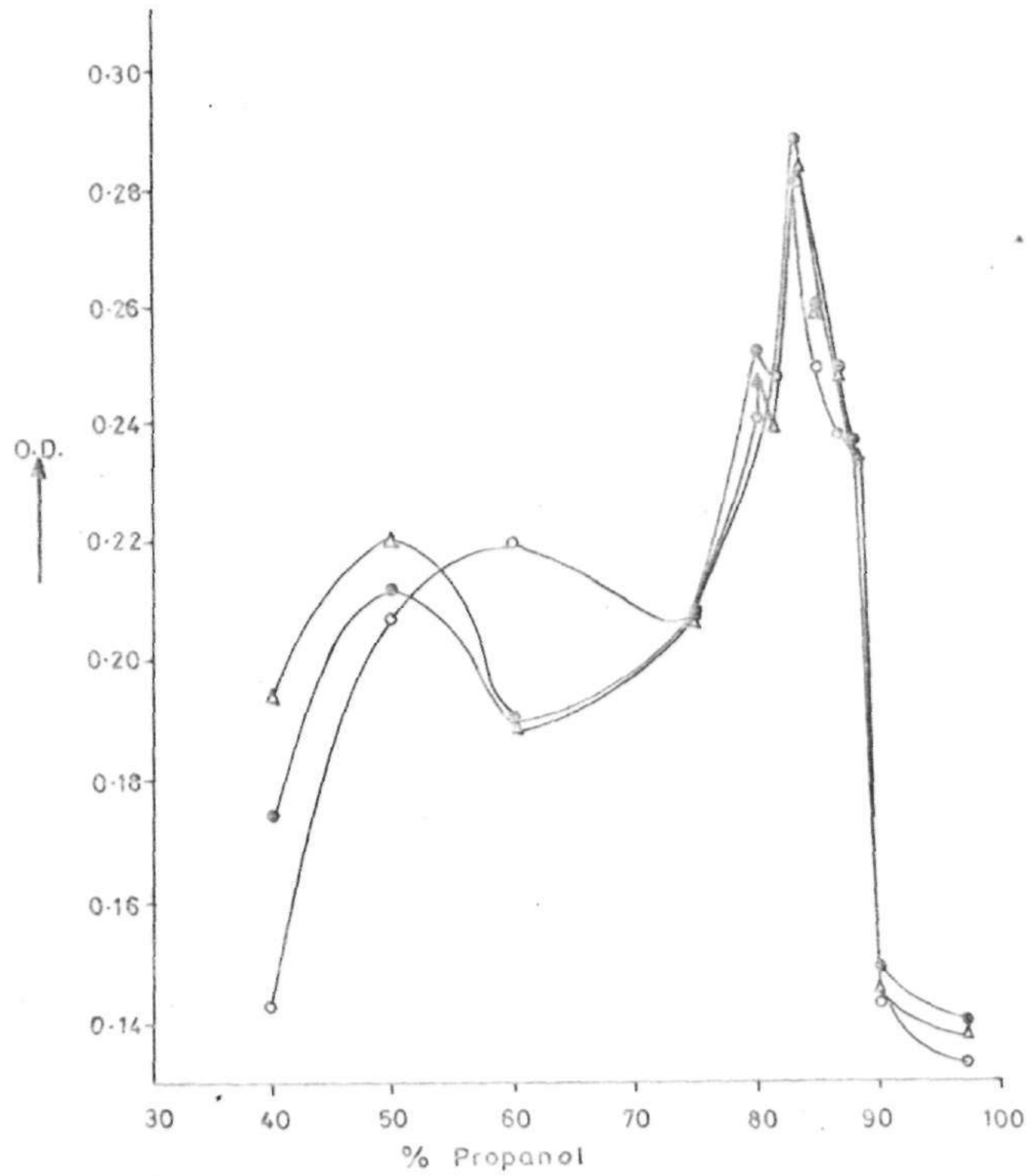


Fig. 19 Absorbance versus solvent composition at selected wavelengths for phenolphthalein

