STUDIES OF SOME PROPERTIES OF LECITHIN MICELLES.

A Thesis presented by

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SUMMARY.

Part 1. Introduction and Theory of Experimental Techniques.

In the introductory section of this thesis, the elucidation of the structure of lecithin, lecithin purification methods (with emphasis on more recent developments) and some routes to its synthesis are outlined. Micellisation and solubilisation in aqueous and non-aqueous solutions are discussed, the effects in aqueous solution being only briefly mentioned, fuller accounts being given of the two properties with respect to non-aqueous solutions and lecithin micellar systems. The structure of myelin is considered, attention being directed to its phosphatide content.

The theories of the two main experimental techniques used, light-scattering and restricted diffusion, are presented. The relationship between the viscosity of solutions of macromolecular particles and the particle shape and solvation is outlined.

Part 2. Experimental, Results, and Discussion.

The development and calibration of a light-scattering photometer are described, together with a new type of cell. An assessment was made of some errors in the light-scattering method of molecular weight determination using a polystyrene fraction in methyl ethyl ketone and in toluene. The total error in a molecular weight determination was dependent upon the solvent, the error range being $\pm$ 8 to 13% ($P = 0.95$). A Rayleigh interference refractometer with a specially adapted cell holder

was used to determine diffusion coefficients.

Light-scattering was used to determine the micellar size of lecithin in a series of eleven solvent systems whose dielectric constant, ϵ, ranged from 2.3 (benzene) to 42.8 (84.0% V/V methanol/water). Over this range the micelles decreased in size from aggregates of 80 monomers in benzene to monomers at ϵ = 29.0 (93.4% V/V ethanol/water); the size then increased to aggregates of 90 monomers at ϵ = 42.8. It was thought that on changing from the non-polar to the polar solvents, the micellar structure was reversed, i.e. the lecithin polar head groups were in the interior of the micelle in the non-polar solvents, and on the exterior where $\epsilon > 29.0$.

To confirm the micellar weights from light-scattering in solvent mixtures, some micellar weights were determined by a combination of diffusion and viscosity results. From the comparison of micellar weights and viscosity intercepts to model structures, it appeared that on increasing ϵ from 2.3, and reaching the range ϵ = 18-25, the micelle structure changed from a bimolecular to a monomolecular leaflet. A similar transition was thought to occur in the more polar solvents where $\epsilon > 29.0$. Interpretation of results in these latter solvents was more difficult due to hydration effects confusing those of asymmetry when considering the viscosity intercepts.

The solubilisation, by lecithin micelles in benzene, of cholic acid and four bis-quaternary ammonium compounds was

investigated. For cholic acid, the ratio of the number of molecules solubilised per molecule of lecithin was 0·64, and methods were suggested for the incorporation of this solubilisate in the micelle. Because of the small solubilisation ratios observed for the bis-quaternary ammonium compounds, no solubilisate-micelle structure was suggested.

A study was made of the interaction of water and lecithin micelles in benzene. Light-scattering and diffusion-viscosity measurements indicated that the number of monomers per micelle remained constant as the micellar water content increased. In such systems it was shown that all the water present could be assumed to be solubilised. Viscosity intercepts showed an increase from 2·87, at zero water content, to a peak of 4·00 at 0·058 g. water/g. lecithin. Further addition of water to the micelles caused a gradual decrease in the viscosity intercept till at 0·33 g. water/g. lecithin the value was 3·33. This latter intercept was characteristic of hydrated spheres having a calculated water content of 0·33g. water/g. lecithin. Comparison to molecular models seemed to indicate that the micelles resembled oblate ellipsoids, and that the addition of water initially increased their asymmetry (up to 0·058g./g.), while further water tended to cause the particles to become spherical. The maximum uptake of water by 1% lecithin solutions in benzene was shown to be 0·33g. water/g. lecithin.

ACKNOWLEDGEMENTS.

I would like to thank Dr. P.H. Elworthy most sincerely for his invaluable help and guidance during the course of this work.

I would also like to thank Professors J.B. Stenlake and J. P. Todd for their interest, the Council of the Pharmaceutical Society for the award of a maintenance grant (1960-61) and a Research Scholarship (1961-62) and Mr. G. Cochrane for building the apparatus.

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CONTENTS.

PART 1: INTRODUCTORY SECTION.

PART 2: EXPERIMENTAL SECTION.

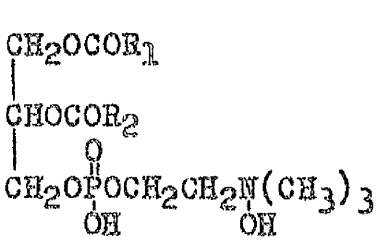
PART 1

INTRODUCTION, AND THEORY OF EXPERIMENTAL TECHNIQUES.

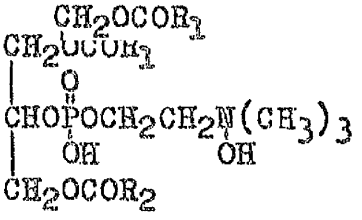
STRUCTURE OF LECITHIN.

The first proof of the existence of complex fatty compounds is generally credited to Fourcroy[1], who worked at the end of the eighteenth century. After the turn of that century Vauquelin[2] became the initial investigator to show the presence of bound phosphorus in the fat-like material of the brain. Following this work, Gobley,[3,4,5] in the mid-nineteenth century, showed that egg yolk contained what is now known as a phosphatide. This phosphatide was later called lecithin, the name being derived from the Greek equivalent for egg yolk. Elucidation of the structure of lecithin then commenced. In 1868 Diakonow[6,7] showed that choline was its nitrogenous base.[8] As a result of his further work, and that of Strecker,[9] it was proved that lecithin contained glycerol and two fatty acids, either similar or different, and that the fatty acids were esterified with the glycerol. Though both these workers agreed that a phosphoric acid molecule was attached both to the third hydroxyl of glycerol and to a choline molecule, they disagreed as to the nature of the phosphoric acid-choline link. Diakonow stated the linkage was via the hydroxyl group of the choline nitrogen, Strecker stating it to be via the other hydroxyl group of the choline. Subsequent work of Hundeshagen[10] and Gilson[11] showed Strecker to be correct.

From this early work, it was clear that lecithin could exist in two forms, these forms arising from the possibility of the phosphate ester grouping being attached either to the α- or the β-carbon of glycerol. This provided workers with the problem of investigating lecithin to try and establish, firstly, which glycerol carbon atom the phosphate ester grouping was attached to, or if it could be attached to either; and secondly, if it was attached to the α- carbon atom, the nature of the stereochemical configuration, there being an asymmetric carbon atom.



α - Lecithin.



β - Lecithin.

As early as 1901, Ulpiani[12] demonstrated optical activity as a characteristic of most samples of naturally occurring lecithin, indicating it to be α- lecithin. Following this work many conflicting conclusions were drawn by workers, the problem not being resolved till the last decade. It was the production of pure synthetic materials and their comparison to the natural material that provided the results showing the existence, in nature, of only the α- form. This comparison of the natural with the synthetic substances has been due largely to the work of Baer and his colleagues since it was they who obtained the synthetic products that were used. Using their own synthetic

Table 1.

Some Recent Fatty Acid Analyses of Egg Lecithin.

Acid	1958 18 Inouye and Noda	1959 20 Tattrie	1960 21 Hanahan Brockerhoff and Barron	1962 22 Hawke
Saturated				
C_{12}				0·05
C_{14}	3·3	trace		0·28
C_{15}				0·07
C_{16}	28·4	35·7	32	29·4
C_{17}				0·2
C_{18}	10·9	14·9	16	12·8
C_{19}				0·25
C_{20}	trace			
Unsaturated				
$C^{'}_{16}$	0·7		1	1·42
$C^{'}_{17}$				0·05
$C^{'}_{18}$	40·2	37·0	30	37·8
$C^{''}_{18}$	8·9	12·4	17	9·4
$C^{'}_{20}$				0·53
$C^{''''}_{20}$	4·1		3	4·0
$C^{''''}_{22}$				2·23

Superscripts indicate the number of double bonds per molecule.

material they established again by comparison to natural products, that the natural material belonged to the L-α-series.[13]

Having obtained the general structure of natural lecithin, there still remained the problem of determining its fatty acid composition. From the results of many attempts to solve this problem, it appeared that the lecithin was a mixture of several lecithins of varying fatty acid composition. To illustrate this, lecithin isolated from egg yolk can be cited as an example.

In 1903[14] egg lecithin was shown to contain the saturated acids palmitic and stearic and the unsaturated acids oleic and linoleic. Since then, numerous workers have stated their findings, and in the light of the more recent work these four acids would seem always to be present. The presence of other saturated and unsaturated acids has been reported less regularly.[15,16]

It was not until the nineteen-thirties that the relative proportions of the individual acids were estimated. From the figures of Reimenschneider, Ellis and Tutis,[16] (palmitic 31·8%, stearic 4·1, oleic 42·6, linoleic 8·2 and clupanodonic 13·3), and those subsequently published,[17,18,19,20,21,22] it appeared that the proportion of palmitic acid was fairly constant at approximately 30% (Table 1). Gas chromatography has been a useful technique for this work. Rhodes[23] has recently shown that by feeding hens a diet that contained a high proportion of unsaturated acids, by

means of cod liver oil, there was an increase in the unsaturation of the lecithin fatty acids. This suggested that the fatty acid composition was variable, depending essentially on the diet. However, it would seem that the proportion of palmitic acid is influenced little by diet.

A further aspect of the structure of lecithin which has only recently been worked out is the distribution of the saturated and unsaturated acids between the α- and β- carbon atoms of glycerol. This has been studied on several occasions [17,18,19,20,24] and it has been known for about ten years that the α- and β- positions are highly specific for certain fatty acids. During this work, the investigators relied on the fact that phospholipase A from snake venom hydrolysed the ester link in the α- position. Obtaining from this hydrolysis an unsaturated acid, they concluded that the α- position was specific for unsaturated acids.

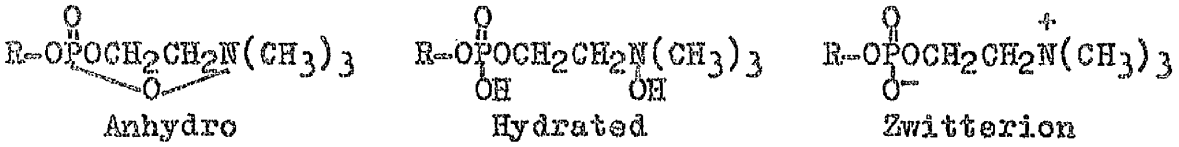
In 1959 Tattrie [20] enzymatically hydrolysed egg lecithin with lecithinase D, obtaining a mixture of α,β- diglycerides. Myristic acid was then incorporated into the free α' position and the resulting triglycerides were hydrolysed with pancreatic lipase. This enzyme specifically releases the fatty acids from the α- and α'- positions, and these released acids were identified as palmitic, stearic and myristic. Contrary to the early findings, this indicated that lecithinase A hydrolysed the β- ester linkage. This latter observation was soon to be supported by Hanahan, Brockerhoff and Barron, [21] and de Haas, Mulder and van

Deenen.[25] The effect of this correction does not detract from the earlier work on the fatty acid positional distribution, it merely reverses the conclusions. Following Tattrie's work, and that of Hanahan[21] which showed only 4% of the β-attached acids to be saturated, Hawke[22] stated that the distribution of palmitic acid between the α-and β-positions almost entirely favours the former. Since the proportion of palmitic is approximately 30% of the total fatty acids present, about 60% of the lecithin molecules have this acid in the α-position. The 40% of the α-positions that remain will then possess the saturated stearic acid and be completed with unsaturated acids. It can therefore be stated that the saturated acids predominate in, and favour, the α-position, while the unsaturated acids favour the β-position.

With the elucidation of the fatty acid composition of egg lecithin it becomes clear that purified natural egg lecithin consists, not of a single chemical entity, but of a mixture of compounds varying in their fatty acid content.

Grun and Limpächer,[26] in 1923, suggested that the phosphoryl-choline grouping of the lecithin could exist in one of two forms - an anhydro or hydrated form. Their further work[27] suggested the anhydro as the more likely. Jukes[28] subsequently rejected the anhydro form on the grounds that the concept of a nitrogen atom with five bonds was not in agreement with the current valency theories. He suggested the formula should be written as a zwitterionic structure substantiating his suggestion by

electometric titration.



Anhydro Hydrated Zwitterion

R= glyceryl difatty acid residue.

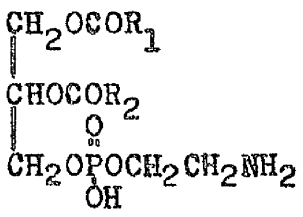
Further evidence for this structure came from dielectric constant studies in three solvents. In alcohol[29] and in water,[30] lecithin solutions had a greater dielectric constant than the solvent, while no change in dielectric constant was found between benzene and lecithin solutions in benzene.

PURIFICATION OF NATURAL LECITHIN.

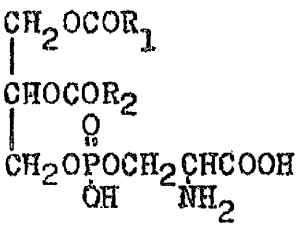
Lecithin is known to be widely distributed in both the animal and vegetable kingdoms. Its extraction from many sources has been the subject of many papers and several texts [31,32,33] and as a result there is a wealth of information on the preparative procedures. It was decided, therefore, that this review of the purification be restricted to lecithin obtained from hens' egg yolk, and in order to emphasise the more recent developments, much more attention has been directed to the work published from the late 1950's onwards than to the earlier findings.

Occurring naturally with lecithin are several other phosphatides whose similar chemical nature and physical properties have provided difficulties in the separation of lecithin. Most closely associated with lecithin are the two other members of the lecithin group, hydrolecithin which contains only saturated fatty acids, and lysolecithin which contains only one fatty acid residue. The other major associated phosphatides may be divided into two groups, the cephalins and the sphingomyelins, each group containing two main members.

The Cephalins.

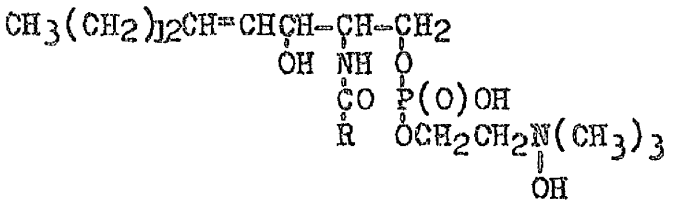


Phosphatidylethanolamine (Cephalin)

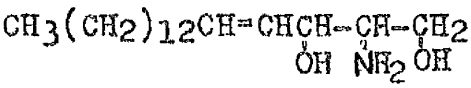


Phosphatidylserine

The Sphingomyelins.



Sphingomyelin



Sphingosine

In addition to these substances many others occur, but in much smaller quantities. Thus there are lysocephalins, acetal phosphatides and many breakdown products or precursers of the above, such as phosphatidic acids.

Solvent Extraction.

Isolation of lecithin by using only solvents is now a method of the past. Due to the very similar solvent characteristics of lecithin and many of the other compounds associated with it in nature, such as those mentioned above, the methods were awkward and tedious.[34,35,36] The fact that chromatography has proved rapid and successful for this separation has also contributed to their falling into disuse. Today, lecithin purification usually consists of a partial solvent fractionation, this being followed by chromatographic techniques.

Initially egg yolks are very often extracted with acetone since lecithin and other phosphatides are almost insoluble in acetone. This fractionation removes such unwanted material as water, fats and sterols. However, it has been shown that considerable quantities of phosphatide are taken up by the acetone in this preliminary extraction stage, as much as 31% of the total

phosphatide available being quoted by some workers.[37] This uptake was credited to the water present in the system. When synthetic lecithins were prepared, an investigation of the acetone solubility of lecithin showed that an increase in solubility was obtained by decreasing the length of the hydrocarbon chains and also by the presence of unsaturation,[38] traces of water also considerably increasing the solubility. This latter fact was borne out by the finding that when freeze-dried egg yolks were used as the starting material, only 4·3% of the total phosphatide was removed by the acetone.[39]

After this initial acetone fractionation, the residue can then be extracted with a suitable phosphatide solvent, usually ethanol. Having therefore obtained an extract of mixed phosphatides, the solvent fractionation method is discarded in preference to the more sensitive chromatographic techniques.

A further problem with solvent extraction methods is that the starting material is very complex. Thus certain substances which by themselves would be insoluble in the extraction solvent may well be extracted by a solubilisation effect. On extracting fresh egg yolks with acetone, the solvent is in effect acetone containing a certain amount of water. Though the solubility of some compounds in wet acetone can be avoided by freeze-drying, mutual solubility effects are more difficult to overcome. However, as was stated earlier, the main difficulty of a pure solvent extraction process is the very similar solvent characteristics of closely related compounds.

This similarity has been overcome by countercurrent distribution.[40,41,42,43] Working with egg lecithin that had been previously purified by column chromatography, it was demonstrated that there was a possibility of further lecithin separation into individual lecithins by means of countercurrent distribution.[43] To obtain this, fifty transfers were done, using as the polar phase a mixture of acetic acid, water and methanol, and as the non-polar phase a petroleum ether-chloroform mixture. Though this technique is rather laborious, it has on its credit side a negligible loss and destruction of solute.

Salt Formation.

As with pure solvent fractionation, the addition of certain compounds to form salts with specific phosphatides and thereby aid their separation, is now becoming outdated. Chromatographic techniques are again the reason for this decline since they yield purer products.

Strecker[9] and Bergell[44] found that phosphatides could be precipitated from alcoholic solution by certain metallic salts, and it was their early work that led to the development of the much used cadmium chloride complex precipitation technique. Levene and Rolf[45], using this method, precipitated the lecithin from an ethanolic egg yolk extract by addition of a saturated solution of cadmium chloride in methanol. The complex was freed of cephalin by shaking with 8-10 separate portions of ether, the purified complex being split with ammonia in methanol. It was usually necessary to repeat the initial cadmium chloride precipitation to obtain lecithin of the required purity.

Pangborn[46,47,48] has on several occasions modified t
procedure. By suspending the lecithin-salt complex
petroleum ether and extracting with 10-12 portions
ethanol, the lecithin was sufficiently pure not to
second precipitation. Complex formation was also o
adding 50% aqueous cadmium chloride to the ethanoli
phosphatide extract, with its subsequent breakdown
chloroform solution by shaking with an equal volume
ethanol, the cadmium chloride being removed in the

In a systematic study of substances thought ca
selectively precipitating diaminophosphatides, Tharn
and Setz[49] found Reinecke acid to be suitable, lecith
cephalin being unaffected.

To effect a separation of lecithin from the ce
Collins and Wheeldon[50] used the structural differenc
amino group of the cephalins. This group was found
with 1-fluoro-2:4-dinitro benzene forming a coloure
compound which was then methylated using diazometha
was unaffected. The egg yolk phosphatides, after t
were separated by chromatography on Hyflo Super-Cel
of the cephalin bands helping to indicate the fract

Chromatography.

It is appropriate to repeat at this point that
utilising a chromatographic procedure for lecithin
preliminary solvent fractionation will almost cer
been carried out. Thus it is essentially a mixture

phosphatides that has to be separated.

(a) Paper Chromatography.

Following the separations of phosphatides on unimpregnated paper,[51,52,53,54] and due to the success of column chromatography, impregnation of the paper with materials similar to those used in columns was shown to effect useful separations. Thus silicic acid was applied to the paper and many improved separations were obtained.[55,56,57,58] The introduction of glass fibre paper which was similarly impregnated[59] also met with success.[60,61] Marinetti[62] has studied the factors that affected the chromatography of phosphatides on silicic acid impregnated paper. From his work, reproducibility of chromatograms was shown to depend mainly upon uniformity of the paper impregnation, the temperature and relative humidity. In addition there was an optimum for the amount of material spotted on the paper since overloading led to elongation of the spots. Because of these and other minor factors, reproducibility was difficult, though the actual separations were good.

Easier reproducibility has been stated to be obtained by the impregnation of glass fibre paper with sodium silicate.[63] Other advantages of sodium silicate over silicic acid were the easier preparation of papers, faster phosphatide separations and the ability to determine lecithin and sphingomyelin quantitatively from solutions. Because of the difficulty of reproducibility of results with silicic acid impregnated paper,

Collier investigated several inorganic metallic salts as possible impregnating agents.[64] Zinc chloride was the most satisfactory of those tested. For the separation of complex lipid mixtures using sodium silicate, an initial chromatogram run with benzene was carried out,[63] the neutral lipids moving with the solvent front, the phosphatides remaining at the origin. After the paper was dried a second run was made using a basic solvent, usually containing pyridine. The previously stationary material then migrated and was separated, any mutual solubility effects that could have interfered having been eliminated by the benzene run. If a greater separation was possible or desired, a further run at right angles to the first two could then be made.

To overcome the difficulty of separating individual lecithins from natural lecithin, Inouye and Noda, before running the chromatogram, converted the natural lecithin to mercuric acetate addition compounds.[18] The mercuric acetate added on to double bonds of unsaturated fatty acid chains, and because the degree of unsaturation varied so did the degree of acetate addition. This resulted in much greater solubility differences between the lecithins in organic solvents, these differences being suggested as a reason for the separation obtained. By such a process five spots were distinguishable from a sample of egg lecithin.

(b) Thin-layer Chromatography.

This technique was developed in 1951 by Kirchner, Miller and Keller,[65] and due to its fairly recent development few

phosphatide separations have so far been attempted. Silicic acid has been the adsorbent used, it being mixed with up to 15% of plaster of Paris to bind it when dried on the plates. Vogel, Doizaki and Zieve[66] have reported a separation of some egg yolk phosphatides. Using a chloroform-methanol-water (80:20:1) mixture as solvent, they separated phosphatidylethanolamine, lecithin, sphingomyelin and lysolecithin from a mixture of the four, in approximately 30 minutes. A separation of some glycerides, free fatty acids and egg yolk derived phosphatides has also been obtained,[67] the phosphatides remaining at the origin. Privett and Blank[68] have found the technique could be used to distinguish between four different lecithins and that they could also be determined quantitatively.

Several of the advantages of this new technique have recently been indicated.[69] On comparison to the older established methods, thin-layer chromatography provides a rapid, efficient separation. Complex mixtures can be resolved in half an hour compared to the several hours required by the other techniques, and in addition sharper separations are obtained. The spots are easier to identify since they tend to be more compact. Plate capacity is such that millegram quantities can be spotted instead of the microgram amounts for paper chromatography. Not least, the technique is simple to operate.

(c) Large-scale Chromatography.

One of the earliest successful adsorption materials was magnesium oxide[70], which removed non-choline containing phosphatides from a mixture of phosphatides. It adsorbed all the phosphatide from solution, but under certain conditions, for example in methanol, the choline-containing phosphatides were eluted, these having a choline/phosphorus ratio of over 0·96.

Alumina was subsequently found to separate more efficiently the same two classes of phosphatide. Hanahan, Turner and Jayko[71] chromatographed a 3% solution of egg phosphatide in 95% ethanol using a column of alumina and recycled the eluted phosphatide solution. This gave a lecithin fraction of over 99% purity, with freedom from breakdown products. Subsequently Hanahan suggested a column loading of 4g. of mixed phosphatide per 125g. of alumina[72], but before all the lecithin fraction was eluted the aminophosphatides started to appear and some lecithin loss resulted. Rhodes and Lea[39], with a loading of 2g. per 40g., applied the mixed phosphatides as a 10% solution in an equal volume chloroform-methanol solvent. On elution with this solvent, the choline-containing phosphatides were rapidly recovered, the adsorbed phosphatides only being eluted on changing the solvent. This enabled all the lecithin, and the adsorbed fraction if desired, to be recovered separately.

In agreement with the early findings of Trappe[73], Renkonen[74]

detected a slow degredation of lecithin on alumina columns. Using a synthetic lecithin and assuming the breakdown to be a first order reaction he showed the degradation to proceed at 1.1% of lecithin per hour at 22°. At 2° this reaction was markedly retarded while the alumina's adsorbing power was unaltered, and he recommended work with this adsorbent to be done at low temperatures.

At the same time as Lea and Rhodes stated silicic acid impregnated paper produced good phosphatide separations they also stated that silicic acid columns could effect the same separations,[55] the separations varying with the source of the silicic acid.[56] Using a single chloroform-methanol solvent mixture, they separated the mixed phosphatides of an egg yolk ether extract, the amino-containing phosphatides being eluted first, followed by lecithin and then lysolecithin. Sharper separations were obtained if the solvent mixture was altered during the run.[39] Wren[75] has described a simple apparatus whereby the composition of the solvent was gradually and smoothly changed, this producing sharper peaks. Renkonen[74] found no degradation of synthetic dipalmitoyl-lecithin by silicic acid. For large-scale lecithin purification, silicic acid now tends to be used only for the final lecithin-lysolecithin separation.

Having obtained a separation of the choline-phosphatides from the amine-phosphatides on non-impregnated paper, Bevan and his colleagues[76] using a column of powdered cellulose, reported the same separation.[77] Lea and Rhodes were unable to confirm this,

obtaining only a phosphatide-free amino acid separation.

The use of an ion-exchange resin for preparative work has been investigated by several workers. Using 'Amberlite MB-3', MacPherson[78] split the cadmium chloride-lecithin complex. Lea, Rhodes and Stoll[56] were unsuccessful in separating lecithin and phosphatidylethanolamine with acidic and basic resins because of the poor capacity of the resins and hydrolysis of the phosphatides. Perrin and Saunders[79], using a mild 'Dowex' bicarbonate resin separated the choline from the non-choline containing phosphatides without hydrolysis, the method being quicker than with alumina.

For preparative work these large-scale methods are now tending to be used almost entirely instead of the cadmium chloride complex method. Having obtained an alcoholic mixed phosphatide extract from the egg yolks, alumina is commonly used to remove the non-choline containing phosphatides. This stage can then be followed by silicic acid chromatography to separate the lecithin and lysolecithin.

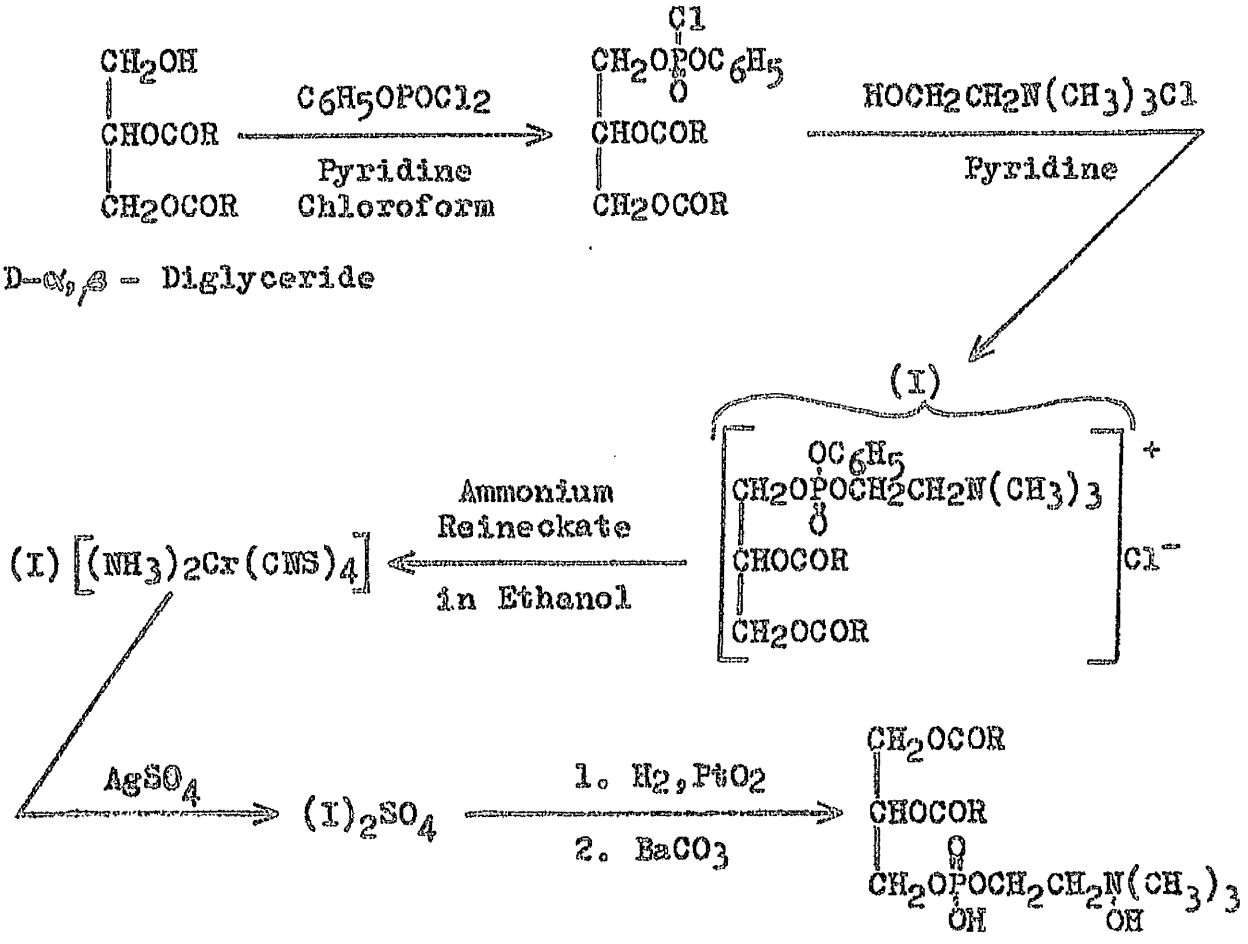
Paper Electrophoresis.

Wallach and Garvin[80] separated lecithin, phosphatidylethanolamine and phosphatidylserine from both natural and synthetic mixtures. At pH 9·8 with a potential gradient of 1 volt/cm. for 30 minutes their respective rates of movement were 0·26, 0·56 and 1·89 mms./hour. The solvent was a mixture of trichlorethylene, methanol and water, whose water content was more than 3%. Zipper and Glantz[81] separated the same phosphatides

using the non-aqueous solvent systems of methanol-pyridine mixtures containing 0.05N sodium acetate with and without glacial acetic acid.

SYNTHESIS.

By starting with a D - α,β - diglyceride, which was prepared by a slightly modified Sowden and Fischer method,[82,83] Baer and Kates[84] in 1950 reported the first synthesis of a lecithin.[13] Soon after this initial work Baer and Maurukas[85] simplified the procedure, replacing the introduction of ammonium reineckate by the use of solubility differences for separating the phosphorylated products. The slower, original procedure, however, had to be resorted to by Baer and Mahadevan[86] when they prepared a series of lecithins with short chain fatty acid radicals, since the solubility differences were too small to allow separations to be made. The original Baer and Kates scheme was as follows:

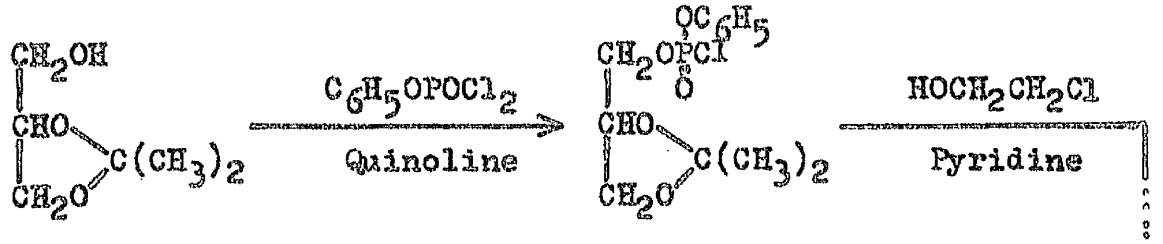


By starting with L- or D,L -α,β- diglycerides the D- or D,L -α- lecithins were obtained.

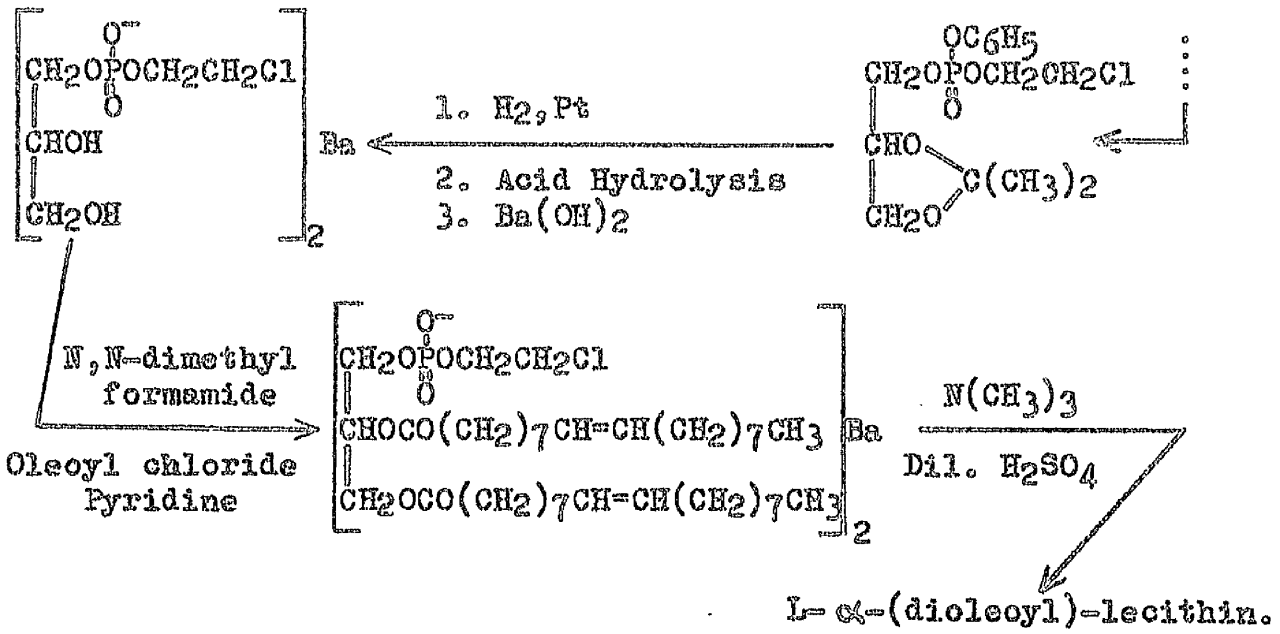
Two years before their lecithin synthesis, Baer and Kates[83] synthesised L-α- glycerylphosphoxylcholine (L-α-GPC) but were unable to acylate it to lecithin. It was not until 1957 that a direct acylation method was successful,[87] the reaction being carried out in amine-free dry solvents instead of pyridine which was first used.[83] The successful acylation however, had the disadvantage of requiring three days to carry out the reaction. By using the cadmium chloride compound of L-α-GPC, Baer and Buchnea[88] found it was possible to acylate to lecithin in two hours.

In the original Baer and Kates synthesis, the final stages included a hydrogenation. Their process, therefore, could only be used to prepare fully saturated lecithins. However, with the acylation of L-α-GPC unsaturated chains could be incorporated.

Before this acylation process had been devised, a successful synthesis for unsaturated lecithins, starting from acetone-glycerol, was reported.[38] Here the reaction scheme was:



D-acetone-glycerol

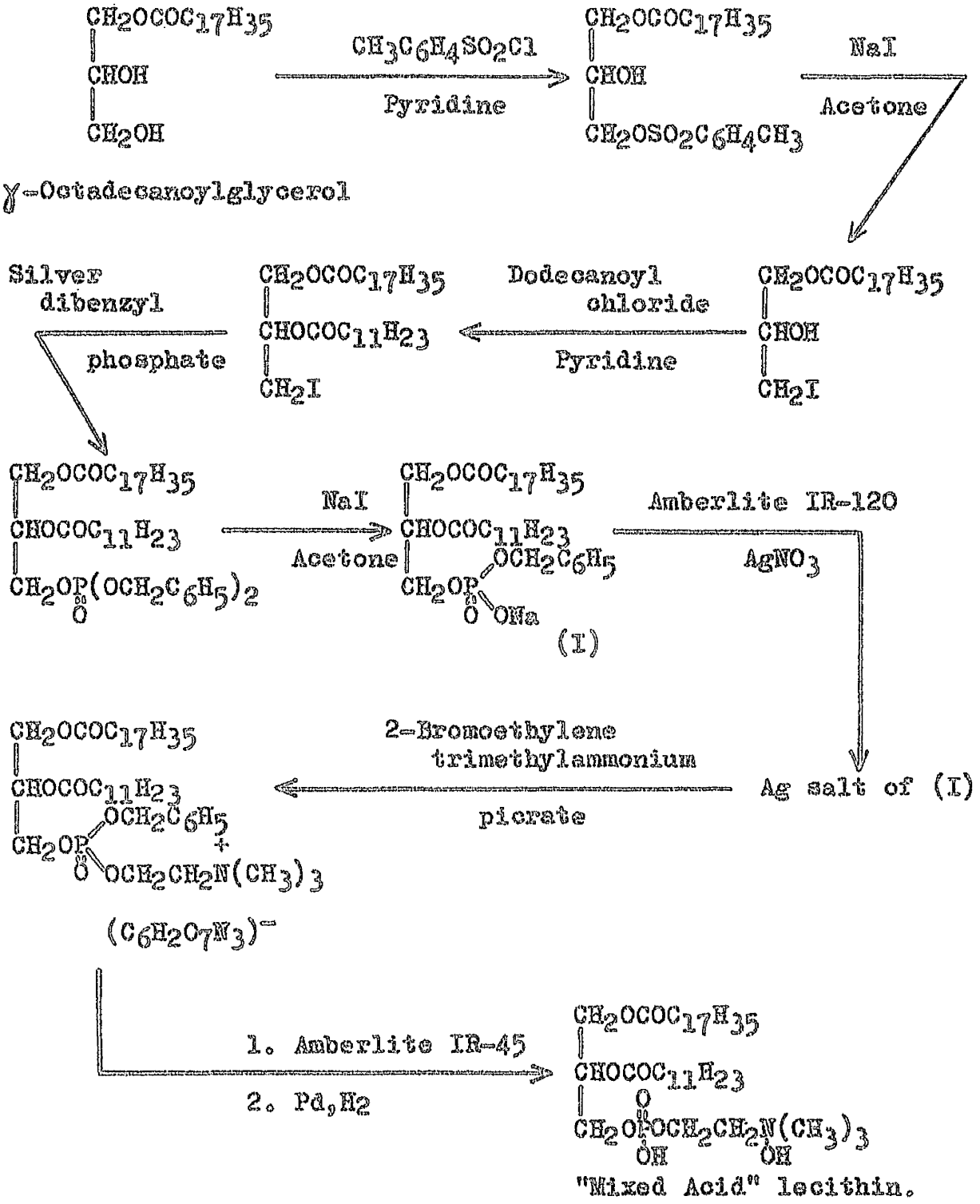


A further route to both saturated and unsaturated lecithins was devised by Hirt and Berchtold[89]. They started from the appropriate diglyceride, and since only saturated diglycerides were available, they could only prepare the fully saturated lecithins. They did, however, indicate that on the availability of unsaturated diglycerides the method could probably be used to prepare the corresponding unsaturated lecithins. This was subsequently found to be possible[90].

Malkin and his co-workers[91,92] have suggested a means of preventing migration with the diglyceride and the resultant formation of unwanted bis-phosphatidic derivatives. This involved the use of a diacyl glycerol-iodohydrin prepared from glycerol-iodohydrin.

In 1960 the Dutch chemists, de Haas and van Deenen, synthesised a lecithin with two differing hydrocarbon chains[93]. Their route to lecithin started with a monoglyceride and

proceeded via a glycerol-iodohydrin compound, the reaction scheme being:

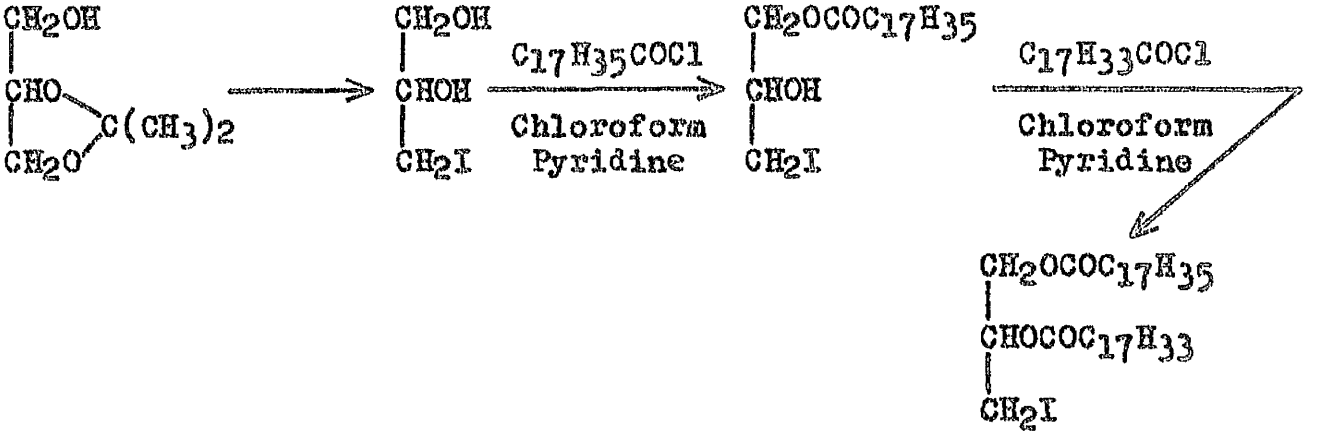


This process was also used to prepare the structural

isomer, γ-dodecanoyl-β-octadecanoyl-α-lecithin.

