THE STABILITY AND DRUG RELEASE CHARACTERISTICS OF MULTIPLE EMULSIONS

by

Andrew Samuel Burbage

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Abstract

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Water-in-oil-in-water (W/O/W) or multiple emulsions have been studied by a number of methods. Bimodal particle size distributions were produced containing oil and multiple droplets. The proportions of these two types of droplets was important as it determined the degree to which the emulsion behaved as a multiple emulsion.

The particle size distributions of the multiple emulsions were determined by optical microscopy. The relative sizes and proportions of the two types of droplets present could be determined from the distributions obtained. The effect of storage on the particle size distributions was followed. The equilibrium effect of an osmotic gradient on the multiple droplets was also determined.

Freeze-etching of samples of multiple emulsions allowed electron microscopy to be performed. This revealed the structure of the internal aqueous droplets and made possible an approximate particle size distribution of these droplets.

The kinetic behaviour of multiple droplets under an osmotic gradient was determined using a Coulter counter. Several rapid particle size analyses were made to follow the change in particle size distributions with time. The rate of change of the distribution enabled a mean diameter for the internal aqueous droplets to be calculated, once certain assumptions had been made. The change of the calculated diameters with storage was followed for a number of emulsions.

Some preliminary experiments were carried out to directly determine the change in distribution of the internal aqueous phase. This was accomplished by using tritiated water as a radio-label.

It was found that the main change on storage of multiple emulsions was diffusion of the internal aqueous droplets into the continuous phase.

It is felt that the change in location of the internal aqueous phase especially under an osmotic gradient will be relevant to the release of drugs from multiple emulsions.

Multiple emulsions - Multiple emulsions
physical stability - Multiple emulsions
drug release characteristics.

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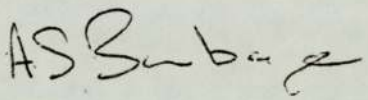
I would also like to thank the Pharmaceutical Society of Great Britain for their financial support during the described research. Finally, I would like to thank Mrs. S. Jones for typing the thesis.

This dissertation is an account of work initiated at the University of Aston and completed at the University of Nottingham under the supervision of Professor S.S. Davis for the degree of Doctor of Philosophy of the University of Aston. All work described herein is original, except where reference is made in the text, and has not been submitted for any other degree.



TO WENDY and

TO MY PARENTS

Chapter 1

1. INTRODUCTION

1.1 Definition of Emulsion

Although there are many definitions of an emulsion, the most comprehensive and generally accepted is due to Becher (1965):

"An emulsion is a heterogenous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets whose diameters, in general, exceed 0.1 μ.m. Such systems possess minimal stability, which may be accentuated by such additives as surface-active agents, finely divided solids, etc."

1.2 Medical Emulsions

The first pharmaceutical use of emulsions is accredited to Galen, the Greek physician, who records the formulation of the first cosmetic emulsion, a cold cream. Since then emulsions have found a wide variety of applications. They have long been used to make mineral and fish oily substances more acceptable. In fact, the current BPC contains many formulae for creams. These are used both to make the topical application of oily substances more acceptable and for the topical administration of drugs.

The emulsion also has some advantage as an oral dosage form and these have been demonstrated by several workers. Lewis et al (1950) found that Vitamin A absorption was increased when given in an emulsion. Feinstone et al (1940), Svenson et al (1956) and Daeschner et al (1957) studied the oral absorption of sulphonamides. They concluded that absorption from a liquid emulsion was more rapid and more complete than from an aqueous suspension. Wagner et al (1966) compared the absorption of indoxole, an oil-soluble anti-inflammatory agent, from various dosage forms. They found that the emulsion and soft elastic

capsule gave equal blood levels, exceeding those of the aqueous suspension and hard gelatin capsule. The drug in the soft elastic capsule was dissolved in the surfactant polysorbate 80. Its high blood levels may be due to the fact that it is already in solution and will readily disperse on wetting.

Carrigan and Bates (1973) and Bates et al (1977) studied the effect of dosage form on the oral absorption of griseofulvin, a water-insoluble antifungal agent. Administration in an emulsion form gave a dramatic increase in the peak plasma concentration of the drug compared to aqueous and oily suspensions although this could be matched by administration as ultramicrosize tablets. They attributed this increase in availability to the inhibition of gastro-intestinal motility and increased gall bladder evacuation caused by the oil. This increased absorption could be related to the amount of oil administered in the emulsion.

Waggoner and Fincher (1971) and Lin et al (1974) studied the effect of HLB on ephedrine release and absorption both in vitro and in vivo. Kakemi et al (1972a,b,c) and Ogata et al (1975) investigated the mechanism of intestinal absorption of drugs from oil-in-water emulsions using a model system. They found that absorption occurred from the continuous phase of the emulsion and that direct absorption of or from the emulsion droplets was negligible. Nakamoto et al (1975) studied the lymphatic transport of a hydrophilic anticancer drug administered in aqueous solution and various emulsions. The water-in-oil emulsion was found to be superior to both oil-in-water emulsion and aqueous solution in that it achieved a higher lymph concentration to plasma concentration ratio at all times by both intra-peritoneal and intramuscular routes. Hashida et al (1977a,b) studied the effect of various dosage forms on the

distribution of radio-active drug and oil tracer molecules in rats. They found that after injection into the tissue studied, either stomach or leg muscle, water-in-oil emulsion was more specific for delivery of both oil and drug into the lymphatic system than oil-in-water emulsion or aqueous solution. This effect was most noticeable in that part of the lymphatic system closest to the site of the injection.

While the use of emulsions as adsorbents has been known for some time (Walsh and Fraser 1943), Kriegstein et al (1974) reported an in vivo use of this property. They found that administration of a fat emulsion could protect experimental animals from the effects of high doses of chlorpromazine.

Jeppsson (1972a,b) has studied the effect of barbituric acids in emulsion form by various routes of administration - i.v., i.p., s.c.. He found that the drug in a lipid emulsion gave a more prolonged release than an aqueous solution of the sodium salt. Jeppsson (1975) found a similar prolongation of action for local anaesthetics. Jeppsson and Ljungberg (1975) studied the anticonvulsant activity of diazepam in solution and emulsion form and found that the emulsion form showed less toxicity and the same duration of action as the solution.

W/O emulsions have been used successfully for the intramuscular administration of vaccines. The emulsified antigen solution provides an enhanced effect due to the delayed release of the active material. This phenomenon has been termed the adjuvant effect (Freund and McDermott, 1942). Careful formulation of the emulsion can further enhance the immunological response. For example, the emulsifier "Arlacel A" (mannide mono-oleate) is sold specifically for use in vaccines because of the enhanced immunological response it produces.

A semi-quantitative study of the effect of the physical properties

of the emulsion on the immunolgical response was made by Berlin (1960). Lazarus and Lachman (1967) have described the formulation, properties and stability of W/O emulsions used as adjuvants. An inverse relationship was found between antibody response and viscosity. Windheuser, Best and Perrin (1970) studied the effect of phase volume and viscosity of the oil and partition coefficient of the drug on the release of drugs from W/O emulsions.

Emulsions can also be used as diagnostic agents in the form of injectable radiopaques. Kunz, Lewis and Sperandia (1965) described in detail the preparation, sterilisation and stability of 50% O/W emulsions of iodized oil, iophendylate injection, and ethiodized oil ("Ethiodol"). Three emulsions of each oil injected i.p. into rats gave an excellent radiopaque outline of the peritoneal cavity. Iophendylate 50% injections injected intrathecally into dogs and into carpal joints of horses were miscible with the cerebrospinal fluid and synovial fluid respectively and demonstrated satisfactory radiopacity.

Arambulo et al (1974-1975) described the use of brominated perfluorocarbon emulsions as radiopaque media. Highly concentrated O/W emulsions were found to be stable and non-irritating and useful in bronchography in humans and animals and in angiography in animal studies.

1.2.1 Emulsions for Intravenous Infusion

Over the past few years, there has been great interest in the use of emulsions for intravenous infusion, with 2 applications in mind—parenteral nutrition and blood substitutes.

Parenteral nutrition is used for patients for whom oral nutrition is difficult. For this, a sterile oil-in-water emulsion of a purified vegetable oil, e.g. soybean oil, is used. These are widely used in

Europe because they have the advantage of providing a large number of calories for a small volume and they have little osmotic effect. Davis (1974) has reviewed the pharmaceutical aspects of these emulsions. They must be non-toxic, stable to both aging and autoclaving and have a particle size less than one micron to avoid the risk of emboli. These requirements are very rigorous and only a few oil/surfactant combinations can meet all the criteria. The most successful is egg lecithin and soybean oil in the commercial product, "Intralipid".

Despite this long-standing interest in parenteral emulsions, little is yet known of the mechanisms of their distribution and elimination from the body. Jeppsson & Schoefl (1974) applied the technique of electron microscopy to observe the distribution of lipid droplets. The emulsifying system appears to be of great importance in both the elimination rate from the blood (Jeppsson & Ljungberg 1975) and the removal site, e.g. Intralipid is removed in the myocardium, splanchnic region, subcutaneous tissue and skeletal muscle (Rossner 1974) whereas other fat emulsions have been found in the reticulo-endothelial system (Diluizio & Riggi 1964, Lemperle & Reichelt 1973). The technique of using radio-active tracers for both oil and drug, described by Hashida et al (1977a,b), could be usefully employed here to give a quantitative picture of fat emulsion distribution in the body.

The kinetics of clearance of fat emulsions can be followed and treated by pharmacokinetic analysis (Hallberg 1965).

The use of fluorocarbon emulsions as blood substitutes is based on the high solubility of oxygen and carbon dioxide in fluorocarbon liquids. This was aptly illustrated by the early liquid breathing experiments of many workers e.g. Clark & Gollan (1966). These involved the immersion of experimental animals in fluorocarbon liquids - although

the lungs became filled with liquid, respiration was still possible. Sloviter and Kamimoto (1967) reported that a fluorocarbon emulsion of 2-3μ in diameter could maintain the electrical activity of a rat brain. Since then many workers have reported the replacement of erythrocytes (Clark et al 1971; Sloviter et al 1970) and blood (Geyer 1970). Fujita et al (1971) showed that the toxic reactions associated with the early fluorocarbon emulsions could be eliminated if the emulsion droplets could be made sufficiently small (less than 0.3μ or less). However, despite all the effort expended on this topic, the only product commercially available is for use with animals only ("Fluosol", Green Cross Corporation, Japan). If such a product became available for human use, its advantages over human blood would be immense: good shelf life, no blood group problems, readily accessible in large quantities and no hepatitis.

1.2.2 Advantages and Disadvantages of Emulsion as Drug Delivery Systems

Despite research into the subject, the use of emulsions for medicinal purposes, especially for administration of drugs, is not extensive. The emulsion that finds widest use in the clinical situation is lipid emulsion for parenteral nutrition. The major obstacle to more extensive use is the lack of stability. As emulsions are disperse systems, they have large surface areas with correspondingly large surface energies. Therefore they are inherently unstable. However this is thermodynamic instability and the time taken for it to manifest itself may be sufficiently long for pharmaceutical purposes. This assumes, of course, an optimisation of stability by judicious choice of the oil/surfactant combination. This is another obstacle to wider utilisation of emulsions - the lack of information about the effect this can have

on emulsion stability which makes it difficult to predict emulsion stability from a knowledge of the composition alone. This is further complicated by the presence of the drug molecules which will exert their own effect on emulsion stability, either alone or interacting with the oil or emulsifier. The use of emulsions is also restricted because of the lack of an accelerated test of stability. Whereas the chemical stability of a drug may be tested by storage at elevated temperatures, there is no easy test for emulsions. In fact the only reliable test for emulsion stability is storage for the required period but this is of course time-consuming.

Despite these drawbacks, emulsions have many advantages over more widely used dosage forms. By oral administration of a drug in an emulsion it is possible to achieve greater absorption for a larger period of time. Similarly, emulsions can be used to achieve absorption of substances not normally absorbed from the G.I. tract e.g. heparin, insulin.

O/W emulsions can also produce sustained release when given parenterally and compared with solutions. Other parenteral uses of O/W emulsions include intravenous nutrition and blood replacement. Intravenous nutrition by fat emulsion is used because it provides a large calorific intake in a small volume of fluid.

W/O emulsions are used as vaccine adjuvants because of the sustained release from them when given intra-muscularly. They can also be used to deliver drugs specifically into the lymphatic system.

1.3 Multiple Emulsions

1.3.1 Definition and nomenclature

In a multiple emulsion, the droplets of disperse phase contain

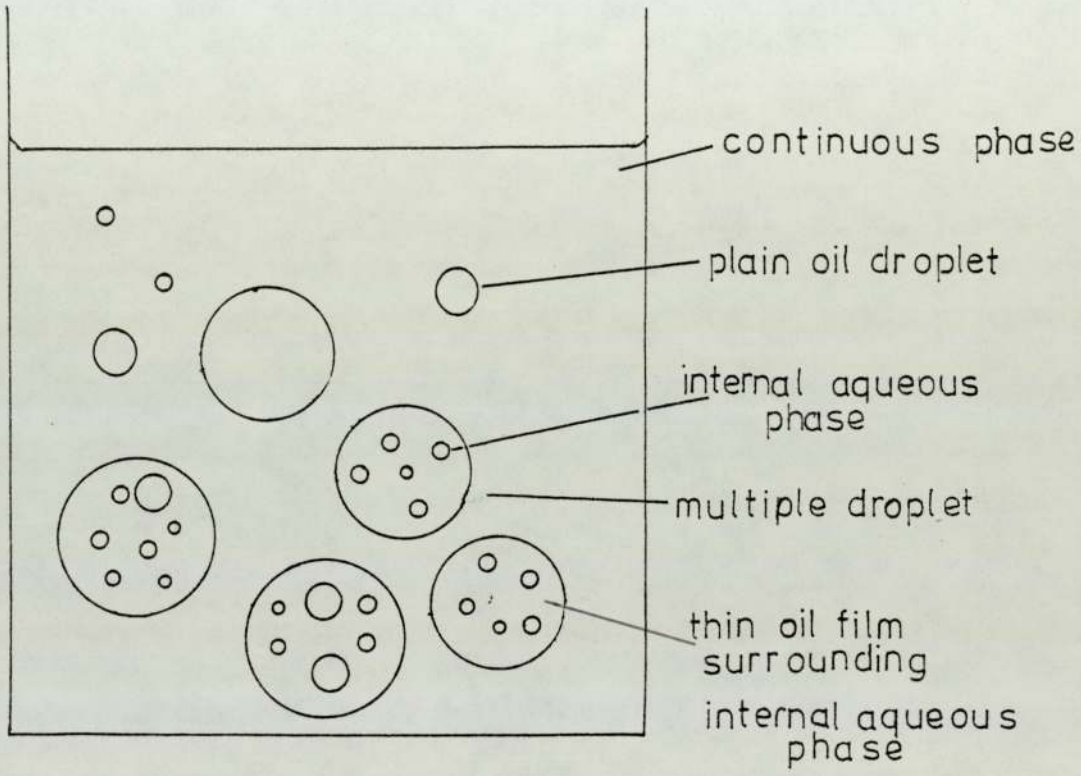


Figure 1.1 Diagram of a multiple emulsion.

Table 1.1. Multiple Emulsions for Drug and Vaccine.

Administration.

Drug or Vaccine	System	Reference
Methotrexate	W/O/W	Elson et al (1970)
Bleomycin	W/O/W	Takahashi et al (1976)
Cytosine arabinoside Vinblastine sulphate	W/O/W	Benoy et al (1972)
Insulin	W/O/W	Engel et al (1968) Schichiri et al (1974, 1975, 1976)
Cysteamine	W/O/W	Gresham et al (1971)
Naloxone	W/O/W	Frank et al (1976)
Ovalbumin	W/O/W	Herbert (1968)
E. coli endotoxin	W/O/W	Hill et al (1973)
Influenza virus	W/O/W	Taylor et al (1969)
Anaerobic coryneforms	W/O/W	O'Neill et al (1973)

smaller droplets of another phase dispersed within them. This secondary disperse phase is usually of similar composition to the continuous phase but physically separated from it. The system of nomenclature adopted for this thesis will be explained by reference to figure 1.1.

Those most widely used in pharmaceutical applications are three-phase systems i.e. water-in-oil-in-water (W/O/W) emulsions or oil-in-water-in-oil (O/W/O) emulsions. However, systems containing up to five phases have been reported (Seifriz 1925).

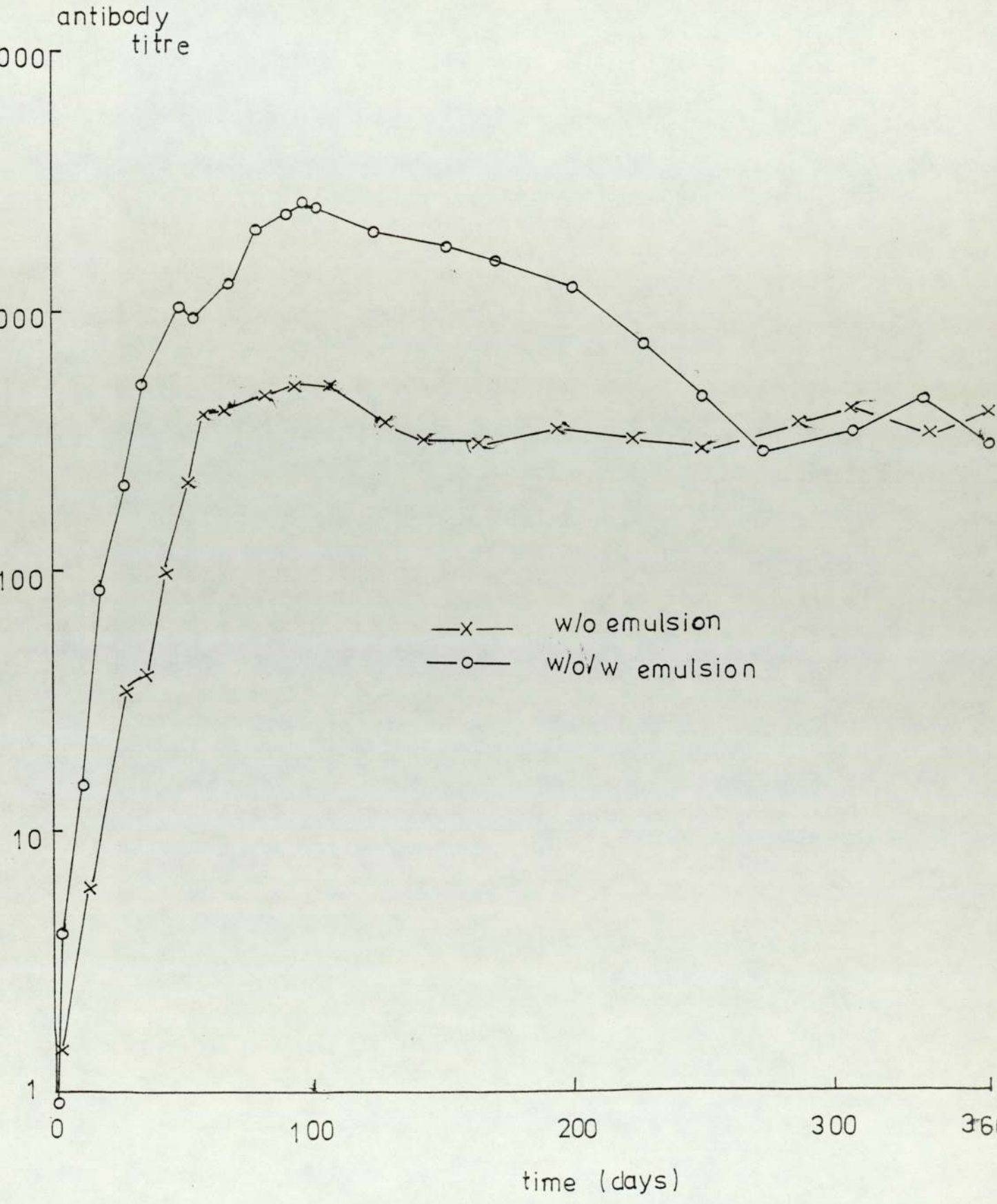
All multiple emulsions referred to in this thesis are W/O/W unless otherwise stated.

1.3.2 Work to date

The principal uses of multiple emulsions are summarised in Table 1.1.

The first reports of multiple emulsions were as "by products" of research into emulsion inversion (Woodman 1929, 1935; Parke 1934; Sherman 1963). The uses of multiple emulsions are listed in Table 1.1. The first use of a multiple emulsion was for the administration of vaccines (Herbert 1965). The usual vaccine adjuvant at that time was a W/O emulsion of an aqueous solution of the vaccine. This gave a sustained release of the vaccine but was difficult to inject because it was so viscous. Herbert re-emulsified this W/O emulsion to give a W/O/W emulsion which was not only easier to inject but gave an even greater increase in antibody titre (Figure 1.2). Since then, further vaccines and antigens have been incorporated into multiple emulsions (Herbert 1967). Taylor et al (1969) reported the use of an influenza vaccine in multiple emulsion form. They found the multiple emulsion gave a higher antibody titre than both an aqueous suspension and a W/O emulsion of the vaccine. O'Neill et al (1973) used a multiple emulsion formulation to incorporate micro-organisms. This was then tested for stimulation of antibody

Figure 1.2 Increase in ovalbumin titre after administering a W/O/W emulsion (Herbert, 1967).



response to human serum albumin.

Much of the early work on multiple emulsions has been published in a patent (NRDC 1967, 1968). A wide range of drugs was studied, mostly in vivo but some studies on in vitro drug release were made. The in vivo experiments demonstrated the property of delayed release, compared with an aqueous solution, but only when the osmolarity of the internal aqueous phase was increased by the presence of a suitable additive e.g. sodium chloride or glucose. The additive would appear to be necessary to counteract the osmotic effect of the body fluids. The in vitro experiments involved the addition of the emulsion to distilled water and measuring the release of an electrolyte such as sodium chloride or sodium salicylate by following the conductivity.

Elson et al (1970) have studied the chemotherapeutic effect of methotrexate in a multiple emulsion in mice. They found that a dose of 3mg/kg in multiple emulsion form gave a longer survival time than 80mg/kg of aqueous solution of methotrexate. The same single dose of 3mg/kg gave a slight greater response than 5 daily doses of 3mg/kg in aqueous solution of methotrexate. They also found that inclusion of sodium chloride was necessary for this sustained release. A concentration of 2% sodium chloride gave the greatest delay while the omission of sodium chloride showed almost the same release characteristics as the control which had no methotrexate. This sustained release was despite the fact that the emulsion had to be used immediately after preparation.

Gresham et al (1971) found that administration of the radio-protective agent cysteamine in a multiple emulsion gave a prolonged protection against the radiation when compared with an aqueous solution.

An interesting application of multiple emulsions is the intestinal absorption of insulin. This was first reported by Engel et al (1968), who found that insulin in multiple emulsion lowered the blood glucose

level in rats and gerbils following intraduodenal administration. Administration to alloxanised rats had the same effect as an aqueous solution. It was suggested that intestinal absorption of insulin may be impaired in alloxanised rats.

Schichiri et al (1974, 1975, 1976) have studied the absorption of insulin from multiple emulsions. Schichiri et al (1974) showed that administration of insulin in multiple emulsion form to the rabbit jejunum caused a significant decrease in blood glucose levels and in excreted glucose when compared to insulin solution. Absorption was not complete as between 0.4% and 1.2% of the administered dose was absorbed. This compared with 0.1% to 0.4% for insulin solution.

Schichiri et al (1975) extended this study to include multiple dosing of alloxan-diabetic rats with insulin in multiple emulsion form given into the jejunum. They found that administration of insulin in multiple emulsion form 3 times daily caused a maintained decrease in blood glucose levels and in glucose excreted. It was found that intra-jejunal administration of 50u/100gm. insulin in multiple emulsion form was equivalent to intramuscular insulin of 1 to 2 u/100gm.

Schichiri et al (1976) have reported the oral administration of multiple emulsion insulin to alloxan-diabetic rats. Dosing with insulin in multiple emulsion over short periods produced a decrease in both blood glucose levels and glucose excretion. It was found that 50u/100g of insulin in multiple emulsion is as effective as 0.5u/100g. of intra-muscular insulin. These studies demonstrated the possibility of using emulsions, especially multiple emulsions, to protect sensitive molecules from degradation in the gastro-intestinal tract. Engel and Riggi (1969) have demonstrated a similar phenomenon for heparin - they obtained intestinal absorption when an O/W emulsion was administered intraduodenally.

Dapergolas and Gregoriadis (1976) recently reported the use of

liposomes to administer insulin intragastrically. They found that liposome-entrapped insulin was effective in reducing blood glucose levels in both normal and diabetic rats. In normal rats, a dose of 1.3 units of liposome-entrapped insulin reduced blood glucose levels to 77% of pre-treatment values after 4 hours and higher doses of 4.2 and 8.4 units extended this effect over 24 hours. In diabetic rats a dose of 1 unit of liposome-entrapped insulin decreased the blood glucose levels to 57% of pre-treatment levels after 4 hours. Higher doses of 2.9 and 5 units decreased the blood glucose levels still further and extended the effect to 24 hours. By comparison, an equivalent dose of 10 units/kg given to normal rabbits caused a maximum lowering of blood glucose levels to about 80% of pre-treatment values. In alloxan-diabetic rats, 500 units/kg of insulin in multiple emulsion form was necessary to cause a decrease in blood glucose to 76% both intra-jujunally and orally. Therefore, it appears that insulin in multiple emulsion form can cause a decrease in blood glucose although a vastly increased dose is required in alloxan-diabetic rats. Engel et al (1968) report a similar impairment of intestinal absorption of insulin in alloxan-diabetic rats, stating also that parenteral insulin administration to such rats causes a hypoglycaemic response. Liposome-entrapped insulin appears to be more effective intragastrically and is not subject to impairment of absorption although diabetes was induced by a different method.

Takahashi et al (1976) have reported the use of multiple emulsions as vehicles for anticancer agents. These were injected directly into the tumour. They found that this produced a higher level of the drug used, bleomycin, in the tumour than an aqueous solution, either intratumoural or intravenous, and this high level was sustained for much longer. This effect was also found in the clinical situation. For squamous cell carcinoma, intratumoural injection of bleomycin in multiple emulsion gave

results superior to those achieved by local injection of an aqueous solution of bleomycin. The dosage regimen for the emulsion was once or twice weekly whereas that for the solution was daily. Similar results were achieved by the use of mitomycin in multiple emulsion form for the treatment of carcinomas.

Matsumoto et al (1976) have recently reported a study on the properties of multiple emulsions. They claim to have prepared multiple emulsions which consist of one water droplet surrounded by a thin layer of oil, although no supporting evidence is given and "in microscopic observation of the W/O/W emulsion the reflected light from the surface of the globules made discrimination between aqueous globules covered with an oil layer and simple oil droplets impossible". The emulsions were prepared containing glucose and, by a dialysis technique, the yield of multiple droplets could be determined. The effect of formulation and manufacturing variables on this yield could then be found. They found that a high yield of multiple droplets could be achieved if the ratio of hydrophobic: hydrophilic emulsifier concentrations was high (10 or greater) and if the volume fraction for the second emulsification was 0.5 or greater. The volume fraction for the first emulsification was found to have little effect on the yield.

A recent publication by Kita et al (1977) used a viscometric technique to assess the stability of multiple emulsions. They reasoned that as internal aqueous droplets are lost by rupture of the thin oil film surrounding each of them, the phase volume of the disperse phase decreases and so the viscosity decreases. Viscosity was measured by cone and plate viscometer. Emulsion viscosities were measured over a period of time and the relative viscosity showed an initial rapid decrease which levelled off to a steady value after several days. From this data, the variation of the phase volume of the internal aqueous droplets with time

could be followed. The main drawback to this method is that the samples must behave as Newtonian fluids, which limits the emulsions which can be tested to those with low phase volumes. Also the presence of additives in the internal droplets increased the relative viscosity and disturbed its relationship with time.

Matsumoto et al (1977) have adapted the technique for preparing multiple emulsions to prepare lipid vesicles. The oil phase consisted of a solution of lecithin and Span 80 in n-hexane. This was then emulsified to produce a W/O emulsion. The n-hexane was evaporated under vacuum. The resulting water-in-lipid droplets were then re-emulsified in an aqueous solution of a hydrophilic surfactant to form a water-in-lipid-in-water emulsion. The emulsion was then dialysed to remove excess hydrophilic surfactant. The authors investigated the effect of various surfactants and phase volume ratio on the yield and stability of the resulting vesicles.

1.3.3 Liquid Membranes

An interesting development of multiple emulsions is the use of liquid membranes for extraction and separation purposes, first described by Li (1968). Typically, they consist of many small water droplets, enclosed in a larger oil droplet, itself dispersed in water. A typical liquid membrane system is shown in Figure 1.3. The major difference from multiple emulsions is that the continuous phase contains no stabilising agent. It is usually a solution of the substance to be extracted, to which is added a W/O emulsion. The oil is dispersed by stirring. When extraction is complete, stirring is stopped and the 2 phases, oil and continuous, rapidly separate. In this case, the oil phase is the liquid membrane but the situation could be reversed. The advantages over other extraction methods are a large surface area (maintained by stirring) and

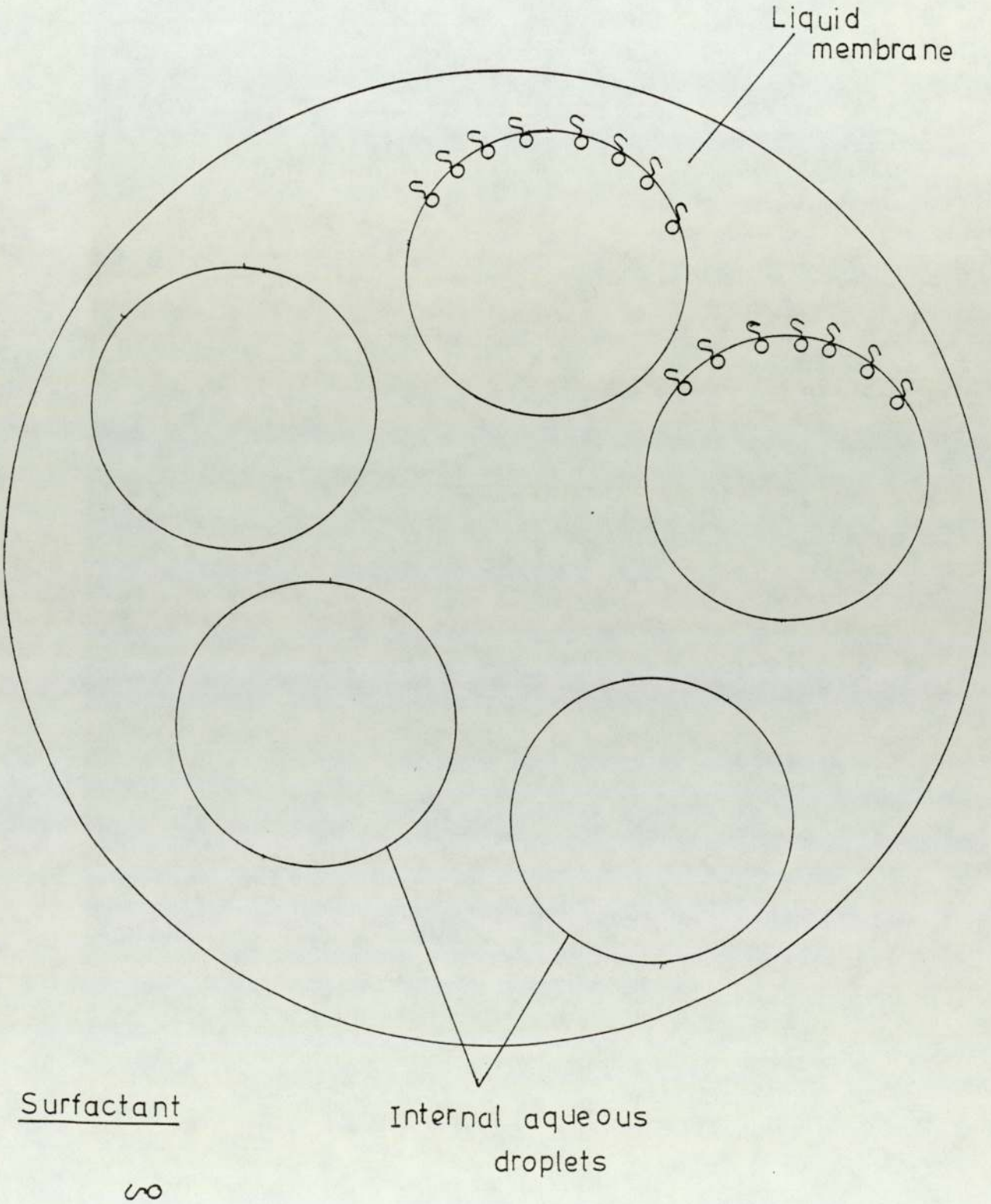


Figure 1.3 Liquid membrane globule.

only a thin liquid film to hinder transport. Various binding and carrier agents can be included in both the internal phase and the liquid membrane phase. The principal applications of liquid membranes are summarised in Table 1.2.

Cahn and Li (1974) reported the use of liquid membrane for the removal of phenol from waste water. The internal aqueous were approximately 1μm in diameter and contained sodium hydroxide. The multiple droplets were up to 2mm in diameter. The phenol could diffuse through the liquid membrane into the internal aqueous droplets down a concentration gradient. Once inside the droplet, it reacted to form the phenolate ion which could not diffuse back. In this way, the concentration gradient could be maintained even while extraction continued. Using this system, a very efficient extraction could be achieved. Using similar systems, a wide variety of substances have been extracted e.g. acetic and citric acids, cupric, mercuric, ammonium, sulfide, nitrate and cyanide ions.

Using the same principle, separation of hydrocarbons, e.g. benzene and cyclohexane, has been achieved (Li 1971). For this application the hydrocarbons to be separated are emulsified in an aqueous surfactant solution. This W/O emulsion was then emulsified in a solvent, in this case hexane, where selective permeation occurred. By recycling, highly efficient separation can be achieved.

An interesting application of liquid membrane technology is the oxygenation of blood (Li and Asher 1973). This is performed in a column. At the bottom is a layer of a fluorocarbon solution. Above this is a constantly circulated layer of blood. Oxygen is bubbled into the bottom of the column, it passes through the fluorocarbon solution. On passing into the blood, the oxygen is covered with a thin liquid membrane of fluorocarbon solution. As this passes through the blood, gas exchange occurs across the membrane, oxygenating the blood and

Table 1.2. Applications of Liquid Membranes

Application	Reference
waste water treatment	Cahn and Li (1974)
Separation of hydrocarbons	Li (1971)
oxygenation of blood	Li and Asher (1973)
treatment of uraemia	May and Li (1972) Asher et al (1978)
emergency treatment of drug overdose	Frankenfeld et al (1976) Chiang et al (1978)

removing carbon dioxide. The gas bubbles pass out at the top of the column, allowing the fluorocarbon to be collected and purified.

Liquid membranes have also been used to encapsulate the enzyme urease with possible application to the treatment of uraemia (May and Li 1972, Asher et al 1975). The liquid membrane in this case included a high molecular weight polyamine as hydrophilic emulsifier. The enzyme remained active when encapsulated and there was no leakage into the continuous phase. The stability of the liquid membrane was demonstrated by incorporating a lethal dose of sodium cyanide into a liquid membrane formulation which was then administered orally to rats. The rats showed no ill effects.

Frankenfeld et al (1976) described an application of liquid membranes for the emergency treatment of drug overdose. In vitro tests were carried out on 2 drugs, phenobarbitone and aspirin, using the principle mentioned above, i.e. salt formation in the internal aqueous phase. The system tested extracted 95% or more drug in five minutes, the viscosity of the oil being the major rate-determining factor. More rapid extraction could be achieved but only at the expense of liquid membrane stability.

Chiang et al (1978) have described an in vitro study of the uptake of six barbiturates by liquid membranes. Drug uptake initially showed first order kinetics and Fick's law was obeyed. The rate constant for uptake was proportional to the proportion of liquid membrane present. The most efficient removal was achieved by a liquid membrane containing pH12 buffer as the internal aqueous phase and using this preparation, 90% of the drug was removed from the donor solution in 10 minutes. The effect of temperature on the uptake was also investigated. An Arrhenius plot enabled the activation energy to be calculated as 10.8 kcal/mole. The presence of bile salts interfered with the transport: 0.5% caused

an initial increase in the uptake rate, followed by a decrease. 2% of bile caused a decrease in drug uptake and at longer times the drug concentration in the donor phase started to increase.

1.3.4 Artificial Cells

Artificial cells were first reported by Chang (1957). Chang (1972) has described artificial cells as "an idea involving the preparation of artificial structures of cellular dimensions for possible replacement or supplement of deficient cell functions". They consist of an aqueous solution or suspension of the active ingredient surrounded by a thin solid membrane, dispersed in an aqueous phase. Thus they only differ from multiple emulsions and liquid membranes in their solid membrane. The membrane may consist of polymer e.g. nylon (Chang 1966), or biological materials, e.g. protein (Chang 1969a). They may also incorporate carrier molecules e.g. Valinomycin (Chang 1969b).

Most of the applications of these systems involve encapsulation of enzyme, cell homogenates or whole cells. The artificial cell, by manipulation of the thickness and composition of the membrane, allows the diffusion of substances in and products out while immobilising the enzymes.

Enzymes that have been incorporated include urease (Chang 1966), carbonic anhydrase (Chang 1964) and asparaginase (Chang et al (1968).

Nylon artificial cells containing erythrocyte haemolysate have been used as red blood cell substitutes (Chang 1964) but their biological half-life is very short (Chang 1965). This is considered to be due to the absence of surface charge. Sulfonated nylon artificial cells survived significantly longer than uncharged cells although their absolute half-life was still short. This could be avoided by use of an extracorporeal blood gas exchange units. This involved the formation of heparin-complex capsules containing erythrocyte haemolysate (Chang 1965).

1.3.5 Other applications

An interesting application of the "multiple emulsion principle" is the product known as "Spindrift" produced by Tioxide. This consists of a ~~slurry of spherical polymer beads containing water vesicles~~ water/styrene/water emulsion in which the styrene is polymerised although the internal aqueous droplets dry to leave vesicles of air. In addition, the vesicles contain titanium dioxide. The internal structure of this product is shown in Figure 1.4. This is a fracture section of a polymer bead showing the vesicles inside.

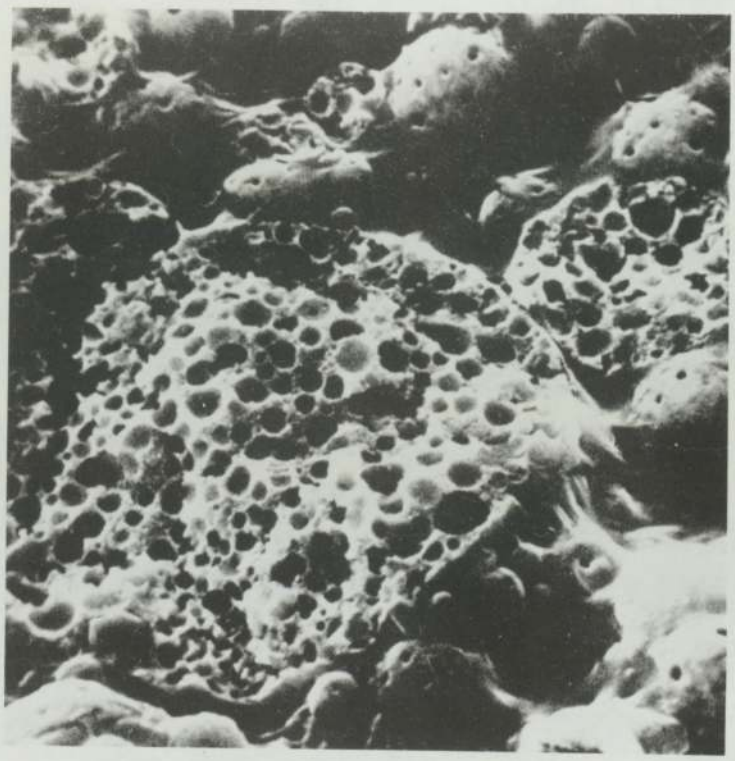
"Spindrift" is used in the paint industry to produce white paint films. Light is scattered by the titanium dioxide particles and this effect is enhanced because they are surrounded by air which has a low refractive index and so increases the difference between itself and the titanium dioxide. The vesicles comprise a large proportion of the bead volume and are of 0.7 to 1.0μm mean diameter. Two versions are available with different polymer bead diameters, namely 5 or 25μm.

1.3.6 Advantages and Problems of Multiple Emulsions.

Aims and Objectives of this study.

A number of papers have described research into multiple emulsions (Section 1.3.2). However, these have been concentrated on the biological and medical aspects of these systems. Little effort has been expended toward an understanding of the formulation and stability of multiple emulsions. Indeed, much more is known about drug release than stability. For instance, several workers have shown sustained release of drugs from multiple emulsions without showing that the emulsion was stable either in vitro or in vivo, for the period of the sustained release.

From a survey of the literature it seemed evident that if multiple emulsions are to find utility as dosage forms it is essential to produce reproducible systems with an acceptable (and known) shelf-life.



Consequently for a W/O/W system one must be able to evaluate the particle size distribution of the internal aqueous phase and its change with time, the particle size distribution of the oil phase and its change with time, and the relative proportion of multiple and non-multiple droplets. Once this data is available, it is then possible to establish the effect of formulation and processing variable on the emulsion properties listed above. This would then permit the reproducible formulation and manufacture of stable emulsions which must be achieved before drug release studies can commence.

As mentioned above, the multiple emulsion principle is also applied in solvent extraction and water purification systems. Therefore, elucidation of the factors mentioned above will be of interest not only in pharmacy and medicine but also chemistry and chemical engineering. There are also the biological applications described below.

Consideration of the diagram of a multiple emulsion (Figure 1.1) illustrates the various factors involved. The normal factors associated with studying a simple emulsion are doubled. Thus, we have two emulsifiers and two aqueous phases to consider. The second and third objectives listed above are relatively easy to achieve with conventional methods, the first is more difficult. For therapeutically viable systems, the internal aqueous droplets will normally be less than 1 micron in size and are contained in the oil droplets of size 10 microns.

There are clearly several advantages to multiple emulsions as dosage forms if the problems outlined above can be overcome. Administration of drugs normally given as W/O emulsions will be facilitated if given as multiple emulsions. They can give higher blood levels and will be easier to handle. Oral administration of drugs in multiple emulsions can result in absorption of drugs not normally absorbed e.g. insulin. They might also find use as models for biological membranes because of

the multiple emulsion structure consisting of two aqueous phases separated by a thin oil layer. They are also more stable and their composition is less rigorously dictated than other membrane models e.g. bilayer lipid membranes. It is therefore instructive at this stage to consider membrane models.

1.3.7 Membrane Models

Examination of the multiple emulsion diagram (Figure 1.1)will show that a multiple emulsion can be considered as consisting of a thin oil layer separating two aqueous phases. This is also a characteristic of biological membranes and we consider that the multiple emulsion may be used as a model for biological membranes. Therefore, some of the work on membrane models will be reviewed, concentrating on two particular models - the black lipid membrane and the liposome.

Biological membranes are complex structures containing many different types of molecules. They are involved in a large number of processes vital to living organisms. This has led to great efforts to elucidate their composition and structure. However, experiments on intact membranes in vivo lead to results which are difficult to interpret. This has naturally led to a search for membrane models. The earliest of these followed the proposal of Danielli and Davson (1935) of a bilayer structure for the plasma membrane. However, the first models were based on proteins, e.g. Schulman and Rideal (1937) used egg albumin and lecithin, but the most successful membrane models have been based on phospholipids. These are "black" lipid membranes (BLM's) and phospholipid vesicles (liposomes).

"Black" lipid membranes (BLM's) were first described by Mueller et al (1962 a,b). They were formed by painting mixed lipids, derived from ox brain, in a chloroform-ethanol mixture onto an orifice in a

polyethylene cup. The membrane will drain and thin until it consists of a bilayer of the constituent lipids. At this point, the membrane is very thin (less than 10nm) and so appears black. The membrane so formed is usually stable for a period of hours at the most and so permits the study of the behaviour of defined composition.

The basic properties of BLM's closely resemble those of natural membranes. For example, the thickness of BLM's is 6 to 9nm, that of natural membranes 4 to 13nm. (Tien and Diana 1968). Properties that are missing from the basic BLM can be produced by the addition of membrane-modifying substances. For instance, inclusion of a modifier of unknown composition known as "excitability inducing material" (EIM) conferred, not surprisingly, excitability (Mueller et al 1964). Inclusion of the cyclic peptide,valinomycin, confers ion selectivity on BLM's (Mueller and Rudin 1967).

The inclusion of these modifiers allows the selected property to be studied in isolation. The property of unmodified BLM's most relevant to the present study is that of water permeability. All BLM's, regardless of composition, show significant water permeability and this has been studied by 2 different methods - exchange diffusion and osmotic flow.

Exchange diffusion follows the flow of isotopically tagged water across the BLM in the absence of an osmotic gradient. The osmotic method creates a concentration gradient across the membrane and measures the net flow of water.