o = 257% • = 258%, Δ = 259%

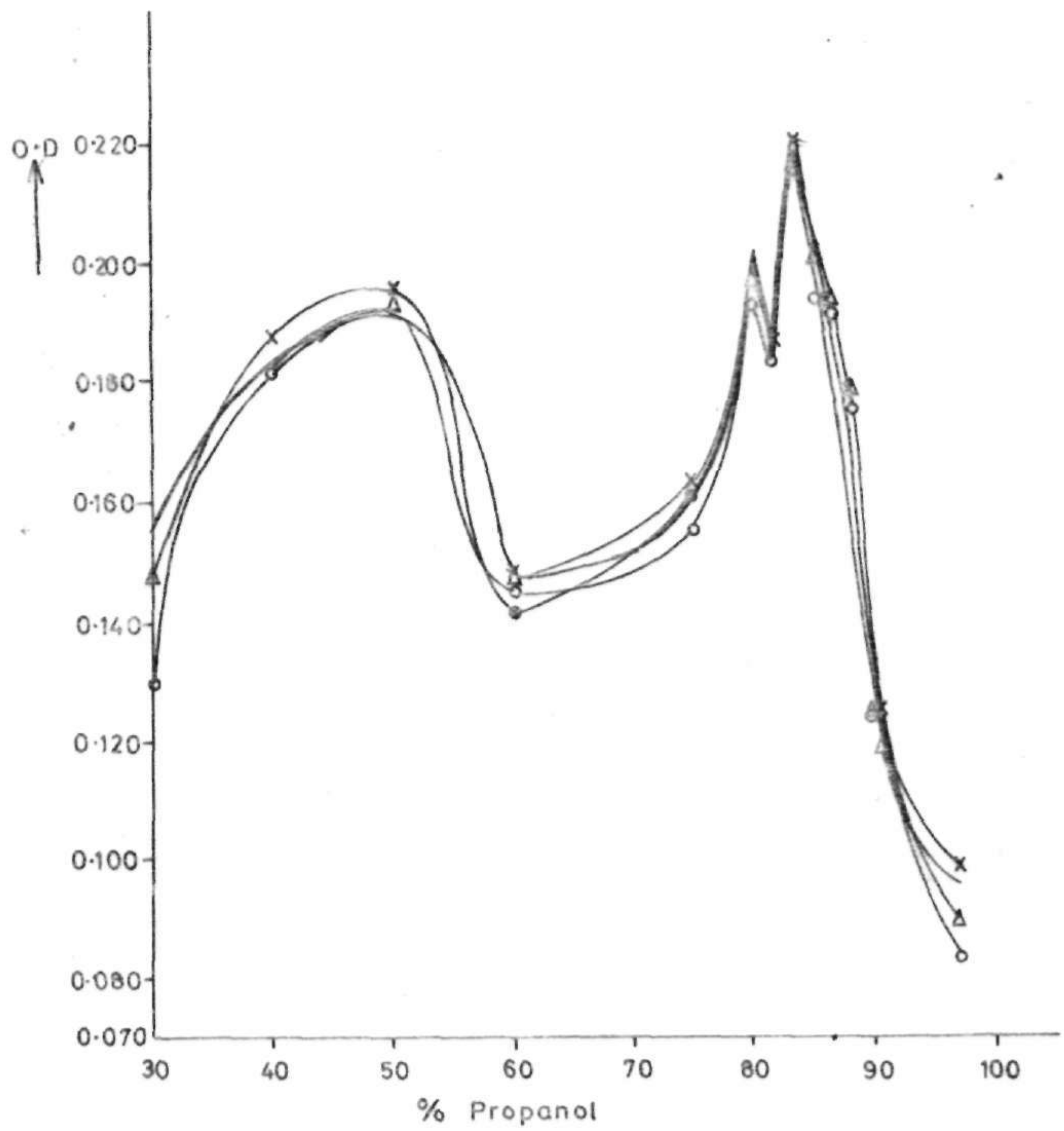


Fig. 20 Absorbance versus solvent composition plot at selected wavelengths for phenolphthalein

• = 288 nm, Δ = 289 nm
x = 290 nm
o = 291 nm

CHAPTER FOUR

DISCUSSION

4.1 THE SPECTROSCOPIC EXPERIMENTS

The absorbance of the solution of phenolphthalein of strength 20 $\mu g/cm^3$ in propan-1-ol at 290 nm wavelength was 0.222 and at 258 nm it was 0.315 (Fig. 14). The absorbances remained at these values at the respective wavelengths on change in solvent composition. Phenolphthalein is not a surfactant and it is reasonable to assume that in propan-1-ol at a concentration of 20 μg per cm^3 it exists as monomers.

The absorbance of a solution of phenolphthalein of strength 20 μg per cm^3 in 83.33 % v/v propan-1-ol in water solvent containing 12 mg of lecithin per cm^3 of solution, was 0.286 at 258 nm (Fig. 15) and 0.220 at 290 nm (Fig. 16). The lecithin was employed at this Concentration for all the kinetic expeeiments. For these runs the absorbances were measured using a blank containing the same concentration of lecithin employed in the kinetic experiments but the phenolphthalein was omitted.

Since the absorbance of the phenolphthalein in the presence of lecithin in the 83.33 % v/v propan-1-ol in water Solvent (figs 15 and 16) is very close to

Elworthy and McIntosh (1964) in their investigation of the effect of solvent dielectric constant on the solution form of the lecithin reported that monomers of lecithin were formed in 93.4 % v/v ethanol in water corresponding to a dielectric constant of 29.0. At concentrations of ethanol higher than 93.4 % v/v in water, inverted micelles of egg lecithin were reported. They also reported that as the concentration of ethanol increased beyond 93.4 % v/v ethanol in water, the aggregation number of the lipid in the reversed micelles also progressively increased and the same trend was observed as the water content increased above that of the solvent composition in which monomers were formed. These findings are consistent with those of Elworthy (1959a) who reported the existence of 'little' inverted micelles of lecithin in benzene. Dervichian (1964) however indicated that the micelles formed in benzene are quite large.

From the work of Akerlof (1932) the dielectric constant of the 83.33 % v/v propan-1-ol in water solvent is 27. Consequently the presence of monomers of lecithin at the spectroscopic peak is consistent with the results of Elworthy and McIntosh (1964). At propan-1-ol in water solvent compositions lower than the spectroscopic peak of 83.33 % v/v propan-1-ol in water, normal micelles in equilibrium with monomers of lecithin exist. At propan-1-ol concentrations higher than the kinetic peak, reversed micelles in equilibrium with monomers of lecithin exist.