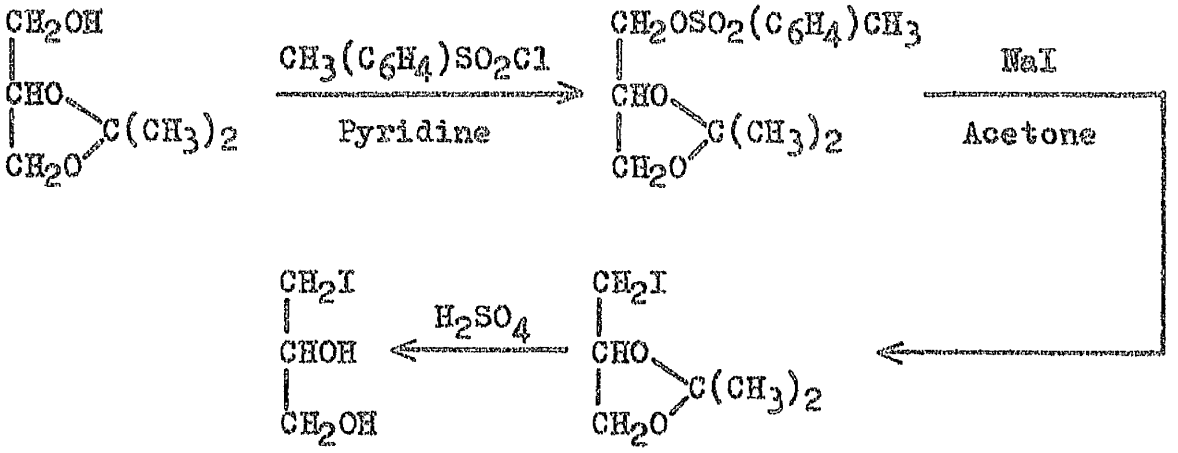
Soon after, there followed a partial synthesis of lecithin possessing one saturated and one unsaturated hydrocarbon chain.[94] Starting from a lecithin with two identical unsaturated hydrocarbon chains, de Haas and van Deenen produced the lysolecithin enzymatically this being then acylated with a saturated acid chloride. The isomer was obtained by hydrogenating the lysolecithin and adding the corresponding unsaturated acid chloride.

Eventually these same workers developed a complete synthesis of such a saturated-unsaturated lecithin, L-α-(β-oleoyl-γ-stearoyl)-lecithin.[95] This work entailed the synthesis, from the acetone-glycerol, of the appropriate diacyl glycerol-iodohydrin. This product was then treated in essentially the same way as the saturated diacyl glycerol-iodohydrin, resulting in the production of the desired lecithin. Their diacyl glycerol-iodohydrin was prepared as follows:



To obtain the glycerol iodohydrin from D-acetone-glycerol,

the method of Baer and Fischer[96] was used:



MICELLISATION.

From the results of work done in the early years of this century it became increasingly obvious that certain types of substances, when in solution and under certain conditions, existed as aggregates of their most simple, single units, or monomers. These aggregates were soon to be called micelles, their aggregation process therefore being micellisation. Work has mainly been directed to micellisation in aqueous solvents, relatively few investigations having been made in non-aqueous media. It is only in the last ten to fifteen years that interest in non-aqueous micellisation has developed.

This phenomenon of micellisation is exhibited by compounds whose structure consists of a non-polar hydrocarbon portion plus a polar grouping. These two parts of the molecule differ in their affinity for water, the hydrocarbon portion having little or no affinity (hydrophobic portion) the polar grouping having a strong affinity (hydrophilic portion). It is this dual, or amphipathic, nature of the molecule that enables it to form micelles.

Micellisation in Aqueous Solution.

Since micellisation in aqueous solution has been comprehensively reviewed by several authors,[97,98,99,100] it will only be briefly mentioned here. More emphasis will be given to micellisation in non-aqueous solution.

(a) Micelle Structure.

Towards the aim of identifying the structure of micelles,

the work of McBain and of Hartley is undoubtedly the most prominent of the early days. McBain[98] postulated the existence to two types of micelle, small spherical micelles existing below the critical micelle concentration (cmc) and lamellar micelles above the cmc. Hartley[97] suggested only one type, which was spherical, and that single molecules were present below the cmc. Today it is known that micelles may adopt any one of several structures, the structure formed depending upon several factors, not the least of which are the nature of the monomer and the solvent and the number of monomers in the micelle. In aqueous micellar systems the polar groups are on the micelle surface.

(b) Micelle Formation.

In their descriptions of the process of micellisation, Debye[101] and also Herzfeld, Corrin and Harkins[102] considered that there were two energy terms of major importance. Firstly, a decrease in free energy was obtained by removing the non-polar paraffin chains from their aqueous environment into the micelle interior. This was termed environmental energy. This decrease in free energy would be maximal when the shielding of the chains from the water was as complete as possible. Secondly, when the molecules were brought together short range repulsive forces between polar head groups came into play and a corresponding free energy increase had to be considered. This was termed repulsive energy. Aggregation would therefore take place if the

environmental energy or, as it is more commonly called, interfacial energy made available from the destruction of the paraffin chain - solvent interfaces is greater than the work to be done against the electrical repulsive energy.[103]

Below the cmc molecules are so distant from each other that the work needed to overcome the repulsive forces to bring the molecules together is greater than the available interfacial energy. On increasing the solute concentration, the interfacial energy per molecule does not alter, but the average distance apart of the molecules is less. Less energy is therefore required to bring the molecules together. With further concentration increase, the state is reached eventually when the available interfacial energy exceeds the repulsive energy and micelles form. In forming, the micelles probably start with a few molecules joining together sufficiently long to form a nucleus, this nucleus then incorporating any colliding molecule till the full micellar size is attained. In aqueous systems, soap micelle size is considered to be limited by the accumulation of charged ions in the micelle, which prevents the approach of further ions. Thus the work needed to add a further unit to the micelle is greater than the energy obtained from shielding the unit's paraffin chain from the solvent.

The foregoing energy and aggregation considerations indicate that the cmc is not a definite concentration, but a concentration range, the range usually being so narrow that it

can only be measured as a single concentration. The cmc defined by Williams, Phillips and Mysels[104] is the concentration of solute at which the concentration of solute in micellar form would become zero if the rate of change in solute micelle concentration with total solute concentration was assumed to be the same at low micellar concentrations as at high micellar concentrations. As obtained practically, therefore, the cmc is the point of intersection of two lines, one line representing the properties of monomers, the other the properties of the complete micelles.

Bury and his co-workers[105,106,107] considered the aggregation process on the basis of the law of mass action. The equilibrium was represented by

$$nS \rightleftharpoons S_n \qquad \text{giving} \qquad K = [S_n] / [S]^n$$

where S and S_n refer to monomer and micelle, n being the number of monomers in the micelle; K is the equilibrium constant and brackets indicate concentrations. Using their relationships to consider a sub-cmc solution of a micelle-forming solute, where n is large (e.g. 50, 60,....), $[S]$ will be small and $[S_n]$ negligible. On increasing the concentration above the cmc, both $[S]$ and $[S_n]$ increase, but since $[S_n]/[S]^n$ is constant, the increase in $[S_n]$, $\Delta[S_n]$, will be much greater than $\Delta[S]$ since $[S]$ is present to the n^{th} power. The larger the value of n, therefore, the greater will be $(\Delta[S_n] - \Delta[S])$ for a given change in solute concentration, and also the smaller will be the solute

concentration change needed to convert the essentially monomeric solute into an essentially micellar solute. Thus the larger the value of n the narrower is the cmc range.

A second approach to the aggregation process treated it as similar to a phase separation,[97] the separation occurring at the cmc. This idea was prompted by the shape of the specific conductivity against concentration graph of a colloidal electrolyte which showed a sharp break at the cmc, a break also being found in the same graph for a salt in water when the salt saturation concentration was reached. In the micellar systems, breaks were also obtained in graphs of colligative properties against concentration. This led to micellar solutions being considered as saturated monomer solutions,[108] such that if there was any loss of monomer from super-cmc solutions (e.g. by adsorption) the monomer loss was replaced from the micellar reserve.

Micellisation in Non-aqueous Solution.

(a) Micelle Structure and Formation.

In aqueous systems the amphipathic nature of certain molecules causes them to form micelles, the micelle surface possessing the polar groupings. In non-aqueous systems this arrangement is reversed, the micelles having the polar groups directed towards their centre. In these reversed micelles, ionic molecules are undissociated and as a result the micelle does not possess a significant net charge. As with aqueous

micellar systems, there is in the non-aqueous a variation in the possible structure of micelles, again dependent upon the prevailing conditions, though one dimension of the micelles usually remains approximately twice the monomer length.

Singleterry,[108] in a comprehensive review of non-aqueous micellisation, has indicated that most of the solutes in such systems have hydrocarbon parts whose cross-sectional area is greater than the corresponding dimension of the polar head. For such molecules, spherical micelles allow the tightest packing of the heads about a core of given volume, this volume being dependent upon the size of the polar grouping. On deviation from spherical shape, the prolate ellipsoid would be the more likely shape, since it provides tighter packing of the heads than the oblate ellipsoid. Where the molecules have polar groups and paraffin chains of the same cross-sectional area, a lamellar micelle is probably the preferred shape. Pilpel[109] has stated that the geometrical factors are of importance in controlling the micelle shape and size of the heavy metal soaps in organic solvents.

Copenhafer and Kraus[110] thought that the aggregation of quaternary ammonium salts in benzene was the result of dipole-dipole interaction between ion pairs, whereas with nonaethylene glycol monolaurate in benzene, hydrogen bonding was probably the mechanism of adhesion. Nelson and Pink,[108,111]

studying copper soaps in toluene, suggested that the dipole interaction was insufficient to account for the aggregation, suggesting that co-ordination bonding played an important role. Pilpel in his recent review[109] of organic solutions of heavy metal soaps discussed the nature and magnitude of the forces involved in micelle stabilisation in organic solvents. He assumed there to be three main factors[112] that contributed to the energy change at micellisation. Firstly there was a change in interfacial energy, ΔE, on aggregation, secondly, dipole-dipole interactions which produced a change, ΔW, in dipole interaction energy, and thirdly, in the case of such complex structures as the heavy metal soaps, there was probably hydrogen, or similar bonding which provided a hydrogen bonding energy factor, ΔH. Considering these three energies together, he denoted the total energy change on micellisation, ΔE_t, by $\Delta E_t = \Delta E + \Delta W + \Delta H$ and by making some assumptions he was able to evaluate ΔE_t, and use the results to show that the micelles existed down to extremely low concentrations. This was in agreement with experimental data.[112] To evaluate ΔE, Pilpel assumed the soap monomer to have a spherical head group (radius R) and cylindrical hydrocarbon tail (length L, radius r) with half the surface of the head group covered by the tail. The exposed head group surface area was $2\pi R^2$, and the exposed hydrocarbon surface area, $2\pi rL$. Spherical micelles were assumed to be formed with

the head groups completely shielded from solvent. Monomers had their complete surface exposed to the solvent. The interfacial energy of the micelle,

$$E_{mic} = 2\pi r\, Ln\gamma_{tt} + 2\pi R^2 n\gamma_{hh} + X$$

where n is the number of monomers in the micelle, γ_{tt} and γ_{hh} the cohesional energy of the tails and heads, and X a factor introduced to allow for some hydrocarbon-solvent contact. On dissociation to monomers, the interfacial energy,

$$E_{mon} = 2\pi\, r\, Ln\gamma_{ts} + 2\pi R^2 n\gamma_{hs}$$

where γ_{ts} and γ_{hs} are the interfacial energies between solvent and the tail and head groups. The difference in these two energies was the interfacial energy change on micellisation,

$$\Delta E = E_{mon} - E_{mic} = 2\pi r Ln(\gamma_{ts} - \gamma_{tt}) + 2\pi R^2 n(\gamma_{hs} - \gamma_{hh}) - X$$

Limiting factors which arrest micelle growth at a particular level have been little discussed in non-aqueous media[108]. It has been suggested that the limit arises due to a balance being obtained between geometrical factors and the attractive polar core forces. Most amphipathic molecules that are soluble in organic solvents generally have hydrocarbon cross-sectional areas greater than that of the polar heads and hence in small micelles the core is best shielded from the solvent when the micelle is spherical. To add a further molecule, the attractive forces between the molecule's polar group and the micelle core must be sufficient to expand the micelle in order to permit this additional molecule to join

Table 2.

Methods Used to Study Micelle Size (Excluding that of Lecithin) in Non-aqueous Solvents.

Reference	Method
116 Arkin and Singleterry	Fluorescence Depolarisation
117, 118 Honig and Singleterry	Fluorescence Depolarisation, Viscosity
119, 120 Kaufman and Singleterry	Fluorescence Depolarisation, Cryoscopy
121 Singleterry and Weinberger	Fluorescence Depolarisation
110 Copenhafer and Kraus	Cryoscopy
122 Rothrock and Kraus	Cryoscopy
123 Martin and Pink	Ebullioscopy
111 Nelson and Pink	Ebullioscopy
124 Debye and Prins	Light-scattering
125 Goniok	Cryoscopy
126 Kitahara	Vapour Pressure Depression
127 Mathews and Hirschorn	Ultracentrifugation
128 Palit and Venkateswarlu	Cryoscopy
129 Sheffer	Osmotic Pressure, Viscosity

the micelle. If the attractive forces are insufficient, the micelle will not grow further.

The law of mass action can again be applied to the equilibrium between monomer and micelle in non-aqueous systems.

(b) Determination of Micelle Size and Shape in Non-aqueous Solvents.

Micelle formation may be detected and the size of the micelles determined by many physico-chemical methods (Table 2). Fluorescence depolarisation has been found capable of determining cmc's at as low as 10^{-7} molar concentrations,[113] but it has the disadvantage of the presence of a dye in the system which can alter the cmc,[114, 115] giving only an approximation of the true cmc.

To estimate micellar shape, light-scattering, viscosity and streaming birefringence methods have been used. These latter two techniques are necessary since other techniques such as diffusion and fluorescence depolarisation provide micellar sizes only of spheres which act in a manner equivalent to the actual, probably non-spherical particles. Thus in many instances the data obtained from two techniques has to be correlated before a true picture of the micellar particle can be obtained.

(c) Factors Affecting Non-aqueous Micellisation.

Several factors have been shown to affect the micellisation of solutes in organic solvents. Working with three α-monoglycerides (C_{10}, C_{16} and C_{18}) in benzene and chlorobenzene,

Debye and Prins[124] showed that increasing the number of carbon atoms in the hydrocarbon chain decreased the micellar weight. Also, the micellar weights were higher in benzene.

For a series of heavy metal fatty acid soaps in toluene the micelle size for any single fatty acid varied with the metallic cation.[111] In contrast, Kaufman and Singleterry,[119] working with a series of dinonylnaphthalenesulphonates whose cations were varied, found little variation in the aggregate weights. Arkin and Singleterry,[116] using benzene solutions of some arylstearate soaps, found that the addition of small amounts of water greatly decreased the viscosity of the very viscous anhydrous systems. This was interpreted as being due to highly elongated micelles becoming spherical. In contrast, the micelle size of some dinonylnaphthalenesulphonates was found to vary little on introducing water.[119] It was suggested that this latter behaviour was due to the micelle size being dependent mainly on the geometry of the acid residue. The size of Aerosol OT micelles has been observed to rise on increasing the hydration of the system.[127]

Temperature has been shown to affect the aggregation of lecithin, since in benzene it has been found that greater aggregation occurs on lowering the temperature.[130,131]

The addition of a second solute to the system, which is insoluble in the solvent by itself but soluble in the presence of the micellar species, affects the micelle size. This

effect will be considered in detail in a later chapter on solubilisation.

Micellisation of Lecithin.

In 1929 the first values of the aggregation of lecithin in certain solvents were published by Price and Lewis.[130] From their ebullioscopic measurements, the observed molecular weights of lecithin in ethanol and benzene were quoted as 797 and 3,388 respectively. This meant that monomers were present in ethanol while in benzene small aggregates were formed. It was not until 1950 that further estimations of the aggregation of lecithin were made. Working with three non-polar solvents, heptane, benzene and isoamyl ether, Faure and Legault-Demare[132] found from vapour pressure studies that lecithin existed as aggregates varying in size from 8,000 to 16,000 depending upon the solvent. On using three solvents that were more polar, glacial acetic acid, ethanol and butanol, the lecithin did not appear to aggregate. No precise details were presented for the micelle size in each solvent, the figures merely referring to the two groups of non-polar and more polar solvents. When a collodion membrane was suspended in the solvent, the lecithin was found to diffuse through it when the more polar solvents were used, but no diffusion occurred with the non-polar solvents. If, however, the lecithin solution in isoamyl ether had 20% v/v butanol added to it, diffusion took place. These results were interpreted as being due to the lecithin forming complex

micelles in the non-polar solvents, and the addition of the butanol to the isoamyl ether serving to decrease the size of the lecithin micelles.

Elworthy,[131] in 1959, published data on the micellisation of lecithin in benzene at 25 and 40°. Using an osmotic pressure technique it was found that at both temperatures lecithin formed small aggregates below a certain critical concentration. On passing this concentration, the smaller micelles aggregated into larger ones. The sizes of the small aggregates were 3,180 and 1,830, and the critical concentrations 0·73 and 0·68g./litre at 25 and 40° respectively. An approximate idea of the micellar size of the larger particles was evaluated assuming the law of mass action applied to the aggregation process. On fitting a mass action equation to the results, values were obtained for the number of small micelles aggregating to form the large micelles. Using these values, the larger micellar weights were found to be 57,000 and 43,000 at 25 and 40°. Comparing these figures to that of Price and Lewis it would seem that the micellar size decreased on increasing the temperature of the system.

In water, lecithin disperses to form sols containing particles of high molecular weight. As early as 1915 the particle size in water was estimated[133] as 350,000. The fact that the particles were large was further indicated later by ultrafiltration[134] and diffusion.[135] From light-scattering data

Robinson,[136] in 1960, measured the particle size as 2.7×10^6 at 20°. This work was done with dilute sols where the concentration was less than 0.3g./litre. No evidence was obtained to indicate the presence of a cmc, even at a concentration as low as 5×10^{-6}g./litre. Following Robinson's work, and working with concentrated sols in the 50 to 150g./litre range, Saunders, Perrin and Gammack[137] obtained microscopically visible particles. By ultrasonic irradiation of these sols, the particles were broken down to give stable micelles of molecular weight 10^7. From preliminary investigations of the more dilute sols, dispersion of the lecithin by ultrasonic irradiation again provided particles of about the same size as that found by Robinson.

These investigations of lecithin in water were all carried out with naturally derived material. Since such preparations were known to consist of a variable mixture of several lecithins, some preliminary investigations of single synthetic lecithins soon followed.[138] Working with fully saturated lecithins, Saunders found that the lecithin with the longest chain that provided a stable aqueous dispersion was dilauryl lecithin. With acids of longer chain length the lecithins were not dispersable. Considering the unsaturated lecithins, dilinoleyl lecithin gave a clear stable sol when ultrasonically irradiated.[137] With dioleyl and stearoyl-oleyl lecithins dispersion was more difficult. They both formed turbid sols and even on irradiation, the clarity

obtained with the dioleyl lecithin was only temporary.

In addition to the size of lecithin in various solvent systems, its structure and shape have been investigated. Faure and Legault-Demare considered that in water the polar grouping of the molecule lay on the outside of the micelle, while in the non-polar organic solvents this grouping was at the micelle centre. Elworthy's work agreed with this for both the small and large aggregates in benzene, while Saunders'[135] work agreed with respect to water.

Using X-ray diffraction studies, Bear, Palmer and Schmitt[139] identified lecithin in both the dry and wet states as existing as bimolecular leaflets. With a molecular model of lecithin, Saunders[135] showed it was possible for the lecithin to assume a configuration where the two fatty acid chains lay parallel to each other while the negatively charged oxygen of the phosphate group lay directly below one of the fatty acid chains and the positively charged nitrogen of the choline group lay below the other. This arrangement whereby the cross-sections of the polar head and hydrocarbon tail were nearly equal would tend to make the lecithin form a laminar micelle. In addition, it was suggested that the micelle could be further stabilised by the choline group of one molecule interacting with the phosphate group of the adjacent molecule. The light-scattering dissymmetry measurements of Robinson indicated that in water the micelles could be rod-shaped, but that the results best fitted

a micelle having the shape of a laminated cylinder of diameter 910Å with a thickness equivalent to a bimolecular leaflet. From the viscosity of the ultrasonically irradiated sols, asymmetry was indicated, and it was calculated that a shape corresponding to an oblate ellipsoid of thickness equal to a single or double bimolecular layer best fitted the results.

In trying to ascribe a structure to lecithin micelles in benzene, Elworthy[140] used viscosity and diffusion techniques. Working at 25° and treating the micelles as ellipsoids, micellar weights of 55,000 (prolate) and 54,000 (oblate) were obtained. He assumed that the micellar structure was either laminar or spherical, these two structures being the generally accepted micellar forms. Then by basing calculations on a molecular model and comparing the figures to the experimental data, the laminar structure was indicated as the more likely.

SOLUBILISATION.

Solubilisation may be defined as the spontaneous solution of a normally solvent-insoluble substance (the solubilisate) by dilute solutions of a second substance (the solubiliser) which exists as micelles in the solvent. As with micellisation, most solubilisation studies have been made in aqueous solutions, the interest in non-aqueous solutions being slower to develop. In solutions of amphipathic compounds, solubilisation usually only begins to become noticeable on reaching the cmc, the effect therefore being directly dependent upon the presence of micelles. However, because of the very small micelles present below the cmc, sub-cmc solubilisation has been detected [141,142] in some cases.

Solubilisation in Aqueous Solution.

Three main solubilisation mechanisms have been suggested.[143] Firstly, the adsorption of the solubilisate on the micelle surface,[144] secondly, its incorporation into the micelle hydrocarbon core,[145,146,147] and thirdly, its orientation in the micelle wall.[148,149,150] Evidence suggests that the first method could be considered as a sub-class of the third since the solubilisates adsorbed show some penetration into the micelle wall.[151,152,153] The latter two methods are considered as the main mechanisms. Several other more specific methods have been suggested, these involving a change in micellar structure on solubilisation,[154] complex

formation [155,156] and ion-exchange. [157]

The factors affecting solubilisation are difficult to generalise due to the uniqueness of many solubilised systems. Some generalisations may however, be made, detailed accounts being given elsewhere. [143,158,143] Klevens stated that most factors which decrease the cmc and increase micellar size will cause increased solubilisation. Thus, considering the chemical nature of the solubiliser, as its chain length increases, on ascending a homologous series, its solubilising power also increases. [143,159,160] A similar chain length change for the solubilisate decreases solubilisation, a decrease also being obtained by decreasing its polarity. [159,161] [143,162] Temperature increase causes increased solubilisation. [160,163,164,165] There are, of course, exceptions to these generalisations.

The concentration of solubilisate in micelles is thought not to increase greatly above a plateau concentration. [99,146,166] If the solubilisate is a solid, the micelles will become and remain saturated, the excess solid remaining as a separate solid phase. If the solubilisate is liquid, that in excess of saturation may either remain as a second (unchanged) phase or it may cause the separation of two phases (aqueous and non-aqueous) each possessing micelles and solubilisate. [143] The entire range of behaviour of the ternary system of water, amphipath and non-aqueous solvent has been described. [158]

Solubilisation in Non-aqueous Solution.

The mechanism of solubilisation in non-aqueous solvents is considered to be similar to the general pattern in water. The solubilisate may therefore enter the micelle core or be orientated in the units forming the micelle. Water is the best example of core solubilisation, while many dyes have structures similar to amphipaths which enable them to fit into the micelle wall. It is the solubilisation of water and dyes that has commanded most attention in non-aqueous solvents.

The fact that water is strongly solubilised by soap in oil was found by Mathews and Hirschorn[127] who obtained a water uptake of 50 molecules per molecule of Aerosol OT. It was suggested that the micelles consisted of a microdroplet of water covered by a monomolecular layer of soap. Sodium dinonylnaphthalene sulphonate in benzene solubilised 10 molecules of water per molecule.[117] Since this latter ratio decreased sharply on increasing the temperature, it was suggested that the water was held by hydrogen bonding rather than as a core droplet. The effect of temperature change has a considerable, but irregular, effect on the solubilisation of water by soap.[167,168,169] Palit[170] found that when soap was dissolved in a mixture of two solvents, it solubilised more water than when dissolved in either single solvent. When present in near trace quantities, water can have a pronounced effect on certain systems. Thus, on adding 0·05% water to benzene solutions of sodium and lithium soaps, Arkin and Singleterry found the viscosity was

greatly decreased.[116] This was assumed to be due to the breakdown of the long thread-like anhydrous micelles to smaller units. In direct contrast, aluminium alkoxide soaps in benzene showed a marked increase in viscosity when small quantities of water were added to the dry system.[171,172] This was explained by small micelles aggregating to long thin threads.

McBain, Merrill and Vinograd[173] effectively solubilised several dyes, including eosin, fluorescein and crystal violet, in benzene, toluene and n-heptane using some fatty acid esters of glycols as solubilisers. Though several of the solubilisers were very selective, solubilisation was enhanced by using solubilisers containing higher fatty acids and by increasing the temperature. Rhodamine B has been used successfully for micelle detection and cmc determination in benzene,[174] since its fluorescence in micellar solutions in benzene is very much greater than that in sub-cmc solutions.[175] Ross, working with several dyes in oil, tested the solubilising action of several materials. Having found mannitan monooleate to have the widest solubilising range, he subsequently found that varying the oleate concentration produced a variation in the degree of dye solubilisation. A minimum was found for three dyes tested, this minimum occurring at approximately the same oleate concentration. This Ross took to be its cmc.

Solubilisation by Lecithin.

It was over fifty years ago that the first observations were made which today would be interpreted in terms of

solubilisation. As early as 1901, Bing,[176] using ethereal and alcoholic phosphatide solutions dissolved various organic and inorganic compounds such as sodium chloride, sodium lactate, strychnine hydrochloride and sugar that were by themselves insoluble in the two solvents. He considered this effect to be the result of a molecular combination. Soon after this a mixed brain lipid extract in chloroform was seen on shaking with an aqueous methylene blue solution to develop a blue colour, the dye being insoluble in chloroform.[177] Further early observations[178] were that in ether, using what was probably a mixed phosphatide preparation, certain other substances that were themselves solvent-insoluble dissolved in the solvent when it contained mixed phosphatide. Examples of such substances included glucose, trypsin and oxides of iron.

Bode and Ludwig[179] have shown that because of lecithin's amphipathic nature it can solubilise in aqueous and non-aqueous solvents. They prepared a series of solutions of varying concentrations of lecithin in chloroform and added excess urea to each. The maximum uptake of urea was found to increase linearly with increasing lecithin concentration, in the range 0·1 to 2% lecithin in chloroform. For cholesterol solubilisation by lecithin in water, a further linear relationship was obtained in the concentration range 0·05 to 0·8% lecithin. The relative ease with which lecithin solubilises cholesterol in water has been noted by other workers.[180] In petroleum ether it has been

stated that neither lecithin nor cholesterol alone were capable of solubilising urea, but when both were present the urea was noticeably taken up.[181] Saunders, Perrin and Gammak[137] ultrasonically irradiated lecithin-cholesterol aqueous sols and obtained a concentration of 10% cholesterol in water in the presence of 20% lecithin. This system formed a clear gel, but when the concentrations were decreased to 8 and 16% no gelation occurred.

On extracting phosphatides from natural sources, using ether and chloroform, many other substances are extracted that are normally insoluble in these solvents. In a study of the solubilisation of several of the likely contaminants, Baer and his colleagues used pure synthetic phosphatides. This work was started using fully saturated α-lecithins, with ether and chloroform as solvents.[182] In chloroform an uptake of sodium chloride, sodium sulphate and glucose was obtained. In ether, the saturated lecithins were insoluble, but in the presence of 1% water in the ether the limited lecithin solubility enabled a slight uptake of sodium chloride to be demonstrated. On changing to the ether-soluble unsaturated dioleyl-lecithin[38] it was found that glucose and saccharose were solubilised in moist and dry ether and chloroform, while sodium chloride and serine were only solubilised in moist ether. From this it was assumed that the solubilising action of lecithin contributed to the unexpected extraction of certain substances in phosphatide purification.

Elworthy has investigated the solubilisation of a series

of twelve dibasic fatty acids in benzene.[183] From this work several trends have been indicated. Thus the acids with an odd number of carbon atoms were solubilised in greater amount than those with even numbers. As the series was ascended, the volume of solubilisate increased though the ratio of molecules of acid to molecules of lecithin decreased from 2·15 for the C_2 acid to 0·629 for the C_{16}. These facts all refer to the large micelles, and since some solubilisation was observed by the small micelles, a correction had to be applied in all cases. In the explanation of a possible method of the packing of the fatty acid molecules, it was assumed that the molecules were at the polar head core of the micelle and that they could protrude into the benzene. Such a protruding molecule would simulate a monocarboxylic acid, this series of acids being benzene-soluble. The difference in degree of solubilisation between the odd and even numbered series was explained on the basis of a different method of packing for each series. Thus considering the cross-section of the micelle as rectangular, the acids of one series packed parallel to the longer side of the rectangle, while the acids of the other series packed at right angles to this.

Water solubilisation has been investigated in three hydrocarbon solvents, benzene, toluene and xylene.[184] With three different concentrations of lecithin, between 0·4 and 6% in each solvent, the uptake of water provided a nearly constant

ratio of weight of water to weight of lecithin. This ratio lay between 0·32 and 0·33 g./g. and was stated to be equivalent to about 14 molecules of water per molecule of lecithin. This solubilisation, which seemed to be independent of the solvent used in the work, was stated to be caused by interaction of the lecithin polar grouping with the water molecules.

Not long after this it was shown that the adsorption of water by dry lecithin was due to the polar phosphorylcholine grouping, since by comparison to lecithin, triolein and tristearin adsorbed poorly.[185] On comparison to the saturated synthetic dipalmitoyl lecithin, natural lecithin containing approximately one double bond per fatty acid was shown to adsorb water better.[186] It was found that at 25°, on saturation of the lecithin with water, a molecule of natural lecithin was associated with 19·5 water molecules, while the synthetic material was associated with 10·5 molecules. This was explained by an increase in the cross-sectional area of lecithin when unsaturation was present, allowing a larger effective area for adsorption at the head groups.

Recently, the solubilisation of the sodium and potassium salts of the dye m-(p-anilino)-phenylazobenzenesulphonic acid by lecithin micelles in benzene has been investigated.[187] The dyes, insoluble in benzene, were taken up by lecithin solutions and ultracentrifugal sedimentation showed them to be associated with the micelles. The amount of dye solubilised was estimated colourimetrically, and the amount of dye solubilised was plotted

against lecithin concentration at both 25 and 40°. All four plots had two breaks, one coinciding with the concentration found by Elworthy for the aggregation of small micelles into large micelles,[131] the other in more dilute solution. These lower breaks were presumed to be the cmc's, their values being, at 25°, 3.3×10^{-4} %, and at 40°, 7.5×10^{-4} % from the sodium dye and 9.8×10^{-4} % from the potassium dye.

MYELIN.

Peripheral nerve fibres are known to consist of two main structural features, these being the axon, or fibre core, and its covering, the medullary, or myelin sheath. The molecular structure of this sheath has not yet been completely determined. However, it seems that the constituent molecules are orientated in relation to the fibre direction suggesting that the sheath, or myelin, has a structural rather than a metabolic function. This suggestion is also borne out by the fact that in abnormal body conditions, such as starvation, the constituent molecules of the myelin do not undergo any change while similar molecules in other parts of the body are affected.

X-ray diffraction studies have proved successful in elucidating the finer structure of myelin.[139,188] Thus on considering a cross-section of the myelin, in the radial direction it was seen to consist of several similar repeating units, these units subsequently being found to be composed of two bimolecular lipid layers plus two layers of protein, the lipid and protein layers alternating with each other. The thickness of the protein and lipid layers have been estimated as 30 and 55Å respectively, the whole unit being 170 - 171 Å thick. These findings for the myelin sheath result in the myelin structure simulating a cell membrane rolled on itself many times. The sheath, or other similar structure such as a phosphatide micellar system, would therefore seem to be a convenient medium with which to study cell

membrane characteristics in vitro.

On analysis, myelin has been shown to contain several phosphatides and cholesterol both in approximately equimolecular amounts, the cholesterol being present as the free alcohol. It was suggested that a stable complex of cholesterol and phosphatide was formed in the myelin.[188] To form this complex, the phosphatide's two fatty acid chains lay parallel, and alongside them lay the cholesterol adhering by Van der Waal's forces. The polar grouping of the phosphatide was curled over enabling its terminal group to lie close to the cholesterol's free hydroxyl group. Where this phosphatide terminal group was an amino group, there was the possibility of hydrogen bonding, with perhaps other less specific ionic relationships for other phosphatide terminal groups. This would help to stabilise the complex. The fact that the thickness of the lipid layer was less than twice the extended length of the average myelin phosphatide was explained by this curling effect, and also by the packing of long chain acids opposite short chain acids.

From the thickness of the protein layer it was thought to consist of two protein monolayers. With the curling of the phosphatide molecules the phosphate group lay next to the protein layer providing the possibility of an association between the two, with perhaps further myelin stabilisation. At present the nature of the protein is unknown.

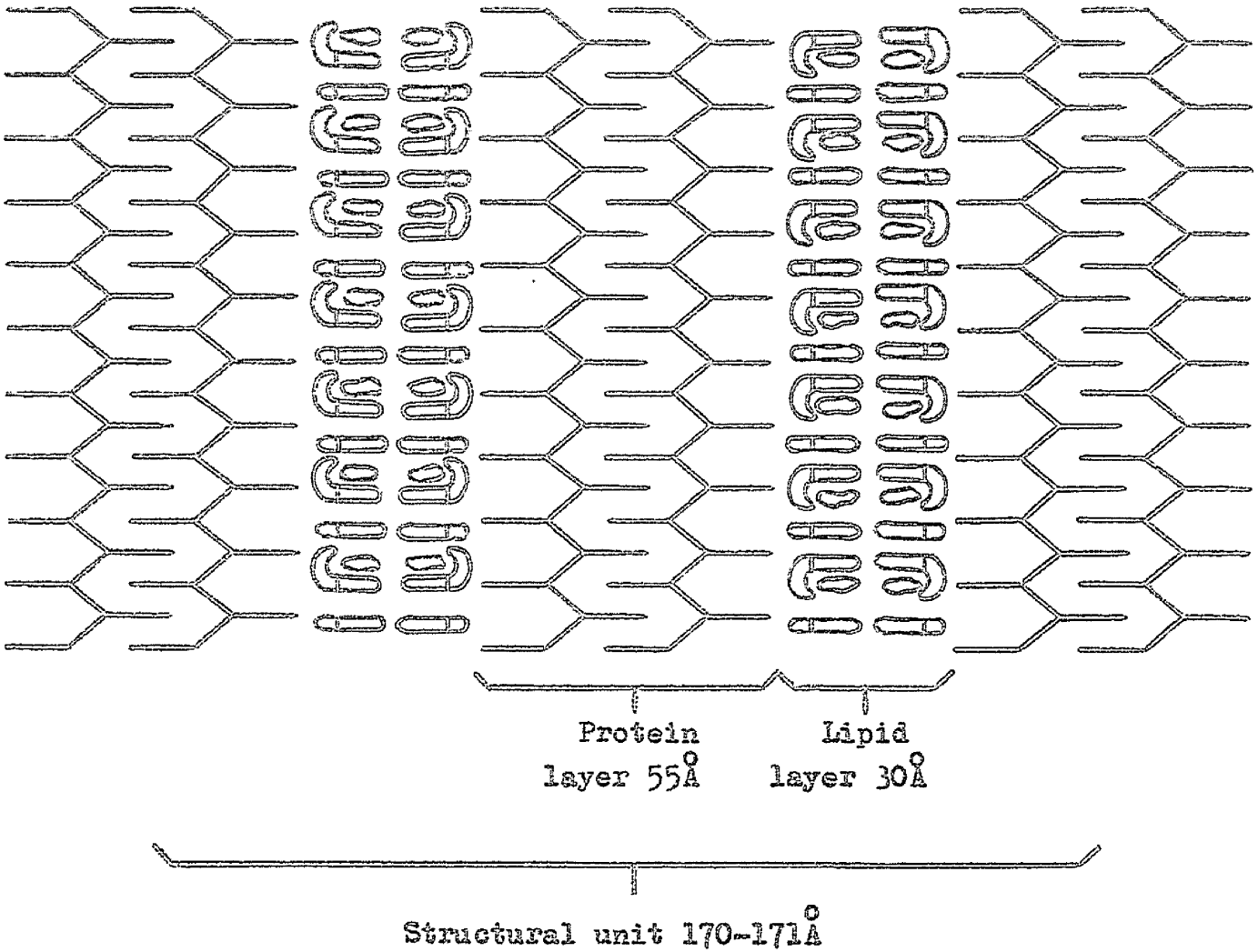


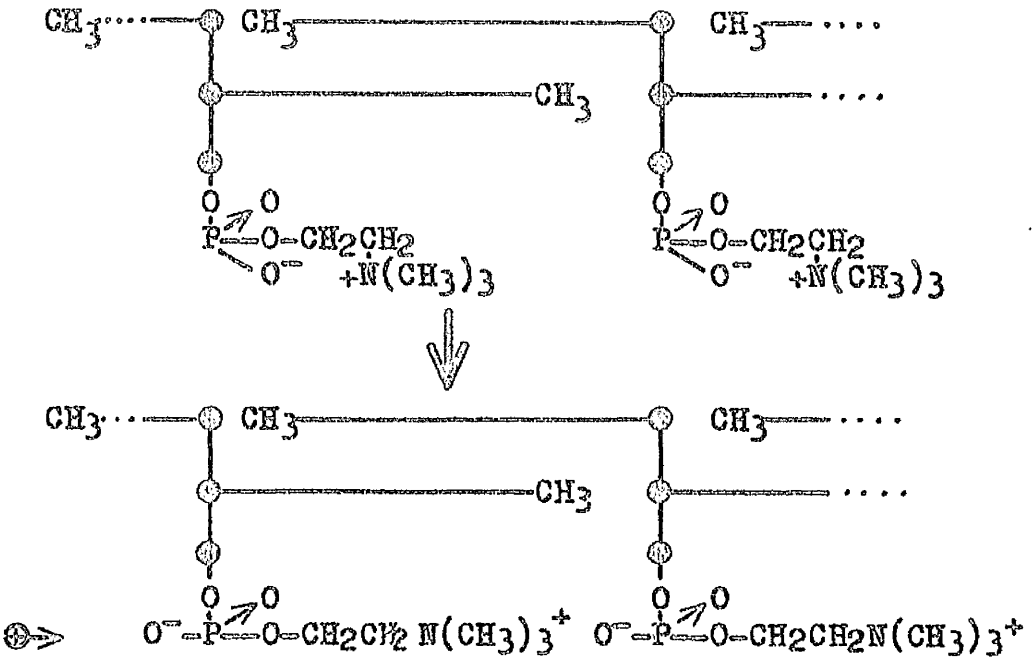
Figure 1.

Peripheral Nerve Myelin Structure.

In addition to protein and lipid, myelin contains some water. The amount of water present is uncertain or very variable, the range of values so far obtained being 30 to 65% by weight.[189] Its method of incorporation into the structural unit is also speculative. From X-ray diffraction data, it would seem to be held in an organised manner at the ionic interfaces aiding the stability of any lipid-protein associations.

