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***Stability of Water-in-Oil-in-Water
Emulsions Formed by Membrane
Emulsification***

A thesis presented in partial fulfilment of the
requirements for the degree of

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Dedicated to

**My Guru
Sri Sri Mata Amritananda Mayi Devi**

Abstract

The main objectives of this study were to determine

- i. The effectiveness of encapsulating whey protein concentrates (WPC) within water-in-oil-in-water multiple emulsions produced by membrane emulsification.
- ii. The effect of the primary and secondary emulsification conditions and membrane operating parameters on the multiple emulsion properties; of particular concern were the yield and physical stability of the emulsions.

The multiple emulsions were prepared by a two-stage emulsification process. The emulsification conditions were varied widely to determine the optimum conditions for the production of multiple emulsions. Ultra-Turrax, ultrasound and valve homogenisation were tried for the preparation of the primary emulsion; an Ultra-Turrax and Shirazu porous glass (SPG) membrane emulsification were used for secondary emulsification.

The standard primary emulsions (water-in-oil) were prepared with 10% of the WPC and 6.4% glucose (as a marker) in the water phase with a water-in-oil volume fraction of 0.25. The oil phase (soybean oil) consisted of 10% hydrophobic emulsifier (Polyglycerol polyricinoleate [PGPR] or Span 80). Typically pre-emulsification was carried out in an Ultra-Turrax at 20500 rpm and the primary emulsification was done in a homogeniser at a pressure of 500/100 bars in the two stages; secondary emulsions were prepared with an SPG membrane of pore size 2 μ m or 3.8 μ m with the dispersed phase (water-in-oil) being pushed through the pores of the membrane at a specific transmembrane pressure (125-150 kPa) and dispersed phase flux into a continuous phase (water with a hydrophilic emulsifier concentration, usually Tween 80, of 1%) flowing through the inside of the membrane at a particular velocity (standard of 1 m/s).

The yield of the multiple emulsions formed was estimated by measuring glucose release using an Advantage Glucose meter.

Unlike Span 80, PGPR was able to form stable o/w emulsions and hence the initial yield of the multiple emulsions varied from 80% at 2.5% to 100% at 10% PGPR. The higher the concentration of water in the inner phase the lower the yield of the multiple emulsions and the higher the droplet size of the primary emulsions. A valve homogeniser gave the best results for primary emulsification. Of the 3 homogenisation pressures (250 bar, 500 bar, 1000 bar) tried, the w/o emulsion produced with 500 bar and 10% PGPR was taken as the standard as this was found to be stable for 6 months without physical damage. A 30% maximum loading of the WPC in the inner water phase was also determined. A further increase may destabilise the process by causing blockage to the membrane pores.

The yield as well as the droplet size of the multiple emulsions was found to increase as the membrane pore-size was increased from 1.4 μ m to 3.8 μ m. Transmembrane pressure and continuous phase velocity did not have much influence on the yield of the multiple emulsions. However an increase in continuous phase velocity increased the opacity of the serum layer formed indicating that an increased amount of smaller droplets were formed. The dispersed phase flux was increased by increases in any of the transmembrane pressure, PGPR concentration and membrane pore-size.

Hydrophilic emulsifiers (whey protein isolate, soy protein isolate and sodium caseinate) did not influence the yield; however the Tween 80 stabilised multiple emulsions showed a smaller droplet size. An increase in temperature from 20 - 50°C resulted in a lower yield as well as a higher droplet size.

The osmotic gradient set up by glucose and WPC in the inner phase of the emulsion resulting in an influx of water from the outer phase causing bulging of the droplets. Sorbitol added at 1.7% in the outer phase gave a high initial yield (100%) as well as a low droplet size (2-3 μm). The cream layer formed as a result of storage was found to decrease with increase in sorbitol concentration (to 5.9%) due to the lower size of the droplets formed.

The key issues identified were to find an alternative to PGPR with lower Accepted Daily Intake (ADI) value without compromising the emulsification properties and to standardise ways to analyse droplet size of w/o emulsions. Overall the study proved that functional ingredients can be encapsulated using stable w/o/w multiple emulsions prepared using SPG membranes under standardised conditions and hence appears to offer promise for manufacture of commercial products.

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1. General Introduction

Emulsions have been of significant importance from very early times. Galen, an ancient Greek physician (131-c.201), referred to the emulsifying power of beeswax (Bennet, Bishop & Wulfinghoff, 1968). Milk, which is the most common naturally occurring emulsion, is in itself an example of the age-old tradition of dependence of man on emulsions. Milk provides man with most of the essential nutrients and therefore the importance of the use of milk and derivatives of milk like butter as an effective food for growth and development of the body tissues can be found in many ancient literatures.

The industrial exploitation of emulsification technology to generate novel food products started in the 19th century with the production of “margarine” in 1870. Since then the 20th century has witnessed many major leaps in the technology and science of emulsions.

An emulsion is formulated to satisfy needs that are otherwise impossible. For example, oil paint, which is an emulsion, can be applied to damp surfaces, something, which is not practical with oil (Bennet, Bishop & Wulfinghoff, 1968). Other examples include the minimisation of fire hazards by emulsifying flammable materials with water. Also the odour and flavour of water insoluble material like cod liver oil is decreased to a great extent by emulsification. The colloidal properties of emulsions also increase the bulk qualities in food products such as mouth feel, appearance and rheology (Dickinson & McClements, 1996). Emulsions are now finding extensive uses in various fields like agriculture as most of the pesticides and insecticides manufactured are emulsions. The pharmaceutical industry is another field which owes a lot to emulsion technology as most of the medicines produced including the “shake well before use” ones are emulsions in nature. The cosmetic industry also utilizes the applications of emulsions extensively.

The production of emulsions or emulsification is considered both a science and an art. This is because the production of emulsions depends on various factors like temperature, speed of mixing, ingredient source and composition etc. An emulsion,

by definition, is a mixture of two immiscible liquids and these liquids, more scientifically called phases, comprise of at least one oil phase and one water phase. Emulsification mainly involves turbulence or violent agitation of the two phases; the most primitive method of emulsification is the mixing together of the two phases, for example, kerosene and water, in a jar. Scientific research of more effective and easier methods of emulsification paved the way for the use of high-speed mixers or blenders for emulsification. This was followed by the invention of colloid mills of various types, which include smooth-surfaced and toothed surfaced colloid mills depending on the type of agitation required. A major boost in emulsification technology was brought about by the invention of the high-pressure valve homogeniser.

Seifritz first reported the existence of multiple emulsions in his literature published in 1925. Although no further work was conducted in the area for the next 40 years, in 1965, Herbert tried to utilise water-in-oil-in-water (w/o/w) emulsions as a new form of antigen adjuvant and in 1968 Engel *et al* tried to immobilise insulin in the inner aqueous phase of a w/o/w emulsion to improve the efficiency of intestinal absorption. Exxon (1972) also tried utilising multiple emulsions as liquid membrane systems for extraction processes (Matsumoto & Kang, 1989).

Membrane emulsification, which is the most recently developed method of emulsification, is being increasingly studied due to the significant advantage of the process over the other emulsification processes. The method involves lower energy than the other high pressure methods and lower shear forces as the emulsification takes place in a laminar flow. Yet the resultant droplet size of the emulsion is very small and the distribution very uniform, a result that cannot be duplicated in any other emulsification system. Due to the low pressure and low amount of shear involved, the process of membrane emulsification makes the formation of multiple emulsions easier. In the formation of multiple emulsions the shear involved in the secondary emulsification step should be as low as possible to retain the inner droplets intact. This, which is considered to be a hurdle if emulsification is carried out in other high-pressure emulsification systems, makes the membrane emulsification system the most suited for production of multiple emulsions. Also the final droplet size in a multiple emulsion can be very efficiently controlled by membrane emulsification by adjusting

the pore-size of the membranes and without increasing the turbulence as in other emulsification systems.

The method of formation of multiple emulsions by membrane emulsification has wide uses in the field of pharmaceuticals, medicine, cosmetics and food. In the pharmaceutical industry, the production of drugs involving controlled release can very well be manufactured by this process. This includes sustained release of narcotic antagonistic drugs, prolonged release of corticosteroids, slow release of Bleomycin, targeted release of anticancer drugs and detoxification of blood (Garti, 1997). Another major use is in the immobilisation of enzymes, where the enzymes can be entrapped or encapsulated in the internal phase. Research was also conducted on the production of pH compartmented w/o/w emulsions where the inner and outer aqueous phases were maintained at two different pH so that the encapsulated material is viable at its optimum pH and the outer pH is compatible with the route of the drug (Tedajo et al, 2001).

As far as the food industry is concerned, studies conducted by Okonogi and co-workers (1994) for the Morinaga Milk Company, Japan has shown the successful production of low fat spreads and oil-in-water-in-oil multiple emulsions by the process of membrane emulsification. Also in the case of water-in-oil-in-water multiple emulsions, water-soluble substances can be entrapped or encapsulated in the internal phase of the emulsion and then slowly released. This process, an example of microencapsulation, is gaining more importance in the food industry due to its potential for encapsulating water-soluble components such as flavours and vitamins. This process is also used in development of low energy foods (Augustin et al, 2001). Encapsulation of an extensive range of ingredients have been reported including flavouring agents, acids, bases and buffers, lipids, enzymes and micro-organisms, artificial sweeteners, antioxidants, preservatives pigments and dyes, essential oils, minerals, amino acids and peptides, vitamins and pro-vitamins and instant starches. Some other products, which involve the principle of microencapsulation, are salted creams (encapsulation of salt) and aromatic mayonnaises (Garti, 1997). Microencapsulation of probiotics is another emerging field. Various techniques of microencapsulation using extrusion and emulsification are being continuously explored (Krasaekoopt et al, 2003). The common methods of encapsulation include

spray drying, spray chilling and spray cooling, extrusion and related processes, fluidised bed coating, coacervation, supercritical fluid spraying, liposome entrapment, inclusions complexation and recently multiple emulsions (Augustin et al, 2001). It is also said that the presence of water in the external phase of multiple emulsions makes the product very palatable thereby improving the taste and the mouth feel. This makes the role of water-in-oil-in-water multiple emulsions potentially very important in food industry.

This project concentrates on the microencapsulation of whey protein concentrates as a model for the production of water-in-oil-in-water multiple emulsions through membrane emulsification. The key objectives were to investigate the effects of emulsion composition (type and concentration of emulsifier, phase composition) and membrane emulsification process parameters on the yield stability of the food emulsions formed.

2. Literature Review

2.1 Food Emulsions

According to Dickinson (1994), an emulsion is defined as an opaque, heterogeneous system of two immiscible liquid phases ('oil' and 'water') with one of the phases dispersed in the other as droplets of microscopic or colloidal size. Emulsions can be broadly classified into two types: simple emulsions and multiple emulsions. The process of making an emulsion (i.e. emulsification) involves the vigorous agitation of oil and water phases in an emulsification device in the presence of a suitable surface-active material. Even though the two liquids involved in the formation of an emulsion are 'oil' and 'water', pure oil and pure water are seldom involved. The oil phase may contain hydrophobic materials like waxes, hydrocarbons and resins, and the water phase may contain hydrophilic materials like salts and sugars (Brennan *et al* 1990). The selection of the emulsifier is a very important step and it should be done depending on the type of emulsion required. The various factors influencing the type of emulsion formed by mixing oil and water are the type of emulsifying agent used, the relative proportions of the phases and the method of preparation of the emulsion (Brennan *et al* 1990).

2.1.1 Simple Emulsions

Simple emulsions are two-phase systems consisting of one water phase and one oil phase. Two types of simple emulsions are recognized: oil-in-water emulsions (o/w) and water-in-oil emulsions (w/o). O/W emulsions have oil as the dispersed phase and water as the continuous phase; milk is a common example. In contrast, w/o has water as the dispersed phase and oil as the continuous phase, e.g. butter or margarine.

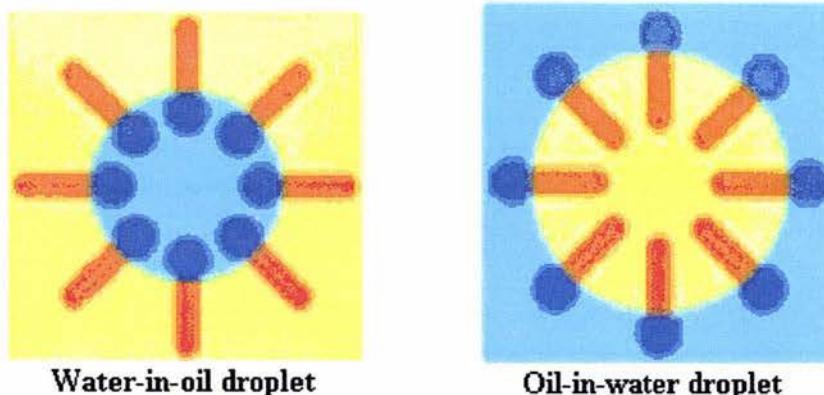


Figure 2.1 Pictures showing the water droplet in w/o emulsion and the oil droplet in an o/w emulsion with the emulsifiers attached. (Danisco website) (http://ingredients.danisco.com/products/detail.jsp?pid=2_1_5)

The two phases may be brought into contact in several ways:

Water may be injected into the oil or vice versa, alternate additions of oil and water can be made, or oil and water can be added and mixed simultaneously (Dickinson, 1994). According to Bancroft's rule, the emulsifier is most soluble in the continuous phase. Hence for producing a stable oil-in-water emulsion the emulsifier used should be hydrophilic in nature and for producing a stable water-in-oil emulsion the emulsifier used should be hydrophobic in nature. This is defined by the hydrophile-lipophile balance of the emulsifier or the HLB number: a hydrophilic emulsifier has an HLB number of >9 and a hydrophobic emulsifier has an HLB number of <9 . Major factors affecting the type and characteristics of the emulsion are temperature, viscosity, dielectric constant, specific gravity, pH, electrolyte concentration, oil type, chain length and co-surfactant concentration (Bennet *et al*, 1968).

Both o/w emulsion and w/o emulsion could be easily recognized by simple tests. For example, a water-soluble dye will tint an o/w emulsion but not the w/o emulsion. Also o/w emulsions spread on the surface of water whereas w/o emulsions coalesce (Bennet *et al*, 1968). O/W emulsions can be diluted with water and they conduct electricity due the presence of an uninterrupted layer of water, but w/o emulsions are thinned with oil and do not conduct electricity (Sutheim, 1947).

2.1.2 Multiple Emulsions

Multiple emulsions are emulsions of emulsions (Dickinson & McClements, 1996), i.e. the dispersed droplets themselves contain small droplets of liquid with the same nature as the bulk continuous phase. Accordingly two types of multiple emulsions are recognized, namely oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions (Figure 2.2).

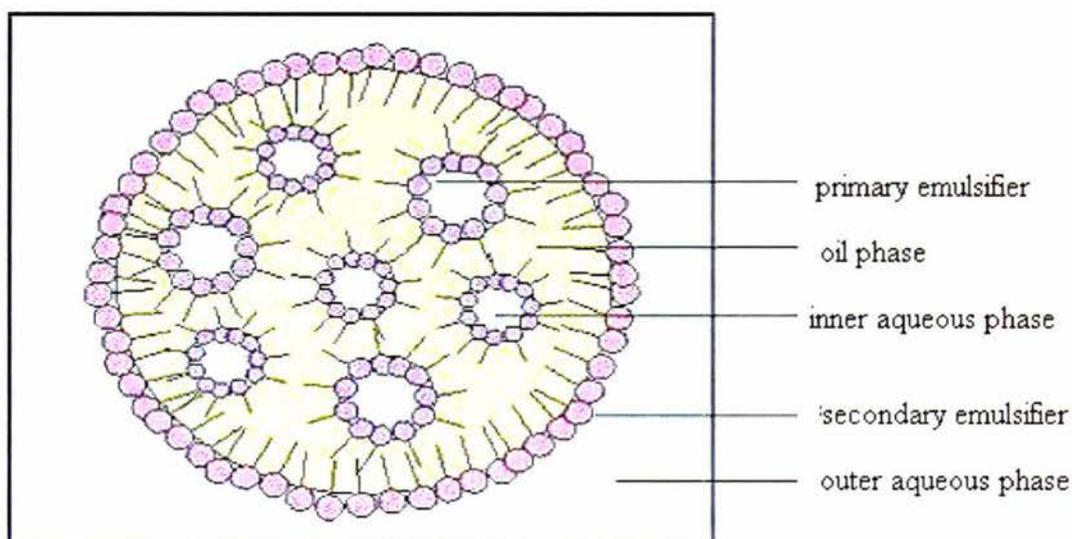


Figure 2.2 Picture showing a w/o/w emulsion droplet with the hydrophobic emulsifier attached to the inner water droplet and the hydrophilic emulsifier attached to the outer oil droplet. (www.foodcolloids.com)
(http://www.foodcolloids.com/dickinson/research_group/mahmood.htm)

According to Matsumoto *et al* (1985), Seifritz first discovered multiple emulsions in 1925. Nowadays multiple emulsions have extensive uses in pharmaceutical industries for controlled release of drugs and in cosmetic industries for production of various cosmetics (Dickinson & McClements, 1996). Owing to the high palatability of w/o/w emulsions, the easiness of controlled release of flavours and production of low calorie food products (e.g. spreads), multiple emulsions are finding increased use in the food industry (Dickinson & McClements, 1996).

Due to the presence of both oil-water interfaces and water-oil interfaces in the same emulsion, multiple emulsions must be prepared by using two different types of surfactants. Both hydrophobic and hydrophilic surfactants should be used for the successful formation of a multiple emulsion. There are three methods by which

multiple emulsions can be prepared (Matsumoto & Kang, 1989): mechanical agitation, phase inversion and two-stage emulsification. Of these, emulsification by the two-stage process is commonly regarded as the most effective for formation of stable multiple emulsions (Dickinson, 1994) and this is the only mechanism considered here.

2.1.2.1 Oil-in-water-in-oil (o/w/o) emulsions

Oil-in-water-in-oil emulsions are multiple emulsions with the internal phase and external phase consisting of oil phases, these being separated by a water phase. An oil-in-water emulsion is first prepared by a suitable emulsification technique in the presence of a hydrophilic emulsifier in the continuous phase (water). The primary emulsion so formed is then further emulsified with oil containing a suitable lipophilic emulsifier as the continuous phase.