Early experiments showed a large discrepancy in the values obtained for the 2 methods. For instance, Hanai et al (1966) reported values of 2 μ/sec for exchange diffusion and 19 μ/sec for osmotic flow. They suggested that this discrepancy could be due to "unstirred layers"

next to the membrane in the exchange diffusion experiments because when exchange diffusion permeabilities were measured simultaneously with osmotic flow, little difference was found. The importance of stirring has since been demonstrated (Cass and Finkelstein 1967).

Haydon (1968) measured permeability of BLM's and cellophane using the same experimental conditions. The thickness of the boundary layers was calculated from the permeability of the cellophane and this value was then used to calculate the true permeability of the BLM. This gave a value close to that for osmotic flow.

Although the main value of BLM's lies in the elucidation of the general properties of membranes, they have been used as models for specific membranes. For instance, BLM's have been used as models for the visual receptor membrane by Tien and Kobamoto (1969). The full mechanism of light conversion in the retina is not fully understood but studies on well-defined model systems may be of value in understanding this process. It was found that a BLM, modified by the addition of one of a number of carotenoids, produced a voltage on exposure to white light. Depending upon the experimental conditions, the voltage/time curve could be altered. In some cases, this curve resembled a biphasic response found in vertebrate retina.

Inui et al (1977) reported the use of BLM's as a model for intestinal absorption of drugs. They determined the permeability of a number of drugs across BLM's generated from egg lecithin and intestinal lipids. Permeability coefficients were much larger than predicted by partition coefficient measurements. Measurements at 2 pH's demonstrated that even ionised molecules of drugs are permeable and that the pH-partition hypothesis cannot explain the permeabilities in this case.

Bangham et al (1965) were the first to show that dilute phospholipid in a salt solution would, on stirring spontaneously form

closed vesicles whose walls consist of bilipid membranes. The walls of these vesicles consist of many concentric bilipid membranes and are said to be multilamellar in nature. However, most of the work on phospholipid vesicles has been performed on unlilamellar vesicles e.g. Huang (1969). These produced from the larger ones by exposure to ultrasonic vibrations and are known as liposomes.

Incorporation of a marker molecule, e.g. radio-actively labelled chlorine or potassium in the solution from which they are formed, results in the presence of the marker in the aqueous core of the liposome. The rate of appearance of the marker in the external phase can be followed, allowing the permeability coefficient to be calculated. By varying the composition, considerable ion selectivity can be achieved. Papahadjopoulos and Watkins (1967) found that in lecithin liposomes with 10%stearylamine the ratio of chlorine/potassium diffusion coefficients was as large as 110.

The water permeability of liposomes can be measured from rates of swelling or shrinking in osmotic gradients (Bangham et al 1967). Values were found of comparable magnitude to BLM's.

Liposomes have also found use as dosage forms. Once a drug has been entrapped in liposomes, it can only be released by leakage across the membrane which is slow or by destruction of the liposome. As a dosage form this gives advantages of reduced reactions, both allergic and immunological, and delayed release. The proposed mechanisms for drug release are fusion (Papahadjopoulos et al 1974) and endocytosis (Gregoriadis 1966). Fusion involves fusion of the liposome membrane with the cell membrane, releasing the drug into the cell. Endocytosis involves engulfment of the liposome by the cells and movement into the lysosome where they are broken down, releasing the drug. This mechanism allows the use of liposomes to deliver enzymes directly into cell lysosomes

(Gregoriadis 1974).

Encapsulation of the anticancer drug, Actinomycin D, in liposomes has led to increased survival rates of leukaemia-bearing mice. (Neerunjun and Gregoriadis 1974).

The same drug in liposomes, when added to tissue culture of Actinomycin-D resistant cells, inhibited RNA synthesis and cell growth (Poste and Papahadjopoulos 1976), whereas addition of free drug at the same concentration did not affect cell growth.

However, there are problems of distribution which must be overcome before liposomes become widely used as a dosage form. Gregoriadis and Ryman (1972) have shown that the liver and spleen are the major sites of liposome uptake. Changing the surface charge of the liposome can have some effect on the distribution (Gregoriadis 1974) as can changing the composition (Rahman et al 1973). A more promising method is to associate the drug containing liposome with immunoglobulins raised against target cells. The immunoglobulins direct the liposomes to the target cell where they release the drug (Gregoriadis 1974 , Gregoriadis and Neerunjun 1975b).

From the preceding outline of model membranes, it appears that the main drawbacks of BLM's are:

they offer a small area for transport,

they are of limited stability,

their composition may only be varied within certain strict limits.

Liposomes offer a large area for transport and greater stability but their composition is still strictly limited to certain phosopholipids. They also have the drawback that marker or drug incorporation is sometimes difficult and unpredictable.

Multiple emulsions, if used in this particular application, would

offer a large area for transport, well-defined stability, ease of incorporation and less strictly defined composition. Transport into the droplets is also possible.

1.4. Emulsion Stability

A stable emulsion has been defined as one in which there is "no observable change with time" (Garrett, 1965). A stable emulsion is very rare and the study of emulsion stability becomes a study of how rapidly the process of instability proceeds. The stability of an emulsion has been defined as the inverse rate of change of the state of dispersion of the disperse phase (Groves, 1970).

Emulsion instability can be caused by flocculation, coagulation, coalesence and molecular diffusion. Flocculation and coagulation both describe the combination of droplets to form into three-dimensional clusters. However, floccules can usually be redispersed by shaking whereas coagulates cannot. Coalescence is the union of two droplets into one. Molecular diffusion, also known as Ostwald ripening, involves the growth of the largest particles at the expense of the smallest. It is due to an effect first noted by Kelvin (1871), namely smaller droplets have a higher vapour pressure than larger ones. The effect is generally significant for droplets of less than 1 micron in diameter.

Breaking of an emulsion involves the two consecutive steps of flocculation or coagulation and coalescence. In its extreme, it involves separation of a layer of the disperse phase.

Creaming occurs when the droplets move due to a density difference between the 2 phases, usually caused by gravity but sometimes by electrostatic or magnetic fields. The rate of creaming follows the Stokes equation at low dilutions. At higher dilutions the qualitative

dependence of creaming rate on the variables should be maintained (Garratt 1962; Greenwald 1955). Creaming itself does not constitute breaking of an emulsion although the tight packing of droplets during creaming makes the processes involved in breaking so much easier.

1.4.1 Assessment of Emulsion Stability

To assess emulsion stability, any property of the emulsion that changes with time may be selected. Each property will change with time at a different rate (Groves 1970), making comparison of emulsions difficult. In fact, the choice of a suitable parameter for the comparison of different emulsions has been discussed several times (Vold and Groot, 1963; Tingstad, 1964; Becher and Griffin, 1965; Kenron, 1966). Lachman (1970) has suggested that emulsion stability may be measured by the "variation of the distribution of sizes of the dispersed droplets with time".

Many methods have been used to measure emulsion stability; some of those relevant to the present work are discussed below.

1.4.1.1 Phase separation

The volume of free disperse phase is used to indicate stability. No specialised equipment is needed but the method is of little use as only grossly unstable emulsions can be assessed in this way (Zografi, 1970). This is because the separated oil is difficult to detect at first.

1.4.1.2 Particle size analysis

Groves and Freshwater (1968) and Walstra et al (1969) have reviewed the methods for emulsion particle size analysis. Optical microscopy seems

to have been the most widely used method but Coulter counter, light reflectance or scattering methods, electron microscopy, sedimentometry and surfactant adsorption have also been used. It is concluded that no one method is adequate to cover the wide range of diameters found in emulsion systems, especially in the sub-micron range.

Experimental data from particle size analysis is usually expressed as the number of droplets in a number of size ranges. This can then be represented by a histogram or frequency distribution (Becher, 1965). Berkman (1935) found that progressive changes in the droplet size distribution could be followed and used as an index of stability. Elworthy and Florence (1967 a,b,c), however, concluded that comparison between emulsions was difficult even when the size distributions were of the same general form. More recently, Lachman (1970) has suggested that emulsion stability may be determined by the variation of the droplet siz distribution with time.

Herdan (1960) noted that many physical systems appeared to obey standard distribution functions, especially the normal or logarithmic-normal distributions. Both of the functions can be represented mathematically by straight line plots (Gaddum, 1945) and from these can be derived the two parameters which uniquely define the distribution; the mean and the standard deviation. The log-normal distribution with its logarithmic scale of diameters is very useful because of the wide range of diameters encountered in some emulsions. Rajagopal (1959) has derived this form of the distribution function from a consideration of the process of emulsion formation. This has been confirmed experimentally by Shotton and Davis (1968) and Davis and Smith (1976). Some slight deviations from the log-normal distribution were reported by Rowe (1965) and Hallworth and Carless (1972) for emulsions stabilised by sodium

dodecyl sulphate.

Many empirical functions have been proposed to fit experimental size distributions of emulsions e.g. Jellinek (1950), Rajagopal (1959), Groves and Freshwater (1968). For data significantly deviant from the log-normal distribution, Rowe (1965) has suggested the use of a "Polydispersity ratio". This is the ratio of the mass median diameter to the number median diameter: 50% of the drops by weight or number have a value greater than the appropriate median.

One of the most popular ways of characterising a particle size distribution is the use of a mean diameter. A number of different mean diameters can be calculated from the experimental data (Becher 1965; Sherman 1968) but opinions differ as to the usefulness of these. Sherman (1968) has suggested that the choice of mean diameter should be related to the property under investigation. For example, if the total surface area for a given volume concentration of disperse phase is required, then the mean volume-surface diameter should be used whereas the mean volume diameter may be more appropriate if viscosity at a high rate of shear is being studied. Because there are so many different diameters, comparison of results is made difficult unless the diameter is specified. With data following certain types of distribution, especially the log-normal, conversion from one diameter to another is possible (Herdan, 1960). This enables direct comparisons to be made.

Use of the distribution by weight or volume often provides useful information (Mahrous and Lemberger, 1968). Total reliance on this distribution can be misleading as it is very sensitive to small numbers of large droplets. Knoechel and Wurster (1959) showed that over 200 days the arithmetic mean diameter, measured every 30 days, increased steadily but the volume-surface and weight mean diameters showed an initial

decrease followed by an increase.

Another popular method of characterising an emulsion is the use of specific interfacial area (S). This is the area per unit weight or volume of disperse phase. Cooper (1937) reported the application of this parameter. It was found to be less sensitive to sampling errors than the mean diameters. King and Mukherjee (1939) used the specific interfacial area to study emulsion stability. They found that, after initial rapid coalescence, the specific interfacial area decreased linearly with time and was proportional to the value of S at time 0. The reciprocal of the rate of the decrease could be used as a coefficient of stability. Similar results have since been reported by several other workers (Jellinek and Anson, 1950; Lawrence and Mills, 1954; Mullins and Becher, 1956 a,b; Knoechel and Wurster, 1959).

Hill and Knight (1965) have developed a theory of coalescence for uncharged spheres of various sizes. This predicts that the reciprocal of S will increase linearly with time, and was tested with the results of earlier workers (King and Mukherjee, 1939; Lawrence and Mills, 1954). Further support was provided by Elworthy and Florence (1967). They found that emulsions stabilised by non-ionic surfactants obeyed the theory although an exponential decrease in S with time was found by Lotzar and Maclay (1943) in emulsions stabilised by gums.

1.4.1.3 Surfactant adsorption

It is possible to calculate the total interfacial area from an analytical estimation of the amount of surfactant adsorbed by the emulsion droplets. The area of interface occupied by each surfactant molecule is required and the average droplet diameter can be calculated from the volume of oil (Cockbain, 1954; Vold and Groot, 1962; Vold and Mittal, 1972).

No information on the particle size distribution can be determined. Also the molecular interfacial area used is not determined for the emulsion under study and so is subject to error.

1.4.1.4 Droplet concentration

This involves the accurate dilution of an emulsion and microscopically counting the number of drops in a known volume. The results may be expressed as the number of drops into which a unit volume of oil is divided or as the droplet concentration in the emulsion. The method was developed by Smith and Grinling (1930) and later modified by Cockton and Wynn (1952). From the first order decay of droplet number with time, Van der Tempel (1953 a,b,c) calculated the rate constant and developed a kinetic theory of coalescence. This theory has been considered by a number of other workers (Elworthy and Florence, 1969b; Hallworth and Carless, 1972; Srivastava, 1964). Elworthy and Florence (1969b) concluded that rates calculated in this manner were more useful for comparison of different systems than size distributions. Use of this parameter is more sensitive, e.g. a 10% change in interfacial area is comparable with a 27% decrease in droplet number (Van der Tempel, 1953b). Some systems have shown a non-linear decrease in droplet-number making the rate constants difficult to measure accurately and of doubtful significance (Hallworth and Carless, 1972, 1973).

1.4.1.5 Accelerated Stability Testing of Emulsions

The purpose of accelerated stability testing is to provide stability data in a much shorter time than that taken by merely storing it on the shelf. For this procedure to be valid, it must only accelerate the process of instability and it should not alter this process.

Accelerated stability testing of emulsions has been reviewed by Groves (1970) and Sherman (1971).

Two main stresses are used to accelerate stability - temperature stress and centrifugal stress. Three methods of inducing temperature stress are used - exposure to elevated or low temperatures and temperature cycling.

Exposure to elevated temperature involves storing an emulsion in an oven maintained at a temperature higher than the normal storage temperature. Stability is assessed by the rate of appearance of separated disperse phase.

Bennett et al (1968) state that "an increase of 10°C in the temperature is considered to double the rate of most reactions. Therefore, three months at 45-50°C is equivalent to one year at 20-25°C for many systems". This may be true but it must be proven that the higher storage temperature merely accelerates the mechanism of instability which operates at the lower temperature. Bennett et al (1968) have stated that "heating lowers the viscosity of the external phase of an emulsion and may cause discontinuities in the film of emulsifying agent. It also changes the solubility balance of the surfactant and facilitates chemical reactions". If this is so, heating does not merely accelerate the normal processes of instability. The emulsion may even invert at the higher temperature due to the altered pattern of emulsifier distribution. This has been further illustrated by the work of Shinoda (Shinoda and Arai, 1964; Shinoda and Saito, 1969).

Exposure to low temperature has been more widely studied due to its application in the refrigeration of milk products. This can cause changes in the solubility pattern of the emulsifier.

When an O/W emulsion is frozen, water crystals in the continuous

phase push the oil droplets more closely together (Young, 1934). This increases the electrolyte concentration causing supercooling of the water and more ice crystals (Lebeder et al, 1962). The pressure of the ice crystals can cause the oil droplets, if still fluid, to elongate and flatten, increasing the area of contact. The lipophilic portions of the emulsifier molecules lose their mobility because of the surrounding ice. The hydrophilic segments are simultaneously dehydrated due to freezing out of water. Thus, on freezing, the droplets are forced together and the interfacial film is weakened. The freezing rate is also important and determines whether a system will recover its original form on thawing.

Temperature cycling involves exposing the emulsion to alternating high and low temperatures. A useful measure of emulsion stability is the number of freeze-thaw cycles the emulsion can withstand, before cracking, under standardised conditions. The factors involved are not fully understood but may well involve a combination of the features of both high and low temperatures. The test is considered to be a more realistic stress than the two preceding ones as products are more likely to be stored under fluctuating conditions.

Centrifugal stress is a widely used technique in emulsion stability testing. Low speeds of up to 3600 rev/min have been used in the dairy industry to accelerate creaming (Merrill, 1953). Cockton and Wynn (1952) used higher speeds of 20,000 rev/min and found that the logarithm of particle number decreased linearly with time. Most emulsion systems are too stable to be affected by this low stress and so the ultracentrifuge has been used for this purpose (Vold and Groot, 1962, 1963, 1964 a,b; Garratt, 1964; Rehfeld, 1964). The method only provides information on the coalescence process. The centrifuging emulsion is in a different physical state from a system under gravitational stress. It is therefore unlikely that a centrifugal method can be used to determine the ageing behaviour of emulsions under normal conditions, especially if the rate-

determining step is flocculation.

The technique may be used as a means of investigating the interfacial barrier around emulsion droplets. By observing the amount of free oil at varying speeds, the pressure required to cause coalescence can be obtained. This gives a measure of the strength of the interfacial barrier (Smith and Mitchell, 1976).

1.4.1.6 Coalescence of a plane interface

The coalescence of single oil or water droplets with the appropriate bulk phase at the oil-water interface has been studied to assess emulsion stability. The method involves measuring the time the droplet is at the interface. Generally the longer the droplet takes to coalesce the more stable the emulsion should be. For instance, Ishida et al (1968) found the single droplet coalescence rates illustrated the stabilising effect of different non-ionic surfactants in emulsions. This appears to be the most useful application of this test - preliminary screening of emulsifiers for further testing. This was illustrated by the work of Davis and Smith (1975) who attempted to correlate the coalescence of a single droplet at a plane interface with bulk emulsion stability for a range of oils. This was not possible as the dominating influence on the single droplet coalescence was the density of the oil i.e. the less dense the oil the shorter the coalescence time. This effect would not apply if just one oil were used and so the method could be used for initial screening of emulsifiers to stabilise droplets of a particular oil.

1.4.1.7 Other methods

Changes in emulsion systems due to instability may be followed by a number of other methods e.g. creaming rate or optical properties

i.e. turbidity, light reflectance (Kennon, 1966; Groves and Freshwater, 1968). However, such parameters are considered of little use because they only measure gross changes in emulsions.

A study of the dielectric properties of an emulsion system can provide information on the changes occurring in an emulsion during its breakdown. This subject has been reviewed by Hanai (1968). The main disadvantage of this method of characterising emulsions is that it can only be used for emulsions of low conductivity i.e. water-in-oil emulsions.

It is possible to follow changes in emulsions with time by following changes in their rheological properties. This is a very sensitive method, capable of detecting very early changes in the state of dispersion whether they are the result of flocculation or coalescence. The only problem then lies in specifying the change that has been measured. Sherman (1963b) studied the effect of particle size on viscosity and was able to predict the viscosity changes in a series of emulsions, both W/O and O/W, from the changes in particle size distributions. Groves (1970) has suggested the use of shearing stress to accelerate instability. However, a problem with this method would be assessing the degree of change or instability. This may explain why this method has not been used although some emulsion systems are designed to breakdown under shearing stress e.g. cutting or rolling emulsions. Micro-electrophoresis may be used to study the surface charge of particles. Surface charge will affect the flocculation rate rather than the rate of coalescence and so will only correlate with emulsion stability if flocculation is rate determining. Jackson and Skauen (1962) found that the electrophoretic mobilities of O/W emulsions prepared from a variety of oils showed some relationship to emulsion bulk stability both at room temperature and at 40°C. Dorle and Rambhau (1972) reported a correlation between Zeta potential and mean globule diameter during ageing. Rambhau (1971) et al found an apparent correlation

between Zeta potential and mean globule diameters during accelerated stability testing of emulsions.

1.4.2 Maintenance of Stability

As stated in Section 1.1, emulsions possess a minimal stability. Unstabilised emulsions coalesce rapidly e.g. King (1941). The driving force for coalescence is the interfacial free energy, and so emulsions may be stabilised by the addition of surface-active agents (surfactants) which concentrate at the interface. The main types of stabilisers used are:

(a) Amphipathic organic compounds such as detergent molecules; these contain hydrophobic and hydrophilic groups.

(b) Macromolecules such as proteins, polymers and polyelectrolytes.

(c) Solid particles partially wetted by both phases.

Cheeseman and King (1940) showed that simple electrolytes can stabilise emulsions but this effect is very slight and is of little practical use.

The exact mechanism of this stabilisation is not fully understood and will depend on the chemical nature of adsorbate and adsorbent (Florence and Rogers, 1971a). The stabiliser may:

(i) decrease the free energy of the system.

(ii) form a physical barrier between droplets.

(iii) affect the electrostatic charge of the dispersed particles.

1.4.2.1 Stabilisation by Surface-Active Agents

1.4.2.1.1 Free Energy Decrease

The adsorption of surface-active agents at the O/W interface reduces the interfacial tension, leading to a reduction in the interfacial

free energy of the system. This was considered to be largely responsible for emulsion stabilisation (Becher, 1965). However, King (1941) found that a low interfacial tension was not, in itself, sufficient for emulsion stability. It has also been reported that emulsions having the same interfacial free energy can have widely different stabilities (Florence and Rogers, 1971a). In fact, even a low interfacial tension can represent considerable free energy resulting in coalescence (Becher, 1962). Lawrence (1952) suggested that a low interfacial tension could destabilise an emulsion due to the reduced tendency of the droplets to remain spherical. It is now generally accepted that reduced interfacial tension indicates interfacial adsorption but it is the nature of the adsorbed interfacial film that determines stability.

1.4.2.1.2 Interfacial Barrier

After two particles have approached to a distance less than the separation corresponding to the potential energy maximum, i.e. when they have coagulated, they draw together due to Van der Waals forces until short range repulsive forces dominate. For emulsion droplets the situation differs as there is some flattening of the drops at small separations. A thin circular film of continuous phase separates the drops. The radius of this film reduces the drainage rate of the continuous phase from between the drops when compared to rigid particles. Coalescence occurs when the film ruptures. Emulsion stability depends on the ability of the adsorbed emulsifier to slow the drainage of this film of continuous phase from between the droplets and its resistance to desorption, displacement, penetration or wetting by the disperse phase.

It is now believed that the interfacial barrier represents a combination of steric, viscous and elastic properties which depend upon the properties of the emulsifier (Florence and Rogers, 1971a).

The presence of the emulsifying agent may present a physical barrier around droplets which enables the droplets to collide or lie in close proximity without coalescing. The earlier studies of adsorbed films (Bancroft, 1915; Harkins, Davies and Clarke, 1917; Fischer and Harkins, 1932), directed toward understanding emulsion type and inversion, regarded the film as a purely mechanical barrier. The stabilisation by finely divided solids and some macromolecules has been assumed to be due to the rigidity of the film (Kitchener and Mussellwhite, 1968). The presence of rigid interfacial films of macromolecules can be demonstrated by withdrawing aged pendant drops of disperse phase into a syringe. Crinkled films appear because of the reduction of surface area in contact with solution (Shotton and White, 1963; Strassner, 1968): Becher (1962) has suggested that any closely packed adsorbed films will give rise to some degree of steric stabilisation. Nevertheless, it is unlikely that surfactants, particularly ionic surfactants, derive much of their stabilising properties from mechanical effects. In fact, Sonntag, Netzel and Underberger (1970) have stated that "in monomolecular adsorbed layers of surfactants, agreement between coalescence stability on the one hand and the mechanical properties..... on the other, would occur only in a few selected instances". A rigid film may not be sufficient to stabilise an emulsion. Elworthy et al (1971) found that hexadecanol enhanced the stability of a nonionic surfactant-stabilised emulsion of chlorobenzene. Hexadecanol alone produced a rigid interfacial film and poor emulsion stability.

A viscous layer around emulsion droplets may prevent thinning of the interfacial film and displacement of the adsorbed surfactant molecules during the collision or contact of two droplets (Davies and Rideal, 1961). Many macromolecular films doubtless exert their stabilising effect by a

combination of viscous and mechanical properties (Kitchener and Mussellwhite, 1968; Srivastava, 1964; Cumper and Alexander, 1950); Blair, 1960). The most important properties of the film are probably its permanence and coherence (Kitchener and Mussellwhite, 1968). However, the precise role played in emulsion stability by interfacial viscosity is poorly understood because it is difficult to measure the relevant properties directly using the presently available equipment (Becher, 1965; Kitchener and Mussellwhite, 1968).

Blakey and Lawrence (1954) demonstrated that a viscous film is not necessary for effective emulsion stabilisation but Sherman (1973) has questioned the use of separated disperse phase in this study as a true measure of coalescence rate.

Kitchener and Mussellwhite (1968) subscribe to the opinion that interfacial viscosity is not a significant factor in emulsions stabilised by surfactants. They argue firstly that a high interfacial viscosity is rarely encountered, except with macromolecular adsorbed films, and secondly that an adsorbed surfactant tends to immobilize the droplet surface and minimize surface flow when two droplets collide.

When two droplets in a liquid environment approach one another, the faces of the droplets are depressed while the medium is squeezed out from between them. Thus the place of closest approach of the two drops is on the line of centres and in an unstabilised system,coalescence will occur at this point as soon as the intervening liquid has drained away. In the presence of a surfactant, the time required for coalescence to occur is greatly increased. This is because a stable layer of continuous phase, or lamella, forms between the flattened surfaces of the droplets. It has been suggested that a "stable" lamella may remain at equilibrium thickness between aggregated or creamed emulsion droplets (Kitchener and

Mussellwhite, 1968).

For lamellae to exist, there must be an element of surface elasticity, since a parallel liquid film cannot arise from attraction and interfacial forces only. Surface elasticity results from a non-uniformity of interfacial tension which can only occur in the presence of an adsorbed surfactant (Kitchener and Mussellwhite, 1968). Florence and Rogers (1971b) have proposed that many emulsion stabilisers do not act by maintaining thick liquid films between droplets but through the stability of thin liquid films due to elasticity. It was suggested that the behaviour of this film, probably the rate of thinning, determines the probability of coalescence.

1.4.2.1.3 Electrical Stabilisation

The theory describing the stabilisation of dispersions by electrostatic forces was first developed by Derjaguin and Landau (1941) and Vervey and Overbeek (1948). It is known as the D.L.V.O. theory or the theory of the electrical double layer. This was developed for lyophobic sols but can be equally applied to emulsions with few alterations.

The electrical double layer consists of a surface charge on the particle and a compensating counter-ion charge accumulated in the liquid, in the neighbourhood of the particle surface. The counter-ions are electrostatically attracted by the oppositely charged surface. However, they also tend to diffuse away from the surface into the bulk solution down the concentration gradient. The result of these two competitive forces, electrostatic attraction and diffusion, results in an equilibrium distribution of counter-ions in which their concentration gradually decreases with increase distance from the surface. The diffuse nature of the counter-ion distribution was recognised by Gouy (1910). It is there-

fore known as the Gouy or diffuse layer. It does not only involve adsorption of counter-ions but ions of the same sign as the surface charge, co-ions, are electrostatically repelled. The diffuse layer therefore involved adsorption of counter-ions and negative adsorption of co-ions. From electrostatic and diffusion theories, it is possible to calculate, as a function of the distance from the surface, the exact distribution of counter- ions, co-ions and the average electrical potential with respect to a point distant from the surface.

The above description of the double layer is only relevant to a single particle. To determine the effect of the double layer on dispersion stability, the interaction of the double layers of two particles as they approach each other must be considered. When two particles approach each other, two long-range forces are produced from the interaction of their double layers: electrostatic double layer repulsion and van der Waals attraction. These long-range opposing forces are of entirely independent origin and so can be evaluated separately. It is the balance of these forces which governs the electrostatic stabilisation, or otherwise, of a dispersion.

The electrostatic double layer repulsion arises from the interference of the Gouy layers as two particles approach each other. This interference changes the distribution of the ions in the double layer. This involves an increase in the free energy of the system and so work is required to bring about these changes, leading to a repulsion between the particles. The variation of the work, or repulsive energy, is plotted as a function of distance a potential curve is obtained. The repulsive potential deceases roughly exponentially with increasing particle separation.

Addition of electrolyte to a particle dispersion compresses the

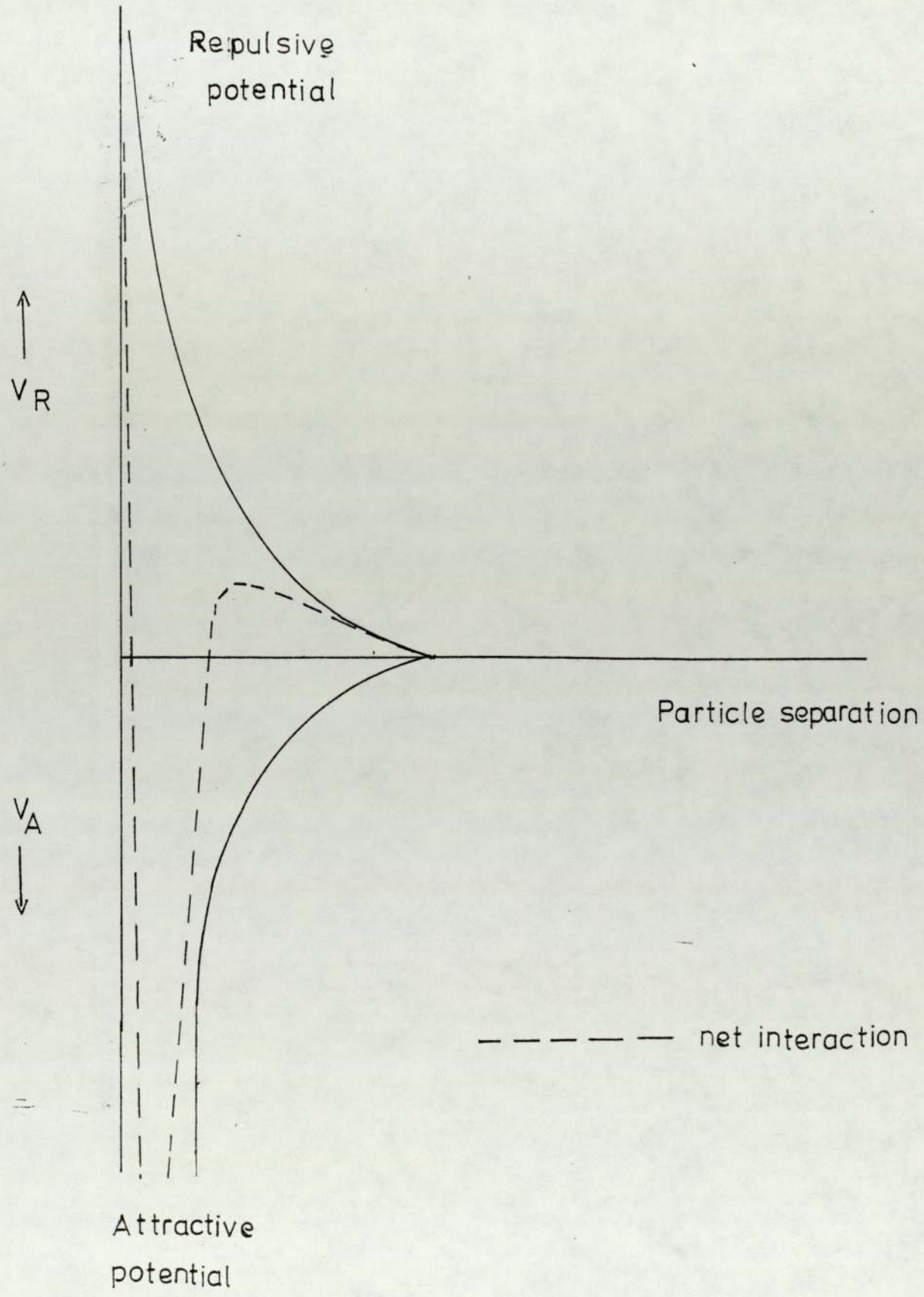
diffuse counter-ion layer toward the surface. Particles with a compressed diffuse layer can approach more closely before the diffuse layers begin to interact. This means that the range of repulsion is reduced.

General van der Waals forces are attributed to charge fluctuations in atoms. This results in an attraction between mutually induced dipoles in interacting atoms. In dispersions, this attraction is of comparable range and magnitude to the repulsive forces. The attractive energy can be expressed as a function of the distance from the surface.

It is therefore possible to determine the effect of particle separation on both repulsive and attractive forces. So the net force on two approaching particles can be found. This is demonstrated in Figure 1.5. The repulsive and attractive forces are shown; also the result of their interaction. It may be seen that the curve for the net interaction shows a deep minimum at short particle separations and a maximum at greater particle separations. The net interaction curve also takes account of some short-range repulsive forces e.g. Born repulsion. The maximum in the net interaction curve causes the electrostatic stabilisation of the dispersion. It acts as a "barrier" over which the particles must pass before destabilisation can occur. The size of the barrier is measured in units of kT (where k = Boltzmann constant; T = absolute temperature). To ensure reasonable stability of a dilute dispersion, the energy barrier should be 15kT. For a more concentrated dispersion, a volume of 25kT may be necessary.

The D.L.V.O. theory described was originally developed for lyophobic sols and requires some alteration for emulsions. Electrostatic instability results in flocculation or coagulation. For a suspension, this is the end of the instability process whereas for an emulsion it can lead to coalescence.

Figure 1.5 Electrical double layer interaction between 2 charged colloidal particles.



The principal difference between a solid particle and an emulsion droplet is that a droplet may possess a double layer on both sides of the interface but this does not appear to have an appreciable effect on electrostatic stabilisation of emulsion droplets.

Workers in the field have tended to assume that W/O emulsions could not be stabilised by electrostatic forces. Albers and Overbeek (1959), however, have pointed out that ionic concentrations of the order of 10^{-10} M are possible in benzene so that the double layer would be considerably extended. Assuming a surface potential of 25mV, an energy barrier of 15kT is present. This would normally impart appreciable stability to the emulsion but the experimental results prove otherwise.

1.5 Transport Processes

Many of the properties of multiple emulsions involve transport of materials across a thin oil film. For instance, instability in multiple emulsions is manifested in transport of the internal aqueous phase into the continuous phase. Drug release from multiple emulsions involves passage of the drug molecules from the internal aqeuous phase into the continuous phase via the oil phase. This section will first consider transport processes in several systems which may be considered analogous to multiple emulsions. Then, transport in emulsions will be considered, considering both studies using emulsions as model systems for solute transport and drug release from emulsions.

1.5.1 Transport processes in specific systems.

1.5.1.1. Liquid Membrane.

Cahn and Li (1974) considered the separation of substances using liquid membranes. The general equation governing the transport across

the oil phase was:

$$\frac{dN}{d\theta} = Dx \text{ area } x \frac{\Delta c}{\Delta x}$$

where $\frac{dN}{d\theta}$ is the quantity of material across a given area of membrane per unit time,

Δc is the concentration gradient of permeating species across the membrane,

Δx is the membrane thickness, and D is the diffusion coefficient of the permeating species. They suggested that this equation could describe two mechanisms of separation across the membrane. The first mechanism requires one of the permeating species to have a higher permeability through the oil phase. This substance will concentrate in the droplet while the substance with the lower permeability will be left in the continuous phase. The second mechanism involves a selective chemical reaction inside the droplet. This keeps the concentration gradient of the reacting species high while the concentration gradient of the non-reacting species soon levels out. This assumes that the non-diffusing reacting species and reaction product cannot pass through the membrane. Cahn and Li (1974) then used the equation above to derive a permeation rate constant from experimental data for both phenol and ammonia removal.

The equation above was derived from consideration of a liquid membrane as a flat surface. Li and Shrier (1972) considered transport into a spherical droplet using the following assumptions:

1. All phases are well mixed.
2. The process is mass transfer limited in the membrane phase.
3. Diffusion through the oil phase occurs only into the outer

droplets. This is equivalent to a droplet containing only one large droplet as shown in Figure 1.6.

4. The oil droplets remain intact once formed.

With these assumptions, transport can be described by a standard diffusion equation (Crank, 1956):

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r}\right) + \frac{2}{r}\left(\frac{\partial c}{\partial r}\right)$$

where c = concentration,

r = radius,

t = time,

and at $r = R$, $c = 0$

$R > Ro$, $c = c_\infty$

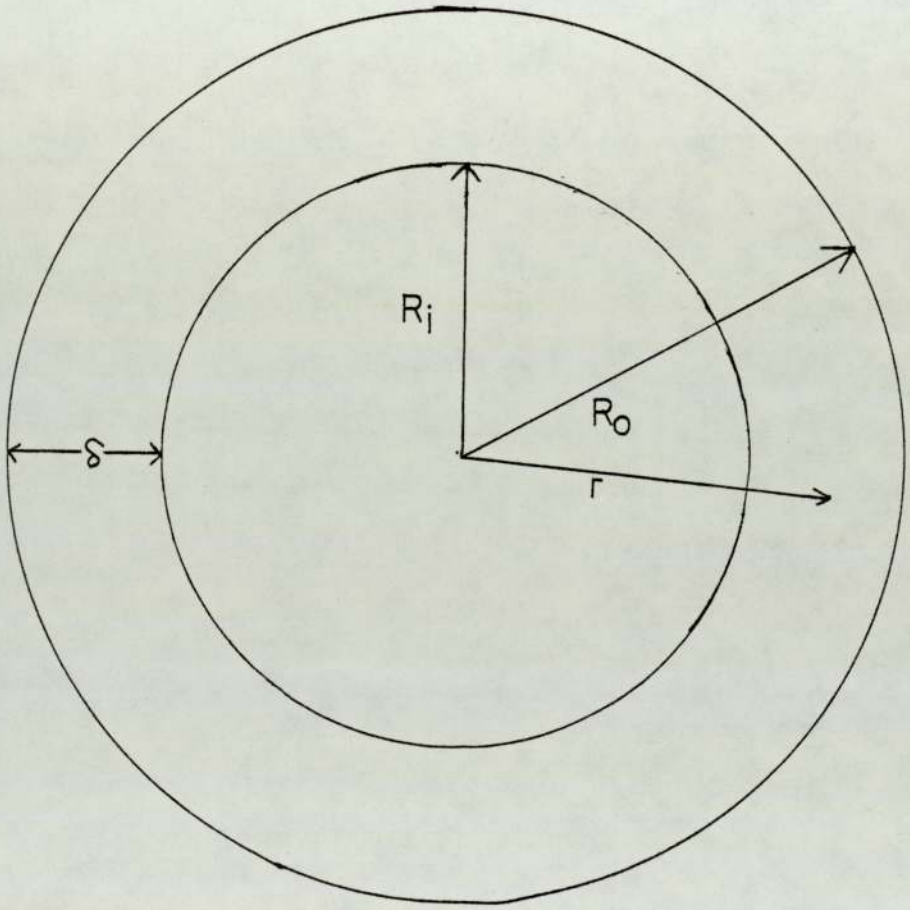
$r = Ro$, $c = Kc_\infty$

Matulevicius and Li (1975) have solved this equation by assuming a particle size distribution, yielding an equation which fitted their experimental data very well. This shows that the assumptions outlined above are valid for their system.

1.5.1.2. Artificial Cells.

Chang and Poznansky (1968) used a stop-flow apparatus to determine the permeability of artificial cells to a range of solutes e.g. urea, sucrose, aspirin. Permeability constants were all approximately 1×10^{-4} cm/Sec. The permeability of tritiated water could not be measured as it equilibrated across the membrane too quickly for the apparatus involved.

Figure 1.6 Model of a liquid membrane globule.



1.5.1.3 Bilayer Lipid Membrane

Two methods have been developed for the determination of the water permeability of BLMs - radiotracer and osmotic. In the case of the radiotracer exchange experiments, the permeability of the BLM was calculated from the equation

$$K = \frac{\Delta C_1 \; V_1}{A \; \Delta t \; (C_2 - C_1)}$$

where ΔC_1 is the change in radiotracer concentration in compartment 1 (Volume = V_1) after time t. A is the membrane area. C_2 and C_1 are average isotope concentrations in compartments 1 and 2 during the time interval Δt.

The permeability of the BLM can be calculated from the osmotic flux experiment using this equation:

$$K = \frac{dV}{dt} \cdot \frac{1}{A \pi}$$

where $\frac{dV}{dt}$ is the volume flux across the membrane, A is the membrane area and π is the osmotic pressure across the membrane. The osmotic pressure due to the impermeant solute can be calculated:

$$\pi = RT \; (g_2 m_2 - g_1 m_1)$$

g and m are the osmotic coefficients and molalities of the solute on the two sides of the membrane. R is the gas constant and T is the absolute temperature.

The equations shown above have been used to calculate the permeabilities of BLMs to water by the 2 different methods. The values obtained from the methods differ and this has been attributed to unstirred

layers present in the isotopic exchange experiments, as has been described earlier (Section 1.3.7). However, the value of performing measurements of water permeability coefficient at a single temperature is generally recognised to be limited (Tien and Ting, 1968).

Price and Thompson (1969) determined the variation of permeability with temperature over the range 7 to 49° C. for BLMs of two different compositions. The results followed a simple Arrhenius equation,

$$Pos = A \exp(-Ea/RT).$$

This enabled the activation energies to be calculated for the 2 different BLMs. Although they had different permeabilities over the whole temperature range, the activation energies were found to be very similar, i.e. 12.7 and 13.1 Kcal/mole. Several models for the mechanism of water transport across BLMs were considered. Two of these involved transport through pores and the calculated activation energies were 4.6 and 4 to 16 K cal/mole. The third model was a solubility - diffusion model. As its name suggests, water moves across the membranes by dissolving in it and diffusing across. The calculated permeability for this model was 10.5 to 12.4 Kcal/mole. The authors concluded that transport of water across a BLM could be best represented by the solubility/diffusion model.

1.5.1.4. Liposomes.

Liposomes can be formed in a wide range of aqueous electrolyte solutions and this of course results in the inclusion of the electrolyte on the inside of the liposome. By placing these liposomes in solutions of different osmotic strengths it is possible to cause the liposomes to shrink or grow, according to the direction of the osmotic gradient.

Bangham et al (1967) described such an experiment. The changes in

volume during swelling or shrinking were followed by a turbidimetric method in which the extinction at 450nm was measured. The initial rate of change of extinction during swelling or shrinking could be used to calculate the osmotic water permeability coefficient. This value is converted to a volume flux $\frac{dv}{dt}$, by means of calibration and inserted in the equation,

$$K = \frac{dV}{dt} \cdot \frac{1}{A\pi} \left(\frac{55.6 \times 22.4T}{273} \right)$$

where A is the area over which transport occurs and π is the osmotic gradient given by $\pi = RT(g_2M_2 - g_1M_1)$. The value obtained was 0.8 μ.sec which is low when compared to the value for BLMs of similar composition, i.e. 8 to 14 μ.sec . This is probably due to the multilamellar nature of the liposomes involved.

The above results were obtained with solutes which could not penetrate the liposome. This means that the liposome lamella is behaving as a semi-permeable membrane. Liposomes, however, are permeable to some solutes and so behave in a different manner.

When liposomes that have been prepared in a non-permeating solute are placed into a solution of a permeating solute of higher osmotic strength than the original solution, there is an initial shrinking as water is drawn out of the liposome. A minimum volume is reached and the time taken is related to the permeability coefficient of the external solute. The volume then begins to increase again as the external solute diffuses into the liposome down its concentration gradient. The growth in volume continues until the solute concentration gradient and the osmotic pressure outside and inside have equilibrated.

1.5.1.5. Cells

(a) Human erythrocytes.

In some ways, the behaviour of red blood cells is similar to that of liposomes, but both behave differently in the presence of solutes which can or cannot penetrate the erythrocyte membrane.

In the presence of an impermeant solute, erythrocytes swell or shrink in the direction of the osmotic gradient. The shrinking behaviour of erythrocytes in the presence of impermeant solutes has been studied by Sha'afi et al (1967). A stop-flow apparatus was used to follow the rapid changes occurring and its construction, performance and calibration are described in detail. Briefly, the equipment consists of two syringes, one containing the erythrocytes and the other the hypertonic electrolyte solution. By means of a motor, the syringes pass their contents through a mixing chamber and into an observation tube. Flow is stopped abruptly and the volume of the red cells was measured by the intensity of the light scattered at 90° to the incident beam. Using this technique, reactions taking a few milliseconds can be measured. The permeability of the red blood cell membrane was calculated. The thickness of the unstirred layer was found to be 5.5 μm.

The determination of the permeability of erythrocytes to a permeating solute was made by Sha'afi et al (1970). The solute used was urea and the apparatus was described above. In a suspension of erythrocytes in an isotonic solution, there is of course no shrinkage. When a permeant solute is added, water moves out of the cell due to the osmotic gradient and so the cell shrinks. At the same time, the permeant solute diffuses into the cell down its concentration gradient but this is opposed by solvent drag due to the water moving out of the cell. A

minimum volume is achieved when the volume of solute diffusing inwards is exactly balanced by the volume of water moving outward due to the remaining osmotic pressure gradient. This then reverses in direction due to the solute in the cell. Water re-enters the cell with the solute. The volume change ends when the solute concentration in the cell equals that in the medium and all gradients disappear. The permeability coefficient can be obtained from the minimum volume and the derivative of the rate of volume change at the minimum.

(b) Cell Cultures.

Ho et al (1972) have considered the use of suspension cultures of mammalian cells for the study of drug transport across membranes. They are of the opinion that, although they are an in vitro system, they are closer to the in vivo system than most other in vitro models. They consider that the flow into a cell can be modelled by considering it to be a sphere covered by a thin lipid membrane. This surrounds the aqueous interior of the cell, which contains the nucleus and other cytoplasmic bodies.

They considered the equations describing the transport of drugs into such a simplified cell under a number of different conditions:

1. Non-steady-state distribution in the heterogeneous cell interior.

2. Instantaneous equilibration in the heterogeneous cell interior.

3. Instantaneous equilibration in aqueous environment with slow simultaneous permeation of drug into the cytoplasmic bodies and nucleus.

For all three models, the steady-state flux of drug in the membrane was represented by:

$$-\frac{dY_{AQ}}{dt} = A \cdot \left\{ Y_{AQ} - \frac{Y_{IN}, b}{Ke,a\ Ke,b} \right\}$$

where Y_{AQ} is the total bulk drug concentration,

$Y_{IN-}b$ is the total drug concentration at the inner edge of the cell membrane,

Ke,a and Ke,b are the effective drug partition coefficients at the outer and inner edges of the cell membrane, respectively,

and A is a coefficient given by:

$$A = \frac{4\pi N a b Pe,M}{V_{AQ}} .$$

Here, N is the number of cells,

a is the radius of the cell,

b is the radius of the aqueous interior,

Pe,M is the effective membrane permeability coefficient,

and V_{AQ} is the volume of the bulk aqueous phase.