From Fig. 19, it is obvious that a number of peaks appear for the absorbances observed between 257 and 259 nm in the various solvents. There is a peak in 60 % v/v propan-1-ol in water solvent system at 257 nm and for the three wavelengths, peaks are observed in the 83.33 % v/v propan-1-ol in water solvent. However both at 258 and 259 nm, a peak is observed in 50 % v/v propan-1-ol in water. A small peak also appears in 80 % v/v propan-1-ol in water for 258 and 259 nm. Moreover in the 288 nm to 291 nm region, three peaks appear over the entire range of solvent composition. (Fig. 20). Peaks are observed for the four wavelengths in the 50 % v/v propan-1-ol in water solvent though the peak in the 60 % v/v propan-1-ol in water solvent has disappeared (cf. Fig. 19). A peak is also observed in 80 % v/v propan-1-ol as is the case for the 83.33 % v/v propan-1-ol in water solvent system. A particular problem arises as to how these peaks should be understood in terms of the presence or absence of aggregates of lecithin.

From Fig. 15, it is evident that there is a change of 2 nm units in the λmax value between the 60 % v/v and 50 % v/v propan-1-ol in water solvent. The red shift can be attributed to the increasing polarity of the environment in which the phenolphatlein is solubilized. Since this observation is consistent with that from Fig. 16 where a slight blue shift exists between 60 % v/v and 50 % v/v propan-1-ol but a slight red shift exists between 50 % v/v and 40 % v/v propan-1-ol, it would seem that the phenolphathlein has a special orientation on the micelle surface. This observation is consistent with that of Burawoy (1930) who in his study of the effect of solvent polarity on the absorption of some aldehydes, ketones and thioketones noted the presence of a short wavelength high intensity band that changed to lower wavelengths as the solvent decreased in polarity C.F. Fig. 15). In addition, Burawoy (1930) noted a change of 2 nm for the absorption maximum of acetone on changing the solvent from methanol to ethanol. The maximum for absorption in the 40 % propan-1-ol solvent also occured at the same wavelength as did the 50 % v/v propan-1-ol solvent system (Fig. 15) but the absorbance value for the former was lower than that for the latter. This is an indication that the phenolphthalein is being partitioned into the micelle interior as solvent polarity increases in this region.

For the longwavelength short intensity band (Fig. 16) there is a shift of 3 nm in changing the solvent composition from 80 % v/v propan-1-ol to 40 % v/v propan-1-ol. This blue shift, as well as the red shift in the 257 to 259 nm region, is consistent with the Frank Condon rule (Fleming and Williams 1966) and with the results of Burawoy (1930). Strangely however, the absorbance in 50 % v/v propane1-ol though lower than that in 80 % v/v propan-1-ol is higher than those in 97 % v/v, 90 % v/v, 75 % v/v and 60 % v/v propan-1-ol (Figs. 15 and 16). In view of these observations, it appears that the lecithin exists as loose aggregates between 80 % v/v and 60 % v/v propan-1-ol. Below 60 % v/v propan-1-ol however, the phenolphthalein which appeared to have been concentrated on the micelle surface between 80 % v/v and 60 % v/v propan-1-ol is desorbed into the bulk solution and the remaining micellar structure rigidifies as a consequence. The increase in absorbance on changing the solvent from 60 % v/v to 50 % v/v propan-1-ol and the decrease in absorbance in changing the solvent still further to 40 % v/v propan-1-ol (Figs. 15, 16 and 17), where the 60 % v/v maximum at 257 nm (Fig. 19) has been left out as an aberration, are consistent with these inferences. The lower absorbances observed in 97 % v/v and 90 % v/v propan-1-ol would tend to indicate that the phenolphathalein is highly adsorbed in the interior of the inverted micelles present in these solvents.

However very small peaks are observed in 80 % v/v propan-1-ol at 258 and 259 nm and between 288 to 291 nm (Figs. 16 and 17). In view of the existence of monomers in 83.33 % v/v propan-1-ol, the peak in 80 % v/v propan-1-ol can be attributed to the formation of a phenolphathalein-lecithin complex. In view of the fact that only in the 83.33 % v/v propan-1-ol in water solvent were unassociated molecules of the lipid present, it appears reasonable to assume that a few molecules of lecithin are associated with a molecule of phenolphthalein in the 81.67 % v/v propan-1-ol in water solvent to form a spectroscopically less exictable complex. Fig. 8 in conjuction with Figs. 16 and 17 bear out this fact.

By relating these inferences to the kinetic data it can be said that in 85 % v/v propan-1-ol in water, lecithin exists as monomers. Reversed micelles of the lipid are present in propan-1-ol concentrations higher than 85 % while in lower compositions of the propan-1-ol, normal micelles exist.

4.2 THE KINETIC EXPERIMENTS

The kinetic behaviour of lecithin under the experimental conditions is divided into four portions (Fig. 8). There is first the portion between 97 % v/v propan-1-ol and the region of the kinetic peak; secondly there is the kinetic peak region; thirdly there is the portion between 60 % v/v propan-1-ol and the region of the kinetic peak and lastly there is the region below 60 % v/v propan-1-ol in water. For the first portion between 97 % v/v propan-1-ol and 90 % v/v propan-1-ol, a fairly constant rate of reaction is exhibited. In consistence with the observations of Elworthy and McIntosh (1964) and Dervichian (1964) this portion contains reversed or inverted micelles in equilibrium with monomers. In view of the low absorbance recorded in this region for the spectroscopic runs (Figs. 15 and 16), it is reasonable to assume that the micelles formed in this region are quite large. This also accounts for the low rate constants recorded for these two solvent compositions especially since the alkali would be partitioned into the bulk solvent. In 85 % v/v propan-1-ol in water monomers of lecithin exist. The high rate constant is consistent with a lack of any hinderance to the attack of the lipid by the base. Between 80 % v/v and 85 % v/v propan-1-ol in water small normal aggregates of lecithin predominate. In view of the small number of monomers present in the aggregates, it is reasonable to infer that the aggregates occur in equilibrium with a high concentration of monomers. Between 80 % v/v and 60 % v/v propan-1-ol in water loose floccules of lecithin, normally oriented, exist in equilibrium with a fairly high concentration of monomers of the lipid. Below 60 % v/v propan-1-ol, the floccules rigidify so that aggregates with an increasing lipophilic core result.