O/W/O emulsions have been used for preparation of low-fat spreads in the food industry where they have proved excellent in flavour and aftertaste (Okonogi *et al*, 1994). However, one of the major problems in their use is the stability of the multiple phase system. The presence of a large proportion of water may lead to separation of the oil and aqueous phases during storage, or the emulsion may undergo phase inversion during production or transit, substantially reducing the product value (Okonogi *et al*, 1994). Another problem is the difficulty in the emulsification procedure.

The emulsifying agent plays a very important role in the stability of o/w/o emulsions. The major advancements with respect to increasing the stability are (Okonogi *et al*, 1994):

- the use of di-glycerides, tri-glycerides or lecithin in the fatty phase, but this, even though it produced a stable product, had the drawback of aftertaste degradation due to undesirable flavour;
- the use of specific amount of protein or macromolecule polysaccharide as stabiliser (or gelling agent), but this also had the drawback of adverse taste and growth of fungi and bacteria; and

- use of specific amounts of suitable emulsifying agents (hydrophobic in case of w/o and hydrophilic in case of o/w) or emulsifier blends.

2.1.2.2 Water-in-oil-in-water (w/o/w) emulsions

As noted above, a water-in-oil-in-water emulsion is one whose internal (W_1) and external phase (W_2) consists of water with an oil phase in between the two, or in other words, the dispersed oil phase in the continuous water phase contains smaller water droplets.

In the two-stage preparation of w/o/w emulsions, the primary w/o emulsion can be prepared under high shear conditions in the presence of a hydrophobic emulsifier (E_1). This can be carried out, for example, in a homogeniser or ultrasonifier. The second step in the formation of the complete w/o/w takes place in the presence of a hydrophilic emulsifier (E_2) and should not be carried out under severe mixing conditions. This is because excess mixing ruptures the drops resulting in a simple o/w emulsion (Garti, 1997). Three main types of morphologies of w/o/w emulsion were proposed by Florence and Whitehill (1981) (Figure 2.3). The type A system consists of multiple emulsions with only one inner droplet, a type B system consists of a large proportion of multiple emulsion droplets containing more than one inner droplet and in a type C system, most of the emulsion droplets contains a substantial number of inner droplets which are flocculated or closely packed (Dickinson and McClements, 1996).

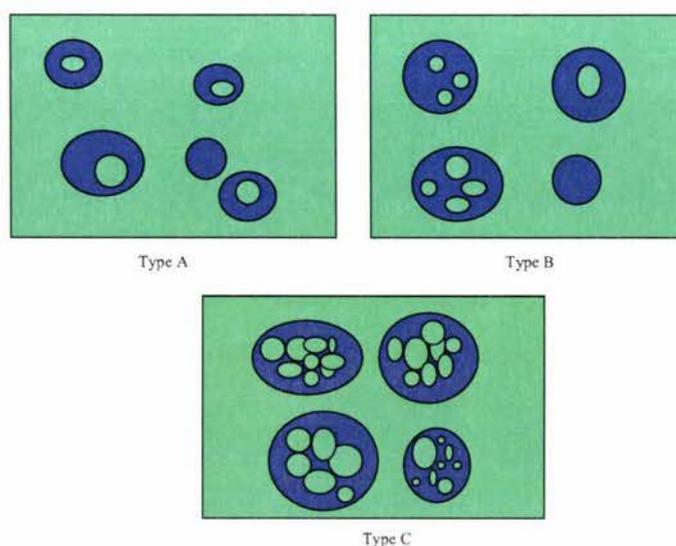


Figure 2.3 Figure showing the three types of multiple emulsions

Usually w/o/w emulsions consist of internal water droplets of 0.5-2 μm and oil droplets of 10-60 μm (Garti, 1997). The use of the right emulsifier is of particular importance in the formulation of a multiple emulsion. Emulsifier combinations with the right HLB values, considering the apparent and weighted HLBs, have to be used (Section 2.2.2.3). It was well established that the stability of the emulsion depends greatly on the emulsifier concentration at the inner and outer phases and that the inner hydrophobic emulsifier (E_1) must be used in greater excess (10 – 30wt% of the inner emulsion), while the hydrophilic emulsifier (E_2) must be used in low concentrations (0.5 – 5 wt%) (Garti, 1997).

2.1.3 Formation of Emulsions

The most important aspect in the formation of any type of emulsion is the disruption of droplets. Two types of forces mainly govern the droplet disruption: the interfacial forces and disruptive forces. The disruption of droplets is opposed by the Laplace pressure, which is the difference in pressure between the convex and concave side of a curved interface and is given by (Walstra, 1993):

$$P = \gamma \left(\frac{1}{r_1} + \frac{1}{r_2} \right) \dots\dots\dots (2.1)$$

where γ is the interfacial tension and r_1 and r_2 are the principle radii of curvature. For a spherical droplet,

$$P = \frac{2\gamma}{r} \dots\dots\dots (2.2)$$

where r is the radius of the spherical droplet. Walstra (1993) has also shown that, in practice, most of the energy input for emulsion formation is dissipated into heat. According to the above equation, as the interfacial forces increases the Laplace pressure increases and hence intense external shear has to be applied for droplet disruption (Mc Clements, 1999).

The action of the disruptive forces during emulsion formation depends on the type of flow (i.e. laminar or turbulent) and the disruptive forces should be such that they

overcome the interfacial forces and also the duration of the forces should be long enough to deform and disrupt the droplet (Schubert & Karbstein, 1994). The disruption of droplets is only possible if the Weber number (Walstra, 1983), which is the ratio of disruptive forces to interfacial forces, exceeds a critical value (close to one)

$$We = \frac{\eta_c Gr}{\gamma} \dots\dots\dots (2.3)$$

where We is the Weber number, η_c is the viscosity of the continuous phase, G is the velocity gradient, r is the droplet radius and γ is the interfacial tension.

In laminar flow, the liquid inside the droplet is set in rotation by the normal and tangential stresses acting on the droplets, which leads to elongation of the droplet resulting in its disruption into several small droplets (McClements, 1999). Under high viscosity conditions this elongational flow (no shear, velocity gradient in the direction of flow) is the most efficient for disruption of droplets (Walstra, 1993). Under low viscosity conditions of the continuous phase, droplet disruption takes place only at high velocity gradients, hence, subjecting the drops to intense turbulent flow satisfies the intense shear requirement for droplet disruption. According to Kolmogorov's theory, in the case of an isotropic turbulent flow, a spectrum of large eddy sizes, (the so called energy bearing eddies) with high velocity gradients are responsible for droplet break-up (Walstra, 1993).

Cavitation is another phenomenon leading to effective droplet disruption in fluids. Here changes in pressure (contraction of liquid when pressure increases and expansion when pressure decreases) bring about the disruption. When the pressure falls below a critical value a cavity is formed, which increases as the pressure increases (expansion), making the surrounding liquid to move into it. On subsequent contraction the cavity collapses creating an intense shock wave causing the droplets in its vicinity to break (McClements, 1999).

2.1.4 Emulsion Stability

Emulsion stability is a kinetic concept (Binks, 1998). The droplets in an emulsion are in continuous motion and as they move they collide with other droplets in the emulsion. If the emulsion is a stable one the droplets may separate after the collision

and there will be no change in the constitution of the emulsion. However this is rather a matter of time: some emulsions may be stable for a few minutes and some for years. In the case of unstable emulsions, after collision the droplets may either stick to each other (flocculation) or they may unite to form larger droplets (coalescence). Due to this increase in size of the droplets they may either settle (sedimentation) to the bottom or rise to the top (creaming) depending on the relative densities of the phases and droplet size (Stoke's Law). The sedimentation rate is directly proportional to the square of the radius and the sedimentation equilibrium is relevant for a droplet size in excess of 0.05µm. The separation rate becomes of practical importance (i.e. according to Stoke's law creaming velocity is directly proportional to the droplet radius) at a size of 1 µm (Friberg, 1990).

The destabilization of emulsions is also called demulsification and five types of demulsification are identified:

- Creaming
- Flocculation
- Coalescence
- Ostwald Ripening
- Phase Inversion

These are considered briefly in the following sections.

2.1.4.1 Creaming

The movement of oil droplets to the top of an o/w emulsion resulting in the formation of separate oil-rich and water-rich layers is called creaming (Figure 2.4). The top oil-rich layer is called the cream. This phenomenon is usually observed in unhomogenised milk kept for some time, where the fat layer is separated from the rest. The same phenomenon occurring in w/o emulsion is called sedimentation (i.e. water droplets collect at the bottom). Creaming is directly proportional to the square of the droplet radius and the velocity of creaming as explained by the Stoke's law,

$$V = \frac{2r^2(\rho_s - \rho_m)}{9\eta} \dots\dots\dots (2.4)$$

where r is the radius of the droplet, ρ_s and ρ_m are the densities of the dispersed phase and dispersion medium respectively and η is coefficient of viscosity of the dispersion medium. Stoke's equation can be modified for a dilute suspension of particles:

$$V = \frac{V_0}{(1 + kx)} \dots\dots\dots (2.5)$$

where V_0 is the Stoke's velocity for simple droplets, x is the volume fraction of the dispersed phase and k is a constant (the value of k is around 5) (Menon & Wasan, 1985).

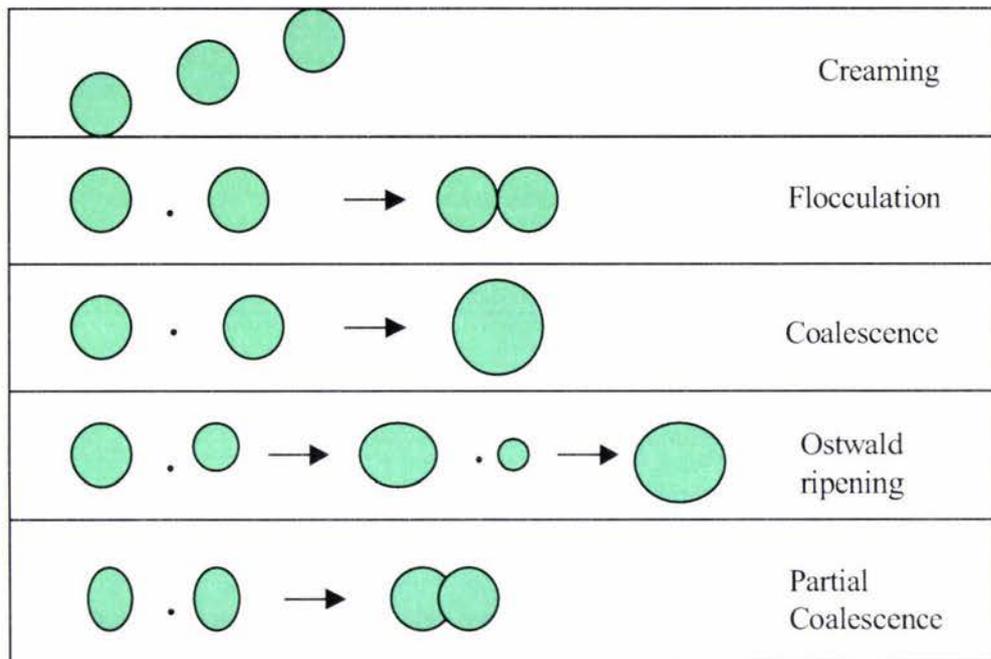


Figure 2.4 Types of physical instability in o/w emulsions (Walstra, 1996).

There are several methods to study creaming, the traditional ones being the measurement of cream height versus time, light scattering techniques and direct sampling for examination of volume fraction and droplet size distribution. Nowadays new methods like conductivity measurements, magnetic resonance imaging and ultrasound methods are also being used (McClements, 1999).

2.1.4.2 Flocculation

As the droplets in an emulsion collide with each other they may aggregate without the rupture of their stabilising layer at the interface and so may exist in pairs or lumps in the emulsion. This phenomenon is called flocculation (Figure 2.4). Flocculation occurs when the kinetic energy released during collision brings the droplets over the repulsive force barrier and into a region where attractive forces operate, causing the

droplets to attach to each other (Bergenstahl & Claesson, 1990). Flocculation can be either reversible or irreversible in nature. Flocculation increases the effective droplet size and hence may lead to creaming. The mutual interaction forces between the droplets govern the rate of flocculation and these forces include the van der Waals forces, the electrical double layer forces and steric forces. The rate of flocculation can be calculated by estimating the number of times the emulsion droplets come into contact and also the time they stay in contact in each case (Binks, 1998). The methods commonly used to study the kinetics of flocculation include photon correlation spectroscopy, turbidimetry, and drop counting methods such as the Coulter counter (Binks, 1998).

2.1.4.3 Coalescence

The droplets in an emulsion may fuse together, after collision, each losing their identity and forming a single large droplet. This phenomenon is called coalescence. The increase in surface/interfacial free energy as a result of breakage of dispersed phase into droplets triggers a thermodynamic instability of the dispersed phase leading to coalescence (Jiao *et al*, 2002). Coalescence may be considered to be the most dangerous physical instability in emulsions as this may lead to complete separation of the phases in the emulsion. It takes place mainly in large drops or aggregates of droplets when the film between the two droplets ruptures (Menon & Wasan, 1985). Various factors affecting coalescence are the thickness of the film (a thick film of about 4-7 μm , including the lipophilic part of the emulsifier, is less likely to be disrupted), interfacial tension (a high interfacial tension minimises the amplitude of surface undulations and thus stabilises the emulsion), droplet size (large droplets coalesce much faster and so a decrease in droplet diameter by a factor of 2 may decrease coalescence rate by 10-100), interfacial viscosity and elasticity (a high viscosity and elasticity will dampen surface undulations and stabilise emulsion films) and presence of particles (particles in continuous phase stabilize an emulsion while that inside the droplets destabilises it.) (Bergenstahl & Claesson, 1990).

2.1.4.4 Ostwald Ripening

Ostwald ripening is also called isothermal distillation or molecular diffusion and it is the result of solubility differences of oil contained within drops of differing sizes

(Binks, 1998). It is caused by the diffusional transport from small droplets into larger droplets as the chemical potential of the liquid in the drops decreases as the droplet radius increases (Bergensstahl & Claesson, 1990). The Kelvin equation predicts that the solubility of a spherical particle increases with decreasing size. The diffused material in the droplet reaches the aqueous phase and then condenses onto larger droplets resulting in an overall increase in the droplet size and leading to a decrease in the interfacial area.

2.1.4.5 Phase Inversion

The sudden change of phase of an emulsion, for instance the change of an o/w emulsion to w/o emulsion, is termed as phase inversion. Phase inversion may be of two types – transitional inversion and catastrophic inversion (Binks, 1998). Transitional inversion is due to the HLB effect of the emulsifier and catastrophic inversion is due to an increase in the proportion of the disperse phase in the emulsion, i.e. in an oil-in-water emulsion, if the concentration of oil increases beyond a certain limit the emulsion changes to water-in-oil emulsion. Catastrophic inversions are not reversible. Temperature is also a determining factor of phase inversion and the effect is more pronounced at lower surfactant concentrations (Binks, 1998).

2.1.5 Stability and Yield of Multiple Emulsions

Since multiple emulsions are complex systems their stability is very important and the destabilization process is more complicated than that of simple emulsions. For instance, a w/o/w oil droplet may coalesce with one or more of the other emulsion droplets resulting in destabilization of the emulsion, or the water phase inside the oil droplets may be liberated due to the rupture of the thin oil film resulting in the mixing of the internal and external water phases (Dickinson & McClements, 1996).

Another possible type of destabilisation is the movement of water either from the inner phase to the external continuous phase or vice versa according to the concentration gradient. This process is called osmotic instability and takes place only when the thin liquid oil film separating the two phases acts as a semi-permeable membrane (Dickinson & McClements, 1996). Water transport rates are affected by

magnitude of the osmotic pressure gradients between phases W_1 and W_2 , the nature and concentration of surfactants used, and the nature and viscosity of the oil phase (Wen *et al*, 2000). In the former case (water moves out of the inner phase), osmosis results in the shrinkage of the inner water droplets, and in the latter case the result is the bulging of the internal water droplets. According to Wen *et al* (2001) three different mechanisms control the water transport in double emulsions depending on the positions of W_1 and W_2 . When W_1 and W_2 are at visual contact water transport occurs mainly through the hydrated surfactant mechanism, i.e.

- surfactants get hydrated at the W_1/O interface
- hydrated surfactants diffuse through the thin oil layer (within a few μm) from W_1/O to O/W_2 interface and
- hydrated surfactants get dehydrated at the O/W_2 interface.

In the case of visually noncontacting W_1 and W_2 , the mechanisms are spontaneous emulsification and reverse micellization, i.e.

- reverse micelles and emulsified water droplets form at the W_1/O interface
- they diffuse through the bulk from W_1/O to O/W_2 interface and
- water is transferred from the reverse micelles and water droplets to the W_2 phase at the O/W_2 interface.

According to Garti (1997) the various methods leading to stability of multiple emulsions can be categorised into three:

1. Stabilisation of the internal interface of the inner emulsion and increasing the viscosity of the inner phase.
2. Selection of proper oil phase and addition of possible carriers, complexants and viscosity builders into it.
3. Stabilisation of outer interface by use of polymeric emulsifiers, macromolecular amphiphiles or colloidal particles for a stronger rigid film.

Suggestions made by Matsumoto (1986), Dickinson *et al* (1994) and Garti (1997) have shown that destabilisation of the multiple emulsion may be prevented by using a high HLB emulsifier (effective water soluble surfactant) in the water phase. Also the

size of the inner water droplets should be very small when compared with the size of the emulsion droplet and also the size of the outer droplet should be sufficiently small so as to minimise flocculation or coalescence. Another method of preventing coalescence is the use of non-adsorbing, water-soluble polymers (hydrocolloids) in the continuous phase to act as thickening or gelling agents. Osmotic destabilisation can also be prevented by the use of oil with low water solubility (Dickinson & McClements, 1996). The stability of the primary w/o emulsion is also very important for the stability of the final w/o/w emulsion and this can be made possible by the formation of very fine droplets by vigorous homogenisation in the presence of a high lipophilic emulsifier concentration (say about 5 – 10% of the oil) (Dickinson & McClements, 1996).

Yield, in the case of a w/o/w emulsion, can be defined as the percentage of the inner water droplets of the primary w/o emulsion (on a volume basis) retained in the final w/o/w emulsion (Dickinson & Hajda, 1996). Yield of a w/o/w emulsion can be determined by adding a suitable marker compound in the water phase of the primary w/o emulsion at a known concentration and then determining its concentration in the outer water phase (Matsumoto *et al*, 1986). The yield of multiple emulsions can be calculated as,

$$Y = \frac{100(M_i - M_o)}{M_i} \dots\dots\dots (2.6)$$

where M_i is the amount of dye encapsulated in the internal phase originally and M_o is the amount of dye in the outer phase (Dickinson & Hajda, 1996).