The authors were aware that, in the case of cells, membrane transport may be due to several processes operating concurrently, i.e. passage through the membrane phase or through pores and Pe,M is intended to account for these different processes.

It was found that in Models 1. and 2. the initial change in the bulk concentration could be described by the same equation:

$$\log Y_{AQ} = \log I - (A/2.303)t,$$

where I is the initial value of Y_{AQ}.

The relations are, of course, different at longer time intervals.

Turi et al (1972) investigated the use such cell cultures studying uptake of cholesterol into Burkitt lymphoma cells. It was found that uptake could best be described by the second model. Further, it was found that inclusion of foetal bovine serum in the cell culture medium reduced the rate of uptake. This was due to binding of cholesterol to

the bovine serum, reducing the amount of free drug available for uptake. The permeability coefficient was found to be inversely proportional to the serum concentration.

These results confirmed those of Rothblat et al (1966, 1968) who found that the presence of phospholipids decreased the absorption of cholesterol into cell cultures.

1.5.2 Transport processes in Emulsions

1.5.2.1. Model Studies

In a series of papers, Brodin and co-workers have investigated the rates of transfer of drug molecules between water and several organic phases (Brodin and Agren 1971, Brodin and Nilsson 1973, Brodin 1974, 1975, Brodin et al 1976).

Brodin and Agren (1971) described the method used in all the studies. A thermostatted glass column was filled with the "continuous phase". Drops of the "disperse phase" were formed by an automatic burette and passed through the column. The drop phase was collected after passing through the column and was continuously monitored using a UV spectro-photometer. The drug to be transported was placed in either the "continuous" or "disperse" phase. Transfer between drops and continuous phase was measured in both directions.

The transport of a solute between two immiscible liquid phases was considered and divided into three steps:

1. Solute is transported to the interface by diffusion and circulation.

2. In the interface, resolvation of solute molecules takes place.

3. Solute is transported from the interface to the bulk of the second phase by diffusion and circulation.

Of the steps described above, 1. and 3. are properties of the bulk phases and only step 2. is a measure of the transport of a molecule between 2 phases. Obviously, one can only study such transport if it is the rate-limiting step and for this to be so, the diffusion and circulation in steps 1. and 3. must be rapid. Also, the transport to or from a moving drop prevents the formation of unstirred layers present in so many diffusion experiments.

The rates of transport for several drugs between cyclohexane and water over a range of pHs were determined. A linear relationship between resolvation rate constant and partition coefficient was found. The resolvation rate constants from water to cyclohexane were seen to vary much more with the partition coefficients than the constants for the reversed process. This is because, for the wide range of structures studied, the possibility of hydrogen bonding is much greater in the water than in the much less polar cyclohexane.

Brodin and Nilsson (1973) applied the same technique to other organic liquids, namely benzene and chloroform. A linear relationship between partition coefficient and resolvation rate constant was found for both liquids. The resolvation rate constants from aqueous to organic phase varied much more with the nature of the organic phase than the rate constants in the opposite direction of transfer. This is due to the solubility of water in the organic phases studied. As the solubility of water increases, so the transferring molecule has to lose less hydrating molecules.

Brodin (1974) extended this technique to a range of more polar organic liquids. Ephedrine was studied in a range of alkanols. A linear relationship between partition coefficient and resolvation rate constant was again found. However, the resolvation rate constants

showed a greater variation and were smaller than in the other liquids studied. This was attributed to the larger variation in the mutual solubility of water and alkanols and by the stronger binding between solute and organic solvent. Several other drugs were studied in the 1-octanol-water system and the relationship between partition coefficient and resolvation rate constant was found to be about the same as in the cyclohexane-water system.

Brodin (1975) studied the effect of surfactants on the interphase transport described above. Phenylbutazone was chosen as a model and its transfer between cyclohexane and water was studied in the presence of surfactants — nonionic, anionic and cationic. In the transfer from an aqueous phase to an organic phase, the presence of the surfactant decreased the resolvation rate constant. This was attributed to a change in the area available to mass transfer. Another factor is the change in circulation within the drops caused by the surfactants. At low surfactant concentrations, a decrease in circulation was observed. At higher concentrations, this effect was reversed. This may be due to disruption of the surfactant layer. Alprenolol transfer from an aqueous to an organic phase was also studied in the presence of surfactants. For this substance, an increase in resolvation rate constant was seen. As the effect of decrease in droplet area was still in effect, this increase was even more remarkable. The increase was observed with all the surfactants studied and so was probably due to some property of the solute itself. As the effect was also seen with chlorpromazine, which is known to be surface-active, it was probably caused by an interaction between alprenolol and the surfactant at the interface.

The transfer in the opposite direction, i.e. from an organic to

an aqueous phase, has also been studied. Because transfer occurred from an organic phase, pH had no effect on the transfer rates and so it proved impossible to separate the observed transfer rate into resolvation rate and circulation rate constants. Also, the observed transfer rates were independent of both solute and surfactant and so it was concluded that the circulation rate constant was rate-limiting in the presence of surfactant.

Brodin, Sandin and Faijerson (1976) determined the transfer rates of methamphetamine and ephedrine for cyclohexane - and 1-octanol-water over a temperature range of 15 to 40° . This enabled the thermodynamic paramaters of the transfer process to be calculated once complicating factors, such as changes in mutual solubility of the solvents with temperature, had been accounted for. Entropies and free energies of activation were calculated by assuming a certain distance over which transfer across the interface takes place.

Yotsuyanagi et al (1973) have described a theoretical treatment of diffusional transport into and through an O/W emulsion with an interfacial barrier at the O/W interface. The purpose of the study was to examine the effect of interfacial resistance on interphase transport in emulsions. The model was described and the effect of various parameters on the flow of a solute into the emulsion was examined.

1.5.2.2. Drug Release.

Stark et al (1958) investigated the influence of surfactants on the release of ions from an emulsified ointment base. For this purpose, an apparatus was designed to simulate in vivo conditions. This consisted of a section of cellulose tubing attached to the apparatus so that leakage did not occur on contact with the circulating fluid. Cellulose

was found to give greater reproducibility. There was no indication of the correlation between these results and those which might be obtained when a living membrane was employed. The compounds incorporated into the ointment were radio-labelled sodium iodide and mercury nitrate. Maximum release was found in bases emulsified with 1% surfactant. Nonionic surfactants gave greater release than anionic or cationic surfactants.

Several authors have described the use of seeded agar plates to monitor the release from emulsions of bacteriostatic or bactericidal substances. Barber et al (1956) studied the release of emulsified mercuric oxide and iodine in this manner. Wood and Rising (1952) investigated the influence of emulsifying agents on the antiseptic activity of various topical preparations using this method. Rossmore and Moore (1966) determined the effect of partitioning of chloramphenicol between the disperse and continuous phases on its release using the seeded agar plate method. O/W emulsions were found to be more effective than W/O emulsions. Diffusion of coloured substances through agar gels has also been used to assess release from emulsions. For example, Lockie and Sprowls (1949) used such a method to follow release from W/O and O/W emulsions as well as grease and mucilage bases. The base was placed over a layer of the agar gel which contained a marker substance which reacted with the released species to give a colour. Patel et al (1961) compared the methods described above, namely seeded agar plates and colour-producing reactions and methods in which the diffusing substance is assayed directly e.g. radio-labelling. Not surprisingly, the direct measurement technique was both more sensitive and accurate.

Higuchi (1964) studied the release of dibutylphthalate from micron-sized emulsion droplets. The particle size distribution was followed

throughout the release study by means of a Coulter counter. The rate of release was found to be proportional to the droplet radius as predicted from theory.

1.6 Plan of Experimental Work.

Following an appraisal of the work described in Section 1.3.2 and summarised in Table 1.1, it was decided that a thorough investigation of all the formulation and processing variables, and their effect on multiple emulsion stability, was necessary before any work on drug release studies could be attempted. To this end, the following programme of experimental work was decided upon.

1.6.1 Formulation.

The formulation and processing variables studied were as follows:

1.6.1.1 method of manufacture — a number of methods were investigated and, from those available, the ultrasonic probe was chosen. This was then used to determine the optimum process conditions which were then followed.

1.6.1.2 Concentration and nature of the emulsifiers — a number of different emulsifiers were chosen and studied over a range of concentrations. Initially, Arlacel 83 and Tween 80 were used to "characterise" the ultrasonic probe. A number of other Tweens and Spans were studied, as well as other emulsifiers e.g. Pluronics, Emulphors.

1.6.1.3 Nature of the oil — Initially light liquid paraffin was used to provide a well-characterised oil. Later, the study was extended

to various vegetable oils, e.g. soybean oil.

1.6.1.4. Osmolarity of internal and external aqueous phases — the effect of altering these osmolarities on the nature and stability of the emulsions formed was investigated.

1.6.2. Stability measurement

A number of methods were used to evaluate the effects of the above variables on the emulsions formed and their stability:

1.6.2.1. Optical microscopy — at first, normal particle size analysis was used to investigate the initial particle size distribution of the emulsions formed and the effect of ageing on this. Later, the effect of altering the osmolarity on the particle size distribution and stability of the emulsions was followed.

1.6.2.2. Electron microscopy — the nature of the internal aqueous droplets could be assessed by the technique of freeze-etching. The technique was later used for particle size analysis of W/O emulsions.

1.6.2.3. Coulter counter — using this instrument, the effect of osmotic gradients on the particle size distribution and stability of multiple emulsions could be determined.

1.6.2.4. Radiotracer technique — by using tritiated water, the flow into and out of the droplets could be followed in the absence of osmotic gradients.

The above is a brief description of the experimental work performed

as part of this thesis. However, besides evaluating stability, it was realised that some of the results could have relevance to drug release from multiple emulsions. Furthermore, it was also realised that the work was of relevance to model membrane studies. These two topics will be considered in more detail below.

Chapter 2

Chapter 2. Materials.

2.1. Water

All water was single-distilled from an all-glass still.

2.2. Oils

All oils were used as received with no further purification.

The majority of emulsions were prepared using Light Liquid Paraffin BP (Fisons S.A., Loughborough). A smaller number of emulsions were prepared using vegetable oils:

cottonseed oil (Croda, Hull)

maize oil B.P. (Evans Medical, Liverpool)

arachis oil B.P. (Evans Medical, Liverpool)

Squalane, a synthetic oil was also used. This was obtained from British Drug Houses Ltd.

2.3. Emulsifying agents.

Table 2.1 lists the emulsifying agents used in this study and their suppliers.

Table 2.1.

Emulsifying agent.	Supplier.
Tween 80	Koch-Light Laboratories
Tween 20	Koch-Light Laboratories
Arlacel 83	Koch-Light Laboratories
Span 80	Koch-Light Laboratories
Span 85	Koch-Light Laboratories
Pluronic F68	Ugine Kuhlmann Limited.
Pluronic L61	Ugine Kuhlmann Limited.
Arlacel A	Sigma

Table 2.1. contd.

Atmos 300	Honeywill-Atlas
Myvacet 9-40	Kodak.

The emulsifying agents were used as received without further purification.

2.4. Miscellaneous materials.

Table 2.2 lists the other materials used in this thesis and their suppliers.

Table 2.2.

Material	Supplier.
Toluene (scintillation grade)	British Drug Houses Limited.
Triton X-100 (scintillation grade)	British Drug Houses Limited.
PPO	British Drug Houses Limited.
DMPOPOP	British Drug Houses Limited.
Formvar (polyvinyl formal)	British Drug Houses Limited.
tritiated water (100mC/ml)	Radiochemical Centre, Amersham.
tetrahydrofuran	Fisons Scientific Apparatus.
sodium chloride	Fisons Scientific Apparatus.
Polyfusors (isotonic saline)	The Boots Company.
"Arcton" 22	Imperial Chemical Industries.

Chapter 3

Chapter 3. Method of Manufacture of Multiple Emulsions

After initial experimentation with a number of methods, the ultrasonic probe was chosen. This was a Dawe Soniprobe, Model 7532A. The W/O/W multiple emulsions were prepared by a two-step emulsification process:

Step 1. A W/O emulsion was prepared by mixing the primary aqueous phase with the oil phase containing the lipophilic surfactant and shaking by hand to achieve a rough dispersion. It is at this stage that any additives to the internal aqueous phase must be added, e.g. osmolarity adjusters (sodium chloride, glucose, etc.), marker molecules (e.g. tritiated water, dyes, etc.), drugs. This dispersion was further emulsified using the probe. The tip of the probe was placed at a set depth beneath the surface of the liquid. This produced a W/O emulsion.

Step 2. The W/O emulsion was then mixed with the secondary aqueous phase containing the hydrophilic surfactant and re-sonicated. This produced a W/O/W emulsion.

The optimum depth of immersion was determined initially by adjusting the depth of immersion until a maximum reading on the output power meter was achieved. By means of a mark on the stand, all subsequent emulsions could be produced with the tip of the probe immersed to the same depth each time. Two such marks were necessary as the second emulsification involved the addition of further liquid, altering the depth of immersion.

All emulsions were emulsified on the maximum power setting of the probe and the degree of emulsification was varied by changing the time of exposure. The optimum time of exposure was determined by the various methods used to assess the emulsions in this study and details are included in the sections on the relevant techniques. However, times of one minute

and ten seconds were chosen for the primary and secondary emulsifications respectively. The emulsions were not cooled during the emulsification procedure which took place in a 250ml plastic beaker. Because of this the temperature rose during the primary emulsification. It was later found that the primary emulsification time could be decreased to thirty seconds without noticeably affecting the W/O or multiple emulsions so formed.

A typical emulsion is :

25 ml distilled water

25 ml 10% $^{w}/_{v}$ Arlacel 83 solution in light liquid paraffin — W/O

50 ml 2% $^{w}/_{v}$ aqueous Tween 80 solution — W/O/W

This signifies that 25 ml of distilled water were emulsified with 25 ml of 10% $^{w}/_{v}$ Arlacel 83 solution in light liquid paraffin to produce a W/O emulsion. This was then emulsified with 50 ml of 2% aqueous solution of Tween 80 to produce a W/O/W emulsion.

Chapter 4

Chapter 4. Optical Microscopy

4.1 Method

The microscope used was a Vickers M15c. Attached to this by means of a monocular tube was a Watson-Barnet 35mm microscope camera.

The procedure adopted to obtain photomicrographs was as follows. A sample of the emulsion was taken and diluted with the continuous phase. Some later experiments also involved the use of sodium chloride in the diluting fluid for osmotic flow determinations. In this case the diluted emulsion was left to equilibrate, usually for 15 minutes. The diluted emulsion was placed on a Hawksley haemocytometer cell of depth 0.1 mm and a cover slip was placed over the slide. Five minutes was allowed for the droplets to cream and the slide was examined. At least 4 fields of each emulsion were photographed. These were chosen at random. Also, any features of interest apparent from further examination of the slide were photographed.

The film finally chosen was 35mm Kodak Tri-X pan. The exposure for each photomicrograph was determined using a Vickers light meter. This was calibrated for the film to be used by photographing the same field over a range of exposure times under the same lighting conditions. The film was developed as described below and the negatives were examined. In this way, the best exposure time for that particular film could be determined.

The exposed film was transferred from the camera to the developing tank in the dark room. The film was developed in Kodak D76 developer diluted 1 in 2 with water for 12 minutes at 20°C. The developer was then removed and the film washed with tap water several times. The film was then fixed for 4 minutes in Ilford Ilfofix fixer. The fixer was then removed and the film was washed under running water for 30 to 60 minutes.

The film was then removed from the developing tank and hung to dry. When dry, the negatives were used to prepare prints. This was done by a Photographic Unit within the University.

The exact magnification was determined by photographing a stage micrometer at each magnification used. This was done for each film exposed. The exact distance between the lines on the micrometer was known, the distance between these lines could be measured on the print and so the magnification could be determined. At the highest magnifications possible on the microscope, the lines of the stage micrometer were too large for the distance between them to be measured accurately. However, the images at these high magnifications were very prone to the slightest vibration and were never really sharp, so this was not too grave a drawback. This procedure was adopted to allow for variations in the enlargement process rather than variations in the microscope.

The particle size distributions of the emulsions were obtained from the prints using a Zeiss TGZ3 Particle Size Analyser. This consists of a circle of light which is adjusted in size until it matches the emulsion droplet on the photograph. A footswitch is then pressed which increases a counter by 1 according to the size of the light spot. There are 48 such counters and the size of the light spot corresponding to each one is known and so it is simple to convert the spot size in millimeters to the droplet diameter in micrometers. The results were, in fact, analysed by computer. A computer programme was available which calculated the diameters from the raw data. It would also fit the data to the normal or Gaussian distribution and calculated mean diameters by number, length, area and volume as well as standard deviation, skew and several other parameters. The distribution by number and volume could be calculated. However, the particle size distributions for multiple emulsions do not fit

a simple normal distribution and so this part of the calculated data was only relevant to the single emulsions in this study. For multiple emulsions, the programme was only used to calculate the diameters from the raw data and the rest of the parameters were derived by hand.

4.2 Results

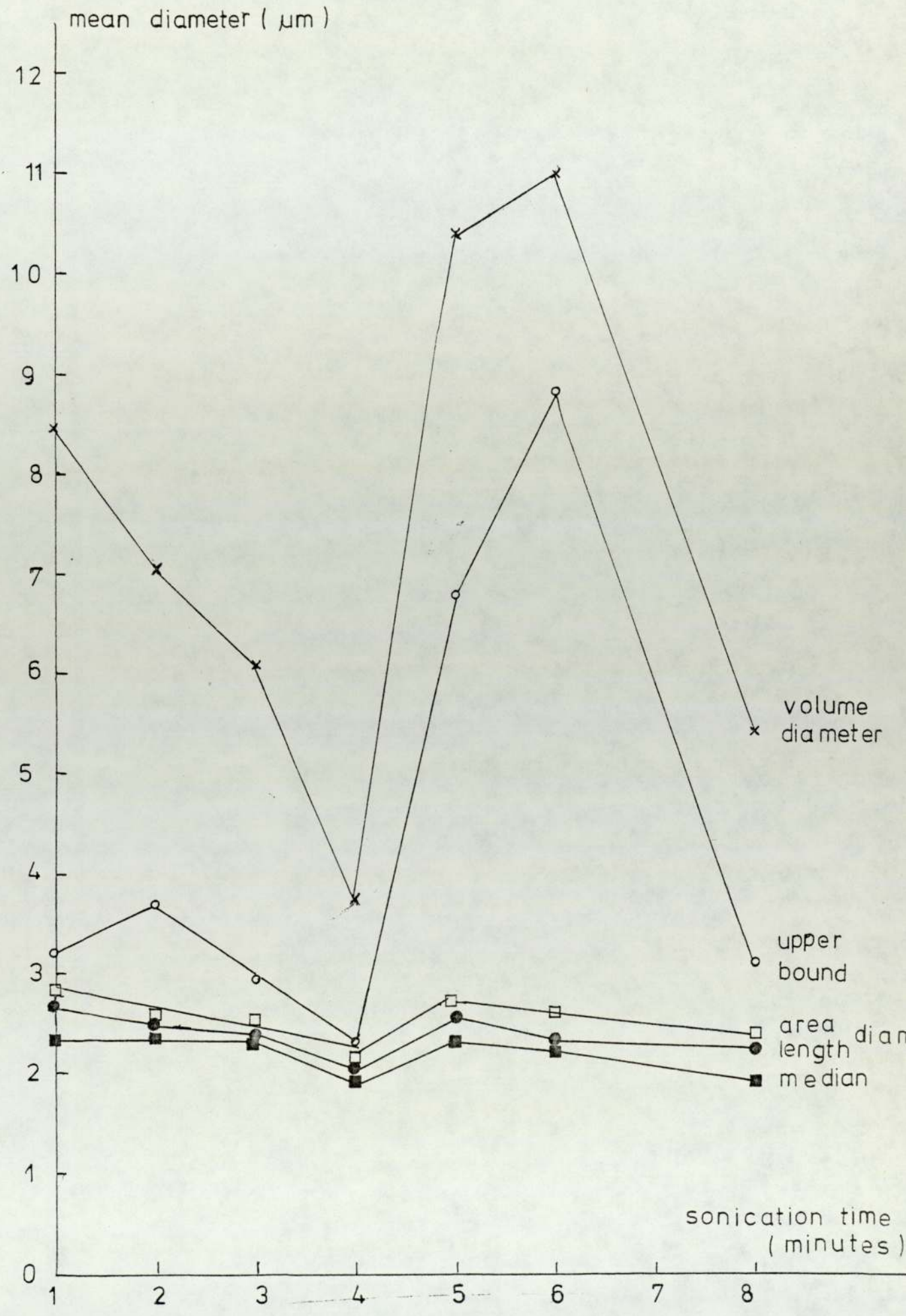
4.2.1 Particle Size Analysis

4.2.1.1 Evaluation of Processing Variables

The choice of the method of manufacture was described above (Chapter 3). Once this choice was made, it was necessary to investigate the effect of manufacturing conditions on the properties of multiple emulsions. This did not involve a full-scale determination of all the parameters affecting the final emulsion but rather took the form of a short-term investigation of these parameters. The purpose of this was to fix the conditions of manufacture so that emulsions having the same constituents had the same particle size distributions. In this way, one set of factors affecting the emulsions manufactured could be removed.

The first experiment undertaken determined the effect of manufacturing conditions on the particle size distributions of an O/W emulsion. With the limitations described above in mind, this was performed as follows. The power control switch was set to full and the probe was immersed to a set depth. The constituents of the emulsion were 50%$^{v}/_{v}$ light liquid paraffin B.P. and 50%$^{v}/_{v}$ of 2%$^{w}/_{v}$ Tween 80 solution. Figure 4.1 shows the results of this experiment. Here, the various mean diameters are plotted against the time of sonication. The values for all the mean diameters show a decrease until 4 minutes sonication and then increase. A decrease is then observed up to 8 minutes sonication which was the longest time studied. The shape of the mean diameter versus time of treatment is peculiar to ultrasonic emulsification and requires further

Figure 4.1 Effect of increasing sonication time on various parameters of O/W emulsions.



comment. During the emulsification, dispersion and coalescence occur simultaneously. The ultrasonic energy causes a breakup of the disperse phase, leading to dispersion, but as the emulsification proceeds, the dispersed droplets are given more energy until coalescence finally dominates and so the mean diameters start to rise. This explains the events in figure 4.1 up to 5 minutes sonication time. After this time, increasing amounts of free oil were observed in the emulsions and this could be due to coalescence of the longer droplets which would lead to a decrease in mean diameter. A further point to note is that while all the mean diameters displayed show the above-mentioned trends, the greatest effects are noticed in the plot of mean volume diameter against time of sonication. Another effect is also obvious from the calculated parameters. Among several statistical measures calculated from the data is the upper bound. This gives an indication of the upper limit of the particle size distribution. For the emulsions in figure 4.1, the upper bound decreases until 4 minutes sonication after which it increases. The 8 minute value is back to the original level. This shows that as the mean diameters decrease, so the "spread" of the distribution decreases and as the mean diameters increase so the "spread" increases again.

This exercise was then repeated for the multiple emulsions. However, particle size analysis of W/O emulsions was not available at the time, so the effect of varying the times of primary and secondary sonication on the properties of the final multiple emulsion was followed. This is illustrated in figure 4.2. which shows the effect of time of primary sonication on the particle size distributions of multiple emulsions. The distributions shown in figure 4.2. all show a break in the curve. The reason for this can be seen by referring to figure 4.3. This shows a typical photomicrograph of a multiple emulsion. Two types of droplets

Figure 4.2 Effect of primary sonication on the particle size distributions of W/O/W emulsions.

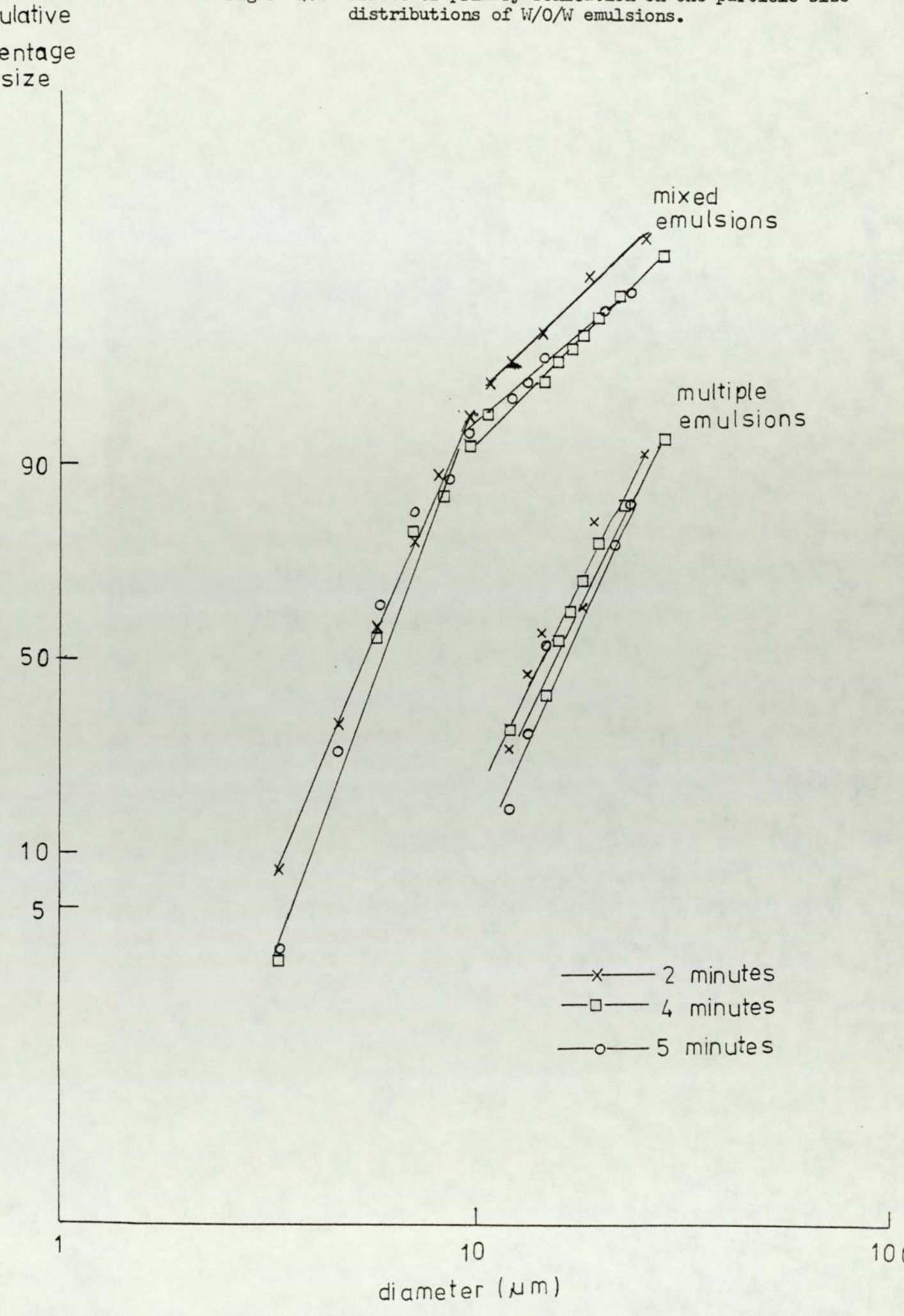
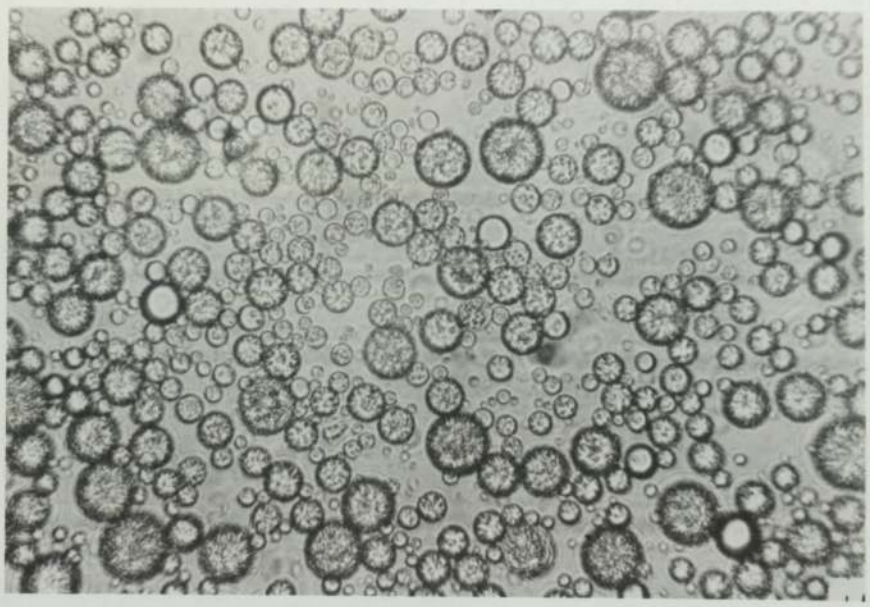


Figure 4.3

Typical photomicrograph of a multiple emulsion.

Bar = 1 μm.



are present. The first are multiple droplets and these have a grey appearance due to the presence of the internal droplets. The difference between this photomicrograph and the diagram (Figure 1.1) is striking - the real system contains many more internal droplets than were shown in the diagram, making the task of sizing them that much more difficult. The second type of droplet present is an oil droplet. So there are two types of emulsions present in figure 4.3 - an O/W and a W/O/W. This is the reason for the break in the particle size distribution of figure 4.2. The two emulsions have different particle size distributions. It is possible to separate these distributions using a graphical technique described by Lewis and Taylor (1967). This is illustrated in figure 4.4. Figure 4.2 also shows the separated distributions for the multiple droplets at the different times of sonication. The effect is very small. Sonicating the W/O emulsions for 2,4 and 5 minutes produces multiple emulsions of geometric mean diameters 15.0μm, 15.5μm and 16.5μm respectively.

The effect of different times of sonication of the multiple emulsions on their particle size distribution was then investigated. Figure 4.5 shows the particle size distributions of the mixed emulsions and the distributions of the corresponding multiple emulsions are shown in Figure 4.6. The effect on the mean diameters is small - sonicating for 10, 20 and 25 seconds produced emulsions with geometric mean diameters of 8.9μm, 8.5μm and 8.5μm. The major effect of this sonication is illustrated by the respective standard deviations - 1.51, 1.44 and 1.41. These values show that, while the mean diameter is affected only slightly by increased sonication, the distribution has become narrower. Further evidence is provided by the values of the upper bounds of these distributions which are 30.32μm, 26.56μm and 20.29μm respectively. The

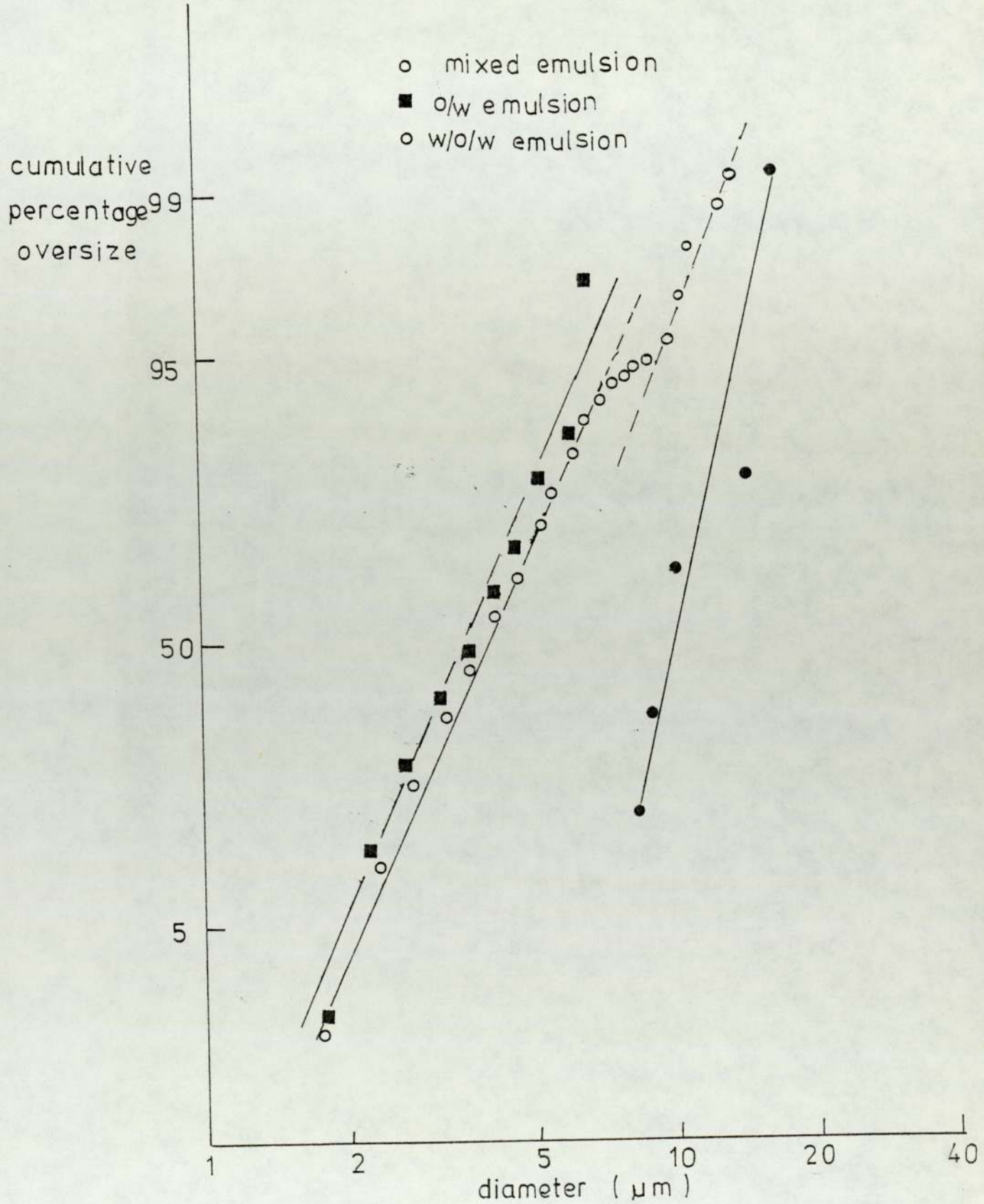


Figure 4.4 The resolution of a bimodal distribution into its two constituent parts by a graphical inflexion method.

Figure 4.5 Effect of secondary sonication on the particle size distributions of multiple emulsions.

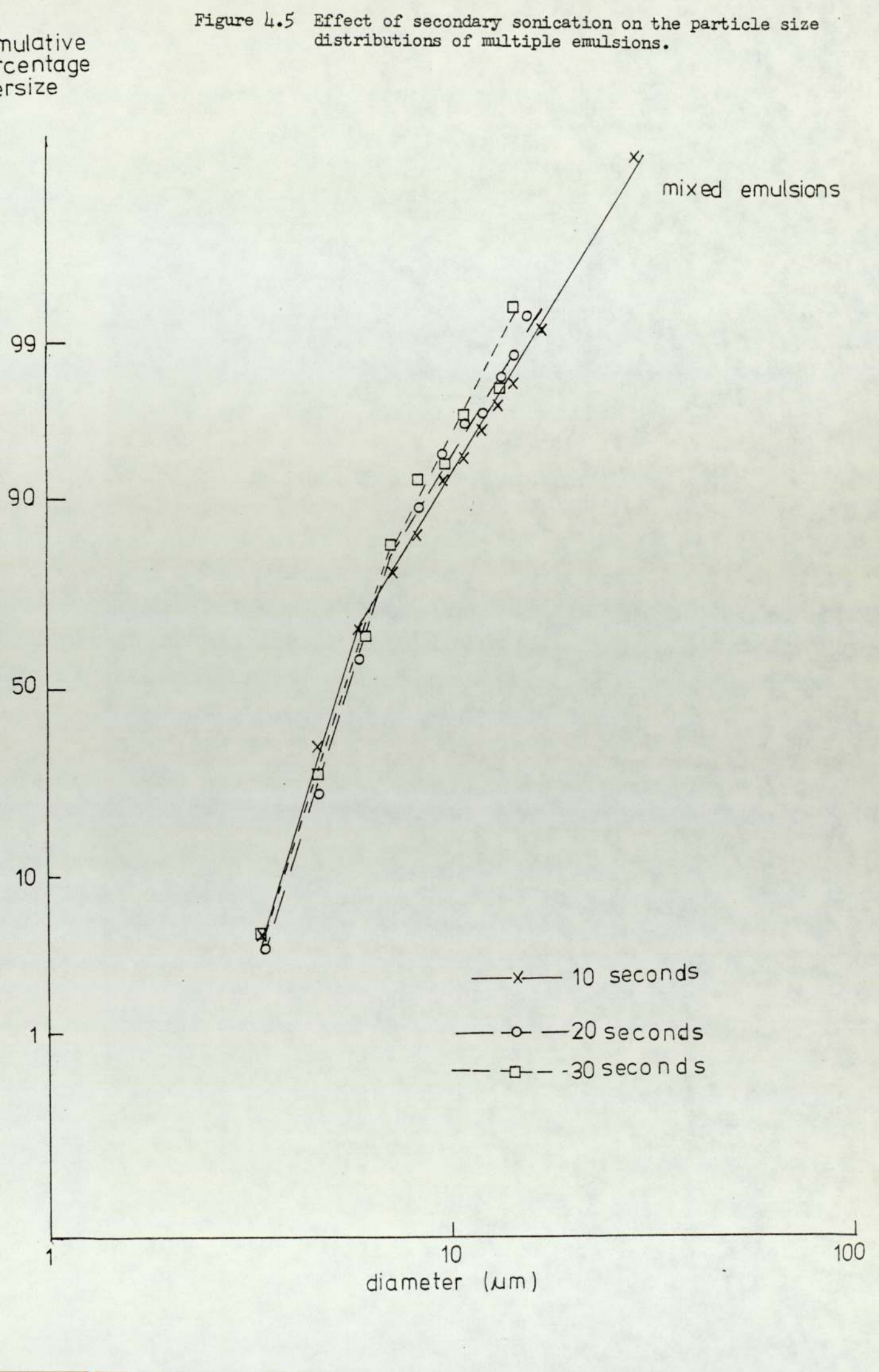
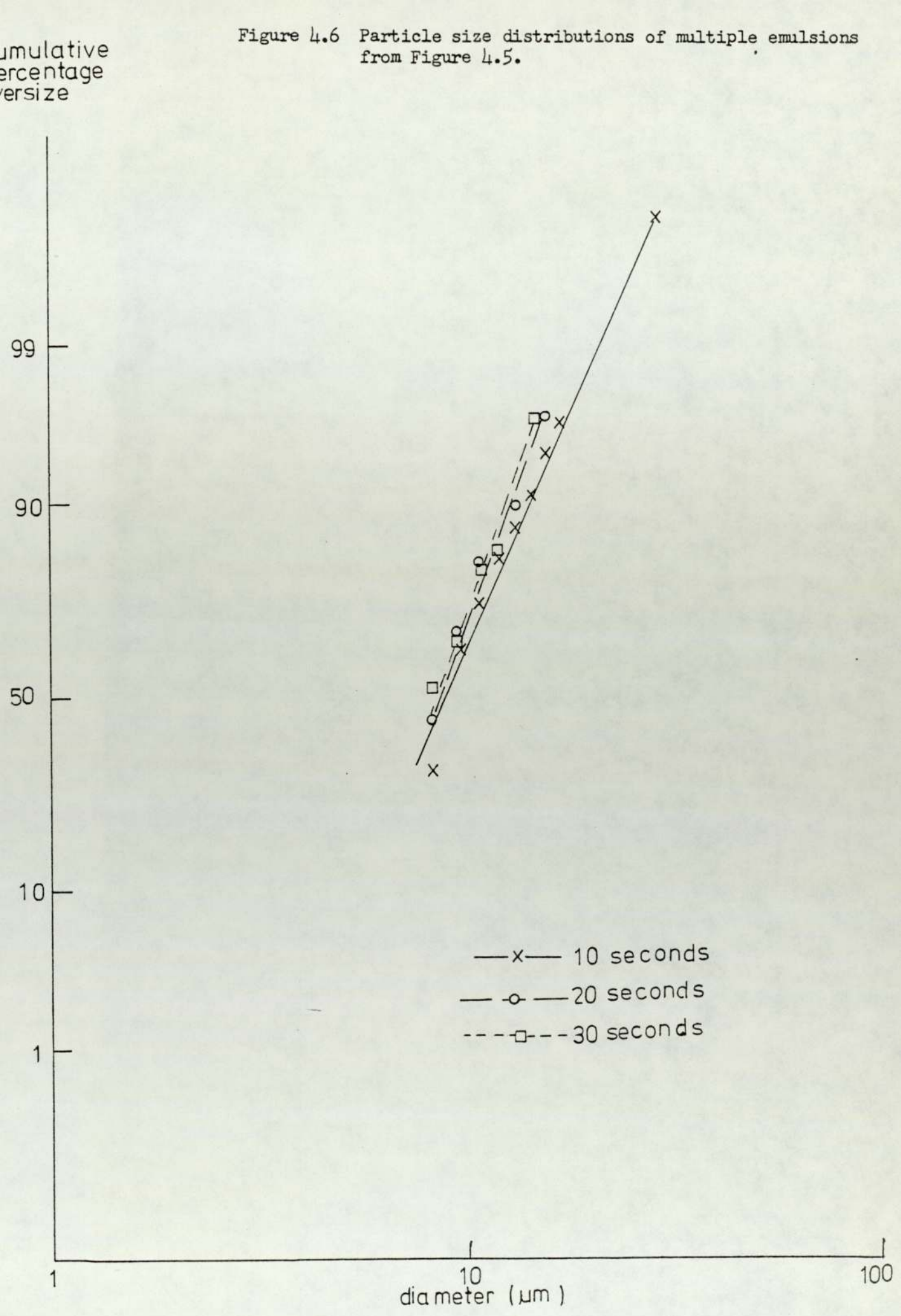


Figure 4.6 Particle size distributions of multiple emulsions from Figure 4.5.



main effect of longer sonication times on the multiple droplets therefore appears to consist of decreasing the size of the multiple droplets, eventually creating oil droplets devoid of internal aqueous droplets. It was therefore decided to use a sonication time of 10 seconds.

4.2.1.2 Evaluation of Formulation Variables.

Once the method of manufacture had been investigated and optimum processing conditions had been found, it was possible to study the effect of formulation variables on the resulting multiple emulsions. Two effects were evident and these are described below. The first effect was on the relative proportions of oil droplets and multiple droplets and the second effect was on the stability of the particle size distributions of the multiple emulsions.

As described earlier, all the multiple emulsions prepared for this study contained 2 separate populations of droplets - oil droplets and multiple droplets. These populations were separated graphically by the method of Lewis and Taylor (1967) and this enabled comparison of such parameters of the distributions as mean diameter, standard deviation etc. In this study, however, the relative proportions of the two types of droplets was just as important as their mean size. This could be evaluated from the data calculated by computer as described earlier. The distribution for the mixed emulsion was plotted and the point of inflexion was then determined. This was the diameter at which the 2 distributions changed over. All droplets larger than this diameter were multiple and from the calculated distributions by number or by volume the percentage of multiple droplets can be calculated. This is illustrated in Figure 4.5. The particle size distribution for the emulsion prepared by 10

seconds sonication shows an inflexion at 5.9μm. The percentage corresponding to this is 64 per cent and so 64 per cent of this emulsion by number are oil droplets and 36 per cent by number are multiple droplets. By this means, the effect of various formulation factors on the formation of multiple emulsions could be followed.

4.2.1.2.1 Effect of Emulsifying Agents.

The effect of the emulsifying agent on the initial particle size distribution was investigated. Initially, the effect of different hydrophilic surfactants was determined. Figure 4.7 shows the particle size distributions of the multiple emulsions produced by three different hydrophilic surfactants: Tween 20, Tween 80 and Pluronic F68. The lipophilic surfactant was constant at 10% Arlacel 83. The multiple emulsions from Figure 4.7 are shown in Figure 4.8. The effect of the different surfactants on the multiple droplets is summarised in Table 4.1.

Table 4.1 Effect of varying the hydrophilic emulsifier on various emulsion parameters.

Emulsifier	Mean diameter of multiple droplets	% of Multiple droplets by number	% of Multiple droplets by volume
2%$^{w}/_{v}$ Tween 20	5.4μm	21.67	74.69
2%$^{w}/_{v}$ Tween 80	12.0μm	17.04	53.00
2%$^{w}/_{v}$ Pluronic F-68	6.6μm	16.32	72.45

Tween 20 gives the smallest multiple droplets and these form the greatest percentage of all the effect of varying concentrations of Tween 20 on the particle size distribution of multiple emulsions was studied.

Figure 4.7 Effect of different hydrophilic emulsifying agents on the particle size distributions of multiple emulsions.