4.2.1 A CONSIDERATION OF THE MODEL WITH RESPECT TO REACTION ORDER

As is evident from Fig. 1, none of the results show constant $t_{\frac{1}{2}}$ values. This implies a clear absence of first order behaviour. In addition, it can be concluded that most of the graphs show two distinct portions. However this distinction is absent in some of the graphs e.g. in 97 % v/v, 90 % v/v and 10 % v/v propan-1-ol in water solvent runs. The plots obtained for the above solvent compositions could not be very clearly distinguished into two separate portions. However it is obvious from a comparison of Fig. 10 with Fig. 9 that the data obtained in 85 % v/v propan-1-ol in water clearly complies with consecutive second order behaviour.

Outside the region of the kinetic peak especially in n-propanol concentrations from 88.33 % v/v in water and above, there is reason to assume the lack of compliance with consecutive second order behaviour. The changes in the calculated values of the concentration of the glycerylphosphorylcholine over time were inconsistent. In 97 % v/v, 90 % v/v, 88.33 % v/v and 80 % v/v propan-1-ol in water, decreases in the calculated concentrations of the glycerylphosphorylcholine were noticed as time progressed. This is unexpected in irreversible consecutive reactions. It would appear that in these solvents, the rate of reaction decreases over time. The decrease is most probably due to a change in the aggregation form and number of the lipid as the reaction progresses.

For the other solvent systems however, estimates of both the first stage and the second stage rate constants have been made (tables 3:1 and 3:2). Hence the model employed for the reaction in this work (page 31) has been a very useful guide.

4.2.2 SOLVENT EFFECT ON THE KINETICS OF THE REACTION:

On the basis of the work by Murto (1962) as reported earlier and the slightly higher nucleophilicity of the propoxide ion relative to the hydroxyl ion, one would assume that in 50 % v/v propan-1-ol/water solvent composition, the propoxide ion concentration is about a third that of the hydroxide ion concentration. The higher nucleophilicity of the propoxide ion does not counteract the basic assumption in the above statement that the propoxide and ethoxide ions show similar nucleophilicity. Hence on purely theoretical grounds one would on the basis of the reported data

$$\bar{O}H + EtOH \longrightarrow H_2O + EtO^- \quad K = 0.66$$
$$\bar{O}H + MeOH \longrightarrow H_2O + MeO^- \quad K = 4.5$$

expect a decreasing reaction rate with increasing water content of the reaction system. However due to the obvious presence of associated structures, deviations arise in view of the differences in the partition coefficients of the propoxide and hydroxide ions between the continuous phase and the dispersed phase.

This is however not the only fact to consider as regards solvent composition. There is a change in dielectric constant as the water content of the solvent increases. This increase in solvent dielectric constant should result in an increased rate of reaction.

The relatively constant values obtained for the rate constant in the regions 97 % v/v to 90 % v/v and 80 % v/v to 60 % v/v propan-1-ol in water may be attributed to the fact that though micellar sizes might vary within these regions, the variations appear to be slight. In addition, it appears that micellar size has no significant effect on reaction rate. The anticipated change in micellar size would be consistent with the observation of Elworthy and McIntosh (1964) which would also explain the various regions from Fig. 8.

Even though no data is available on the influence of the solvent composition on the solution form of the lysolecithin, it would seem, in view of its higher hydrophilicity, that the peak observed in the 81.67 % v/v solvent (table 3:2) was obtained in a solution containing monomers of lysolecithin. Above the 81.67 % v/v propan-1-ol in water solvent system, inverted micelles of lysolecithin predominate and below this solvent composition normal micelles appear to predominate in equilibrium with monomers of the lysolecithin. The lower values of the rate constants for the lysolecithin appear to be due to two reasons. First is the ionization of the secondary or primary alcoholic function of lysolecithin or glycerylphosphorylcholine due to the fairly high base concentration encountered in the kinetic experiments. Banks (1978) has estimated a pKa value of about 13 for the secondary alcoholic functional group of lysolecithin. The ionization of the alcoholic function would lead to a net negative charge on the lysolecithin molecules and on the various molecular aggregates formed. The net negative charge would lead to an electrostatic repulsion of the base from the molecules and aggregates resulting in a decreased rate of reaction. It is interesting to note that the lesser rate of reaction for the lysolecithin has been recorded inspite of the fact that the lysolecithin exhibits a lower aggregation number than the lecithin. This is due to the higher hydrophilicity of the lysolecithin, and the fact that a relative catalytic effect seems to be operative in micellar lecithin.

4.2.3 COMPARISON OF THE KINETIC DATA FROM THE CURRENT WORK WITH THOSE FROM PREVIOUS WORKS.

The graph of rate constant against solvent composition presented in Fig. 8 shows a flattening between 50 % v/v and 80 % v/v propan-1-ol in water.