Dickinson & McClements (1996) reported that fresh w/o/w emulsions with w/o volume fraction ($\Phi_{w/o}$) = 0.2, 8 wt% lipophilic emulsifier in the oil phase, w/o/w volume fraction ($\Phi_{w/o/w}$) = 0.2, 0.125 wt% caseinate as hydrophilic emulsifier gave yields of 96% based on the loss of polymeric dye marker (violet Poly R-478 or yellow Poly T-128) from the inner water droplets. Any reduction in the amount of lipophilic emulsifier used for the formation of the primary water-in-oil emulsion reduced the yield of the final w/o/w multiple emulsion and also decreased yield on storage (Dickinson & McClements, 1996). They also reported that a higher viscosity of the oil phase increased the yield of the multiple emulsions with higher lipophilic emulsifier concentration. This was attributed to the increased resistance to the

disruptive shear forces of second homogenisation stage, which is believed to be responsible for much of the release of the primary emulsion droplets.

Apenten & Zhu (1996) introduced a concept of effective minimum concentration (EMC), which states that there is a minimum necessary level of hydrophilic and lipophilic surfactants that should be added to produce a multiple emulsion with high yield and stability. They studied the effects of Span 80 and Tween 20 concentrations on the stability and yield of w/o/w multiple emulsions using kerosene as the oil phase and glucose as the marker compound. Span 80 concentrations of 0.25 – 10% w/w were investigated. They found that a Span 80 concentration of 0.5% was enough to give a yield of >80%, but this resulted in low stability. A concentration of 2.5% w/w was taken as the EMC as this gave an increased yield (>85%) as well as stability. The Tween 20 concentration was fixed at 1% w/w as it was found that increasing the Tween 20 concentration did not improve the yield of the emulsions, but resulted in lower droplet size thereby improving the stability with respect to creaming. Dickinson *et al* (1991) found that the incorporation of a marker compound (glucose) in the preparation of protein stabilised w/o/w emulsions caused an influx of water from the external phase to the internal phase according to the concentration gradient established. This, however, instead of resulting in an increase in droplet size resulted in its decrease, which was explained as due to the rupture of the thin oil film at the interface.

Dickinson *et al* (1993) experimented with a method for encapsulation and controlled release of nutrients like L-tryptophan and vitamin B₂ in food systems. They used Span 80 and Tween 20 as the hydrophobic and hydrophilic emulsifiers, respectively, but also incorporated BSA (0-1 wt%) in the aqueous phase of the primary w/o emulsion. A high yield of 95-98% was obtained and the w/o emulsion mean droplet size was 20 µm. They confirmed that the presence of BSA forms a more viscoelastic mixed adsorbed layer that prevented the inner w/o emulsion droplet from coalescence.

Dickinson *et al* (1994) studied flavour release from protein stabilised w/o/w emulsions. The emulsions were stabilised with Span 80 and sodium caseinate and the inner aqueous phase contained the flavour compound butan-1-ol. They found that the presence of butanol reduced the emulsion droplet size by 15% in a 7-day period

making the depletion of the inner aqueous phase quite clear. To stabilise the emulsion the only possible way suggested was the incorporation of protein or hydrocolloid into the internal aqueous droplets but no results of this treatment were reported.

The two possible mechanisms of transport across the oil phase are reverse micellar transport and diffusion across a very thin lamella of surfactant (Kita et al, 1978). In ionisable matter, controlling the dissociation is an effective method of controlling the diffusion through the oil phase, as the transport rates depend on the nature of the entrapped material (dissociation constant), on the oil and on the pH of the aqueous phase (Garti, 1997). Garti & Magdassi (1986) showed that not only ionisable matter, but also water molecules and non-electrolyte water-soluble matter could easily migrate through the oil membrane without affecting the double emulsion stability and that the rate of micellar dissociation through the oil phase is the rate (of release) determining step. Also the rate of release could be varied by changing the nature of oil, its viscosity and also by addition of carriers.

Similar results were obtained for Garti *et al* (1994) in experiments with 10wt% Span 80, 0.5 wt% BSA and 5wt% Span 80-Tween 80. They found that BSA (a polymeric emulsifier) and Span 80 (a monomeric emulsifier) formed a strong and thick-gelled film preventing the rupture of the oil membrane, thereby increasing the yield and stability of w/o/w emulsions. Also the presence of BSA in the inner phase had a greater effect on slowing NaCl release than the presence of BSA (0-0.1 wt%) in the outer phase. Garti (1997) reported that BSA in the inner phase results in slower transport rates as a result of two factors: binding the hydrophobic Span 80 and formation of reverse micelles, thickening the interfacial film and formation of thin surface lamellae at the interface.

Work done by Law *et al* (1984) on the interfacial polymerisation of modified polyxamer surfactants and in the interfacial complexation of polyxamer co-polymeric non-ionic surfactants and proteins like albumin has helped in increasing the stability of w/o/w systems by the formation of polymeric film or macromolecular complexes across the o/w interfaces through interfacial interaction between macromolecules like albumin and nonionic surfactants.

Work done by Omotosho *et al* (1986) on the effect of osmotic gradient on the stability of w/o/w emulsions proved that an increase in Span 80 concentration from 1-5% with 0.2% BSA increased the number of internal water droplets (with mean diameters 2.05 – 2.15 μm) from 1-4 to 20-50. However a 10% concentration of Span with 0.2% BSA resulted in a large number of flocculated droplets. To determine the effect of osmotic gradients between internal and external aqueous phases on release of NaCl, w/o/w multiple emulsions containing 1.25% NaCl in the inner aqueous phase were prepared and it was observed that there was an influx of external aqueous phase resulting in the thinning of the oil film, which in turn led to the increased release of NaCl through the oil layer. In contrast, this problem was not seen in emulsions osmotically balanced with sorbitol in the external aqueous phase.

The studies conducted by Omotosho *et al* (1986) using various types of oils (octane, dodecane, hexadecane, cyclohexane, toluene and isopropyl myristate) and two different types of markers – NaCl and 5-fluorouracil (5-FU) helped to establish the relationship between the droplet size and release rates and also the nature of the marker compound and the release rates. They found that solute is released fastest from w/o/w emulsions containing isopropyl myristate as the oil phase and the slowest from emulsions with octane as the oil phase. The mean droplet diameter in the former was 2.1 μm and in the latter was 12.6 μm due to smaller (25.38 mN m^{-1}) o/w interfacial tension in the former than the latter (50.13 mN m^{-1}). The increased release rate was attributed to the reduction in droplet size as this increases the surface area exposure. Also 5FU was released at a faster rate than NaCl because of its greater lipophilicity.

As mentioned earlier stability of a w/o/w emulsion can be affected by factors like flocculation and coalescence leading to oil membrane rupture, osmotic instability and instability due to diffusion across the oil membrane. Various methods tried by different researchers were discussed and in short, using a high concentration of a low HLB emulsifier in the oil phase, oil with low water solubility and a high HLB emulsifier in the outer aqueous phase can increase the yield stability of a w/o/w emulsion.

2.2 Food Emulsifiers

All emulsifiers are surfactants or surface-active agents, i.e. compounds that adsorb at an interface to lower the surface (interfacial) tension in an emulsion. This lowers the cohesive force between the droplets of the emulsion and thereby improves its stability (Bennet et al 1968). An emulsifier should also be able to prevent recoalescence by a combination of surface activity and structure formation at the newly formed interfaces (Dickinson, 1994). According to Bergenstahl (1997), the effects of an emulsifier are:

- 1) reduction in surface tension
- 2) increase in bubble lifetime
- 3) increase in emulsifiability
- 4) changes in aggregation rate, sediment volume and crystallization properties of emulsions.

In the United States, food emulsifiers are broadly divided into GRAS (21CFR184) (generally recognized as safe) and direct food additives (21CFR172). Substances affirmed as GRAS usually have less stringent regulations attached to their use, e.g. lecithin and monoglycerides. But direct food additives may be allowed only in certain specific foods at low maximum allowable levels, e.g. polysorbates and polyglycerol esters (Hasenhuettl, 1997). The European Economic Community (EEC) regulates the use of food additives by dividing them into four annexes (Hasenhuettl, 1997). Annex 1 (similar to GRAS list 21CFR 182) consists of additives which can be used anywhere except in natural or standardized processed foods. Annexes 2 and 3 are similar to direct food additive regulations. Annex 4 consists of a separate listing for solvents and solubilising agents unlike the USFDA. Therefore, trade between the United States and EEC should be done with caution as some specific emulsifiers like those derived from polyglycerols are permitted in the US up to decaglycerol, but limited to tetraglycerol in the EEC (Hasenhuettl, 1997).

2.2.1 Emulsifier Structure

The structure of an emulsifier is characterised by a hydrophilic 'head' group, which prefers to associate with the water phase and a lipophilic 'tail', which favours the oil

phase (Figure 2.5). Polar head groups present in a variety of functional forms may be incorporated to produce anionic (carries a negative charge when dissociated in water, e.g. soap, alkyl sulphates), cationic (carries a positive charge when dissociated in water, e.g. alkyl dimethyl ammonium chloride), amphoteric (carries both positive and negative charge when dissociated in water) or non-ionic surfactants (do not ionise in solution). Examples of the four types of surfactants are,

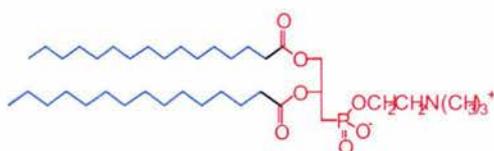
Anionic (Sodium dodecyl sulphate)



Cationic (Cetyl pyridinium bromide)



*Zwitterionic/Amphoteric (lecithin)
(Dipalmitoylphosphatidyl choline)*



Nonionic (polyoxyethylene (4) lauryl ether)

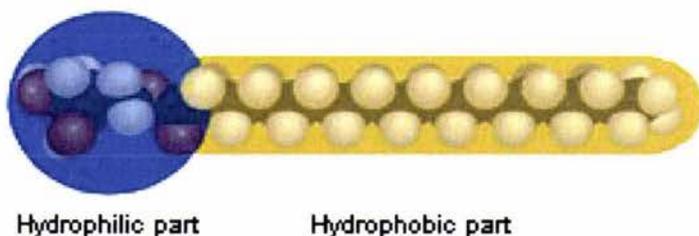


Figure 2.5 The structure of an emulsifier molecule (Danisco website http://ingredients.danisco.com/products/detail.jsp?pid=2_1_5)

During solubilisation, the surfactant system may pass through a series of phases in a particular sequence. They are:

- reverse micelles
- reverse hexagonal phase
- lamellar phase
- hexagonal phase
- micellar solution
- molecular solution

These phases may collectively be called liquid crystalline phases and they depend on the temperature, concentration and chemical composition of the surfactant. Of the

various liquid crystalline phases the most suited for a food grade emulsion is the lamellar phase and this consists of bimolecular lipid layers in one dimension alternating with layers of water between the polar groups (Becher, 1985). Span 80 (sorbitan mono oleate) and PGPR (polyglycerol polyricinoleate) are the commonly used hydrophobic emulsifiers and Tween 20, Tween 80 (polyoxyethylene sorbitan monooleate), whey protein isolates (WPI), soy protein isolates (SPI) and sodium caseinate are the commonly used hydrophilic non-ionic emulsifiers in food systems.

2.2.1.1 Span 80 (S-6760) (Source – www.sigmaaldrich.com)

Span 80, or sorbitan monooleate, is a non-ionic hydrophobic emulsifier. It consists of approximately 75% oleic acid (C18:1) with the rest primarily of linoleic (C18:2), linolenic (C18:3) and palmitic (C16:0) acids. The water content is not more than 1%. Other emulsifiers belonging to the Span family are Span 85, Span 65, Span 60, Span 40 and Span 20. The structure of Span 80 is given in Figure 2.6

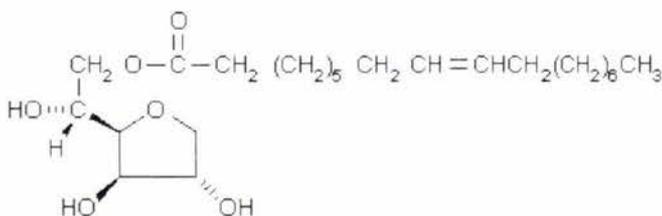


Figure 2.6 Structure of Span 80 molecule (Sigma Aldrich website) (http://www.sigmaaldrich.com/cgi-bin/hsrun/Distributed/HahtShop/HAHTpage/HS_StructureImage)

2.2.1.2 PGPR (Palsgaard 4125)

(Source - http://www.palsgaard.dk/Pages/Product_areas/Palsgaard4125.htm)

The main components of PGPR or polyglycerol polyricinoleate (Palsgaard 4125) are castor oil and glycerol. Castor oil is obtained from castor plant (*Ricinus communis*) and is high in hydroxyl acid and ricinoleic acid. Castor oil and glycerol are polycondensed and then esterified to get polyglycerol polyricinoleate. The acceptable daily intake (ADI) is 0-7.5 mg/kg body wt and it is considered GRAS. PGPR is usually used in chocolate coated bars and chocolate novelties with kid appeal. Other emulsifiers in the family are Palsgaard 4150 and Palsgaard 4445. Of these Palsgaard 4150 possesses no off-flavour and is highly concentrated, thus making it suitable to be administered at lower dosage levels than Palsgaard 4125. The structure of PGPR is given in Figure 2.7

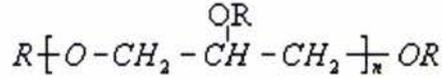


Figure 2.7 Structure of PGPR molecule (EFEMA Index of Food Emulsifiers, Nov 1999)

(www.cefic.be/sector/efema/9911foodemulsi.pdf)

where n = degree of polymerisation of glycerol (average >1)

R = H or a fatty acyl group derived from polycondensed ricinoleic acid.

2.2.1.3 Tween 80 (P-8074) (Source – www.sigmaaldrich.com)

Tween 80 is otherwise called poly oxyethylene sorbitan monooleate and is a non-ionic hydrophilic emulsifier consisting of approximately 70% oleic acid and the rest of linoleic, linolenic, palmitic and stearic acids (Figure 2.8). Also present are trace amounts of P, Cl, SO₄, Al, NH₄, Ca, Cu, Fe, K, Mg, Na, Pb and Zn.

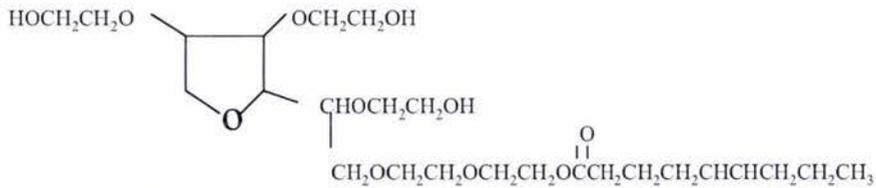


Figure 2.8 Structure of Tween 80 molecule (Sigma Aldrich website)

[http://www.sigmaaldrich.com/cgi-](http://www.sigmaaldrich.com/cgi-bin/hsrun/Distributed/HahtShop/HAHTpage/HS_StructureImage)

[bin/hsrun/Distributed/HahtShop/HAHTpage/HS_StructureImage](http://www.sigmaaldrich.com/cgi-bin/hsrun/Distributed/HahtShop/HAHTpage/HS_StructureImage)

Other forms of Tween are Tween 85, Tween 65, Tween 60, Tween 40 and Tween 20.

2.2.1.4 Sodium Caseinate

Skim milk is acid coagulated at pH 4.5 and at a temperature of 46°C to obtain a curd, which is then neutralised by addition of NaOH. This is then pasteurised and spray dried to form commercial casein or sodium caseinate (Tonberg *et al*, 1997). Creamer & Barry (1975) reported that sodium caseinate has a molecular weight of 250,000Da and consists of particles of about 10nm. The major components are α- and β-caseins. The solubility of caseinates depends on pH (completely soluble at pH >5.5), salt content and type of cation (Tonberg *et al*, 1997). According to Fox & Mulvihill (1983), caseinates have high heat stability with a 3% solution stable without gross aggregation when heated to 140°C at pH 7. Caseinates are capable of adsorbing onto

the o/w interface thus acting as a surfactant stabilising the emulsion. β -caseins are preferentially adsorbed in o/w emulsions made at sodium caseinate concentrations of $< 2\%$ of the water phase and α_{s1} -casein adsorbed preferentially at higher levels of sodium caseinate (Srinivasan *et al*, 2000). However Dickinson *et al* (1997) proved that caseinate concentrations of $\geq 2\%$ produce less stable emulsions due to association of oil droplets on the surface forming a network or depletion flocculation.

2.2.1.5 Whey Protein Isolates (WPI)

Whey protein isolates are the most pure form of whey proteins produced commercially with a protein content $>90\%$. Commercial WPI is usually manufactured by microfiltration and spray drying of cheese whey. Being the most pure form, a higher amount of protein is available for a given quantity of WPI resulting in more surface coverage (protein load at the o/w interface) and hence better emulsification capacities. The whey proteins include four major proteins; β -lactoglobulin ($\sim 59\%$, M.W. $\sim 18,600$), α -lactalbumin ($\sim 22\%$, M.W. $\sim 14,400$), bovine serum albumins (M.W. $\sim 67,000$) and immunoglobulins (M.W. $\sim 160,000$) (Tornberg *et al*, 1997).

2.2.1.6 Soy Proteins

Soybeans are the most important protein source of vegetable origin. They can be classified according to ultracentrifugal analysis into 2S, 7S, 11S and 15S fractions, out of which, the 7S (M.W. $\sim 180,000$ - $210,000$) and 11S (M.W. $\sim 350,000$) are the major ones (Tornberg *et al*, 1997). The association-dissociation reactions of soy proteins are influenced by pH, salt addition and temperature (Tornberg *et al*, 1997). Soy protein isolate (SPI) can be prepared from defatted soybean flakes by extractions with deionised water, precipitation at pH 4.6, washing the curd with deionised water, neutralising to pH 7.0 and spray drying.