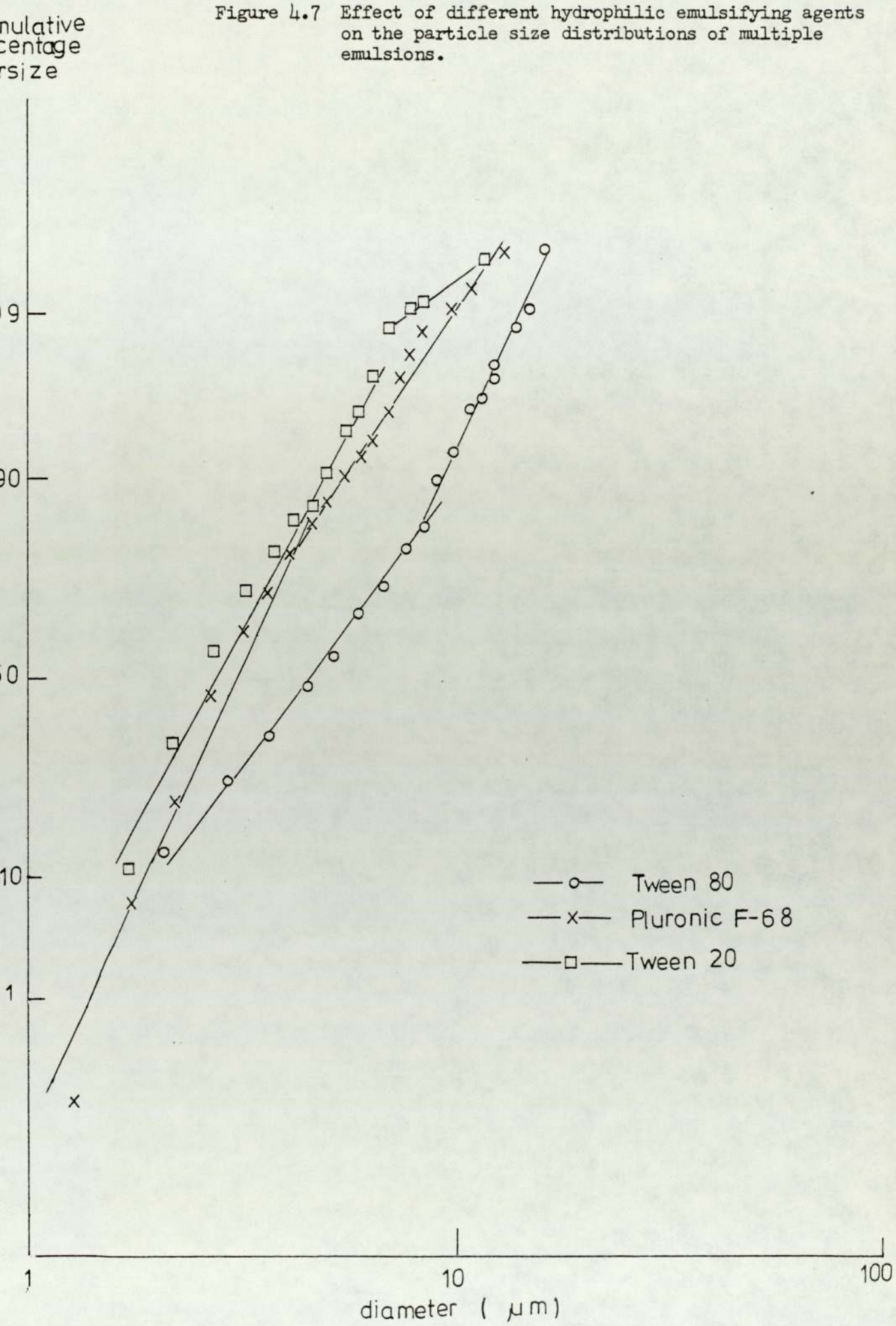
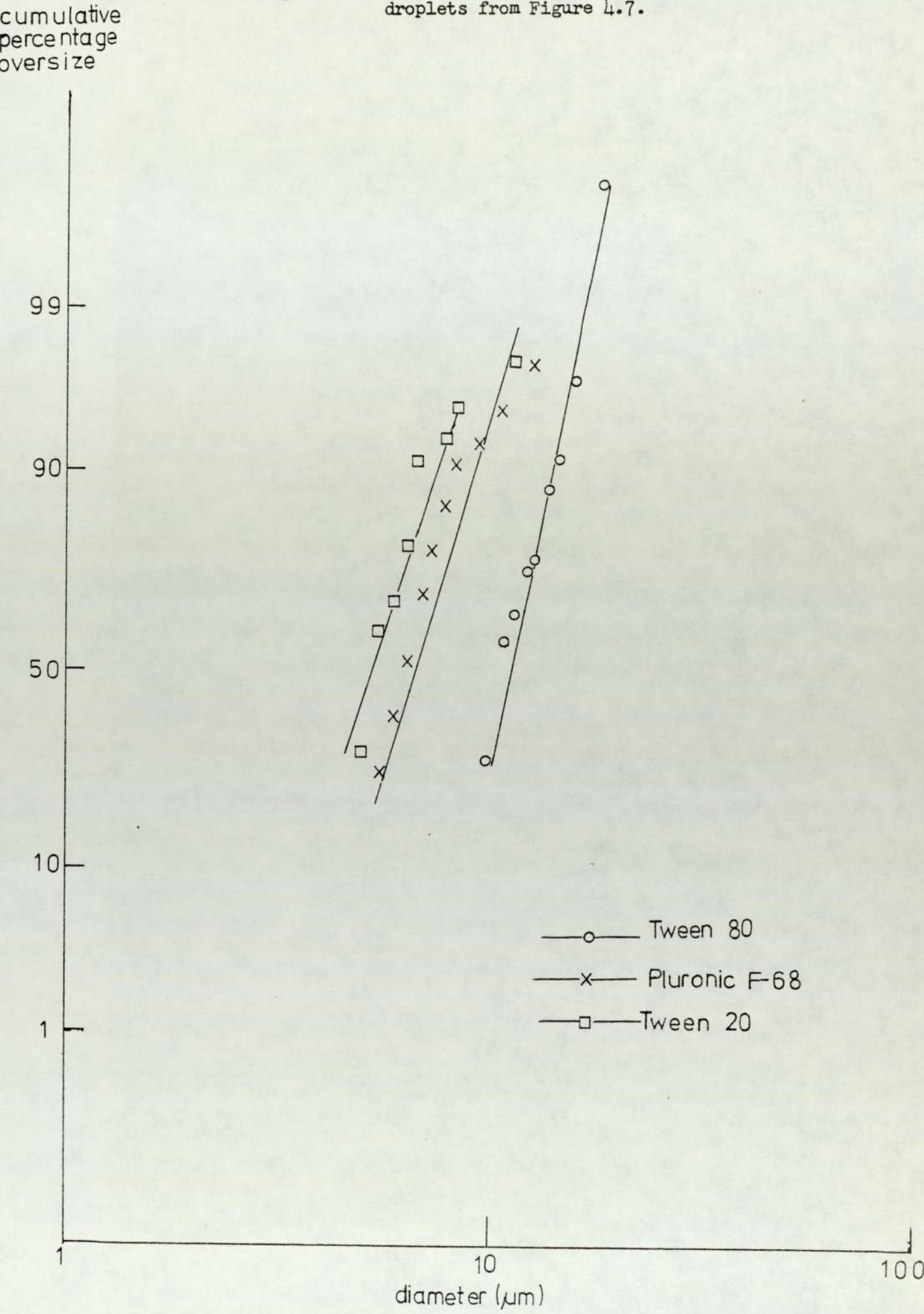


Figure 4.8 Particle size distributions of the multiple droplets from Figure 4.7.



The effect of ageing of these emulsions is seen in Figure 4.9. This shows the particle size distribution of the multiple droplets of the emulsion stabilised by Pluronic F-68. The multiple droplets have decreased in size from a mean of 6.6μm to 5.6μm over a period of 43 days. Over the same time, the percentage by volume has fallen from 72.45% to 51.14%. This is caused by diffusion of the internal aqueous phase into the continuous phase.

Figure 4.10 shows the effect of ageing of an emulsion stabilised by Tween 20. In this instance, the mean droplet diameter has increased from 5.10μm to 10.25μm during 30 days.

It appears, therefore, that two opposing processes are involved in the ageing of multiple emulsions. This is illustrated in Figure 4.11 which shows the ageing of a multiple emulsion. There is an initial rapid fall in mean diameter followed by a more gradual increase over a longer period. This can be explained by the visual evidence. The multiple droplets are subject to competing processes. The first is diffusion of the internal aqueous droplets into the continuous phase: this causes shrinking of the multiple droplets. The second is coalescence of multiple droplets causing an increase in size of the multiple droplets. The first process causes the multiple droplets to shrink by losing water while the oil remains constant. The second process causes the multiple droplets to grow in size by increasing the oil phase while the aqueous phase remains constant. Of course, the processes occur simultaneously during the ageing of an emulsion although one may dominate. This is the situation in Figure 4.11. The diffusion process is the major one in the initial decline while coalescence predominates in the subsequent rise.

Figure 4.12 shows the effect of varying the Tween 20 concentration on the mean diameter of the multiple droplets formed. The graph shows

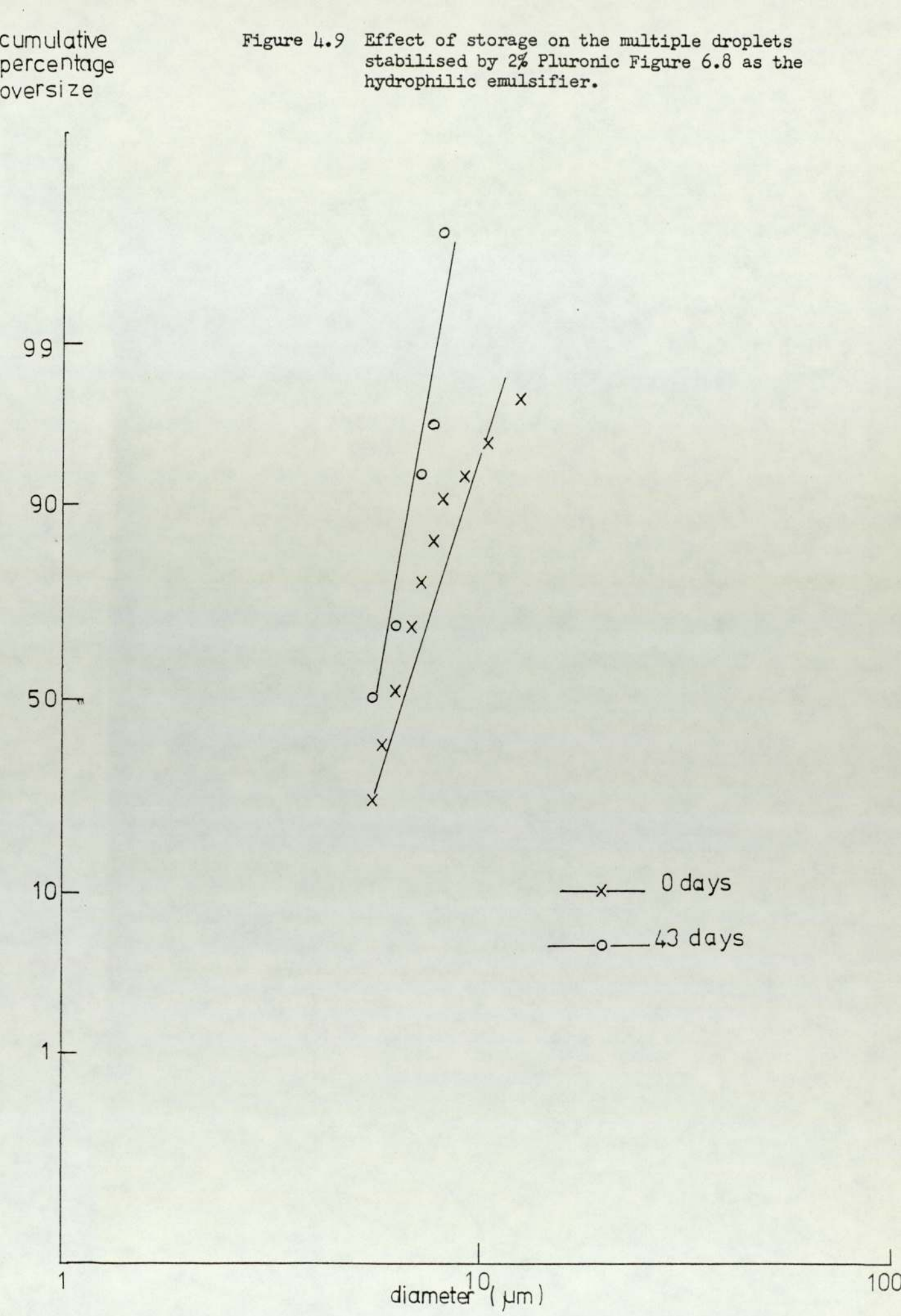


Figure 4.9 Effect of storage on the multiple droplets stabilised by 2% Pluronic Figure 6.8 as the hydrophilic emulsifier.

Figure 4.10 Effect of ageing on multiple emulsions stabilised by 2% Tween 20 as the hydrophilic emulsifier.

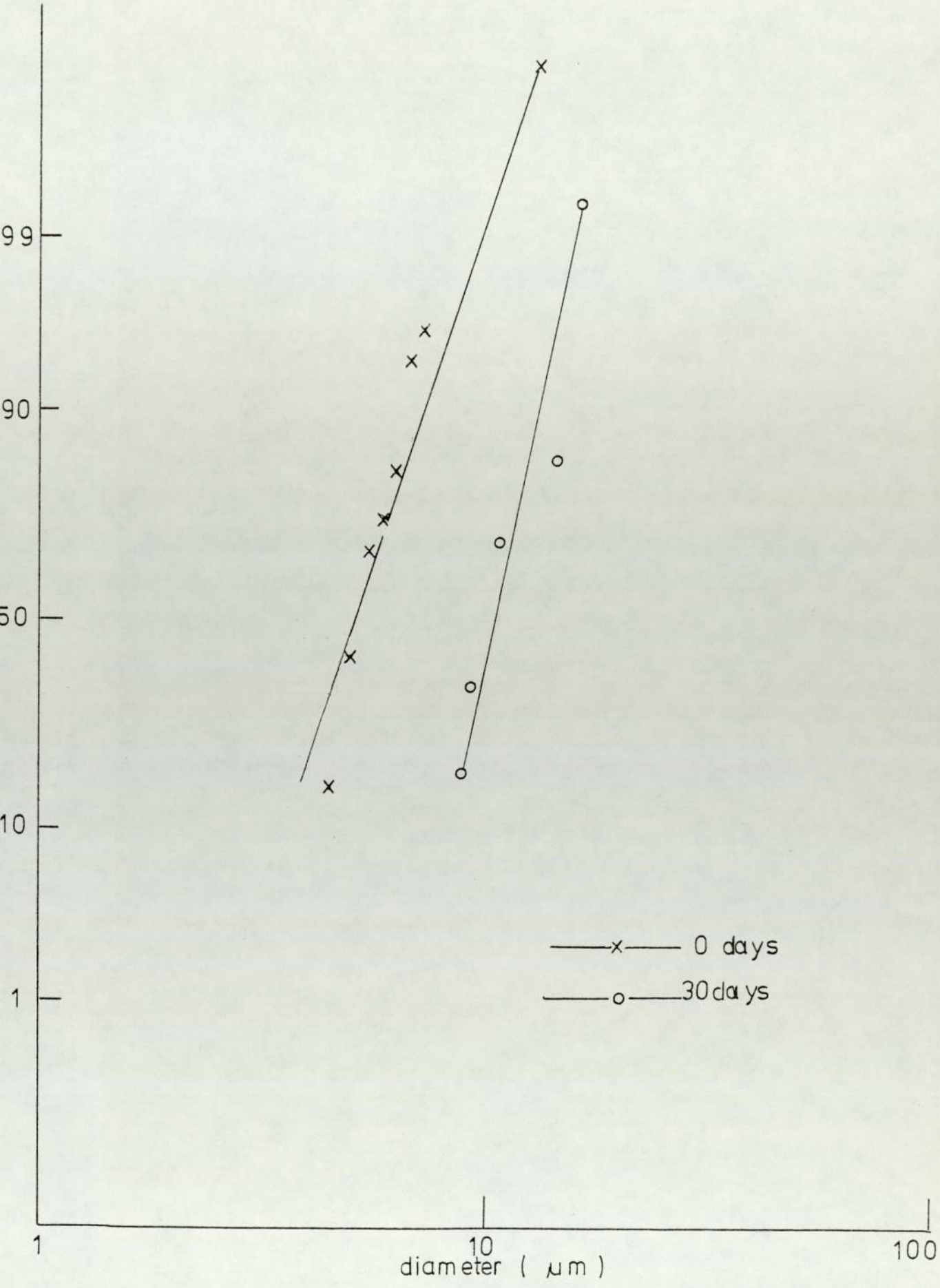


Figure 4.11 Ageing of multiple droplets stabilised by Tween 80 as the hydrophilic emulsifier.

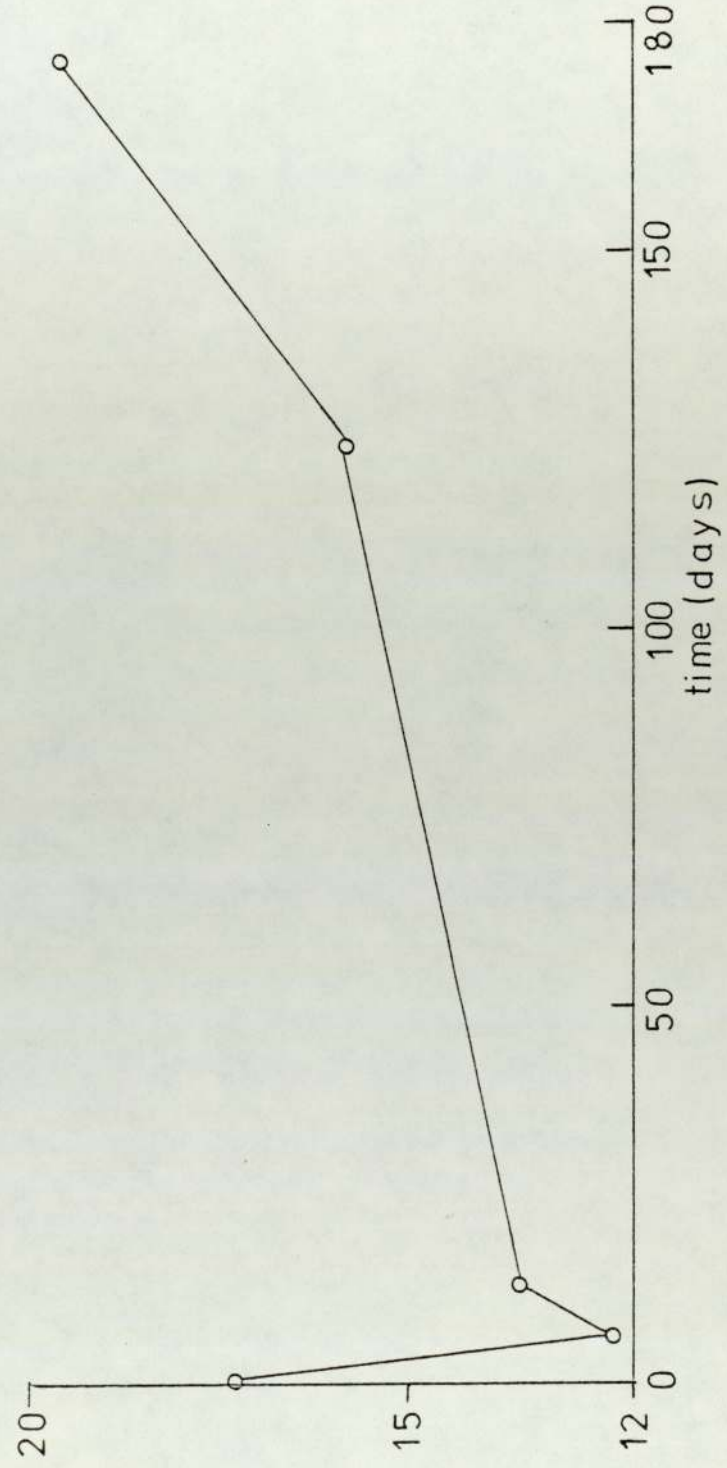
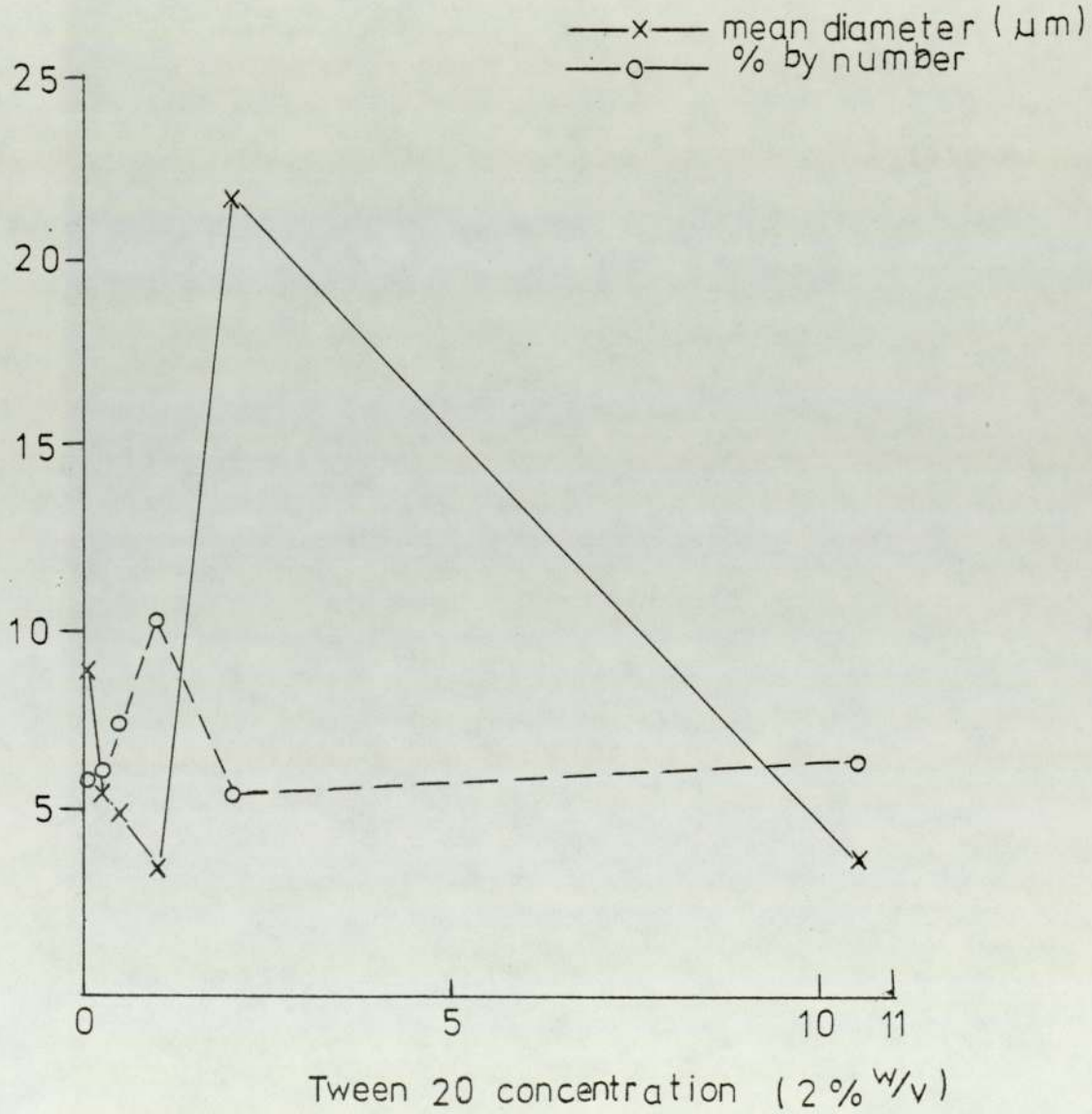


Figure 4.12. Effect of Tween 20 concentration on mean diameter and yield of multiple droplets.



an unusual biphasic pattern with a peak of 1% Tween 20 concentration. Emulsions with approximately equal diameters can be formed from Tween 20 concentrations of 0.1, 0.25, 2 and 10.5%. This trend is mirrored in the plot of percentage by number against Tween 20 concentration, although this shows a greater variation with concentration. The maximum of mean diameter at 1% Tween 20 concentration is matched by a minimum of percentage by number at the same concentration. The greatest percentage of multiple drops is formed at the concentration of 2%. The cause of the fall in mean diameter at low values of surfactant concentration is not known.

The effect of the lipophilic surfactant concentration on the particle size distribution of multiple emulsions is illustrated in Figure 4.13. The particle size distributions of the mixed and multiple emulsions are shown. The effect is not straight-forward but the trend may be more easily discerned in Table 4.2.

Table 4.2. Effect of varying the lipophilic emulsifier concentration on various emulsion parameters.

Emulsifier concentration	Mean diameter of multiple droplets	% by Volume of multiple droplets
2.5% Arlacel 83	8.1μm	53.07
5.0% Arlacel 83	4.2μm	69.65
10.0% Arlacel 83	5.3μm	74.70

The effect of increasing the Arlacel 83 concentration is to increase the yield of multiple droplets. The reason for this appears to be simple: increasing the lipophilic surfactant concentration increases the number of internal aqueous droplets that can be stabilised, especially during the process of formation.

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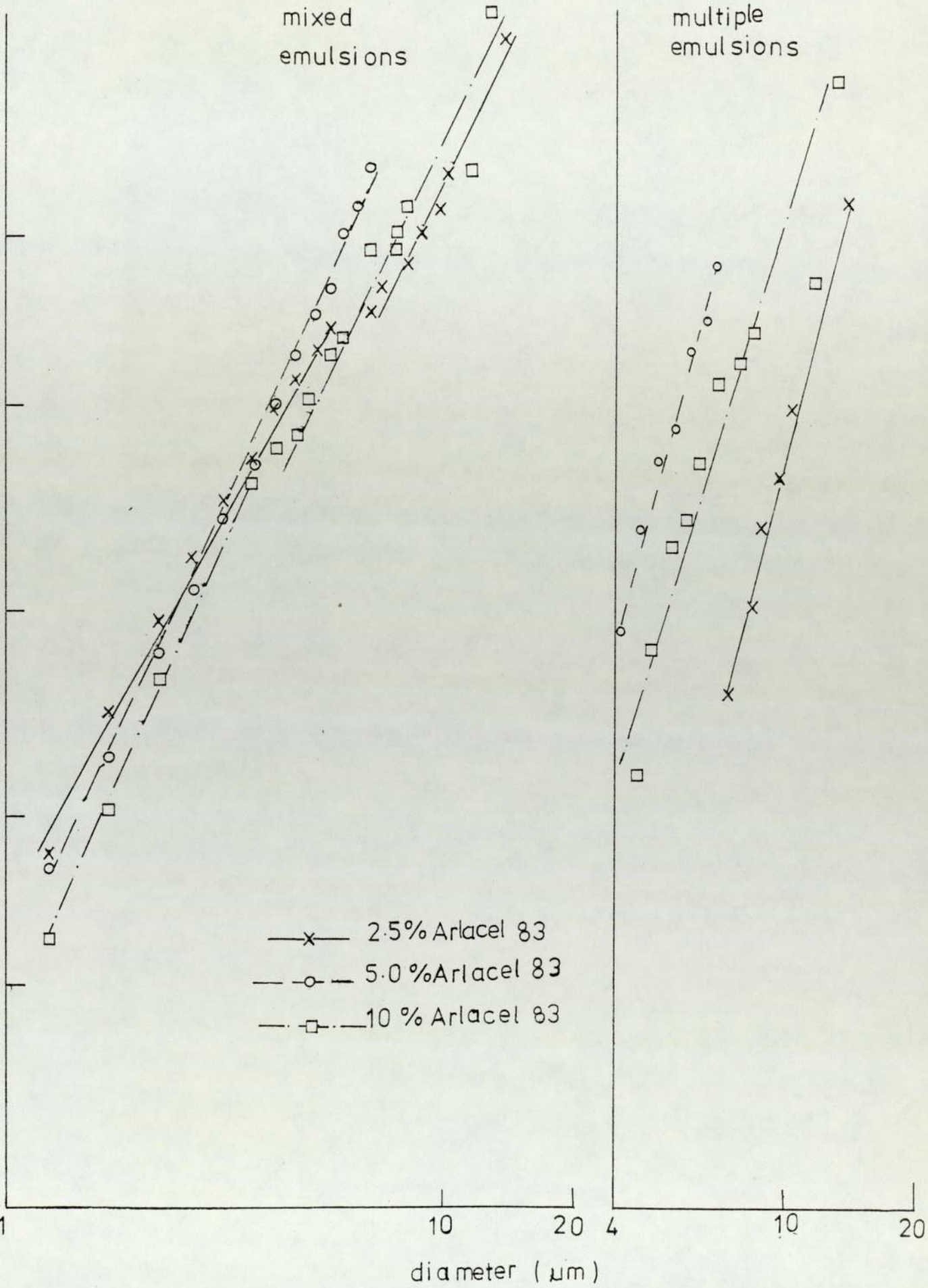
Figure 4.13. Effect of Arlacel 83 concentration on the particle size distributions of multiple emulsions.



4.2.1.2.2 Effect of phase volume ratios.

In a multiple emulsion which consists of 3 phases, there are 2 phase volume ratios. The first is the proportions of water and oil in the first emulsification. The ratio of water in this W/O emulsion will be denoted by $\phi_{w/o}$. The second phase volume ratio is the proportion of W/O emulsion and external aqueous phase. The ratio of the W/O emulsion in this second emulsification will be denoted by $\phi_{w/o/w}$.

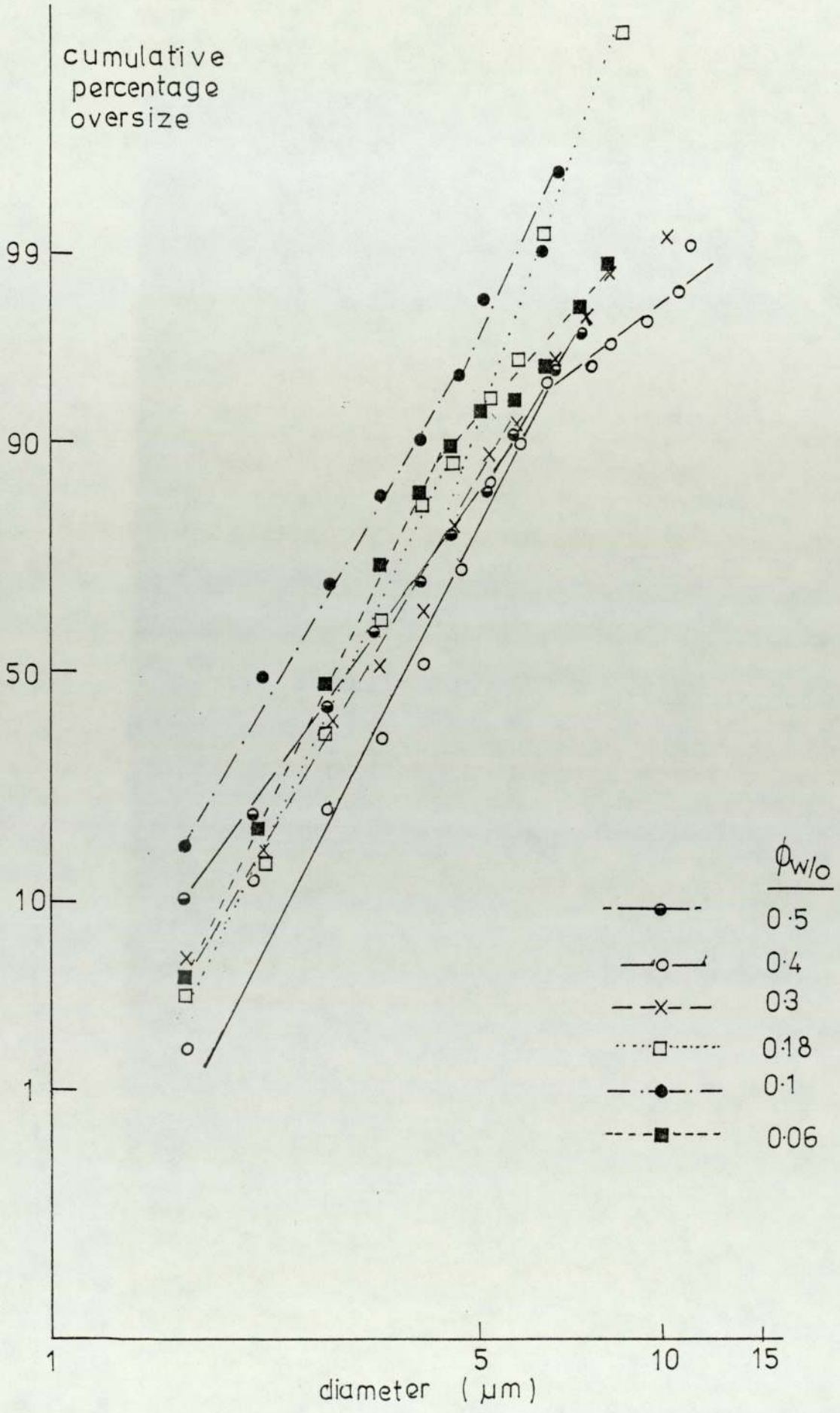
The effect of varying $\phi_{w/o}$ on the emulsion particle size distributions is seen in Figure 4.14. This shows the effect of $\phi_{w/o}$ on emulsions stabilised by 10% Atmos 30 and 2% Tween 20 as lipophilic and hydrophilic emulsifiers respectively. The value of $\phi_{w/o/w}$ was constant at 0.5 throughout this experiment. No obvious pattern emerges from this graph. However, a trend emerges from the derived data in Table 4.3

Table 4.3 Effect of varying $\phi_{w/o}$ on various emulsion parameters.

$\phi_{w/o}$	Mean Diameters of Multiple Droplets	% by number of multiple droplets	% by volume of multiple droplets
50.0	7.1μ	16.1	60.9
40.0	9.5μ	10.1	53.8
30.0	8.1μ	7.6	47.7
18.2	5.6μ	6.5	30.7
10.0	6.4μ	5.0	31.9
6.0	6.8μ	11.5	51.1

The trend is to a minimum at a value of $\phi_{w/o}$ of 10%. The first part of the trend, i.e. the decrease of all 3 parameters with decreasing $\phi_{w/o}$, is easily understood. As $\phi_{w/o}$ decreases, less water is available to form internal aqueous droplets and so the number and volume of the multiple droplets decreases. The increase in the number and volume of

Figure 4.14 Effect of varying $\phi_{w/o}$ on the mean diameter and yield of multiple droplets.



multiple droplets as $\phi_{w/o}$ decreases from 10 to 6%. This is not an isolated instance, however. This has also been observed during Coulter counter studies for this thesis and will be more fully discussed in Chapter 6.

4.2.1.2.3 Effect of the nature of the oil phase.

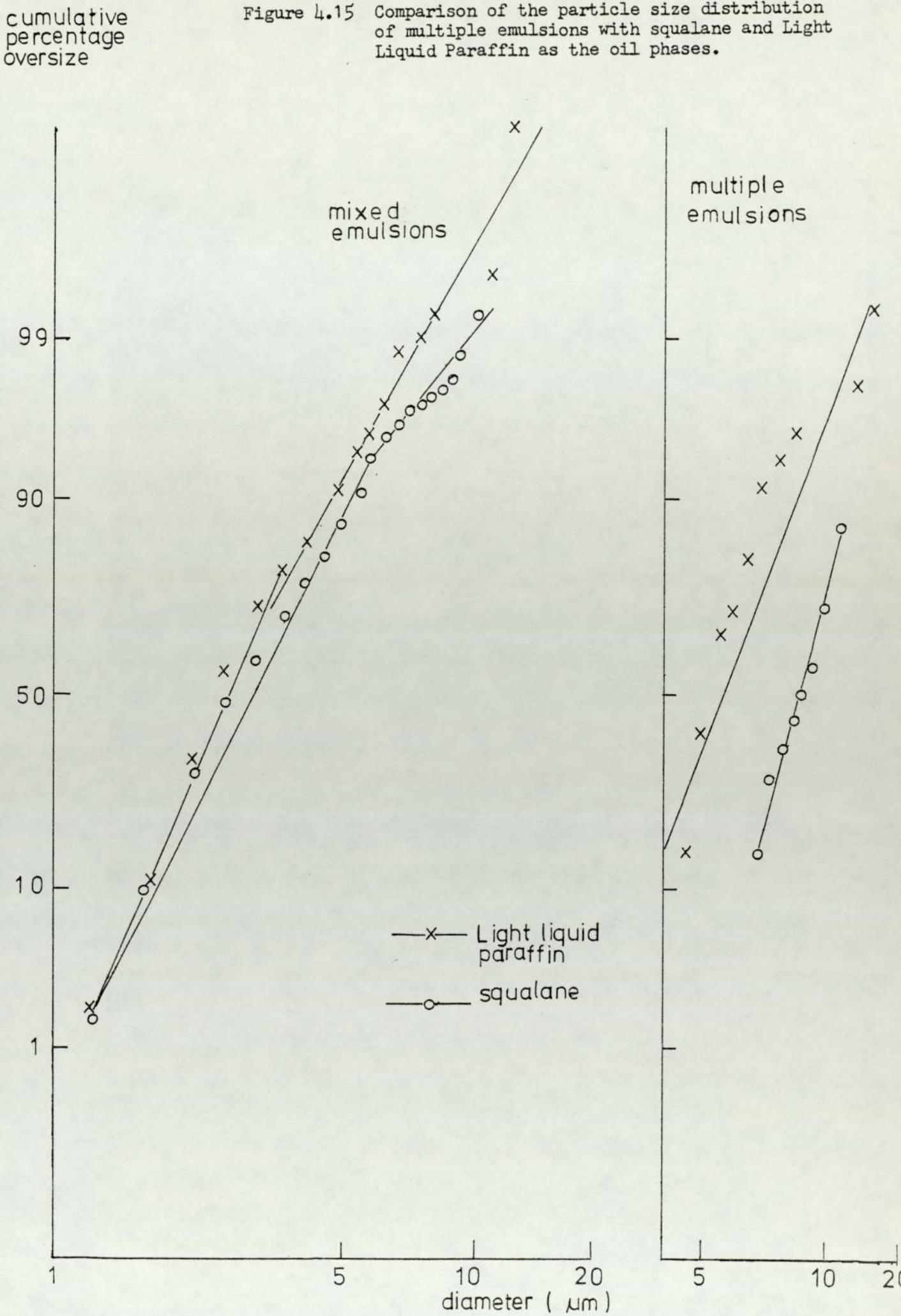
The effect of altering the nature of the oil phase on the particle size distributions of multiple emulsions is shown in Figure 4.15 which compares multiple emulsions containing light liquid paraffin or squalane. Squalane is 2,6,10,15,19,23 - hexamethyl tetracosane: a saturated analogue of squalene which is a vegetable oil. Squalene is liable to autoxidation because of its unsaturation but squalane is stable.

The mean diameter of the multiple emulsion formed from light liquid paraffin is 5.5μm whereas the multiple emulsion formed from squalane has a mean diameter of 8.4μm. The emulsion formed from light liquid paraffin has a greater yield. The percentages by volume of multiple droplets are 74.69% and 50.69% for light liquid paraffin and squalane respectively.

It was then decided to investigate the effect of a number of other vegetable oils on the particle size distribution of multiple emulsions. A number of emulsions were prepared from cottonseed oil and corn oil, stabilised by Arlacel 83 and Tween 20 as lipophilic and hydrophilic emulsifiers respectively. However, it was not possible to produce multiple emulsions using these materials. Following this discovery a number of other emulsifiers were used in combination with the vegetable oils. Mulgofen BC-420, Pluronic L-61, Arlacel A, Atmos 300 and Myvacet 9-40 were used as lipophilic emulsifiers. No multiple emulsions were formed with the stabilisers either.

The reason for the failure to produce multiple emulsions from vegetable oils and a number of emulsifiers is not known. However, this

Figure 4.15 Comparison of the particle size distribution of multiple emulsions with squalane and Light Liquid Paraffin as the oil phases.



does not mean that it is impossible to form multiple emulsions from vegetable oils. What is clear is that for the oil/emulsifier combinations used in this study the manufacturing conditions were not correct. It may be that multiple emulsions formed from these materials are less stable and are destroyed by the violent emulsifying conditions used. Alternatively, the correct emulsifier for the oil was not found.

4.2.1.2.4 Effect of the osmolarity of the internal aqueous phase.

The effect of the osmolarity of the internal aqueous phase was investigated by preparing emulsions containing sodium chloride solution as the internal aqueous phase. Figure 4.16 compares the particle size distributions of emulsions containing water and a 5% sodium chloride solution as the internal aqueous phases. The emulsion containing 5% sodium chloride solution as the internal aqueous phase was diluted for photomicrography in 5% sodium chloride solution. This was necessary to prevent a change in the particle size due to the presence of an osmotic gradient between the internal and external aqueous phases. Therefore, the unperturbed particle size distribution is seen. This is very important in the systems in this study due to the fact that they consist of 2 aqueous phases separated by an oil phase. The importance of eliminating the osmotic gradient between the 2 phases and the results of not doing so will be demonstrated later in this chapter.

The effect of the sodium chloride in this particular instance is to decrease the mean diameter of the multiple droplets. The emulsion with 5% sodium chloride has a mean diameter of the multiple droplets of 3.9μm and they comprise 81.0% of the volume of the disperse phase. Multiple droplets of a similar emulsion with sodium chloride have a mean diameter of 5.5μm and they comprise 74.7% of the volume of the disperse phase.