A peak is observed in the region between 80 % v/v and 90 % v/v propan-1-ol in water. The values of the apparent second order rate constants range from 2.856×10^{-2} to $83.36 \times 10^{-2}\ dm^3 mole^{-1} s^{-1}$ between 10 % v/v and 97 % v/v propan-1-ol in water.

Using the data from previous works, a calculation of the apparent second order rate constants has been done. The results obtained are presented in Table 4:1. As can be observed from this table the apparent second order rate constants range from $1.4 \times 10^{-3}\ dm^3 mole^{-1} s^{-1}$ to $74.1 \times 10^{-2}\ dm^3 mole^{-1} s^{-1}$. From the work of Elworthy and McIntosh (1964), it is highly likely that in Dawson's largely ethanolic solvent (Fig. 8) monomers in equilibrium with a very low concentration of dimers and perhaps trimers would have predominated. This would be consistent with his rate constant of $74.1 \times 10^{-2}\ dm^3 mole^{-1} s^{-1}$ as compared to the value of $86.36 \times 10^{-2} dm^3\ mole^{-1} s^{-1}$ obtained for 85 % v/v propan-1-ol in water (table 3:1) in the present work. Hence the results from the present work are in reasonable agreement with those from previous works.

Problems arise however, with the results of Baer and Kates (1959) and Marriott (1969). Noting that Baer and Kates' solvent consisted of 8 % v/v carbon tetrachloride, 10 % v/v water and 82 % v/v methanol, one would expect that synthetic lecithin would exist

Table 4.1 Rate constants obtained by previous workers

Reference	Substrate	Temperature	Base concentration (molar)	Solvent composition % v/v	Pseudo first order constant s^{-1}	Lipid concentration (molar)	Apparent second order rate constant $dm^3 moles^{-1}$
Baer and Kates (1950)	Lecithin (synthetic dipalmitoly compound)	37	Sodium hydroxide 2×10^{-1}	Cabon tetrachloride ...8 Water..10 methanol . . 82	2.8×10^{-4}	2.04×10^{-2}	3.4×10^{-3}
Marrinetti (1962)	Lecithin (natural compound)	37	Sodium methoxide 1.7×10^{-1}	Chloroform . .67 methanol ...33	2.4×10^{-2}	6.4×10^{-3}	1.412×10^{-1}
Hauser and Dawson (1967)	Lecithin monolayer	37	Sodium alkoxide 1.7×10^{-1}	Water .100	3.7×10^{-4}	-	2.2×10^{-3}
Marriott (1969)	Natural lecithin	35	Potassium sodium and lithium hydroxides 1.8×10^{-1}	Chloroform ..4 methanol .70-92 Water. 4-26	$4.7 - 8.2 \times 10^{-4}$	$0.8 - 1.1 \times 10^{-3}$	3.6×10^{-3}
Alexander and Rideal (1937)	Dipalmitin monolayer	37	1.7×10^{-1}	Water. 100	5.0×10^{-4}	-	3.6×10^{-3}
Jellinek and Gordon (1951)	1-monostearin		1.7×10^{-1}	Water .. 25 Acetone. 75	7.1×10^{-2}	-	4.176×10^{-1}

(Contd')

Table 4.1 Rate constants obtained by previous workers

Reference	Substrate	Tempe-rature	Base concentra-tion(molar)	Solvent composition % v/v	Pseudo first order constant s^{-1}	Lipid concentraticn (molar)	Apparent second order rate constant $dm^3 mol^{-1} s^{-1}$
Banks (1978)	Lecithin (synthetic)	37	Sodium potassium and lithium hydroxides 1.7×10^{-1}	Propan-1-ol 1-ol .. 50 Water . 50	2.7×10^{-2}	$6 - 8 \times 10^{-4}$	1.588×10^{-1}
Dawson (1966)	Natural lecithin	37	Sodium hydroxide 2.7×10^{-2}	Ethanol .. 82 Carbon tetrachloride. 8.7 water .9.8	2×10^{-2}	2×10^{-3}	74.1×10^{-2}

as monomers and hence a far higher rate of reaction would have been expected. The inconsistence is worsened by the fact that there was a decrease of at least 24 % w/v in the base concentration during the reaction and hence the reactions did not comply with pseudo first order conditions. Secondly Baer and Kates assumed that the reaction occurred in one step. In view of the largely methanolic solvent employed by Marriott (1969) (table 4:1 page 94) one would expect fairly large aggregates of the natural compound in his reaction system and hence a rate constant about three hundred times greater than that reported. Marriott too, assumed that the reaction was one step. In view of the rate constant value reported and the observation that the $t_{\frac{1}{2}}$ value for the deacylation of lecithin and lysolicithin were quite close (Marriott, 1969), it would seem that the author employed an impure sample of the egg lecithin.

As for the rate constant for the deacylation of lysolecithin, no data is available except that of Banks (1978). He obtained an apparent second order rate constant of about 1.588×10^{-1} $dm^3 mole^{-1} s^{-1}$. From the present work the rate constant in 50 % v/v propan-1-ol in water, was 1.19×10^{-2} $dm^3 mole^{-1} s^{-1}$ which is about fourteen times less than that reported by Banks who also used the same solvent system. Since Banks employed egg lysolecithin, it is difficult to understand the lack of agreement between the current work and his.