2.2.2 Classification of Emulsifiers

Emulsifiers can be classified based on

- Solubility
- Phase Inversion
- Hydrophile-Lipophile balance

2.2.2.1 Solubility concept

This concept cited by Bergenstahl (1997) was put forward by Bancroft (1913) and the underlying principle is that the solubility of the emulsifier determines the type of emulsion, i.e. an oil soluble emulsifier will produce an oil-continuous emulsion (w/o) and a water soluble emulsifier will produce a water-continuous emulsion (o/w) (Bergenstahl, 1997). For example, sorbitan esters (Spans) are oil-soluble and hence oil-continuous (hydrophobic) whereas ethoxylated sorbitan esters (Tweens) are water soluble and hence water-continuous (hydrophilic).

2.2.2.2 Phase Inversion Concept

The principle here is that emulsifiers tend to change their behavior (i.e. from oil-soluble to water soluble and vice-versa) depending on temperature. For instance, the hydrophilicity of ethoxylated surfactants (e.g. ethoxylated sorbitan esters or Tweens) declines with increase in temperature, as a result of which it changes from a water soluble surfactant at low temperature to an oil soluble one at high temperature (Bergenstahl, 1997).

2.2.2.3 Hydrophile-Lipophile Balance (HLB)

The concept of the HLB value was introduced by Griffin (1949) and involves assigning a calculated HLB value to every surfactant depending on its solubility. The number and the polarity of the polar groups in a surface-active molecule determine its solubility. The high HLB values are assigned for water-dispersible surfactants and hence they are suitable for making o/w emulsions (Table 2.1). Low HLB values are for oil-dispersible surfactants that are suitable for forming w/o emulsions; extreme high and low values are not functional (Hasenheuttl & Hartel, 1997). Even though the solubility of surfactants (Refer 2.2.2.2) and hence the HLB value depends on temperature, the temperature dependence factor is omitted during its calculation, which is also considered as one of the limitations of this system. According to Friberg et al (1990):

$$HLB = 7 + \text{sum of hydrophilic groups} - \text{sum of lipophilic groups} \dots \dots \dots (2.7)$$

Emulsifiers with the appropriate HLB value to be used in a system can be obtained by blending correct proportions of suitable emulsifiers. This can be done by utilizing Equation 2.7 or by using the software made available by <http://pharmacal.tripod.com/ch17.htm>.

Emulsifier	HLB Number	Nature
PGPR	2	Hydrophobic
Span 85	1.8	
Span 65	2.1	
Span 80	4.3	
Span 40	6.7	
Tween 85	11.0	Hydrophilic
Tween 80	15.0	
Tween 20	16.7	

Table 2.1 Table showing the HLB numbers of commonly used emulsifiers (www.pharmacal.tripod.com) (<http://pharmacal.tripod.com/ch17.htm>)

2.2.3 Surface Activity

Surface activity is the most important property of any surfactant. It involves the adsorption of surfactant molecules at the oil-water interface reducing the interfacial tension. To display surface-activity the emulsifier needs to have certain properties (Bergentahl, 1997):

- 1) It has to be in a non-crystalline form when in contact with water.
- 2) It should have a reduced stability in water due to the long hydrophobic part.
- 3) It has to interact with water through polar interactions.
- 4) It should have a low molecular weight to reduce the effect of reduced entropy when it adsorbs.
- 5) It has to have a reduced solubility in an oil environment due to presence of polar groups at the interface.

Emulsifiers with high melting points will adsorb to surfaces only at a particular temperature called the Kraft's temperature. Due to the presence of hydrophobic parts, most emulsifiers tend to aggregate under aqueous conditions at a particular concentration above the critical concentration called the critical micelle concentration

(CMC) (Tanford, 1973). Figure 2.9 shows the variety of structures that can be formed by surfactants under appropriate conditions.

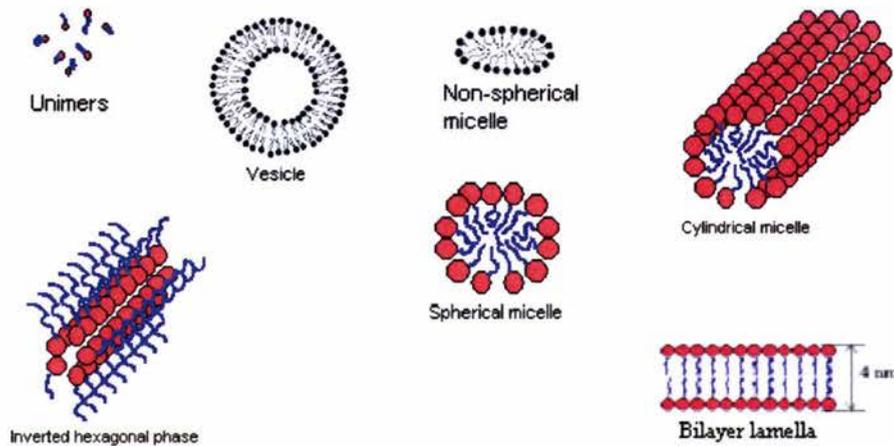


Figure 2.9 Typical structures formed due to self-association of surfactant molecules. (McClements, 2000).

2.2.4 Surface Active Forces in Emulsion

2.2.4.1 Van der Waals forces

According to Hamaker (1937) and de Boer (1936), van der Waals forces are forces between molecules and particles caused by the interactions between permanent and induced dipoles and other multipoles (Bergenstahl & Claesson, 1990).

2.2.4.2 Electrostatic double layer forces

The counter-ion concentration between two identically charged surfaces increases as they approach each other in an electrolyte solution. The increased osmotic pressure at the mid-point of the two surfaces generates a repulsive force. This force is called the electrostatic double layer force. The adsorption of proteins, ionic surfactants or other polyelectrolytes causes the surface charge on food emulsions (Bergenstahl & Claesson, 1990).

2.2.4.3 Hydrophobic forces

When two hydrophobic molecules are brought close to each other the total contact area between the molecule and the aqueous phase decreases and this gives rise to hydrophobic interactions. These forces are entropy driven at room temperature and enthalpy driven at elevated temperatures (Bergensstahl & Claesson, 1990).

2.2.4.4 Repulsive hydration forces

Repulsive hydration forces are a result of the removal of water molecules solvating the interfacial polar groups at an oil-water interface.

2.2.5 Role of Emulsifiers

The role of an emulsifier can be broadly classified into two parts; i.e. formation of new droplets and their protection against recoalescence. The emulsifier acts according to both static and dynamic (diffusion-induced) interactions (Walstra, 1983). The roles of emulsifiers during emulsion formation are (Table 2.2):

FUNCTION	STATIC	DYNAMIC
Destabilise the interfaces	Interfacial tension	Diffusion to and across the surfaces.
Stabilise the droplets	Repulsive surface forces	Diffusion to the interfaces.

Table 2.2. Table showing the role of emulsifiers during emulsion formation (Bergensstahl, 1997).

For a triglyceride/water system, the presence of an emulsifier can reduce the interfacial tension from about 30 mN/m to between 1-10 mN/m. During emulsification droplets are formed rapidly and hence the emulsifier molecules should adsorb to the surface of the droplets as rapidly as the formation of droplets. Water-soluble particles in the oil phase also diffuse into the water phase during emulsification creating disturbances, which in turn contribute to emulsion formation (Bergensstahl, 1997). Different emulsifiers lower the interfacial tension to a different degree (Figure 2.10) thereby reducing the energy requirement for attaining a particular droplet-size (Walstra, 1993).

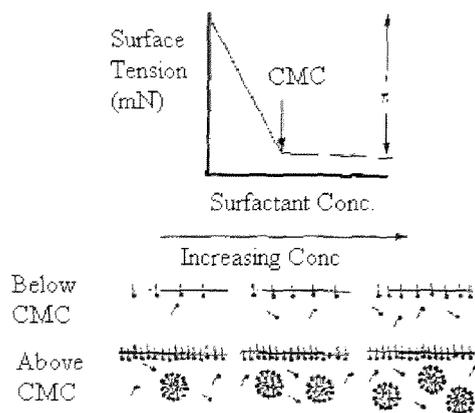


Figure 2.10 Dependence of surface tension on the concentration of the emulsifier. (Mc Clements, 2000).

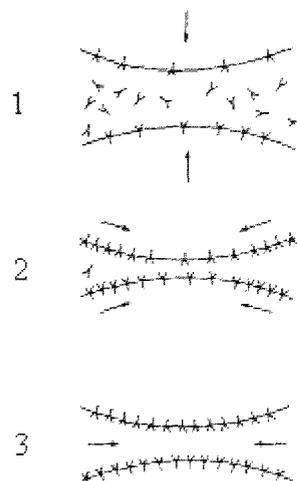


Figure 2.11 Gibbs-Marangoni effect on approaching droplets (Walstra, 1993).

As the emulsion droplets move close to each other high interfacial tension gradients are developed where the film is thinnest between the droplets due to the low amount of surfactants present. The gradient results in the movement of surface to the direction of highest interfacial tension. Repulsive interactions are generated to stabilise the emulsion from recoalescence (Gibbs-Marangoni effect) (Figure 2.11) resulting in streaming of the liquid, which drives the droplets away from each other (Walstra, 1993, Bergenstahl, 1997).

2.2.6 Biopolymers as Surfactants

The most important biopolymer emulsifiers include proteins and polysaccharides, and their functional properties in food products are determined by their molecular characteristics (McClements, 1999). Limited hydrolysis of proteins results in the production of protein hydrolysates, which are found to be excellent emulsifiers (Ramkumar *et al*, 1999). Most of the biopolymers possess sufficient polar groups so that when they are added to the emulsion they will dissolve in the aqueous phase to exhibit their functional properties. The mechanism of adsorption of biopolymers to the interface of water and oil is mainly due to the presence of both polar and non-polar groups in the molecule. When the biopolymers are dissolved in water the polar groups are located in the water phase and the non-polar groups have a tendency to

move away due to the hydrophobic interactions. This is made possible by the molecule locating itself to the oil phase, which in turn helps in forming the emulsion by reducing the interfacial tension (McClements, 1999). Also the biopolymers forms films on the interface after adsorption thereby preventing the coalescence of newly formed droplets. This is made possible by steric repulsive forces and also by electrostatic repulsion (which depends on pH) in the case of charged biopolymers. Thus polymeric emulsifiers selected should have the following properties. They should: (Garti, 1997)

- 1) be amphiphilic in nature
- 2) have the ability to adsorb effectively
- 3) have the ability to provide full coverage over the surface
- 4) exhibit good solubility in the dispersion medium (good stabilizing ability) & good solubility in the dispersed medium (good anchoring ability).

2.3 Emulsification Processes

As discussed above the purpose of emulsification equipment is to mix the dispersed phase in the continuous phase resulting in the production of a fine, uniformly dispersed and stable emulsion. This can be achieved in many ways. The most common methods are discussed below. Other methods, which are not that common, include jet homogenisation and hydroshear (liquid whirl) homogeniser. Another method of emulsification, which is gaining high popularity nowadays and is our primary concern, is “membrane emulsification”. This will be addressed in detail in the next section.

2.3.1 Mixers

Mixers are of several types and they use shearing force for droplet disruption. They have a poor efficiency in terms of emulsion stability and the oil droplets produced typically has a diameter of $>10\ \mu\text{m}$ (Tornberg *et al* 1990). The slow speed paddle agitator, pan and Z-blade mixer and rotating vessels like the butter churn are some examples of this type (Schubert & Karbstein, 1994). High-speed mixers are another

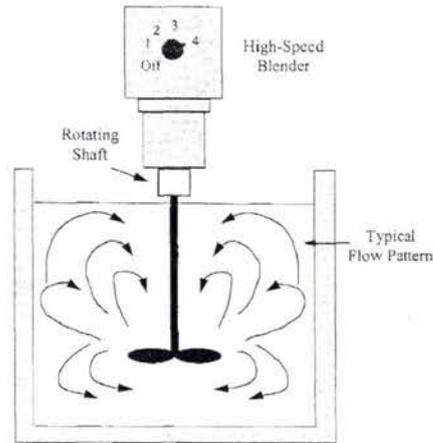


Figure 2.12 High-speed blender (McClements, 1999)

form of mixers and one among them is the turbine or propeller-type high-speed mixer (Figure 2.12). They are mainly used for low viscosity systems. The principle involved is the same as that of simple mixers, i.e. they operate by a shearing action on the food at the edges and the tips of the blades (Fellows, 1988). The energy input is relatively low and so they produce coarse emulsions.

2.3.2 Colloid Mill

The colloid mill is a type of rotor-stator device mainly suitable for emulsification of high viscosity liquids. Here the emulsification is carried out as the particles are disrupted by a very high shearing force between the conical gap of the rotor and stator (Figure 2.13). The rotor and stator may have either a smooth surface or a rough surface. Disruption takes place in laminar flow in smooth surfaced units and in rough surfaced machines droplet disruption is in turbulent flow. Gap width, rotor speed and flow rate are decisive (Schubert & Karbstein, 1994) in emulsification of this type and the droplet size so obtained is about 2 μm (Walstra, 1983). The commonly used Ultra-Turrax, also called a turbo mixer, is one such rotor-stator device that is capable of giving a globule size of around 5 μm . (Tornberg *et al* 1990)

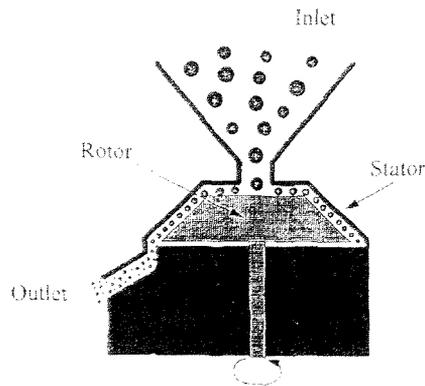


Figure 2.13 Colloid Mill (McClements, 1999)

2.3.3 Toothed Disc Dispersing Machines

This is another rotor-stator device that consists of one or more pairs of concentrically arranged split discs of various designs of which only one rotates. Here turbulence is the key to effective disruption and the emulsion is repeatedly subjected to stress resulting from tangential acceleration and deceleration (Schubert & Karbstein, 1994).

2.3.4 High Pressure Valve Homogenisers

Pressure homogenisers are one of the most widely used systems of emulsification owing to the high degree of efficiency (Figure 2.14).

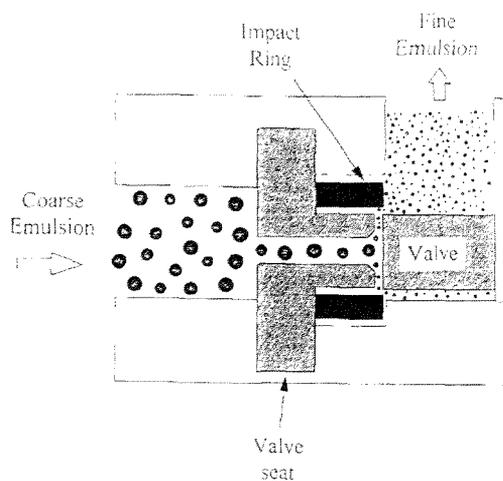


Figure 2.14 High pressure valve homogeniser (McClements, 1999)

A high pressure or valve homogeniser consists of a high-pressure pump operating at 10–70 MPa (Fellows, 1988), which is fitted with a discharge valve. The valve provides an adjustable gap of 15-300 μm (Brennan *et al*, 1990). The emulsion is pumped through the discharge valve at these high pressures, which results in high velocities of about 8400 m/s (Fellows, 1988). This in turn results in high turbulences leading to very powerful shearing forces. The shearing forces, together with the cavitation effect due to collapse of air bubbles, and in some cases the breaker ring together impart the necessary force for the formation of a stable uniformly dispersed emulsion. In some cases, in order to prevent the agglomeration of droplets, the emulsion formed may be subjected to another stage of homogenisation of lesser pressure (two-stage systems). There is an optimum time for emulsification below which an unstable emulsion results and above which the protective film may be lost due to excessive agitation.

2.3.5 Microfluidiser

Microfluidisers, also called jet-dispersing systems are emulsification systems in which the two streams of the feed are pumped into a chamber at high pressure (16000 psi) and at a high velocity (160 m/s) (Brennan *et al*, 1990) (Figure 2.15). The resulting intense shear and turbulence releases energy and causes cavitation. The emulsion so formed is said to have very tiny droplets of the order of less than 1 μm .

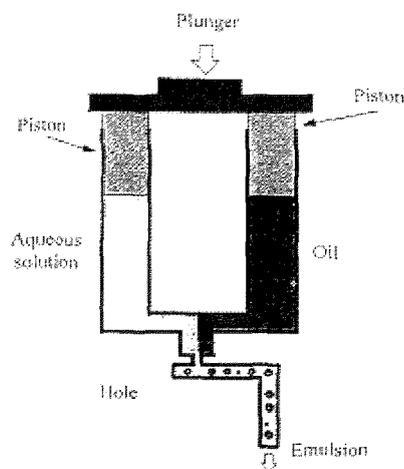


Figure 2.15 Microfluidiser (McClements, 1999)

2.3.6 Ultrasonic Emulsification Devices

Ultrasonic waves are high frequency sound waves and they produce alternate tension (rarefaction) and compression in low viscosity liquids when irradiated. Rarefaction creates voids in the liquid and high-pressure compression of these results in their collapse. This process is called cavitation and results in intense shear fields that promote emulsification. There are three common methods of generating ultrasonic waves: mechanical systems, systems employing magnetostrictive oscillators and piezoelectric crystal oscillators (Brennan *et al*, 1990). A common form of mechanical ultrasonic generator used for food emulsification is a liquid whistle (Figure 2.16).

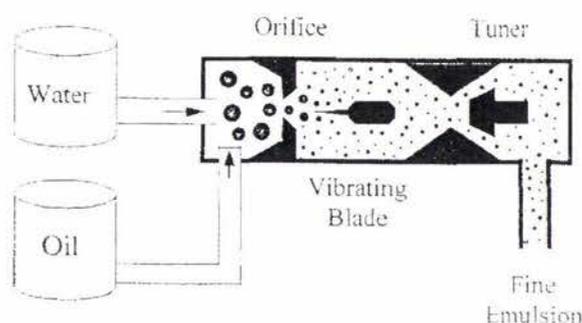


Figure 2.16 Liquid Whistle (McClements, 1999)

The liquid premix is passed through a nozzle at high pressure (50-200 psi) by means of a gear pump and the liquid coming out is forced on to the leading edge of a blade with wedge shaped edges and results in its vibration. The blade resonates at its natural frequency and imparts ultrasonic frequency to the liquid causing cavitation. Ultrasonic sound waves in the range 18-30kHz are used for emulsification and emulsions typically have droplet sizes of 1-2 μm (Fellows, 1988). This type of homogeniser has been used in the preparation of ice creams, salad creams, peanut butters, artificial creams, baby foods etc (Brennan *et al*, 1990). Piezoelectric transducers are used in bench top ultrasonic homogenisers and consist of a piezoelectric crystal within a protective metal casing, which is tapered at the end. The high intensity electrical wave applied to the transducer causes the piezoelectric crystal to rapidly oscillate and generate an ultrasonic wave, which is radiated from the tip of the transducer generating intense pressure and shear gradients causing the liquid to break up into smaller fragments (Mc Clements, 1999).