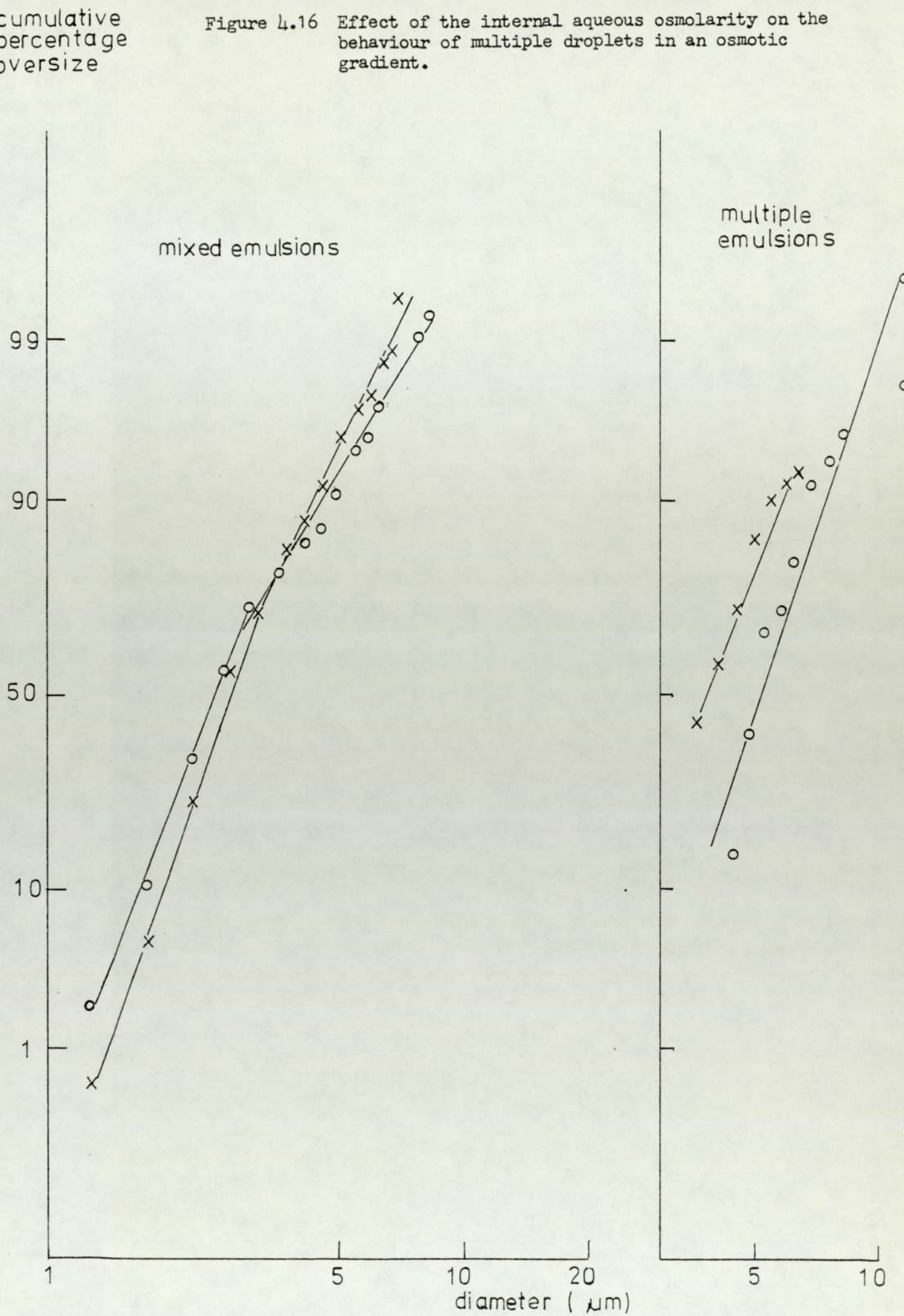
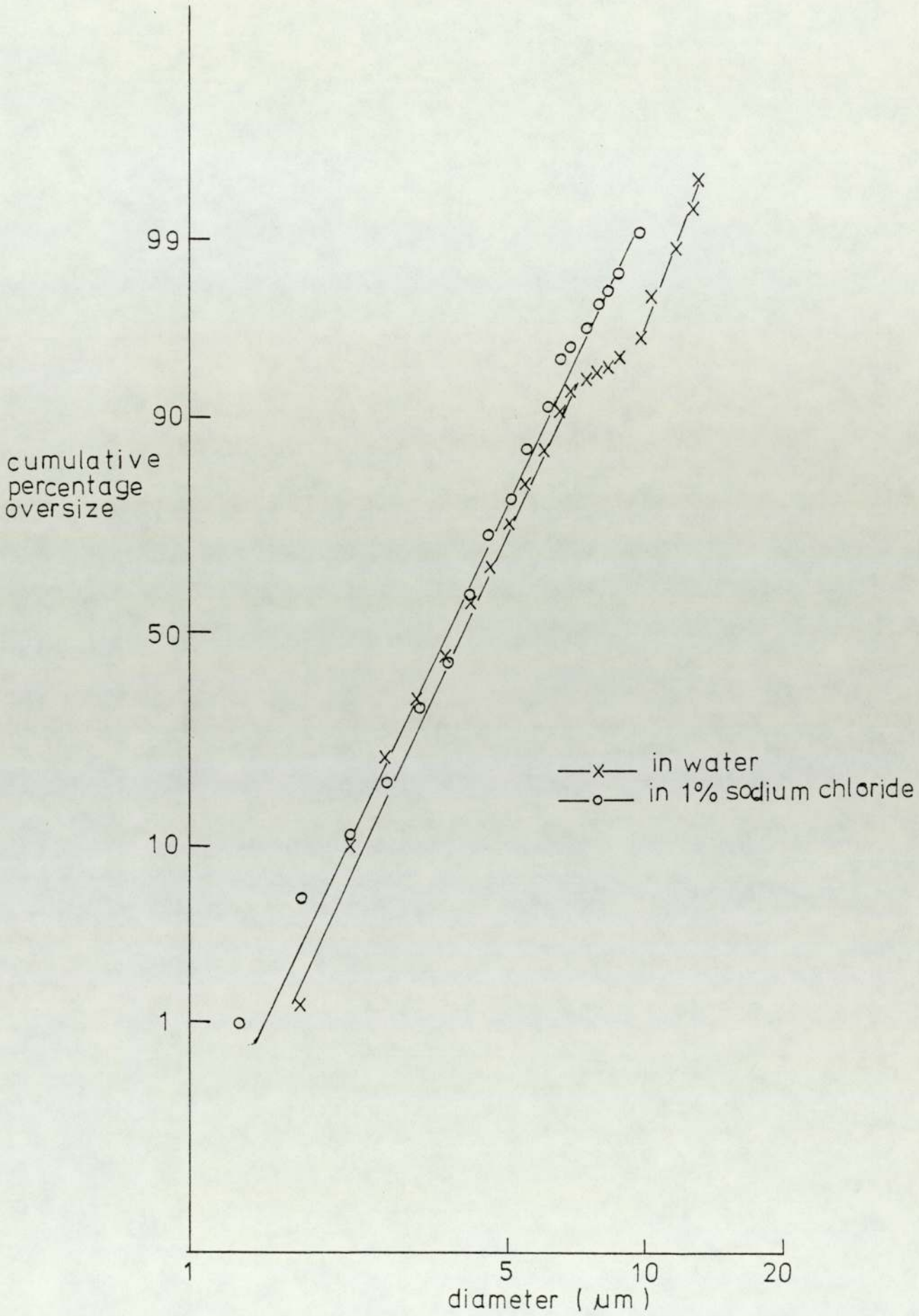


Figure 4.16 Effect of the internal aqueous osmolarity on the behaviour of multiple droplets in an osmotic gradient.

Figure 4.17 Effect of the external aqueous phase osmolarity of the particle size distribution of a multiple emulsion.



4.2.2 Behaviour of Multiple Droplets in an Osmotic Gradient.

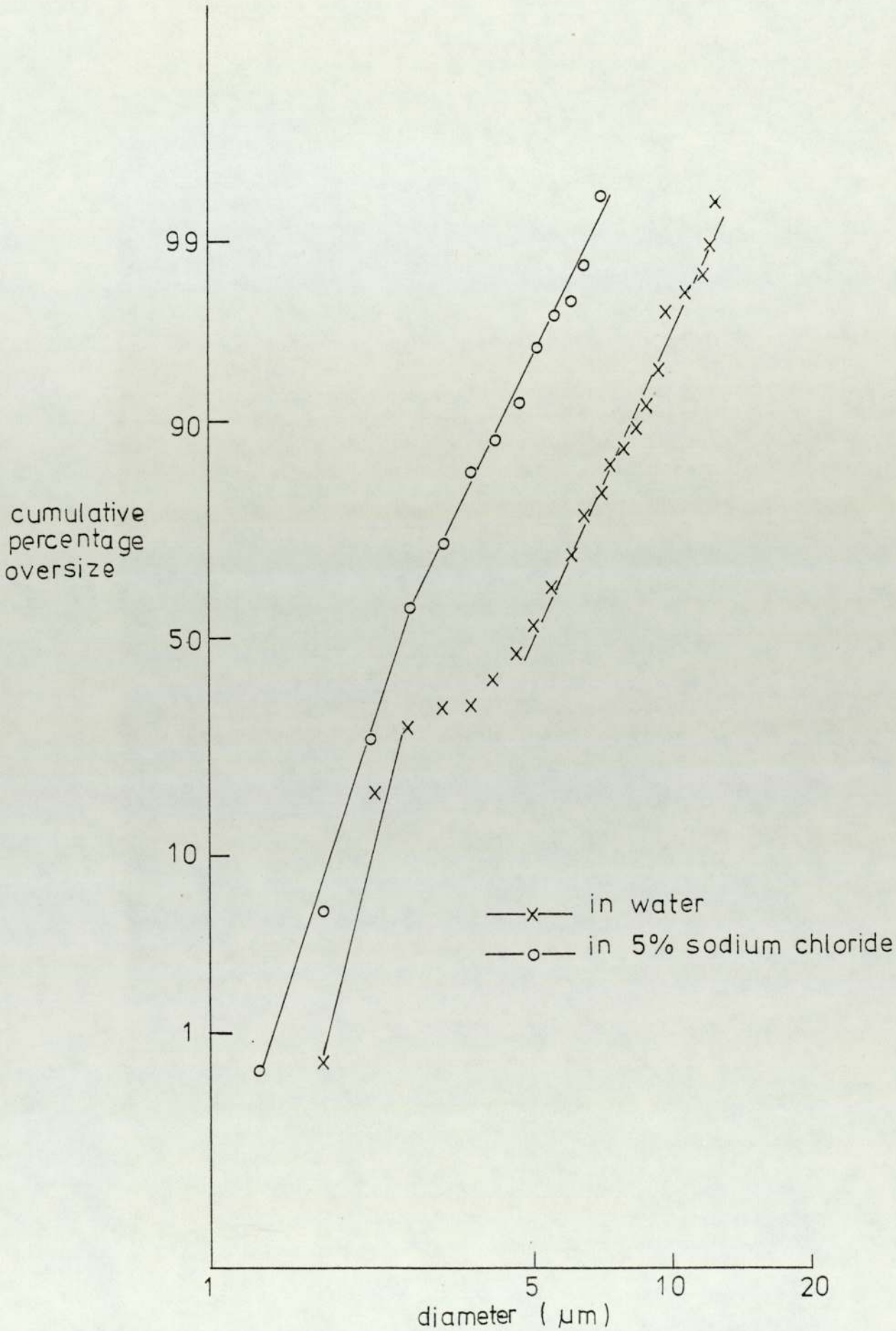
Multiple emulsions consist of two aqueous phases separated by a thin oil phase. The oil can act as a semi-permeable membrane. This means that water flow can occur across the oil phase in the presence of an osmotic gradient. This presents another method of assessing the state of multiple droplets.

Figure 4.17 shows the effect of adding 1% sodium chloride solution to a multiple emulsion. When the emulsion is diluted with water, i.e when there is no osmotic gradient, the normal bimodal particle size distribution is obtained. However, when the emulsion is diluted with the electrolyte, a unimodal distribution is obtained, indicating the disappearance of the multiple droplets. This is because the internal droplets have been drawn out by the osmotic effect of the electrolyte solution.

An osmotic gradient in the opposite direction is shown in Figure 4.18. The emulsion contains 5% sodium chloride solution as the internal aqueous phase. The particle size distribution of the emulsion when it was diluted with 5% sodium chloride solution is bimodal. When this emulsion is diluted with water, the multiple droplets swell and the bimodal nature of the distribution is greatly exaggerated. This confirms that the point of inflexion has correctly separated the multiple and oil droplets.

The osmotic gradient in a multiple emulsion is not simply due to the difference in solute concentrations between the aqueous phases. The fact that the solute is contained within droplets alters the gradient. This is described as the Kelvin effect. The vapour pressure of the droplet is increased above the vapour pressure of the bulk phase. This is due to the curvature of the droplet. The increase in vapour pressure is inversely proportional to the radius of the droplet. The effect is negligible for droplets of diameter greater than 1 micron. For droplets

Figure 4.18 Effect of the osmolarity of the internal aqueous phase on the particle size distribution of a multiple emulsion.



smaller than this, the effect becomes significant. In fact, even droplets of pure water can produce an osmotic effect due to their small size. This would mean that, for an osmotic balance, the solute concentration in the continuous phase would need to exceed the solute concentration in the internal aqueous phase.

Figure 4.19 shows the effect of different concentrations of external electrolyte on the particle size distributions of multiple droplets. There appears to be a critical value of electrolyte concentration below which the electrolyte has little effect on the multiple droplets. Above the critical electrolyte concentration, the osmotic shrinking described above commences. In this instance, the critical concentration lies between 0.005% and 0.01% sodium chloride.

Figure 4.20 shows the effect of ageing on an emulsion containing 5% sodium chloride as the internal aqueous phase. At time 0, the droplets swell from a mean of 5.9μm to 7.0μm. After 81 days, the unswollen droplets have increased in size from 10.20μm and swell, in water, to 11.5μm. Although the unswollen droplets had increased in size after storage, the proportion of aqueous phase had decreased. This is shown in the amounts of swelling which decreased during storage. This effect is also shown in the proportions of oil and multiple droplets. Initially the multiple droplets in the unswollen emulsion comprised 28.8% of the total disperse phase volume. This increased on swelling to 91.2%. The figures for the stored emulsion were 48% before and 51% after storage.

The kinetic behaviour of multiple emulsions in an osmotic gradient will be illustrated in Chapter 6.

4.3 Discussion

The particle size analysis by optical microscopy of multiple emulsions has been described. The technique of splitting the particle

Figure 4.19 Effect of a range of external aqueous phase osmolarities on the particle size distribution of a multiple emulsion

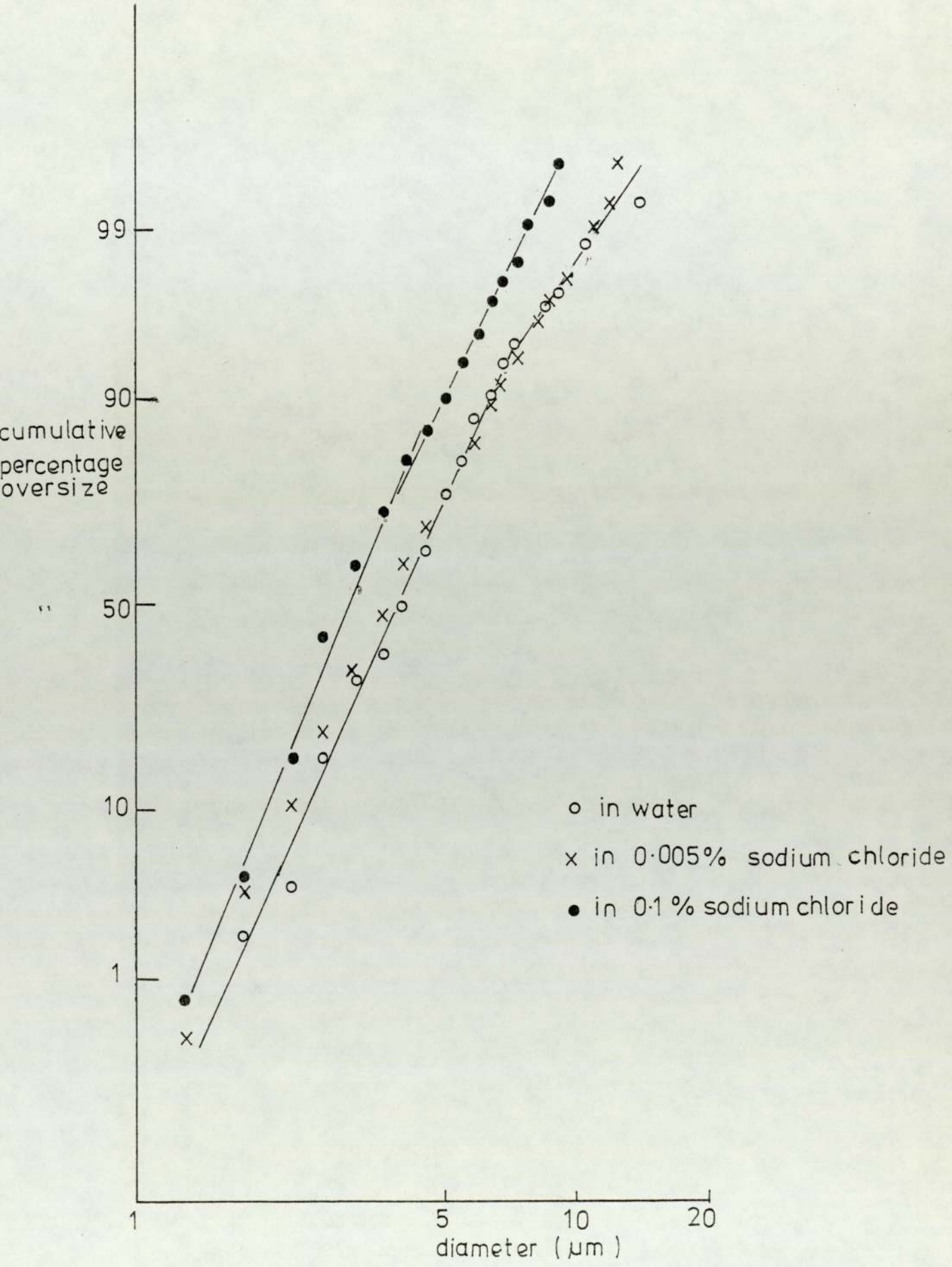
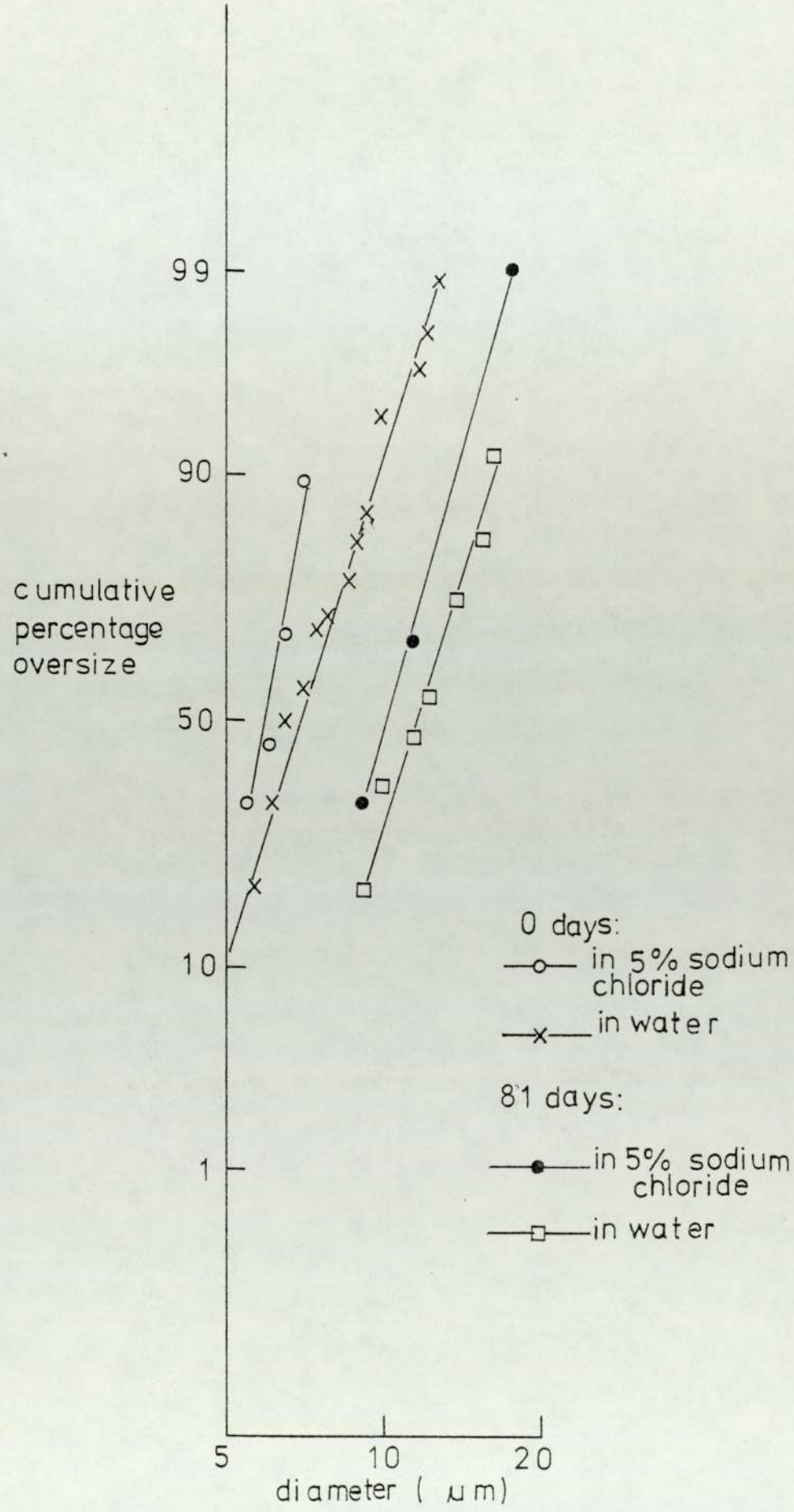


Figure 4.20 Ageing of multiple droplets containing 5% sodium chloride as the internal aqueous phase — change of behaviour under an osmotic gradient.



size distribution into its constituent populations has enabled the effect of various formulation factors to be evaluated.

Ageing of multiple emulsions seems to occur by two processes — diffusion of the internal aqueous into the continuous phase and coalescence of the oil droplets. The first effect causes a decrease in the mean diameter while the second causes an increaase.

The behaviour of the multiple droplets under equilibrium in an osmotic gradient has also been illustrated. The shrinking or swelling depending on the direction of the gradient, enables the multiple droplets to be distinguished from the oil droplets, whose dimensions do not later in the osmotic gradient. There appears to be a critical electrolyte concentration necessary to induce shrinking. This appears to be related to the multiple droplet diameter. Other workers (Davis and Gostling, 1977) found that multiple droplets greater than 20μm in diameter were unaffected by electrolyte concentrations of 0.45% .

Chapter 5

Chapter 5 - Electron Microscopy

5.1 Method

5.1.1 Freeze-etching

Freeze-etching was performed using a Balzers BA 360M machine. This was in the Botany department of the University of Nottingham.

One drop of the emulsion was placed on a gold stub. This was frozen rapidly in Arcton 22 and stored under liquid nitrogen.

Four such stubs were placed on the cold table of the freeze-etch device at -150°C. The bell-jar cover was lowered and the bell-jar was evacuated to better than 10^{-5} torr vacuum. The cold table was allowed to rise to -100°C while the knife was cooled to -150°C. The knife was a single edged razor blade. When the vacuum and temperature were correct, the samples were planed down using the knife until clean surfaces were obtained. The surfaces were etched for 1 minute by placing the knife over them. Because the knife was at -150°C and the sample at -100°C, water evaporated from the sample. The knife was moved away.

Platinum and carbon were evaporated onto the etched surfaces from an angle of 45°. The evaporated film thickness was monitored using a thin-film monitor. Evaporation was stopped when a pre-determined thickness was reached. This was known to be the optimum film thickness for electron microscopy. A film of carbon was applied to the samples from directly above.

The bell-jar was brought back to atmospheric pressure. The gold stubs, complete with replicas, were removed from the cold table. The replicas were removed from the sample by floating off in a solvent. The type of emulsion which had been treated determined the solvent. In this work, two types of emulsions were studied by freeze-etching: W/O and

W/O/W. W/O emulsion replicas were floated off in tetrahydrofuran; W/O/W emulsions were treated with distilled water. The solvent was changed several times and after soaking, the replicas were picked up on formvar-coated electron microscope grids and dried.

The described course of treatment produced, on average, one replica out of the four samples on the cold table. The greatest loss was caused by the cutting step - as the sample was struck by the blade, it would shoot off the stub. This was caused by air between drop and the stub lessening the adhesion. Flaws in the drop produced loss of part of the drop when the blade struck. This was less extreme and a smaller replica could still be obtained. In fact, the few replicas produced often broke into small pieces but they could still be picked up and examined.

5.1.2 Electron microscopy

The replicas were examined using an AEI EM6B electron microscope. They were examined initially at low magnifications and these were increased to observe features of interest in more detail. Selected areas of the replica were photographed. Plate film was used. Each film cradle held six plates and when these had been exposed, the microscope had to be brought to atmospheric pressure before the cradle could be removed. The exposed plates were developed immediately. A cradle with unexposed plates was put in the microscope which was then re-evacuated before further exposures could be taken.

Some of the replicas showed black areas. These were excess platinum and carbon left on the replica. They were present because of the short washing time. This was thought to be better than the extensive break-down of the replicas caused by longer washing times.

5.2 Results

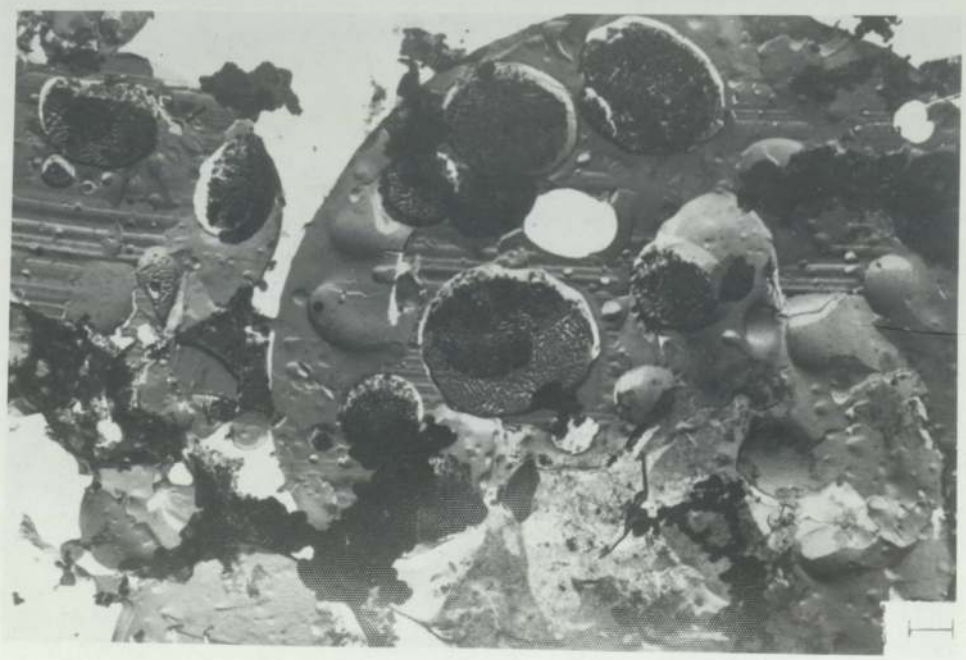
5.2.1 W/O/W emulsions

The photomicrographs in Chapter 4 (Optical Microscopy) show a number of multiple emulsions. Their main feature is the two types of droplets present: oil droplets and multiple droplets. Freeze-etching was used in an attempt to reveal the nature of the internal aqueous phase of the multiple droplets. Figure 5.1 is an electron micrograph of a multiple droplet at low magnification. It includes the main features of electron micrographs of multiple droplets.

During the facture of the sample, the line of fracture does not exactly match the path of the blade. As the blade passes through the sample, the fracture line takes the path of least resistance. Clearly, there are three possibilities. The fracture path will pass over, through or under a droplet. If the water droplet is just passed over, it may just be visible in the plane of the replica. If the fracture path passes through the droplet, the interior is visible. If the path passes under the droplet, the cavity it occupied is left empty.

All these possibilities are evident in Figure 5.1. The water droplets which have been fractured show their characteristic pattern. This is caused by ice crystals formed during the freezing process. Another common feature evident in this photomicrograph is etching of the water droplets.

The foregoing description of the freeze-fracture process also illustrates some of its limitations in this particular application. Fracture may occur through any chord of the droplet. Herdan (1960) has described a correction factor to be applied to the mean diameter of droplets which have been cut through. This applies to droplets where their distribution in the horizontal plane is random. This is not the case for freeze-fractured emulsion droplets: the fracture plane follows



the line of least resistance, the position of the droplets in the sample may be affected by the freezing and etching may also change the diameter of the exposed droplets. Therefore, accurate particle size analysis by freeze-etching is not possible. An approximate estimation of the size of the internal aqueous droplets is possible, however. The real value of freeze-etching lies in revealing the structure of the internal aqueous droplets.

Figure 5.2 shows several emulsion droplets. Some of these contain no internal water droplets: these may be oil droplets or multiple droplets which contained no water droplets in the plane of the replica. Areas of the electron micrograph show scuff marks on the oil phase. These are caused by the blade as it passes through the sample and indicate the direction of its movement. The value of particle size analysis from freeze-etched electron micrographs was described earlier. However, the internal aqueous droplets can be sized very approximately. They appear to be about 1 micron in diameter or less.

Figure 5.3 is a low magnification micrograph. One multiple droplet and many apparently oil droplets are visible. The scuff marks from the passage of the blade are again visible. The wide range of diameters of oil droplets compared to the multiple droplet is evident. Although it is unwise to draw conclusions from so few droplets, this is similar to the pattern seen by other techniques (Chapters 4 and 6). The characteristic etched pattern of the internal aqueous is also seen in areas of the continuous phase. This is obscured to some degree by the "debris" on the replica which was caused by the minimal replica washing described above.

The next four electron micrographs are higher magnification pictures showing in greater detail the structure of the internal aqueous

Figure 5.3.

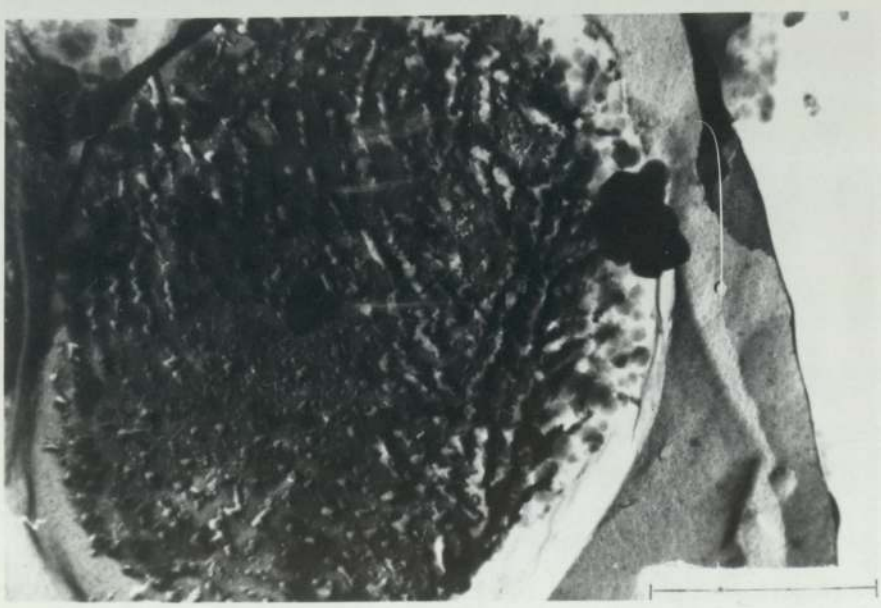
Electronmicrograph of emulsion droplets

Bar = 1 μ.m.

Figure 5.4.

Electronmicrograph of internal aqueous droplets

Bar = 1 μ.m.



droplets. Figure 5.4 and 5.5 show internal aqueous droplets from Figure 5.2. The most obvious feature is the pattern of ice crystals caused by the freezing. Other workers (Eley et al, 1976) have been able to show micrographs including detail of the interfacial structure. No such detail can be seen in the Figures 5.4 and 5.5.

Figure 5.6 shows several internal aqueous droplets from Figure 5.1 under greater magnification. Figure 5.7 shows further detail of internal aqueous droplets.

5.2.2 W/O Emulsions

Figure 5.8 is a low magnification electron micrograph. It shows the typical features of electron micrographs of W/O emulsions. There are several water droplets present which have been cut through. This has revealed the pattern of the ice crystals. The pattern differs from that seen in the multiple emulsions (Section 5.2.1). The crystals in the water droplets of the W/O emulsions in this study are much smaller and show a more regular pattern. There are also droplets present which the blade has passed over. Marks in the continuous phase caused by the passage of the blade are also evident. The effect of the etching process is particularly noticeable due to the difference in level of the two phases.

The effect of etching is also evident in Figure 5.9 which is at a greater magnification. This micrograph reveals part of the interface in greater detail. Here the interface has been exposed by etching.

Figure 5.10 shows the typical micrograph produced when the fracture plane passes over or under the emulsion droplets. No internal detail of the droplets is revealed but the overall shape of the droplets is still visible.

Figure 5.5.

Electronmicrograph of internal aqueous droplets

Bar = 0.1 μ.m.

Figure 5.6.

Electronmicrograph of internal aqueous droplets

Bar = 0.1 μ.m.

Figure 5.7.

Electronmicrograph of internal aqueous droplets

Bar = 1 μ.m.

Figure 5.8.

Electronmicrograph of a water-in-oil emulsion

Bar = 1 μ.m.

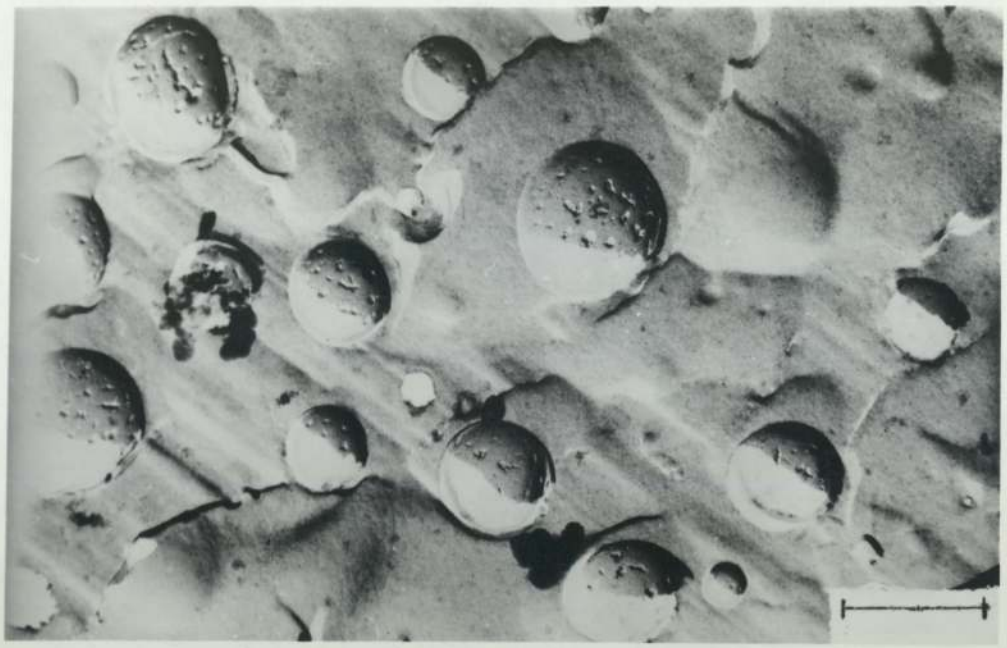


Figure 5.9.

Electronmicrograph of a water-in-oil emulsion

Bar = 0.1 μ.m.

Figure 5.10

Electronmicrograph of a water-in-oil emulsion

Bar = 1 μ.m.

However, fracture often does not occur clearly. This is illustrated in Figure 5.11. Fragments of several droplets are visible.

Figure 5.12 shows the fusion of part of two droplets. This may be part of droplet coalescence which has been interrupted.

5.3 Discussion

The greatest value of freeze-etching is obvious from an examination of the preceding electron micrographs. This is to reveal the general structure of the droplets in W/O/W and W/O emulsions. The existence of the internal droplets of multiple emulsions has been confirmed. Some of the structure of the interface of the W/O emulsions has been revealed.

As a method of particle size analysis, freeze-etching is less reliable. Nevertheless, a very approximate estimation of mean droplet diameter is still possible. This allows a corroboration of the relative sizes found by other methods; that is the multiple droplets are generally larger than the oil droplets. The size of the internal aqueous droplets can also be estimated. It appears that these droplets are about one micron or less in diameter. This estimate is similar to that made by optical microscopy (Chapter 4).

The micrographs of the W/O emulsions in this study resemble those of Eley et al (1976) although the patterns found in the oil phase in their study are not present in the emulsions described herein.

Figure 5.11.

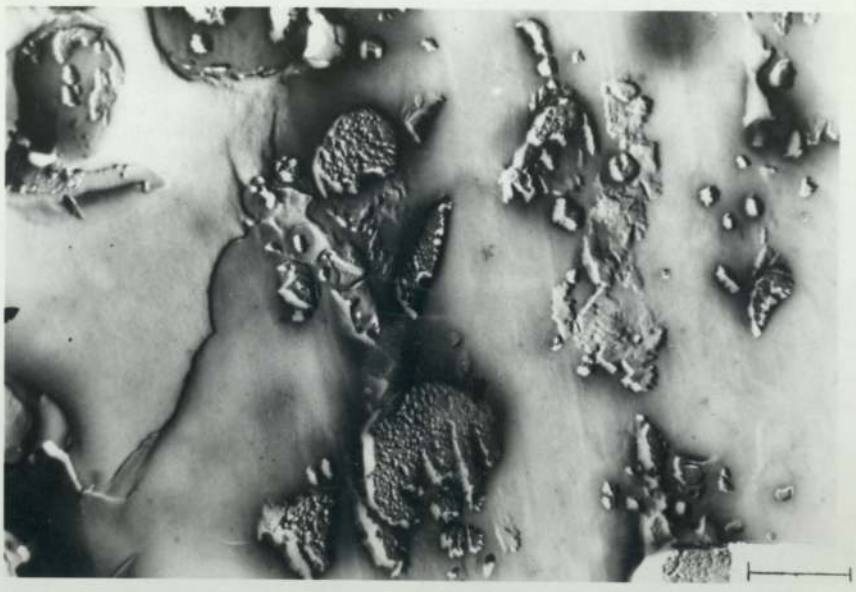
Electronmicrograph of a water-in-oil emulsion

Bar = 1 μ.m.

Figure 5.12.

Electronmicrograph of a water-in-oil emulsion

Bar = 0.1 μ.m.



Chapter 6

Chapter 6 — Coulter counter

6.1 Introduction

It was found that the thin oil film around the internal aqueous droplets could act as a semi-permeable membrane. This meant that transfer of water could occur across the oil film under the influence of an osmotic gradient. The Coulter counter was chosen as a useful instrument to study this behaviour. The electrolyte necessary for the operation of the Coulter counter provided the osmotic gradient and the changes in the water content of the internal aqueous droplets was followed by the changes in the particle size distribution.

6.2 Method

A model TA Coulter counter was used. The orifice diameter was 200μ. The instrument was calibrated using a 18.71μ polystyrene divinyl benzene latex. The electrolyte used was generally 0.18%$^{w}/_{v}$ sodium chloride solution but some experiments were performed using 0.45%$^{w}/_{v}$. The sodium chloride solutions were prepared by dilution of 0.9% sodium chloride solution Polyfusors with distilled water to give the required concentration. The diluted sodium chloride solution was filtered under positive pressure of nitrogen through a 0.8μ Millipore membrane filter.

The following procedure was adopted for particle size analysis. The instrument was switched on and allowed to warm up. The instrument was then adjusted to the calibrated settings. Fresh electrolyte was placed in the instrument beaker. The background count was checked to ensure that there was no particulate contamination or electrical interference, which seemed to be particularly prevalent when the lower electrolyte concentrations were used. A known volume of emulsion was then added to the beaker by means of a Gilson Pipetman automatic pipette. The volume to be added was determined empirically and was adjusted so that counting

was rapid enough to ensure that the experiment did not take too long but not so rapid that coincidence levels were excessive. A reading of 0.1 on the concentration index meter was found to be satisfactory on both these points.

Once the emulsion had been added to the beaker, counting was immediately started. The model TA Coulter counter has a facility to measure the amount of information displayed and to indicate when sufficient particles have been counted to give an accurate particle size distribution. When the minimum number of particles necessary had been counted, counting was stopped and the distribution was plotted out. The instrument was reset and counting was restarted. This was usually repeated until no further change in the distribution occurred. In this way several particle size distributions could be produced for the same sample in less than five minutes. The time factor was the main reason for using 0.18% sodium chloride solution to produce the osmotic gradient — use of a 0.9% solution produced shrinking which was more rapid and which only allowed one or two distributions to be produced in the time taken for equilibration.

The results are plotted in the form of a histrogram as relative percentage against equivalent spherical diameter. Figure 6.1 shows a typical series of histograms obtained for a multiple emulsion. The larger peaks around 60μ shrinks as time elapses whereas the peaks at 10μ and less increase in size. This increase is due to the shrunken multiple droplets which are of this diameter.

Once the histograms have been obtained the percentage values can be converted to absolute volumes because the volume of emulsion added is known. Thus the total volume represented by the multiple droplets can be calculated. The values of volume are plotted against time after addition to the electrolyte. The experimental procedure is repeated

Figure 6.1 Change in the bimodal distribution of a multiple emulsion under an osmotic gradient — determined by Coulter counter.

several times for fresh samples of the emulsion. All the volume against time are plotted. A typical plot is shown in Figure 6.2. This illustrates the shrinking of the multiple droplets. Also shown in the same Figure are plots for O/W and aged multiple emulsions. These illustrate that no change in volume is obtained from both these emulsions. The O/W emulsion had no internal droplets and so had no possibility for shrinking. The multiple emulsion has been stored and the water originally contained in internal aqueous droplets had diffused into the continuous phase.

Once the experimental results have been obtained and plotted, the water flux across the oil phase can be determined. The flux is the slope of the curve at any time. It is more meaningful, however, to take the slope from the initial shrinking process. This gives an estimate of the water flux across the unperturbed system. A number of calculations can be performed when the value for the water flux is known.

The permeation of water through the oil phase from internal to external aqueous phase under the influence of an osmotic gradient is controlled by the outer layer of the internal aqueous droplets. The multiple droplet can therefore be considered as a large double liquid droplet. This is illustrated in Figure 6.3. The rate of change of volume with time can be calculated using an equation of the form (Sha'afi et al, 1967):

$$\frac{dV}{dt} = - \frac{D \quad A \quad \Delta\pi}{\delta \qquad RT} \qquad (6.1)$$

where D is the diffusion coefficient of water in the oil phase,

δ is the thickness of the diffusion layer,

A is the total surface area of the oil droplets,

$\Delta\pi$ is the osmotic gradient and R and T have their usual meanings.

$\frac{D}{\delta}$ is also termed the permeability coefficient for osmotic transport (Pos).

Figure 6.2 Change in multiple emulsion volume in an osmotic gradient (0.18% sodium chloride)

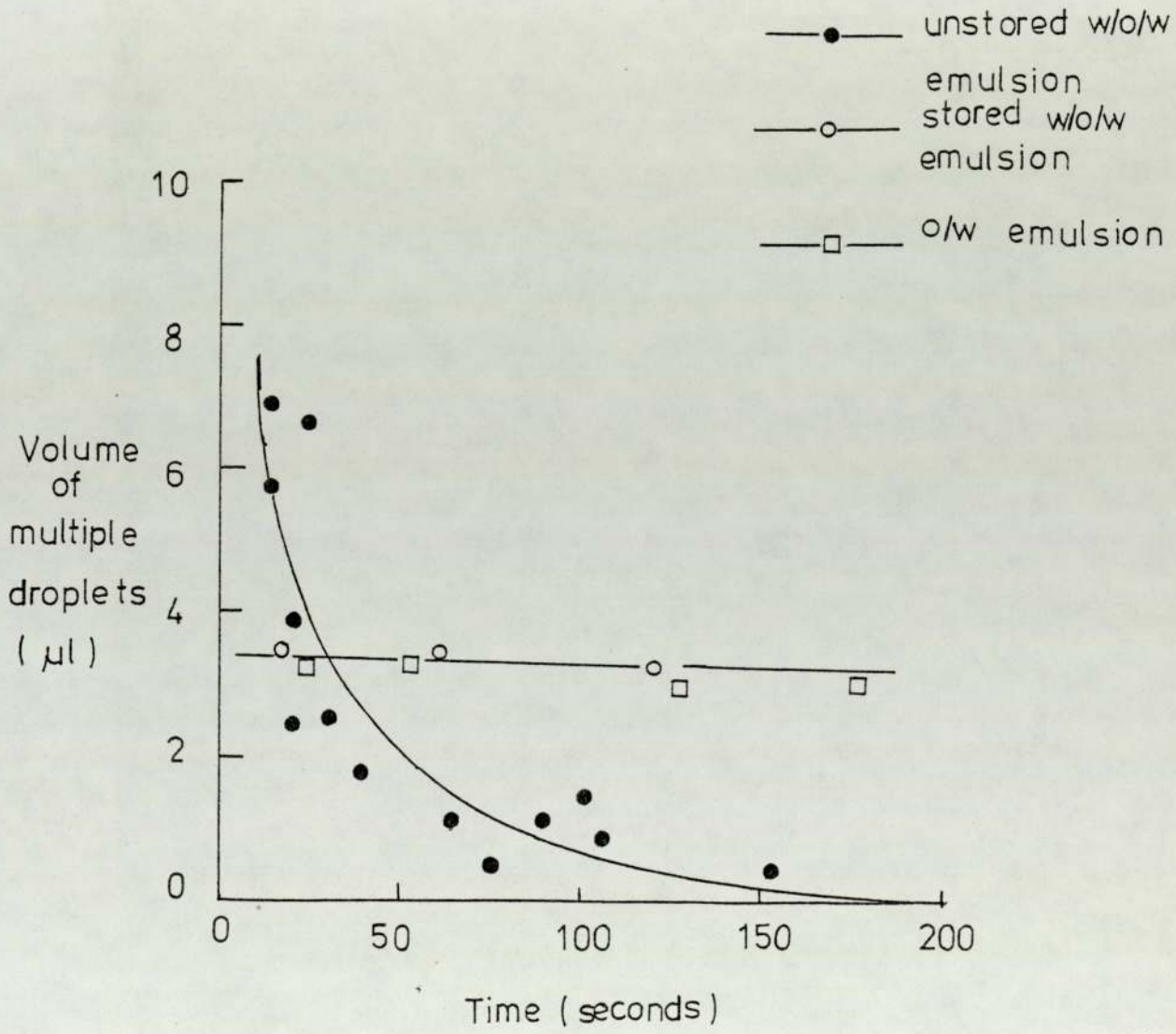
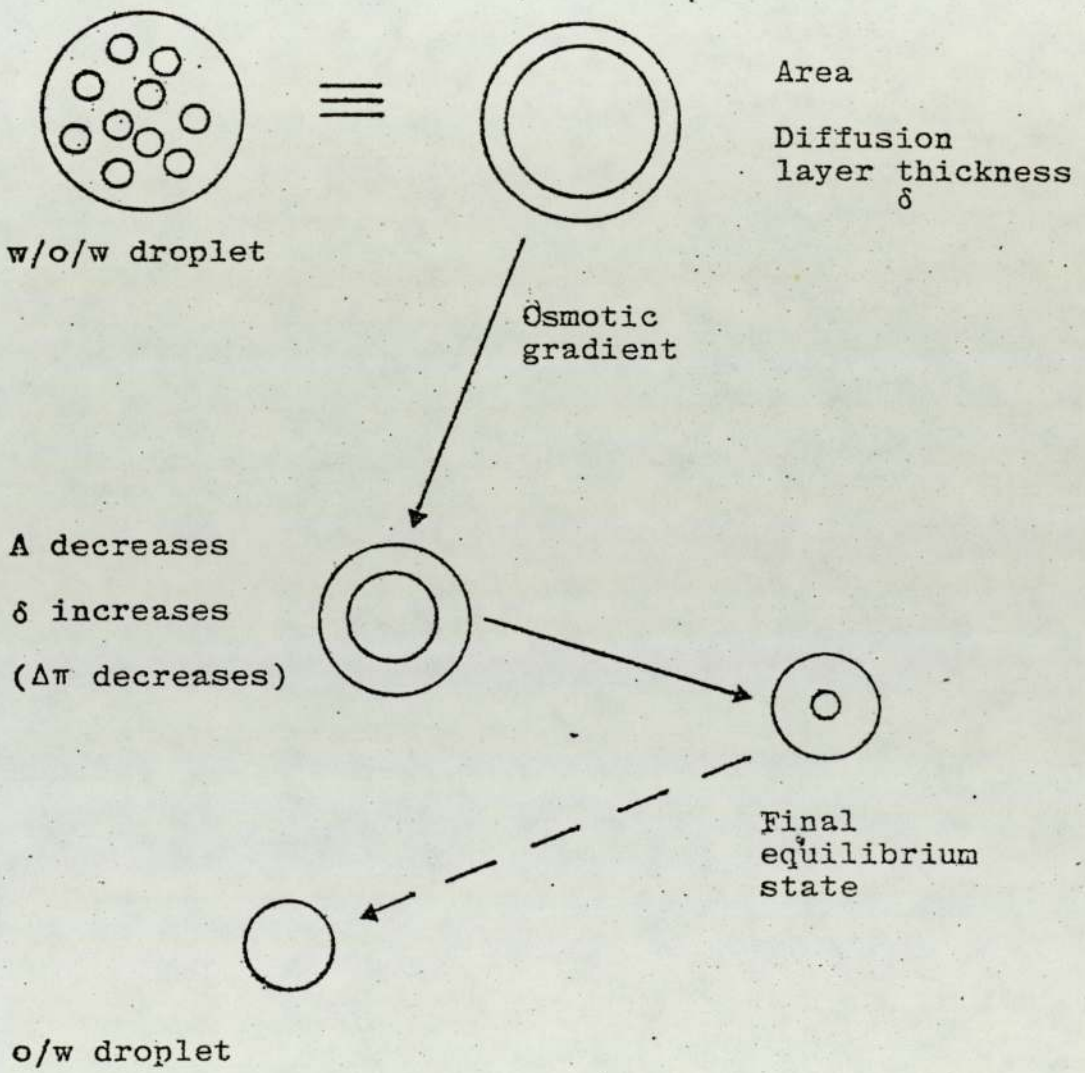


Figure 6.3

SHRINKAGE OF MULTIPLE EMULSION DROPLETS



For an osmotic gradient created by a single solute species,

$$\Delta \pi = vRT\phi M,$$

where v is the number of ionic species produced by the solute e.g. 2 for sodium chloride,

ϕ is the osmotic coefficient

and M is the molality. When the internal aqueous phase contains no electrolyte and when the emulsifier for the original W/O emulsion is non-ionic and contained almost exclusively in the oil phase then

$$\Delta \pi = 2RT\phi M.$$

The value of D for the sodium chloride can be obtained from Robinson and Stokes (1959).