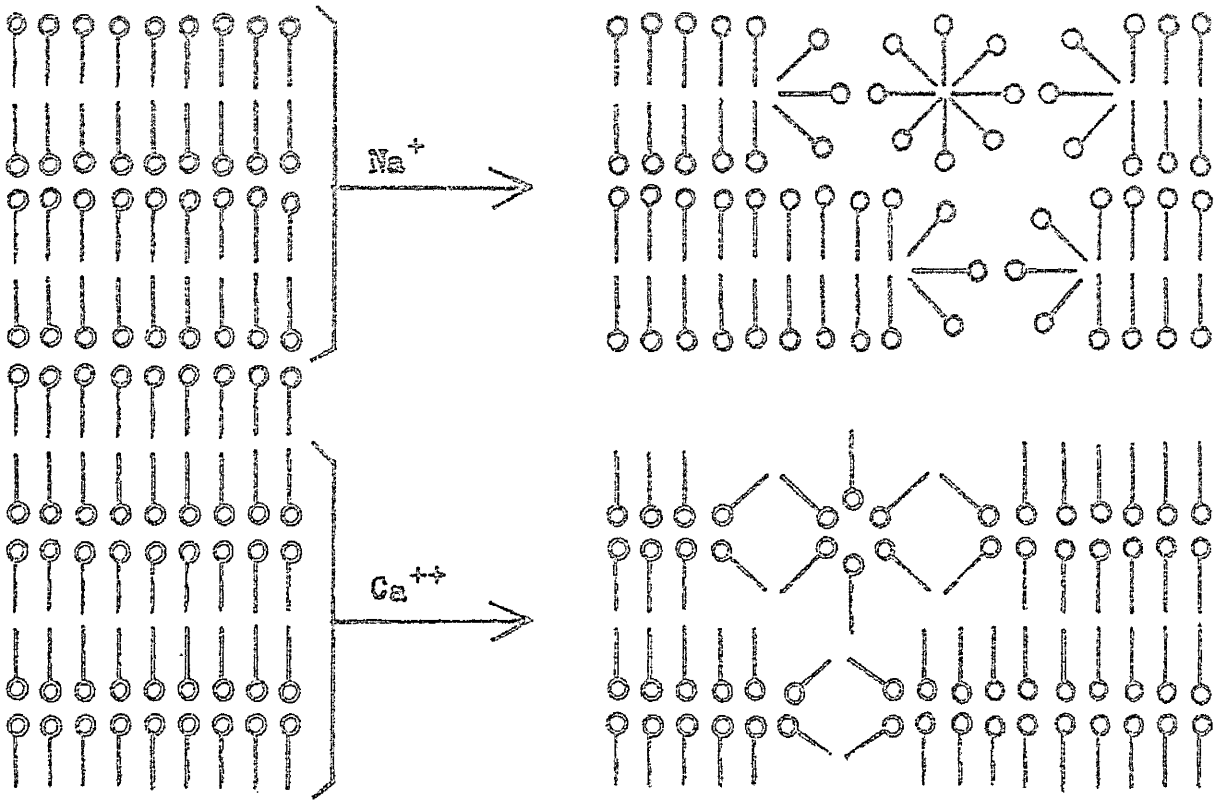
Johnson, McNabb and Rossiter,[190] in an analysis of the lipid of peripheral nerve, obtained cerebroside, cholesterol and phosphatide in a molecular ratio of 1:2:2. On further examination the phosphatide was found to be mostly sphingomyelin, with some cephalin and lecithin. Acetal phosphatide, inositol phosphatide, ganglioside, strandin and proteolipids have also been extracted from myelin and identified, however their exact roles in the sheath are still unknown. In the light of the structural data available, peripheral nerve myelin was represented diagramatically by Finean[191] (fig.1).

Hirt and Berchtold[192] have suggested that the conduction of nervous impulses could be carried out by lecithin. This was based on the transformation of the lecithin, during conduction, from an intramolecular zwitterionic form to an intermolecular form. This was represented thus:



The conduction of a single impulse involved a slight movement of the zwitterionic charge carrier, the $-O^{-}-N(CH_3)_3^{+}$ structure. This movement was used to explain why the velocity of nervous conduction was slower than metallic conduction. It was also thought to be a possible explanation for the nerve only conducting impulses of a certain intensity, and the conduction only taking place when the impulse exceeded a certain intensity minimum.

Wolman and Wiener [193] very recently investigated the effects of high concentrations of sodium and calcium ions on myelin structure. Peripheral myelin, after homogenisation with 1M sodium chloride formed an 'oil in water' emulsion, and extraction with water yielded about 50% of the myelin cerebroside, phosphatide and cholesterol. Homogenisation with 0·5M calcium chloride gave a 'water in oil' emulsion which yielded little



Phosphatide molecule: Polar head

Hydrocarbon tail

Protein layers have been omitted

Figure 2.

A possible Mechanism of Cell Membrane Phosphatide Rearrangement in the Presence of Sodium and Calcium Ions.

lipid on aqueous extraction. This change in the hydrophilic-lipophylic balance of the phosphatides (the emulsifying agents) with ionic environmental change, prompted the assumption that in vivo, ions and any other factors capable of effecting the same change in the phosphatide could have a similar action on the cell membrane phosphatide.

A possible mechanism of cell permeability, based on these findings, was suggested. Thus a factor producing a change of an area of cell membrane into an 'oil in water' emulsion would produce aqueous channels through which the cytoplasm could communicate with the outside (fig. 2). A change to a 'water in oil' emulsion might allow lipid-soluble substances to cross the cell wall. In an equilibrated area, the lipid and aqueous layers are parallel and the inside of the cell does not communicate with the outside.

It was further suggested that the bending of the phospholipid layer, from the equilibrated state, in the direction of a 'water in oil' emulsion would produce an increased density of charges on the surface, this charge density increase perhaps being related to nerve impulse transmission.

THEORY OF EXPERIMENTAL METHODS.

A. LIGHT-SCATTERING.

Since the theory of light-scattering has been reviewed by a number of authors,[194-200] only a brief outline will be presented.

(a) Dilute Gases.

Here, a number of independent particles are considered, each particle being small compared to the wavelength of light. The incident electric field (strength, $\mathcal{E}$) induces an oscillating dipole (moment, p), in each particle, which acts as a source of scattered light. The magnitude of the dipole moment is dependent on the polarisability, α, so,

$$p = \alpha\mathcal{E} \qquad (1)$$

On substituting equation (1) in the equation for the electric field of a light wave,

$$\mathcal{E} = \mathcal{E}_0 \cos 2\pi(\nu t - x/\lambda) \qquad (2)$$

$$p = \alpha\mathcal{E}_0 \cos 2\pi(\nu t - x/\lambda) \qquad (3)$$

where $\mathcal{E}_0$ is the maximum amplitude, ν the frequency, λ the wavelength, t the time and x the location along the line of propagation. The field strength of the scattered radiation is proportional to d^2p/dt^2, to $1/r$ (r is the distance from dipole to observer) and to $\sin\theta_1$ (θ_1 is the angle between the dipole axis and the line of observation). Dividing by $\bar{c}^2$ for dimensional correctness ($\bar{c}$ is the velocity of light), from equation (3) the field strength of scattered light is,

$$\mathcal{E}_s = \frac{4\pi^2\nu^2\alpha\mathcal{E}_0\sin\theta_1\cos 2\pi(\nu t - x/\lambda)}{c^2 r} \qquad (4)$$

Squaring equation (4) to give the intensity of scattered light (i_s) and equation (1) to give the intensity of incident light (I_0),

$$\frac{i_s}{I_0} = \frac{16\pi^4\alpha^2\sin^2\theta_1}{\lambda^4 r^2} \qquad (5)$$

The polarisability of a medium is related to its dielectric constant (ϵ) and hence to its refractive index (n), [201]

$$\epsilon - 1 = n^2 - 1 = 4\pi N\alpha \qquad (6)$$

Expressing equation (6) in terms of specific refractive index increment (dn/dc),

$$\alpha^2 = c^2(dn/dc)^2/4\pi^2 N^2 \qquad (6a)$$

where N is the number of particles per c.c. and c the concentration in g./ml. For the gas molecular weight (M) and Avogadro's number ($\bar{N}$), $M/\bar{N} = c/N$, therefore rewriting equation (5) for N particles and introducing equation (6a),

$$\frac{i_s}{I_0} = \frac{4\pi^2 c(dn/dc)^2 M\sin^2\theta_1}{\lambda^4 \bar{N} r^2} \qquad (7)$$

Equation (7) is for polarised incident light, hence for unpolarised light,

$$\frac{i_\theta}{I_0} = \frac{2\pi^2(dn/dc)^2 Mc(1+\cos^2\theta)}{\lambda^4 \bar{N} r^2} \qquad (8)$$

where θ is the angle between the line of observation and the direction of the incident light.

(b) Scattering from Solutions.

For a solute in a medium of refractive index n_0, equation

(6) is,

$$n^2-n_o^2 = 4\pi N\alpha \qquad (9)$$

which leads to

$$\frac{i_\theta}{I_o} = \frac{2\pi^2 n_o^2 (dn/dc)^2 Mc(1+\cos^2\theta)}{\lambda^4 N r^2} \qquad (10)$$

Equation (10) only applies to an ideal solution. Where the scattering elements are fixed in space (as in a transparent crystal), destructive interference between scattered rays results. In liquids and solutions, fluctuations of composition occur, and considering the excess scattering of a solution over that of pure solvent, a concentration fluctuation (δc) can be regarded as responsible for the scattering. There is also a corresponding fluctuation in polarisability ($\delta\alpha$). Thus from equation (5), considering an element of solution, of volume ψ, such that $N = 1/\psi$,

$$\frac{i_s}{I_o} = \frac{16\pi^4 (\overline{\delta\alpha})^2 \sin^2\theta_1}{\lambda^4 r^2 \psi} \qquad (11)$$

The fluctuation in polarisability can be expressed in terms of concentration fluctuation to give, for unpolarised incident light,

$$\frac{i_\theta}{I_o} = \frac{2\pi^2 \psi n^2 (dn/dc)^2 (1+\cos^2\theta)(\overline{\delta c})^2}{\lambda^4 r^2} \qquad (12)$$

The concentration fluctuation can be related to the change of free energy with concentration,

$$(\overline{\delta c})^2 = kT/(\delta^2 G/\delta c^2)_{T,P} \qquad (13)$$

and to the chemical potential (μ_1) and partial molar volume of solvent ($\overline{V_1}$),

$$\left(\frac{\partial^2 G}{\partial c^2}\right)_{T,P} = -\frac{\Psi}{c\bar{V}_1}\left(\frac{\partial \mu_1}{\partial c}\right)_{T,P} \qquad (14)$$

Introducing equations (13) and (14) into (12) then gives

$$\frac{i_\theta}{I_o} = \frac{2\pi^2 n^2 (dn/dc)^2 (1+\cos^2\theta)c}{\lambda^4 r^2 \left[-(1/\bar{V}_1 kT)(\partial\mu_1/\partial c)_{T,P}\right]} \qquad (15)$$

As the dependence of solvent chemical potential on solute concentration is given by

$$\mu_1 - \mu_1^o = -RT\bar{V}_1 c_2\left(\frac{1}{M_2} + Bc_2 + Cc_2^2 + \ldots\right) \qquad (16)$$

where B and C are the second and third virial coefficients respectively,

$$\frac{i_\theta}{I_o} = \frac{2\pi^2 n_o^2 (dn/dc)^2 (1+\cos^2\theta)c}{\lambda^4 N r^2 (1/M + 2Bc + 3Cc^2 + \ldots)} \qquad (17)$$

substituting n_o for n since their difference is negligible in dilute solution.

Defining Rayleigh's ratio, R_θ, as

$$R_\theta = \frac{r^2 i_\theta}{I_o(1+\cos^2\theta)} \qquad (18)$$

and the turbidity, T as

$$T = 16\pi R_\theta/3$$

equation (17) becomes

$$\frac{Hc}{T} = \frac{1}{M} + 2Bc + 3Cc^2 + \ldots \qquad (19)$$

where the optical constant, H is

$$H = \frac{32\pi^3 n_o^2 (dn/dc)^2}{3\lambda^4 N} \qquad (20)$$

Equation (19) gives the relationship between the measurable turbidity and optical constant and the molecular weight and virial coefficients at finite concentrations. The second virial coefficient can be interpreted in terms of molecular properties.[202]

(c) Depolarisation.

Having, so far, only considered small optically isotropic particles, if small anisotropic particles are considered, a correction to the observed scattering may have to be introduced because of random particle orientation. When a dipole is induced in an optically isotropic particle, the dipole is aligned parallel to the electric vector of the incident light. Due to the isotropy of the particle, no matter its orientation, the dipole and electric vector remain parallel. In the case of anisotropic particles, the dipole is unlikely to be parallel to the vector (due to random orientation and anisotropy) this being liable to cause excess scattering which would have to be corrected for when using the above equations to determine M.

For an isotropic particle scattering from incident unpolarised light, the induced dipole can be resolved into two independent dipoles that are aligned one at $\theta = 90^{\circ}$ in the horizontal plane and the other at $\theta = 90^{\circ}$ in the vertical plane. On viewing the scattered light at $\theta = 90^{\circ}$, the former dipole will contribute no scatter. (If scatter existed it would have been horizontally polarised.) The light scattered by the particle will therefore

all be vertically polarised. It is the ratio of the intensities of the horizontally and vertically polarised scattered lights that is the depolarisation (ρ), the ratio being used to correct for any increase in scatter due to anisotropy.

For an isotropic particle the ratio is zero. An anisotropic particle whose induced dipole is not aligned parallel to the incident light has a ratio which is usually greater than zero. Cabannes [203] has related the excess scattering of anisotropic particles to the depolarisation, such that the correction factor (Cabannes' factor) for the Rayleigh ratio is $(6-7\rho)/(6+6\rho)$.

(d) Scattering from Large Particles.

When the size of the solute particle exceeds $\lambda/20$ it can no longer be considered as a point scatterer since destructive interference results from rays scattered by different parts of the particle. The interference is absent at $\theta = 0$, and increases as θ increases. The particle scattering factor $P(\theta)$, is defined as,

$$P(\theta) = \frac{\text{Scattered intensity for large particle}}{\text{Scattered intensity in the absence of interference}} \qquad (21)$$

For a particle of σ scattering points,

$$P(\theta) = \frac{1}{\sigma^2} \sum_{i=1}^{\sigma} \sum_{j=1}^{\sigma} \frac{\sin \mu r_{ij}}{\mu r_{ij}} \qquad (22)$$

where $\mu = (4\pi/\lambda_1)\sin\theta/2$, $\lambda_1 = \lambda/n_o$ and r_{ij} is the distance between two scattering elements. $P(\theta)$ can be related to the radius of gyration of a particle (Rg), giving as a limiting expression,

$$\mathop{\mathcal{L}}_{\theta \to 0} 1/P(\theta) = 1 + \frac{16\pi^2 Rg^2 \sin^2\theta/2}{3\lambda_1^2} \qquad (23)$$

Equation 19, for large particles, becomes

$$\mathop{\mathcal{L}}_{c \to 0} \frac{Hc}{T_\theta} = \frac{1}{MP(\theta)} = \frac{1}{M}\left(1 + \frac{16\pi^2 Rg^2 \sin^2\theta/2}{3\lambda_1^2}\right) \qquad (24)$$

The Zimm method is convenient for treating data on large particles. Hc/T_θ is plotted against $(\sin^2\theta/2+kc)$, where k is an arbitrary constant. The lines at constant angle are extrapolated to $c = 0$, and equation (24) applies to the zero concentration line so produced. The lines at constant concentration are extrapolated to $\theta = 0$, producing a line to which equation (19) applies, with a slope of $2B/k$. The intercepts of both the zero angle and concentration lines are $1/M$. The radius of gyration can be interpreted in terms of molecular shape,[204] and is one of the most useful quantities obtained from light-scattering. Complete expressions for $P(\theta)$ have been calculated for variously shaped models[197,205,206] (e.g. rod, coil, sphere) and Zimm plots can be used to give an idea of molecular shape.

A second method of treatment for results from large particles is by measuring the dissymmetry, Z_θ, where

$$Z_\theta = \frac{i_\theta}{i(180-\theta)} = \frac{P(\theta)}{P(180-\theta)}$$

Usually Z_{45} is measured. After a choice of molecular model has been made, the appropriate correction to the 90° scattering is made to enable equation (19) to be used to determine the molecular weight. The characteristic dimensions of the particles can also be determined from Z_{45}.

(e) Three Component Systems.

Where a solute is present in a mixture of two solvents, interaction between solute and one solvent component may cause additional composition fluctuations (i.e. excluding those for two component systems). The probability of such fluctuations can be calculated[207,208,209], and for non-electrolytes the activity coefficients can be expressed as a power series whose coefficients can be related to various terms, e.g. G_{10}, etc., in the equation

$$\frac{H_2 c_2}{\Delta T} = \frac{1}{M_2}(1+G_{10}c_1+G_{01}c_2+G_{20}c_1^2+G_{11}c_1c_2+G_{02}c_2^2) \qquad (25)$$

where c_2 is the concentration of macromolecular solute in the binary solvent, c_1 the concentration of additive solvent in both the solution and binary solvent (in g./g. of primary solvent) and ΔT the difference in turbidity between solution and solvent mixture. Also,

$$H_2 = 32\pi^3 n_o^2 (dn/dc_2)^2 / 3N\lambda^4 \rho_o \qquad (26)$$

where (dn/dc_2) is measured in the binary solvent, and ρ_o is the

mass of primary solvent per unit volume of solution.

Experiments have been done by Blaker and Badger[210] on nitrocellulose in acetone-water mixtures in which a specific adsorption of water by the macromolecules was shown. The use, therefore, of equation (19) in such a solvent may lead to incorrect molecular weights. A correct value can only be expected if the two solvents had the same refractive index or if the refractive index of the solvent mixture was independent of solvent composition.[211]

(f) Molecular Weight Averages.

When a particle size is obtained for such as a micellar solute or fractionated polymer, the size is usually that of an average of all the varying-sized particles considered. These size distributions can be treated statistically to provide several average values depending on the method of calculation.

The number average molecular weight, $\bar{M}_n$, may be expressed by:

$$\bar{M}_n = \sum N_i M_i / \sum N_i$$

where N_i is the number of particles of type i present, M_i being their molecular weight. This summation is for all types of molecules present.

The weight average molecular weight, $\bar{M}_w$, is given by:

$$\bar{M}_w = \sum W_i M_i / \sum W_i$$

where W_i is the weight of species i present.

For a homogeneous mixture $\bar{M}_w = \bar{M}_n$. Their ratio is a useful indication of the degree of polydispersity of a system. Further statistical averages are also obtainable. Thus by rewriting

$$\bar{M}_n = \sum N_i M_i / \sum N_i$$

and

$$\bar{M}_w = \sum N_i M_i^2 / \sum N_i M_i$$

a higher average, the z - average, can be denoted by

$$\bar{M}_z = \sum N_i M_i^3 / \sum N_i M_i^2$$

If the molecular weight is evaluated by methods using colligative properties, $\bar{M}_n$ is the average obtained. The light-scattering technique yields $\bar{M}_w$.

B. VISCOSITY.

For dilute colloidal solutions where the solute is in the form of rigid spheres, the Einstein expression for the relationship between the viscosities of solution (η) and solvent (η_o) is

$$\eta = \eta_o(1+2.5\phi) \qquad (27)$$

where ϕ is the solute volume fraction. This may be rewritten in the form

$$(\eta_r - 1)/\phi = \eta_{sp}/\phi = 2.5 \qquad (28)$$

where $\eta_r = (\eta/\eta_o)$ is the relative viscosity and η_{sp} the specific viscosity.

Among the assumptions made to derive the equations, Einstein assumed that the solutions were sufficiently dilute that no solute-solute interactions occurred, and it was subsequently shown [212,213]

that the equations were valid up to solute concentrations of about 3%. On the basis of equation (28), if (η_{sp}/ϕ) is plotted against ϕ, the intercept at $(\eta_{sp}/\phi)_{\phi=0}$ is 2·5 [214,215]

Following this work, investigations were made of possible adaptations of the Einstein equation that would hold for rigid non-spherical particles. This led to the evaluation of the Simha relationship [216] for ellipsoids

$$\eta = \eta_0(1+\nu\phi) \quad \text{or} \quad \eta_{sp}/\phi = \nu \qquad (29)$$

where ν was a function of the axial ratio of the ellipsoid and was equal to 2·5 for spheres and larger than 2·5 for ellipsoids. Simha [216] also derived ν in terms of axial ratio for both the prolate and oblate ellipsoids, the prolate being obtained by rotating an elipse about its major axis, the oblate by rotation about the minor axis. Mehl, Oncley and Simha, [217] on the basis of the Simha relationships, then evaluated ν for both ellipsoids having axial ratios of 1 to 300. When the ellipsoid axial ratio is large, the prolate simulates a long thin rod, the oblate simulating a flat circular disc.

An experimental value of the viscosity intercept, $(\eta_{sp}/\phi)_{\phi=0}$ represented by $[\eta']$, can thus be compared to the values of Mehl, Oncley and Simha and the particle axial ratio determined. The axial ratio is a useful factor for helping to assign a shape to the particle and hence in constructing models of the particle. On deviating from a spherical shape, the particles are considered to become ellipsoidal, such that they approximate to either a

prolate or oblate ellipsoid.

The above Einstein and Simha relationships have been modified to apply to a solvated solute. Solvated particles act hydrodynamically as particles of macromolecular solute plus solvating liquid and have higher values of $[\eta']$ than the same unsolvated solute particles. Thus for spherical solvated particles, $[\eta'] > 2{\cdot}5$. To apply to solvated particles, Oncley [218] derived the relationship

$$\eta_{sp}/\phi = \nu(1+w/\bar{v}\rho) \qquad (30)$$

which for a spherical solvated solute may be written as

$$\eta_{sp}/\phi = 2{\cdot}5(1+w/\bar{v}\rho) \qquad (31)$$

where w is the weight (in g.) of solvating liquid per g. of solute, ρ the density of the solvating liquid and $\bar{v}$ the partial specific volume of the solute. Thus for a spherical solvated solute of known $[\eta']$, the extent of its hydration may be calculated. Also, for solutes of known solvation and $[\eta']$, ν values may be obtained. To fully characterise a solvated asymmetric solute, either the degree of solvation or asymmetry would have to be estimated by another technique since both factors are included in $[\eta']$.

C. TRANSLATIONAL DIFFUSION.

(a) Fick's Laws.

If in a solution there is an unequal solute concentration in different parts of the solution, solute will flow from the

high concentration areas to those of low concentration till the concentration throughout the solution is constant. Translational diffusion is this flow of solute.

According to Fick's first law, the amount of substance diffusing in direction x across an area A in time dt is proportional to the concentration gradient dc/dx. Thus

$$dm \propto A\frac{dc}{dx}\,dt \qquad \text{or} \qquad dm = -DA\frac{dc}{dx}\,dt$$

where D is the diffusion constant. On eliminating dm from this relationship, Fick's second law is obtained. Thus if two planes of unit area and perpendicular to the concentration gradient are considered, one at x and the other very near x at (x+dx), the flow of matter per unit time through the planes will be

$$-D\frac{dc}{dx} \qquad \text{and} \qquad -D\frac{dc}{dx} - \frac{d}{dx}\left(D\frac{\partial c}{\partial x}\right)dx$$

The flow difference is therefore

$$\frac{d}{dx}\left(D\frac{\partial c}{\partial x}\right)dx$$

Thus the rate of change of concentration between the planes is

$$\frac{\frac{d}{dx}\left(D\frac{\partial c}{\partial x}\right)dx}{dx} = \frac{d}{dx}\left(D\,\frac{\partial c}{\partial x}\right)$$

which can also be represented as $\partial c/\partial t$. Thus

$$\frac{\partial c}{\partial t} = \frac{d}{dx}\left(D\frac{\partial c}{\partial x}\right)$$

Assuming D to be independent of concentration, we get

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \qquad (32)$$

which is Fick's second law. On evaluating c, x and t experimentally, Fick's second law can be used to obtain D.

(b) Restricted Diffusion.

This may be considered as a characteristic of a diffusion system existing between two plates such that material is unable to pass through the plates. Considering a restricted diffusion system whereby the distance between the two plates is ℓ, such a system has the conditions, firstly, that $(\partial c/\partial x) = 0$ at $x = 0$ and $x = \ell$ (i.e. at the plates) for all values of t, and secondly, that the initial concentration $c = f(x)$. It now remains to solve equation (32) under these conditions.

Thus for the concentration at height x, [219]

$$c = c_0 + \sum_{n=1}^{n=\infty} A_n \exp(-Dtn^2\pi^2/\ell^2) \cos n\pi x/\ell$$

where $c_0 = \frac{1}{\ell}\int_0^{\ell} f(x)dx$ and $A_n = \frac{2}{\ell}\int_0^{\ell} f(x)\cos(n\pi x/\ell)dx$.

From this solution it can be seen that when $t = \infty$, $c = c_0$, where c_0 is the average initial concentration, and hence a further condition of restricted diffusion is met.

After the fashion of Harned and Nuttall, [220] if concentrations were measured at $x = \ell/6$ and $x = 5\ell/6$, the concentration difference between the two levels would be

$$c_{\ell/6} - c_{5\ell/6} = \sum_{n=1}^{n=\infty} A_n \exp(-Dtn^2\pi^2/\ell^2)(\cos n\pi/6 - \cos 5n\pi/6) \qquad (33)$$

For even values of n, $\cos n\pi/6 = \cos 5n\pi/6$, and also for odd values of n, $\cos n\pi/6 = -\cos 5n\pi/6$. Thus the cosine terms with even values of n disappear while those containing the odd values contract to $2\cos n\pi/6$. On enumerating $2\cos n\pi/6$, values are obtained of $\sqrt{3}$ for $n = 1$, 0 for $n = 3$, $-\sqrt{3}$ for $n = 5$, etc. Equation (33) thus becomes

$$c_{\ell/6} - c_{5\ell/6} = A_1' \exp(-Dt\pi^2/\ell^2) + A_5' \exp(-Dt\pi^2/\ell^2) + \ldots \quad (34)$$

where the constants A_1', A_5', ... contain the evaluated cosine terms. It will be noticed that the term where n=3 has disappeared, this being due to the convenient choice of $x=\ell/6$ and $x=5\ell/6$. As a result, also of this choice, the second and subsequent terms on the right hand side of equation (34) are very much smaller than the first term and if a short delay is observed, these former terms become negligible. Equation (34) can then be reduced to

$$c_{\ell/6} - c_{5\ell/6} = A_1' \exp(-Dt\pi^2/\ell^2)$$

which on differentiation becomes

$$\frac{d}{dt} \ln(c_{\ell/6} - c_{5\ell/6}) = -\pi^2 D/\ell^2 \quad (35)$$

Thus on plotting $\ln(c_{\ell/6} - c_{5\ell/6})$ against time, a straight line is obtained of slope $-\pi^2 D/\ell^2$. Since the difference in concentrations was proportional to the difference in the reciprocal resistances for electrolyte solutions, Harned plotted the logarithm of this difference against time. In the subsequent diffusion work, by the same reasoning, a plot is made of the logarithm of the difference in interferometer reading against time.

(c) Diffusion and Molecular Properties.

For translational motion of spherical particles of large dimensions relative to surrounding solvent molecules, Stokes expressed the frictional coefficient (f) of each particle as

$$f = 6\pi\eta r$$

where r is the particle radius and η the solvent viscosity. On expressing r in terms of molecular weight and partial specific volume of the particle,

$$f = 6\pi\eta(3M\bar{v}/4\pi N)^{1/3} \qquad (36)$$

where $\bar{v}$ is the reciprocal of the solute density (i.e. partial specific volume).

Since from Fick's first law it can be shown that [221]

$$D = RT/Nf \qquad (37)$$

where R is the gas constant and T the absolute temperature, substitution of equation (36) in equation (37) gives, for spherical particles,

$$D_0 = \frac{RT}{6\pi\eta N(3M\bar{v}/4\pi N)^{1/3}} \qquad (38)$$

where D_0 is the diffusion coefficient

To obtain D_0 for non-spherical particles, a frictional coefficient correction factor has to be included in the relationship in order that a true molecular weight be calculated. The need for this correction factor, or frictional ratio, arises because of solvation or deviation from a spherical shape. Thus the frictional ratio, f/f_0, may be represented by

$$f/f_0 = (f/f_e)(f_e/f_0)$$

where the ratio is split into the solvation and asymmetry factors. Considering asymmetry, the frictional ratio (f_e/f_0) has been shown to be related to the axial ratio of prolate and oblate ellipsoids. Thus by using the value of $[\eta]$ from viscosity, it is possible to derive f_e/f_0 [222]. Tables have been prepared

containing this data [223] assuming that the deviation of $[\eta']$ from 2.5 is due only to asymmetry.

This viscosity intercept can also be interpreted as the solvation frictional factor, f/f_e, by assuming the deviation of $[\eta']$ from 2.5 is caused by solvation. Thus from equation (31) [218] and the relationship

$$f/f_e = (1+w/\bar{v}\rho)^{1/3}$$

it can be seen that

$$f/f_e = ([\eta']/2.5)^{1/3}$$

Thus for particles that are either solvated or asymmetric, or both, the particle molecular weight is calculated from equation (38) in its modified form:

$$D_o = D(f/f_o) = \frac{RT}{6\pi\eta N(3M\bar{v}/4\pi N)^{1/3}}$$

Having obtained the molecular weight of a hypothetical unsolvated spherical particle, a guide to the shape or solvation of the particle may be obtained by calculating f_o and comparing it with the observed value of f from equation (37). f/f_o can be treated in terms of deviation from spherical shape, solvation or a combination of both factors.

PART 2.

EXPERIMENTAL, RESULTS AND DISCUSSION.

MATERIALS.

Lecithin.

This was prepared from fresh egg yolks by the following general method.[224] The yolks of 12 eggs were dropped into about 300mls. acetone and the mass was stirred for 15 minutes after which time the extract was filtered and discarded. This extraction was repeated twice, final acetone traces being removed by rapidly sucking 100mls. of ethanol through the residue. The residue was then stirred in 400 mls. ethanol for about 2 hours, and filtered. The filtrate, which contained the extracted phosphatides, was retained and restirred for a further 2 hours in the presence of 100g. alumina powder (B.D.H., for chromatographic adsorption analysis). The alumina adsorbed the unwanted amine phosphatides, leaving lecithin and lysolecithin in solution. The absence of the amine phosphatides in solution was demonstrated by evaporating a 2 ml. portion to dryness and dissolving the residue in 1 ml. chloroform. On boiling with 2 mls. 0.3% ninhydrin solution in this solvent no purple colour was obtained. If colour appeared the solution was restirred for half an hour with a further 30g. of alumina, and then retested. When the test gave no colour, the mixture was filtered and the alumina washed with 100mls. ethanol. The washings were ninhydrin-tested, and if amine-free, were bulked with the previous filtrate.

This ethanolic solution of lecithin and lysolecithin was

then evaporated under vacuum on a water-bath at 40°. The residue was taken up in a minimum of ether and the ethereal solution poured into 250 mls. of cold acetone. After standing in the cold for 15 minutes the supernatant liquid was decanted from the precipitated phosphatides and evaporated at not more than 40°. The remaining phosphatides present in this solution were reprecipitated in acetone from ether. The combined precipitates were dried under vacuum and weighed.

This lecithin-lysolecithin mixture was chromatographed on silica gel (B.D.H., for chromatographic adsorption), using 4g. per g. phosphatide mixture and 25% V/V methanol in chloroform as solvent. For a column load of 12g. phosphatide, 400 mls. solvent eluate were collected and evaporated at not more than 40°. When the solvent was removed the residue was dissolved in a minimum of ether and precipitated in cold acetone as previously described. Finally the lecithin was dried and kept in the dark in a vacuum dessicator over anhydrous calcium chloride.

A test chromatogram on silica gel indicated that 400 mls. of solvent was sufficient to provide a good yield of lecithin. Further elution was uneconomical due to the very small quantity of lecithin that was obtained. Analysis figures for the various lecithin samples used in the subsequent work are presented in Table 3.

Table 3.

Analysis Figures for Lecithin Samples.

Lecithin Sample	N%	P%	Iodine N°.
A	1·75	3·82	73
B	1·77	3·81	60
C	1·81	3·88	41
D	1·86	3·80	52
E	1·86	3·69	51
F	1·88	3·90	62

Synthetic Lecithin.

L-α-(dipalmitoyl)-lecithin ($[\alpha]_D^{25}$, 55,225) (L. Light & Co. Ltd.) was purified by chromatography on silica. Analysis gave N, 1·9% and P, 4·1%. Calculation of N and P for $C_{40}H_{82}NO_9P$ gave the same figures.

Solvents.

Benzene. AnalaR benzene was purified by fractional crystallisation, drying with sodium and fractional distillation. It was stored over sodium and had n_D^{25}, 1·4979 (1·4981).

Toluene. AnalaR toluene was sodium dried, fractionally distilled and stored over sodium and had n_D^{25}, 1·4940 (1·4941).

Carbon Tetrachloride. AnalaR carbon tetrachloride was dried over anhydrous calcium chloride, filtered and fractionally distilled. It had n_D^{25}, 1·4604 (1·4603).

Methanol and Ethanol. These were refluxed with magnesium

and iodine and distilled. Methanol had n_D^{15}, 1·3315 (1·3306) and ethanol n_D^{20}, 1·3619 (1·3614).

n-Butanol and n-Hexanol. After drying with anhydrous potassium carbonate and filtering, they were fractionally distilled. n-Butanol had n_D^{15}, 1·4020 (1·4012), and n-hexanol n_D^{20}, 1·4191 (1·4179).

n-Heptane. n-Heptane ASTM (Fluka Inc.) was used as supplied and had n_D^{25}, 1·3862 (1·3851).

Water. This was distilled once from a seasoned Manesty still.

The figures in parenthesis are literature values of refractive index.[226,227]

Additional Materials.

Ludox. Fresh "Ludox H.S." colloidal silica (Durham Chemicals Ltd.) was used.

Cadmium Potassium Iodide. After drying over anhydrous calcium chloride it analysed correctly ($CdI_2.2KI.2H_2O$) for iodine content when the iodine was released and titrated with potassium iodate using starch as indicator.[228]

Polystyrene. Samples were kindly supplied by the National Chemical Laboratory and Monsanto Chemicals Ltd.

Quaternary Ammonium Compounds. Hexamethonium iodide, decamethonium iodide, hexadecamethonium iodide and decamethonium bromide were obtained from Dr. P.H. Elworthy. They had been purified for use in conductance work,[229] all having purities of over 99·5%.

Cholic Acid. On recrystallisation from methanol and drying, the melting point was 198·5°(195°)[230], and the equivalent weight by titration 419 (409).

Glycine and Potassium Chloride. AnalaR analytical reagents were used.

Tropaeolin OOO (B.D.H.). This was recrystallised twice from water.

A. Lamp

B. Lightproof box

C. Cell

D. Shutter

F. Light filter

H. Light shield

L. Lens

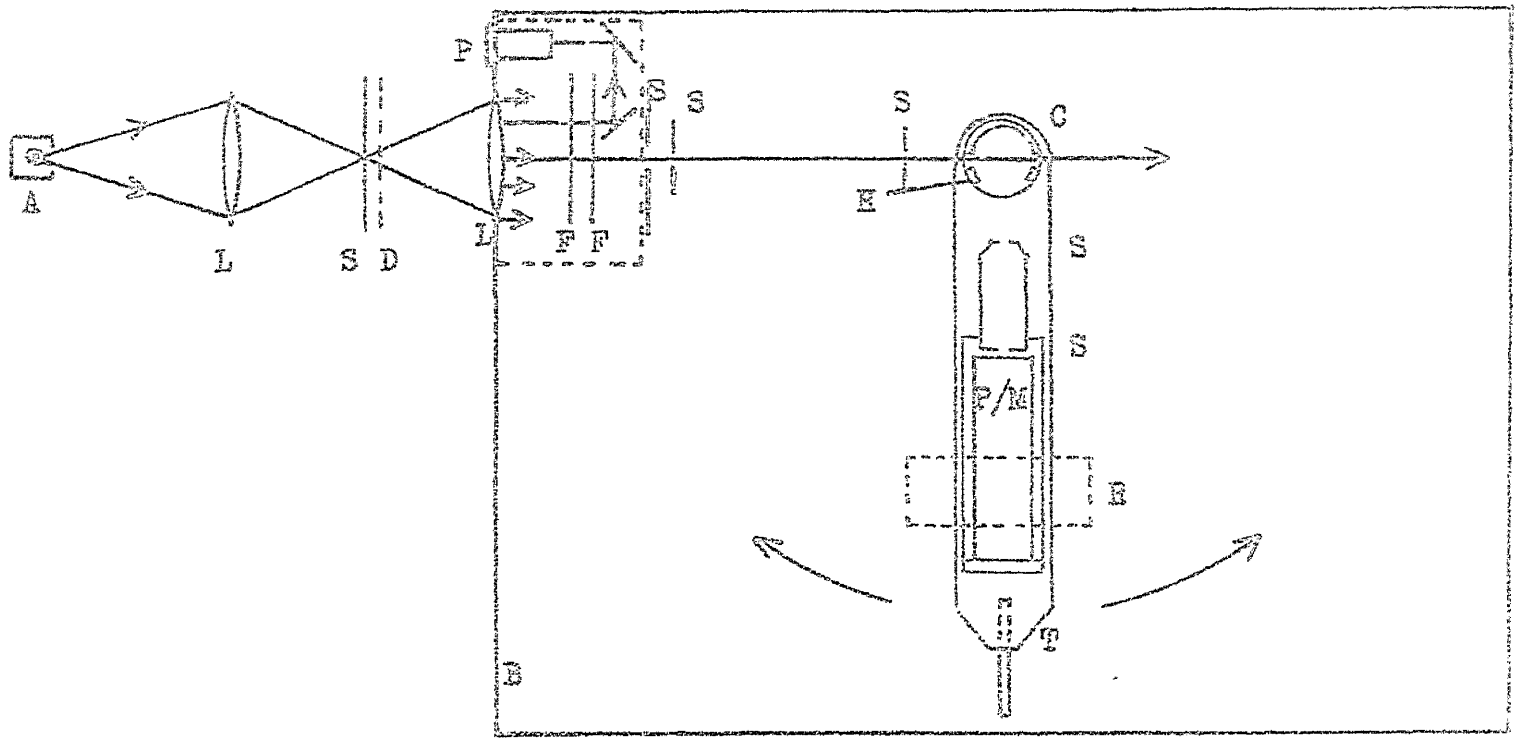
P. Photocell

P/M. Photomultiplier tube

R. Photomultiplier resistance chain

S. Slit

T. Tufnol arm



Light-scattering Photometer Optical System.

THE LIGHT-SCATTERING PHOTOMETER.

Development and Description.

Light from a 250 watt mercury vapour lamp (Osram, type ME/D) was made parallel for passage through the cell by passing it through an optical system composed of a series of slits and lenses, as shown in fig. 3. The green line (5461 Å) was isolated by means of an interference filter together with a neodymium glass to remove completely traces of yellow light. Final collimation of the beam, before it entered the cell, was achieved by passing the incident light through two beam-defining slits 2x25 mms. in size and 32 cms. apart, the nearer slit being 10 cms. from the cell entrance window. The resulting beam passing through the cell measured 2x26 mms.

The light source power was stabilised by a voltage stabiliser ('Advance' Voltstat, type CVN 250 A) to minimise lamp intensity fluctuations. Any further small intensity fluctuations could be automatically compensated for since part of the light from the lamp was fed to a photocell (EMI, type 25110) which, if necessary, could be readily incorporated in the circuitry in such a way as to mask lamp intensity fluctuations.