2.4 Membrane Emulsification

2.4.1 Background

Membrane emulsification is a relatively new technique, having originated about 10 years ago, and has possible applications, which are attracting much research interest in the fields of pharmaceuticals, cosmetics and food. The process involves emulsification by utilizing a low pressure (typically about 200kPa) to force the dispersed phase through the pores of a microporous membrane into the continuous phase, which flows across the membrane with a uniform velocity (Joscelyne & Tragardh, 1999). The distinguishing feature of the process is that the resulting droplet size is controlled primarily by the choice of the membrane and not by the generation of turbulent droplet break-up (Joscelyne & Tragardh, 2000).

2.4.2 Principle of Membrane Emulsification

Membrane emulsification is the process by which two immiscible liquids (oil and water) are mixed by forcing one of them (dispersed phase) through the small pores of the microporous membrane into another (continuous phase) by application of external pressure (Katoh *et al*, 1996, Kandori, 1995, Joscelyne & Tragardh, 2000). This emulsification process is aided by the presence of an emulsifier in the continuous phase. The membrane so used should have a uniform pore size distribution and tolerable mechanical strength (Kandori, 1995). Both simple and multiple emulsions can be prepared by this method. In the case of simple emulsions, the dispersed phase is passed through the micropores of the membrane into the continuous phase containing the suitable emulsifier (hydrophilic in the case of o/w emulsion and hydrophobic in the case of w/o emulsion) (Figure 2.17).

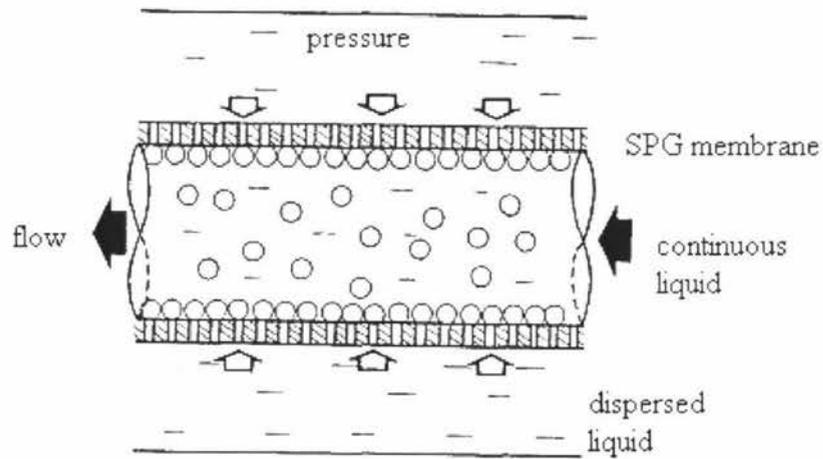


Figure 2.17 Membrane emulsification of a simple emulsion (Kandori, 1995)

In the case of multiple emulsions, typically the primary emulsion is first made by one of the methods described above and this is then passed through a membrane into the outer continuous phase containing the required quantity of suitable surfactant (hydrophilic in the case of w/o/w emulsion and hydrophobic in the case of o/w/o emulsions). It is possible to form the primary emulsion by membrane emulsification and Kandori (1995) proposed a system of two concentric membranes for the continuous production of a multiple emulsion. (Figure 2.18).

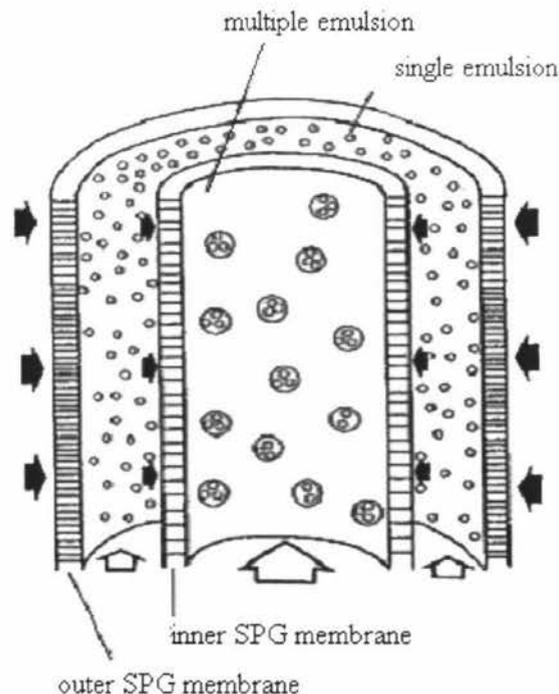


Figure 2.18 Membrane emulsification of multiple emulsions (Kandori, 1995)

2.4.3 *Relative merits of membrane emulsification* (Joscelyne & Tragardh, 2000; Katoh *et al* 1995)

The key advantages of membrane emulsification over other emulsification methods have been described as:

- Simplicity of operation.
- Requiring very low amount of energy.
- Requiring a comparatively low quantity of surfactant.
- Providing a narrow droplet-size distribution.
- Formation of a monodispersed emulsion.
- Offering control of the droplet size through selection of the pore-size.
- Giving preparations of a stable emulsion even with a highly concentrated dispersed phase.

These contrast with some limitations that have been identified for conventional emulsification methods (Williams *et al*, 1998):

- The droplet size and size distribution cannot be controlled in all cases.
- The energy utilisation is very poor in the case of conventional emulsification systems, which causes a significant increase in the manufacturing cost.
- Reproducibility on a single piece of equipment is a major problem and the quality of the product varies from one manufacturing vessel design to another even on the same manufacturing scale.
- Scaling-up is another problem leading to inflexibility of the manufacturing equipment resulting in additional cost arising from the under-utilisation of expensive equipment.

2.4.4 *Droplet formation and detachment*

According to Schroder & Schubert (1999), droplet formation is initiated when the dispersed phase, on reaching the membrane surface, is pressed through the pore by the net external pressure applied. The permeate on reaching the other side forms into a half-sphere shaped droplet at first and then increases in size until it reaches a critical

volume. The droplet becomes unstable and the droplet neck diameter decreases, finally resulting in detachment of the droplet from the pore.

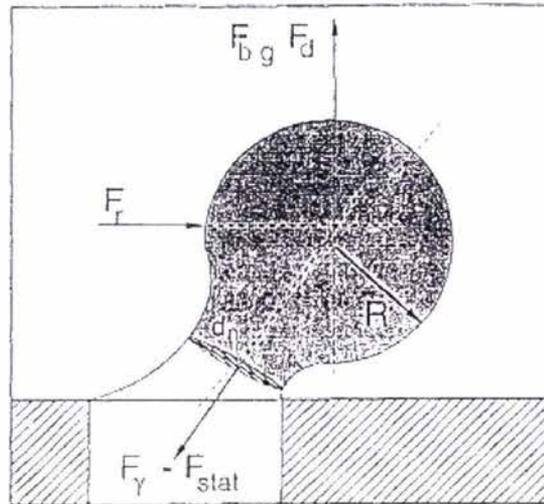


Figure 2.19 Droplet detachment from the membrane surface (Schroder & Schubert, 1999). Forces acting at a single droplet. F_γ =interfacial tension force; F_{stat} =dynamic effect of the pressure difference between the phases; F_r =flow resistance force; F_d =dynamic lift force; F_{bg} = buoyancy and gravitation force; d_n =neck diameter; R = drop radius.

As shown in the Figure 2.19, the forces controlling the detachment of the droplet from the membrane pore are (Rayner & Tragardh, 2002):

- Interfacial tension force (F_γ) due to dispersed phase adhesion around the edge of the pore opening
- The dynamic effect of pressure difference between the phases (F_{stat}) due to pressure difference between the dispersed phase and the continuous phase at the membrane surface
- The flow resistance force (F_r) due to continuous phase flowing past the droplet parallel to the membrane surface
- The dynamic lift force (F_d) due to the asymmetric velocity profile of the continuous phase near the droplet.

The force due to buoyancy and gravitation can be neglected. The flow resistance force and the dynamic lift force (proportional to the wall shear stress of the continuous phase) should be greater than the interfacial tension force and the dynamic effect of the pressure difference between the phases (proportional to the neck diameter) for the effective detachment of the droplet from the membrane surface (Schroder & Schubert, 1999).

2.4.5 Membrane emulsification systems

The process of membrane emulsification can be divided into two based on the working of the apparatus.

2.4.5.1 Batch method

In the batch method (Figure 2.20), the dispersed phase is pressed through the pores of the membrane using pressurised N₂ or other inert gas, or by other suitable means (e.g. pumping). The continuous phase containing the emulsifier is circulated constantly at a particular velocity or is simply mixed. An approximate transmembrane pressure (Section 2.5.2) is maintained throughout the process and the process is continued until the entire dispersed phase is exhausted. The process is then stopped and the multiple emulsion formed is collected (Williams *et al*, 1998; Joscelyne & Tragardh, 2000).

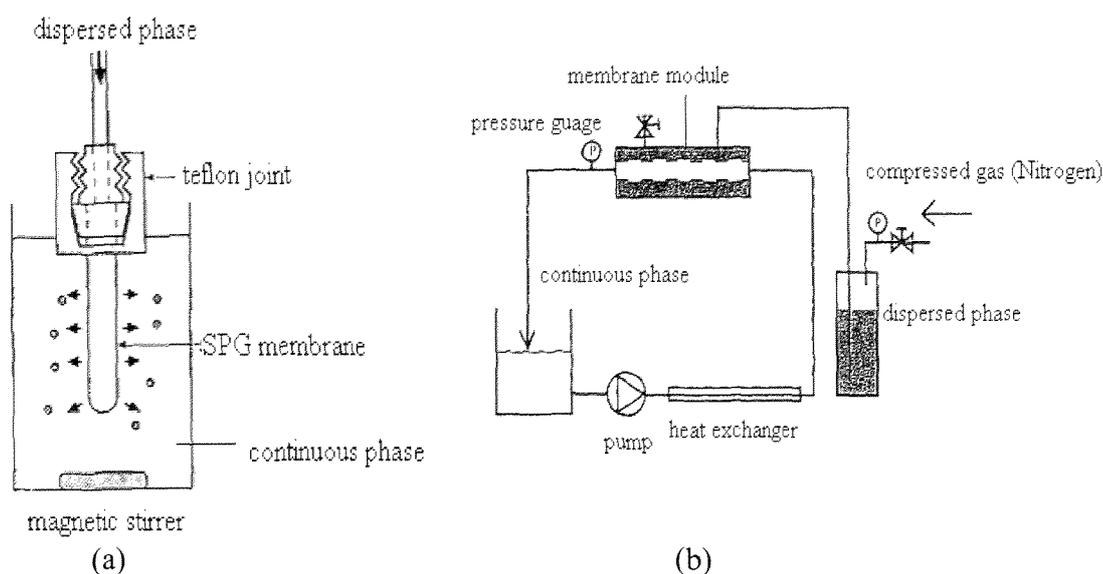


Figure 2.20 Batch method of membrane emulsification (Kandori, 1995; Joscelyne & Tragardh, 2000)

2.4.5.2 Continuous Method

The emulsification process in the continuous method is carried out in much the same way as in the batch method, but in the final stage instead of stopping the process when the emulsion concentration reaches the desired level (e.g. 25 wt%), a fixed

volume of the product is bled out and an appropriate amount of the continuous phase is fed into the continuous phase tank so that the emulsion is diluted to a concentration of 20 wt%. Then the process is continued (Figure 2.21). This is analogous to the feed and bleed operation in ultrafiltration plants (Williams *et al*, 1998).

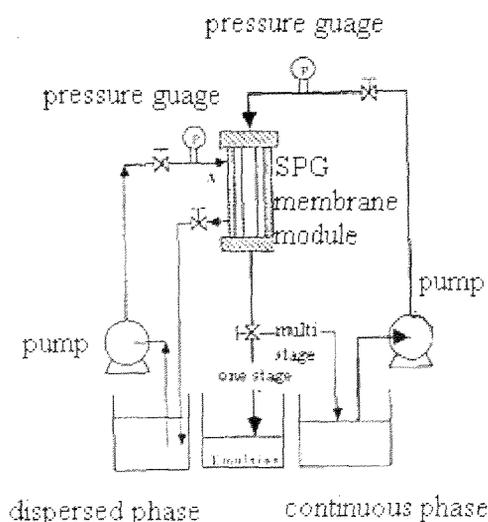


Figure 2.21 Continuous method of membrane emulsification (Kandori, 1995)

2.5 Factors Affecting Membrane Emulsification

The main aim of the process is to obtain a stable emulsion with a narrow droplet-size distribution. Hence all the factors affecting the membrane emulsification process should contribute towards these goals and each will be discussed in detail in the following sections.

2.5.1 Membrane

2.5.1.1 Types of membranes

Membranes can be of different types based on their make: (Williams *et al*, 1998; Kandori, 1995)

1. Ceramic membranes (e.g. aluminium or titanium oxide)
2. Stainless steel
3. Polymeric (track-etched)
4. Microporous glass membranes (MPG)

Of these, ceramic and MPG membranes have been most widely studied. Other possible types of membranes suggested by Rayner & Tragardh (2002) are laser-drilled stainless steel sheets and inorganic microfiltration membranes made using laser interference lithography and silicon micro-matching technology.

1) Ceramic Membranes (Williams et al, 1998)

The most common ceramic membrane element is a tubular sintered alumina substrate (composed of a blend of fused alumina and fine-grained calcined alumina) with one or more alumina coatings on the internal surface. Both the substrate and alumina membrane coating have a controlled pore-size distribution with the average pore size of the substrate being 5-10 times that of the membrane coating. The substrate provides the necessary strength for the surface membrane and is extruded to form a body, which is then passed through a ram extruder and die to form single or multi-channel tubes. These tubes are then fired to high temperature and clipped to required lengths. The tubes are next immersed in an aqueous solution of calcined alumina for internal surface coating. The pore-size (pore-size distribution) is tested by bubble point test equipment and is typically in the range 0.2-1.4 μm for food applications.

2) Microporous Glass Membranes (MPG) (Kandori, 1995)

Nakashima and Shimizu (1989) developed the Shirazu Porous Glass (SPG) membranes, which are the most widely used form of MPG membranes. They are made from $\text{CaO-Al}_2\text{O}_3\text{-B}_2\text{O}_3\text{-SiO}_2$ type glass and Shirazu (a material available from certain volcanic ashes). The steps involved in the formation of SPG begin with the dissolution of the lime and boric acid with Shirazu, and additives like Al_2O_3 and SiO_2 , at 1200-1300°C. After formation the material then undergoes heat treatment at 600-800°C and acid treatment at 50-80°C. It is then desilicified to form SPG. Another type of microporous glass is the PSG (Porous Silica Glass) in which the process (the Corning process) is almost the same, but does not include lime and Al_2O_3 as major components.

2.5.1.2 Surface type of membranes

The most important principle underlying the selection of an appropriate surface type for the membranes is that the dispersed phase should not wet the membrane pores. This means that for the preparation of o/w emulsion a hydrophilic membrane should be used and for the preparation of w/o emulsion a hydrophobic membrane should be used. Nakashima *et al* (1991) have shown that if a hydrophobic membrane is used for the preparation of o/w emulsion it will result in a polydispersed emulsion with larger average droplet size than when the same process is done with a hydrophilic membrane. Conversely if a w/o emulsion is prepared using a hydrophilic membrane, the resultant droplet size is less than the pore size of the membrane (Joscelyne & Tragardh, 2000).

Hydrophilic membranes can be rendered hydrophobic, thus making them suitable for emulsification of w/o emulsions, by suitable silane-coupling surface treatments. However, this may create problems in food industry where such agents are banned (Joscelyne & Tragardh, 2000). An acceptable alternative has been provided by Katoh *et al* (1996) who discovered the method of pre-soaking the hydrophilic membrane in the oil phase before emulsification to render it hydrophobic. Okonogi *et al* (1994) showed that when a w/o emulsion is prepared with a hydrophilic membrane pre-soaked in the fatty phase, this resulted in a more uniform and stable emulsion than that produced with a hydrophobic membrane.

2.5.1.3 Pore-size of membranes

The membrane pore-size is a very important factor in determining the droplet-size of the final emulsion, as the membrane pore-size and droplet size of the emulsion typically show a linear relationship for a given set of operating conditions (Joscelyne & Tragardh, 2000):

$$D_d = x D_p \quad \dots\dots\dots (2.8)$$

where D_d is the diameter of the droplet in μm , D_p is the diameter of the pore in μm , and x ranges between 2-10.

Experiments conducted by Kandori (1995) showed that the droplet size increased with increasing the pore-size (Figure 2.14). Several other researchers including Sotoyama *et al* (1999), Katoh *et al* (1996), Mine *et al* (1996), Schroder & Schubert (1999), Williams *et al* (1998) and Shiomori *et al* (1995) conducted experiments to determine the relationship between droplet size and pore size of the membranes. Results obtained were similar in all cases (Figure 2.22). According to Katoh *et al* (1996) $D_m = 5D_p$, where D_m is the average pore size of the membrane and D_p is the average droplet diameter of the emulsion measured with a centrifugal particle size analyser (Houba Ltd., CAPA-700). However, Joscelyne & Tragardh (1999) observed that droplet size did not depend on membrane pore-size for a 2% emulsifier concentration, but at 8% concentration particle size decreased with decreasing pore-size.