A value for D, the diffusion coefficient of water in the oil phase, of 4×10^{-5} CM^2 sec can be obtained from the data presented by Schatzberg (1965).

A, the surface area of the multiple droplets, can be calculated from the Coulter counter data.

A value of $\frac{dV}{dt}$ may be measured from the Coulter counter measurements at any storage time.

Therefore, the only unknown in Equation 6.1 is δ, the diffusion layer thickness.

The phase volume ratio of the initial W/O emulsion is known, it is possible to compute the mean diameter and the number of monosize water droplets necessary to give this film thickness. The mean diameter is a derived, not a measured, parameter but may still be used to assess the behaviour on storage of the multiple emulsions.

6.3 Results

6.3.1 Comparison with optical microscopy

Apart from its main use in this work for studying the transport of

water from multiple emulsion droplets, particle size analysis is also possible. As the diameters are rapidly shrinking the best estimate must be the first histogram plotted. Figure 6.4 compares the particle size distributions for the multiple droplets of one emulsion as determined by optical microscopy and by Coulter counter. The distributions differ considerably. However, the diameters determined by microscopy are projected length diameters and those from Coulter counter analysis are equivalent spherical diameters. Herdan (1960) described equations for inter-conversion of mean diameters by length, area and volume. The relevant equation is:

$$\ln dv = \ln d + 3\ln^2 S.$$

dv is the mean volume diameter, d is the mean length diameter and S is the standard deviation. The standard deviation should remain constant for all distributions if they are log-normal. Applying this equation to the curves in Figure 6.4 a converted value for the mean diameter by Coulter count of 10.64 μ . This lies close to the actual diameter, 10.50 μm indicating that in this case the first particle size distribution plotted gives an accurate figure for the mean particle diameter.

6.3.2 Reproducibility

The reproducibility of the technique was investigated. Two emulsions of identical formulae were prepared. These were then subjected to Coulter counter analysis using the method described above. The results are shown in Figure 6.5. The volume of multiple droplets is plotted against time of exposure to the osmotic gradient. Points are plotted for several duplicate experiments of each emulsion. The points correspond well. The water flux can be calculated by taking the tangent to the curve at any desired point. It is usual to take the gradient from the first part of the curve. This gives the value for the flux when the system is least perturbed by the

Figure 6.4 Comparison of the particle size distribution of a multiple emulsion determined by Coulter counter and microscopy.

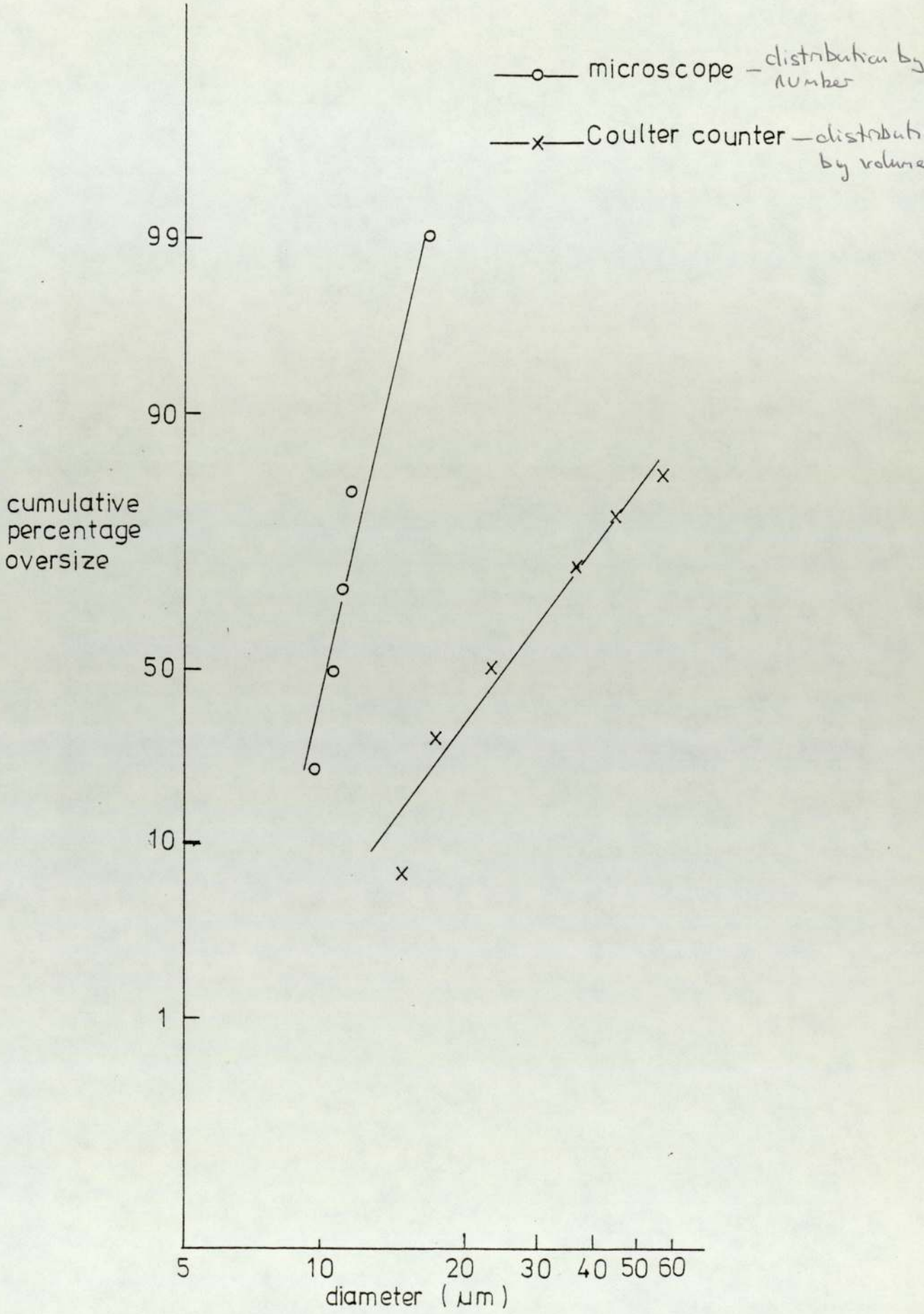
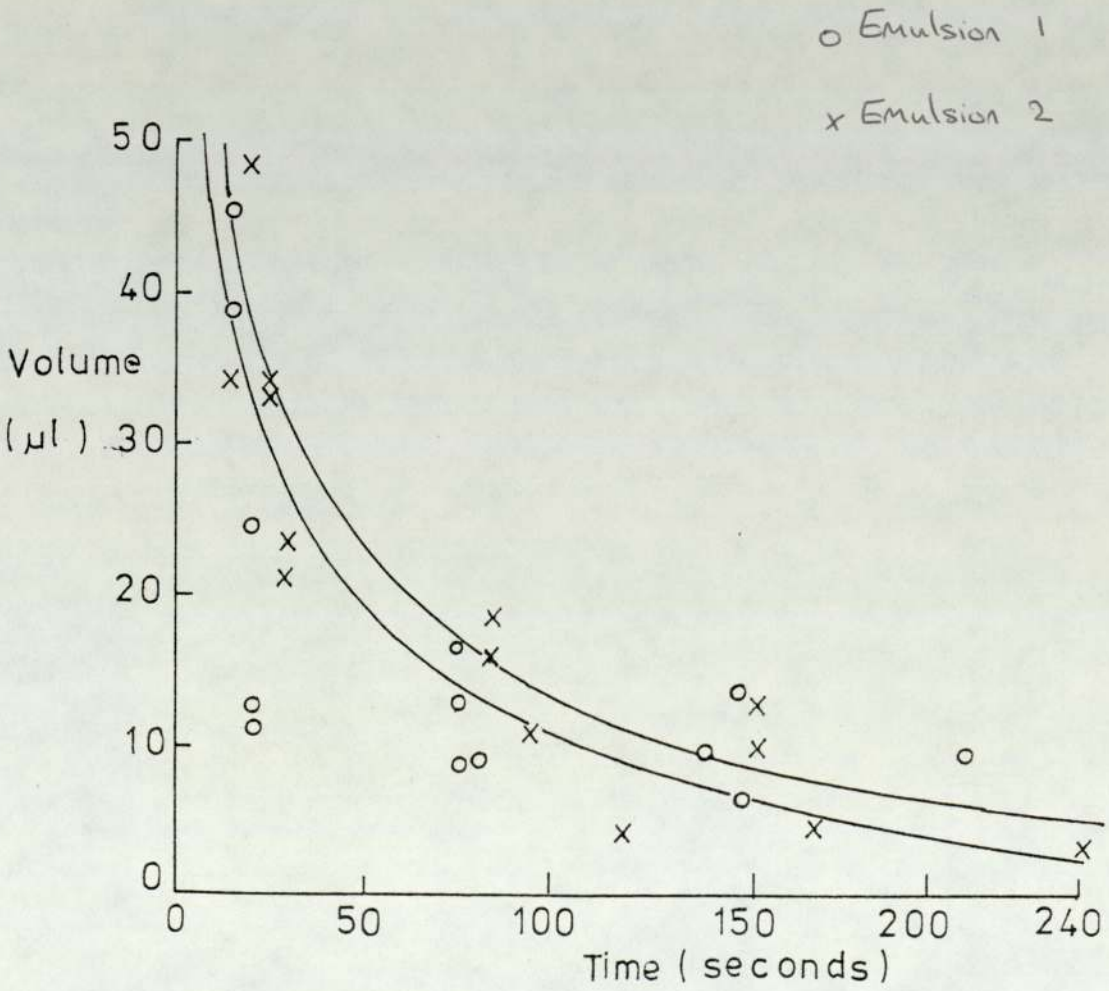


Figure 6.5 Behaviour of similar multiple emulsions in an osmotic gradient.



experimental conditions. The initial values for the water flux for the two emulsions compared in Figure 6.5 are 1.90 x 10^{-4} and 1.43 x 10^{-4} cm /sec. The relevant values for the osmotic permeability coefficients are 5.92 x 10^{-4} and 6.27 x 10^{-4} μ/sec. The values for the diffusion layer thicknesses were calculated as described above to be 0.014μ and 0.016 μ and those for the calculated radii of the internal aqueous droplets were 0.022 μ and 0.026μ. These values are to all intents and purposes the same

6.3.3 Formulation Factors Affecting Initial Multiple Emulsion

In this section, the effect of various formulation factors on the properties of the initial emulsion will be shown.

The most striking property of the multiple emulsions examined by Coulter counter was the variation in the proportion of multiple droplets. This was measured as the percentage by volume of the larger diameter peak of the biphasic particle size distribution. Figure 6.6 shows the effect of varying the phase volume ratio of the W/O emulsion, W/O, on the proportion of multiple droplets produced. The trend of a biphasic pattern of yield of multiple droplets was also seen on examination by optical microscopy (Chapter 4). However, the reason for this effect is not clear. Apparently continuous phase is drawn into the oil droplets during the second mixing process. The extreme of this effect was noted when emulsions prepared with no added internal aqueous phase showed a second peak of multiple droplets in the particle size distribution as determined by the Coulter counter. The difference between this situation and an O/W emulsion was that the multiple emulsion with no added internal aqueous contained a lipophilic emulsifier in the oil phase which could stabilise any water droplets formed during manufacture or subsequently.

The effect of other factors can also be compared in a similar manner. For example, Tables 6.1 and 6.2 show the yield of multiple droplets

produced by varying the lipophilic emulsifier in 2 different oils.

Table 6.1 illustrates the effect in light liquid paraffin while Table 6.2 shows the data for cottonseed oil. With light liquid paraffin, Arlacel 83 produced the greatest volume of multiple droplets at concentrations of 10 and 20% . However, in cottonseed oil, Arlacel A is superior to Arlacel 83 at 20% concentration. This is due to the different natures of the two oils and their chemical compatibility with the emulsifiers. For instance, an emulsion stabilised with 10.4% Span 85 in squalane produced a low yield of multiple droplets (3%) and this was discovered to be because Span 85 is almost insoluble in Squalane.

Table 6.3 illustrates the effect of increasing the hydrophilic emulsifier concentration on the yield of multiple droplets. The increase in emulsifier concentration causes an increase in the yield of multiple droplets. The concentrations necessary to produce high yields are much greater than the concentration needed to give a monomolecular layer over all the droplets. Therefore, the mechanism of stabilisation must be more complex.

Figure 6.7 shows the effect of varying the phase volume ratio of the second emulsion on the yield of multiple droplets. Increasing the phase volume ratio decreases the yield. The results from Figures 6.6 and 6.7 have been combined and replotted in Figure 6.8. This shows the relationship between the total amount of water present in the emulsion (in both the internal and continuous aqueous phases) and the yield of multiple droplets. For water contents of greater than 55%, there is a direct relationship between water content and yield. This suggests that during manufacture, the violent mixing means that the 2 aqueous phases can be exchanged. In fact, some continuous phase must be included inside the oil droplets. The increase in yield with decreasing water content below 50%

Figure 6.6 Effect of $\phi_{w/o}$ on the yield of multiple droplets.

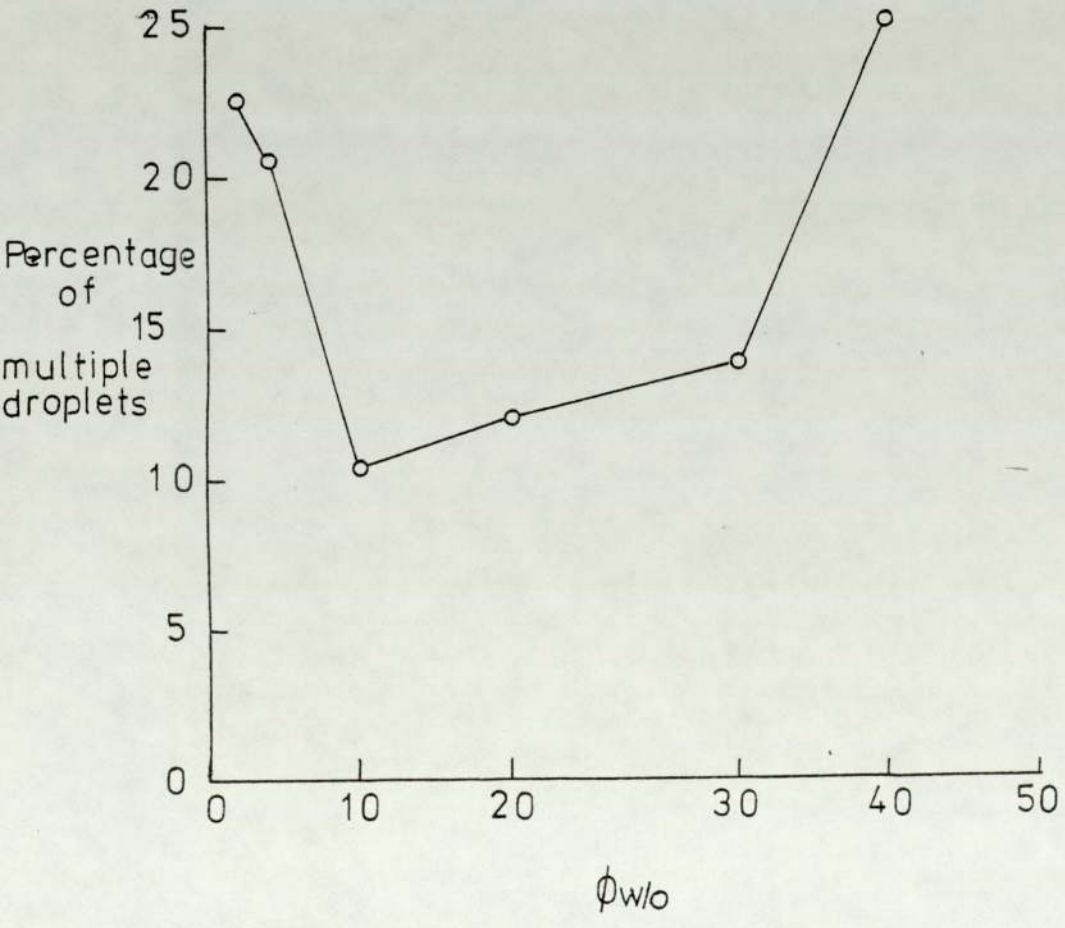


Figure 6.7 Effect of $\phi_{w/o/w}$ on the yield of multiple droplets.

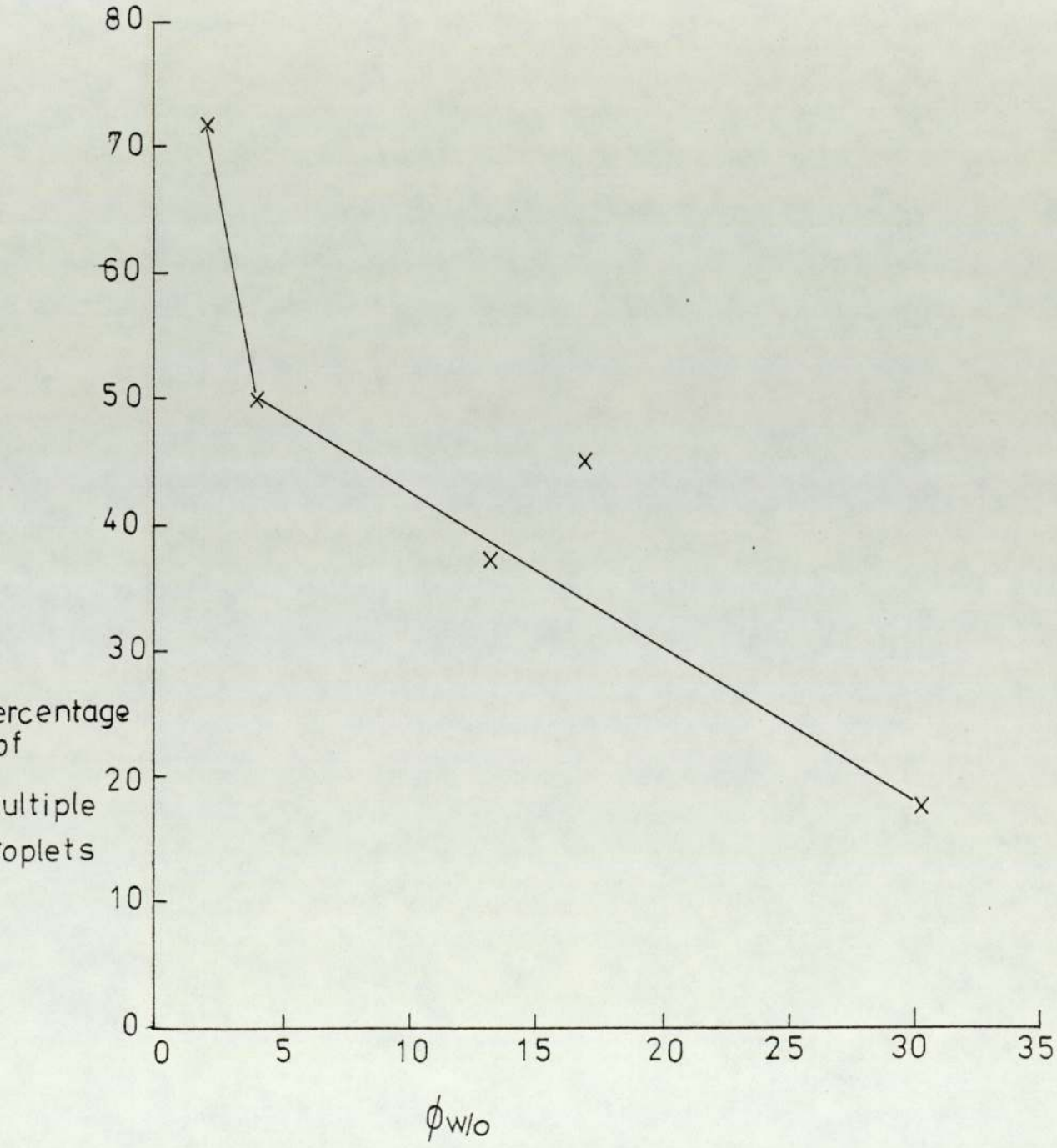


Figure 6.8 Effect of total water content on the yield of multiple droplets.

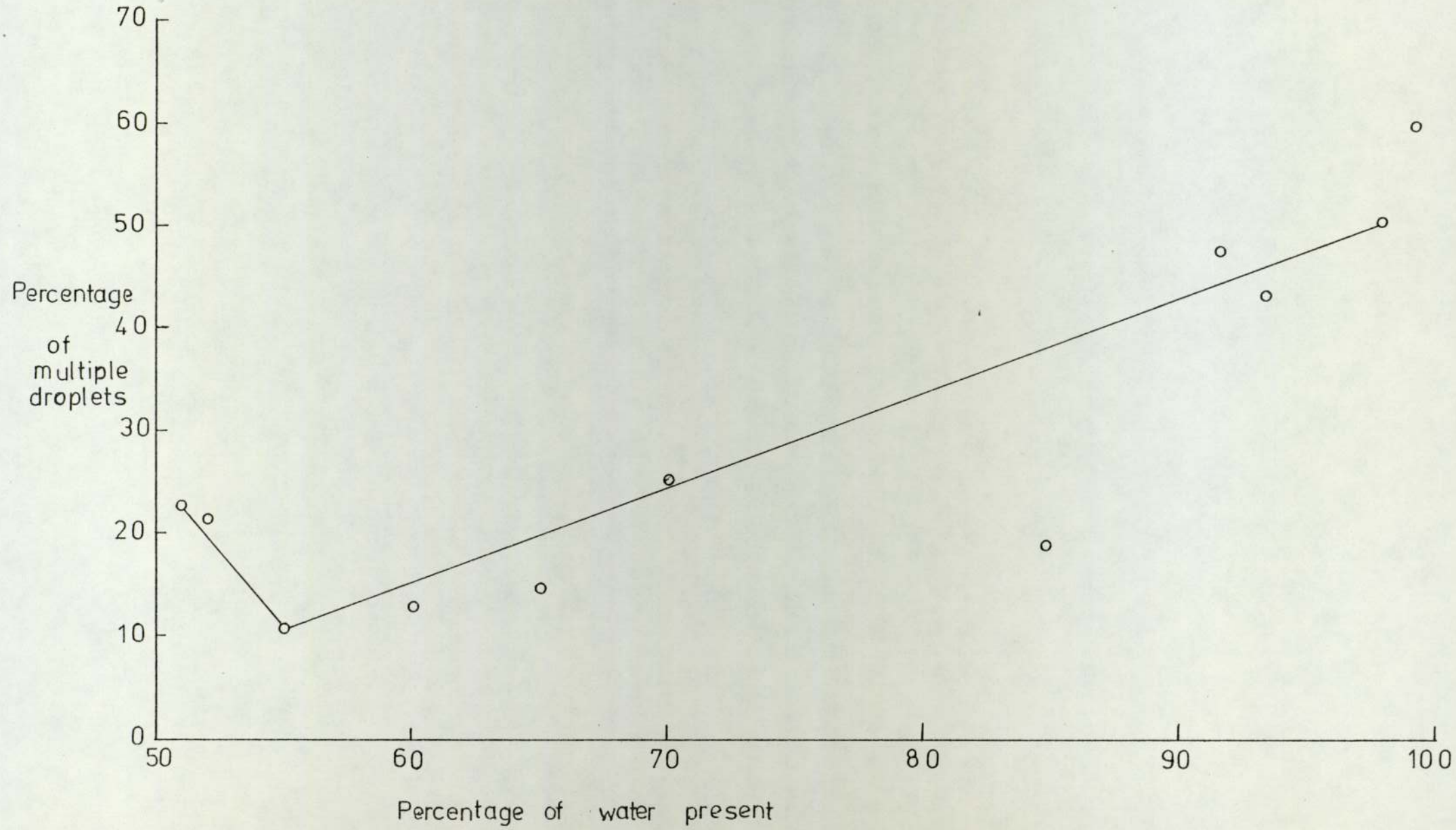


Table 6.1 Effect of different lipophilic emulsifiers on percentage volume of multiple droplets produced with light liquid paraffin as the oil phase.

Lipophilic emulsifier	% volume of multiple droplets
10% Arlacel 83	20.0
10% Atmos 300	8.0
20% Arlacel 83	25.3
20% Arlacel A	6.5
20% Atmos 300	6.25
20% Myvacet 9-40	14.6
30% Span 80	30.0

Table 6.2 Effect of different lipophilic emulsifiers on the percentage volume of multiple droplets produced with cottonseed oil as the oil phase.

Lipophilic emulsifier	% Volume of multiple droplets
20% Arlacel 83	12.7
20% Arlacel A	17.0
20% Atmos 300	8.3
20% Myvacet 9-40	0

Table 6.3 Effect of hydrophilic emulsifier concentration on percentage volume of multiple droplets.

Tween 20 concentration ($\%^{w}/_{v}$)	Percentage volume of multiple droplets
1.0	15.0
2.0	53.0

water content has been described previously.

6.3.4 Storage of Multiple Emulsions

The yield of multiple droplets is very useful for an initial assessment of formulation factors but the rate of change of the characteristics of the droplets is just as important. One way to evaluate the change using data determined on the Coulter counter is to calculate the diffusion layer thickness and droplet diameters. Table 6.4 shows these values calculated for a series of emulsions with varying values of $\phi_{w/o}$. The trend for the initial values of the calculated droplet diameter may be more clearly seen in Figure 6.9. The calculated diameter appears to be related to $\phi_{w/o}$ i.e. as the water content of the original W/O emulsion increases, so the derived diameter increases. With one exception the diffusion layer thickness is broadly constant for the initial emulsions. The effect of storage of the multiple emulsions is also shown in Table 6.4. The values of both δ and d increase with storage.

Table 6.5 shows the effect of $\phi_{w/o/w}$ on the yield of multiple droplets. The effect of storage is also seen. The yield is related to $\phi_{w/o/w}$ in the manner described earlier i.e. the yield is proportional to the total amount of aqueous phases present in each emulsion. The high values for total amount of water present in the emulsions in Table 6.5 of course produce high yields of multiple droplets. However, these droplets are less stable and after 32 days the emulsions have all lost all the multiple droplets and are O/W emulsions.

6.4 Discussion

The use of the Coulter counter to characterise multiple emulsions has been illustrated. By measuring the rate of change of the particle size distribution in an osmotic gradient, several parameters amy be calculated to describe the changes of multiple emulsion during storage. These fit in

Figure 6.9 Effect of $\phi_{w/o}$ on the calculated diameter of the internal aqueous droplets.

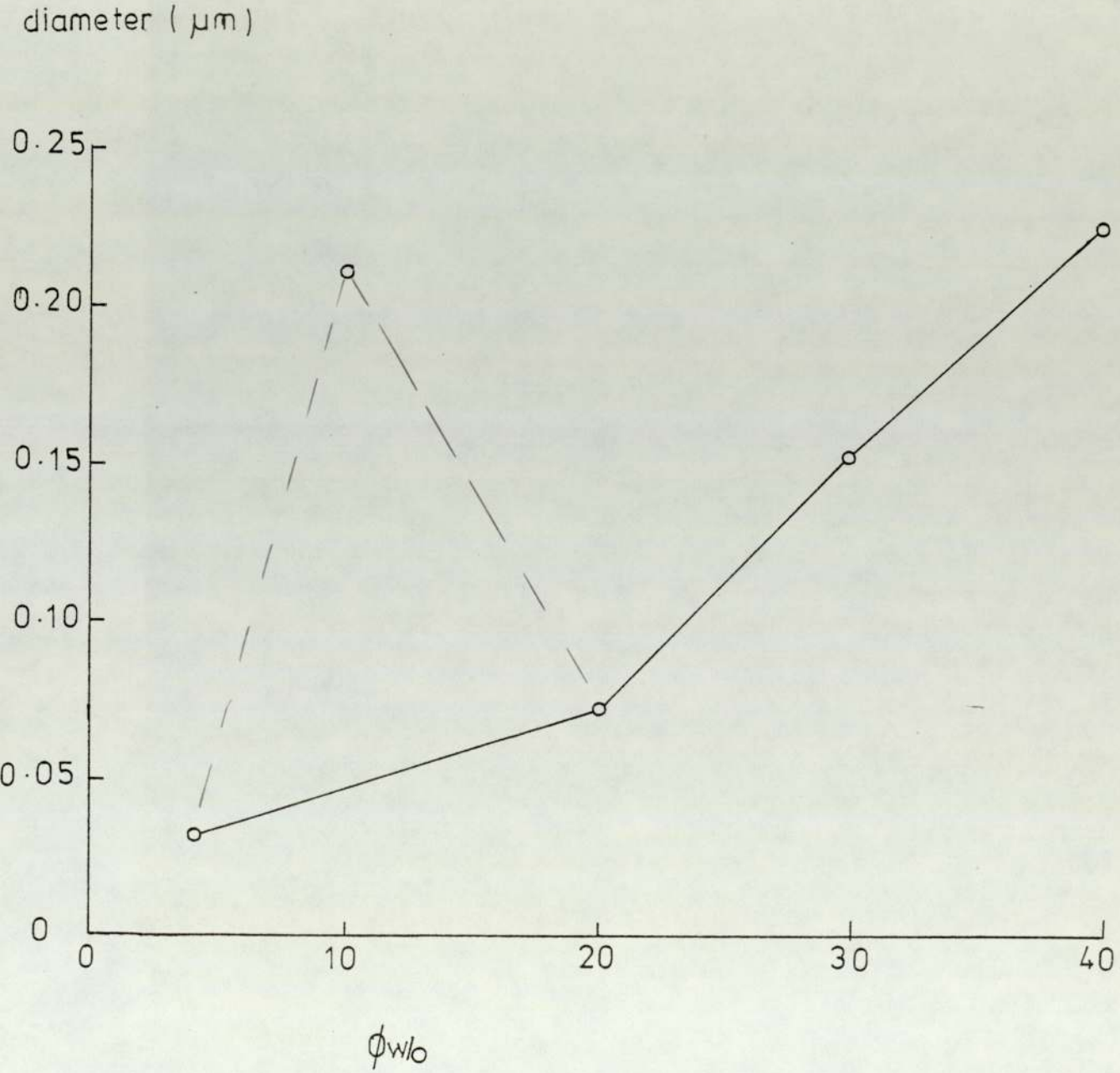


Table 6.4 Change in calculated values of δ and d (in microns) for emulsions with differing values of $\phi_{w/o}$

$\phi_{w/o}$		Storage Time (days)			
		0	4	32	60
0.02	δ	N.D	0.05	0.39	2.11
	d	N.D	0.02	0.13	0.35
0.04	δ	0.06	0.03	0.10	0.05
	d	0.03	0.01	0.05	0.01
0.10	δ	0.21	0.02	0.03	0.04
	d	0.21	0.02	0.03	0.04
0.20	δ	0.04	0.09	0.09	0.23
	d	0.07	0.16	0.15	0.22
0.25	δ	N.D	0.02	0.04	0.04
	d	N.D	0.03	0.09	0.20
0.30	δ	0.06	0.08	0.10	0.47
	d	0.15	0.21	0.27	1.30
0.40	δ	0.06	0.09	0.09	0.17
	d	0.23	0.38	0.41	0.73

N.D = not determined

Table 6.5 Effect of varying $\phi_{w/o/w}$ on the yield of multiple droplets and the variation with storage time.

$\phi_{w/o/w}$	% Volume of multiple droplets		
	0 days	5 days	32 days
2.2	59.0	18.7	0
4.2	50.1	15.7	0
7.4	5.25	9.4	0
13.4	37.0	25.5	0
17.0	45.0	11.6	0
30.4	17.1	12.5	0

well with the results from other techniques. The loss of the internal aqueous droplets on ageing may be followed using the derived parameters. The diffusion layer thickness increases with time. This is due to loss of the internal aqueous droplets increasing the distance between the aqueous droplets. The calculated diameters also increase with time. This is due to coalescence of the internal aqueous droplets which still occurs as in a W/O emulsion which is not enclosed in an oil droplet.

The Coulter counter may also be used to measure the relative volumes of oil and multiple droplets. This ratio can then be used to compare various manufacturing and formulation variables. The greatest effect on the yield was produced by varying the proportion of the aqueous phases within the emulsions. However, these emulsions with the greatest proportion of multiple droplets are not as stable as those with lesser yields. This may be due to the low oil proportion in those emulsions with high yields. The low oil level is of course associated with low amounts of lipophilic emulsifier. Therefore, a large volume of water droplets may be formed but there is not enough surfactant present to stabilise the droplets and so they rapidly coalesce and pass into the continuous phase.

The effect of the proportion of water on the proportion of multiple droplets formed has several implications;

Drug incorporation may be complicated. If a portion of what was added as continuous phase is present in the internal aqueous droplets, the concentration of drug and any other additive will be affected. This will affect the release rate.

Hydrophilic emulsifier may be included in the internal aqueous droplets. This might affect the stability of the internal droplets. In fact this may be the reason for the low stability of those emulsions prepared with high water contents. Also, the presence of micelles in the internal aqueous phase would affect drug release rates.

This interchange between internal and continuous aqueous phases may be due to the violent mixing method employed. If less vigorous agitation was employed, less exchange might occur.

Chapter 7

Chapter 7 - Radiotracer Experiments

One of the properties of multiple emulsions that is of interest is the behaviour of the internal aqueous phase. The proportion of aqueous droplets in the oil phase is an important variable that is affected by all the formulation and manufacturing factors discussed in previous chapters. The rate at which the internal aqueous phase migrates into the continuous phase determines, to a large extent, the stability of the multiple emulsion. The techniques described in previous chapters only indirectly assess the behaviour of the internal aqueous phase by changes in particle size distribution. In particular, the Coulter counter method measures the behaviour under a stress, induced by an osmotic gradient. It seemed desirable to find a method which would observe the behaviour of the internal aqueous phase without perturbing the system. Several marker molecules were considered but practical problems were encountered. In any case, the behaviour of marker molecules might not match that of the aqueous phase. Therefore, the most suitable marker for the aqueous phase was tritiated water. It would mark the aqueous phase, it was easy to measure accurately and it would not perturb the emulsion.

However, this study was only designed to serve as an introduction to the method. The intention was to try out the technique to see if the behaviour of the internal aqueous phase could be characterised by tritiated water and perhaps to illustrate the factors that would need investigation by later studies.

7.1 Method

The transfer of tritiated water into and out of multiple emulsion droplets was studied using a perspex diffusion cell. This is shown in Figure 7.1. It consisted of a hollow perspex cylinder which could be

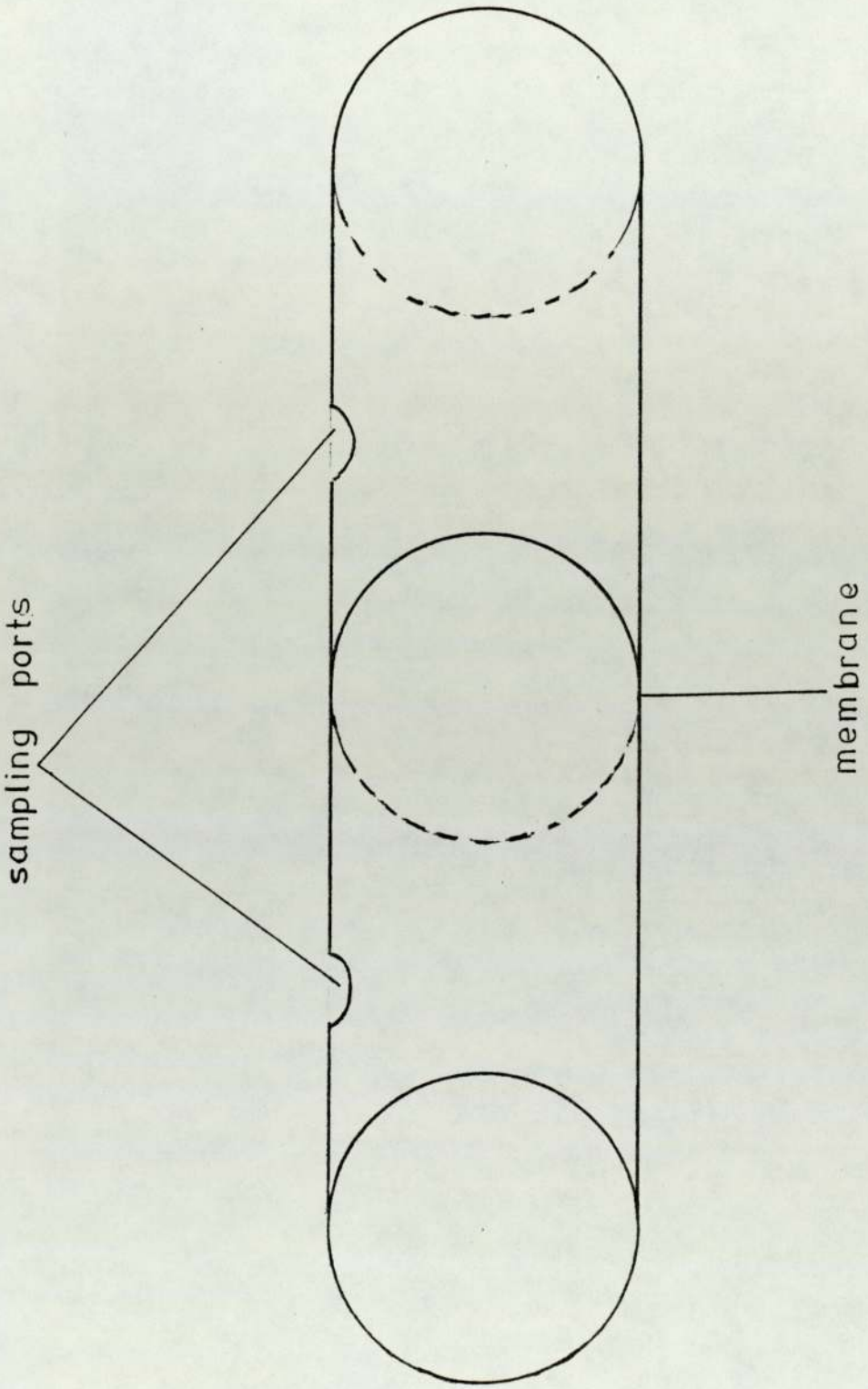


Figure 7.1 Diagram of the diffusion cell used in the radiotracer experiments.

divided into 2 equal compartments by means of a membrane. Each compartment could be sampled separately by means of small ports.

The experimental system involved placing a sample of emulsion in one compartment and distilled water in the other.

The labelled water was placed in the internal aqueous droplets of the emulsion, the continuous phase of the emulsion or in the distilled water compartments, according to the type of transport to be studied. The 2 compartments were filled with the appropriate fluids and the clock was started. Samples were taken from the distilled water compartment at time intervals. Sampling was by means of a Gilson Pipetman automatic pipette. The use of this pipette allowed an accurately known small volume to be sampled. The radiation in the sample was then assayed using a liquid scintillation spectrophotometer. The scintillation cocktail used consisted of:

Toluene (scintillation grade)	2L.
Triton X-100	1L.
2,5 - Diphenyloxale (PPO)	15g.
1.4-Di-2-(4-methyl-5-phenyloxazoly) benzene (DMPOPOP)	300 mg.

This scintillation cocktail could take up to 10% of its own weight of water. In fact, it was found that the most reproducible results were obtained when 1ml of distilled water was added to 10ml of cocktail. Because sample volumes were less than 10μl, 1ml of distilled water was

added to each vial before counting.

The liquid scintillation spectrophotometer was operated using an Automatic External Standard method of assessing the degree of quenching of each vial. After counting the sample, a known amount of a radioactive standard was introduced into the counting chamber. Counting was restarted and the ratio of actual counts to theoretical counts was the AES ratio. A quench correction curve had been constructed for the instrument and this allowed the counting efficiency to be read off directly against the AES ratio. The number of disintegrations per minute could be calculated from the number of counts per minute. The quench correction curve is shown in Figure 7.2. A calibration curve for tritiated water was constructed by determining the number of counts for known amounts of tritiated water. This curve enabled disintegrations per minute to be converted into the amount of radioactivity present in the vial. The calibration curve is shown in Figure 7.3.

7.2 Results

The choice of membrane for transport studies is important. It must allow rapid equilibration of the permeant species in comparison with the rate of release or uptake from the emulsion droplet. The property of the emulsion under study, either uptake or release, must be the rate-limiting factor. To determine the effect of the chosen membrane on the rate of equilibration, an experiment was conducted in which tritiated water was placed in one compartment and its rate of appearance in the distilled water in the other compartment was monitored. The results of a typical experiment for a membrane prepared from dialysis tubing are shown in Figure 7.4. Equilibration was complete in less than 30 minutes. This was considered to be sufficiently rapid in comparison with the times taken for radio-labelled emulsions. Therefore, the membrane used in all

Figure 7.2 AES ratio quench correction curve for the liquid scintillation spectrophotometer.