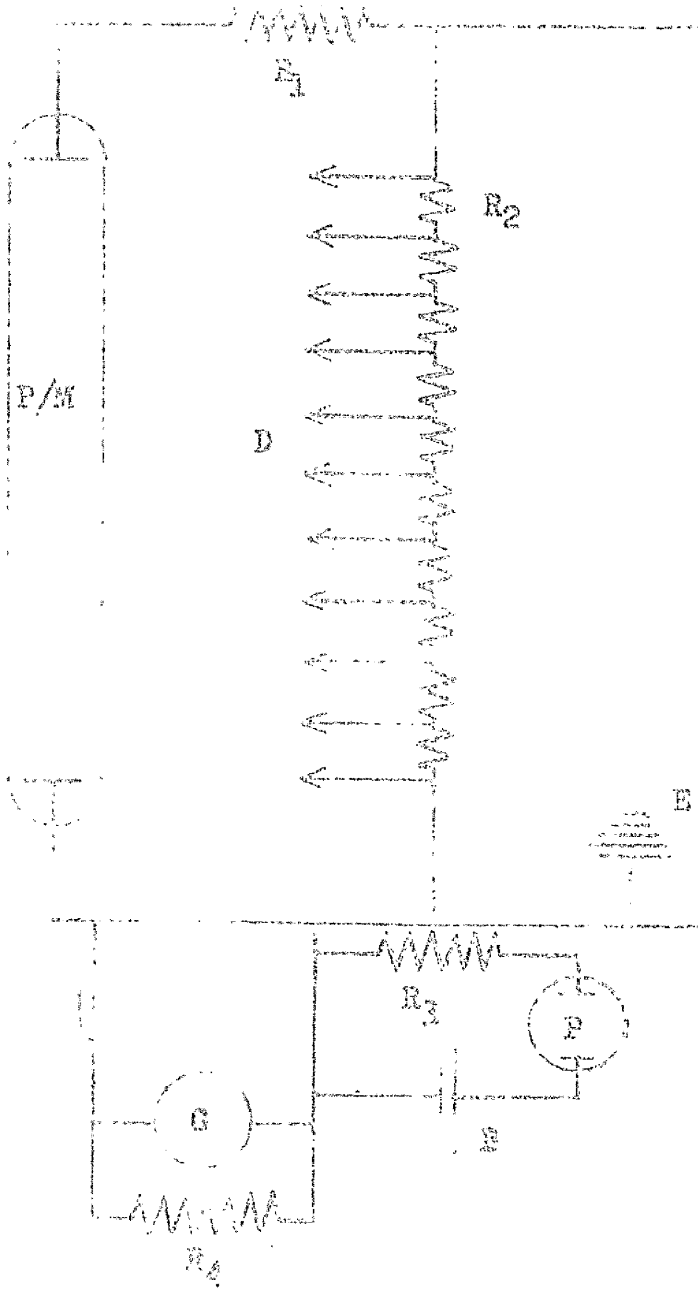
The scattered light was received by an eleven-stage photomultiplier (EMI, tube type 6097B) whose signal was recorded by a d'Arsonval galvanometer and 50 cm. scale. The

Plate 1.

voltage to the photomultiplier was supplied by two power supply units (Siemens Ediswan, type R1184) in series. Though capable of supplying 1100 volts each, their total output was always restricted to 1500 volts. At higher voltages than 1500 volts, photomultiplier dark current effects made the readings difficult to obtain.

Before reaching the photomultiplier, the scattered light passed through two slits 3x10 mms. in size and 8 cms. apart. The slit at the photomultiplier end window was adjacent to, but not touching, the window. The dual slit system ensured correct viewing of the beam passing through the scattering solution. The photomultiplier was housed in a cylindrical metal tube. The entire unit, including the photomultiplier resistance chain, was mounted on a broad tufnol arm which rested on a tufnol board, the arm being pivoted beneath the centre of the cell giving a very smooth movement of the photomultiplier around the cell. Dark current was reduced by raising the photomultiplier tube wall to cathode potential and covering it with insulating tape. The entire photometer is illustrated in Plate 1, the optical and electrical systems being presented in figs. 3 and 4. To reduce stray light, part of the apparatus was enclosed in a lightproof blackened box.

Initially, work was directed towards improving the
231
cell and cell holder design in order to reduce stray



B. 60v. Battery

D. To dynodes

E. Earth

G. Galvanometer

P. Photocell

P/M. Photomultiplier tube

R_1. 2megn. Resistance

R_2. 1megn. Resistance chain

R_3. 1,000n. Resistance

R_4. 5,000n. Resistance

Light-scattering Photometer Electrical Circuit.

light. The cell was a simple rectangular all-glass cell of 5 cm. optical path length and 5x1 cm. base which sat in a black painted rectangular cell holder. The holder had a curved perspex viewing window and glass entrance and exit windows. It was thought that the main causes of stray light were the four windows through which the incident beam passed during its passage through the cell and cell holder. In addition, though the cell holder was completely blackened, the two long walls of the cell were glass and could therefore act as reflecting surfaces.

Two factors soon emerged from the early work. Firstly, less stray light was found using 1x8 cm. cells than with 1x5 cm. cells. Secondly, from tests with a flat glass window attached to the cell holder, it was found necessary to have a curved viewing window through which the scattered light left the cell holder, the curve being concentric with the arc made by the photomultiplier when viewing the angular scattered light. Using the longer cell and a suitably curved perspex viewing window, work was directed towards reducing the stray light found in the 8cm. cell.

To try and reduce stray light by removing a region of sharp refractive index change, the holder was filled with liquid paraffin whose refractive index was approximately equal to that of the glass of the cell. No appreciable

improvement was found and, as it was more convenient, water continued to be used. Directing attention then to the four reflecting windows mentioned above, and also to the long walls of the cell, various modifications were tested. Firstly, a cell holder entrance window of polished perspex was used, perspex enabling the window to be attached to the holder, near its corners, by four screws. 'Bostic' power adhesive was smeared between the holder and window to ensure a watertight joint. With such an arrangement, the entrance window was aligned exactly at right angles to the incident beam by adjusting the tightness of the four screws. A reduction in stray light resulted.

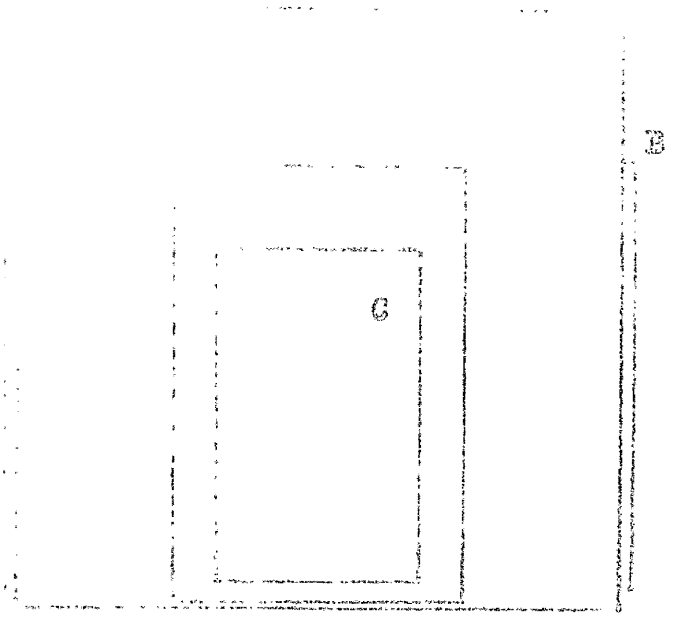
Incident beam reflection from the cell holder exit window was next investigated. In order to reduce this to a minimum a thin glass window, cut from a microscope slide, was substituted for one similar to the screw-attached perspex entrance window. This, however, was found unsatisfactory due to the difficulty of obtaining the 90° angle between the beam and window since it could not be held directly by aligning screws. The glass exit window was then set at approximately 45° to the beam, (the angle not being critical) the reflections leaving the cell via an angled piece of polished perspex, the perspex also holding the glass in place. This, too, was found to have no advantage

over the perspex exit window whose angle to the beam could be adjusted, and therefore both windows were of the latter design.

When adapted to accommodate 8 cm. cells the cell holder, inside both end windows, had blackened channels 1·5 cms. long through which the beam had to pass before entering and after leaving the cell. The presence of these channels reduced stray reflections. On attaching the windows to the inner ends of the channels an increase in stray reflections was again obtained. This indicated that these channels reduced stray reflections arising from the cell holder end windows. The effect of placing a third beam-defining slit directly in front of, or directly behind, the cell entrance window served only to increase unwanted light.

To try and further reduce unwanted light inside the cell, part of the exterior of the cell was painted with blackboard paint, this preventing any stray light present in the cell holder from entering the cell. The entire back face of the cell was painted and also the front face, from each end window inwards leaving a central area such that scattered light could still be viewed between 45 and 135°. This provided a drop in stray scatter, eliminating reflections from the cell holder walls. There was still, however, the interior glass surface of the cell, this being a very likely source of unwanted light. This was strikingly seen when blackened brass plates were put inside

Figure 5.



(A) Entrance window

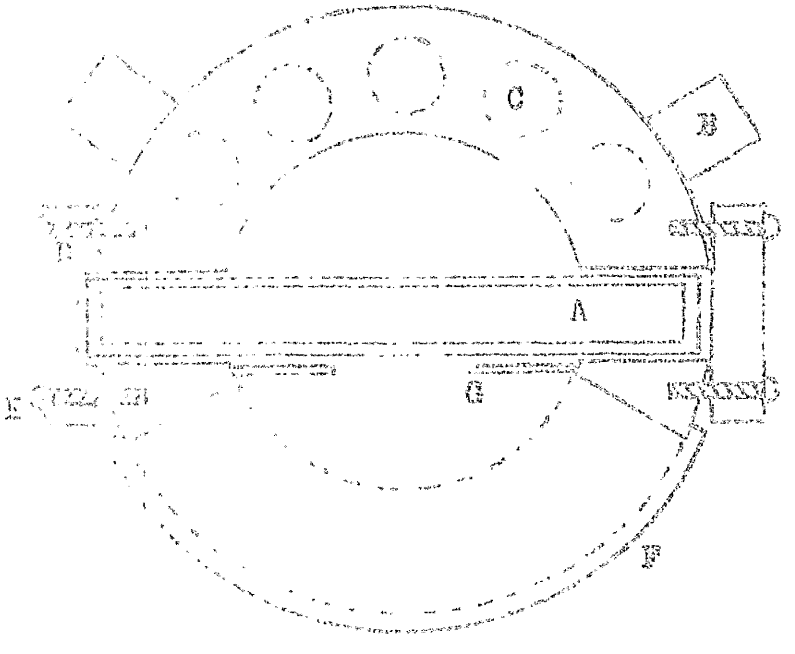
(B) Exit window

(C) Viewing window

Actual size

Rectangular Brass Cell.

Figure 6.



(A) Cell in holder

(B) Input pipe for circulating water

(C) Vertical tube in back wall

(D) Perspex entrance window

(E) Window aligning screw

(F) Carved viewing window

(G) Brass plate

Actual size

Cylindrical Brass Cell Holder with Glass Cell.

the cell to cover the same area of glass as that covered by the paint. With the brass plates in position, Z_{45} values of 1.06 to 1.00 could be obtained consistently for liquids such as toluene, 0.5% aqueous potassium chloride solutions and dilute Ludox solutions.

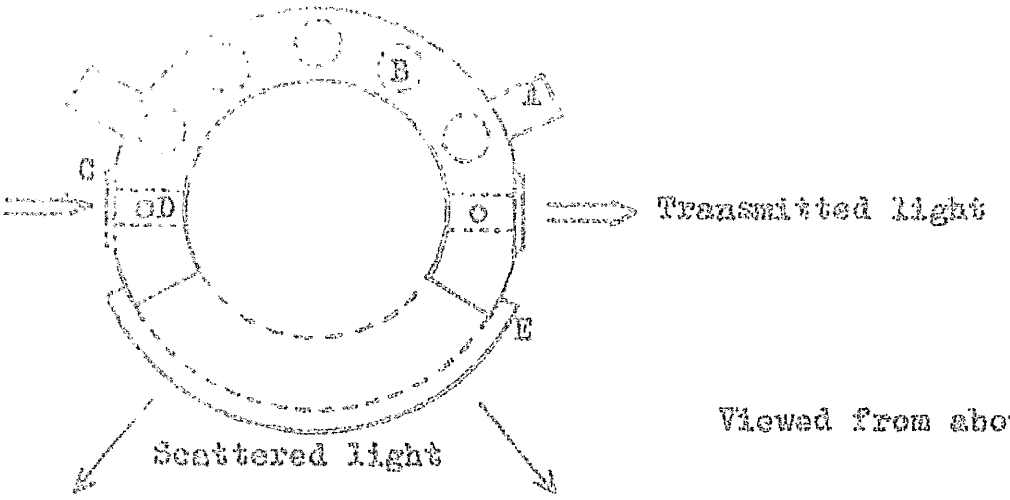
Since these good dissymmetries were readily repeatable with the brass plates in the cell, the glass cell was replaced by a rectangular brass cell with glass windows (fig. 5). Before attaching the windows, the brass was blackened by the Relonol process, the windows being held by 'Araldite'. This cell was 7 cms. long and 9 mms. wide, these dimensions enabling a smaller volume of solution to be used than that required by the 8 cm. all-glass cell. As purely a test cell, promising results were obtained. Though possessing the advantages of entrance and exit channels 2 cms. long and a matt black inner surface, the narrowness of the cell made it very difficult to align at right angles to the incident beam.

During the testing of this cell, an all-glass cell of similar size was obtained, the cell being constructed with optically ground glass faces accurately angled at 90° to each other. To house this cell a new cell holder was designed (fig. 6). This cell holder which was made from a solid brass rod, was cylindrical in shape and had incorporated in its design the features found advantageous from the earlier tests. Thus it had a curved viewing window, perspex adjustable entrance

and exit windows with entrance and exit channels inside the windows and a brass plate to restrict photomultiplier viewing to just over the range 45 to 135°. Its entire brass surface was Rolonol-blackened. A further feature was that the inside of the back wall was concave since it was thought that this extra volume might serve as a light trap for stray reflections. Interconnecting vertical tubes were cut in the back wall to facilitate thermostating.

After preliminary tests with this new cell holder and glass cell, the curved back wall was identified as a useful light trap. However, the glass back face of the cell still remained as a surface for reflections. At this stage it was thought that if stray light was to be further reduced it would be wise to use a cell that did not have a glass back face. On filling the cell holder with water, the cell not being in the holder, surprisingly little stray light was found. Most of the stray scatter that was present arose from passage of the incident beam through the 0·25 inch thick perspex of the windows. This thickness was necessary to make the windows robust and to prevent them curving when screwed to the cell holder. This test, in the absence of the cell, further suggested that a reduction in unwanted light could be credited to the concave back wall and to its black matt surface. With these factors in mind and with the encouraging results obtained with water alone in the cell holder, it was thought possible to design a cell of

Figure 1.



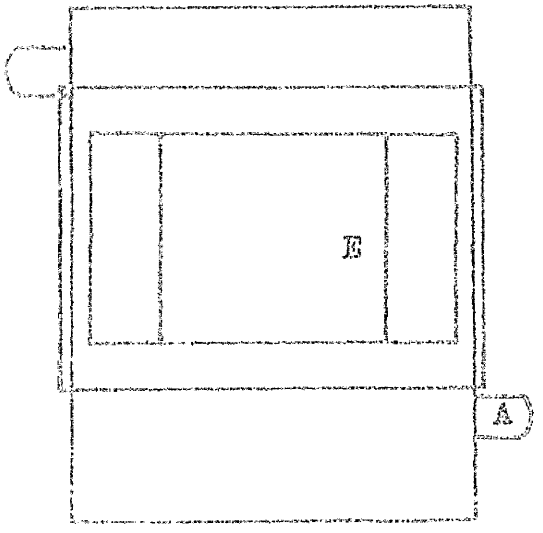
(A) Input pipe for circulating water

(B) Vertical tube in back wall

(C) Incident beam entrance window

(D) Incident beam entrance channel with air escape hole

(E) Curved viewing window



Actual size

Front view

Brass Light-scattering Cell.

similar shape to the cell holder, but much smaller in size, that could stand in air rather than in a water bath. The advantage of such a cell would be that the number of windows through which the incident and scattered light would have to pass would be halved, this immediately reducing unwanted reflections. Also there would be no refraction effects due to water surrounding the cell. A cell, cylindrical in shape, was thus made and was the cell used for the light-scattering studies.

This new cell, (fig. 7) which was also made from a solid cylinder of brass, had two parallel flattened faces to accommodate the windows through which the incident beam passed. These flat faces were 13mms. broad, this leaving sufficient space to attach the entrance and exit windows, the entrance and exit slits being 4mms. broad. The two flat windows were cut from microscope slide glass, the glass being 1mm. thick. The curved viewing window, having to be more robust, was made from 2mms. thick glass, the curvature of the inside of the glass being the same as that of the outside of the cell. Above each of the 9mms. long entrance and exit channels was a small hole 2·5mms. in diameter which enabled trapped air to escape from the channels when the cell was filling with solution. A series of vertical tubes was cut in the solid back wall of the cell, with the tubes connected to enable water to circulate and provide thermostating. The tubes were covered at the top of the cell

by a brass arc and at the base by a circular base plate.

Before attaching the windows, the entire cell was blackened, again by the Relonol process, this providing the necessary black matt surface. The blackening on the outside of the cell, where the windows were to be attached, was rubbed off before attaching the windows since this permitted stronger adhesion of the windows. The windows were attached, again using 'Araldite' and curing at 37° overnight. This slow low-temperature curing to harden the adhesive was necessary because of the difference in coefficients of expansion of glass and brass. No strain lines or breaking of the glass occurred on cooling from 37° as did occur on cooling from higher temperatures.

In the photometer the cell sat in a 7mms. deep depression cut from a piece of tufnol. This seating was made such that the cell fitted neatly into it and also that the centre of the cell was directly above the pivot of the photomultiplier arm. When liquids were in the cell and when the beam was passing through it, slight incident beam reflections were obtained from the entrance and exit windows. These reflections appeared on the metal surrounding the last beam-defining slit. On rotating the cell in its seating, a point was reached where both these reflections coincided with the incident beam. At this point the entrance and exit windows were at 90° to the beam and beam reflection was masked by the beam itself. The rotation of the cell to this position provided a simple method of cell alignment

and enabled the cell to be placed in exactly the same position for successive readings.

With external and internal diameters of 48 and 28mms. the cell required 35 to 40mls. solution before readings could be satisfactorily obtained. This disadvantage of having to use fairly large volumes of solution was, however, far outweighed by the fact that stray light was negligible. The broad curved viewing window of the cell permitted Z_{45} values to be obtained, while the entrance and exit channels were of sufficient length that the points where the incident beam struck the glass windows could not be seen. To prevent the possibility of light travelling from the last beam-defining slit to the photomultiplier by reflection from the surface of the viewing window, a metal plate was placed parallel to the incident beam, between the slit and the cell.

For the earlier work on the micelle formation by lecithin in four alcohols, a low sensitivity spot galvanometer was used. To increase the signal received by this galvanometer, the output signal from the photomultiplier passed through an amplifier, the circuit of which was based on that described by Ottewill and Parriera.[232] This enabled the voltage being fed to the photomultiplier to be kept below 1500 volts and still provide full-scale galvanometer readings. By keeping this input voltage minimal, a much smaller noise to signal ratio was obtained for the photomultiplier which decreased the annoying flicking of the

galvanometer needle whilst taking readings. All electric leads near the photomultiplier were screened to reduce unwanted electrical pick-up by the photomultiplier. On changing to the more sensitive d'Arsonval galvanometer and 50cms. scale, the amplifier was discarded since the signal from the photomultiplier was sufficient in itself to provide full-scale deflections when the input to the photomultiplier was still below 1500 volts. The slight movement of the galvanometer needle which still remained was effectively damped by placing a resistance across the galvanometer terminals. This galvanometer, without the amplifier, was used in the subsequent work.

The presence of dust in solutions for light-scattering has to be avoided since it acts as a source of scatter with a resultant error in the scattering intensity. All solutions were therefore filtered into the cell through a No.5 sintered glass filter to remove the dust. It was found that for the systems studied one or two filtrations were generally sufficient. No decrease in the scattering intensity was found on repeating the filtrations, an increase being found after four or five repetitions. When the cell was filled, it was covered with a flat, blackened brass lid to prevent the access of dust while the solution scattering was measured. All readings were made at 20 ± 0.5^{o}.

Molecular Weight Determinations.

In using the light-scattering technique to evaluate

molecular weight, three main factors have to be determined. They are

(a) the specific refractive index increment,

(b) the depolarisation of the scattered light, and

(c) the Rayleigh ratio, or turbidity.

This last factor is evaluated by using a calibrated light-scattering standard such as a perspex block. By comparing the scattering of the solutions to that of the standard, the measurements being made at 90°, the turbidity of each solution was obtained.

Two other factors, the dissymmetry and the cone angle correction, have also to be obtained for molecular weight determinations. The dissymmetry was measured as the solution scattering ratio at 45 and 135°, the solvent scattering at these angles being subtracted. The cone angle correction was necessary due to the difference in refractive indices of the media through which the scattered light passed on travelling between the incident beam and the photomultiplier end window. Since the photomultiplier did not see past the edges of the beam, and since calibration was by aqueous Ludox solutions, this correction factor was equivalent to the square of the refractive index of the scattering solution divided by the square of the refractive index of water.

(a) The Specific Refractive Index Increment, dn/dc.

Since this factor is squared when used to evaluate

molecular weight from light-scattering, its measurement had to be made with precision. To obtain the required accuracy a Rayleigh interference refractometer and 1cm. optical path length cell (Hilger and Watts, type M154) were used, the cell being enclosed in a water-jacket (type M322) and thermostated to 20 ± 0·01°. The light source was a Pointolite lamp, its light being made monochromatic before entering the refractometer by passage through filters similar to those used for the incident beam of the photometer.

In the determination of dn/dc, care was taken to use sufficiently dilute solutions for comparison to the solvent or two solutions of sufficiently small refractive index change such that the location of the zero order band was unambiguous and below the first region of any band shifts. Thus for a solute whose dn/dc was approximately 0·1ml./g., the maximum concentration difference between the two compartments in the cell was restricted to about 0·5%. When a solvent was used whose rate of evaporation was too great to enable accurate readings to be obtained, the cell compartments were covered with thin glass lids cut from coverslip glass of a microscope slide. This restricted the evaporation sufficiently to permit consistent readings to be obtained, the thin glass enabling the lid of the water jacket to be tightly closed. The stoppered cell used in the diffusion work was not available at the time the dn/dc values in volatile solvents were being obtained.

The technique used for measurements was similar to that
234
described by Bauer, Fajans and Lewin for monochromatic light. This consisted firstly of obtaining the zero reading, r_o, with solvent or the same solution in both compartments. This reading was obtained by matching the zero order band of the two patterns, this band having colourless edges, using white light and then inserting the filters before accurate matching. One of the cell compartments had its contents replaced by a solution of different concentration and the band patterns were approximately rematched using white light. The filters were then inserted and the patterns matched exactly. This gave a reading, r. The micrometer scale was then turned slowly towards r_o, the number of bands passing the reference pattern zero order band being counted, till r_o was reached. The band patterns were then exactly matched at the band nearest r_o. If the number of bands counted was F, and the third reading r', the scale reading equivalent to one band was $(r'-r)/F = r_f$. Thus the fraction of a band between r' and r_o is $(r'-r_o)/r_f = f$, and the total number of bands resulting from the refractive index difference, dn, between the two compartments is $F + f = \Delta F$. Since the refractive index difference is given by

$$n_2-n_1 = (\lambda/d).\Delta F$$

where λ is the wavelength of light (cms.) and d the optical path length of the cell (cms.), the specific refractive index increment is given by $dn/dc = 5461 \times 10^{-8}.\Delta F/\Delta c$

where Δc is the solute concentration difference between the two compartments, in g./ml.

The interferometer was checked using sodium chloride solutions as standards, good agreement being obtained on comparison to published data.[235] The error in $(dn/dc)^2$ was subsequently estimated as $\pm$ 1%.[236]

(b) Depolarisation.

This was determined by isolating and measuring the intensities of the horizontally and vertically polarised components of the scattered light. The two components were separated by inserting a polaroid disc cut with one edge parallel to one axis of transmission, in the path of the scattered light. The contribution of the solvent to both components was subtracted from the values for solutions. Since some high depolarisations are reported, particular care was taken in checking the technique.

To establish if the direct ratio of the two measurements was equal to the depolarisation, the sensitivity of the photomultiplier to both components was determined. For this sensitivity check, the cell was replaced by a small torch bulb which was connected in series to a 1·5 volt battery, a 1000 ohm potentiometer and a switch. The light from this bulb was sufficiently weak to allow the photomultiplier to view it directly. The two polarised components of the light emitted by the bulb were then measured at several settings of the potentiometer,

the mean ratio of the components being a measure of the photomultiplier's sensitivity.

During the work with the four aliphatic alcohols as single solvents, the sensitivity check showed the photomultiplier to be more sensitive to the horizontal component. From the results of the check, it was found that the inequality was rectified by reducing subsequent horizontal component values by a factor 0·864. Using this correction factor the depolarisations of benzene and toluene gave 0·41 and 0·42 respectively, in good agreement with literature values (0·41 and 0·43).[237,238]

On completion of the work in the alcohols, the photometer optical system was subjected to several minor readjustments. From a further sensitivity check, the ratio was found to be unity, no correction needed, therefore, to be applied to the remainder of the depolarisation values. Again the values of 0·41 and 0·42 were obtained for the depolarisations of benzene and toluene.

If fluorescence is present in a system, photomultiplier readings include both the solute scattering and the fluorescent effect. For such a system a correction is necessary. All the systems studied were therefore tested for fluorescence,[239] sharp cut-off filters being used which excluded the 90° scattered light from the photomultiplier but allowed fluorescent light, of longer wavelength, to pass. Three 'Kodak' glass filters were

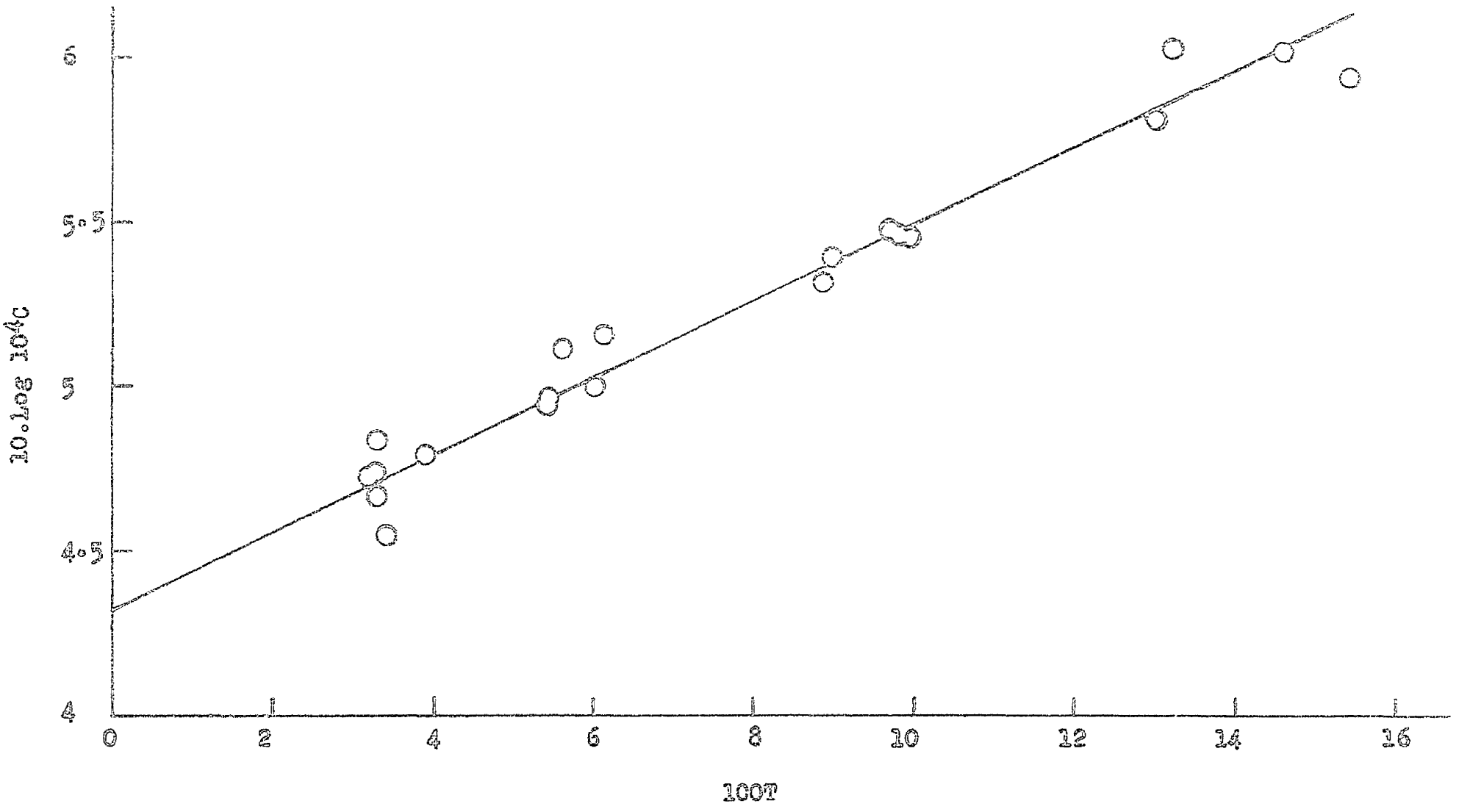
successively used for each system, their wavelengths of maximum transmission being 575, 605 and 675 mμ. All had negligible transmission at 550mμ. No fluorescence was observed in any of the systems studied.

(c) Calibration.

As a light scattering standard, a polished perspex block was used, the apparatus being calibrated by means of this reference scatterer. To ensure that the block always occupied the same position in the photometer, two guide-pieces of tufnol were attached to the tufnol cell seating. Calibration was done, using Ludox solutions, by the absolute method of measuring both the scattering and optical density of solutions of various concentrations.

Solutions of Ludox of sufficient concentration to give a precisely measured optical density had S_{90} values which were too large for direct comparison with the perspex standard. The scattering from the Ludox was measured in comparison with that from a rectangular glass cell, coated with aluminium paint. A set of perspex blocks of scattering intermediate between this standard and the normal perspex block enabled comparison to be effected, and the low turbidity standard to be calibrated. Repeated checks in comparing the standards were made, the ratio of the scattered light of the weakest to the strongest being $122{\cdot}2 \pm 0{\cdot}2$. Little error arose in this comparison.

The Ludox solutions, whose concentrations lay between 1



Plot for the Evaluation of the Calibration Constant.

and 7%, were ultracentrifuged at 25,000g. for twenty minutes, then filtered directly into two 4cms. absorption cells and the scattering cell. The depolarisation and Z_{45} of each solution were measured. Readings on carefully clarified water were also made. Twenty Ludox solutions were used in this calibration, the data being treated to obtain the calibration constant C at zero turbidity according to Maron and Lou.[240] From the relationships

$$T = 16\pi R_{90}/3 = C.S_{90} = 2.303D/L$$

where T is the turbidity, D the optical density and L the liquid path length of the absorption cell, a value of $C = 2.71 \times 10^{-4} \pm 0.12$ was obtained (fig.8) after extrapolation to zero turbidity. A depolarisation of 0.011 and Z_{45} of 1.04 were found and these values were used to evaluate the independent values of C correctly for each Ludox solution.

This calibration was carried out jointly with Macfarlane.[241]

As a check on the calibration constant the turbidities of three organic liquids were measured (Table 4). In addition, the molecular weights of two polystyrene fractions in toluene were determined. For the N.C.L. sample the weight was found to be 390,000, the Monsanto sample giving 490,000. The quoted molecular weights were 390,000 and 500,000 respectively.

Table 4.

Turbidities of Organic Liquids.

Liquid	Turbidity 10^5cm.$^{-1}$	Literature Value [237,238,242]
Carbon tetrachloride	9.6	9.5 - 9.7
Benzene	27.2	27.3 -27.5
Toluene	29.6	29.5

It was stated earlier that the optical system was readjusted after working with the four alcohols. The calibration constant of 2.71×10^{-4} was that obtained after the final adjustments and was therefore used for all systems other than the four alcohols.

For the lecithin weight in the alcohols, the calibration constant used was 3.28×10^{-4}, it also having been determined by the method described above. Using this constant, the N.C.L. polystyrene gave a weight of 370,000, and benzene and toluene gave turbidities of 27.0 and 29.9×10^{-5}cm.$^{-1}$ respectively.

Statistical Assessment of some Light-scattering Errors.

Stacey[243] has stated that the usual degree of accuracy of a light-scattering molecular weight determination is ± 5%, the figure being dependent upon the system under investigation. Robinson and Saunders[244] have found an overall accuracy of ± 7% in their determination of the micellar weight of lysolecithin in water. For a synthetic non-ionic detergent in water, Elworthy and Macfarlane[236] found the accuracy of the $(c/T)_{c=0}$ factor to be

$\pm$ 8%, resulting in a total error in M in excess of $\pm$ 10%, this rather high error being thought to be due to the characteristic difficulty of removing suspended dust from such solutions. Since aqueous solutions containing salts and also organic solvents and solutions are easier to clarify than pure aqueous solutions, systems of these types were examined.

The error present in the calibration constant was analysed from the twenty values used for the logC against T plot. Since both variables are subject to error, the values were divided into three non-overlapping groups and analysed according to Davies.[245] The analysis is presented in Appendix 1, the error in C being $\pm$ 4·4% (P = 0·95).

To investigate the errors in $(c/T)_{c=0}$ and $(Z_{45})_{c=0}$ the Monsanto polystyrene fraction was studied in toluene. Ten measurements (Table 5) gave

$$(c/T)_{c=0} = 1{\cdot}53 \pm 6{\cdot}1\% + 708c \pm 3{\cdot}3\% \quad (P=0{\cdot}95)$$

and $(Z_{45})_{c=0} = 1{\cdot}463 \pm 3{\cdot}3\%$ giving $1/P(\theta) = 1{\cdot}351 \pm 2{\cdot}6\%$ (P=0·95). To obtain the error in Z_{45}, the individual results were plotted as $1/(Z-1)$ against c since this gave a linear relationship with c.

Table 5.

Data for Polystyrene in Toluene Analyses.

c(g./1000mls.)	1·506	1·883	2·109	2·560	2·673
c/T	2·63	2·93	2·94	3·36	3·43
Z_{45}	1·323	1·295	1·284	1·265	1·273
c	3·464	4·619	5·121	6·025	6·251
c/T	3·93	4·82	5·10	5·86	5·95
Z_{45}	1·218	1·196	1·185	1·164	1·167

Due to the high errors in both factors and the large slope of the c/T against c plot, a similar investigation of the Monsanto polystyrene was made in methyl ethyl ketone (MEK). Again, from ten measurements (Table 6):

$$(c/T)_{c=0} = 0{\cdot}488 \pm 2{\cdot}0\% + 53{\cdot}4c \pm 5{\cdot}9\% \quad (P=0{\cdot}95)$$

and $(Z_{45})_{c=0} = 1{\cdot}398 \pm 0{\cdot}9\%$ giving $1/P(\theta) = 1{\cdot}304 \pm 0{\cdot}8\%$ $(P=0{\cdot}95)$

Table 6.

Data for Polystyrene in MEK Analyses.

c(g./1000mls.)	0·5	1·0	1·5	2·0	2·5
c/T	0·514	0·549	0·564	0·593	0·624
Z_{45}	1·368	1·347	1·336	1·303	1·291
c	3·0	3·5	4·0	4·5	5·0
c/T	0·636	0·674	0·708	0·733	0·752
Z_{45}	1·268	1·262	1·250	1·239	1·229

The polystyrene analyses in toluene and MEK are given in Appendices 2 and 3 respectively. Table 7 summarises the errors in the light-scattering factors investigated.

Table 7.

Errors in some Light-scattering Factors (P=0·95).

Factor	Error
Calibration Constant	± 4·4%
$(c/T)_{c=0}$ toluene	± 6·1%
$(c/T)_{c=0}$ MEK	± 2·0%
1/P(θ) toluene	± 2·6%
1/P(θ) MEK	± 0·8%

There is a striking difference between the precision of the intercepts in toluene and MEK. In the analysis, the mean value of c/T was subject to random error, so was the slope of the c/T against c plot, while the error in c was negligible. When the slope was small, as in MEK, error in its measurement contributed little to the total error in $(c/T)_{c=0}$. Therefore for a precise determination of molecular weight by light-scattering, a "poor" solvent is the correct choice.

While the limits of error in C are large, its subsequent use in evaluating turbidities and micellar weights would indicate that its value is reasonably accurate. It would seem therefore that if the Maron and Lou procedure is adopted, a sufficient number of points yield an accurate value of C. The error was thought reasonable in view of the small optical

densities which were measured (all below 0·25) and the blank optical density of filtered water of about 0·03.

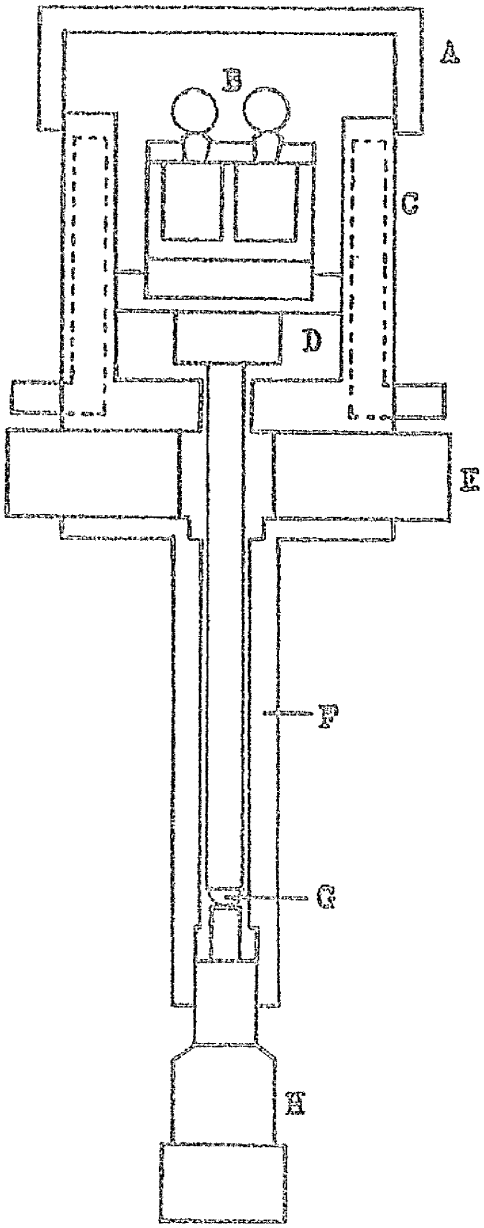
Including the errors of 1% in both $^{236}(dn/dc)^2$ and the depolarisation, the total error in micellar weight will be ± 12 to 13% in a "good" solvent and ± 8 to 9% in a "poor" one. The molecular weights of polystyrene in toluene and MEK were 500,000 and 470,000 respectively.

To circumvent the lengthy calibration procedure using Ludox, it is possible to calibrate the photometer using secondary standards. Considering the turbidities of benzene and carbon tetrachloride, their values were obtained as means of six and three measurements, the limits of error being ± 1·5 and ± 2·1% respectively. As a further possible secondary standard, a 30% W/V aqueous solution of cadmium potassium iodide gave a turbidity of $1·17 \pm 0·01 \times 10^{-4} cm.^{-1}$, as a mean of seven measurements. Thus from known values of their turbidities, benzene, carbon tetrachloride, aqueous solutions of cadmium potassium iodide or other suitable secondary standard could be used conveniently to calibrate a photometer.

VISCOSITY.

Viscosities of solutions relative to solvent were determined in a suspended-level dilution viscometer (Polymer Consultants Ltd.) at 20±0·01°. The relative viscosities were reproducible to ± 0·2%.

Figure 9.



(A) Lid

(B) Interferometer cell

(C) Hollow wall of cell holder

(D) Cell table

(E) Base plate of interferometer

(F) Brass tube

(G) Ball bearing

(H) Micrometer head

Half size

Cell and Cell Housing for Diffusion Measurements.

THE DIFFUSION TECHNIQUE.

Diffusion coefficients were obtained by the technique of restricted diffusion in a similar manner to that of Harned and Nuttall.[220] These workers measured the concentration difference between two levels of the diffusion system in terms of conductance, whereas refractive index differences have been used in the present work.

The basic apparatus was a Rayleigh interference refractometer (Hilger and Watts, type M154) which was adapted for diffusion measurements (Plate 2, fig. 9). The two-compartment cell (B) sat on a small level brass table (D) fitted with two tufnol guide-pieces to locate the cell. Attached to the table base was a brass rod, 12 cms. long, with a steel ball-bearing incorporated at its end(G), the rod passing through the interferometer platform. This rod rested in a brass tube (F) which was secured to the base of the interferometer platform. The internal diameter of the tube was such that the brass rod, 9 mms. in diameter, fitted neatly into it and could be raised and lowered in it with negligible lateral movement. A micrometer head (H) was attached at the lower end of the brass tube, the ball-bearing resting on the micrometer head. It was thus possible to move the cell vertically, in a slow, smooth manner, a known distance through the optical system.