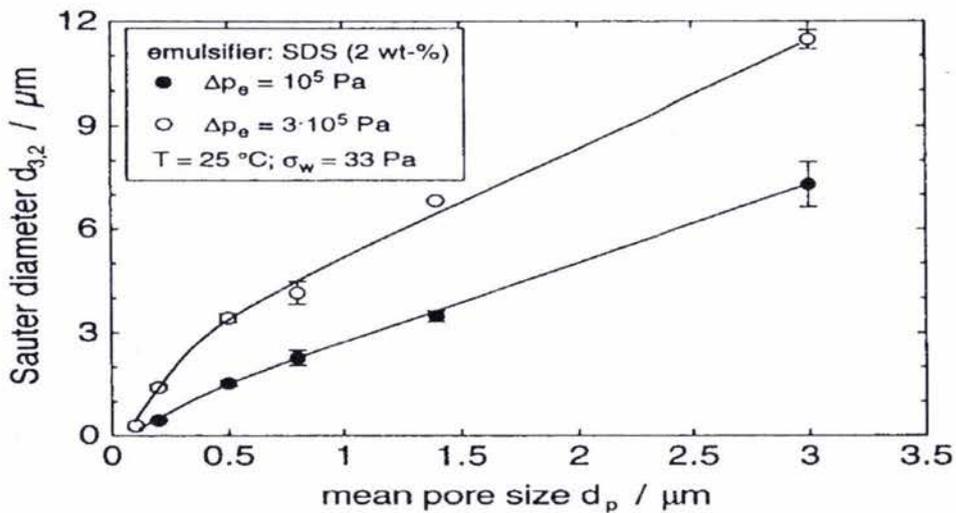


Figure 2.22 Influence of mean pore size and effective transmembrane pressure on mean droplet diameter $d_{(3,2)}$, wall shear stress=33Pa, $T=25$ degree C. (Schroder & Schubert, 1999)

2.5.1.4 Membrane pore-size distribution

Monodispersed emulsions can only be produced if the membrane pore-size distribution is sufficiently narrow as the presence of small number of coarse pores will lead to a bimodal distribution (Joscelyne & Tragardh, 2000). The actual pore size may also be important: Kandori (1995) found that membranes with pore sizes 0.98 μm and 2.70 μm gave a monodispersity ratio ($U = d_{(4,3)}/d_{(3,0)}$), where 'U' is the monodispersity ratio, ' $d_{(4,3)}$ ' is the weight moment mean diameter and ' $d_{(3,0)}$ ' is the

number volume mean diameter (Refer 3.4.3) close to 1 whereas the membrane with pore size 4.70 μm gave a broad size distribution.

2.5.1.5 Membrane porosity

Porosity determines the distance between adjacent pores and has also been shown to be directly proportional to droplet size (Figure 2.23). At higher porosities the distance between adjacent pores is less and so there is a greater chance of droplet coalescence at the membrane surface before the droplets detach (Joscelyne & Tragardh, 2000; Rayner & Tragardh, 2002). As the distance between adjacent pores increases the porosity decreases; this decreases the droplet size formed and results in a more stable emulsion. Hence it is desirable that the pores are uniformly located on the membrane surface so that the maximum possible distance between two adjacent pores is ensured for a given porosity (Williams *et al*, 1998). The distance between two pores is recommended to be at least six times the pore diameter to avoid contact and thus avoid coalescence between two neighbouring droplets (Abrahamse *et al*, 2001). Schroder *et al* (1998) found that a droplet-size to pore diameter ratio of more than 1.6 for a membrane porosity of 0.3 led to a significant degree of coalescence. However too low a porosity results in reduced dispersed phase flux, which may make emulsion production economically unattractive (Joscelyne & Tragardh, 2000).

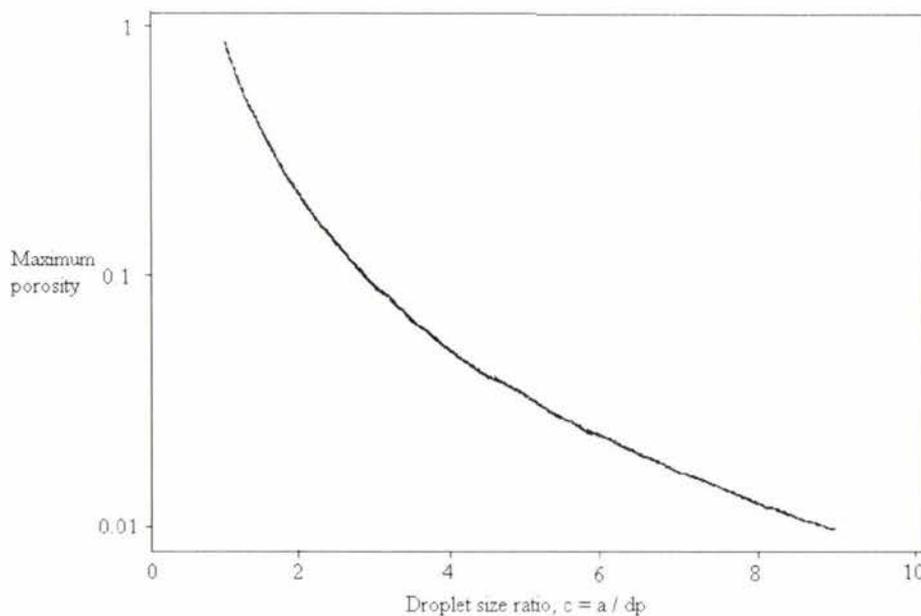


Figure 2.23 The maximum porosity for the membrane surface vs. the droplet size (ratio) requirement for the product. (Williams *et al*, 1998).

2.5.2 Transmembrane Pressure and Dispersed Phase Flux

The average transmembrane pressure is calculated:

$$\Delta P = P_d - \frac{(P_{c,1} + P_{c,2})}{2} \dots\dots\dots (2.9)$$

where P_d is the pressure of the dispersed phase outside the membrane, $P_{c,1}$ and $P_{c,2}$ are the pressures at both ends of the membrane module. There is a minimum transmembrane pressure required for the permeation of the dispersed phase through the membrane, and is called critical pressure (Williams *et al*, 1998), i.e.

$$P_c = \frac{4\gamma}{d_p} \dots\dots\dots (2.10)$$

where γ is the interfacial tension and d_p is the pore diameter. A slight modification to this formula is proposed by Joscelyne & Tragardh, (2000):

$$P_c = \frac{4\gamma \cos\theta}{d_p} \dots\dots\dots (2.11)$$

where θ is the contact angle between the dispersed phase and membrane surface. An increased transmembrane pressure results in an increased dispersed phase flux (Nakashima *et al*, 1991). According to Williams *et al* (1998), the maximum transmembrane pressure should be applied to obtain the highest emulsification rate under processing conditions and the maximum practical pressure is usually 2-10 times higher than the minimum transmembrane pressure. But if the pressure is too high it will result in jetting of the dispersed phase through the pores resulting in formation of an unstable emulsion (Joscelyne & Tragardh, 2000).

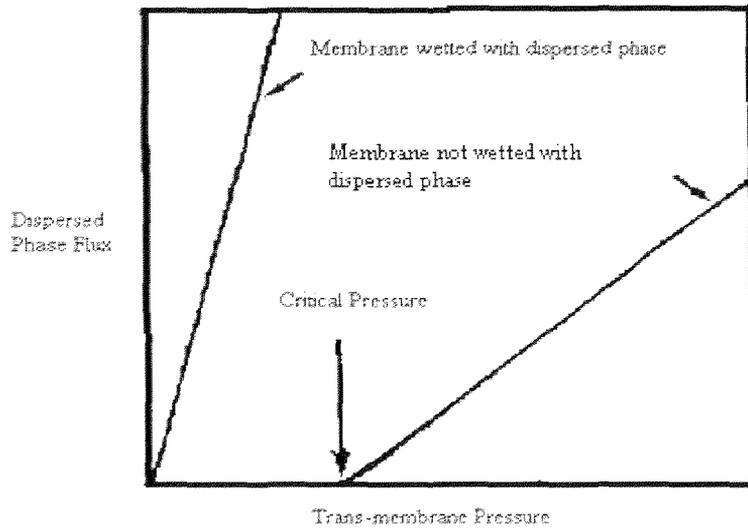


Figure 2.24 Relationship between transmembrane pressure and dispersed phase flux. (Nakashima et al, 1991, Membrane Emulsification Operation Manual).

According to Schroder and Schubert (1999), the larger the effective transmembrane pressures the larger the droplets (Figure 2.25). Also when the transmembrane pressure is increased the number of pores taking part in the emulsification process is increased. Consequently an increased permeability and flux through the membrane are seen with the increase in transmembrane pressure. For an effective transmembrane pressure of 100 kPa the number of active pores was estimated as approximately 10% of the total number of pores (Schroder & Schubert, 1999).

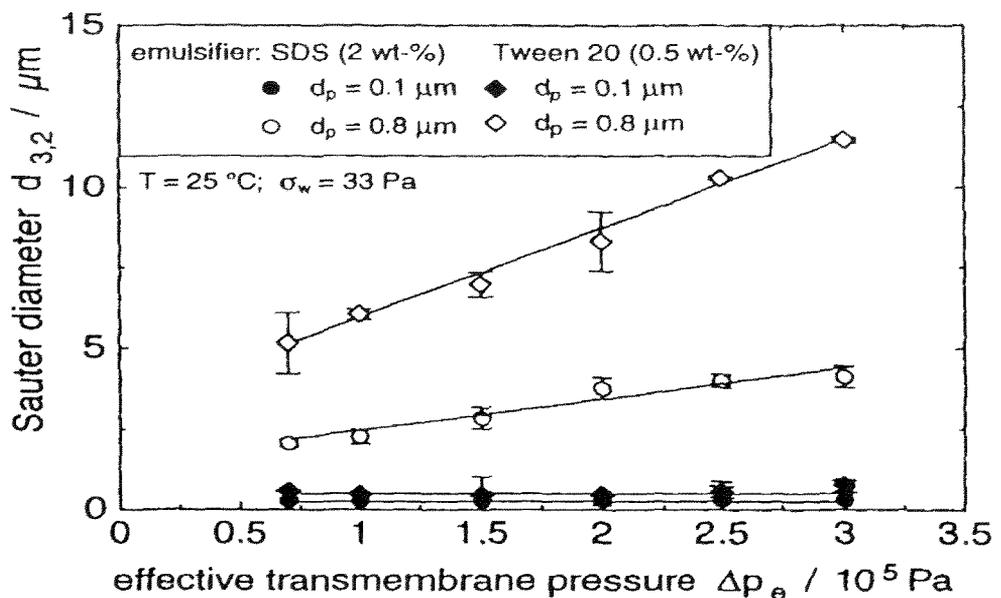


Figure 2.25 Influence of emulsifier and effective transmembrane pressure on mean droplet size for different mean pore sizes, wall shear stress = 33Pa, T=25 °C.

Their experiments with two types of emulsifiers, namely sodium dodecyl sulphate (SDS) (2 wt%) and Tween 20 (0.5 wt%), showed that emulsions stabilised with these emulsifiers and produced using membrane of 0.1 μm gave uniform droplet sizes irrespective of the variation in transmembrane pressure. This was explained as due to the large reduction in the dynamic interfacial tension under these conditions.

According to Darcy's Law the dispersed phase flux is proportional to the pore-size of the membrane and the effective transmembrane pressure. The dispersed phase flux can be calculated as (Schroder & Schubert, 1999)

$$J_d = \frac{B_o}{\eta_d} \cdot d_p^2 \cdot \frac{p_e}{L} \quad \dots\dots\dots (2.12)$$

where B_o is the modified permeability as determined by the porosity and number of active pores, η_d is the viscosity of dispersed phase d_p is the droplet-size and p_e/L is the pressure gradient. Suzuki *et al* (1994) showed that the mean droplet diameters of corn oil-in-water emulsions was decreased to about 1.5 times the mean pore-size by increasing the dispersed phase flux. In the study conducted by Joscelyne and Tragardh (1999), for the production of an o/w emulsion using ceramic membranes, the largest flux ($\sim 200 \text{ kg m}^{-2} \text{ h}^{-1}$) was observed for 0.5 μm membrane at an effective transmembrane pressure of 20kPa and decreased with decreasing pore-size. The formation of larger droplets at higher fluxes is mainly due to the inability of the emulsifier molecules to rapidly adsorb to the interface. The variation of flux with time is due to the variation in transmembrane pressure and also due to the variation in the permeation of the pores in the membrane. In their study it was observed that at higher pressures (250 kPa) the flux increased with time and reached steady state only after 10 minutes at $140 \text{ kg m}^{-2} \text{ h}^{-1}$; at lower pressures (e.g.100 kPa) the flux was nearly constant at $15 \text{ kg m}^{-2} \text{ h}^{-1}$.

In a further study on producing o/w emulsions, the oil flux ranged from 2–20 $\text{l m}^{-2} \text{ h}^{-1}$ using 0.2 μm to 40 $\text{l m}^{-2} \text{ h}^{-1}$ for 0.8 μm hydrophilic membranes and in the case of w/o emulsions the water fluxes were 2300 and 200 $\text{l m}^{-2} \text{ h}^{-1}$ for 1 and 0.5 μm hydrophilic membranes pre-soaked in oil phase to render them hydrophobic (Joscelyne & Tragardh, 2000). According to Ban *et al* (1994), increased dispersed phase flux in the

case of o/w emulsion can be obtained from pre-treatment of membrane by immersion for 20 hours in aqueous phase and this may be due to better filling of membrane pores due to lowering of the interfacial tension.

2.5.3 Dispersed phase concentration

Katoh *et al* (1996) plotted the relationship between the concentration of oil phase and the resulting droplet size in the production of o/w emulsions using corn oil and de-ionised water. They observed that a monodispersed emulsion could be prepared and the droplet size remained constant even at a high concentration of 20vol%.

2.5.4 Velocity of the continuous phase

Williams *et al* (1998) found that the cross-flow velocity can be utilised to vary the effect of the pore-size on droplet size. They selected four different velocities (1.12, 2.49, 4.34, 5.09 ms^{-1}) and found that the droplet size decreased as the cross-flow velocity increased, with the effect of cross-flow velocity diminishing with the lowering of droplet size. The droplets formed at the surface of the membrane are detached under the influence of the flowing continuous phase; this velocity is normally between 0.8 and 8.0 ms^{-1} (Joscelylene & Tragardh, 2000). The influence of the cross-flow velocity is best expressed in terms of wall shear stress. Shubert & Schroder (1997), in their experiments with ceramic membranes, found that a wall shear stress of >2 Pa was needed for 0.1 and 0.5 μm membranes and >20 Pa for 0.8 μm membrane to obtain the minimum droplet size. Coalescence at the membrane surface can be expected if the wall shear stress is smaller because of the increase in droplet size. Joscelylene & Tragardh (1999) investigated the effect of wall shear stress on emulsion droplet size and found that the droplet size becomes smaller as the wall shear stress increases and that the influence is much greater for wall shear stresses $< \sim 30$ Pa.

Muschiolik *et al* (1997) found that the droplet size was halved when the wall shear stress was increased from 0.3 to 1.5 Pa using a 0.5 μm membrane. Katoh *et al* (1996) showed that monodispersed o/w emulsions using SPG membranes could be produced

only if the minimum wall shear stress was 0.48 Pa. Figure 2.26 shows the general trend although the absolute values of droplet size as a function of wall stress may vary with differences in membrane set up, transmembrane pressure, oils and emulsifiers used.

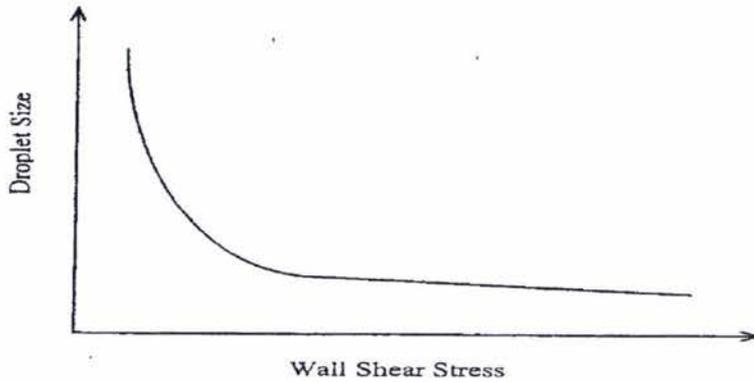


Figure 2.26 Effect of wall shear stress on droplet size for o/w emulsions (Joscelyne & Tragardh, 2000).

2.5.5 Surfactant type and concentration

As stated earlier, depending on its type and concentration, the roles of the surfactant or emulsifier can be divided into two:

- 1) Lowering the interfacial tension thereby lowering the minimum emulsification pressure and hence reducing the required energy input.
- 2) Stabilising the emulsion by preventing droplet coalescence or aggregation.

Different types of surfactants can be used for emulsification depending on whether o/w or w/o emulsion must be stabilised. Natural or Synthetic surfactants are available; natural surfactants are preferred in food systems but effectiveness, cost, sensory and regulatory acceptability will determine the type of surfactant used. Few studies have compared a range of emulsifiers for the same system and process conditions and hence it is difficult to generalise.

2.5.5.1 Effect of surfactants on o/w emulsions

Nakashima et al (1991) found that emulsification using sodium dodecyl sulphate (SDS) at a concentration much less than the critical micelle concentration resulted in a monodispersed emulsion due to the lowering of minimum emulsification pressure.

Katoh et al (1995) investigated the effects of various emulsifiers in producing stable corn oil/kerosene emulsions. The emulsifiers tried included synthetic (SDS) and natural (sucrose esters (SE), polyglycerol esters (PE), and sodium caseinate). Monodispersed emulsions were prepared in all cases but when the concentration of sodium caseinate was increased it resulted in a decrease of droplet size, whereas an increase in concentration of all other emulsifiers resulted in constant droplet sizes.

Ban et al (1994) studied the preparation of o/w emulsions using poly(oxyethylene) hydrogenated castor oil (EC-n) (n = 5-50) as the emulsifier. Various HLB values (9.8 to 12.8) of EC-n were prepared and the smallest emulsion droplet formed was for the HLB value of 12.8 at 10% total surfactant concentration (Joscellyene & Tragardh, 2000).

The study conducted by Sunder et al (2000) investigated the interactions between different natural surfactants phospholipids (hydrolysed and unmodified deoiled lecithin) with proteins (partially heat denatured whey protein concentrate [WPC] and undenatured whey protein isolate [WPI]) and salt (NaCl) in o/w emulsions. O/W emulsions with 10wt% sunflower oil, 1-2% whey proteins, 0.39-0.78% phospholipids and 0.5-1.5% salt were prepared using a high-pressure laboratory homogeniser. It was found that the effect of protein-phospholipid combinations on the emulsion properties depended greatly on the protein-salt ratio and the emulsification temperature. An increase in protein content (WPC & WPI) independent of lecithin concentration, and also an increase in lecithin concentration for a given protein-lecithin combination, reduced the droplet size and increased the stability of o/w emulsions, but the latter strategy produced larger droplets than the former. An increase in the NaCl content of the emulsions containing WPC & WPI produced less stability due to electrostatic double layer repulsion, often leading to aggregation. It was found that the use of unmodified deoiled lecithin and whey protein (undenatured or heat modified) in the water phase resulted in a lower interfacial pressure and thus the presence of lecithin

(hydrolysed), together with whey protein, contributed to the formation of smaller oil droplets than with whey protein alone.