The results as reported by Hauser and Dawson (1967) and Alexander and Rideal (1937) are inconsistent with those obtained from the present work. This is not suprising in view of the fact that no work has been carried out in pure water in this present work.

It is evident from the results that an inhibitory effect on the rate of reaction appears to be operative in the lecithin micelles. However from the present data, it can be infered that the effect of the inhibition varies from one solvent system to the other and the precise mechanisms involved might differ in the various solvent systems.

CHAPTER FIVE

CONCLUSION

From the kinetics of the deacylation of lecithin and the spectroscopic experiments, the following conclusions can be reached -

1. In view of the spectrum obtained in the spectroscopic experiments, lecithin at a concentration of 1.5×10^{-2}M exists as inverted or reversed micelles in propan-1-ol in water concentrations higher than 85 % v/v. In 85 % v/v propan-1-ol in water solvent system lecithin forms a solution containing monomers of the lipid. In propan-1-ol in water concentrations lower than 85 % v/v, lecithin forms normal micelles

2. Since the solution form of the lipid depends on the solvent composition and since this itself is closely related to the dielectric constant, micellization of lecithin in propan-1-ol in water systems can be said to depend on the dielectric constant of the solvent.

3. That the rate constant for the deacylation of lecithin varies with the composition of the propan-1-ol in water solvent system and hence with the solution form of the lipid. The rate constant for the deacylation process is maximal in 85 % v/v propan-1-ol for which it's value is 8.636 $dm^3 Mole^{-1} S^{-1} \times 10^{-1}$ for the deacylation of lecithin. The rate constant then decreases as the percentage of

propan-1-ol in the solvent increases. Below 85 % v/v propan-1-ol in water solvent composition, a decrease in the rate constant is observed.

4. The rate constants for the deacylation of lysolecithin are less than those of lecithin in the corresponding solvents. This is inconsistent with the observation of Banks (1978).

5. In view of the fact that no work was done on solvent compositions higher than 97 % v/v propan-1-ol and less than 10 % v/v propan-1-ol in water, further investigation in these solvent regions could be helpful.

6. Since the spectroscopic experiments show interesting but inconclusive results in the region of solvent composition between 80 and 81.33 % v/v and 50 - 60 % v/v propan-1-ol in water, further spectroscopic work should be carried out to investigate these regions.

7. In view of the amphiphilic nature and complete non-toxicity of lecithin and the micellization which it undergoes in various solvents, lecithin aggregates in water and various other solvents could be useful for

   i) The solubilization of poorly water soluble drugs like phenobarbitone, vitamin A and the oil soluble vitamins and many other drugs.

ii) stabilization of drugs susceptible to degradation in water like vitamin A and various non-water stable esters and amides due to a micellar inhibitory effect on the degradation reaction.

iii) the preparation of artificial membranes for the invitro study of various biopharmaceutic drug characteristics.

iv) preparation of some emulsions and creams.

APPENDIX

TABLES SHOWING THE CONCENTRATION OF SODIUM HYDROXIDE AGAINST TIME FOR THE VARIOUS SOLVENT SYSTEMS EMPLOYED

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
97	0	3.00
	4.5	2.21
	10.5	2.02
	16	1.90
	22	1.89
	30.33	1.73
	40.5	1.67
	60	1.56
	90	1.34
	120	1.05
	183	0.88
	240	0.72
	360	0.24
90	0	3.00
	2.5	2.21
	6.167	1.78
	11	1.61
	14.50	1.58
	21	1.43
	30.75	1.33
	45	1.20
	60.167	0.93
	120	0.87
	240	0.18

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
88.33	0	3.00
	+ 2.50	2.21
	5.00	1.85
	6.167	1.78
	11	1.61
	14.50	1.58
	21	1.43
	30.75	1.33
	45	1.20
	60.167	0.93
	120	0.87
	240	0.18
86.67	0	3.00
	+ 2.00	1.93
	5.00	1.61
	10.00	1.39
	15.00	1.31
	20.00	1.25
	30.00	1.19
	40.00	1.18
	61.00	1.14
	120.00	0.87
85.00	0	3.00
	+ 2.0	1.69
	4.83	1.53
	10.25	1.43
	20.33	1.28
	25	1.20
	30.83	1.18
	40.33	1.06

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
	50.42	1.02
	60	1.00
	90.42	0.85
	153	0.73
	185	0.54
	240	0.51
	360	0.21
83.33	0	3.00
	+ 1.00	2.31
	5.00	1.71
	10.00	1.40
	20.00	1.24
	40.00	1.14
	60.00	1.07
	80.00	1.03
	90.00	1.01
81.67	0	3.00
	+ 2.00	2.10
	4.33	1.66
	15.00	1.27
	21.33	1.17
	31.42	1.[illegible]5
	60.00	0.91
	128.00	0.79
	162.00	0.77
80.	0	3.00
	2.00	2.22
	5.00	1.61
	10.00	1.49
	17.20	1.38
	22.30	1.27

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
	50.42	1.02
	60	1.00
	90.42	0.85
	153	0.73
	185	0.54
	240	0.31
	360	0.21
83.33	0	3.00
	+ 1.00	2.31
	5.00	1.71
	10.00	1.40
	20.00	1.24
	40.00	1.14
	60.00	1.07
	80.00	1.03
	90.00	1.01
81.67	0	3.00
	+ 2.00	2.10
	4.33	1.66
	15.00	1.27
	21.33	1.17
	31.42	1.[illegible]5
	60.00	0.[illegible]1
	128.00	0.79
	162.00	0.77
80.	0	3.00
	2.00	2.22
	5.00	1.61
	10.00	1.49
	17.20	1.38
	22.30	1.27