The cell and its table were enclosed in a water-jacket. The two light-transmitting faces of the jacket were made from

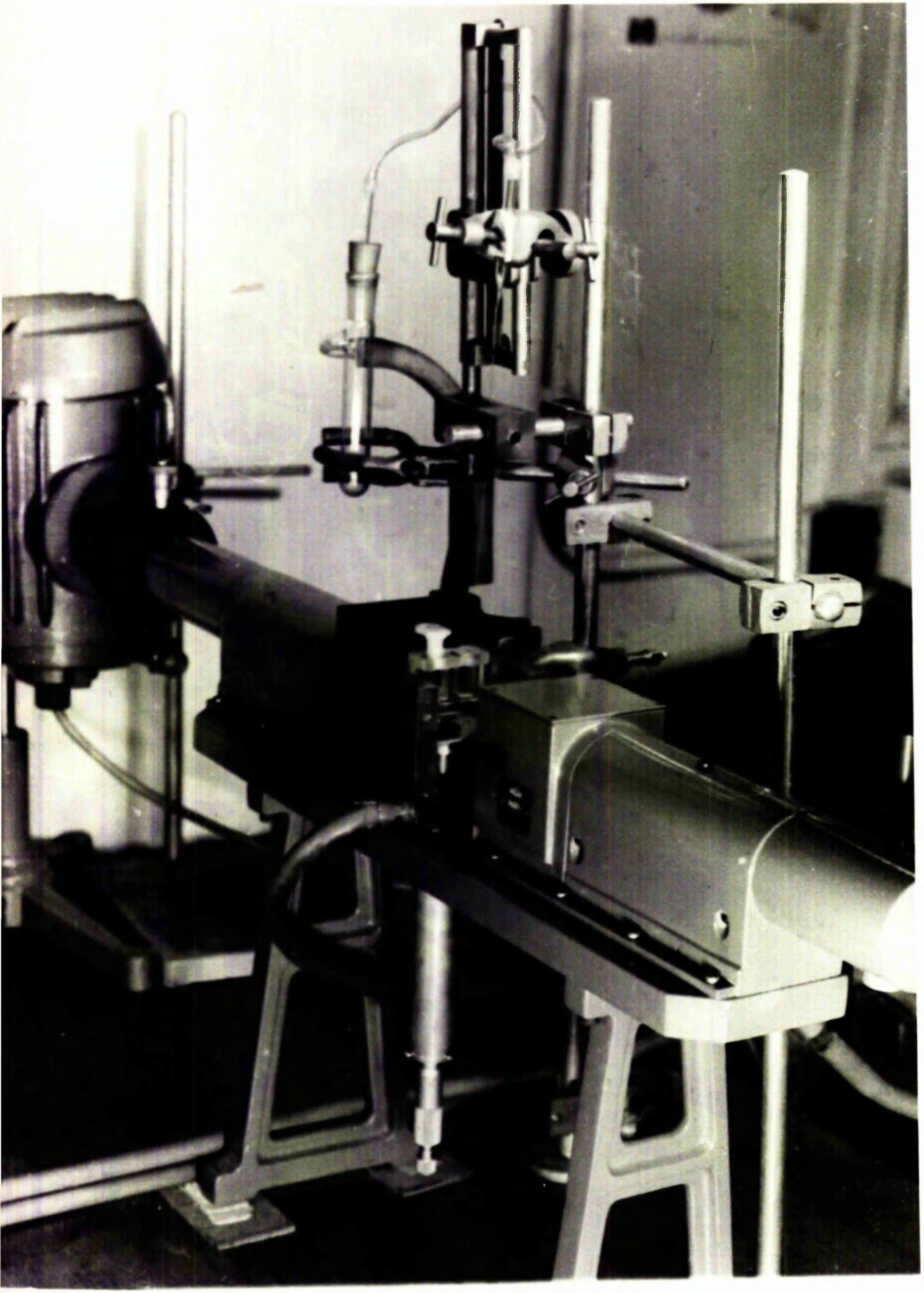


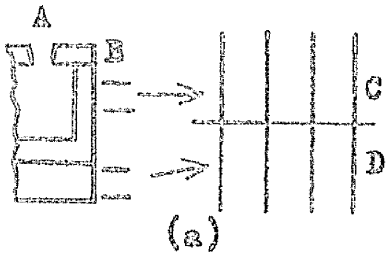
Plate 2.

tufnol, each side having two vertical slits to allow the passage of the beams. The two other faces and the base were brass, the faces having a series of connecting vertical tubes through which the circulating thermostated water passed. The base had one tube through which the water passed from one brass face to the other. The water-jacket was covered by a tufnol lid. The width of the cell table was such that it just fitted between the brass side-walls of the water-jacket. This prevented any rotation of the table, and hence the cell, restricting movement to the vertical plane.

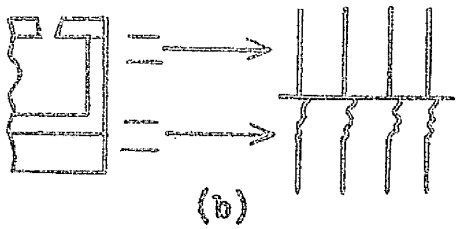
Concentration values were required to be measured at levels in the diffusion compartment equivalent to 1/6th its height from its base and top. The micrometer scale (reading to 0·0001 in.) was therefore calibrated such that these levels could be accurately located on the upper band pattern. For this calibration the following procedure was adopted (fig. 10).

The cell, of liquid path length 1cm., was lowered using the micrometer, till the top of the upper band pattern began to disappear from view, cut out of light being due to the opaque top of the compartment of the cell (fig. 10b). The micrometer reading, x, was noted. Lowering continued till the upper pattern just completely disappeared, (fig. 10c), giving a micrometer reading, y. The cell was then raised till the base of the compartment just appeared at the bottom of the upper

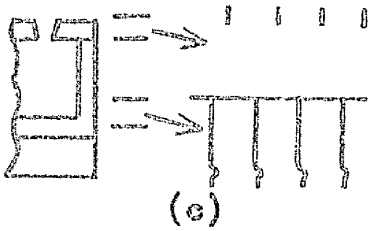
Figure 10.



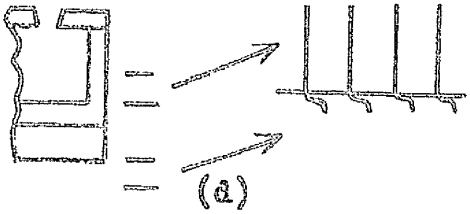
Normal interferometer band pattern



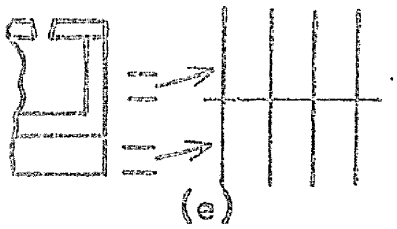
Micrometer reading, x

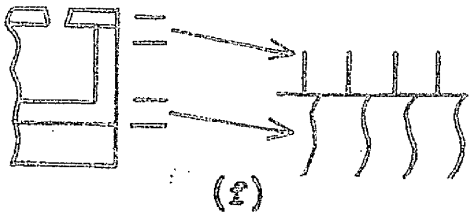


Micrometer reading, y



Micrometer reading, z



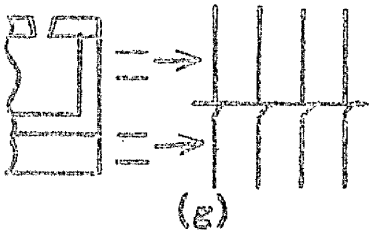


(A) Cell

(B) Regions forming the band patterns

(C) Upper band pattern (cell empty)

(D) Lower band pattern (cell empty)



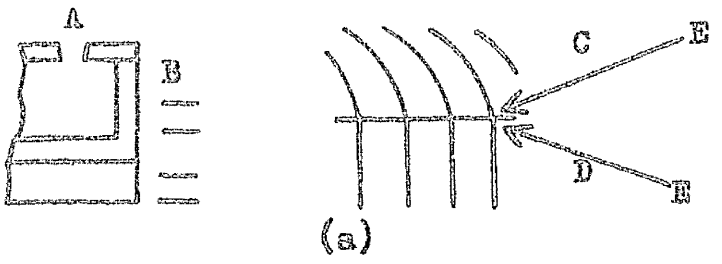
Calibration of Diffusion Apparatus Micrometer.

pattern (fig. 10d). This gave reading z. Thus the compartment height, in terms of micrometer reading was given by (y-z), and 1/6th its height by (y-z)/6.

Location of the lower compartment level. On lowering the cell a distance (y-z)/6, from z, the interference pattern of liquid at the level (y-z)/6 from the base was obtained at the base of the upper band pattern (fig. 10e). The micrometer reading at this position was given by z+(y-z)/6. At this level, the refractive index was obtained as the drum reading when the base of the upper band pattern matched the lower band pattern (fig. 11a).

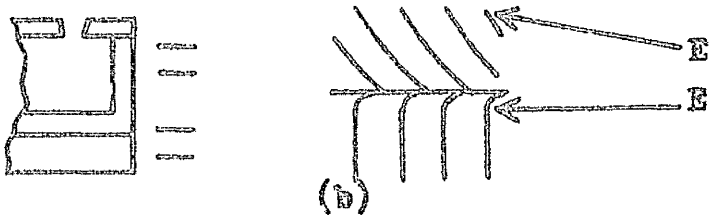
Location of the upper level. By a similar method it was possible to obtain the interference pattern of the liquid at the level (y-z)/6 from the top of the compartment, at the base of the upper band pattern. However, in this position, due to the overall position of the cell, the lower reference band pattern was irregular (fig. 10f). It was arranged, therefore, that the cell be raised such that the required interference pattern be at the top of the upper band pattern, in which position the lower reference band was visible (fig. 10g). The required micrometer reading was then given by x-(y-z)/6. Here, the drum reading was obtained by comparing the top of the upper band pattern with the lower reference pattern (fig. 11b). In order to take readings at both levels, the vertical movement of the cell was 0·1982 in.

Figure 11.



(a)

Patterns observed when obtaining lower level reading



(b)

Patterns observed when obtaining upper level reading

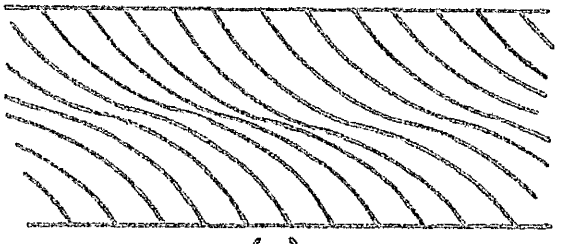
(A) Cell

(B) Regions forming the band patterns

(C) Upper band pattern (Diffusion system in cell)

(D) Lower band pattern (Diffusion system in cell)

(E) Pattern levels matched to obtain drum reading



(c)

Entire upper band pattern of diffusion system

Band Patterns of Diffusion Systems.

Though it was initially awkward to compare the top of the upper band pattern to the lower reference pattern, with practice the reading was obtained nearly as easily and as quickly as that of the other liquid level. In all cases, individual readings were obtained as mean values of four separate readings. The time was taken at the start and end of the reading at each level and two mean times were calculated. These two mean times were then used to evaluate a further mean time, this third mean being used for the plot against the logarithm of the drum reading difference. At the end of each diffusion run, the cell was shaken and the final concentration was obtained from the resulting drum reading and calibration graph.

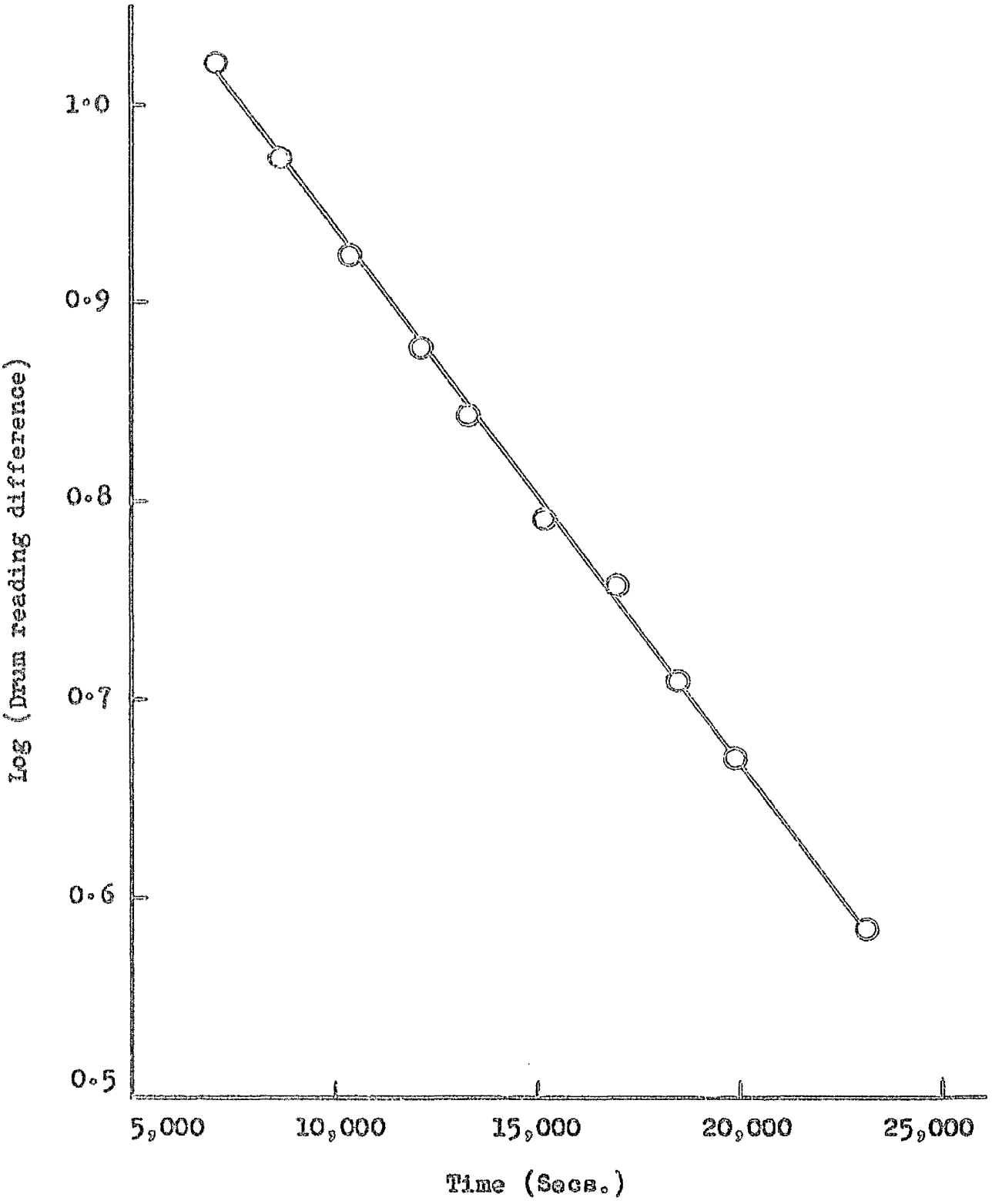
To prepare the diffusion system various techniques were used. The most common was that of filling the diffusion compartment with the more concentrated solution till the meniscus was half way up the stopper neck. A fine pipette was then lowered gently into the solution till its tip, which was curled upwards, was at the level where the boundary was required at zero time. The pipette was secured in this position to prevent movement. The contents of the compartment were then slowly sucked out, using a vacuum pump with an air leak, while the volume in the compartment was maintained by the addition, by hand pipette, of a more dilute solution, or solvent. This replacement was done very carefully to avoid drops falling

through the solution and disturbing the boundary. To help prevent this, a small metal baffle was placed in the cell neck. When the boundary reached the level of the pipette tip, the pipette was carefully removed and the cell stopper put firmly in place.

The less common method of boundary formation was to three-quarter fill the compartment with the more dilute solution, and add the second solution gradually from a hand pipette, the pipette tip resting on the base of the compartment. When the cell was full, the pipette was removed and the cell stoppered. This method produced less sharp boundaries, but sharpness was not a prerequisite for restricted diffusion.

When in place, the base of the cell stoppers lay flush with the top of the cell compartment. To ensure the compartments were completely sealed, the stoppers and necks were lightly greased. For the aqueous test systems 'Apiezon' grease was used; however, because it was alcohol soluble, a silicone stopcock grease had to be used for the alcohol systems. Care was taken to ensure that no silicone was deposited on the optical faces of the compartments. Unfortunately the silicone grease was soluble in benzene and could not be used in the presence of this solvent. A further complication was that the ungreased stoppers did not effect a complete enough seal to prevent evaporation. To overcome this, plastic stoppers from 'E-Nil' volumetric flasks were shaped to fit the compartment necks and were found

Figure 12.



Potassium Chloride Test Diffusion System.

to effect the required seal. For most of the diffusion systems the second cell compartment contained solvent, but in some cases the refractive index change was too large, the solvent having to be replaced by a solution of known concentration, to provide readings within the drum scale.

To test the apparatus, an aqueous potassium chloride and two aqueous glycine solutions were diffused into water, at 25°. The values obtained for the diffusion coefficients are tabulated below and compared to literature figures (Table 8). In all three cases, after an initial time lapse, a straight line was obtained, according to equation (35), on plotting the logarithm of the difference in drum reading between the two levels in the cell compartment, against time (fig. 12). In the subsequent micellar weight studies, an initial time lapse of one to two days was necessary for the attainment of the restricted diffusion conditions.

Table 8.

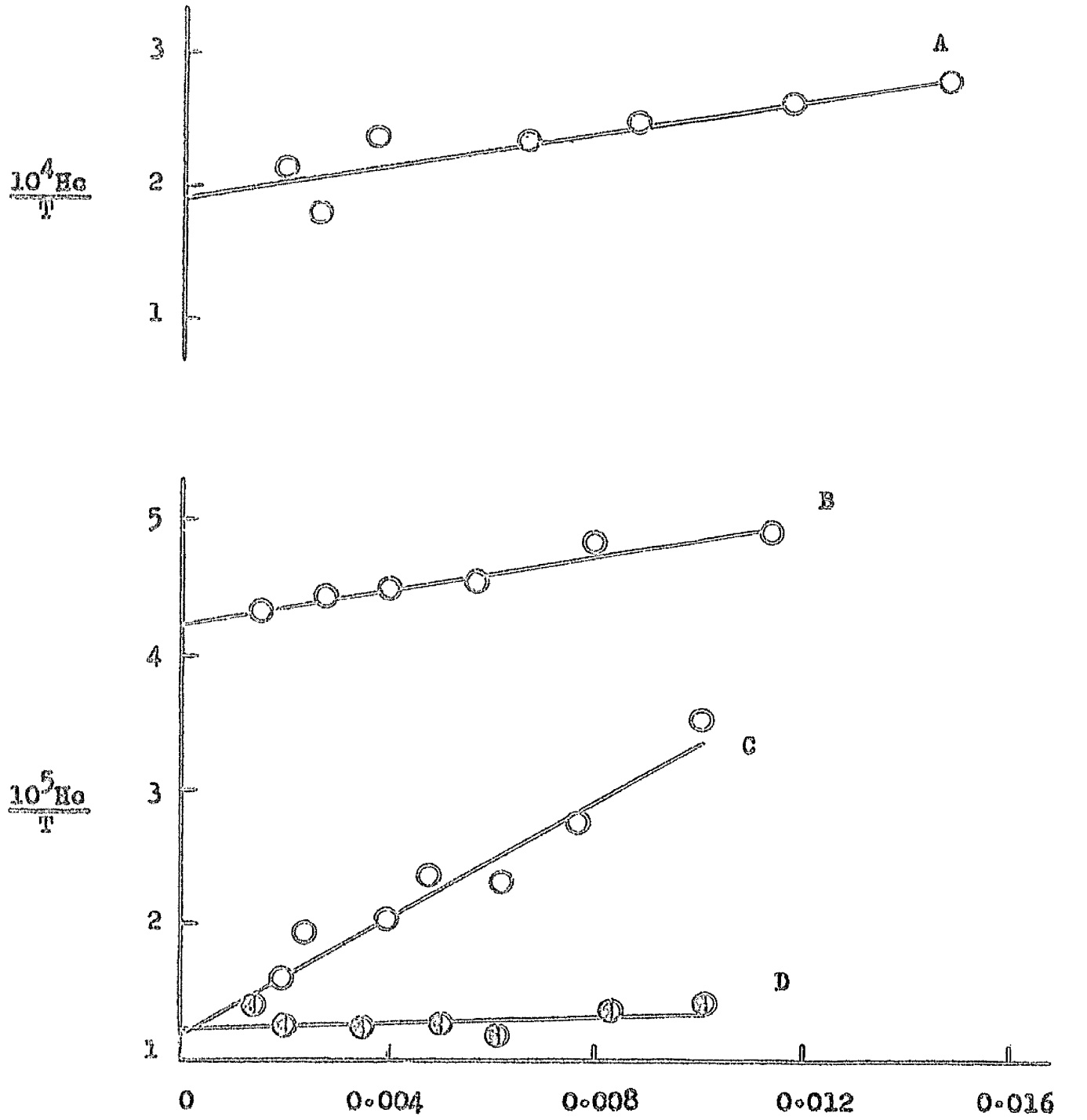
Test Diffusion Systems at 25°.

Solute	Final Concentration	$D.10^5$ $cm.^2 sec.^{-1}$	Literature Values [246, 247]
KCl	0·1N	1·82	1·85
Glycine	0·25%	1·06	1·058
Glycine	0·25%	1·07	1·058

With lecithin as solute, each diffusion system gave a slope of the logΔR against time plot which provided a value of D. When this value was plotted against the final concentration of the system, extrapolation to zero concentration gave the diffusion

coefficient from which the solute micellar weight was calculated. Apart from the aqueous test systems, diffusion was measured at $20 \pm 0.1^{\circ}$.

Figure 13.



A. Methanol B. Ethanol C. Hexanol D. Butanol

Light-scattering Results as Hc/T Against c.

THE EFFECT OF SOLVENT DIELECTRIC CONSTANT ON MICELLISATION BY LECITHIN.

Micellisation in some Aliphatic Alcohols.

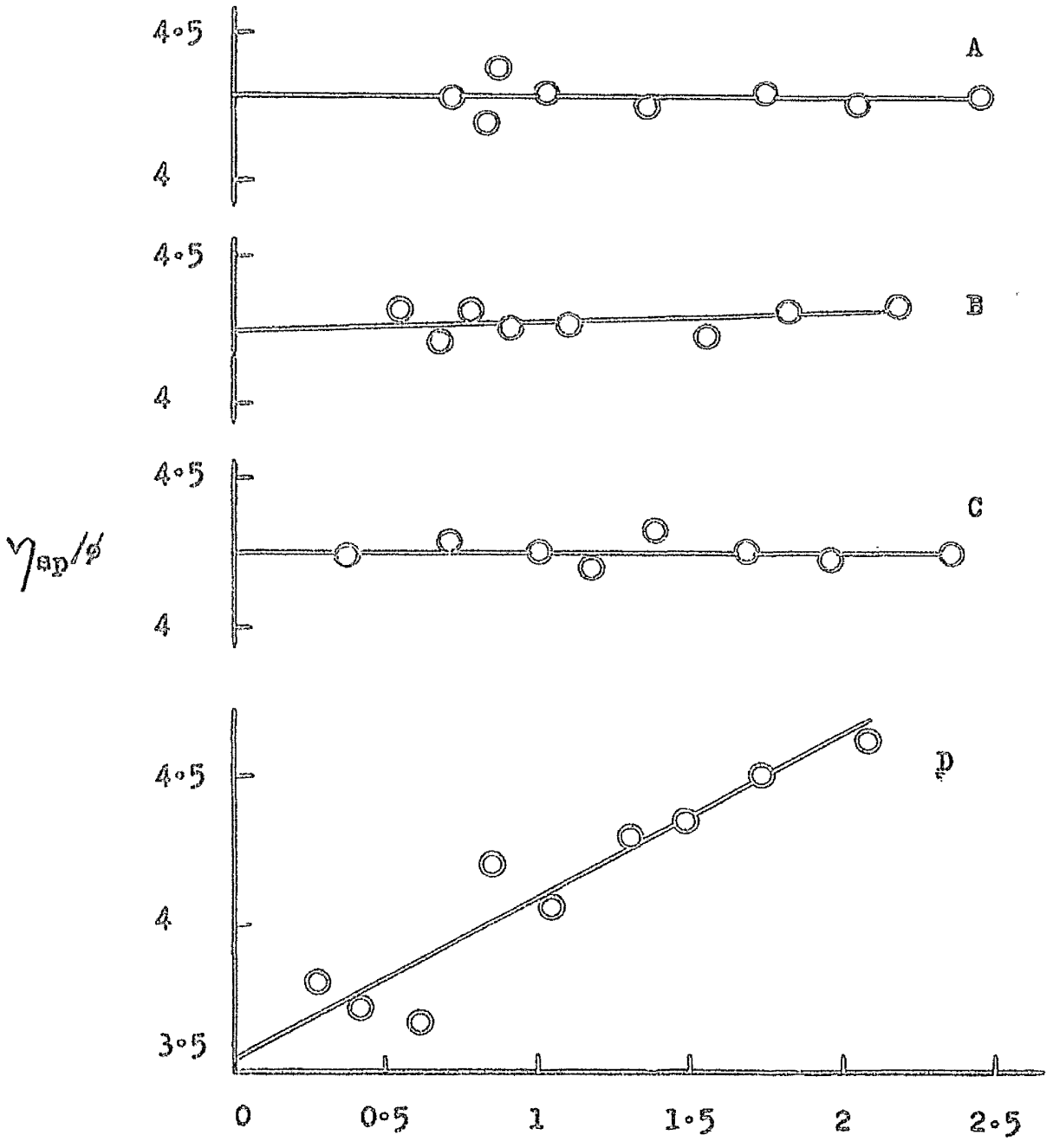
To continue the study of the micelle formation by lecithin in organic solvents, it was decided to study lecithin in organic solvents which should provide intermediate conditions between water and benzene. A series of aliphatic alcohols was chosen, varying in dielectric constant, ϵ, from methanol (ϵ=33·6), via ethanol and butanol, to n-hexanol (ϵ=14·3), the constants for benzene and water being 2·3 and 80·4 respectively.[248]

Results.

For the determination of micellar weights the light-scattering technique was used, the results being given in Table 9 and fig. 13. In the calculation of micellar weight, the Cabannes factor was applied to the Ho/T value at zero concentration. Graphs of concentration against turbidity gave straight lines passing through the origin after subtracting the solvent turbidity from the turbidity of each solution. There were no breaks characteristic of cmc's. If cmc's were present they were at too low concentrations to be detected by light-scattering.

An idea of micellar shape was obtained from viscosity measurements, the results being presented also in Table 9 and in fig. 14.

Figure 14.



A. Methanol B. Ethanol C. Butanol D. Hexanol

Viscosity Results.

Table 9.

Light-scattering and Viscosity Data.

Solvent	Methanol	Ethanol	Butanol	Hexanol
ϵ_{20}	33·6	25·3	17·8	14·3
Micellar Weight	2300	7100	18000	22000
Monomers/micelle	3	9	24	28
Depolarisation	0·345	0·476	0·552	0·509
dn/dc (ml./g.)	0·135	0·118	0·087	0·071
z_{45}	1·01	1·00	1·00	1·00
$(\eta_{sp}/\phi)_{\phi=0}$	4·29	4·26	4·26	3·56

No differences were observed between the properties of the two lecithin samples, A and B, that were used.

Discussion.

It can be seen that the micelle size increases on passing from methanol to hexanol, that is, increasing as the value of ϵ decreases.

This trend is continued in benzene ($\epsilon = 2·3$) where the micellar weight has been stated[131] as 57,000 at 25°. Further, in benzene it has been suggested that the micelles have the structure of a bimolecular leaflet with the polar heads tucked inside the micelle.[140] It would seem therefore that the bimolecular leaflet structure is developed with the micellar growth on passing into solvents less polar than methanol. The development of this structure will be described later. In water, the type of micellar structure is a reversal of that in benzene, the

hydrocarbon chains being inside the micelles.[136,137] The lecithin-methanol system would therefore appear to represent an approximate half-way point between the type of micelles formed in aqueous systems and those present in the higher alcohols and benzene.

The micellar size in benzene decreased from 57,000 at 25° to 43,000 at 40°.[131] This trend may explain why in ethanol, at 20°, small micelles were found while monomers have been reported as being present at the boiling point.[130] The micellar weight in methanol agrees well with that of 2,000 obtained by diffusion.[249]

A discussion of the use of the viscosity data, in conjunction with the micellar weights, for comparison to model structures will be made later when the simultaneous consideration of further work can be made.

Micellisation in some Further Solvent Systems.

To extend the previous work, micellisation was studied in solvents having a wider range of dielectric constant.

Results.

For comparison with the results in the alcohols, the micellar weight was measured in benzene, a 79·7%V/V methanol/benzene mixture (ϵ = 21·1)[250] and in various methanol/water and ethanol/water mixtures where ϵ ranged from 29·0 to 42·8.[250] Light-scattering and viscosity studies were made of all systems, diffusion studies being made of four mixed solvent systems.

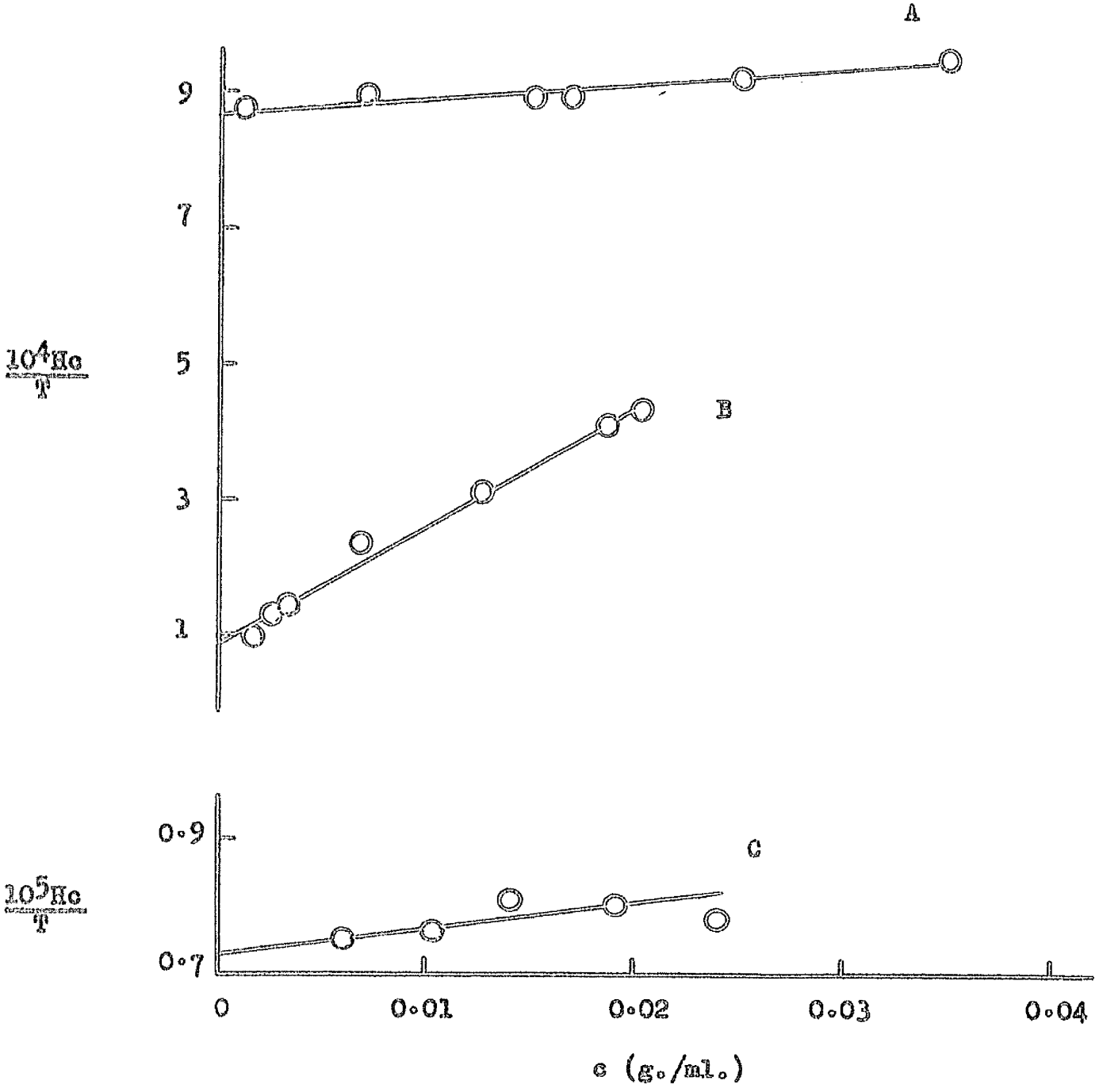
For the light-scattering results in binary solvents, the reciprocal of the intercept of the H_2c_2/T_2 against c_2 plot was designated $M_{approx.}$, the correct micellar weight only being given from equation (25) if the interaction coefficients were small or if the two solvents had similar refractive indices. T_2 and c_2 refer to the micellar species, the turbidity at the cmc itself having been subtracted from the experimental results in the usual manner. In the normal use of the light-scattering equation for binary solvents, M_2 for polymer solutions can be determined in a single solvent and the interaction terms evaluated in mixed solvents. However, for micelles, M_2 may alter as the solvent composition is changed, so there is the added difficulty that $M_{approx.}$ must be checked by evaluation from another method. Determination from diffusion-viscosity measurements indicates that the value of $M_{approx.}$ evaluated as described is substantially correct.

[210]

The light-scattering results, uncorrected for depolarisation, are given in figs. 15 and 16, final molecular weights being corrected for depolarisation and dissymmetry (Table 10). The cmc of lecithin in benzene, previously and in the present work, was too small to be detected, only the higher association limit between small and large micelles being found. The larger micellar weight was therefore determined accordingly. All the results in single solvents showed that the cmc was too small to be detected by the light-scattering techniques. However, the presence of

[131,140]

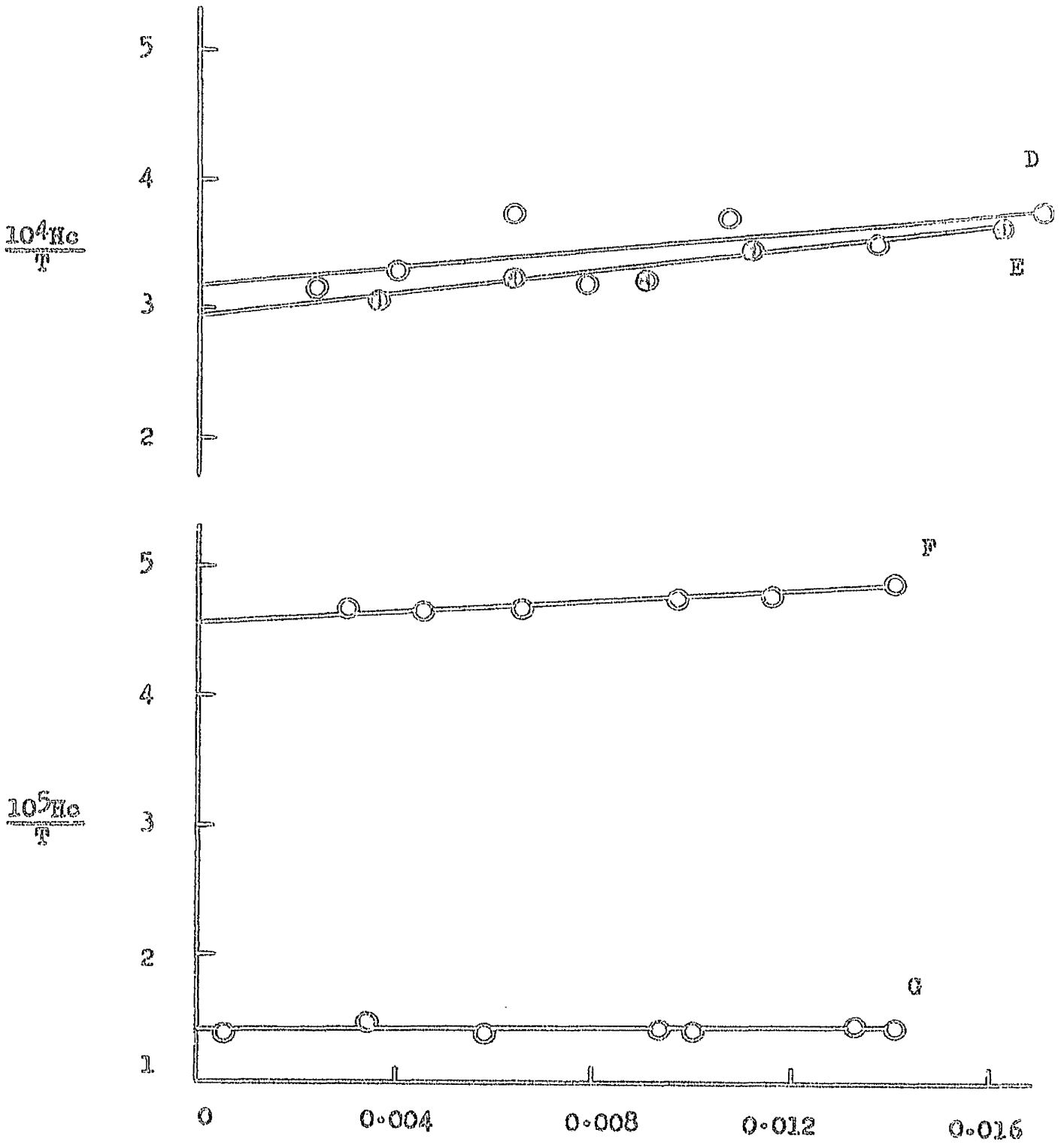
Figure 15.



A. 93·4% Ethanol/water B. 79·7% Methanol/benzene C. Benzene

Light-scattering Results as Hc/T Against c.

Figure 16.



D. 93% Methanol/water E. 80% Ethanol/water

F. 70% Ethanol/water G. 84% Methanol/water

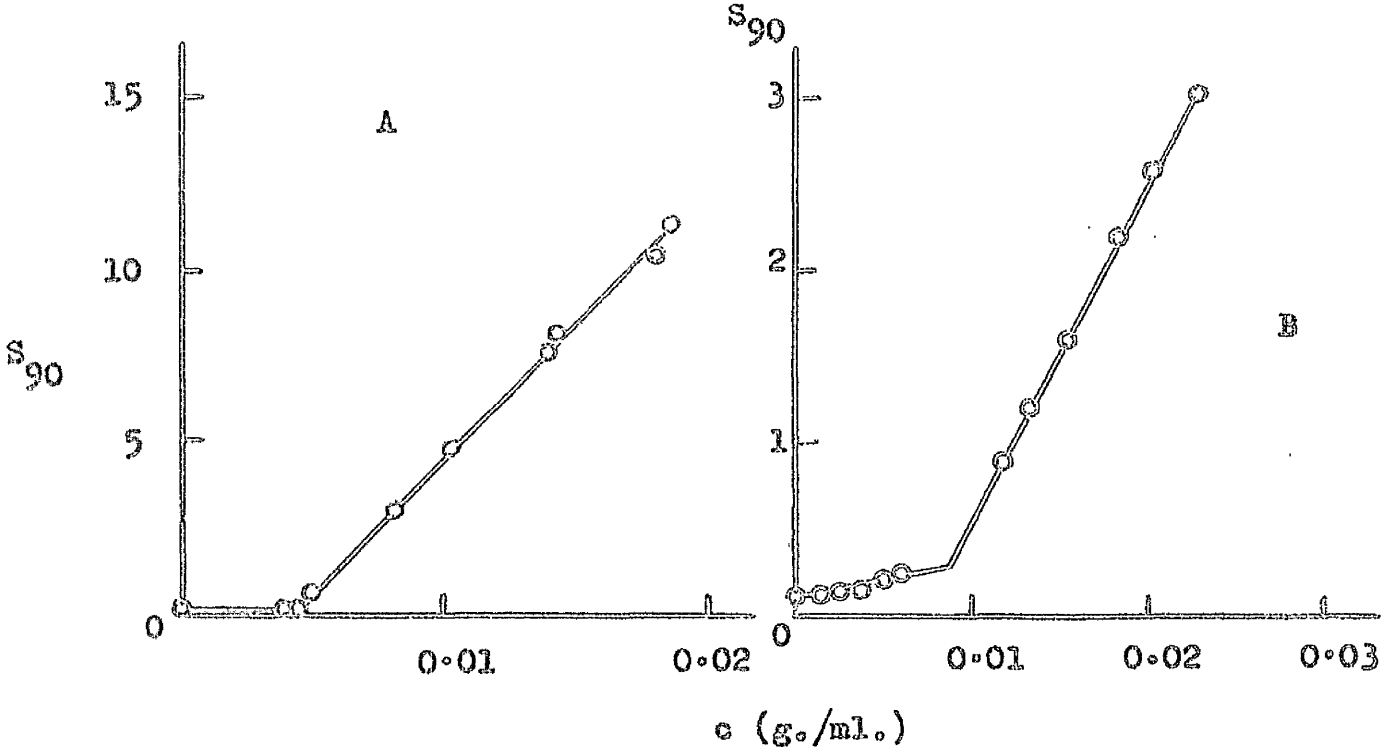
Light-scattering Results as Hc/T Against c.