Research has been conducted to study the combined effect of emulsifiers and emulsification systems on production of stable emulsions. Mushiolik et al (1997) studied the influence of two types of whey protein concentrates (WPC 1, containing a high portion of free SH-groups, which is a native whey protein, and WPC 2, containing a lower portion of free SH-groups, that is more cross-linked) in the production of o/w emulsions using membrane emulsification (emulsion 1), blade homogeniser (emulsion 2) and mini-high-pressure homogeniser (emulsion 3). It was found that the more hydrophobic WPC 1 reduced the interfacial tension at the oil phase in a shorter time than WPC 2. It was also found that the pre-heating of WPC 1 and WPC 2 solutions (70°C, 10 min) enhanced the surface activity by inducing unfolding of the globular protein, thereby reducing the average droplet size using the blade homogeniser. High-pressure homogenisation using WPC 1 resulted in a decrease in solubility of protein (denaturation), resulting in a larger droplet size for droplets stabilised with WPC 1 when compared with that of WPC 2. By varying high shear energy inputs, the surface activity of whey protein concentrates could be changed depending on the degree of protein denaturation. However, MPG emulsification with membrane pore size of 0.5 µm and low shear energy input produced no significant difference in droplet size between WPC 1 and WPC 2. In contrast, emulsification with membranes of pore size 0.19 µm resulted in a smaller average droplet size for emulsions stabilized with WPC 1, which was attributed to the higher surface activity of this emulsifier.

Schroder & Schubert (1999) investigated the effect of surfactant and transmembrane pressure on droplet size. They found that SDS adsorbed quickly onto the surface of newly formed droplets while adsorption of Tween 20 was much slower. Consequently the droplet diameters with Tween 20 were about twice the size of droplets stabilised with SDS. Unfortunately, SDS is not a food grade emulsifier and cannot be adopted into food systems.

2.5.5.2 Effect of surfactants w/o emulsions

Kandori et al (1991) prepared monodispersed w/o emulsions using hydrophilic SPG membranes and PE-64 as the surfactant and they found that the size of the droplets produced was smaller than the average pore size. Here the interfacial tension was almost nil and the water droplets are stabilised by the steric repulsion of the elastic adsorbed layers of PE series surfactant. The role of interfacial tension was later confirmed by using various types of PE-n ($n = 61,62,64$) and SPG membranes of various pore sizes and it was identified that an interfacial tension of $< 1\text{mN/m}$ is required for the production of a stable monodispersed emulsion.

Sotoyama et al (1999) produced w/o emulsions using membrane emulsification. The microscopic observation of the emulsion droplets stabilised by a hydrophobic emulsifier added at 1% (w/w) and 3% (w/w) concentration revealed that a few collapsed dispersed droplets were detected in the former case whereas no collapsed droplets were observed in the latter case.

In the preparation of simple emulsions by Mine et al (1996), stable w/o emulsions were not obtained using egg phosphatidyl choline (PC) alone as a surfactant, but when egg PC was combined with polyglycerol esters of polycondensed ricinoleic acid (PGCR), the resultant emulsion was fine with a sharp droplet size distribution. In the case of o/w emulsions, a stable emulsion was not formed when PC was dispersed in the water phase due to clogging of the membrane with PC micelles. It was reported that the presence of a surface charge on the membrane resulted in the adsorption of the surfactant molecule onto the membrane surface if the functional group of the surfactant molecule possesses a charge opposite to that of the membrane surface, resulting in the formation of an unstable simple emulsion. Thus in the case of MPG membranes, which have strong hydrophilic charge due to the presence of silanol groups, SDS, which is amphipolar in nature, is often used as the surfactant. It was also found that lysophosphatidyl choline (LPC) is a more suitable emulsifier than PC for membrane emulsification due to the presence of a highly charged ionic head group. The stability of simple o/w emulsions was studied by comparing an o/w emulsion prepared by using a membrane ($D_m = 1.36\ \mu\text{m}$) and a polydispersed emulsion prepared with a disperser; the emulsion was composed of 1% egg PC and

20% soyabean oil and was stored for 6 weeks at 5°C. There was slight creaming but no coalescence in the first case, whereas there was significant coalescence and droplet breakdown in the second case, leading to a wide and heterogeneous droplet size distribution.

2.5.6 Temperature, viscosity and pH

The viscosity of the dispersed and continuous phases can be considerably affected by the operating temperature, which can also affect the solubility and phase inversion temperature of the emulsifier (Joscelyne & Tragardh, 2000). The selection of emulsifier is therefore often dependent on the operating conditions. Overall a higher temperature is preferred for increasing the fluidity of the dispersed phase (which increase the flux) and also for increasing the solubility of the emulsifier.

Investigations of Katoh *et al* (1996) proved that an increased viscosity of the continuous phase led to an increased emulsion droplet size of w/o food emulsions and also resulted in a reduced flux.

Sotoyama and Asano (1999) conducted a study to determine the rheology of o/w food emulsions prepared using membrane emulsification. Corn oil-in-deionised water emulsions (the viscosity of deionised water was adjusted to 3.7 mPa.s) were prepared with polyglycerol esters as the emulsifier and carrageenan was used as the stabiliser. The viscosity measurements were done using a vibration viscometer (DJV 5000, Chichibu Onoda Co. Ltd). All the emulsions prepared were found to be monodisperse (even though the mean droplet sizes were in the range 5-30 μm) and this was confirmed by micrographs obtained immediately and 1 month after emulsification. The total surface area of the dispersed droplets was calculated and it was found that as the droplet size increased, the relative viscosity of emulsions (η/η_0) decreased, and as the total surface area of droplets increased the relative viscosity increased. (η is the emulsion viscosity and η_0 is the viscosity of the continuous phase).

The membrane surface properties are often pH dependent and the membranes may exhibit an iso-electric point at a given pH where the surface has no net charge (Joscelyne & Tragardh, 2000). Huisman *et al* (1999) determined the iso-electric points of a number of ceramic membranes to be between pH 5.2 and >8. The positive

and negative charges of the membranes above or below the iso-electric point result respectively in adsorption of the surfactant molecule to the membrane surface if the functional group of the surfactant molecule possess a charge opposite to that possessed by the membrane surface. This may have disastrous consequences for the emulsification process, often leading to formation of large droplets and unstable emulsions.

The structure of a multiple emulsions provides the potential to separately adjust the pH of the phases. Tedajo *et al* (2001) studied the effect of maintaining the two aqueous phases (internal and external) in a w/o/w multiple emulsions at different pH values. For the experiments, three homogenous and consistent multiple emulsions, one with no acidic species (EM0), the second with lactic acid (0.1M in 100g H₂O) (EM1) and the third with hydrochloric acid (0.2M in 100g H₂O) (EM2) in the internal phases, were prepared. It was found that for EM2, the external phase pH decreased just after preparation and for EM1 the pH of the external phase was constant over time. From this they assumed that there was a rapid transport of HCl from the internal phase to the external phase (Fickian diffusion) in the case of EM2 indicating rapid reduction in yield, in contrast the decrease in pH for EM1 was slower showing a slower reduction in yield.

2.6 Comparison of emulsification processes

Due to the wide variety of devices available, comparing data from the literature is not easy. Tornberg *et al* (1990) identified the need for a standardised emulsification procedure and developed a protocol based on isothermal, continuous, recirculation system where flow velocity was controlled. As emulsification is dependent on new surface creation and this requires energy input, system performance can also usefully compared based on the energy or power densities, which are given by

$$E_v = P_v \cdot t = \frac{P}{V} \dots\dots\dots (2.13)$$

where E_v is the energy density, P_v is the power density, P is the power input, V is the volume flow rate and t is the mean residence time of the droplets is the dispersing zone (Schubert, 1997). (Figure 2.27 & Table 2.3)

Using the standardised procedure Tornberg *et al* (1990) found that the Ultra-Turrax or Turbo-Mixer had the poorest emulsifying efficiency in terms of the creaming stability of the emulsions formed. Comparing other emulsification systems, the ultrasonic device showed higher emulsifying efficiency at lower power input. The valve homogeniser also showed an increased emulsifying efficiency, but in this case the power input was high (Tornberg *et al*, 1990). Tornberg *et al* (1990) also showed that there were differences in the droplet size and PSD width of emulsions formed in a sonifier with those obtained in a valve homogeniser. For this study sodium caseinate dispersed in 0.2M NaCl at pH 7 was used. It was found that sonication gave the smaller fat globules and thereby a larger fat surface areas than valve homogenisation at the same power input.

However Walstra obtained opposing results to those of Tornberg *et al* (1990). He compared average droplet size, expressed as $d_{(4,3)}$, as a function of net energy input for diluted paraffin oil-in-water emulsions produced in various machines. Walstra found that, at the same energy input, the valve homogeniser was superior to a sonifier in giving droplets of lesser diameter. This difference may be due to many reasons. One such identified is the number of passes, i.e. the number of passes in the case of Walstra's experiment was 1 and in the case of Tornberg's were 10. Another reason may be that Walstra plotted droplet size against energy input whereas Tornberg plotted droplet size against power input. Also the average droplet diameter $d_{(4,3)}$ (Walstra) is more susceptible to polydispersity than $d_{(3,2)}$ (Tornberg) as $d_{(4,3)}$ increases more with PSD width.

As noted above, turbulence is the determining factor for droplet disruption in rotor-stator systems (Schubert, 1997). In toothed disc dispersing machines, high energy densities are produced only if the emulsions have a very high viscosity. However even under these conditions a uniform small droplet size cannot be produced and therefore high-pressure homogenisers and jet dispersing systems are preferred for this purpose (Schubert, 1997). High pressure dispersion systems provide more effective droplet disruption than colloid mills due to the combined effect of cavitation and the fact that power densities are greater for the former at the same energy density and volume flow rate. This is because of the low volume of the dispersing zone.

Okochi & Nakano (1997) conducted a study to compare the processes of membrane emulsification and stirring in the preparation of w/o/w emulsions. The ratio of internal phase to oil phase to external phase was 1:4:5. In the case of stirring the particle size depended on the rotation rate and the particle size distribution (PSD) was found to be broader whereas in the membrane method the particle size depended on pore size and the PSD was sharp and homogenous. Also the standard error of droplet size was found to be smaller and viscosity slightly lower in the case of the membrane method. Regarding entrapment efficiency, they found that drug release was more controlled in emulsions prepared by the membrane method and the surfactant concentration required for the membrane method was less due to the more uniform adsorption of surfactants to the interface.

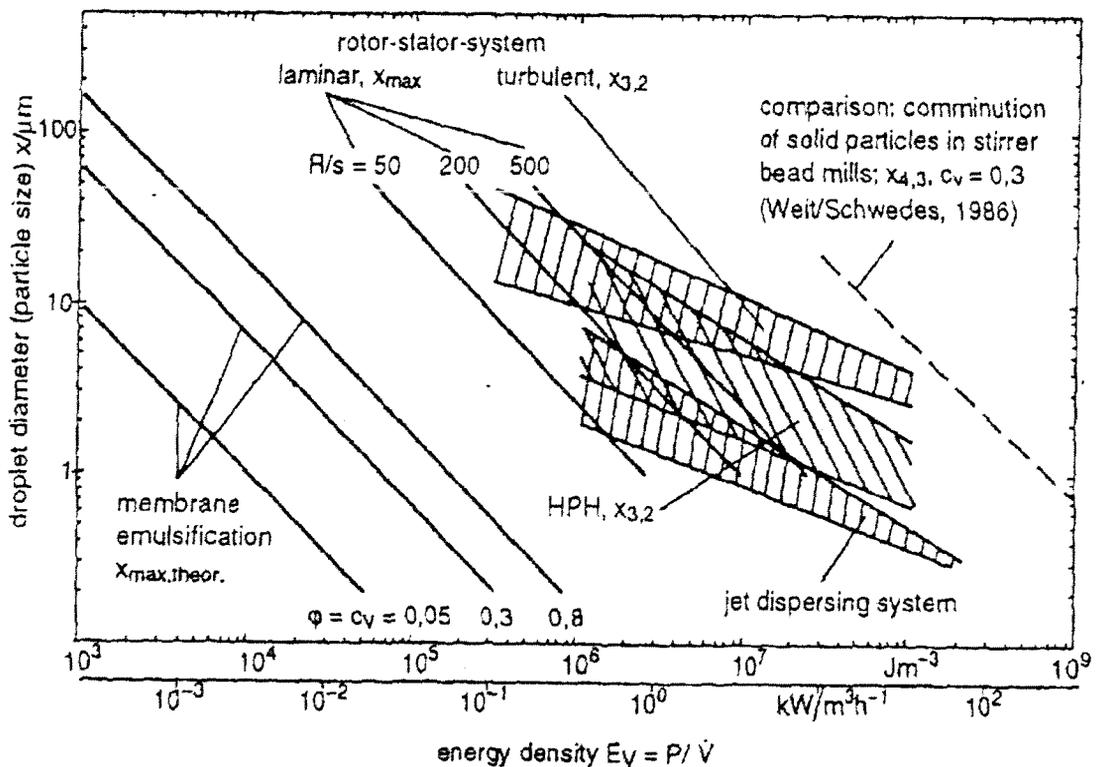


Figure 2.27 Comparison of different continuous emulsification equipment (Schubert, 1997). HPH: high-pressure homogeniser. Viscosity of the continuous and the dispersed phase $\eta_c = \eta_d = 100 \text{ mPa}\cdot\text{s}$; density of the continuous and the dispersed phase $\rho_c = \rho_d = 1000 \text{ kg/m}^3$; interfacial tension $\gamma = 10 \text{ mN/m}$; $\phi =$ oil volume fraction; $c_v =$ volume of solids / volume of suspension; $x_{4,3} =$ volume mean diameter; $x_{\text{max,theor}}$ and x_{max} calculated from experimental data; $x_{3,2}$ and $x_{4,3}$ experimental results; $R =$ rotor radius; $s =$ gap size.

In smooth colloid mills, where laminar flow is utilized, other than energy density the ratio of the viscosities of the dispersed phase and emulsion is of utmost importance in determining the effective disruption of droplets. This ratio should be between 0.1 and

1 for effective results (Schubert, 1997). In membrane emulsification the required energy level is very low in comparison with the conventional methods and the droplet size is mainly determined by the pore-size and size distribution of the membrane. The mean droplet size in membrane emulsification is a function of energy density, volume fraction of the dispersed phase and the energy needed to pump the continuous phase (Schubert, 1997).

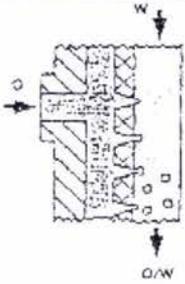
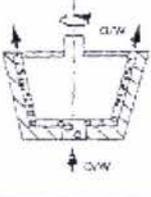
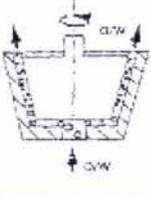
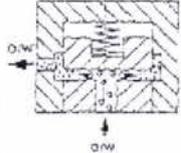
Emulsification systems	Membrane emulsification	Rotor-stator system		High pressure equipment	
		Smooth	Toothed	High-pressure Homogeniser (HPH)	Jet dispersing system
					
Dispersing mechanism	Droplet formation on capillaries	Laminar shear flow	Turbulence	Turbulence, cavitation	Turbulence, cavitation
Droplet size	$X = C_1 \Phi E_v^{-1} + C_2$	$X_{3,2} = C E_v^{-b}$ ($E_v = P/V = \Delta p$)			
		b=1	b=0.2-0.4	b=0.6(0.4-0.9)	b=0.6
E_v / Jm^{-3}	$< 10^3 - 10^6$	$10^3 - 10^5$	$10^5 - 10^8$	$10^6 - 10^8$	$10^8 - 2 \cdot 10^8$
t_{res} / S	1	0.1 - 1		10^{-3}	10^{-4}
P_v / Wm^{-3}	$< 10^3 - 10^6$	$10^3 - 10^6$	$10^6 - 10^9$	$10^9 - 10^{11}$	$10^{10} - 10^{12}$
Controlling variable	η_d	η_d / η_o (0.1-1)	$\gamma(t)$:adsorption kinetics		
	Capillary design wettability		η_d =viscosity of the dispersed phase		Valve design
Variable without influence			Φ = oil content		
			η_c = viscosity of the continuous phase		
			Tooth design		

Table 2.3 Comparison of different continuous emulsification equipments (Schubert, 1997).

2.7 Fouling and cleaning of membranes

Membranes, due to their small pores, pose a greater challenge as far as cleaning is concerned and fouling of membranes often affects the overall performance of emulsion formation. This may lead to a reduced dispersed phase flux and increased transmembrane pressure due to the reduced participation of membrane pores in the emulsification process due to clogging. The factors effecting membrane fouling include membrane zeta potential, surface charge density and hydrophobicity (Huisman, 1999). Fouling can occur by plugging of pores by macromolecules or particles, or by filter cake build-up. Joscelyne & Tragardh (1999) reported that they cleaned their membranes by first treating them with 1% NaOH followed by rinsing with deionised water and then treating with 2% Divos 100 for 12 hours at 70°C. They also reported that CIP systems were ineffective in cleaning the membranes and hence they dismantled the rig after every experiment to clean the membrane manually. In experiments conducted by Huisman *et al* (1999), it was clear that the membrane zeta potential decreased after cleaning the membrane with a detergent (Ultrasil 10). When proteins are used as the emulsifier, water entering the pore may cause fouling as the proteins can stick to the hydrophilic groups on the membrane surface thereby making the pore hydrophobic. This can interrupt the droplet formation process and may even lead to the blockage of the pore (Abrahamse *et al*, 2001).

2.8 Summary

Membrane emulsification, owing to its simplicity in principle, ease in operation, lower consumption of energy and the efficiency in controlling the droplet size of the emulsions formed, shows a huge potential for the production of high performance emulsions. This technology appears ideal for the production of multiple emulsions due to the low shear involved in the secondary emulsification stage and hence could be exploited for the microencapsulation of food ingredients provided adequate yield stability can be guaranteed.