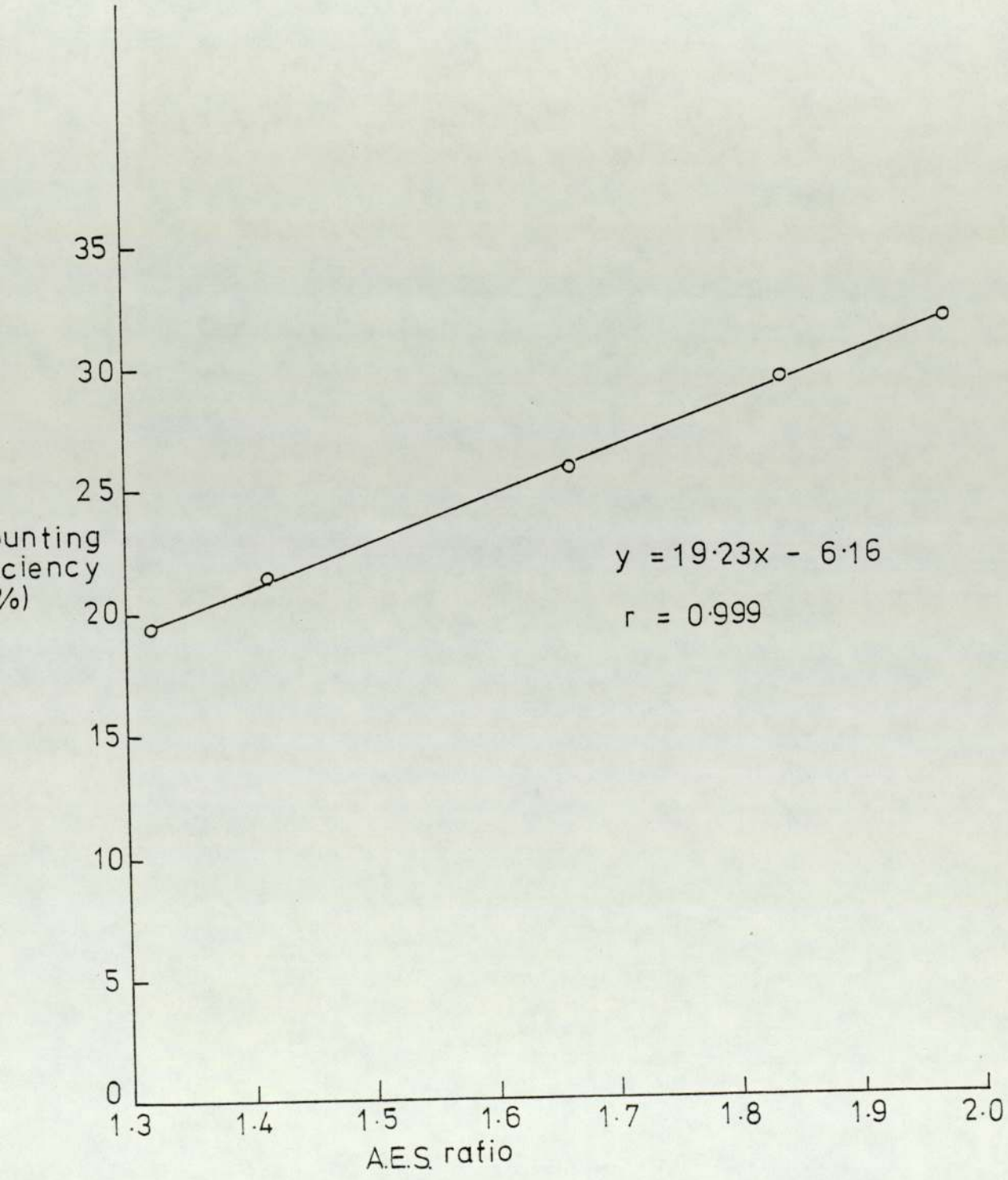


Figure 7.3 Calibration curve for tritiated water.

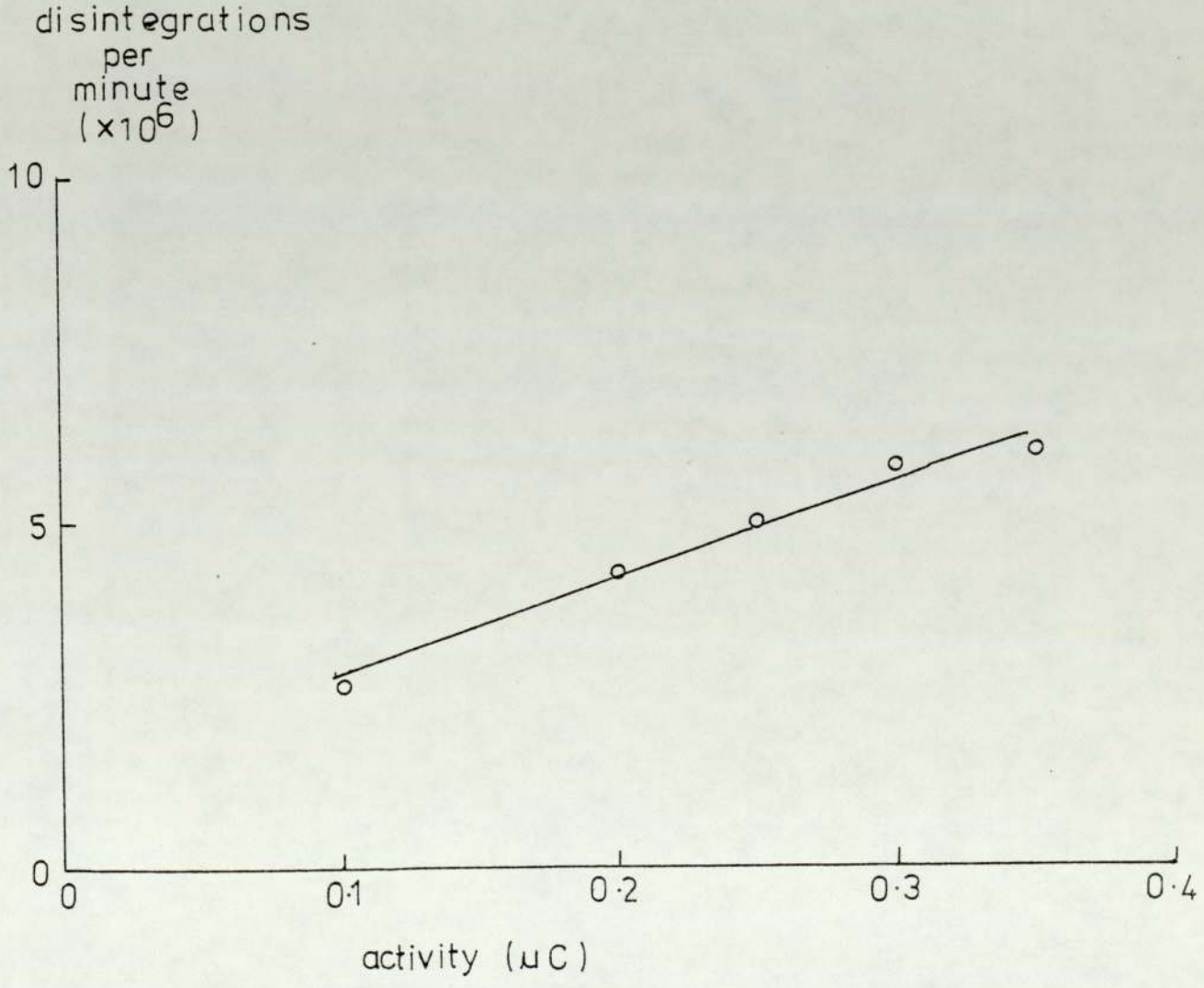
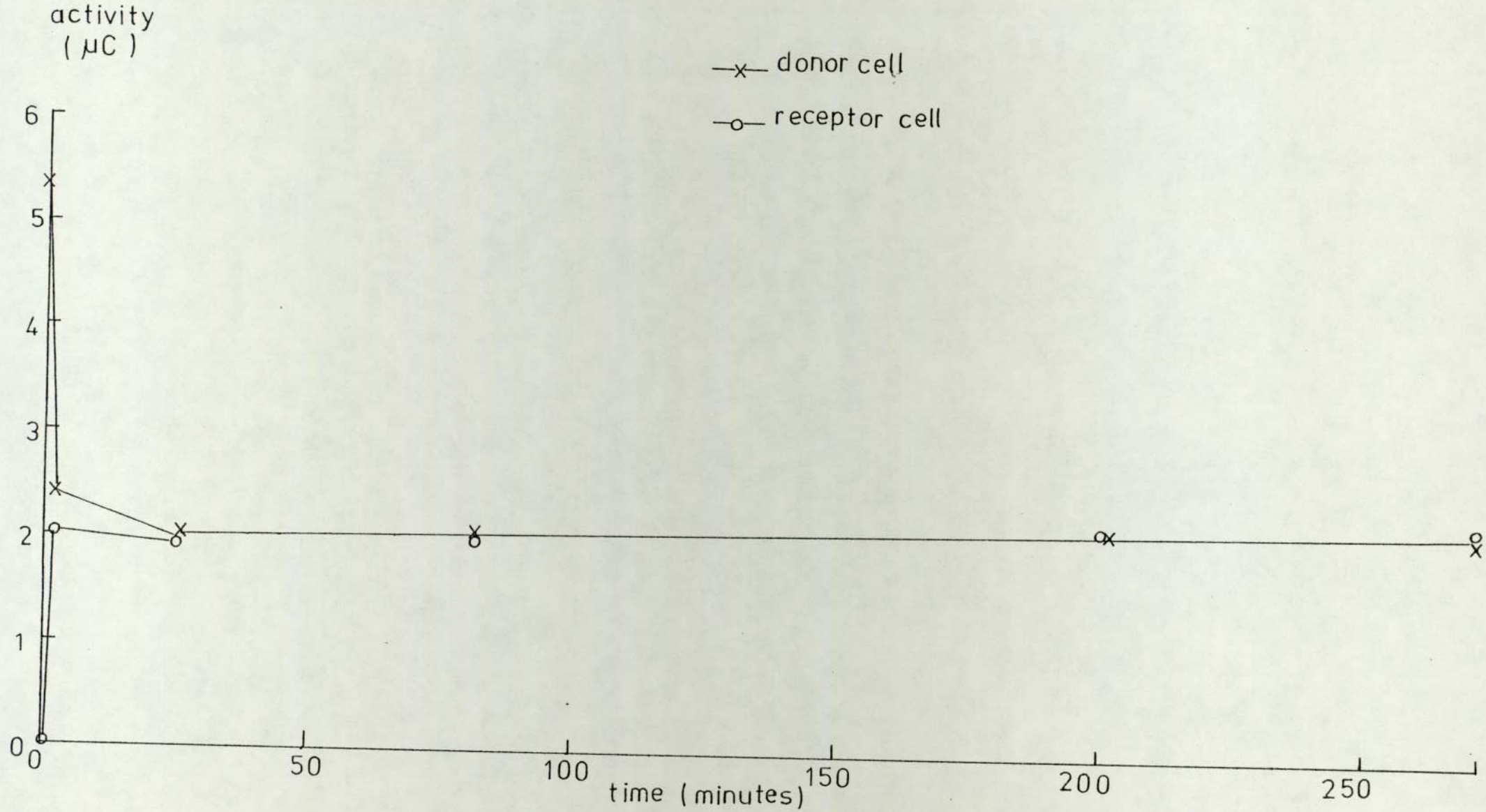


Figure 7.4 Diffusion of tritiated water across a membrane of dialysis tubing.



subsequent studies was dialysis tubing.

Figure 7.5 shows the typical sort of curve for the 3 different types of sample. The curve for the control shows the transport of labelled water across the membrane. The only barrier is the membrane and so rapid equilibration occurs. The curve for the O/W emulsion shows the barrier to transport provided by the presence of the droplets. In this case, of course, the tritiated water was placed in the continuous phase of the emulsion. However, equilibration is still complete in 85 minutes. The curve for the W/O/W emulsion shows an even greater delay. The biphasic release pattern seemed to be a common property of many of the multiple emulsions studied. The reason for this behaviour will be discussed below. Figure 7.5 illustrates the typical release patterns of the emulsions studied. The diagram also illustrates the way in which an O/W emulsion could be used to assess the decrease in release produced by the presence of the tritiated water in the internal aqueous phase.

Figure 7.6 shows typical release curves for an O/W emulsion. The release rate appears to be dependent on the concentration remaining and so the concentration in the receptor cell reaches an equilibrium value when the concentrations of the tritiated water are equal in both compartments.

Figure 7.7 shows a typical release curve for a multiple emulsion. A biphasic release pattern is observed. The release rate for the initial more rapid phase was 0.0033 μC/minute. This is close to the rate of release from the O/W emulsion shown in Figure 7.6 which was 0.0035 μC/minute. The release rate for the second less rapid phase was 0.0017 μC/minute. It appears that the two different release rates can be related to the two different types of aqueous phase in a W/O/W multiple emulsion. The initial release rate appears to be from the external aqueous phase

Figure 7.5 Release of tritiated water from O/W and multiple emulsions.

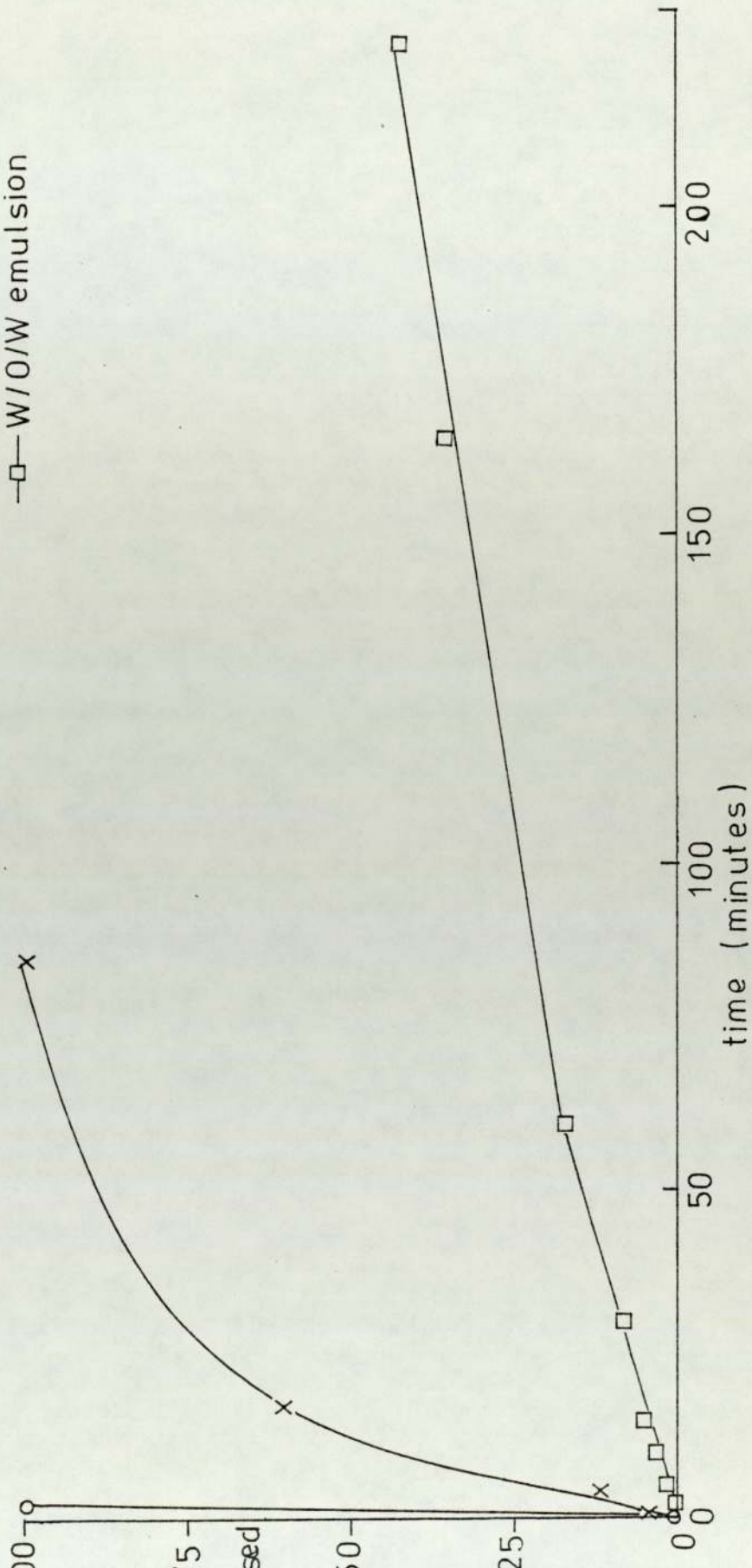


Figure 7.6 Release of tritiated water from an O/W emulsion.

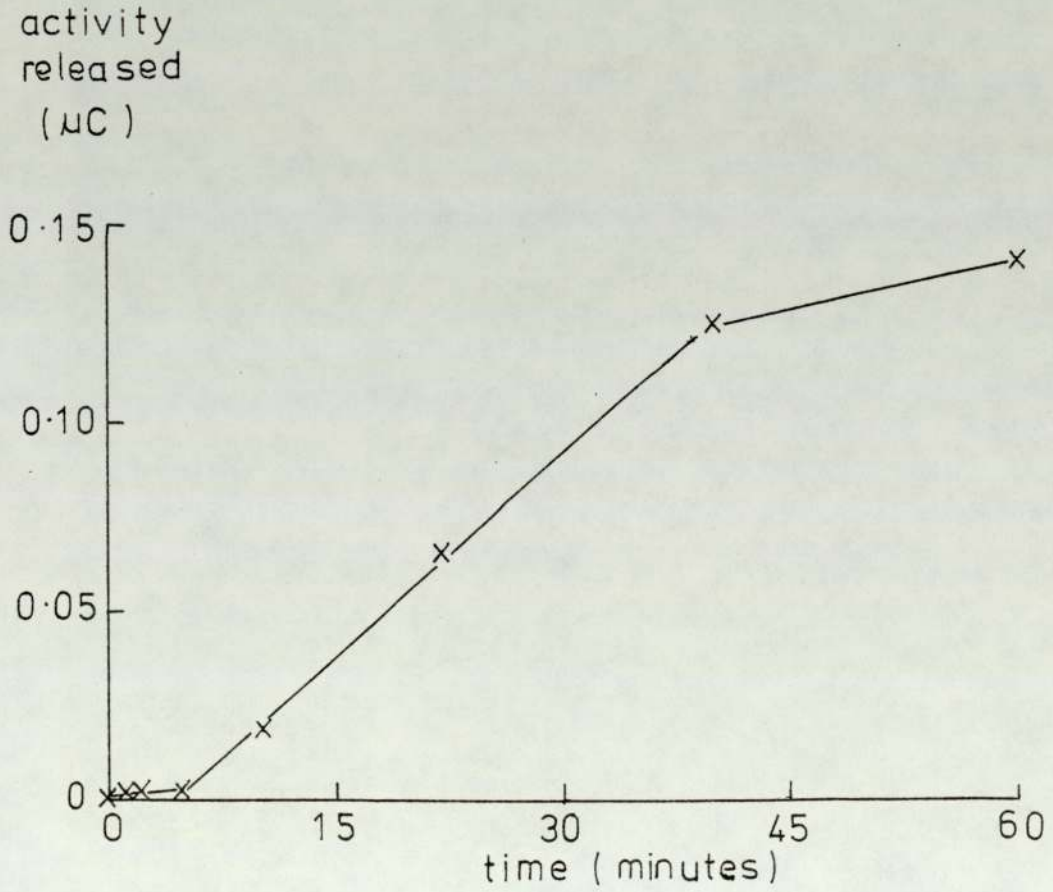
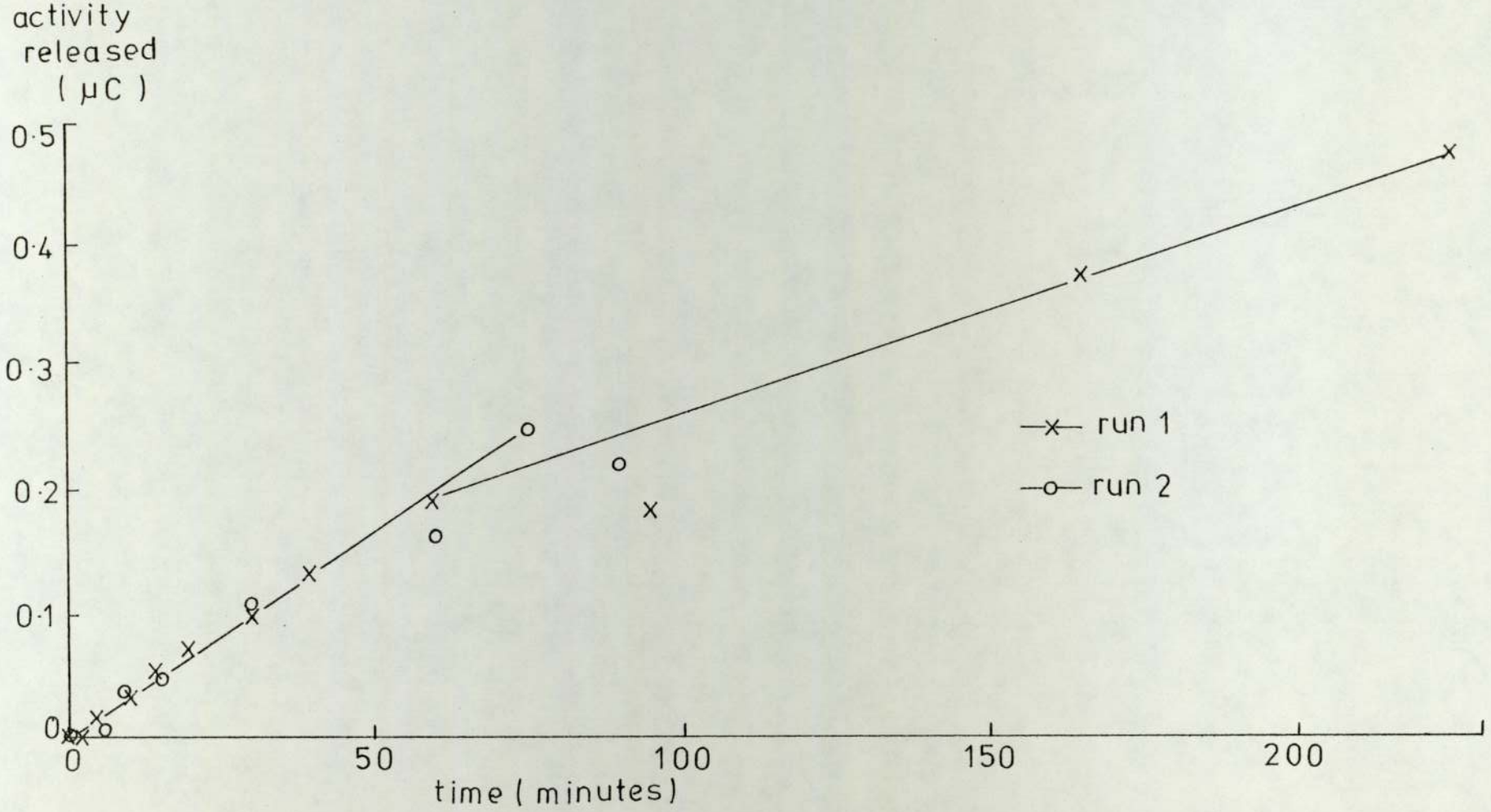


Figure 7.7 Release of tritiated water from a multiple emulsion.



and this explains why the release rate for this phase is close to the release rate for an O/W emulsion. The second phase appears to be caused by the release from internal aqueous droplets. This rate is lower than the initial rate because diffusion across the oil phase is also necessary.

However, it was decided that the preparation of radio-active emulsions caused a restriction on the use of such an emulsion after its manufacture. Therefore, the possibility of "challenging" the emulsion with a radiotracer was investigated. This would allow a small sample to be "challenged", leaving the remainder uncontaminated.

One method to provide a challenge using tritiated water uses the apparatus described above. However, the emulsion under study is placed in the receptor compartment and tritiated water is placed in the donor compartment. The compartments are stirred and the donor cell is sampled to follow the change in concentration with time. A typical uptake curve for an O/W emulsion is shown in Figure 7.8. After a rapid equilibration, the concentration remains constant. The release curve for a W/O/W emulsion is seen in Figure 7.9. There was a rapid initial fall in the concentration in the donor cell. This was followed by further slower uptake until an equilibrium value is reached.

The release pattern can also be studied by adding tritiated water to a sample of the emulsion. The release rate can be studied after sufficient time has been allowed for equilibration of the tritiated water within the multiple emulsion. This is illustrated in Table 7.1. The table shows the initial rate of release of tritiated water from 2 typical emulsions.

Figure 7.8 Uptake of tritiated water by a multiple emulsion.

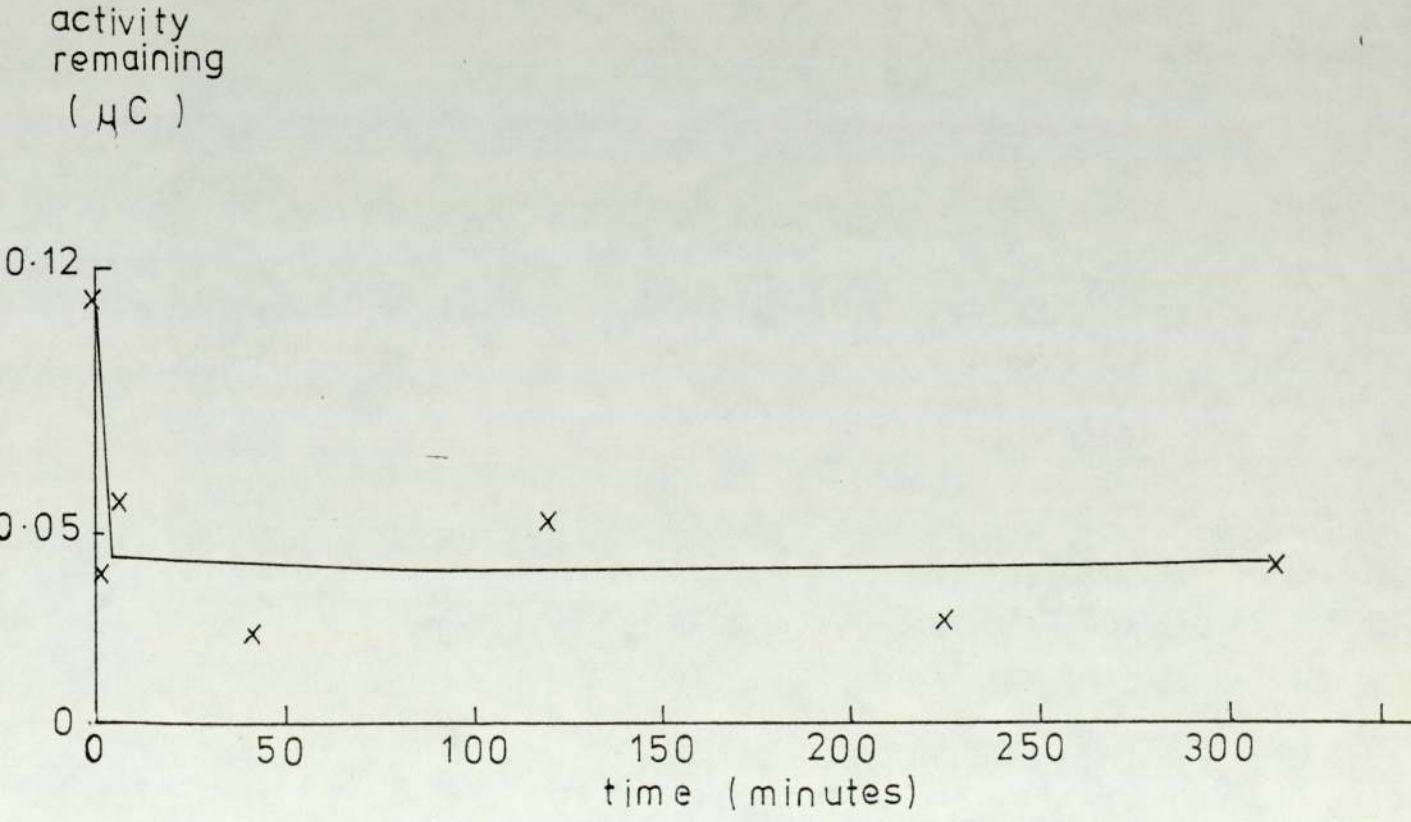


Figure 7.9 Uptake of tritiated water by a multiple emulsion.

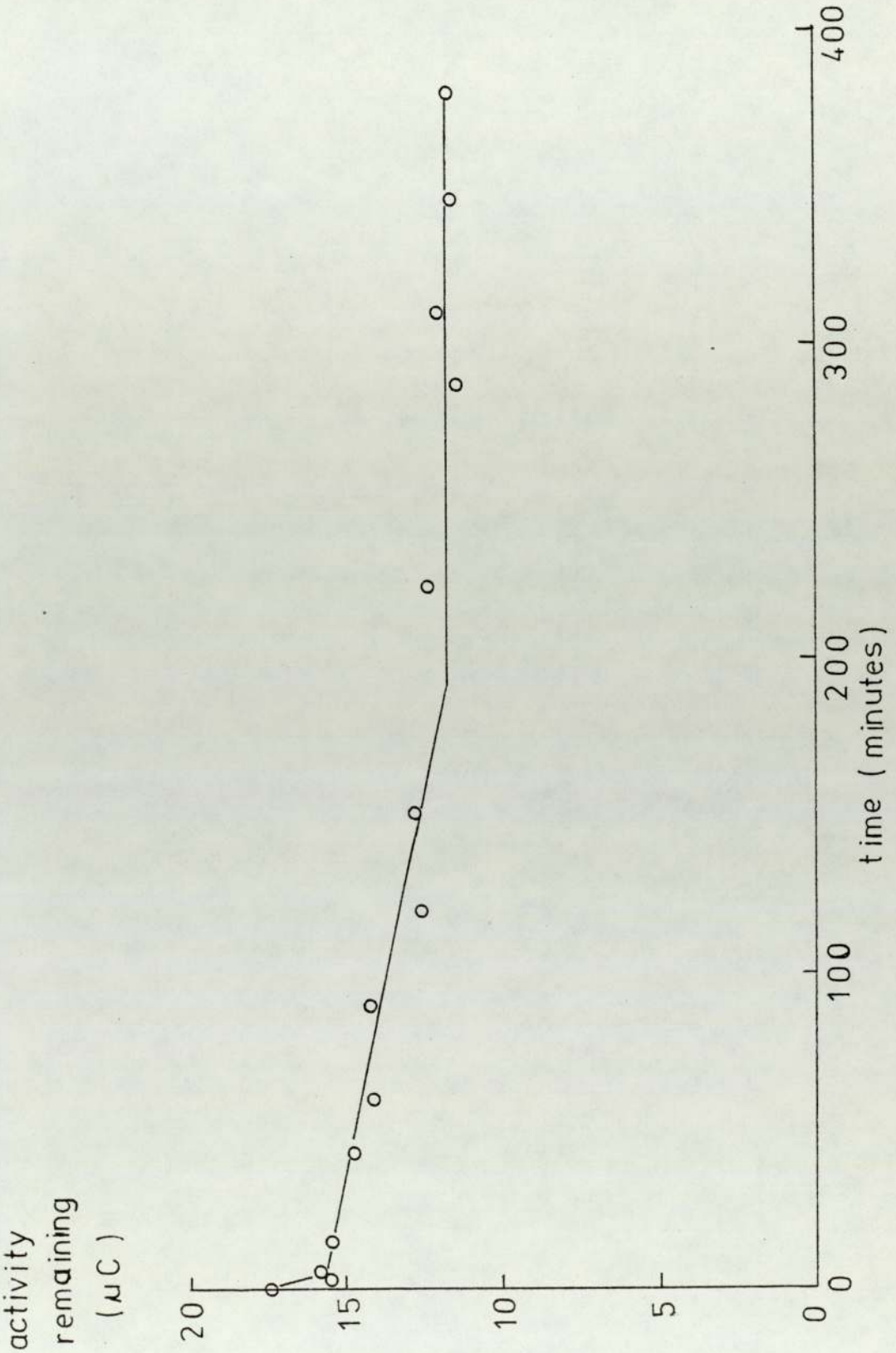


Table 7.1. Release from multiple emulsions

Time after addition (days)	Initial release rate (μC/minute)	
	Emulsion 1	Emulsion 3
0	0.0038	0.0015
1	0.0053	0.0040
2	0.0075	N.D.
7	N.D.	0.0045
8	0.0097	N.D.

(N.D. means rate not determined at this time)

Once equilibration has been achieved within the emulsion, the release rate increased with increasing storage time. This indicates that as the emulsions aged, the proportion of radio-labelled water in the continuous phase increased. This type of behaviour has been observed with other techniques and is also described in the relevant chapters.

7.3 Discussion

The characterisation of multiple emulsions using radiotracer techniques has been described. The rate and extent of release of the tritiated water were used to determine the multiple character of emulsions. The ageing of multiple emulsions can also be described by the change in the release rates with time.

The rapid equilibration of tritiated water throughout the multiple emulsion indicates that transfer of water between the internal aqeuous

phase and the continuous phase is rapid. The film of oil and emulsifier molecules separating the two aqueous phases did not act as an impermeable barrier.

The release curves only have been plotted and no further characterisation of the emulsions from the data has been attempted. This section of the study was only intended to be of a preliminary nature. However, it serves to illustrate clearly some of the possibilities of this method. Some of the experiments that need to be carried out to characterise multiple emulsions are:

1. To challenge multiple emulsions with known quantities of tritiated water to determine the volume of the internal aqueous droplets. To follow the change of this property.

2. To use tritiated water to measure the rate of uptake and release of tritiated water. This can be used to characterise the surfactant-oil-surfactant films which can be varied in composition.

3. To follow the rate of release of tritiated water, or other labelled molecule, under a stress e.g. an osmotic gradient. This can be used to determine the rate and extent of drug release from multiple emulsions.

Chapter 8

Chapter 8 - Conclusions

8.1 Conclusions from Present Study.

The previous chapters have described various methods for the characterisation of multiple emulsions. This chapter will discuss the results contained in earlier chapters and compare the results of this study with those of other works.

Multiple emulsions of the W/O/W type prepared by the method outlined in Chapter 2 have been shown to consist of two types of droplets - oil droplets and multiple droplets. In other words, the emulsions prepared were not completely multiple in nature. Therefore, an important property of such emulsions is the proportion of multiple droplets present. In the present work, the proportion of multiple droplets was calculated from the particle size distribution determined by optical microscopy and by Coulter counter. The effect of a number of formulation factors on the yield of multiple droplets was evaluated.

One of the factors affecting the yield of multiple droplets was the phase volume ratio of the 3 phases involved. A relationship between the yield and the proportion of aqueous phases in the emulsion was found. This means that the 2 different aqueous phases used to prepare multiple emulsions are not separate phases but are interchangeable. When the W/O emulsion is added to the continuous phase and these are mixed, droplets of "continuous phase" are trapped in the oil droplets. In this way, a large volume of multiple droplets may be produced from a W/O emulsion with a low $\phi_{w/o}$. Conversely, some of the disperse phase from the W/O emulsion will be present in the continuous phase of the final W/O/W emulsion.

Inclusion of a portion of the continuous phase in the internal aqueous droplets will also have an effect on the stability of the emulsion. The entrapped continuous phase will include the hydrophilic emulsifier. This

means that a mixed film of lipophilic and hydrophilic emulsifiers will be formed at the interface of internal droplets. The presence of hydrophilic emulsifier in the internal aqueous droplets may have a destabilising effect if it is present in large enough amounts. The degree of destabilisation will be governed by the relative amounts of hydrophilic and lipophilic emulsifiers present in the interfacial film which is, of course, related to the ratio of concentrations in oil and continuous phases. This could be the explanation of a phenomenon observed by Matsumoto et al (1976). These workers found that the yield of multiple emulsions was related to the ratio of the weights of lipophilic to hydrophilic emulsifiers present in the emulsion. This relationship held for a number of hydrophilic emulsifiers in combination with Span 80. For Tween 20, the optimal weight ratio was approximately 20. A similar result was also found in the present study. For a fixed concentration of Tween 20 of 2%, increasing the lipophilic emulsifier concentration, and hence the lipophilic/hydrophilic emulsifier concentration ratio, increased the proportion of multiple droplets formed. The amount of the increase, however, was dependent on the nature of the lipophilic emulsifier. In fact, increasing the lipophilic emulsifier concentration in one case decreased the yield of multiple droplets. This is obviously due to the interaction between the surfactant and the oil. Different oil phases produced different orders of yields with the same range of surfactants. Solubility in the oil is therefore not the only criterion to be considered when selecting the oil and surfactants. A technique such as single droplet coalescence, described in Chapter 1, may be useful in screening surfactants with a particular oil without preparing a series of emulsions and examining this stability.

An interesting result found by Matsumoto et al (1976) is that in the multiple emulsions formed in their studies, "each aqueous phase globule forms a single droplet surrounded by an oil layer.....". This is in contrast to the results from the electron microscope examination of this study.

Those multiple droplets that were examined all contained several internal water droplets in the plane of the replica and probably contained many more. If a multiple emulsion could be formed in which the internal aqueous droplets were only separated from the continuous phase by a single thin oil layer then this would have advantages in drug release studies and in studies using multiple emulsions as model membranes.

The existence of the two separate aqueous phases was also illustrated by the radiotracer experiments. The release of tritiated water showed a biphasic release curve, showing that the aqueous phases behave differently in the transport of tritiated water from them. The initial more rapid release rate corresponds to release from the continuous aqueous phase. The later release corresponds to release from the internal aqueous droplets. Of course, release from the internal droplets must occur through the continuous phase. In the second slower phase of release, the release from the internal aqueous droplets is rate-limiting and is slower because there are more barriers to release.

The biphasic pattern of release from multiple emulsions was also observed by Brodin et al (1978) for release of naltrexone from O/W/O emulsions. This effect could be exploited in producing a multiple emulsion for drug delivery. The initial rapid release could be used to provide a loading dose while the slower release could be used to maintain the levels of the drug.

When multiple emulsions are stored, breakdown occurs by two mechanisms -

1. diffusion of the internal aqueous droplets into the continuous,
2. coalescence of the oil droplets.

The results of particle size analysis show that these two mechanisms occur in sequence rather than simultaneously. Multiple emulsions are characterised by a decrease in the mean diameter of the disperse phase followed by an increase - these phases seem to correspond to the mechanisms outlined above. However, the 2 processes cannot be completely mutually

exclusive. Mechanism 1 will predominate in the early stages of breakdown and mechanism 2 in the late stages but there may be an intermediate period when neither mechanism dominates.

The driving force for the diffusion of the internal aqueous droplets into the continuous phase is the reduction of interfacial area and so reduction of interfacial free energy. Another mechanism operates at the same time - coalescence of internal aqueous droplets. This is seen in the calculated diameters from the data from the Coulter counter. The calculated diameters increased with storage. If diffusion had been the sole mechanism of breakdown, then the diffusion layer thickness would have increased but the calculated diameters would have decreased. The increase in calculated diameters indicates that coalescence had also occurs. This fact is not surprising, however, because initially the water droplets are packed into the oil droplets. The fact that they survive for as long as they do illustrates the excellent stabilising effects of the lipophilic surfactants used. The coalescence of the internal aqueous droplets was also noticed visually. When the multiple emulsions were first formed, the internal aqueous phases consisted of a large number of small droplets. At longer time periods, some emulsion could be observed to consist of a few large droplets as the internal aqueous phase.

The effect of osmotic gradients on the diffusion of internal aqueous phase into the continuous phase is important. The oil layer separating the 2 aqueous phases can act as semi-permeable membrane and so passage of water across this oil layer can occur under the influence of an osmotic gradient. If the agent increasing the osmotic pressure is placed in the internal aqueous droplets then the droplets will swell as water passes into them. If the gradient is large enough the droplets will burst.

If the osmotic gradient forming agent is placed in the continuous phase, then the internal aqueous droplets will pass into the continuous phase. This effect was utilised to characterise multiple emulsions by the Coulter

counter. However, in parallel work using the microscope, it was found that the minimum electrolyte concentration necessary to induce osmosis was directly related to the size of the oil droplets in which the aqueous droplets were contained. In other words, the larger the droplets the greater the concentration necessary to induce movement of the water.

These results have some relevance to the parenteral use of multiple emulsions. If no additive is present in the internal aqueous droplets, then the electrolytes in the body fluids will cause the internal aqueous phase to diffuse out rapidly. To counteract this, an agent may be placed in the internal aqueous phase to increase its osmotic pressure. In this way, the rate of release into the biological fluids could be selected. However, the size of the multiple droplets will also have an effect on the release rate in an osmotic gradient. It may be possible to produce multiple droplets of such a diameter that no internal additive is needed to prevent the rapid release of the internal aqueous phase. Mixing of multiple droplets of different sizes would allow the release of medicament over a long period.

8.2 Suggestions for Further Work.

Several different methods for the study of the behaviour of multiple droplets have been demonstrated. The following topics are recommended as deserving further study by the techniques described above and by other methods as appropriate.

The method of formation of multiple droplets requires further elucidation. Results from the present work suggest that multiple emulsion formation is not a simple re-emulsification of a W/O emulsion. This has important implications in the use of multiple emulsions as drug delivery systems. If the two aqueous phases, internal and continuous, can intermix during manufacture, drug incorporated into the W/O emulsion will not be completely located in the internal aqueous phase. Therefore the amount

of interchange of the aqueous phases will need to be determined before the multiple emulsion can be used as a dosage form.

Study of the mechanism of formation of multiple emulsions will also provide support for the suggestion of other workers that multiple emulsions prepared in their laboratory consist of a single water droplet surrounded by a thin layer of oil. If multiple emulsion such as this can be prepared, they would have greater value because they would be easier to characterise than multiple emulsions in which the oil droplets contained many internal aqueous droplets.

The primary mechanism for the breakdown of multiple emulsions is the diffusion of the internal aqueous phase into the continuous phase. Further study is necessary to determine the most effective way of delaying or preventing this diffusion. Different emulsifying agents might be successful in providing a more effective interfacial barrier, especially if they form an interfacial layer of liquid crystals. Modifications might also be made to the oil phase. Inclusion of a monomer in the oil phase would allow polymerisation after the multiple emulsion had been formed. The oil phase could be prepared from an oil which was solid at room temperature. Preparation of the multiple emulsion would take place above the melting point of the oil which would solidify on cooling down to room temperature - a similar approach has been applied to liposomes which are more stable if the phospholipid transition temperature is above room temperature.

The use of biological materials in the preparation of multiple emulsions would allow their use as models of cells and biological membranes. Even so, further study of their permeability characteristics may provide valuable information on the behaviour of biological systems.

Once the above work on the formation and formulation of multiple emulsions has been completed, experiments will be required to determine the in vivo drug release characteristics of multiple emulsions. An important factor affecting the rate of drug release will be the osmolarity

of the internal aqueous phase. If the emulsion is stable, the rate of drug release will be largely governed by the osmotic gradient. It may be possible to use this effect to choose the rate of release.

8.3 Possible Applications of Multiple Emulsions as Drug Delivery Systems

Multiple emulsions were first studied as sustained release parenteral preparations. This application is still possible. The emulsion will need to be stable and the osmolarity of the internal aqueous phase will be important.

Oral dosage of labile molecules, e.g. insulin, heparin, may be possible. The multiple emulsion will protect the molecule from the digestive enzymes. Whether absorption would be enhanced by enclosure within a multiple emulsion remains to be seen as not enough is known about the intestinal absorption of emulsions.

An important possible application is local injection of toxic therapeutic agents. An example might be intra-tumoural injection of an anti-cancer agent or intra-articular injection of an anti-arthritic drug.

Multiple emulsions may also find use in a liquid membrane type of extraction system. For example, treatment of drug overdosage may be possible using the multiple emulsion to concentrate the toxic agent against its concentration gradient.

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The Particle Size Analysis of Multiple Emulsions (Water-in-Oil-in-Water)

S. S. Davis and A. S. Burbage

Pharmacy Department, University of Nottingham,
University Park, Nottingham NG7 2RD, UK

INTRODUCTION

A multiple emulsion is an emulsion in which the dispersed phase contains smaller droplets of another phase dispersed within it. This secondary disperse phase is usually of similar composition to the continuous phase but is physically separated from the continuous phase. Multiple emulsions containing tertiary and quaternary disperse phases have been reported.[1]

For example, a water-in-oil-in-water emulsion (w/o/w) consists of water droplets inside oil droplets dispersed in an aqueous continuous phase, and an oil-in-water-in-oil (o/w/o) emulsion consists of oil droplets inside water droplets in an oily continuous phase. Such multiple emulsion systems can be made by re-emulsifying the appropriate single emulsion system. Multiple emulsions may also be produced when an emulsion undergoes phase inversion. In this case the number of internal droplets is relatively small.[2,3]

The particle size analysis of the oil droplets in a w/o/w system is a relatively simple matter. However, the analysis of internal water droplets presents a much more difficult problem. This paper describes a variety of techniques for the characterization of the particle size of multiple emulsion droplets.

Multiple emulsion systems were first described in detail by Herbert[4] in 1965 as useful vehicles for the administration of vaccines. Up until that time the normal approach was to employ a w/o emulsion with the antigen contained in the aqueous dispersed phase. This type of emulsion system gave good antibody response due to the slow release of the antigen but it was highly viscous and difficult to inject. Herbert re-emulsified the w/o emulsion to produce a less viscous w/o/w system. This was not only easier to inject but gave rise to a further improvement in antibody titre. The methodology has been subsequently refined to provide vehicles not only for vaccines but also for drugs.[5]

TABLE 1
Multiple emulsions for drug and vaccine administration

Drug or vaccine	System	Ref.
Methotrexate	w/o/w	6
Bleomycin	w/o/w	7
Cytosine arabinoside vinblastine sulphate	w/o/w	8
Insulin	w/o/w	9, 10
Cysteamine	w/o/w	11
Naloxone	o/w/o	12
Ovalbumin	w/o/w	4
E. coli endotoxin	w/o/w	13
Influenza virus	w/o/w	14
Anaerobic coryneforms	w/o/w	15

Multiple emulsions have also been used as liquid membranes for extraction purposes.[16] A binding agent or carrier molecule is contained in the secondary dispersed phase and the technique involves the preparation of a w/o emulsion which is then mixed with the phase containing the substance to be extracted. Usually there is no hydrophilic surfactant present so that when extraction is completed, stirring is stopped and the w/o emulsion separates rapidly. Applications of liquid membranes have included extraction of phenol from waste water,[17] separation of hydrocarbons,[18] treatment of uremia[19] and treatment of drug overdosage.[20]

In spite of this widespread interest in multiple emulsions and liquid membranes, little effort seems to have been directed towards an investigation into the stability and particle size analysis of such systems. Various qualitative estimates have appeared in the literature:

'The emulsions found were finely dispersed but relatively unstable.'[3]
'The diameters of the w/o droplets in water were in the range 0.6 to 2 μm.'[7]
'It should be noted that many smaller droplets, approximately 1 μm in diameter, are encapsulated within each [oil] globule.'