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
40	0.00	3.00
	+3.00	2.40
	6.00	1.94
	10.00	1.66
	15.00	1.48
	20.00	1.37
	30.00	1.22
	35.00	1.18
	41.10	1.01
	60.40	0.83
	91.00	0.82
	138.00	0.75
	180.00	0.71
	300.00	0.58
	420.00	0.37
	540.00	0.14
30	0.00	3.00
	3.00	2.55
	7.50	1.96
	10.00	1.89
	15.33	1.61
	27.67	1.46
	35.00	1.29
	45.00	1.18
	50.00	1.03
	75.00	0.91
	100.00	0.85

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
	25.[illegible]0	1.24
	32.50	1.23
	40.00	1.1[illegible]
	61.10	1.03
	92.00	0.80
	123.00	0.64
	180.00	0.5[illegible]
	240.00	0.54
	300.00	0.49
	360.00	0.26
75	0.00	3.00
	+ 2.00	2.31
	4.50	1.75
	8.50	1.57
	14.05	1.24
	20.00	1.23
	30.33	1.22
	63.17	1.11
	120.00	1.01
	200.00	0.75
	260.00	0.65
	312.00	0.4[illegible]
	380.00	0.35
	540.00	0.05

Percentage Propan-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
60	0.00	3.00
	+ 2.50	2.25
	5.00	1.89
	10.00	1.56
	15.00	1.37
	20.00	1.25
	25.00	1.17
	39.50	1.08
	52.00	0.99
	71.00	0.92
	92.33	0.80
	123.00	0.73
	180.00	0.37
	240.00	0.35
	360.00	0.17
	420.00	0.08
50	0.00	3.00
	+ 2.50	2.42
	5.00	2.02
	10.00	1.65
	15.00	1.48
	20.00	1.37
	25.00	1.28
	30.00	1.22
	40.00	1.15
	60.00	0.98
	97.40	0.74
	120.00	0.51
	210.00	0.50
	376.00	0.22
	480.00	0.04

Percentage Propen-1-ol v/v	Time in minutes	Molar concentration of hydroxide $\times 10^{-2}$
20	0.00	3.00
	+ 2.00	2.63
	10.00	2.43
	20.00	2.20
	40.00	1.96
	50.00	1.91
	60.00	1.85
	80.00	1.77
	90.00	1.75
	100.00	1.64
	150.00	1.46
	200.00	1.36
	250.00	1.30
	300.00	1.25
	450.00	1.16
	550.00	1.13
10	0.00	3.00
	5.00	2.68
	10.00	2.66
	15.00	2.50
	20.00	2.44
	40.00	2.42
	60.00	2.415

+ Values of concentration read off from the concentration versus time plot.

REFERENCES

Akerlof G. (1932), Journal of the American Chemical Society, Vol. 54, pp. 4125 - 4139.

Alexander A. E., Rideal E. K. (1937), Proceedings of the Royal Society, Series,A, vol. 163, pp 70 - 89.

Baer E., Kates M. (1948), Journal of Biological Chemistry, vol. 175, pp. 79 - 88.

Baer E., Kates M. (1950), Journal of Biological Chemistry, vol. 185, pp. 615 - 625.

Bangham A. D., Dawson R. M. C. (1959), Biochemical Journal, vol. 720, pp. 486 - 492.

Bailly O., Gaumme J. (1926), Journal de Pharmacie et de Chimie, vol. 22, pp. 793 - 795.

Bailly O., Gaumme J. (1935), Journal de Pharmacie et de Chimie vol. 22, pp. 22 - 32.

Banks F. B. (1978), "The Kinetics of the deacylation of Phospholipids under alkaline conditions". Unpublished Ph.D. dissertation School of Pharmacy, Liverpool Polytechnic.

Banks F. B. (1983), Journal of Chromatography, vol. 254, pp. 247 - 255.

BDH Laboratory Catalogue (1981 - 1982), Export Edition, pp. 276.

Bloor W. R. (1943), Biochemistry of fatty acids and their compounds the lipids, Reinhold publishing corporation, New York through E.R.M. Kay (1966), Biochemistry (An Introduction to Dynamic Biology), The Macmillan Company, New York, pp. 113 - 114.

Burawoy A. (1930), Berichte der bunsen gesellschaft fur physikalische Chemie, vol. 63, pp. 3155 through Jaffe' H.H. Orchins M. (1964), Theory and Application of Ultraviolet Spectroscopy, John Wiley and Sons, Inc. New York, pp. 186.

Charlot G., Tremillion B. (1969), Chemical Reactions in Solvents and Melts, Pergamon press, Oxford, pp. 273 - 297.

Davies J. T., Rideal E. K. (1963), Interfacial Phaenomena, Academic press, Chapter 6.

Dawson R. M. C. (1954), Biochimica et Biophysica Acta, vol. 14, pp. 374 - 379.

Dawson R.M.C. (1960), Biochemical Journal, vol. 75, pp. 45 - 53

REFERENCES

Dawson R.M.C. (1966), Biochemical Journal, vol. 98, vol. 35c - 37c.

Dervichian O.G. (1964), Progress in Biophysics and Molecular Biology, vol. 14, pp. 263 - 342.