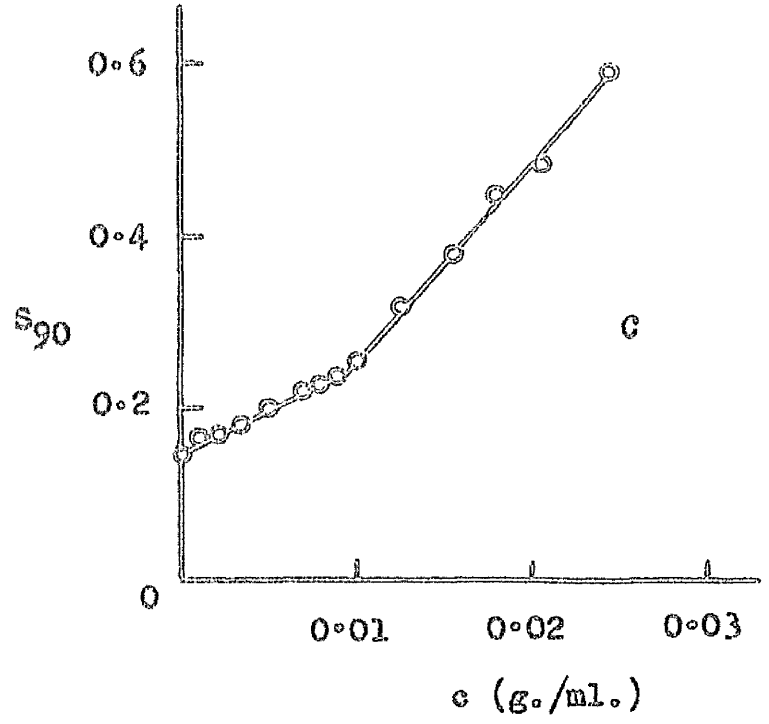
water in methanol or ethanol caused a distinct cmc to develop, as shown by the S_{90} against c curves of figs. 17 and 18.

Several methods were tried to check the cmc values obtained by light-scattering. When the refractive index difference between solvent and solution in 70% ethanol/water was plotted against solution concentration, no abrupt change in slope was observed, even at the light-scattering cmc. Using the bubble pressure method of Sugden[251], the surface tensions of both 70% and 80% ethanol/water mixtures did not differ significantly from lecithin solutions in these solvents. With the Wilhelmy plate technique[252] and the 70% system, again the difference in solution and solvent surface tensions did not indicate the presence of a cmc. To try and solubilise a dye in the lecithin micelles in 80% ethanol/water, a finely powdered indigo vat dye (soluble in benzene, insoluble in water and solvent) was shaken with several solutions till their optical densities remained constant. There was no marked change in optical density between micellar and monomeric solutions of lecithin, and thus no confirmation of a cmc was possible. Nevertheless, the breaks in the S_{90} against concentration curves are sharp and distinct.

Apart from the 84% methanol/water system (all solvent mixture percentages are V/V), the observed dissymmetries were close to unity, indicating that no dimension of the particles exceeded 270Å ($\lambda/20$). It was difficult to clarify solutions in this methanol/water solvent, and this may have been

Figure 17.





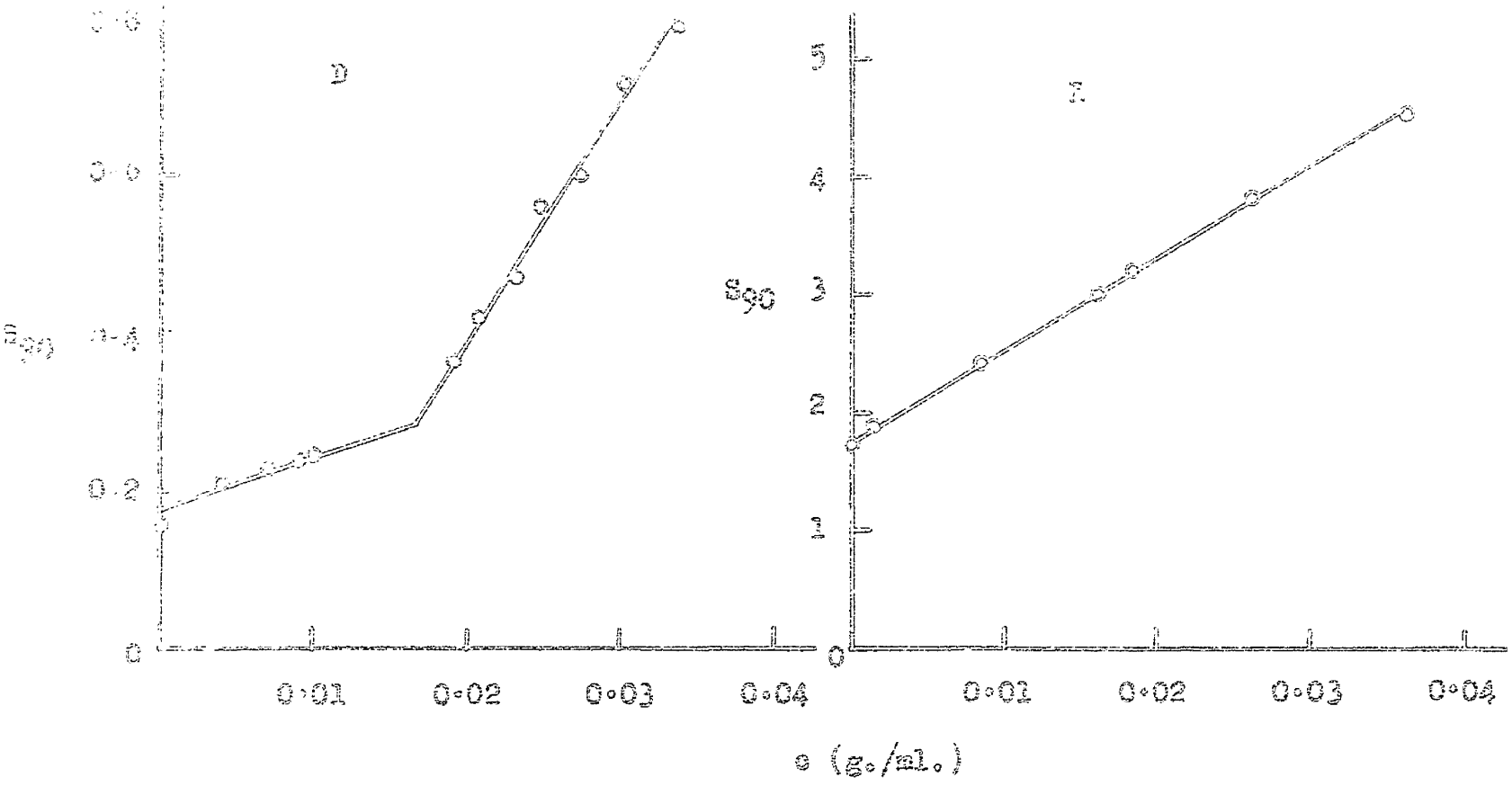
A. 84% Methanol/water

B. 70% Ethanol/water

C. 80% Ethanol/water

Light-scattering Results as S_{90} Against c.

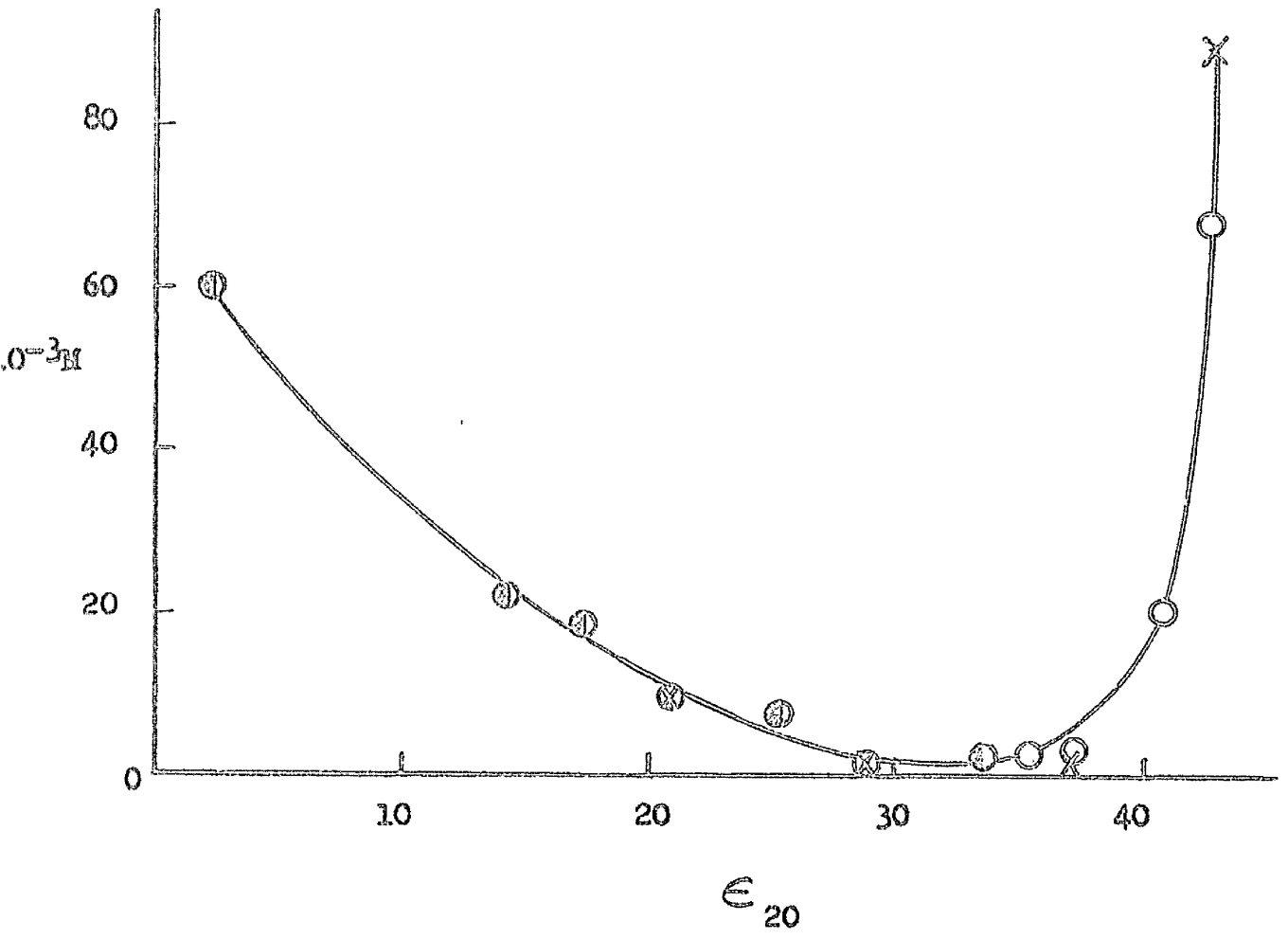
Figure 18.



D. 93% Methanol/water E. 93·4% Ethanol/water

Light-scattering Results as S_{90} Against c.

Figure 19.



◉ Light-scattering in Single Solvents

○ Light-scattering in Mixed Solvents

X Diffusion-viscosity

The Effect of Solvent Dielectric Constant on the Micellar Weight of Natural Lecithin.

responsible for the slight dissymmetry observed. High values for the depolarisation were observed, continuing the trend previously noted with the aliphatic alcohols. For naturally occurring lecithin, the depolarisation decreases as ϵ increases; in water small depolarisations (0·055) were found by Robinson.[253]

Table 10.

Light-scattering Results.

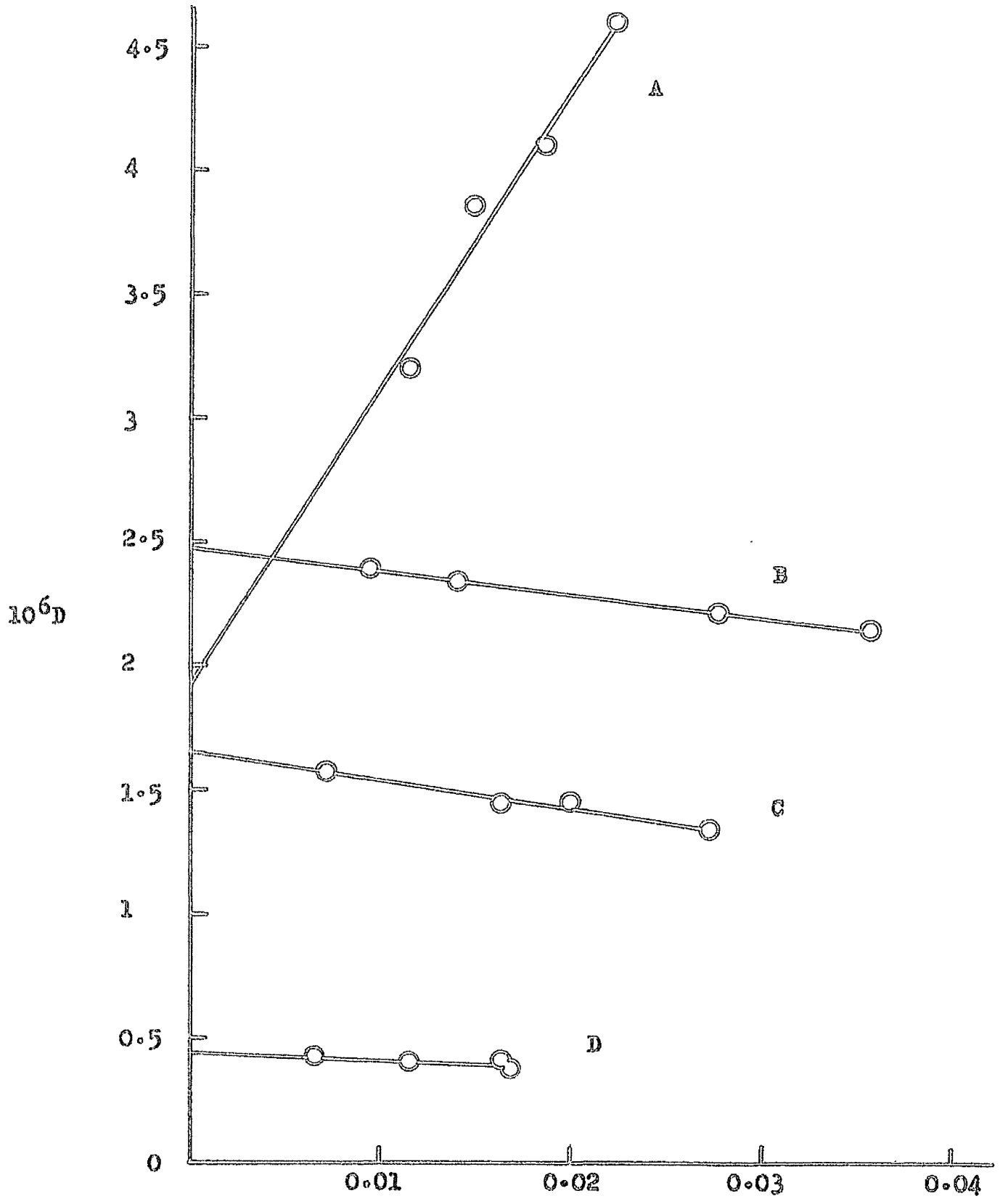
Natural Lecithin.

Solvent	$\epsilon_{20°}$	$10^{-3} M_2$	n	ρ	dn/dc	Z_{45}	cmc(g./ml.)
Benzene	2·3	60	80	0·360	-0·038	1·06	0
79·7% MeOH/Benzene	21·1	9·0	12	0·125	0·119	1·00	0
93·4% EtOH/H_2O	29·0	0·7	1	0·232	0·114	1·00	>0·036
80·0% EtOH/H_2O	35·5	2·6	3	0·137	0·107	1·01	0·0095
93·0% MeOH/H_2O	37·3	2·7	4	0·063	0·140	1·04	0·0167
70·0% EtOH/H_2O	40·8	20	27	0·031	0·112	1·00	0·0088
84·0% MeOH/H_2O	42·8	68	90	0·013	0·139	1·11	0·0045
Synthetic Lecithin							
79·7% MeOH/Benzene	21·1	1·1	1-2	0·053	0·109	1·00	
EtOH	25·3	0·82	1	0·070	0·122	1·00	>0·0089
MeOH	33·6	0·89	1	0·159	0·155	1·00	>0·009

For Table 10, n = number of monomers in micelle, M_2 = $M_{approx.}$ for solvent mixtures, MeOH = methanol and EtOH = ethanol.

From Table 10 and the plot of ϵ against M and M_2 (fig. 19), monomers appear to be present in the 93% ethanol/water mixture (ϵ = 29·0), and changing the solvent dielectric constant from

Figure 20.



(A) 79·7% Methanol/Benzene (B) 93% Methanol/Water

(C) 93·4% Ethanol/Water (D) 84% Methanol/Water

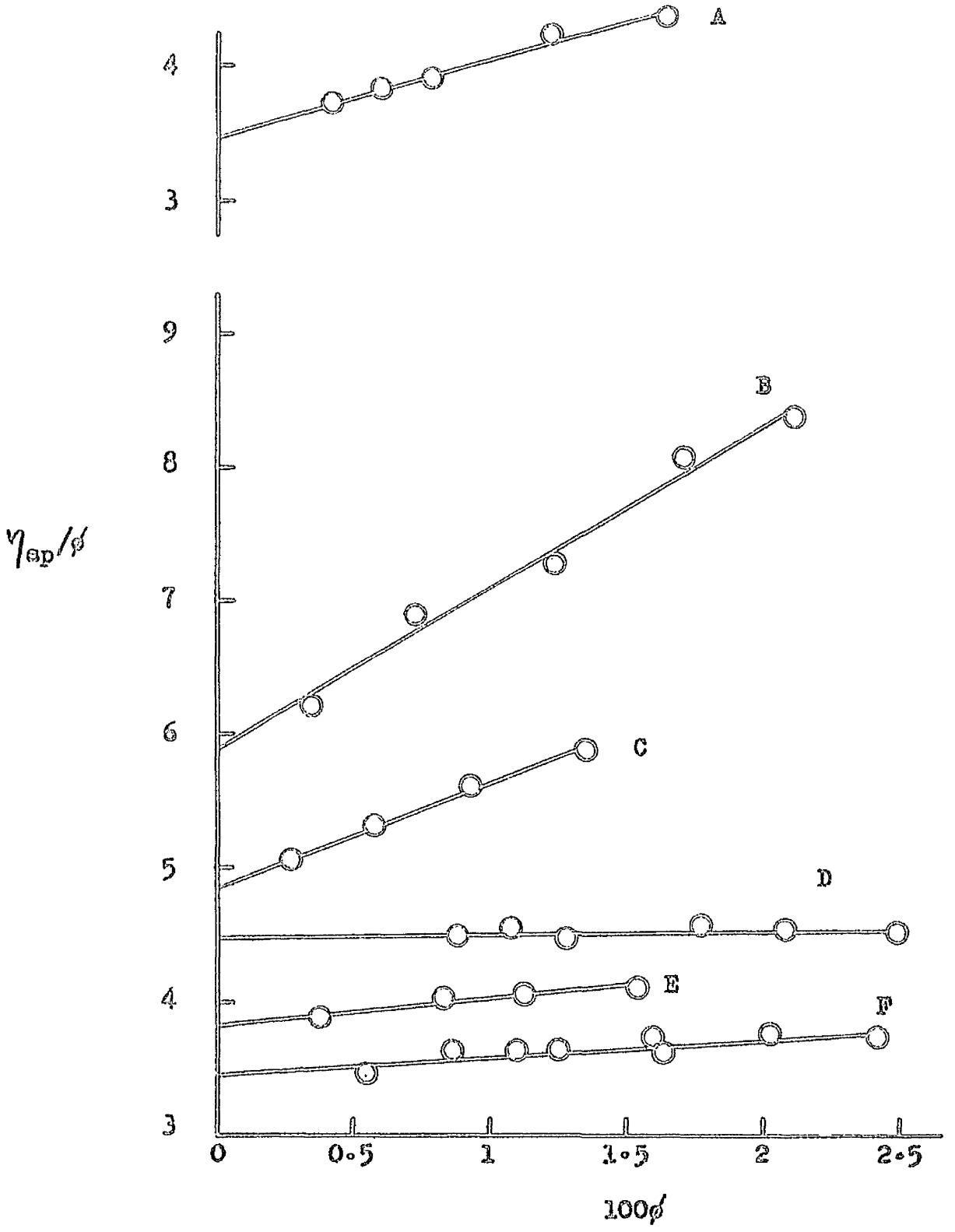
Diffusion Results.

this value causes micelles to develop. In this solvent system the cmc is greater than 0.036g./ml., since this was the most concentrated solution whose S_{90} was measured and no break was found in the S_{90} against c graph. In general, the cmc decreases as the dielectric constant is increased. No detectable cmc was found for lecithin in pure water.[253]

As a means of checking the approximate values of micellar weight found by light-scattering in mixed solvent systems, diffusion measurements were made (Table 11, fig. 20). The lowest concentration in the diffusion cell was maintained above the cmc where micelles are present, so that it was reasonable to assume that only micelles were diffusing. Following the procedure of Stigter, Williams and Mysels,[254] the diffusion coefficients were extrapolated to the cmc (or to zero concentration in the case of the 93·4% ethanol/water system).

For the viscosity results (Table 11, fig. 21) where cmc's were present, the viscosity of the solution at the cmc was used as the 'solvent' viscosity, and the volume fraction of solute at the cmc was subtracted from the total volume fraction in the evaluation of η_{sp}/ϕ. Similar procedures have been used before.[140, 255] The viscosity intercept was used to calculate the frictional coefficient ratio of the micelle, considering the micelle, in turn, as a prolate and oblate ellipsoid (in the absence of solvation) and as a spherical solvated particle. The micellar weights were then calculated for the three alternative cases

Figure 21.



(A) 80% Ethanol/Water (B) 84% Methanol/Water

(C) 70% Ethanol/Water (D) 79·7% Methanol/Benzene

(E) 93% Methanol/Water (F) 93·4% Ethanol/Water

Viscosity Results.

(M_2pro., M_2ob., M_2solv.). In the alcohol/water systems water was assumed to be the solvating liquid, and methanol in the methanol/benzene system.

Table 11.

Diffusion and Viscosity Results.

Natural Lecithin.

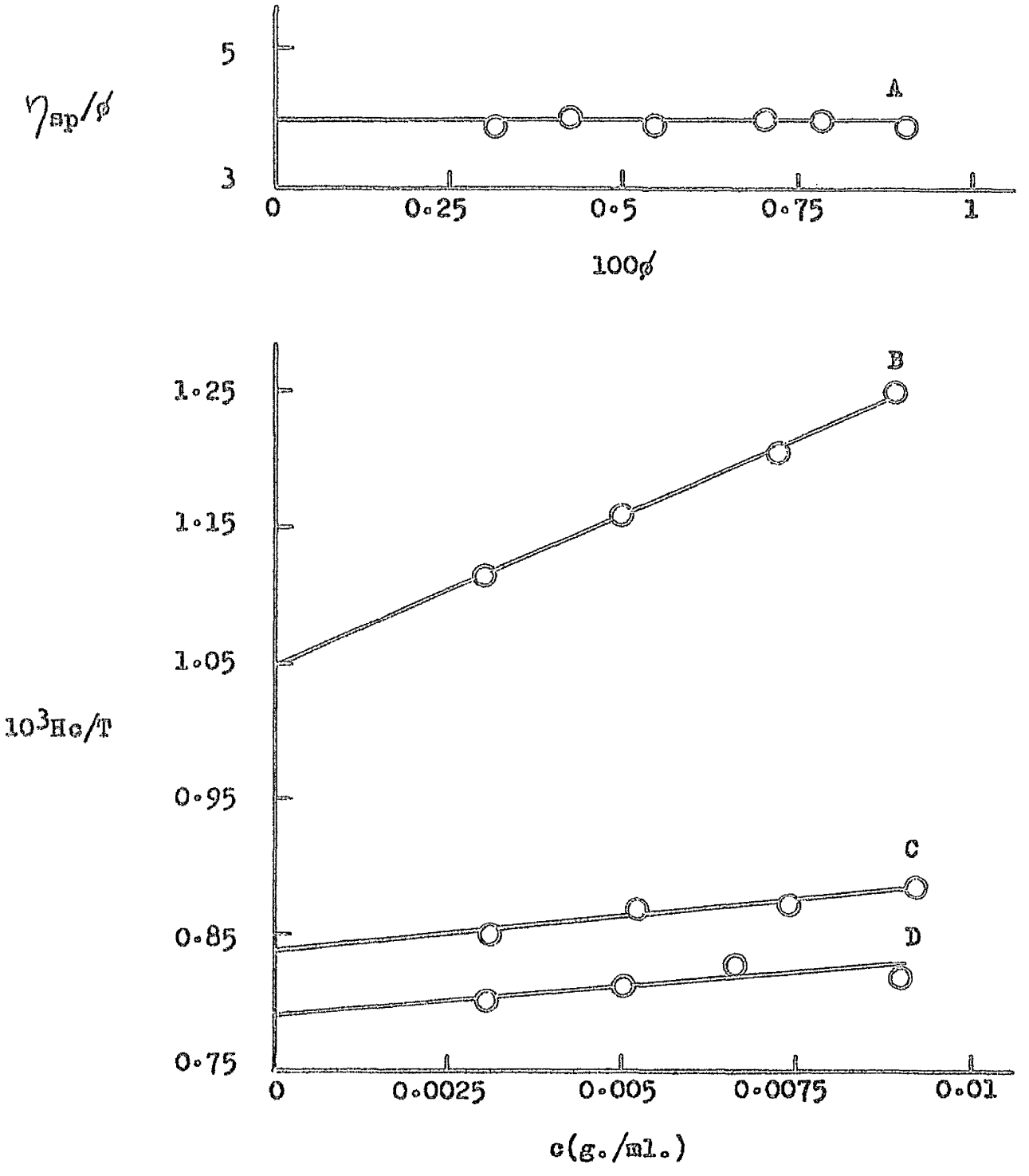
Solvent	$(\eta_{sp}/\phi)_{\phi=0}$	$10^6 D$ ($cm^2 sec^{-1}$)	$10^{-3} M_2$ pro.	$10^{-3} M_2$ ob.	$10^{-3} M_2$ solv.
79·7%MeOH/Benzene	4·48	1·92	10·0	9·2	8·9
93·4%EtOH/H_2O	3·47	1·66	1·1	0·98	0·96
80·0%EtOH/H_2O	3·36				
93·0%MeOH/H_2O	3·84	2·48	1·9	1·8	1·7
70·0%EtOH/H_2O	4·87				
84·0%MeOH/H_2O	5·89	0·441	99·1	87·0	83·7

Synthetic Lecithin.

EtOH	3·96

Comparison of the diffusion coefficients of potassium chloride and glycine with known values indicates an error of $\pm 2\%$, so in view of the potential errors present in the experimental measurements, the agreement between M_2 obtained from the two methods is good. In the alcohol/water systems there is not much difference between the refractive indices of the two solvent components, and presumably any specific adsorptions of one component makes little difference to the observed turbidity. In the case of the methanol/benzene system, there

Figure 22.



Viscosity (A) Ethanol

Light-scattering (B) Ethanol (C) 79.7% Methanol/Benzene

(D) Methanol

Viscosity and Light-scattering Results for Synthetic Lecithin.

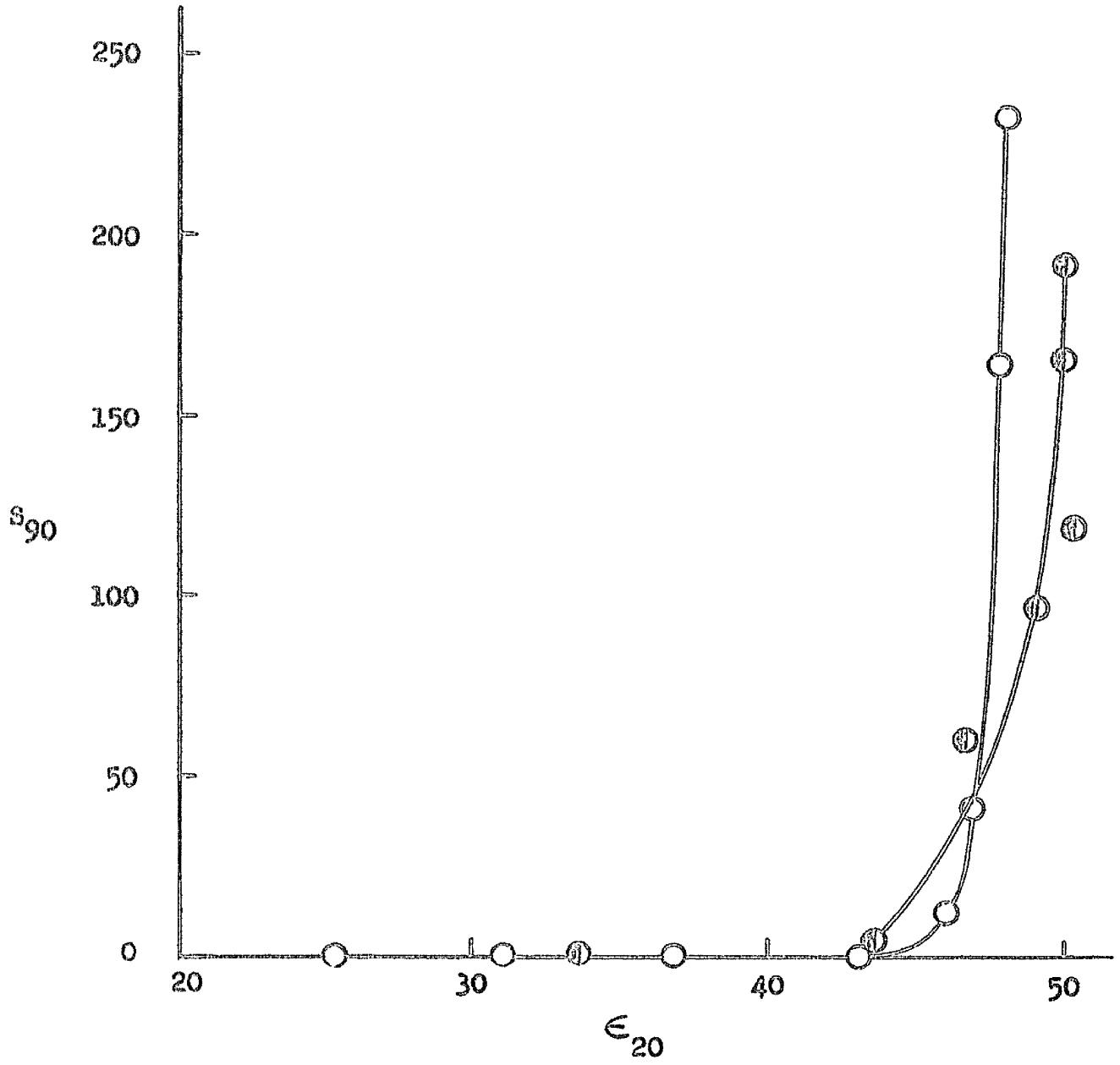
is a considerable difference between the refractive indices of the two components, and the agreement between the micellar weights from the two methods indicates no specific adsorption is occurring. Also the micellar weight found in benzene (at 20°) agrees well with those of 55,-57,000 found before[131,140] at 25°.

A few results are presented for synthetic dipalmitoyl-lecithin (fig. 22), showing that in methanol and ethanol monomers are present up to the highest concentrations studied. A slight association was noted in the methanol/benzene mixture.

Attempts were made to obtain micellar weights of natural lecithin in solvents of lower dielectric constant than benzene. These were unsuccessful due to the ease of precipitation of the solute from such as iso-octane (ϵ = 1·9),[248] n-heptane and n-heptane containing 10 and 20% benzene (ϵ = 1·97-2·03).[248,256] For the same reason, a study of the synthetic material in benzene could not be undertaken.

In 60% ethanol/water (ϵ = 46·5), the reluctance of natural lecithin to dissolve or stay in solution prompted an investigation of the limit of ϵ such that solutions could readily be formed without ultrasonic dispersion. Thus a series of 0·1% W/V lecithin solutions and sols in methanol and ethanol aqueous mixtures were prepared. The S_{90} of each was measured without previous filtration, and the values plotted against ϵ (fig.23). Separate plots for methanol/water and ethanol/water mixtures were very similar, both showing negligible change in S_{90} till the ϵ = 43-47 range,

Figure 23.



Plot of S_{90} Against ϵ for 0·1% Lecithin in some Methanol/Water (●) and Ethanol/Water (○) Mixtures.

where a rapid increase was observed. For this reason the choice of alcohol/water mixtures for micellisation studies was limited to those of $\epsilon<43$.

The two lecithin samples used (C and D) again showed no difference in properties.

Discussion.

Association of single molecules into micelles will occur if this change reduces the total free energy of the system. Changes in interfacial energy at the solute/solvent interface, and in electrostatic forces, and dipole-dipole interactions must be considered. If h is the area of the polar head group of the monomer, t is the area of the hydrocarbon tails, and the superscripts s and m represent single molecules and micelles respectively, then the interfacial energy for n monomers is

$$n(h^s \gamma_{hs} + t^s \gamma_{ts})$$

γ_{hs} and γ_{ts} being the interfacial energy per unit area between the head group and solvent, and the tails and solvent, respectively. For a micelle of n monomers, the interfacial energy is

$$n(h^m \gamma_{hs} + t^m \gamma_{ts}).$$

The change of interfacial energy on forming the micelles is

$$\Delta E = n\gamma_{hs}(h^s - h^m) + n\gamma_{ts}(t^s - t^m).$$

Considering the non-aqueous side of fig. 19, between $\epsilon = 2$ and $\epsilon = 30$, where a decrease in micellar size with increasing dielectric constant occurs, a small value of γ_{ts} would be expected, due to the miscible nature of these solvents with paraffin chains,

and therefore it would be expected that the first term on the right of the above equation is largely responsible for the ΔE on micellisation. A high interfacial energy between the head groups and solvent could be reduced by forming a micelle of the bimolecular leaflet type, with the head groups in the centre of the micelle. Further important factors for micellisation in non-aqueous solvents have been shown to be dipole-dipole interactions between head groups, and the possibility of hydrogen bond formation,[109,110,111] and it is possible that these factors may be more important than interfacial energy considerations in the ϵ = 10-20 region. This will be discussed in more detail below.

In the ϵ = 30 region, monomers are present over the concentration range studied, indicating that both γ_{hs} and γ_{ts} γ_{ts} are small, and that other interactions are absent. A further increase of dielectric constant will begin to make γ_{ts} larger, hence promoting the formation of micelles where the hydrocarbon chains are placed in the interior and the polar groups on the outside, this being the conventional micellar structure in water. It seems apparent from the results on the right hand (aqueous) side of fig. 19 that the interfacial energy considerations are most important, the energy saving of placing the hydrocarbon chains out of contact with solvent being the significant factor for micelle formation.

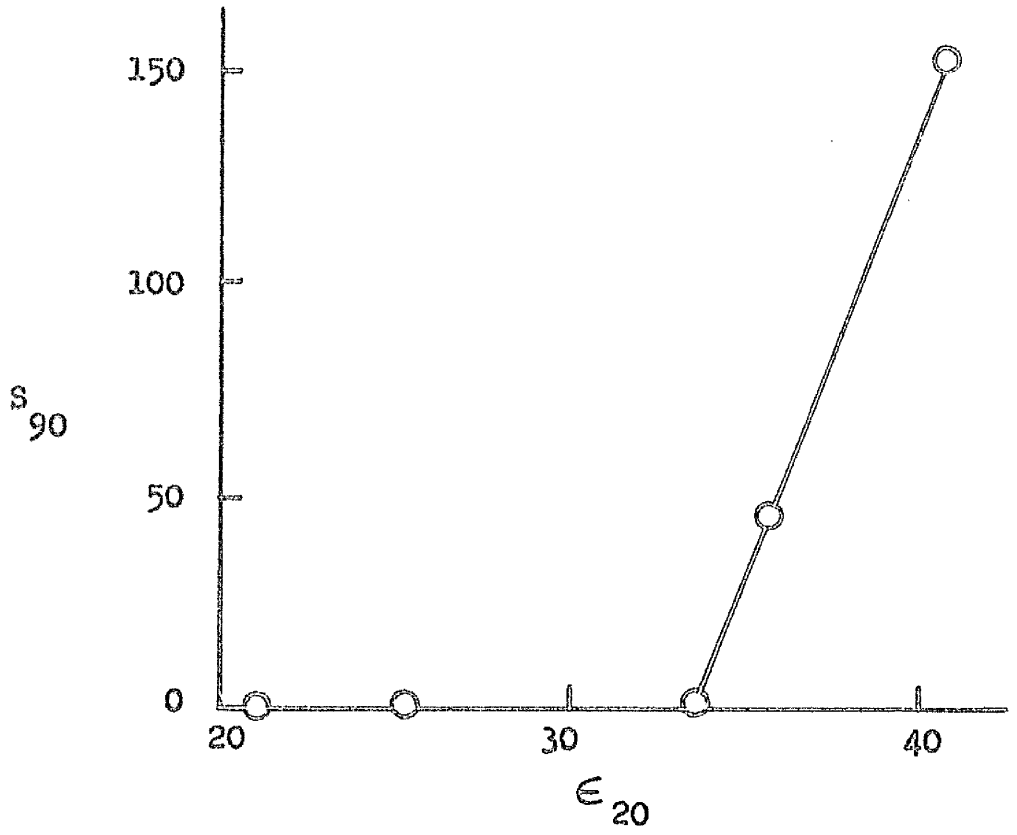
It is interesting to note that the alcohol/water systems are characterised by the presence of monomers, and of cmc's in most

cases. We may compare methanol ($\epsilon = 33{\cdot}6$), where the cmc was present at too low a concentration to be detected by light-scattering, with the 93·4 and 80% ethanol/water systems ($\epsilon = 29{\cdot}0$ and $35{\cdot}5$ respectively), both of which show pronounced cmc's. These cmc's may be increased by greater ionisation of the polar head groups giving greater repulsions between the head groups, due to the interaction of water with these groups; the cmc would be shifted to a higher concentration by the presence of water. As more water is added to the alcohol/water mixtures the general decrease in cmc is probably due to the increased hydrophobicity of the monomers under these conditions.

Because of the agreement, firstly, between the methanol/water and ethanol/water systems when the S_{90} values of the 0·1% solutions and sols were plotted against ϵ, and secondly, between the lecithin micellar weights in methanol (2,300), 80% ethanol/water (2,600) and 93% methanol/water (2,700) where the solvent dielectric constant range was narrow (33·6–37·5), it would appear that the micellar weights would be the same in either a methanol/water or ethanol/water mixture of the same dielectric constant, though the proportion of water in the two solvents was different.

Saunders[257] has shown that a lecithin containing only saturated fatty acids was not dispersible in water, while natural lecithin was dispersible. In the light of this, and the few results presented for the synthetic dipalmitoyl-lecithin,

Figure 24.