Herbert[5] found that it was desirable from a stability point of view for w/o/w emulsions to have dispersed oil globules of average diameter 10–20 μm (measured by microscopy). The average diameters of the second disperse aqueous phase were preferably in the range 1–7 μm. More recently Matsumoto *et al.*[21] have claimed to have prepared w/o/w emulsions in which each aqueous phase globule forms a single droplet surrounded by an oil layer and the two emulsifying agents. However, no direct evidence for the formation of such systems was given and they observed that 'in microscopic observation of the w/o/w emulsion, the reflected light from the surface of the globules made discrimination between aqueous globules covered with an oil layer and simple oil droplets impossible'.

If multiple emulsions are to find use as pharmaceutical dosage forms it is highly desirable to produce reproducible systems that have an acceptable shelf-life. Consequently for a w/o/w emulsion one must be able to evaluate the following:

(i) The particle size distribution of the internal aqueous phase, and its change with time.

(ii) The particle size distribution of the oil phase and its change with time.
(iii) The relative proportions of multiple (w/o/w) and non-multiple (o/w) droplets.

To this end we have examined a variety of sizing methods. Objectives (ii) and (iii) are relatively simple to achieve. Objective (i), the sizing of submicrometre water droplets contained in oil droplets, is much more difficult.

EXPERIMENTAL

Materials

Light liquid paraffin was obtained from Fisons, Loughborough. Atmos 300, Tween 20, Tween 80 and Arlacel 83 were obtained from Honeywill-Atlas, Carshalton, and were used without further purification. Water was distilled from an all-glass still. The photographic film used, FP4, and developer, D76, were from Kodak, London, and the fixer, Ilfofix, came from Ilford Ltd., Ilford.

Methods

Preparation of multiple emulsions

W/o/w multiple emulsion systems were prepared by a two-step emulsification process. A water-in-oil emulsion was prepared by mixing the primary aqueous phase with the oil phase containing the lipophilic surfactant and shaking by hand to achieve a rough dispersion. This mixture was then further emulsified using a Dawe Soniprobe (Model 7532A) at maximum output (usually for 30 s) with the tip of the ultrasonic probe placed just beneath the surface of the liquid. The resultant w/o emulsion was then mixed with the secondary aqueous phase combining the hydrophilic surfactant and sonicated (usually for a further 10 s). Typical emulsion systems were as follows:

25 cm^3 water
25 cm^3 10% w/v Arlacel 83 solution in light liquid paraffin } w/o } w/o/w
50 cm^3 20% w/v Tween 80 solution

25 cm^3 water
25 cm^3 Atmos 300 solution 10% w/v in light liquid paraffin } w/o } w/o/w
50 cm^3 2% w/v Tween 20 solution

Particle size analysis

A variety of experimental methods have been used to size the external and internal droplets in w/o/w emulsion systems. A typical photomicrograph of a w/o/w system is shown in Fig. 1. The oil droplets are clearly visible and can be sized using conventional methods such as the light microscope (note that some oil droplets appear to be devoid of internal water droplets and two different populations of emulsion droplet, w/o/w and o/w, are to be expected).

The internal water droplets can be seen within the larger oil droplets. These droplets are 1 μm or less in size and cannot be measured using the light microscope. It was apparent that freshly prepared emulsions contained a large proportion of multiple oil droplets packed with smaller water droplets, and on storage the number of multiple

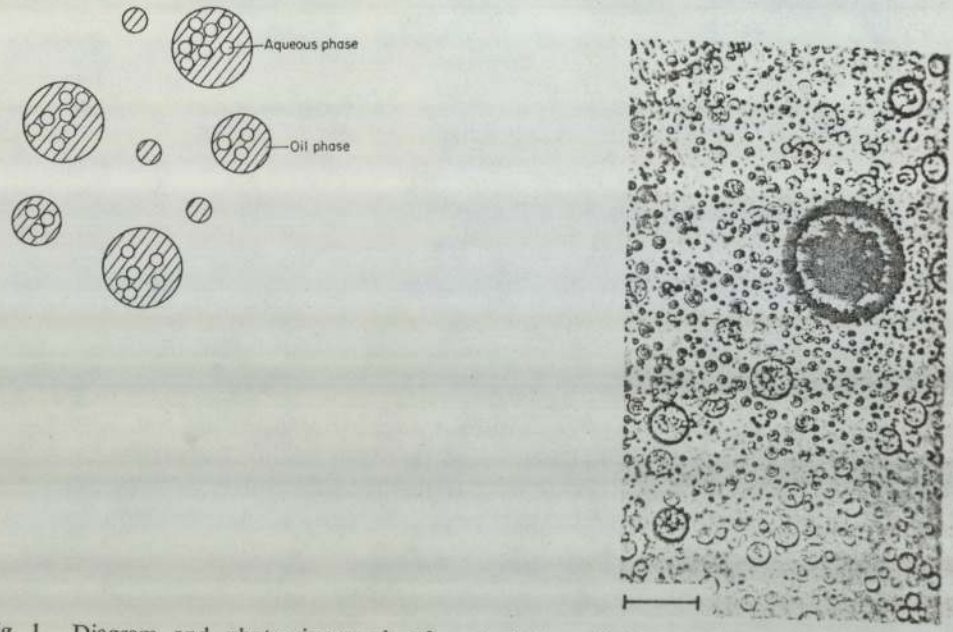

Fig. 1. Diagram and photomicrograph of a typical multiple emulsion system. The bar represents 10 μm.

droplets and their water droplet content both decreased. Two methods to characterize such systems and to quantify the various changes that take place with time have been investigated:

(i) Direct observation of internal water droplets using a freeze etching/electron microscope method.
(ii) Indirect observation of water flux under an osmotic gradient using the Coulter Counter.

Light microscope

A sample of the emulsion to be examined was diluted using the appropriate continuous phase and placed on a haemocytometer cell (Hawskley) of depth 0.1 mm. After allowing time for droplets to cream, the slide was examined using a Vickers MI5c microscope. At least four fields of each emulsion chosen at random were photographed. Any other features of interest were also photographed.

The exposed film was processed in D76 developer and Ilfofix fixer. Development time was 12 minutes at 20 °C. The particle size distribution of the emulsion was determined using a Zeiss TGZ3 particle size analyser. The results were analysed by computer program which calculated particle size distribution by number and by volume, mean length, volume and area diameters, and standard deviation.

Electron microscope

Freeze-etching of w/o/w emulsions was performed using a Balzers BA360M machine. One drop of the emulsion was placed on a gold stub. This was frozen rapidly in Arcton 22 and stored under liquid nitrogen.

Four such stubs were placed on the cold table of the freeze-etch device maintained at −150 °C. The bell-jar cover for the cold table was lowered and evacuated to better than 1 Pa vacuum. The temperature of the cold table was allowed to rise to −100 °C

while the knife (a single-edged razor blade) was cooled to −150°C. When the correct vacuum and temperatures had been achieved, the samples were then planed down using the knife until good clean surfaces were obtained. These surfaces were then etched for 1 minute by placing the knife over them. This caused water in the sample to evaporate. The knife was then moved away and platinum and carbon were evaporated onto the etched surfaces from an angle of 45°. The thickness of this film was checked by using a thin-film monitor. A film of carbon was then applied to the samples. The bell-jar was brought back to atmospheric pressure, the gold stubs were removed and the replicas floated off in distilled water. After soaking overnight, the replicas were then picked up on electron microscope grids coated with formvar.

The replicas were then examined using an AEI EM6B electron microscope. Micrographs were taken of features of interest.

Coulter Counter

A model TA Coulter Counter fitted with a 200-μm diameter orifice tube was used for particle size analysis. The tube was calibrated using 18.71-μm latex in the appropriate strength of sodium chloride solution (generally 0.18% w/v sodium chloride but some experiments were performed using 0.45% w/v sodium chloride). The sodium chloride solutions were prepared by dilution of 0.9% sodium chloride (Polyfusor) with distilled water to give the required concentration and filtered through 0.8-μm Millipore membrane filter, under positive pressure (N_2).

The placing of a w/o/w multiple emulsion droplet into an osmotic gradient created by sodium chloride electrolyte results in the transport of water from the internal phase to the exterior and the subsequent change in size of the emulsion droplet. The extent and rate of this change in size depend on the size and number of internal water droplets and can be studied with the Coulter Counter using the following technique.

The Coulter Counter was set up with a beaker of clean electrolyte and the background reading was recorded. A known volume of a multiple emulsion was added by means of a Gilson Pipetman, to give a concentration index reading, i.e. coincidence level, of about 0.1, and counting started immediately. When sufficient data for a particle size distribution had been accumulated, counting was stopped and the distribution was plotted out. Counting was then restarted after resetting the instrument. This was repeated until no further change in the distribution occurred. In this way several particle size distributions could be obtained for the same sample in a period of less than five minutes.

The volume of multiple droplets can be determined and when volume is plotted against time of contact with saline an exponential curve is obtained. The points were fitted by computer to the following equation using a standard library routine:

$$\text{Volume} = x_1 \exp(-x_2 \text{ time}) + x_3 \qquad (1)$$

The routine calculates the parameters x_1, x_2 and x_3 for the data points given.

RESULTS AND DISCUSSION

Microscopy

The examination of a photomicrograph of a typical multiple emulsion demonstrates two major characteristics (Fig. 1). Firstly, the internal aqueous droplets are very

small, many are less than 1 μm in diameter, and each multiple droplet contains many of these internal droplets. Their very small size and the fact that they are enclosed within an oil droplet make their particle size analysis very difficult. Secondly, it will be noticed that there are droplets present which apparently contain no internal aqueous droplets, that is plain oil droplets. This means that there are two types of droplets present, o/w and w/o/w. We require to know the proportion of multiple droplets and their change with time.

It is difficult to separate the two types of droplets physically, and particle size analysis of all oil droplets will give the distribution for the mixture of droplets. Because the different types of droplets have different mean diameters and standard deviations, a bimodal distribution will result. This distribution when plotted on log probability paper is composed of two linked straight lines. Each straight line represents a distribution, but it is difficult to follow the behaviour of the individual distributions as changes in one affect the other.

We are interested in the behaviour of the multiple droplets and thus it is useful to separate the two constituent distributions. This may be achieved using a graphical inflexion technique[22] which involves determining the point of inflexion, then expanding the portion of the graph to 100% and plotting it out. This then allows direct comparison of the two particle size distributions. Figure 2 illustrates the graphical inflexion technique applied to a multiple emulsion system. Once the distributions have been separated in this way, it is easy to compare directly the distribution of the multiple droplets for different emulsions and so evaluate the effect of different formulations and the effect of ageing.

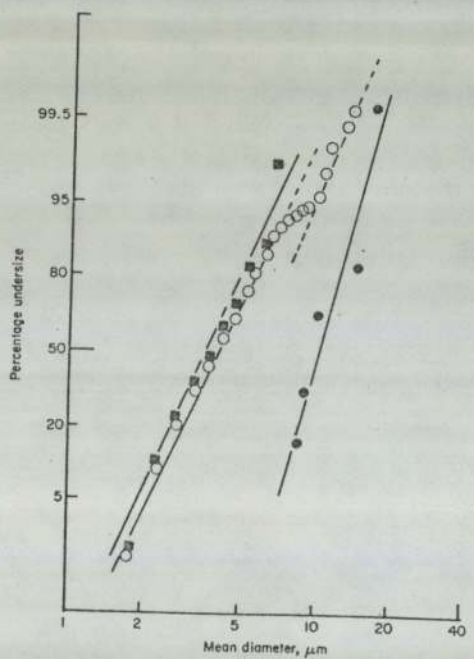

Fig. 2. The resolution of a bimodal distribution into its two constituent parts. Multiple emulsion formulation: 25 cm³ water; 25 cm³ 30% w/v Arlacel 83 in light liquid paraffin; 50 cm³ 2% w/v Tween 20. First sonication 30 s, second sonication 10 s unstored system. ○ – mixed emulsion; ■ – o/w droplets; ●—w/o/w droplets.

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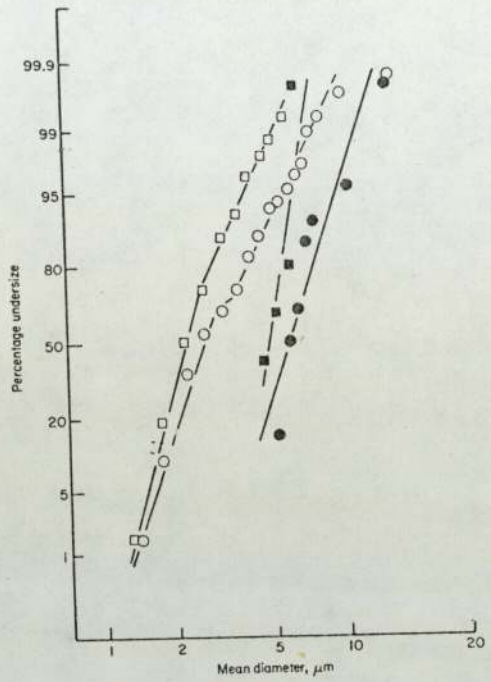

Fig. 3. The effect of secondary sonication on the particle size distribution of an unstored multiple emulsion. Formulation: 30 cm^3 water; 20 cm^3 10% w/v Arlacel 83 in light liquid paraffin; 50 cm^3 2% w/v Tween 20 solution. First sonication period 60 s. Second sonication period: 10 s (○ – mixed emulsion, ● – w/o/w); 20 s (□ – mixed emulsion, ■ – w/o/w).

The use of this technique is illustrated in Fig. 3, which shows the size distributions of the droplets for two emulsions with the same composition varying only in the time of secondary sonication. The longer the time of sonication the smaller the droplets. The distribution of w/o/w droplets is also shown in Fig. 3. The emulsion sonicated for 30 s has a smaller mean diameter (5 μm) than that sonicated for 10 s (6 μm). In addition, the former has a much narrower distribution (standard deviation $\sigma = 1.14$) than the latter ($\sigma = 1.36$).

The distributions for the same emulsions after 28 days' storage were also examined. The most striking change was that the emulsions sonicated for 30 s no longer appeared to have any multiple droplets.

One interesting property of multiple emulsion is that, on ageing, the multiple droplets usually decrease in size. For example, Fig. 4 shows the distributions for an emulsion after 0 and 42 days' storage. It can be seen that the distribution of the single droplets remains almost unchanged but the multiple droplets decrease in size. The diameter changes from 6.4 to 5.8 μm and σ from 1.34 to 1.12 in 42 days.

The more prolonged ageing of coarser multiple emulsion systems is shown in Fig. 5, where the mean diameter is plotted against time (days). During the initial stages of storage the mean diameter falls as internal water droplets are lost from the multiple oil droplets. After longer times the mean diameter increases due to droplet coalescence.

Particle size analysis using the microscope has also been used in an alternative approach to distinguish between multiple droplets and simple oil droplets. This involves sizing samples of the emulsion diluted with continuous phase and with varying

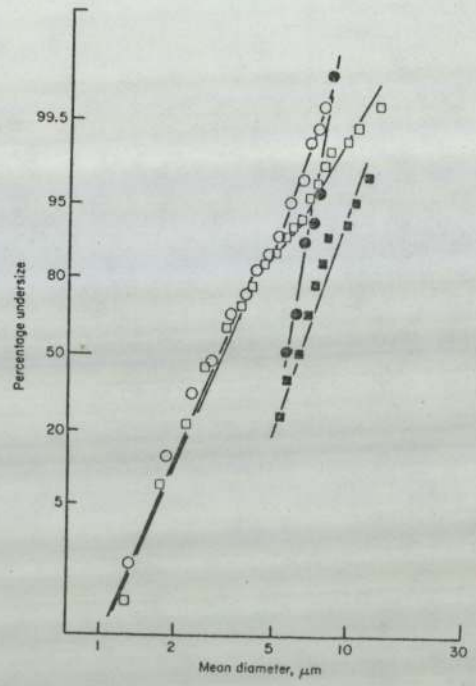

Fig. 4. The effect of storage on the particle size of a multiple particle. Formulation: 20 cm³ water; 30 cm³ 10% w/v Arlacel 83 in light liquid paraffin; 50 cm³ 2% Pluronic F-68. First sonication period 60 s, second sonication period 10 s. Unstored system: □ – mixed emulsion; ○ – w/o/w. System stored 42 days: ■ – mixed emulsion; ● – w/o/w.

Fig. 6. The effe
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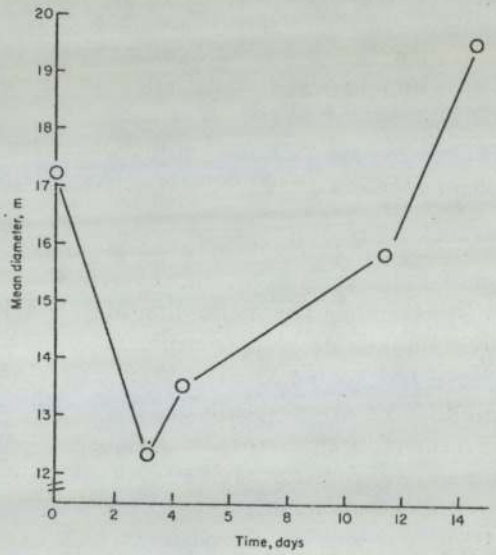

Fig. 5. Ageing of a multiple emulsion system. Formulation: 15 cm³ water; 35 cm³ 10% w/v Arlacel 83 in light liquid paraffin; 50 cm³ 2% w/v Tween 80 solution prepared using hand homogenizer.

Fig. 7. The eff
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■ – 0.001% w/

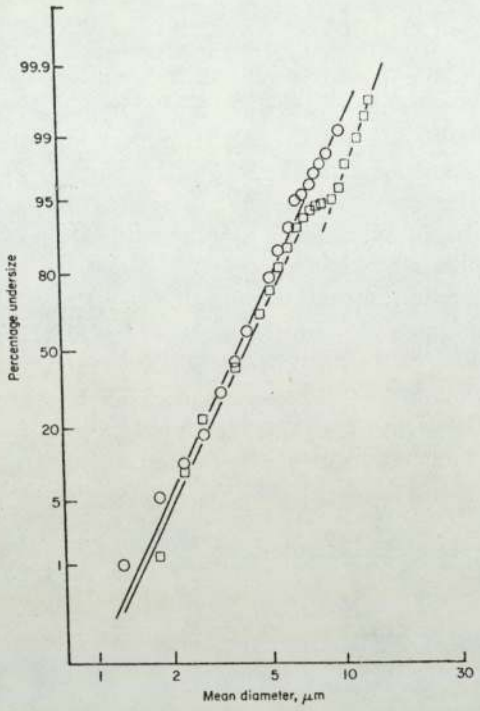

Fig. 6. The effect of external electrolyte on the particle size distribution of a multiple emulsion system. Formulation and preparation as for Fig. 2. □ – emulsion diluted with water; ○ – emulsion diluted with 1% w/v sodium chloride solution.

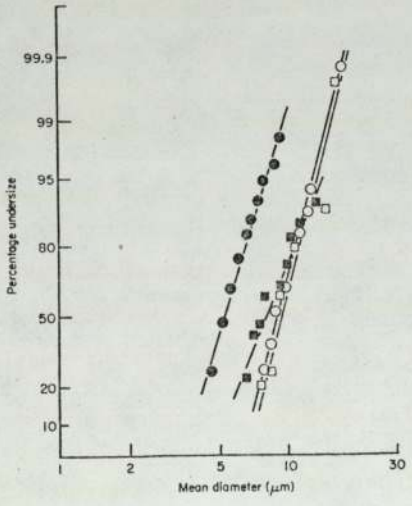

Fig. 7. The effect of electrolyte on the particle size distribution of w/o/w emulsion droplets. Formulation as for Fig. 4. First sonication 60 s, second sonication 10 s. Emulsion stored for 30 days. □ – water; ■ – 0.001% w/v sodium chloride; ○ – 0.005% w/v sodium chloride; ● – 0.1% w/v sodium chloride.

concentrations of sodium chloride solution. The sample diluted with continuous phase represents the unperturbed situation. In sodium chloride solution, the small aqueous droplets inside the multiple droplets are drawn out, under an osmotic gradient, the oil phase acting as the semi-permeable membrane. This causes the multiple droplets to shrink whereas the simple oil droplets are unchanged (Fig. 6). The particle size distribution obtained in continuous phase is typically bimodal, having contributions from single and multiple droplets. The distribution obtained in 1% sodium chloride at equilibrium solution demonstrates how the multiple droplets have shrunk in size.

Figure 7 shows the effect of different concentrations of sodium chloride solution on the multiple droplet distribution of the same emulsion. There appears to be a threshold concentration of sodium chloride below which little or no change of size occurs. This lies between 0.005% w/v and 0.1% w/v *for this particular emulsion*. The threshold concentration appears to vary with *droplet diameter*. The larger the multiple emulsion droplets the higher the concentration of sodium chloride required to effect shrinkage.

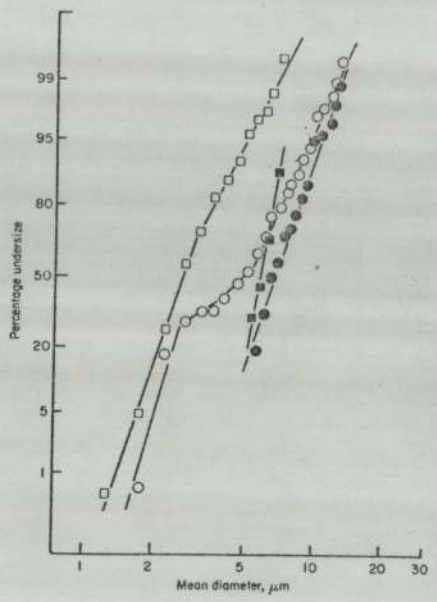

Fig. 8. The effect of electrolyte in the internal aqueous phase of a w/o/w emulsion on particle size distribution. Formulation: 20 cm³ 5% w/v sodium chloride solution; 30 cm³ 10% w/v Arlacel 83 in light liquid paraffin; 50 cm³ 2% w/v Tween 20 solution. First sonication 60 s, second sonication 10 s. Emulsion sized in water: ○ – mixed emulsion; ● – w/o/w. Emulsion sized in 5% w/v sodium chloride: □ – mixed emulsion; ■ – w/o/w.

The size of internal water droplets may be increased by using an osmotic gradient. For example, during the emulsification procedure the initial w/o emulsion can be prepared with saline in the aqueous phase. Upon preparation of the multiple w/o/w system, water is transported from external to internal phase. Figure 8 shows the change in size distribution when a multiple droplet, containing 5% saline in the internal aqueous phase, is allowed to reach equilibrium with an external continuous phase containing water or 5% saline.

Electron microscope

Electron micrographs of a typical multiple emulsion system are given in Fig. 9. The emulsion comprised 25 cm^3 of distilled water and 25 cm^3 of 10% Arlacel 83 in light liquid paraffin as the w/o emulsion. This was re-emulsified in a 2% aqueous solution of Tween 80. The equivalent electron micrograph for the original w/o emulsion is very similar.

The internal water droplets can be clearly seen within the individual oil droplets and can be sized easily. Note that some oil droplets appeared to be devoid of any internal water droplets. The appearance of the internal water droplets differs depending

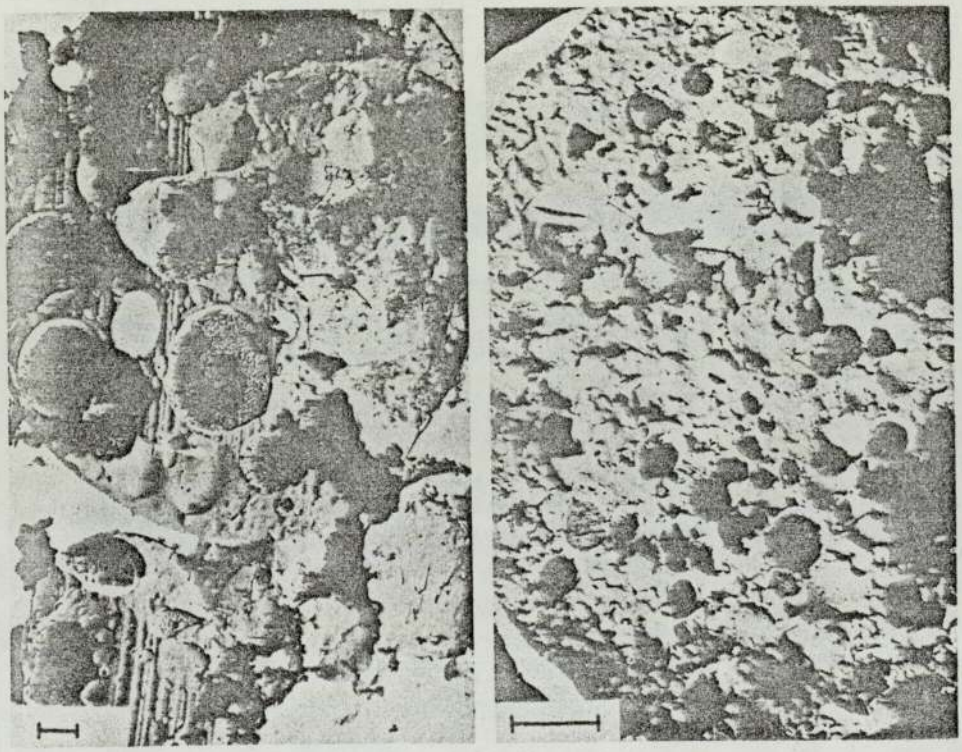

Fig. 9. Electron micrographs of a typical multiple emulsion system. The bars represent 1 μm.

whether individual frozen droplets were cut through during the fracture process (dark granular appearance) or whether the fracture plane passed around a droplet (concave or convex morphologies). Further details of this method have been published elsewhere.[22]

Although the freeze-fracture electron microscope technique provides a clear record of the particle size and distribution of water droplets in the internal phase, it is time consuming and costly. Consequently the method is not suited to routine stability studies, and alternative methods that exploit the semi-permeable nature of the oil phase have been examined.

Coulter Counter

When a w/o/w multiple emulsion droplet is placed in an osmotic gradient, the oil droplets containing internal aqueous phase droplets can shrink or grow depending upon the osmolarities of the external and internal aqueous phases. In these studies the internal phase comprised water without added electrolyte and the external aqueous phase was 0.18% saline. Consequently the w/o/w droplets shrink in size. This shrinkage is demonstrated in Fig. 10, which shows particle size histograms for a w/o/w emulsion

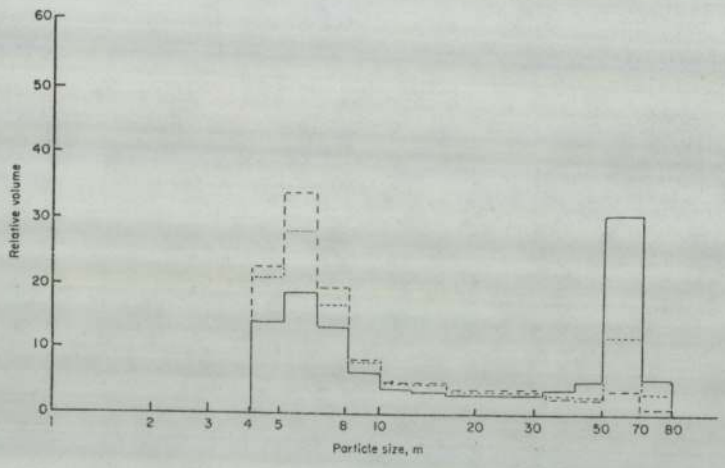

Fig. 10. The change of particle volume of a multiple emulsion in an osmotic gradient (Coulter Counter).

The rate of particle shrinkage can be followed by studying the change in volume of the w/o/w droplets. Three different situations are shown in Fig. 11: multiple emulsion (freshly prepared), a multiple emulsion (after storage) and a sample of o/w emulsion. at different times after being placed in the osmotic gradient. It is clear that initially there is a bimodal distribution of droplets (o/w and w/o/w droplets) but, on contact with saline, water is transferred from internal to external aqueous phase. The proportion of large particles decreases and the proportion of small particles increases.

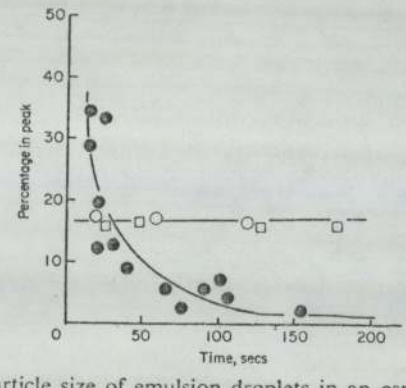

Fig. 11. The change in particle size of emulsion droplets in an osmotic gradient (0.18% saline). ● – unstored w/o/w emulsion. ○ – stored w/o/w emulsion. □ – o/w emulsion.

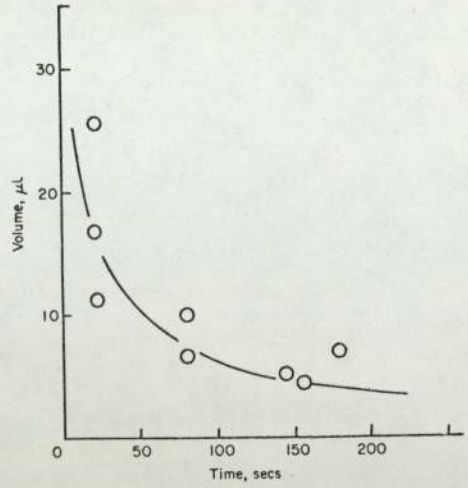

Fig. 12. The change in the volume of w/o/w droplets in an osmotic gradient (0.18% saline). Unstored emulsion. Formulation: 30 cm^3 water; 20 cm^3 10% w/v Arlacel 83 in light liquid paraffin; 50 cm^3 2% w/v Tween 80.

The multiple emulsion after storage, when viewed under the microscope, was found to be devoid of internal water droplets. Thus the Coulter Counter technique can be used to follow storage stability of multiple emulsion systems. Two additional shrinkage curves are shown in Figs. 12 and 13, where the data are expressed in terms of volume changes.

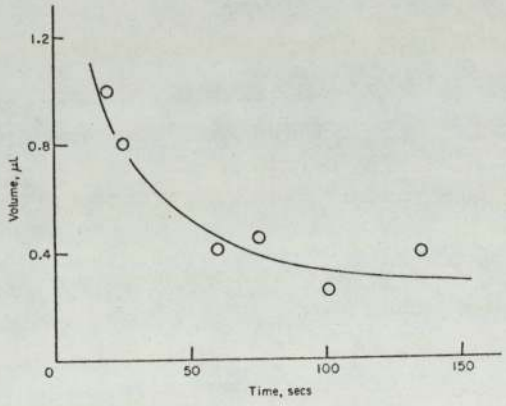

Fig. 13. The change in volume of w/o/w droplets in an osmotic gradient (0.18% saline). Emulsion stored 60 days. Formulation: 10 cm^3 water; 40 cm^3 10% w/v Arlacel 83 in light liquid paraffin; 50 cm^3 20% w/v Tween 20.

The permeation of water through the oil phase from internal to external aqueous phase under osmotic gradient is being considered using the following model.

The diffusion of water through the oil phase will be controlled by the outer internal aqueous droplets only. Consequently the actual multiple system is equivalent to a large double liquid droplet (Fig. 14).

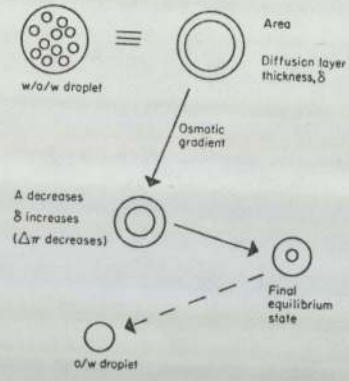

Fig. 14. Shrinkage of multiple emulsion droplets.

The rate of change of volume with time can be approximated using an equation of the form[24]

$$\frac{dV}{dt} = -\frac{DA}{\delta}\frac{\Delta\pi}{RT} \tag{2}$$

where D is the diffusion coefficient of water in the oil phase, δ is the thickness of the diffusion layer, A is the surface area of the oil droplets, $\Delta\pi$ is the osmotic gradient, and R and T have their usual meaning. The ratio D/δ is also termed the permeability coefficient for osmotic transport (P_{os}). (It is assumed that unstirred layers of water do not contribute significantly and that hydrostatic pressure effects are minimal.[25])

For an osmotic gradient created by a single solute species

$$\pi = vRT\phi m \tag{3}$$

where v is the number of ionic species produced by the solute (two in our case for sodium chloride), ϕ is the osmotic coefficient (given by Robinson and Stokes[26]), and m is the molality.

When the internal aqueous phase contains no electrolyte and when the emulsifier

TABLE 2
Stability parameters for w/o/w emulsions obtained from osmotic gradient technique

Parameter	Storage time (days)		
	0	32	60
dV/dt ($mm^3\ s^{-1}$)	6.9×10^{-5}	3.7×10^{-5}	1.9×10^{-5}
Mean volume diameter (μm), w/o/w droplet	50.3	49.7	47.3
Mean number diameter (μm), w/o/w droplet	19.7	19.4	18.5
δ (μm)	0.053	0.10	0.17
Mean number diameter d (μm), internal water droplet	0.35	0.39	0.66

Formulation: 20 cm^3 water; 30 cm^3 2% w/v Arlacel 83 in light liquid paraffin; 50 cm^3 2% w/v Tween 20 solution.

for the original w/o emulsion is non-ionic and is contained almost exclusively in the oil phase, then

$$\Delta\pi = 2RT\phi m \tag{4}$$

A value of D of 4×10^{-5} cm^2 s^{-1} can be obtained from the data presented by Schatzberg.[27]

In order to characterize the multiple emulsion droplets at any given storage time, a value of dV/dt is measured from the initial part of the volume–time plot.

The mean surface area A of the multiple emulsion droplets can be obtained from the Coulter Counter data.

Thus, the only unknown in Eqn. (2) is the thickness of the diffusion layer.

Knowing the quantity of water within each multiple droplet (obtained by comparing the volume of the unshrunk droplet (extrapolated) with the volume of shrunken droplet or by assuming that the phase volume of internal water droplets approximates to that in the initial w/o emulsion), we can compute the mean diameter and number of equivalent monosize water droplets that will give this film thickness. The change of the various parameters with time may then be followed and used to assess storage stability (Table 2).

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DISCUSSION

Dr M. J. Groves (Pharmacy, Chelsea College, University of London) suggested that the emulsions described here may be rather less than ideal as drug delivery systems since the internal water was apparently so mobile. Professor Davis disagreed since the use of multiple emulsions for drug delivery is a somewhat different problem. The release of the drug from the internal aqueous phase will depend on at least three different factors. The first is the size of the drug molecule itself since if it is large the material will be unable to diffuse through the membranes involved. Smaller molecules may well partition but this could be advantageous since drug in the external aqueous phase would provide a starting dose for the patient who would then be sustained with drug from the internal phase. In any case, many drugs are weak acids or bases so that by adjustment of the pH of the internal phase the drug could be kept mainly in the ionized form which is poorly transportable across the oil layer. The second approach would be to use emulsifying agents that produced thick interfacial films, possibly liquid crystalline in nature, and these would certainly retard drug transport. The third approach, which was more of an encapsulated system, is to modify the oil phase by the use of a polymerizing agent or using a high melting-point oil and cooling the multiple emulsion after manufacture.

Electron Micrography of Water-in-Oil-in-Water Emulsions

Multiple emulsions such as water-in-oil-in-water systems (w/o/w) may be prepared by reemulsifying the corresponding single emulsion (w/o). Recently, there has been interest in using such emulsions as vehicles for drug administration (1, 2) and as liquid membranes in solvent extraction processes (3).

A photomicrograph of a typical w/o/w emulsion system is shown in Fig. 1. The oil droplets are self-evident and these can be sized using conventional methods such as the light microscope or Coulter Counter. The proportion of single and multiple droplets can also be determined in many cases. However, the internal water droplets are much more difficult to observe since they are often less than 1 μm in size and are enclosed within oil droplets. Matsumoto *et al.* (4) have reported the existence of w/o/w droplets where it was not possible to see the inner water droplets under the light microscope.

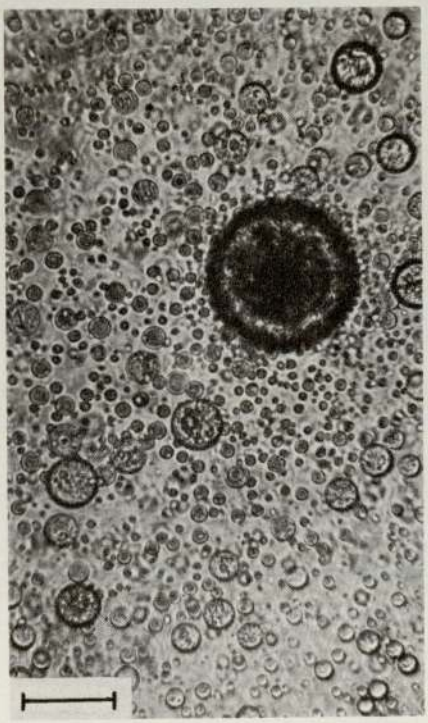

FIG. 1. Photomicrograph of a multiple emulsion. Bar = 1 μm.

The successful application of w/o/w emulsions in various technologies will depend on the production of stable reproducible systems. Consequently, knowledge about the particle size distributions of the internal aqueous droplets and their change with time is essential, and we are investigating a number of alternative methods of particle size analysis (5). We describe here a direct method using freeze-etching and the electron microscope based on the approach described by Eley *et al.* (6) for the size analysis of w/o emulsions.

MATERIALS AND METHODS

Multiple emulsions are normally prepared in two stages, first, a water-in-oil emulsion which is then re-

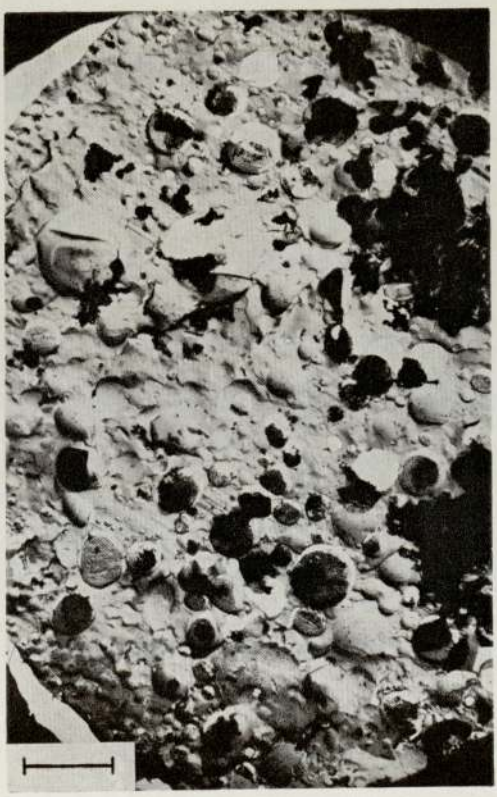

FIG. 2. Electron micrograph of a multiple emulsion. Bar = 1 μm.

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FIG. 3. Electron micrograph of a multiple emulsion. Bar = 1 μm.

FIG. 4. Electron micrograph of a multiple emulsion. Bar = 1 μm.

emulsified to produce a multiple emulsion. The multiple emulsion described in this paper comprises 25 ml of distilled water and 25 ml of 10% Arlacel 83 in light liquid paraffin as the water-in-oil emulsion. This was reemulsified in a 2% aqueous solution of Tween 80. Dispersion was effected by a Dawe Soniprobe Type 7532A; dispersion times were 1 min for the water-in-oil emulsion and 10 sec for the multiple emulsion.

Samples of the multiple emulsion were freeze-etched using a Balzers BA360M freeze-etcher following the method of Moor and Muhlethaler (7).

Samples were fractured and etched at −100°C. They were then shadowed with carbon and platinum–carbon. Replicas were cleaned by distilled water and left overnight soaking in sodium hypochlorite solution. They were then rinsed in distilled water and mounted in "Formvar"-coated grids. The replicas were then examined using an AEI EM6B electron microscope to obtain electron micrographs.

RESULTS AND DISCUSSION

Emulsion droplets in the replicas were photographed, and some of these electron micrographs are shown in Figs. 2–6. Figures 2 and 3 show typical electron micrographs of multiple emulsion droplets. The internal water droplets within the individual oil droplets are clear and can be sized easily. Three different appearances are found. When water droplets are cut through during the fracture procedure a dark granular appearance (ice crystals) is found. Higher magnification of such droplets is shown in Figs. 4 and 5. In some cases the fracture process occurs at the surface of an internal water droplet to leave either a depression where a droplet has been or an exposed droplet surface. A fracture plane passing over water droplet surfaces is shown in Fig. 6.

Figure 3 shows four smaller oil droplets that appear to be devoid of internal water droplets; certainly within the plane of the replica.

SUMMARY

The internal droplets of a multiple emulsion (w/o/w) are difficult to size due to their small size and enclosure in oil drops. It is possible to observe internal water droplets by a freeze-etching technique employing the electron microscope.

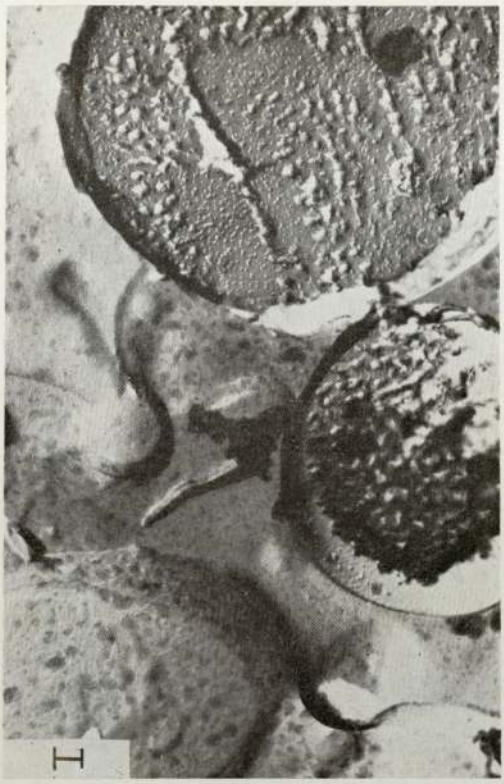

FIG. 5. Electron micrograph of a multiple emulsion. Bar = 0.1 μm.

FIG. 6. Electron micrograph of a multiple emulsion. Bar = 0.1 μm.

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S. S. DAVIS
A. S. BURBAGE

Pharmacy Department
University of Nottingham
University Park
Nottingham NG7 2RD
United Kingdom

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THE PARTICLE SIZE ANALYSIS OF MULTIPLE EMULSIONS

S.S. Davis, T.S. Purewal and A.S. Burbage, Pharmacy Department, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.

Water in oil (w/o) or oil in water (o/w) emulsions can be further emulsified to produce multiple emulsion systems (w/o/w and o/w/o). These emulsions, that contain one disperse phase inside another disperse phase, have been used as sustained release parenteral dosage forms (Gresham & others, 1971) and as liquid membranes in solvent extraction and enzymology (American Chemical Society, 1976). We are presently studying the stability of w/o/w emulsions formulated using liquid paraffin as the oil phase and nonionic surfactants (Tween 80 and Arlacel 83) as stabilizers.

Size analysis of the dispersed oil phase is a relatively simple problem and conventional methods such as light microscopy and the Coulter Counter have been employed with success. Some of the oil droplets contain small water droplets whereas others do not and this results in a bimodal size distribution. The size characteristics of the two types of emulsion particle and their change with time can be resolved using a graphical inflexion method (Lewis & Taylor, 1967).

Size analysis of the internal water droplets presents a more difficult problem. The average size of the particles is less than 1 micron and conventional methods such as light microscopy are inapplicable. Two alternative techniques have been adopted. A direct measurement of water droplet size has been achieved with a freeze-etching, carbon-platinum replication method using the electron microscope (Eley & others, 1976). However, this procedure is expensive and time-consuming and is not suited to routine size analysis in stability studies. Thus, a second method has been developed based on the semi-permeable nature of the thin oil films that separate the water droplets and the external aqueous phase.

In an osmotic gradient (provided by 0.18% saline) there is a net flow of water from internal to external aqueous phase such that the multiple droplets gradually shrink in size. The rate and degree of shrinkage is related to the surface area and volume of the internal aqueous phase, and can be measured using the Coulter Counter TA Mark I. This instrument provides a full particle size analysis (16 channels) in about 10 seconds).

Fresh multiple emulsions, containing many small water droplets, change in size in a different way to aged multiple emulsion droplets that contain fewer, larger water droplets. The water permeability of thin films can also be measured in the absence of an osmotic gradient by exchange diffusion using labelled water (^{3}H and ^{18}O) (Hanai & Haydon, 1966).

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