Diakanov (1867), Hoppe- Seyler. Med. Chem.. Untersuch, vol. 2, pp. 221 through Elworthy P.H., Sauders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1001 - 1,081

Diakanov (1868 a), Ibid, vol. 3, pp. 405 through Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1001 - 1,081

Diakanov (1868 b), Zentr. Med. Wissench, vol. 2, pp. 434 th through Elworthy P. H., Saunders L. (1956), Journal of Pharmacy and Pharmacology vol. 81, pp. 1001 - 1,081.

Elworthy P. H. (1959 a), Journal of the American Chemical Society, vol. 81, pp. 813 - 817.

Elworthy P.H. (1959 b), Journal of the American Chemical Society, vol. 81, pp. 1951 - 1956.

Elworthy, P.H. (1960), Journal of the American Chemical Society, vol. 82. pp. 139 - 144.

Elworthy P.H., McIntosh D. S., (1964), Kolloid Zeitschrift und Zeitschrift Polymere, vol. 195, pp. 27 - 34.

Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1001 - 1,081.

Fendler, J. H. (1976), Accounts of Chemical Research, vol. 9, pp. 153 - 161.

Fishegold and Chain (1935), Proceedings of the Royal Society series B., vol. 117, pp. 239 through Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1001 - 1,081.

Franks F., Ives D. J. G., (1960), Quarterly Review, (London), vol. 40, no. 1, pp. 1 - 41.

Frost A. A., Schwemer W. G., (1952), Journal of the American Chemical Society, vol. 74, pp. 1268 - 1273.

Fujiwara Y., Masuoka M., Urakami C. (1967), Bulletin of the Chemical Society, Japan, vol. 40, no. 1, pp. 230 - 231.

Furth, (1923), Ann. Physik. vol. 70, pp. 63 through Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1001 - 1,081.

Garrett E. R. (1967), Advances in the Pharmaceutical Sciences, Academic Press, vol. 2, Chapter 1, pp. 1 - 94.

REFERENCES

Gerrard W., Macklen E. D., (1959), Chemical Review, vol. 59 pp. 1105 - 1123.

Hanahan D. J. (1952), Journal of Biological Chemistry, vol. 195, pp. 199 - 206.

Hanahan D. J. (1954), Ibid, vol. 211, pp. 213.

Hauser H.; Dawson R.M.C. (1967), Biochemical Journal, vol. 105, pp. 401 - 407.

Hauser H., Guyer W., Spiess H., Pascher I., Sundell S. (1980), Biochemistry, vol. 19, pp. 366 - 373.

Hauser H., Phillip M.C., Levine B. A., Williams R.J.P. (1976), Nature, vol. 261, pp. 390 - 394.

Ian F., Duddley H. W., (1966), Spectroscopic Methods in Organic Chemistry, McGraw Hill Publishing Company, London, pp. 12 - 14

Jellinek H. H. G., Gordon A. J. (1951), Applied Chemistry, vol. 1, pp. 185 - 188

Jukes (1934), Journal of Biological Chemistry, vol. 28, pp. 965, through Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1,001 - 1,081.

Kuhn, Hauser, Brydowna (1935), Berchte der bunsen gesellschaft fur Physikalische Chemie, vol. 68, through Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 81, pp. 1,001 - 1,081.

Levitt L. S., Levitt B. W. (1971), Tetrahedron, vol. 27, pp. 3777 - 3783.

Marrinetti G. V. (1962), Biochemistry, vol. 1, no. 2, pp. 350 - 353.

Marriott, P.H. (1969), Journal of Pharmacy and Pharmacology vol. 21, pp. 137 - 144.

Martin A. N., Swarbrick J., Cammarata A. (1969), Physical Pharmacy, Lea and Febiger, Philadelphia, pp. 462 - 463.

Martindale W. (1977), The Extra Pharmacopoeia, The Pharmaceutical Press, London, pp. 1339.

Murto J., Suomen (1962), Kemistilhti, vol. 35B, pp. 157 - 161.

REFERENCES

Osipov L. I., (1962), Surface Chemistry, Reinhold Publishing Corporation, New York through Banks F. B. (1978) "The Kinetics of the deacylation of Phospholipids under alkaline Conditions". Unpublished Ph.D. dissertation, School of Pharmacy, Liverpool polytechnic.

Osol A. Hoover, J. E. (1975), Remington's Pharmaceutical Sciences,"Mack Publishing Company, Pennsylvania, pp. 276.

Perrin J. Saunders L. (1960). Journal of Pharmacy and Pharmacology, vol. 12, pp. 253.

Phillips M. C., Finer E. G., Hauser H. (1972), Biochimica et Biophysica Acta, vol. 290, pp. 397 - 402

Rawlins, E. A. (1979), Bentley's Textbook of Pharmaceutics. Published by Cassell Limited, London, pp. 151.

Singleton W.S., Gray M.S., Brown M. I., White J. L., (1965), Journal of the American Oil Chemical Society, vol. 42, pp. 53 - 56.

Stretcher (1868), Annals of Chemistry and Pharmacy, vol. vol. 148, pp. 77, through Elworthy P.H., Saunders L. (1956), Journal of Pharmacy and Pharmacology, vol. 01, pp. 1,001 - 1,081.

Strickland K.P. (1963), writing on "The Biogenesis of Natural products", Pergamon Press, pp. 104.

Tewari, Mehrotra, Vishnoi (1976), A textbook of Organic Chemistry, pp. 736.