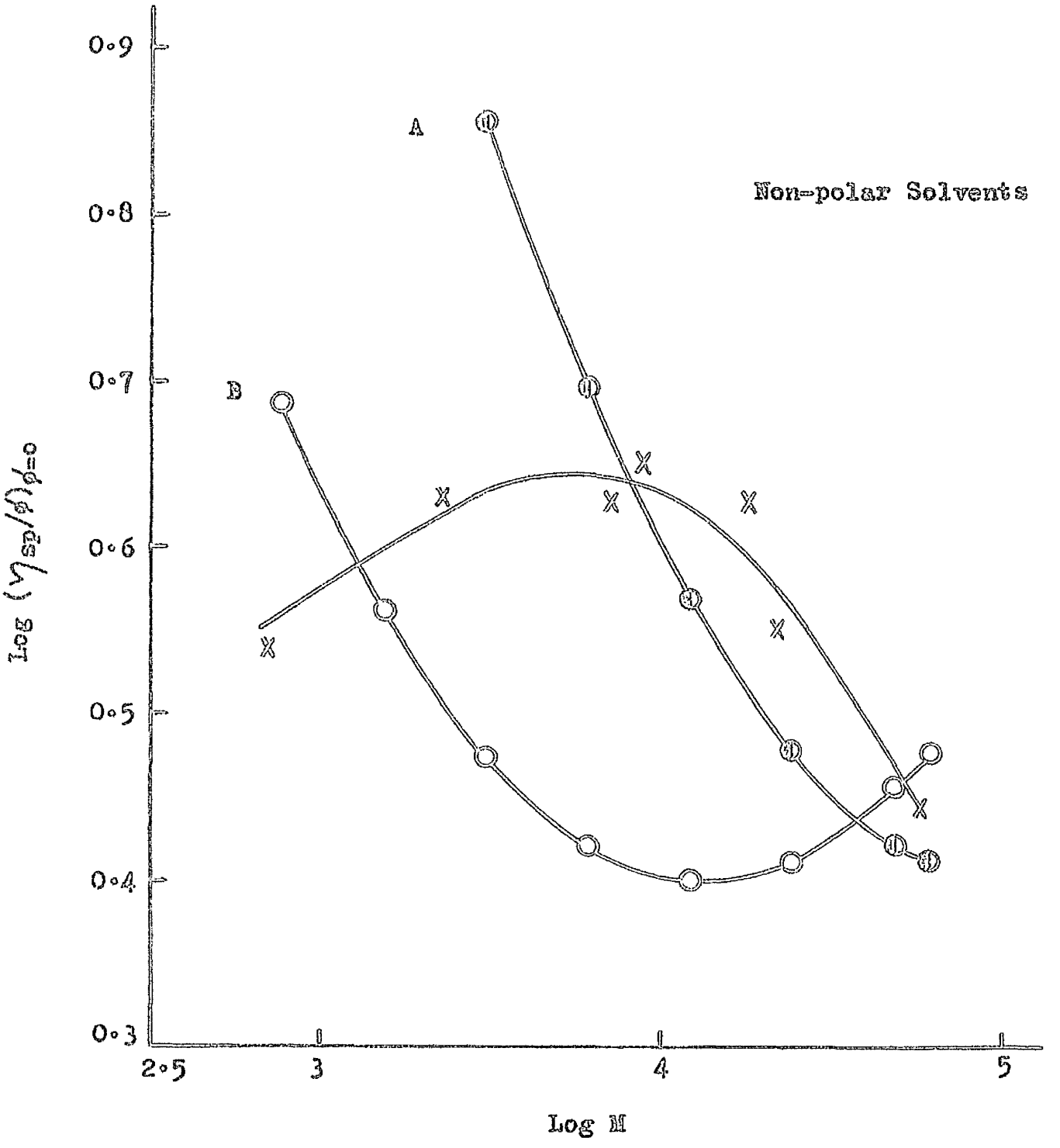
Solvent	ϵ	S_{90}
79·7% Methanol/Benzene	21·1	0·5
Ethanol	25·3	0·2
Methanol	33·6	0·2
80% Ethanol/Water	35·6	40·5
70% Methanol/Water	40·8	152

Plot of S_{90} Against ϵ for 0·1% Synthetic Lecithin.

including also a plot of the S_{90} values of 0·1% solutions and sols in various solvents (fig. 24), an indication is obtained that the micellar weight-dielectric constant curve for the synthetic lecithin may have a narrower 'U' form than fig. 19.

Some idea of micellar structure can be gained from micellar weights and viscosity intercepts. Considering first the non-aqueous side of fig. 19, in general $(\eta_{sp}/\phi)_{\phi=0}$ decreases as M_2 increases. Previous results in benzene[131, 140] indicated that unsolvated micelles were present in this solvent. On assuming that this behaviour is general on the non-aqueous side of fig. 19, the viscosity results can be interpreted in terms of micellar asymmetry. The dimensions of the lecithin monomer used before[140] were length 35Å and head group area 55Å^2, from molecular models. Allowing a gap of 2Å between the two sheets of polar groups in the centre of a bimolecular leaflet micelle, Simha's shape factor, ν, can be calculated as a function of the number of monomers in the micelle. One dimension of the micelle remains fixed at a length of 72Å in these calculations, and the other two dimensions increase as monomers are added. An equal growth in the two dimensions at right angles to the 72Å dimension is assumed, as this gives more effective shielding of the polar head groups from the solvent than growth in one dimension. Fig. 25 shows experimental and calculated results as log (viscosity intercept) against logM_2 plots. For micelles containing more than 16 monomers, the calculated growth (A, fig. 25) is reasonably in accordance with experimental results. For the smaller

Figure 25.



X Experimental values

O Theoretical values of unsolvated monomolecular leaflet

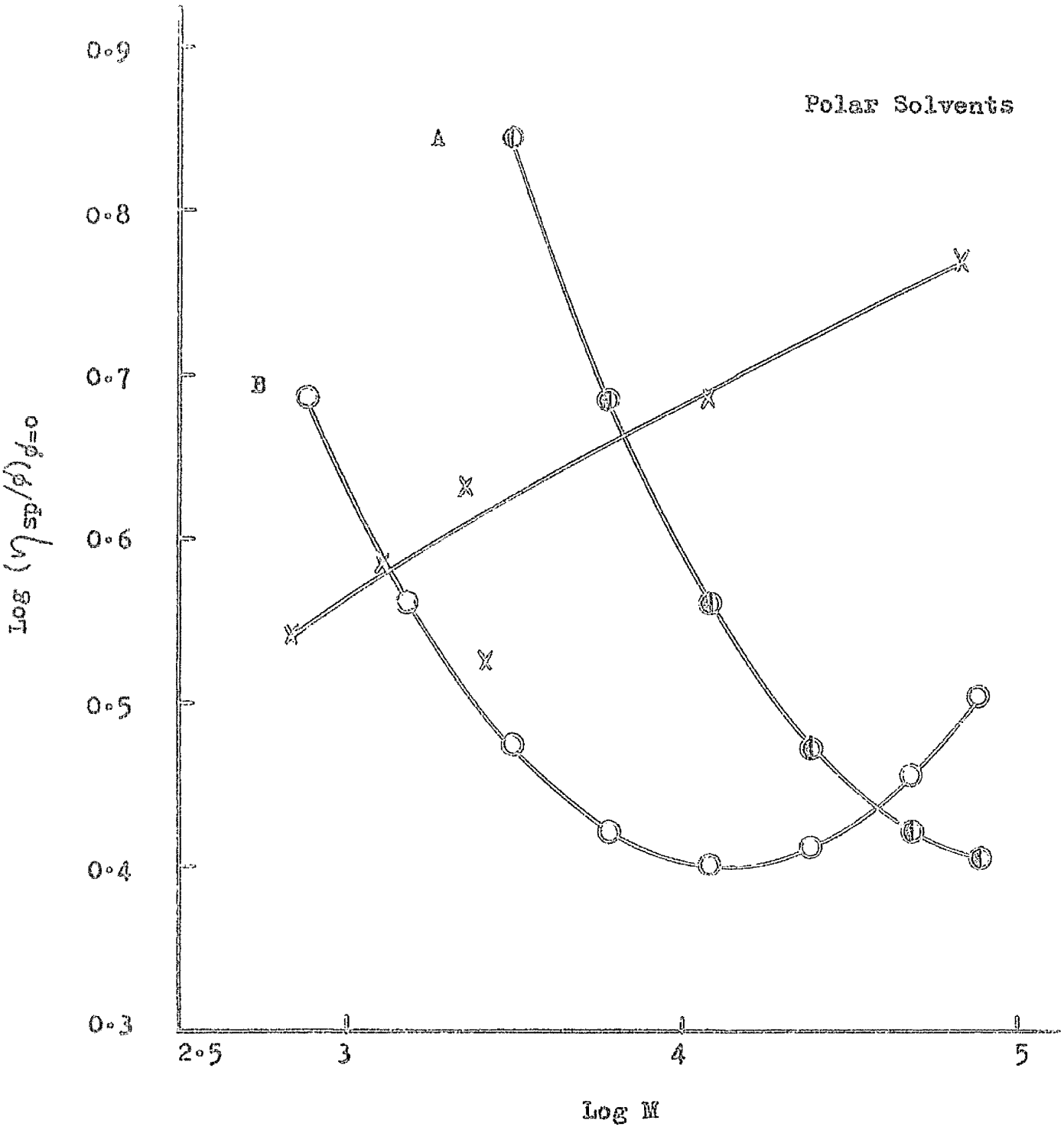
Ⓘ Theoretical values of unsolvated bimolecular leaflet

Plots of Log $(\eta_{sp}/\phi)_{\phi=0}$ Against Log M.

micelles, the bimolecular leaflet model is too asymmetric. Calculations of ν and M_2 on the assumption of a monomolecular leaflet gives curve B, fig. 25. In the methanol or ethanol systems, where the micelles contain a small number of monomers, there is better agreement between experimental results and calculations for the monomolecular model, than for the bimolecular model. These results indicate that a change in micellar structure from a monomolecular leaflet to the bimolecular type may well occur as the dielectric constant is decreased, that is, in the 8,000-16,000 micellar weight region, where ϵ = 18-25. For monomolecular leaflets there is no effective shielding of the head groups from the solvent, but dipole-dipole interactions and hydrogen bonding between neighbouring molecules in the leaflet are possible, and may be the main reason for micelle formation. In the region of dielectric constant where bimolecular leaflets are present (below ϵ = 18), effective shielding of polar head groups from the solvent occurs, and repulsive forces between these groups and the solvent are likely to be additional factors for micelle formation.

For the lecithin monomer, using the 35Å and 55Å^2 dimensions as before, ν is 4·86, a value much higher than the experimentally determined 3·47. For better agreement with the experimental figure, the hydrocarbon tails may separate slightly at their free end giving a V-shaped particle. It may be that the monomer is too small a particle to comply with the Simha relationships.

Figure 26.



X Experimental values

○ Theoretical values of unsolvated monomolecular leaflet

◑ Theoretical values of unsolvated bimolecular leaflet

Plots of Log $(\eta_{sp}/\rho)_{\rho=0}$ Against Log M.

Considering micellar structure on the aqueous side of fig. 19, there is a steady increase of log (viscosity intercept) with log M_2 (fig. 26). On this side of the curve the hydrocarbon tails should be present in the interior of the micelles; the micellar structure is explored by calculations for the same models as used on the non-aqueous side, the length of the bimolecular form being taken as $2 \times 35 = 70$Å. The calculated values for the viscosity intercept and M_2 are given in fig. 26 for bimolecular leaflets (A) and monomolecular leaflets (B). There is a considerable discrepancy between experimental and calculated results, the calculated viscosity intercepts being too small. As the experimental results were obtained in solutions containing water, it seems likely, in view of the tendency of the phosphoryl-choline head group to hydrate, that this discrepancy is due to hydration.[185]

Bimolecular leaflets will only be formed when the interfacial energies between the solvent and the hydrocarbon chains are less for this type of structure than for the monomolecular leaflet, other factors being equal. The areas of the hydrocarbon chains exposed to the solvent can be calculated for the mono and bimolecular leaflets as a function of the number of monomers present. The difficulty in these calculations is to assess the length of the lecithin molecule occupied by the hydrocarbon chain, as the fatty acid composition of natural lecithin is variable. For a hydrocarbon chain

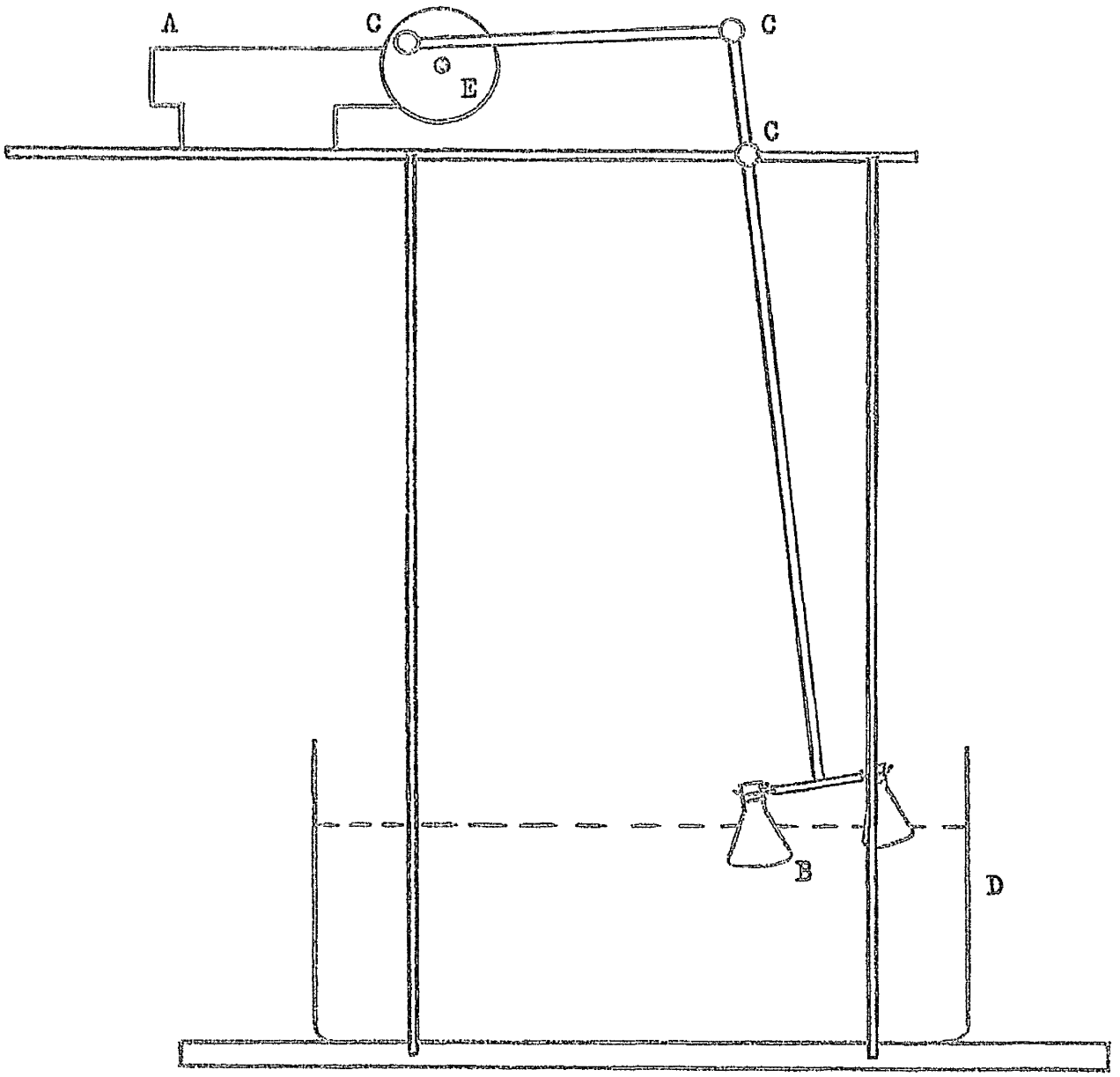
length of 20Å, micelles containing less than 16 monomers have a smaller hydrocarbon/solvent interfacial area if arranged in a monomolecular micelle; the situation is reversed when they contain more than 16 monomers. Corresponding figures for the 'change over point' between monomolecular and bimolecular leaflets for hydrocarbon chain lengths of 25 and 30Å were found to be 25 and 36 monomers respectively. It is felt that the 25Å hydrocarbon length is the most probable from figures for the average fatty acid composition of lecithin[17,20,21]. The results of this type of calculation are somewhat sensitive to the model chosen for the micelle. If it is considered as a monomolecular leaflet with an area at the polar head groups of $55nÅ^2$, and at the ends of the hydrocarbon tails as $(2 \times 16)nÅ^2$, the micelle then resembles the section of a cone. Calculations on this type of model, allowing two cone sections to be placed together end to end to form the bimolecular leaflet, show that for hydrocarbon lengths of 25 and 30Å the change over points are 50 and 65 monomers, respectively. A bimolecular leaflet model in which the ends of the hydrocarbon chains from each half of the leaflet interpenetrate was also explored, but no better agreement with the experimental log (viscosity intercept) against $\log M_2$ graph resulted.

It seems likely that the micelles initially formed with increase of dielectric constant on the aqueous side of fig. 19 are monomolecular in type, and that bimolecular leaflets develop

at values of $\log M_2$ of 4·3–4·6. No exact decision on the type of micelles can be made, due to the effects of hydration on the viscosity intercepts confusing those of asymmetry. Calculations of hydration using $\bar{v}$ values for monomolecular leaflets gave values of approximately 1g.water/g. lecithin. While this value is rather larger than that found from vapour pressure adsorption studies[185] (0·44g./g.), it is of the correct order.

Tables 9 and 10 show a gradual increase in depolarisation with increase in dielectric constant. From the earlier results in non-polar solvents it was tentatively suggested that the trend might be a consequence of the hydrocarbon chains lying roughly parallel to one another, and allowing a much greater polarisation to occur along the axis of the hydrocarbon chains. It would seem that this suggestion would not apply in polar solvents where a similar, but inverted, hydrocarbon chain arrangement was found. At present, therefore, no satisfactory explanation for this trend can be presented. Since the micellar weights obtained by diffusion agree with those from light-scattering, the depolarisations appear to be true.

Figure 27.



(A) Driving motor (B) Flask

(C) Axle (D) Water bath

(E) Slowly rotating wheel

Flask Rocking Apparatus.

SOLUBILISATION BY LECITHIN IN BENZENE.

The peripheral nerve myelin structural unit described by Finean [188,191] contained two bimolecular phosphatide leaflets. In benzene, lecithin forms bimolecular leaflet micelles, and it has been suggested [183,187] that this system might provide a useful model of part of nerve myelin. Solubilisation of materials by lecithin in benzene might, therefore, indicate the ability of the myelin phosphatide to permit the same materials to pass through the phosphatide layers. The solubilisation of some dibasic fatty acids and some dyes by lecithin in benzene have previously been described [183] [187].

Cholic Acid.

The lecithin solution and some finely powdered dry acid were continuously and gently shaken (fig. 27) till equilibrium was reached, 5 - 7 days being needed. The materials were contained in 150ml. conical flasks having slightly concave internal bases to enable a marble to roll in the flask and stir the contents. Six flasks were shaken simultaneously, the stoppered flasks being sealed with 'Parafilm' and dipping into a water bath at $20 \pm 0{\cdot}1^{\circ}$. To determine when equilibrium was reached, samples were withdrawn periodically from one flask, two successive concordant assays indicating equilibrium. The remaining solutions were then assayed.

To assay a solution, 8 - 10mls. were removed from the flask and centrifuged at 3000 r.p.m. for 30 minutes in an M.S.E. angle

centrifuge. About 5mls. of the clear supernatant solution were removed and weighed. The solution, of known weight, was evaporated to dryness under reduced pressure at 35 - 40°, and the residue was dissolved in a mixture of 20mls. methanol and 5mls. water. This solution was titrated with N/50 aqueous sodium hydroxide, using phenolphthalein as indicator. A blank titration of 20mls. methanol and 5mls. water enabled the titre of lecithin and cholic acid to be isolated. The 5 mls. water were necessary to sharpen the titration end-point satisfactorily.

To reduce the lecithin titre, a methanolic solution of lecithin was deionised using a column of 'Bio-Deminrolit' mixed bed resin. After evaporation of the methanol, the lecithin was recovered by precipitation from ether by acetone as described earlier. Lecithin sample E was used in this work, its analysis data, after ion-exchange chromatography, being given in Table 3.

Several lecithin solutions were assayed as for the solubilisation systems, the resulting plot of titre against weight of lecithin being a straight line through the origin. Subsequent lecithin titres of solubilisation systems were therefore obtained by extrapolation from this plot. The small titre of cholic acid dissolved in benzene was also determined. Thus by subtracting the titres of lecithin, solvent and dissolved cholic acid (15 - 30% of the total titre) from the assay titre of a solubilisation system, the cholic acid titre was isolated.

Results.

With lecithin solutions of concentration below 0·073% (the concentration at which small micelles aggregate to the large micelles at 25°[131]) no solubilisation was detected. The results for lecithin solutions, of concentration above 0·073%, are given in Table 12, the molecular weight of lecithin being calculated from its analysis data. The mean value for the ratio of the number of molecules of acid solubilised per molecule of lecithin, M_s/M_l, was 0·636.

Table 12.

Solubilisation of Cholic Acid by Lecithin in Benzene.

Total lecithin concentration (Moles/Kg. solution) x 10^3	9·08	18·15	21·87	27·20	36·38	45·24
Lecithin concentration as large micelles (Moles/Kg. solution) x 10^3	7·62	16·65	20·34	25·65	34·82	43·66
Solubilised cholic acid concentration (Moles/Kg. solution) x 10^3	4·66	10·55	13·41	16·07	23·21	26·90
M_s/M_l	0·612	0·634	0·659	0·627	0·667	0·616

Discussion.

The sterols form very stable surface films at the air/water interface, where a variety of molecular orientations are

found. Thus where there is a polar group (usually -OH) on position 3 only, and a hydrophobic chain on position 17 (of the steroid ring), alignment is vertical to the interface, the horizontal cross-sectional area being about 35-45 Å^2. For sterols with more than one hydrophilic group (e.g. one at position 3 and a second elsewhere), the alignment tends to be less vertical, the tendency being dependent upon the separation and hydrophilic nature of the groups. In the extreme, the sterol will lie horizontally, this allowing polar groups at maximum separation both to be in contact with the water. Apocholic acid is aligned horizontally due to its hydrophilic groups, these being a carboxyl group at the end of the side-chain on position 17 and two hydroxyl groups at positions 3 and 12.

It would seem therefore, that in the lecithin micelles, cholic acid would lie between the polar sheets and be orientated to favour maximum association between its polar groups and the lecithin monomer polar heads. An approximately vertical alignment of the acid between the sheets would favour this arrangement. From the $M_s/M_l = 0.636$ ratio, micelles containing 80 monomers would contain about 50 cholic acid molecules. If the acid horizontal cross-sectional area is taken as 40 Å^2, the maximum polar sheet surface area required for close packing (assuming no change in cross-sectional area on close packing) would be 2000 Å^2. For a micelle having 40 monomers per monomolecular leaflet, and a polar group cross-sectional area

of 55A^2, the surface area of each polar sheet is 2200A^2. The agreement of these two figures would suggest that the cholic acid could lie in the micelle with its carboxyl group adjacent to a monomer polar group of one leaflet and its number 3 position hydroxyl group adjacent to a monomer polar group of the other leaflet.

It may be that the cholic acid molecules are horizontally aligned at the micelle polar core and that they tend to associate with the polar groups of one leaflet only. For such an arrangement, the acid could lie either between the two lecithin leaflets or between the polar groups of monomers in the same leaflet. The former would cause separation of the two leaflets, the latter, separation of monomers in the same leaflet. Assuming the cholic acid molecule to be 20A long and 40A^2 in cross-section, and considering its cross-section to be square or circular, the surface area of lecithin covered by a horizontally aligned cholic acid molecule would be about 130A^2. For 50 such molecules, the area would be 6500A^2. Since the polar sheet surface area is 2200A^2, the acid could be accommodated by forming a trimolecular acid layer. Also, since one cholic acid molecule would cover more than one monomer head group, there is the possibility of association between lecithin polar groups and cholic acid hydroxyl groups at positions 7 and 12 on the steroid ring.

Assuming the lecithin polar group to be approximately square

in section (from molecular models), it would be possible for two cholic acid molecules (each of side 20Å) to lie between two rows of five lecithin monomers (each row of length about 40Å). Expanding such an arrangement in one dimension, 2n acid molecules would lie alongside 10n lecithin monomers. Exp alongside $[(5n/2)+5]$ r direction, 2n acid molecules would lie alongside $[(5n/2)+5]$ monomers. Expanding in both directions simultaneously would produce an intermediate association level. Since from experimental data, n = 25 for each monomolecular leaflet of 40 monomers, this method of incorporating the cholic acid would seem inadequate.

With the limited results available, it is not possible, at present, to identify which way the cholic acid is incorporated in the micelle.

Bis-quaternary Ammonium Compounds.

Four compounds, hexamethonium, decamethonium and hexadecamethonium iodides and decamethonium bromide, were studied. The solubilisation technique was as described for cholic acid.

When the solubilisation system reached equilibrium, it was assayed by removing 8-10mls. and centrifuging as before. 5mls. of the supernatant solution were removed and weighed and about 10mls of ion-exchanged water were added gradually to the solution, shaking the mixture between each addition. The benzene was evaporated slowly, under reduced pressure, to form an aqueous lecithin sol. A 10ml. column of 'Zeo-Karb 225' was prepared

and washed with ion-exchanged water, the last 100 mls. washings being retained. The sol containing the previously solubilised compound was then washed through the column with more water till a second 100 mls. fraction was obtained. The column was finally washed to produce a third 100 mls. fraction. The three fractions were titrated with N/50 aqueous sodium hydroxide, using phenolphthalein. The solvent titre was the mean titre of the first and third fractions, and by subtracting this mean value from the titre of the second fraction, the titre due to solubilisate and lecithin was obtained. When varying strengths of lecithin sols were similarly assayed, again the plot of titre against weight of lecithin was a straight line through the origin. Subsequent lecithin titres were extrapolated from this plot, enabling the titre due to solubilisate to be isolated. Test assays of known mixtures of solubilisate and lecithin sols in water yielded over 99% solubilisate, 10 mls. of the resin being found sufficient for 0·056 m.eq. of solubilisate. None of the four compounds was detected as soluble in benzene.

Results.

(a) Hexadecamethonium Iodide.

No solubilisation by the small micelles was detected, the solubilisation by the large micelles being summarised in Table 13. The mean value of M_s/M_l was 0·025.

Table 14.

Solubilisation of Hexamethonium Iodide by Lecithin in Benzene.

Total lecithin concentration (Moles/Kg. solution) x 10^3	0·52	1·04	7·23	15·35	20·94	30·54	34·01	43·59
Total solubilisate concentration (Moles/Kg. solution) x 10^3	0·029	0·064	0·784	1·216	2·224	2·186	3·438	2·93
Lecithin concentration as large micelles (Moles/Kg. solution) x 10^3	—	—	5·80	13·86	19·42	28·99	32·45	42·01
Solubilisate concentration in large micelles(Moles/Kg. solution) x 10^3	—	—	0·700	1·128	2·134	2·095	3·346	2·84
M_s/M_l	0·056	0·061	0·121	0·081	0·110	0·072	0·103	0·06

Table 13.

Solubilisation of Hexadecamethonium Iodide by Lecithin in Benzene.

Total lecithin concentration (Moles/ Kg. solution) x 10^3	9·06	22·61	31·63	38·38	45·12
Lecithin concentration as large micelles (Moles/ Kg. solution) x 10^3	7·60	21·08	30·08	36·82	43·54
Solubilisate concentration (Moles/Kg. solution) x 10^3	0·180	0·623	0·654	0·879	1·005
M_s/M_l	0·024	0·030	0·022	0·024	0·023

(b) Hexamethonium Iodide.

Solubilisation by the small micelles was found, and the values of M_s/M_l for lecithin solutions of concentration greater than 0·073% were corrected for this effect. Results for lecithin solutions below and above 0·073% concentration are given in Table 14, the mean values of M_s/M_l for the small and large micelles being 0·059 and 0·093 respectively.

(c) Decamethonium Bromide and Iodide.

Neither compound was measurably solubilised.

Discussion.

From a previous study[183] of the solubilisation of a series of dibasic fatty acids by lecithin in benzene, it was suggested that the acids lay in the micelle in one of two ways, and for the acids that lay in a similar manner, the M_s/M_1 ratio decreased as the fatty acid chain length increased. Since the ratio for hexamethonium iodide is greater than that for hexadecamethonium iodide, it was thought that the ratio for decamethonium iodide would fall between these two values. Since this was not observed, and due to the very small observed ratios for the two substances solubilised, a small solubilisation ratio of the decamethonium compound may be masked by experimental error. The very small observed ratios do not permit the postulation of methods of incorporation of solubilisate in the micelles other than to suggest that it probably lies between the polar sheets, as opposed to lying parallel to the monomer hydrocarbon chains. It is thought that it would be worthwhile continuing the solubilisation study of these water-soluble materials in lecithin micelles in benzene where the micelles contain some water. Such hydrated micelles would more closely resemble nerve myelin phosphatide than the dry lecithin/benzene system. Prior to such a study, the effect of water on lecithin micelles in benzene was investigated.

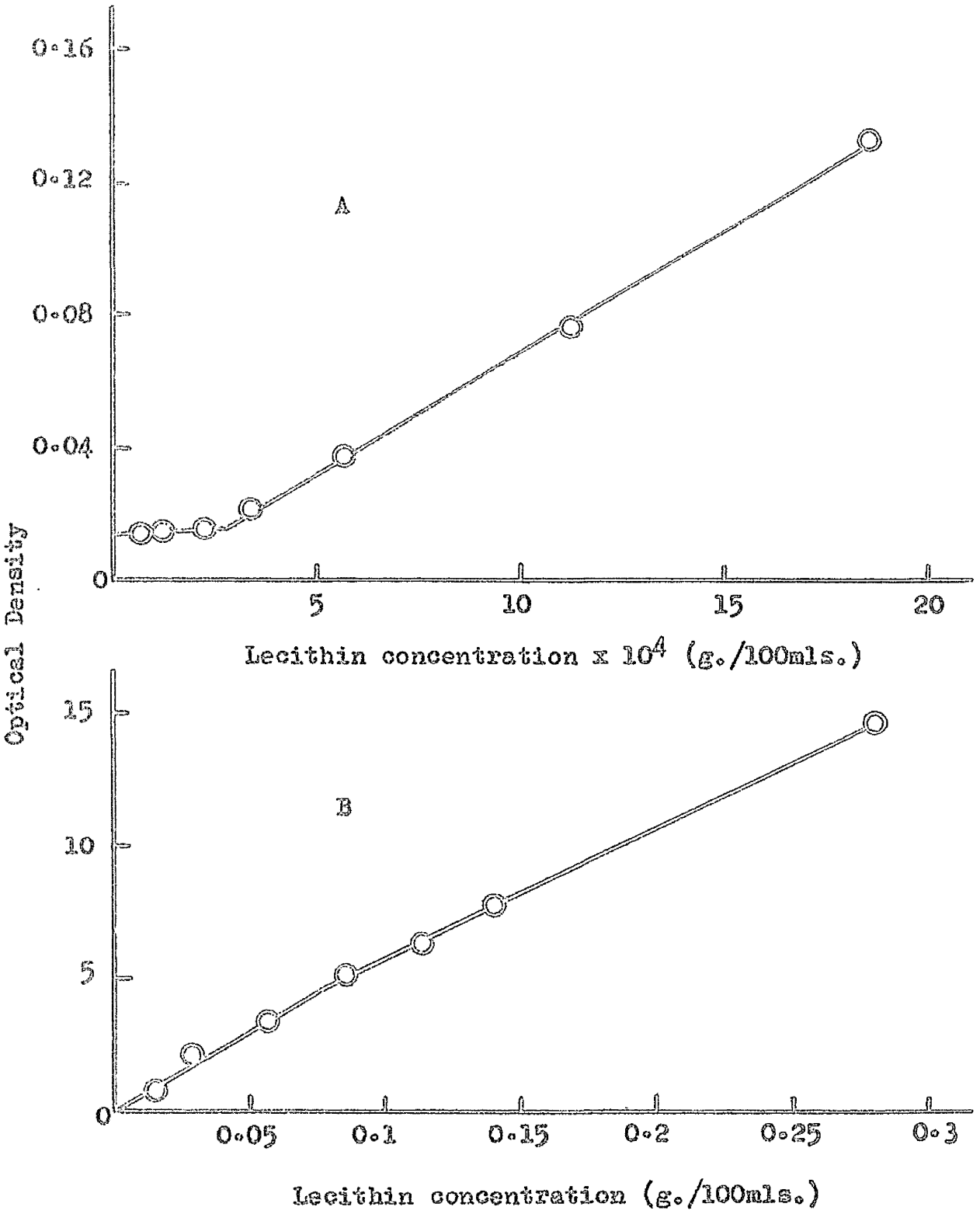
THE INTERACTION OF WATER WITH LECITHIN MICELLES IN BENZENE.

From water vapour adsorption studies on dry lecithin, the association of water and lecithin has been shown to occur mainly at the polar head groups.[185] Demchenko[184] found the maximum quantity of water solubilised by lecithin in benzene, toluene and xylene to be independent of the solvent used, the maximum uptake being 0·33g./g. lecithin. Since it was thought that a more realistic biological model of part of nerve myelin would be achieved by incorporating water in the micelles, a study was made of the interaction of water and lecithin micelles in benzene.

Lecithin sample F was used, it having been deionised similarly to sample E. To investigate the aggregation of lecithin in benzene at 20°, the solubilisation of tropaeolin 000 was studied using the solubilisation technique previously described. Equilibrium was reached in 3 days. To assay the systems, their optical densities were measured at 483 mμ. in 1 cm. cells, against benzene, in a spectrophotometer (Hilger and Watts, Uvispek). Dye solubility in dry benzene was negligible.

Water was incorporated in the micelles either by shaking the lecithin solution with a weighed quantity of water in a sealed flask or by allowing dry lecithin to adsorb the required amount of water vapour with subsequent solution of the wet lecithin in dry benzene. No differences in behaviour were observed between solutions made by the two methods. To determine the maximum water uptake, the optical density of 1% lecithin

Figure 28.



(A) Determination of the cmc of lecithin in benzene

(B) Determination of the second aggregation concentration

Solubilisation of Tropaeolin OOO by Lecithin Micelles in Benzene.

solutions containing various amounts of water were measured at 546 mμ. Vapour phase equilibrium experiments with lecithin solutions, and benzene with added water, were carried out by connecting the two flasks containing the solutions with a U-shaped adaptor.

Results and Discussion.

From the solubilisation of tropaeolin 000, the cmc of lecithin in benzene at 20° was determined as $2{\cdot}6\times10^{-4}$% (fig. 28). Using this value and those of Blei and Lee[187] of $3{\cdot}3\times10^{-4}$% at 25° and $8{\cdot}6\times10^{-4}$% (mean value) at 40°, for substitution in the standard relationships of the two phase model for micelle formation[261],

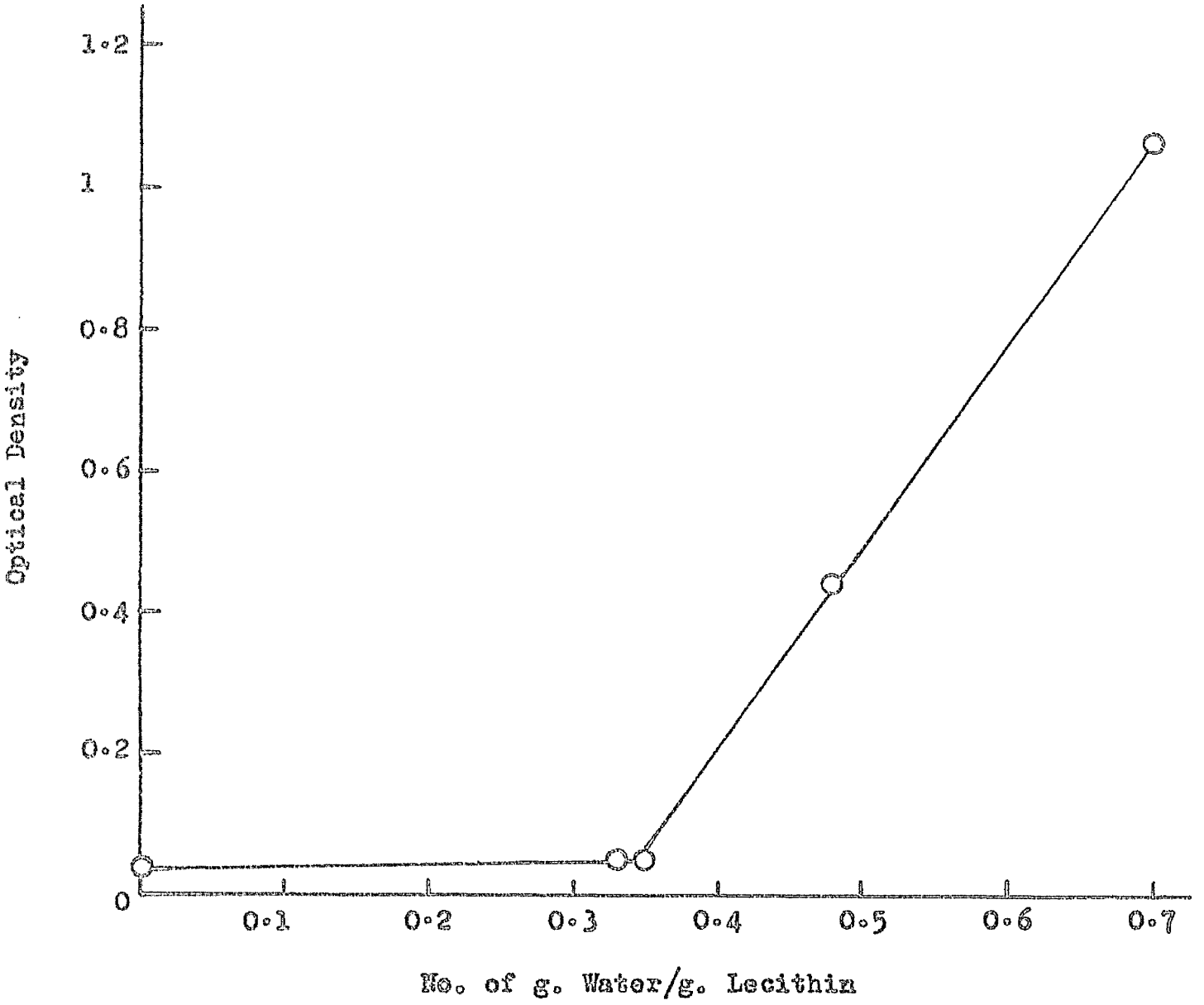
$$\Delta H_m = -2{\cdot}303RT^2(\partial \log \text{cmc}/\partial T) \qquad \text{and} \qquad \Delta S_m = \Delta H_m/T$$

values were obtained for the heat and entropy of micellisation, ΔH_m and ΔS_m, of -10k. cal. $mole^{-1}$ and -35 cal. $deg.^{-1} mole^{-1}$ respectively. These values are large compared with those of ionised detergents in water. The large negative ΔS_m value indicates that a large ordering of monomers occurs on entering the micelle, this perhaps being related to the micellar hydrocarbon chain arrangement. To permit further conclusions to be drawn, further cmc determinations would have to be made. The aggregation of small into large micelles occurs at 0·075% (fig. 28), which compares favourably with that of 0·073% at 25°. Since the present interest is in the large micelles, it was this second aggregation concentration which was used as

previously described, to correct the viscosity results. Only small corrections were necessary, and since irreproducible results were obtained for the dye solubilisation in micelles containing water, the value obtained in the dry state was used for all systems.

It is necessary to gain some idea of the location of the water in the lecithin/benzene system since there is the possibility of a significant partition between the micelles and the surrounding medium. Also, the interpretation of the viscosity results depends to some extent on knowing the location of the water. Since lecithin is very hygroscopic, it would seem likely that nearly all the water would be associated with the lecithin. To try and establish the position of the water, water-saturated benzene was equilibrated, via the vapour phase, with a 3% solution of lecithin in dry benzene. After 6-8 days the presence of water in the benzene was no longer detectable, indicating a transfer into the micelles in the second phase. Further tests indicated that there was no water loss from the system, transfer from water-saturated to dry benzene giving the correct equilibrium distribution. Also, water-saturated benzene in vapour contact with lecithin containing 0·15g.water/g.lecithin had no detectable water at equilibrium. If the partition of water favoured benzene rather than lecithin micelles in benzene, a significant amount of water would be expected to remain in the benzene when in vapour contact with a micellar system. Since

Figure 29.



The Effect of Water in 1% Lecithin Solutions in Benzene upon the Solution Optical Density.

at equilibrium, water could not be detected in the benzene, the equilibrium existing between the water in the micelles and the water in the benzene around the micelles very much favoured the former position and it would seem reasonable to consider all the solubilised water as part of the micelles. The maximum uptake of water by 1% solutions of lecithin in benzene was 0·33g./g. lecithin (fig. 29) which agrees well with the value of Demchenko.

Micellar weights were obtained by light-scattering for systems containing 0·0536g. and 0·1047g. water/g. lecithin (Table 15, fig. 30). Solutions containing 0·15g. or more water/g. lecithin could not be satisfactorily clarified either by filtration or centrifugation. The results, uncorrected for depolarisation, were plotted as $H(c-c_1)/(T-T_1)$ against $(c-c_1)$ where c is the total concentration of lecithin plus water, c_1 the second association concentration of lecithin in benzene and T_1 the turbidity at c_1. Since the water seemed completely associated with the micelles, it seemed reasonable to treat the system as essentially a two-component system, this treatment having been used before for the light-scattering of solubilised systems.[262] Z_{45} values close to unity indicated that no dimension of the micelles exceeded $\lambda/20$.

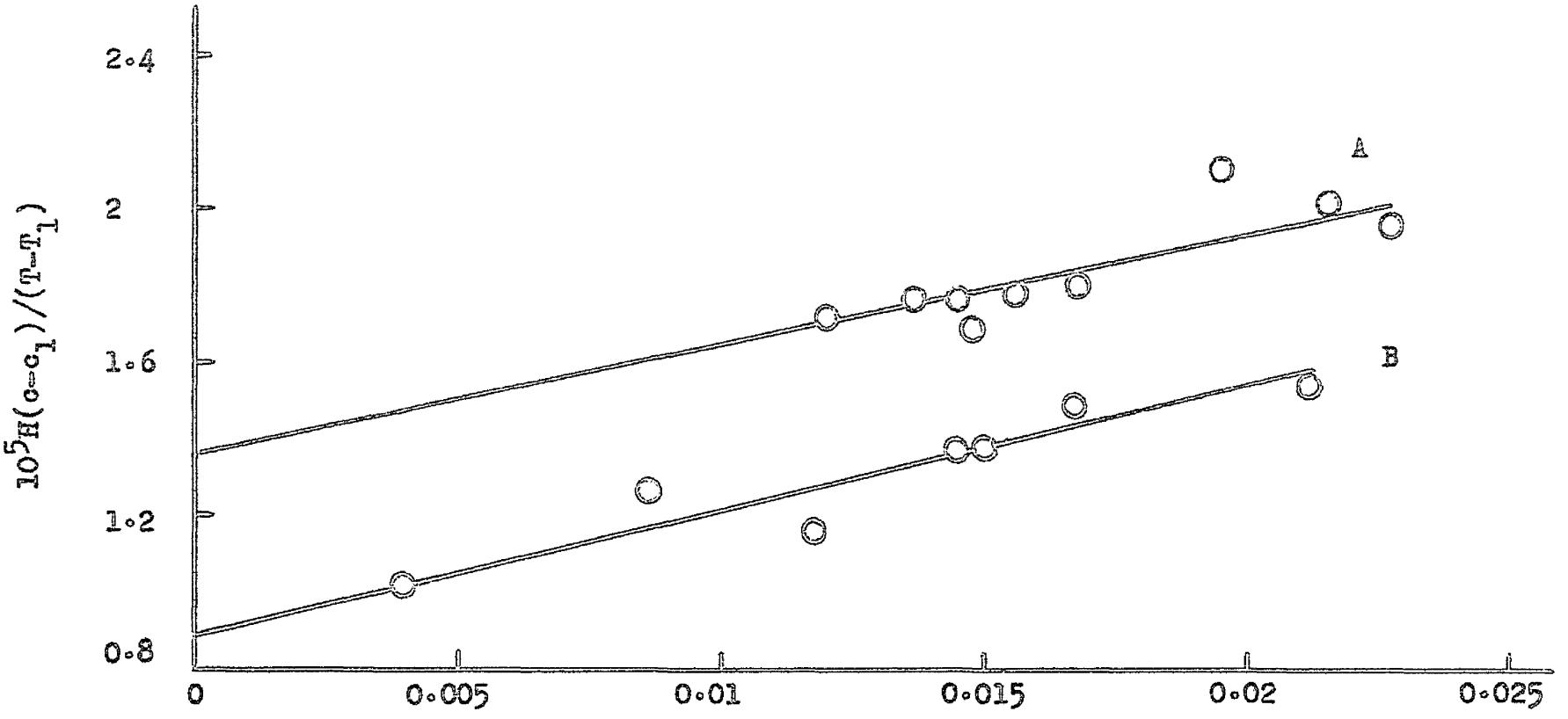
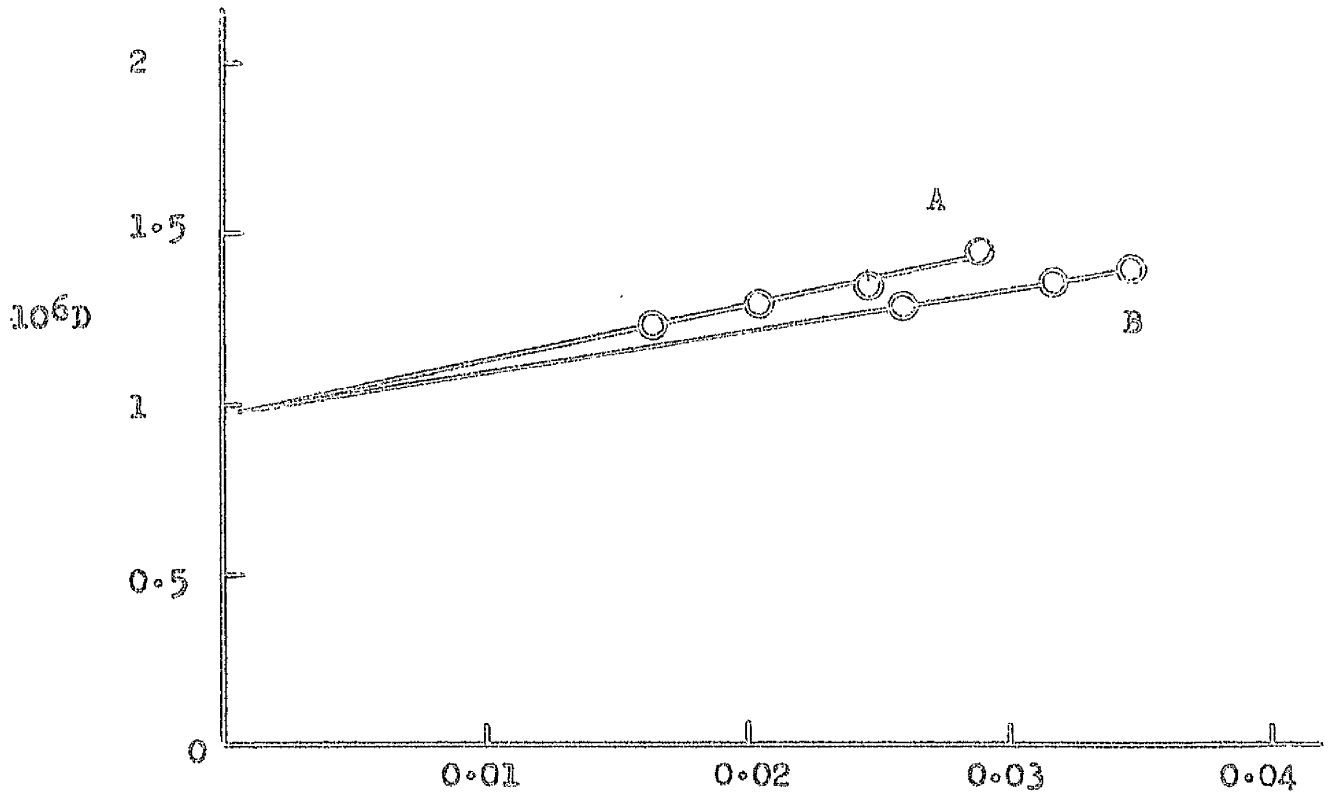


Figure 30.

(A) 0.1047g. Water/g. Lecithin (B) 0.0536g. Water/g. Lecithin

Light-scattering Results as $H(c-c_1)/(T-T_1)$ Against $(c-c_1)$.

Figure 31.



(A) 0·0536g. Water/g. Lecithin (B) 0·2550g. Water/g. Lecithin

Diffusion Results.

Table 15.

Light-scattering Results for Solubilisation of Water by Lecithin in Benzene.

No. of g. water/ g. lecithin	$10^{-3}M$	$10^{-3}M_{lecithin}$	ρ	dn/dc	Z_{45}
0·0536	59	56	0·280	-0·052	1·00
0·1047	65	58	0·050	-0·058	1·00

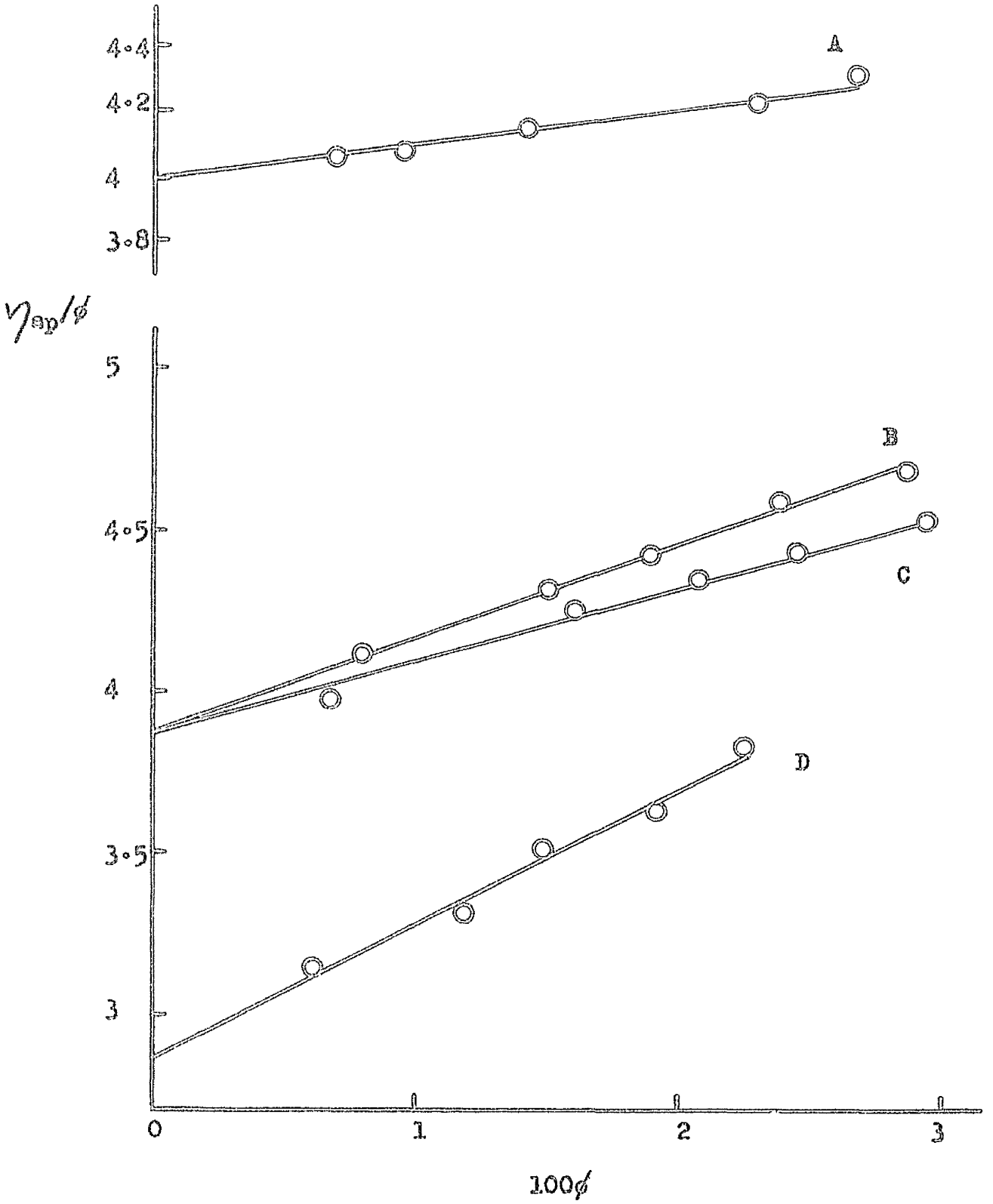
Because the systems had small specific refractive index increments, $(T-T_1)$ values were very small. This, together with the ± 8-13% error in light-scattering molecular weights, made checks on the light-scattering micellar weights necessary. Thus the diffusion coefficients of systems containing 0·0536g. and 0·2550g. water/g. lecithin were measured (fig. 31) and by using further viscosity data (figs. 32 and 33, Table 16), the three alternative micellar weights, M_{pro}, M_{ob}, and M_{solv} were determined (Table 17). For the 0·2550g. water/g. lecithin system it will be shown later that M_{solv} provides the closest calculation to the nature of the micelle, while for the 0·0536g./g. system, M_{ob} is closest.

Table 16.

Viscosity Results.

No. of g. water/ 100g. lecithin.	0	1·40	4·22	5·78	8·41	11·2	16·4	24·6	31·8
$(\eta_{sp}/\phi)_{\phi=0}$		2·87	3·82	3·86	4·00	3·94	3·89	3·89	3·76	3·43

Figure 32.

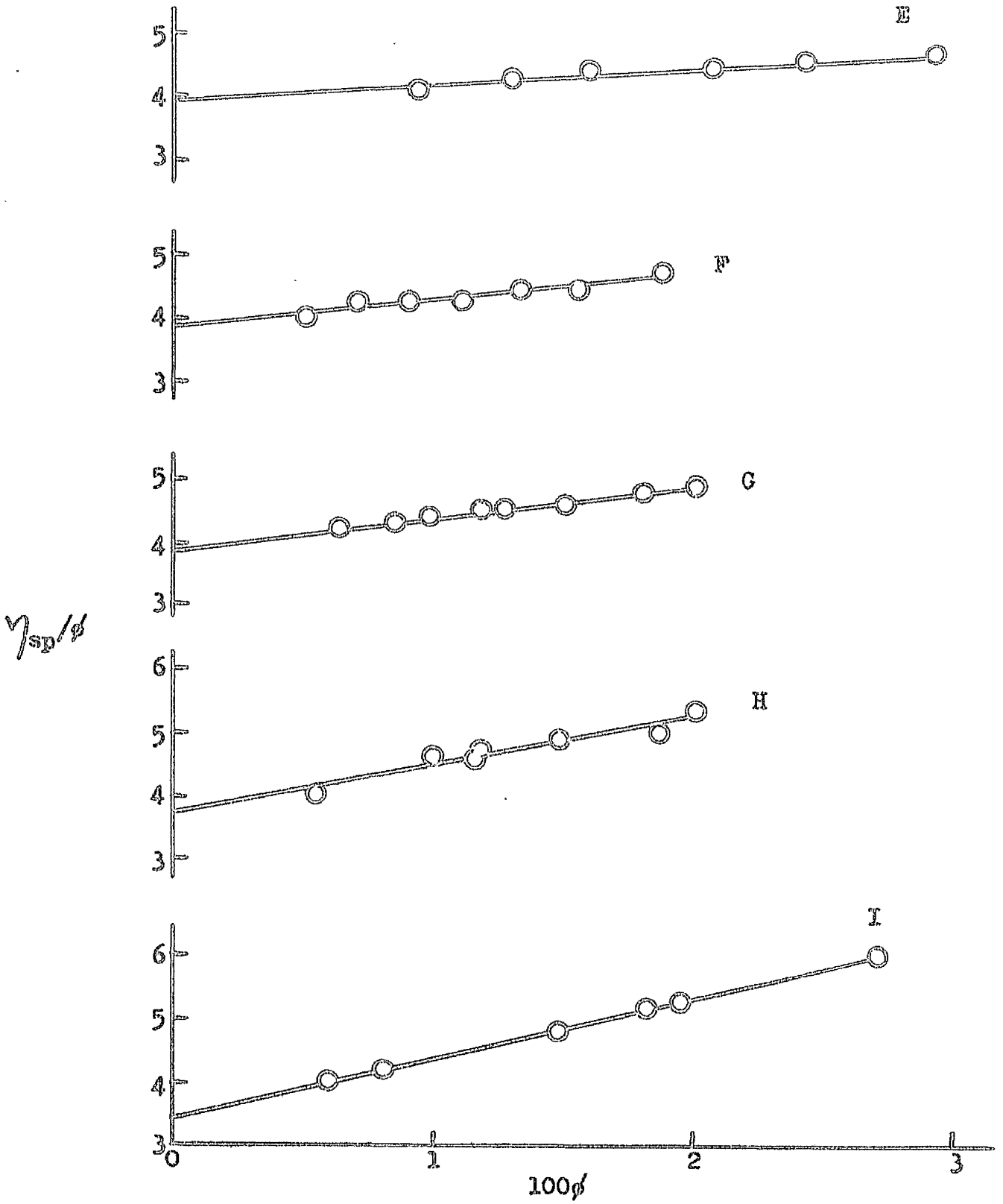


(A) 0·0578g. Water/g. Lecithin (B) 0·0140g. Water/g. Lecithin

(C) 0·0422g. Water/g. Lecithin (D) 0 g. Water/g. Lecithin

Viscosity Results.

Figure 33.

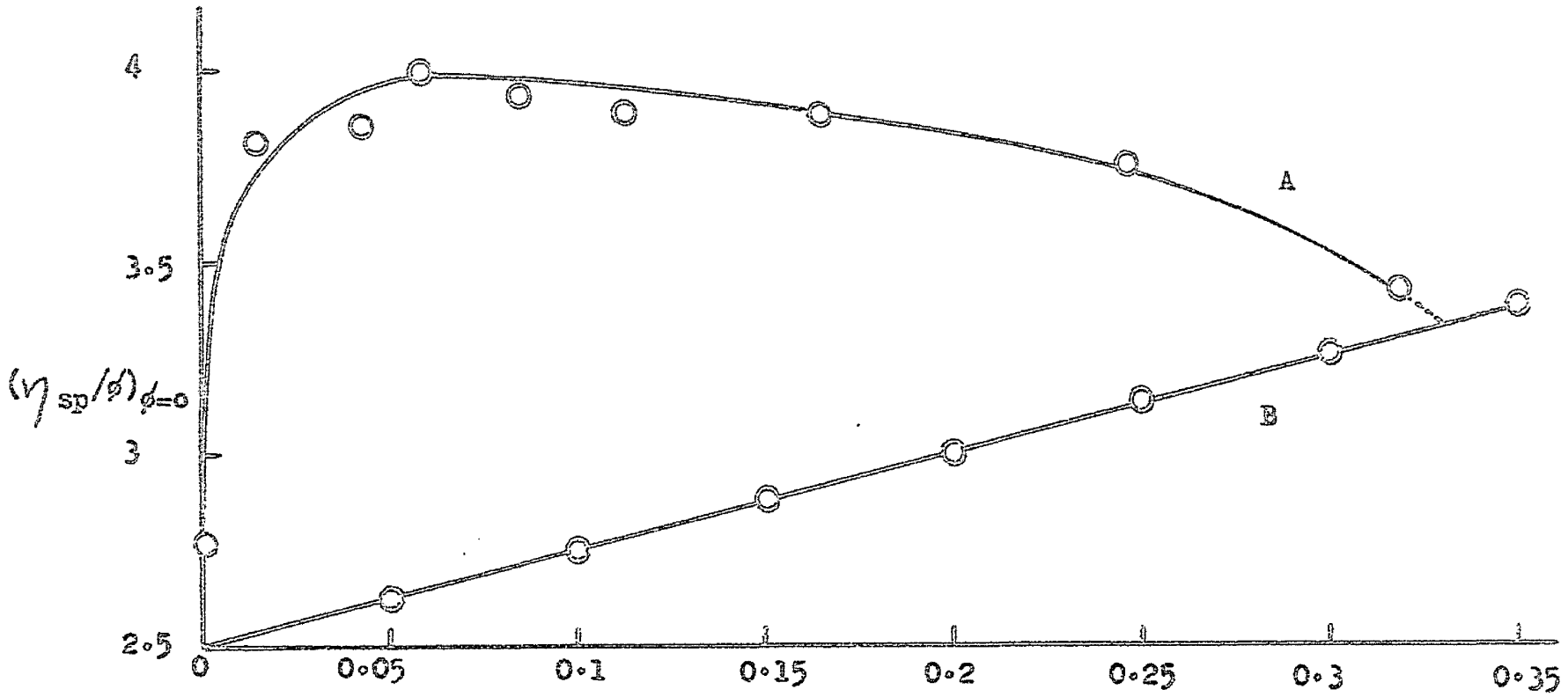


(E) 0·0841g. Water/g. Lecithin (F) 0·112g. Water/g. Lecithin

(G) 0·164 g. Water/g. Lecithin (H) 0·246g. Water/g. Lecithin

(I) 0·318g. Water/g. Lecithin

Viscosity Results.



(A) Experimental values (B) Theoretical values

Figure 14.

Comparison of Experimental Viscosity Intercepts with Theoretical Values for Hydrated Spheres.

Table 17.

Diffusion-Viscosity Results.

No. of g.water/g.lecithin	$10^{6}D$	$10^{-3}M_{pro}$	$10^{-3}M_{ob}$	$10^{-3}M_{solv}$
0·0536	0·976	68(64)	64(61)	63(60)
0·2550	0·962	74(55)	71(53)	71(53)

In Table 17 the figures in brackets represent the micellar weights of lecithin in the micelles.

Micellar weight increases with increase in solubilised water. For dry lecithin, M=60,000, and within experimental error, the micellar weights here correspond to 60,000 plus solubilised water. This indicates that the number of monomers per micelle remains reasonably constant as the micellar water content increases.

The viscosity intercepts, $(\eta_{sp}/\phi)_{\phi=0}$ (represented again by $[\eta']$) in figs. 32 and 33 increase from 2·87 at zero water content to a peak value of 4·00 at 0·058g. water/g. lecithin and then decrease on adding further water. At 25°, and zero water content, $[\eta'] = 2·78$, which is close to the value found here at 20°. [140]

Using equation (30) where

$$[\eta'] = (\eta_{sp}/\phi)_{\phi=0} = \nu(1+w/\bar{v}\rho)$$

viscosity intercepts were calculated for spherical particles using w as the number of g. water solubilised/g. lecithin. On comparing these latter values to the experimental $[\eta']$ values (fig. 34), it is seen that this treatment does not account for the observed behaviour of the viscosity intercepts at low water contents, indicating that micellar shape changes occur in this

region. At 0·33g. water/g. lecithin it appears that the micelles are spherical, since a short extrapolation of the experimental curve (fig. 34) to this water content gives $[\eta']$ = 3·34, the theoretical water content of such an $[\eta']$ value for hydrated spheres being 0·330g. water/g. lecithin. This latter amount of water would fill a spherical cavity of radius 19·8Å in the micelle centre. Taking the head group area of lecithin as 55Å^2, and arranging 80 monomers so that the head groups cover the surface of a sphere, the radius of the sphere is 18·7Å. The reasonable agreement between these figures indicates that this is probably the maximum amount of solubilisate which can be incorporated in the micelle.

Assuming, as in the calculations above, that no regular layering of solubilised water occurs and that the micelles are unsolvated with respect to benzene (which seems reasonable from preceeding work[140] and from the viscosity results at 0·33g. water/g. lecithin), an idea of the changes in micellar shape occurring on the addition of water may be gained from models of lecithin micelles. Molecular models show that the polar head group can be represented as a block 7x8Å^2 in cross-section, and using also the length of half the bimolecular leaflet as 36Å, it is possible to calculate $[\eta']$ for prolate and oblate ellipsoidal micelles of 80 monomers.

When water is absent, the short semi-axis, b, for the

prolate ellipsoid is given by $b = (56 \times 40/\pi)^{\frac{1}{3}}$ Å, and since $a = 36$ Å, the axial ratio and hence $[\eta']$ can be evaluated. Here, $[\eta'] = 2{\cdot}54$, which is rather lower than the experimental value. An estimate of $[\eta']$ can be made for the 0·058g. water/g. lecithin system, this system providing the highest experimental $[\eta']$. There are two principal ways in which the water can be incorporated. Firstly, it may lie in the gap between the polar sheets and, in so doing, increase the a dimension. 0·058g. water/g. lecithin corresponds to 2·4 water molecules per monomer. Allowing a sheet of water molecules, six molecules thick, to lie between the lecithin polar sheets, $[\eta'] = 2{\cdot}87$, a value much lower than the experimental 4·00. Secondly, the water may be incorporated between the polar heads in each sheet, thereby increasing the micelle cross-sectional area, causing an increase in b. The summation of the polar head areas and water molecule areas gives $[\eta'] = 2{\cdot}62$. A third possibility is that both types of micellar expansion may occur, the changes balancing one another with respect to asymmetry.

Water vapour adsorption studies on natural lecithin showed[185] that 2·5 water molecules/lecithin molecule represented the completion of first layer adsorption. This figure agrees reasonably well with the water content giving the peak value of $[\eta']$. It was also shown, in interpreting the transport properties of lysolecithin, that this first layer might fit into cavities in the polar head group without significantly increasing the

effective monomer length.

The consideration of oblate ellipsoids is more difficult since decisions have to be made on the geometry of the sheet of polar head groups. Solubilisation[183] indicated that the length of the sheet of polar groups was three times greater than its width. Taking the shorter dimension (2a) as 4x8 = 32Å, then b' = 44·6Å, and using the mean of b' and 36Å to give an average value of b, $[\eta']$ becomes 3·09 for dry lecithin. The incorporation of six rows of water molecules between the two polar sheets gives $[\eta'] = 3·5$, while increasing the area of the sheets on incorporating the same amount of water gives $[\eta'] = 3·6$. These calculations for oblate models are tentative since they are sensitive to the exact geometry of the lecithin head group, and also have the drawback that the model does not exactly fit the oblate shape. However, they do provide a guide to the development of micellar asymmetry.

It would seem that the micelles resemble oblate ellipsoids, and that the addition of water initially increases their asymmetry. Above 0·058g. water/g. lecithin, the micelles tend towards a more spherical shape since $[\eta']$ decreases with increase in water content. This change may represent rearrangement of micellar structure, since to prevent contact between the polar heads (with their associated water) and benzene, as the volume of water in the micelle centre increases, the micelle shape will tend to become spherical, reaching this shape when its water content becomes 0·33g. water/g. lecithin.

BIOLOGICAL ASPECTS OF THE PRECEEDING WORK.

It is interesting to consider the results in the light of possible function of lecithin as a component of the cell membrane. The centre of the membrane is believed to consist of a bimolecular leaflet of phosphatide material.[263] Where this structure is free of adsorbed protein,[189] changes in membrane permeability could be caused by a local alteration of the dielectric constant of the water adjacent to the phosphatide which could bring about a rearrangement of the phosphatide structure. Electrolytes have been shown to decrease the dielectric constant of water considerably.[264.] Thus a large decrease in dielectric constant could be obtained from a high transient, local concentration of ions, which would cause the disintegration of a small area of the membrane and allow transport of materials across it.[193] Recently, it was found that high ionic concentrations have an effect on phosphatide double layers which suggested ionic change as a possible cell permeability mechanism.

Lecithin micelles in benzene are a convenient model of the in vivo phosphatide bimolecular leaflet structure. However, with water incorporated between the two polar sheets of the lecithin micelles a better resemblance is obtained to the phosphatide structure found by Finean in nerve myelin. It is thought that this second model, containing water, has promise as a further useful model of the in vivo phosphatide leaflets.

APPENDIX 1.

Estimation of Error in Calibration Constant.

	$10\log 10^4 C = y$	$100T = x$
(1)	4·54	3·41
	4·66	3·30
	4·72	3·31
	4·72	3·18
	4·79	3·90
	4·83	3·28
	4·94	5·43
(2)	4·79	5.46
	5·00	6.03
	5·11	5·59
	5·16	6·12
	5·32	8·86
	5·39	8·95
(3)	5·46	9·94
	5·47	9·84
	5·48	9·69
	5·82	13·03
	5·94	15·44
	6·02	14·59
	6·03	13·19

The twenty values used for the log C against T plot were divided into three non-overlapping groups as shown.

For the twenty values,

$\sum x = 152·54$ $\quad \bar{x} = 7·627$

$\sum y = 104·37$ $\quad \bar{y} = 5·2185$

For group (1) values (subscript 1),

$\sum x_1 = 25·81$ $\quad \bar{x}_1 = 3·687142$

$\sum y_1 = 33·20$ $\quad \bar{y}_1 = 4·742857$

For group (3) values (subscript 3),

$\sum x_3 = 85·72$ $\quad \bar{x}_3 = 12·245714$

$\sum y_3 = 40·22$ $\quad \bar{y}_3 = 5·745714$

The slope of the line between (x_1, y_1) and (x_3, y_3) is given by

$$b = (\bar{y}_3 - \bar{y}_1)/(\bar{x}_3 - \bar{x}_1) = 0·117175$$

Thus, for the line,

$$y - \bar{y} = b(x - \bar{x})$$

giving

$$y = 4·3248 + 0·1172x$$

Also:

$$\sum(y^2) = 548{\cdot}8739 \qquad (\sum y)^2/N = 544{\cdot}65485$$

$$\therefore \sum(y^2) - (\sum y)^2/N = 4{\cdot}21905$$

$$\sum xy = 831{\cdot}7599 \quad \sum x \sum y/N = 796{\cdot}02999$$

$$\therefore \sum xy - \sum x \sum y/N = 35{\cdot}72991$$

The error variance (σ^2) of x and y is given by

$$\sigma_y^2 = k^2 \sigma_x^2 = \frac{\left[\sum(y^2) - (\sum y)^2/N\right] - b\left(\sum xy - \sum x \sum y/N\right)}{N-2} = 0{\cdot}001800$$

$$\sum(x^2) = 1477{\cdot}0610 \qquad (\sum x)^2/N = 1163{\cdot}42258$$

$$\therefore \sum(x^2) - (\sum x)^2/N = 313{\cdot}63842$$

Also, $$\sigma_x^2 = \left[\sum(x^2) - (\sum x)^2/N\right]/(N-2) - \left(\sum xy - \sum x \sum y/N\right)/b(N-2)$$

$$= 0{\cdot}483926$$

$$k^2 = \sigma_y^2/\sigma_x^2 = 0{\cdot}003719576$$

The variance of b, $$V(b) = \frac{\sigma_x^2(k^2+b^2)^2}{k^2\left[\sum(x^2) - (\sum x)^2/N\right] + b\left(\sum xy - \sum x \sum y/N\right)}$$

$$= 0{\cdot}000027525$$

The variance of a predicted value of y, for a given value, x_k, of x is

$$V(y_k) = k^2\sigma_x^2/N + (x_k - \bar{x})^2 V(b) + b^2\sigma_x^2(1+1/N)$$

For $x_k = 0$, $\quad V(y_k) = 0{\cdot}008668$

$\therefore$ Standard error of $y_k = 0{\cdot}09310$

The 95% confidence limits for y_k are $\pm$ 0·09310 x 2·11 = $\pm$ 0·1964 for 17 degrees of freedom.

The limits of error of y_k, at x = 0, are therefore 4·325 $\pm$ 0·196, giving

$$C = 2{\cdot}71 \times 10^{-4} \pm 0{\cdot}12 \times 10^{-4} = 2{\cdot}71 \times 10^{-4} \pm 4{\cdot}4\%.$$

APPENDIX 2.

Polystyrene in Toluene.

1. Estimation of Error in $(c/T)_{c=0}$.

The data used is contained in Table 5, where $c/T = y$ and $c = 10^3 x$.

$$\sum x = 0{\cdot}036211 \quad \bar{x} = 0{\cdot}0036211 \quad \sum y = 40{\cdot}95 \quad \bar{y} = 4{\cdot}095$$

$$\sum xy = 0{\cdot}16794257 \qquad \sum x \sum y/N = 0{\cdot}148284045$$

$$\therefore \sum xy - \sum x \sum y/N = 0{\cdot}019658525$$

$$\sum(x^2) = 0{\cdot}000158894859 \qquad (\sum x)^2/N = 0{\cdot}00001311236521$$

$$\therefore \sum(x^2) - (\sum x)^2/N = 0{\cdot}000027771207$$

Regression coefficient,

$$b = \left[\sum xy - \sum x \sum y/N\right] / \left[\sum(x^2) - (\sum x)^2/N\right] = 707{\cdot}87$$

Thus, for the regression equation of the line, $y - \bar{y} = b(x - \bar{x})$

$$y = 1{\cdot}532 + 707{\cdot}9x$$

$$\sum(y^2) = 181{\cdot}6293 \qquad (\sum y)^2/N = 167{\cdot}69025$$

Sum of squares due to regression,

$$b(\sum xy - \sum x \sum y/N) = 13{\cdot}915680$$

Sum of squares about regression,

$$\sum(y^2) - (\sum y)^2/N - b(\sum xy - \sum x \sum y/N) = 0{\cdot}02337$$

Variance about regression,

$$s^2 = 0{\cdot}02337/(N-2) = 0{\cdot}002921$$

Variance of regression coefficient,

$$0{\cdot}002921 / \left[\sum(x^2) - (\sum x)^2/N\right] = 105{\cdot}2$$

Standard error of regression coefficient = 10·26

The 95% confidence limits for the regression coefficient are

$$\pm 10{\cdot}26 \times 2{\cdot}31 = \pm 23{\cdot}70$$

The limits of error of the regression coefficient are therefore

$$707 \cdot 87 \pm 23 \cdot 70 = 708 \pm 3 \cdot 3\%$$

The variance of the regression estimate is given by

$$S^2\left[1/N + (x_k - \bar{x})^2 / \sum(x-\bar{x})^2\right]$$

where x_k is any value of x.

Thus for $x_k = 0$, the variance of the regression estimate

$$= 0 \cdot 002921(0 \cdot 1 + 0 \cdot 4722) = 0 \cdot 001671$$

$\therefore$ Standard error of regression estimate = 0·04088

The 95% confidence limits for the regression estimate are

$$\pm 0 \cdot 04088 \times 2 \cdot 31 = \pm 0 \cdot 0944$$

The limits of error of y, at x = o, are therefore $1 \cdot 532 \pm 0 \cdot 094$ giving

$$(c/T)_{c=0} = 1 \cdot 532 \pm 6 \cdot 1\%$$

2. Estimation of Error in $1/P(\theta)$.

From the data in Table 5, Z_{45} values were plotted as $1/(Z-1)$ against c (Table 18).

Table 18.

Data for Polystyrene in Toluene Dissymmetry Analysis.

c(g./1000 mls.) = 10^3x	1·506	1·883	2·109	2·560	2·673
$1/(Z-1)$ = y	3·10	3·39	3·52	3·78	3·66
10^3x	3·464	4·619	5·121	6·025	6·251
y	4·58	5·10	5·41	6·10	5·99

$\sum x = 0 \cdot 036211 \quad \bar{x} = 0 \cdot 0036211 \quad \sum y = 44 \cdot 63 \quad \bar{y} = 4 \cdot 463$

$\sum xy = 0 \cdot 17925825 \quad \sum x \sum y/N = 0 \cdot 161609693$

$\therefore \sum xy - \sum x \sum y/N = 0 \cdot 01764856$

As for the estimate of error in c/T, $\sum(x^2) - (\sum x)^2/N = 0{\cdot}000027771207$

Thus the regression coefficient = 635·50, giving for the equation of the line $y = 2{\cdot}162 + 635{\cdot}5x$

Since at $x = 0$, $y = 2{\cdot}162$, $(Z_{45})_{c=0} = 1{\cdot}463$

$\sum(y^2) = 210{\cdot}5211 \qquad (\sum y)^2/N = 199{\cdot}18369$

Sum of squares due to regression = 11·215659880

Sum of squares about regression = 0·1217

Variance about regression = 0·01521

For $x_k = 0$, variance of regression estimate =

$$0{\cdot}01521(0{\cdot}1 + 0{\cdot}47214) = 0{\cdot}008702$$

∴ Standard error of regression estimate = 0·09328

The 95% confidence limits for the regression estimate are

$$\pm 0{\cdot}09328 \times 2{\cdot}31 = \pm 0{\cdot}21548$$

The limits of error of y, at x = 0, are therefore 2·162±0·215, giving

$(Z_{45})_{c=0} = 1{\cdot}463 \pm 3{\cdot}3\%$ and $1/P(\theta) = 1{\cdot}351 \pm 2{\cdot}6\%$.

APPENDIX 3.

Polystyrene in Methyl Ethyl Ketone.

1. Estimation of Error in $(c/T)_{c=0}$.

The data used is contained in Table 6, where $c/T = y$ and $c = 10^3 x$.

$$\sum x = 0{\cdot}0275 \quad \bar{x} = 0{\cdot}00275 \quad \sum y = 6{\cdot}347 \quad \bar{y} = 0{\cdot}6347$$

$$\sum xy = 0{\cdot}0185555 \qquad \sum x \sum y/N = 0{\cdot}01745425$$

$$\therefore \sum xy - \sum x \sum y/N = 0{\cdot}00110125$$

$$\sum(x^2) = 0{\cdot}00009625 \qquad (\sum x)^2/N = 0{\cdot}000075625$$

$$\therefore \sum(x^2) - (\sum x)^2/N = 0{\cdot}000020625$$

Thus the regression coefficient = 53·393, giving for the equation of the line $y = 0{\cdot}4880 + 53{\cdot}39x$

$$\sum(y^2) = 4{\cdot}087547 \qquad (\sum y)^2/N = 4{\cdot}0284409$$

Sum of squares due to regression = 0·0587957375

Sum of squares about regression = 0·0003104

Variance about regression = 0·00003880

Variance of regression coefficient = 1·881

∴ Standard error of regression coefficient = 1·371

The 95% confidence limits for the regression coefficient are

$$\pm 1{\cdot}371 \times 2{\cdot}31 = \pm 3{\cdot}167$$

The limits of error of the regression coefficient are therefore

$$53{\cdot}39 \pm 3{\cdot}167 = 53{\cdot}39 \pm 5{\cdot}9\%$$

For $x_k = 0$, variance of regression estimate = $0{\cdot}000031575(0{\cdot}1 + 0{\cdot}36667)$

$$= 0{\cdot}00001811$$

∴ Standard error of regression estimate = 0·004255

The 95% confidence limits for the regression estimate are ±0·004255 x 2·

$$= \pm 0{\cdot}00983$$

The limits of error of y, at x = o, are therefore $0{\cdot}4880 \pm 0{\cdot}0098$, giving

$$(c/T)_{c=0} = 0{\cdot}4880 \pm 2{\cdot}0\%$$

2. Estimation of Error in $1/P(\theta)$.

Using the data in Table 6, Z_{45} values were plotted as $1/(Z-1)$ against c (Table 19).

Table 19.

Data for Polystyrene in MEK Dissymmetry Analysis.

c(g./1000 mls.) $= 10^3x$	0·5	1·0	1·5	2·0	2·5
$1/(Z-1) = y$	2·72	2·88	2·98	3·30	3·44
10^3x	3·0	3·5	4·0	4·5	5·0
y	3·73	3·82	4·00	4·18	4·37

$$\sum x = 0{\cdot}0275 \quad \bar{x} = 0{\cdot}00275 \quad \sum y = 35{\cdot}42 \quad \bar{y} = 3{\cdot}542$$

$$\sum xy = 0{\cdot}10513 \quad \sum x \sum y/N = 0{\cdot}097405$$

$$\therefore \sum xy - \sum x \sum y/N = 0{\cdot}007725$$

As for the estimate of error in c/T, $\sum(x^2) - (\sum x)^2/N = 0{\cdot}000020625$

Thus the regression coefficient = 374·54 giving for the equation of the line

$$y = 2{\cdot}512 + 374{\cdot}5x$$

Since at x = o, y = 2·512, $(Z_{45})_{c=0} = 1{\cdot}398$

$$\sum(y^2) = 128{\cdot}3714 \qquad (\sum y)^2/N = 125{\cdot}45764$$

Sum of squares due to regression = 2·89332150

Sum of squares about regression = 0·02044

Variance about regression = 0·002555

For x_k = o, variance of regression estimate = 0·002555(0·1 + 0·36667)

= 0·001192

$\therefore$ Standard error of regression estimate = 0·03453

The 95% confidence limits for the regression estimate are

$$\pm 0{\cdot}03453 \times 2{\cdot}31 = \pm 0{\cdot}0798$$

The limits of error of y, at x = o, are therefore $2{\cdot}512 \pm 0{\cdot}080$, giving

$$(Z_{45})_{c=o} = 1{\cdot}398 \pm 0{\cdot}9\% \text{ and } 1/P(\theta) = 1{\cdot}304 \pm 0{\cdot}8\%.$$

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