The review of the various aspects of producing simple and multiple w/o/w emulsions through membrane emulsification shows that a lot of challenges need to be overcome to produce stable emulsions. It is clear that the stability of the primary w/o emulsion

is critical in the stability of the final w/o/w emulsion. Hydrophobic emulsifiers with an appropriate HLB value and in appropriate concentration, the percentage by weight of water in the w/o emulsion and also the type and conditions of primary emulsification are the most important factors that need to be taken into consideration to produce a stable w/o emulsion. Hence research should be conducted for defining the optimum conditions for each parameter stated above for formation of multiple emulsions by membrane emulsification.

Production of a stable w/o/w emulsion requires a suitable hydrophilic emulsifier in appropriate concentration (most likely 1 wt%) in the outer phase and also a suitable emulsification step that will not result in the breakage of the primary emulsion droplets leading to reduced encapsulation efficiency. Membrane emulsification appears to be a very suitable option to achieve this. However relatively few studies have been made to determine optimum process parameters integrating the primary emulsification step on yield and stability of multiple emulsions.

Another key issue that also needs to be addressed is the method for yield estimation of the multiple emulsions. This needs to be performed with a suitable marker in the inner water phase of the w/o/w emulsion and the influence on the performance of the emulsifier, process and stability of the emulsion need to be understood. Droplet size estimation of both the primary and secondary emulsions will also be of utmost importance as the stability of the emulsions depends to a great extent on the droplet size distribution. In particular gravity separation (sedimentation in the case of w/o and creaming in the case of w/o/w) is affected by the droplet size. Since membranes to be used are of $>1 \mu\text{m}$ pore-size, the multiple emulsions formed will be of mean droplet size $>1 \mu\text{m}$ and so will be susceptible to creaming. In view of these considerations, the key objectives of this work were identified as to study

- The effect of the primary and secondary emulsification conditions and membrane operating parameters on the multiple emulsion properties with particular concern to the yield and physical stability of the emulsions for encapsulation of WPC.

3. Materials and Methods

3.1 Constituents of the Emulsion

3.1.1 Oil

Soya oil containing antioxidants E319, E320, E330 purchased from Davis Trading, Palmerston North, New Zealand was used for all experiments.

3.1.2 Water

Reverse osmosis water was used for all experiments.

3.1.3 Whey Protein Concentrate (WPC)

ALACEN 3423 was donated by Fonterra Research Centre, Palmerston North, New Zealand and was added in the range of 1 - 30 wt% to the internal phase.

3.1.4 Emulsifiers

The hydrophobic emulsifiers used were PGPR and Span 80. PGPR (polyglycerol polyricinoleate, E476, Palsgaard 4125) was obtained from Palsgaard, Denmark, supplied by Hawkins Watt, Auckland, New Zealand. Span 80 (sorbitan monooleate, S-6760) was obtained from Sigma Chemical Company, USA.

Hydrophilic emulsifiers used were Tween 80, WPI, SPI and Sodium Caseinate. Tween 80 (polyoxyethylene sorbitan monooleate, P-8074) was obtained from Sigma Chemical Company, USA. WPI (whey protein isolate) (ALACEN 895) and sodium caseinate (ALANATE 180) were supplied by New Zealand Milk Products, New Zealand Dairy Board, Wellington, New Zealand. SPI (soy protein isolate, FP 940) was obtained from Columbit New Zealand Limited, Auckland, New Zealand.

3.1.5 Glucose

Analytical grade anhydrous D glucose (Ajax Chemicals, Australia) and (Riedel-de Haen AG, Germany) was used as the marker in the internal phase.

3.1.6 Sodium Azide

Research grade sodium azide (SERVA, Boehringer Ingelheim Bioproducts, Germany) was used at 0.02 wt% in the internal phase as well as the external phase as a preservative for long-term storage trials.

3.2 Equipment

3.2.1 Membrane Equipment

The experimental rig used is shown in Figure 3.1.

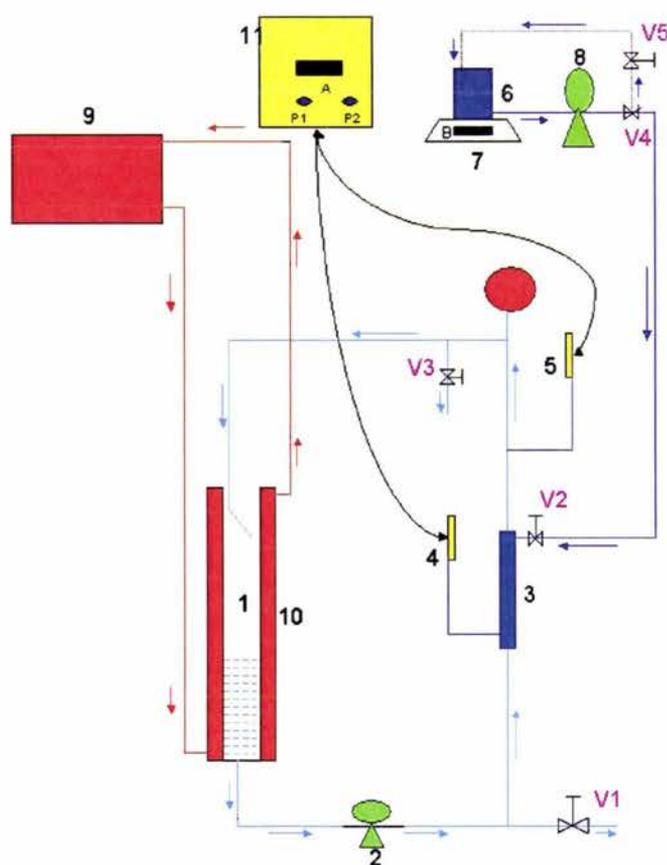


Figure 3.1 Schematic diagram of the experimental rig used for membrane emulsification.

- 1 --- Stainless steel container for circulating the continuous phase and in turn holding the w/o/w multiple emulsion.
 - 2 --- Rotary lobe pump, Sew Eurodrive, New Zealand with a maximum of 349 revolutions per minute and is used for circulating the continuous phase.
 - 3 --- Membrane rig made of stainless steel with SPG membrane inside.
 - 4 --- Pressure gauge for determining the dispersed phase pressure.
 - 5 --- Pressure for determining the continuous phase pressure.
 - 6 --- Dispersed phase container.
 - 7 --- Electronic balance for measuring the dispersed phase flux.
 - 8 --- Peristaltic pump, Masterflex, Cole-Parmer Instrument Co, Chicago, USA fitted to a Solid State Masterflex Controller also from Masterflex, Cole-Parmer Instrument Co, Chicago, USA for pumping the dispersed phase into the membrane rig.
 - 9 --- Hot water bath for producing the hot water to be circulated through the hot water jacket.
 - 10---Hot water jacket (stainless steel)
 - 11---Control Panel, used for controlling the speed of the continuous phase and also for determining the transmembrane pressure.
 - 12---Thermocouple attached to a digital thermometer, Extech Instruments for determining the temperature of the continuous phase.
- A --- Pressure display (P_1 & P_2)
 B --- Weight display of the electronic weighing machine.
 P_1 & P_2 shows dispersed phase pressure and continuous phase pressure respectively.
 V_1 & V_3 are drain valves.
 V_2 is a valve for letting in the dispersed phase.
 V_4 is a three way valve for adjusting the dispersed phase pressure and
 V_5 is a pin valve for adjusting the back flow of the dispersed phase.

The tubes for the transport of continuous phase are made of stainless steel and the tube used for pumping the dispersed phase is a Masterflex 96400-16.

3.2.2 Method of operation of the membrane equipment

At first the power terminals were switched on and then the dispersed phase was placed on the electronic balance (7) with the suction tube of the peristaltic pump (8) immersed in the dispersed phase. The peristaltic pump was switched on so that enough dispersed phase was present in the membrane rig (3) to build the pressure up to 50kPa, which in turn was the pressure developed when the continuous phase was circulated at 1m/s. (In the experiments with varying continuous phase velocities the initial pressure of the dispersed phase was adjusted accordingly). For the dispersed phase to permeate the membrane the applied pressure should be greater than the sum of the average pressure in the continuous phase, P_{cts} and the capillary pressure, P_{cap} (Rayner & Tragardh, 2002). The continuous phase poured into the collecting vessel

(1) at the start was then circulated at the desired velocity by adjusting the speed of the pump (2). The dispersed phase pressure builds up as the experiment proceeds due to continuous pumping of the dispersed phase. The pressure was maintained at the desired level by adjusting the back-flow of the dispersed phase through the valve V_4 by adjusting the pin valve V_5 attached to V_4 . Membrane emulsification was continued until all the dispersed phase was passed through the membrane into the continuous phase. The multiple w/o/w emulsion was collected by opening the valve V_1 . During the process the pressure of the dispersed phase (P_d) and the pressure of the continuous phase (P_c) were noted for calculating the transmembrane pressure (ΔP).

$$\Delta P = P_d - P_c \dots\dots\dots (3.1)$$

Also the average disperse phase flux (J) through the membrane pores was determined by noting the total time taken (t) for the process to complete.

$$J = \frac{V}{A \times t} \dots\dots\dots (3.2)$$

where V (kg) is the amount of dispersed phase used for the process and A is the area of the membrane (m^2).

Monitoring the electronic balance gave an indication of the flow rate of the dispersed phase and also indicated stoppages due to clogging or breakage of lines.

In some experiments, the continuous phase was pre-warmed at the required temperature and the hot water jacket (10) was used to maintain the temperature during w/o/w emulsion formation. For this a hot water bath (9) was used to produce water at a higher temperature ($80^\circ C$) and this was circulated through the stainless steel jacket.

3.2.3 Membranes

Shirazu porous glass (SPG) membranes purchased from SPG Technology Co. Ltd, Miyazaki, Japan were used. The pore sizes tried were $1.4\mu m$, $2\mu m$ and $3.8\mu m$. Other characteristics of the membrane were as shown in Table 3.1.

- Diameter of the membranes 0.01m
- Length of the membranes 0.125 m
- Cross-Sectional Area $0.786 \times 10^{-4} \text{ m}^2$
- External Surface Area 0.003925 m^2

The other physical properties of SPG membrane include (K.Kandori, 1995);

Porosity	50 – 60%
Pore Volume	$0.4 – 0.6 \text{ cm}^3/\text{g}$
Specific surface area	$0.2 – 8.0 \text{ m}^2/\text{g}$
True density	$2.5 \text{ g}/\text{cm}^3$
Thermal expansion coefficient	$60 \times 10^{-7} \text{ K}^{-1}$
Heat resisting temperature	650 deg C
Compressive strength	$2000 – 3000 \text{ kgf}/\text{cm}^2$
Bending strength	$710 – 840 \text{ kgf}/\text{cm}^2$

Table 3.1 Table showing the physical properties of the SPG membrane.

3.2.4 Ultra-Turrax

The Ultra Turrax used was a Heidolph Diax 600 obtained from Heidolph Elektro GmbH, Germany.

3.2.5 Homogenisers

Two homogenisers were used: an APV 2000 and a Rannie lab unit.

The APV 2000 (Invensys APV Products, Denmark) bench top unit had a maximum operating pressure of 2000 bar (29000psi or 200MPa) in the first stage and 1000 bar (14500psi or 100MPa) in the second stage. The normal capacity is 11L/Hr and the hold up volume is 13 ml. Emulsions with viscosities higher than 500 cP can be processed and the maximum processing temperature is 100° C. The homogenising valve is made of ceramic and the pump valve seats are of tungsten carbide.

Some emulsions were also prepared by a 2-stage Rannie lab (pilot plant) homogeniser, (Rannie, Denmark. This had a capacity of 100 L/h and a maximum homogenisation pressure of 600 bars in the first stage.

3.2.6 Sonicator

The sonicator used for w/o emulsification in some experiments was a Virsonic Digital 475 Ultrasonic Cell Disrupter (VirTis Company, USA). This used a piezoelectric transducer operating at 20 kHz and could deliver up to 475W of ultrasonic power.

3.2.7 Advantage glucose meter

Glucose was used as the marker for estimating the yield stability of the w/o/w emulsions. Glucose was added to the internal phase of the w/o/w emulsion at 6.4 wt% and the concentration in the outer phase was measured using an Advantage Plus Pack Blood Glucose System (Roche Diagnostics (NZ) Ltd, Auckland, New Zealand). A drop of the sample was placed on the yellow spot of the test strip, which was then inserted into the glucose meter and the reading noted. The system works on the principle of biamperometry, i.e. the glucose dehydrogenase enzyme in the test strip converts the glucose in the sample to gluconolactone. The reaction creates an electrical current, which the meter interprets and shows the result on the digital screen in mmol/L.

3.2.8 Polarimeter

ADP 220 Polarimeter (Bellingham + Stanley Limited, UK) was used during the development of the glucose assay.

3.2.9 Water activity meter

Water activity of the samples containing known concentrations of glucose, sorbitol and NaCl was measured at 25°C using a Decagon water activity meter (Decagon Devices Inc, USA).

3.3 Methods

3.3.1 Preparation of Primary Emulsions

The first step in the preparation of the multiple w/o/w emulsions was the preparation of the primary w/o emulsion, which was preceded by a pre-mixing step.

3.3.1.1 Pre-mixing of primary emulsions

The pre-emulsification step was done to distribute the water droplets thoroughly in the continuous phase (oil in this case) before the actual emulsification step. The desired proportion of inner phase (water), oil, and emulsifier (hydrophobic) was weighed. The marker (glucose) was added to the inner phase at 6.4 wt%. The dispersed phase (water + glucose) was then added drop by drop by means of a peristaltic pump (Model Masterflex, Cole-Parmer Instrument Co, Chicago, USA fitted to a Solid State Masterflex Controller also from Masterflex, Cole-Parmer Instrument Co, Chicago, USA) at a flow rate of around 5 ml per minute. The pre-emulsification was done by means of an Ultra-Turrax operating at 9,500rpm.

3.3.1.2 Primary emulsification by ultra-turrax

In the initial experiments involving Span 80 as the hydrophobic emulsifier, the Ultra-Turrax was tried as a primary emulsification device. The dispersed phase (water) fraction of the primary w/o emulsion was 25% w/w and the amount of hydrophobic emulsifier (Span 80) was 10% w/w. The emulsification was carried out at 20,500 rpm for 5 minutes.

3.3.1.3 Primary emulsification by sonicator

The pre-mixed emulsion was placed under the probe of the sonicator. The emulsification was carried out at different power levels (1 – 10) and at different time periods (1, 2 and 3 minutes), and the LCD reading (% of total power) and the temperature rise due to the excess energy input in each case were noted. The energy input was calculated by the formula

$$E = 475W \frac{LCD}{100} (\%) \dots\dots\dots (3.3)$$

Different concentrations of Span 80 and different concentrations of the inner phase were also tried.

3.3.1.4 Primary emulsification by APV 2000 homogeniser

The APV 2000 was primarily used for the emulsification of small volumes of the primary w/o emulsion. Samples of 200 ml were processed in each run. The runs were conducted to find out the effects of the concentration of the inner phase, the concentration of the hydrophobic emulsifier (PGPR), the number of passes of the homogeniser and also the pressure of homogenization. In every run one or more of the above variables was changed. Accordingly five different inner phase concentrations of 20wt%, 25wt%, 30wt%, 40wt% and 50wt% of the primary w/o emulsion, four different emulsifier concentrations of 2.5wt%, 5wt%, 7.5wt% and 10wt% of the oil phase, one, two and three passes of the homogeniser and three different pressures of 250/50 bars, 500/100 bars and 1000/200 bars were employed for the first and second stages respectively. The appropriate composition of the pre-mixed w/o emulsion was passed through the APV 2000 homogeniser (usually 2 times) to get the final w/o emulsion.

3.3.1.5 Primary emulsification by Rannie homogeniser

The Rannie homogeniser was used to prepare primary emulsions with both Span 80 and PGPR as the hydrophobic emulsifier. With the Span 80, the first stage pressure was set at 240 bars and the second stage pressure at 40 bars. Different concentrations of the inner phase were investigated. The Rannie was used mainly to prepare bulk volumes of primary w/o emulsions in the range of 800 ml to 1L in the case of PGPR as the hydrophobic emulsifier. This was mainly for experiments to investigate the effect of different outer phase concentrations and of different types of hydrophilic emulsifiers in the outer phase of the multiple w/o/w emulsions. In this case the first stage pressure was set at 500 bars and the second stage pressure was set at 100 bars. The pre-emulsified w/o emulsion was passed through the Rannie two times to obtain the final w/o emulsion.

3.3.2 Preparation of Multiple Emulsions

3.3.2.1 Emulsification with Ultra-Turrax

The Ultra-Turrax method of preparing w/o/w multiple emulsions was used in the initial stages of the research. In this method, the prepared w/o emulsion was emulsified with water containing a suitable concentration of the hydrophilic emulsifier. The speed was set at 8,000 rpm and the time at 3 minutes as a higher speed and time was found to lower the yield of the emulsions formed. This method was also used to study the osmotic movement of water across the oil layer in w/o/w multiple emulsions.

3.3.2.2 Membrane emulsification

SPG membranes were the main mode of secondary emulsification in the research. The primary emulsion was used as the dispersed phase and was added so that this comprised 20% by mass of the final multiple emulsion. The dispersed phase was forced through the SPG membrane of desired pore-size at the desired transmembrane pressure and the dispersed phase flux was measured by noting the change in volume with time. The continuous phase generally containing 1 wt% Tween 80 (general case) was circulated at the desired velocity through the inside of the membrane. The process was continued until all the dispersed phase was exhausted and the w/o/w multiple emulsion was formed. The method of formation with description of the apparatus is detailed in section 3.2.2.

3.3.2.3 Cleaning of membranes

After the emulsification process, the equipment was circulated with hot water at 50° C for 5 minutes with the membrane in the rig. The water was flushed out and a second batch of hot water containing 0.06 wt% of Koch UltraClean 2 cleaning agent (Koch, USA), with the pH adjusted to 11 by adding 2M NaOH, was circulated through the equipment with the membrane in the rig. At approximately 3-minute intervals the disperse phase inlet valve (V_2) was opened to drain a portion of the solution. This was continued for 10 minutes, and then the entire solution was drained out. The membrane was taken out from the rig, cleaned manually in hot water and

soaked overnight in 0.06 wt% UltraClean 2 solution (pH 11). Before the start of every run the flux was checked with hot water at 50°C.

3.4 Particle Size Analysis

The particle size of the primary w/o emulsion was determined using a Malvern Ultrasizer and the images were obtained by means of a confocal microscope. The particle size of the secondary w/o/w multiple emulsions were determined by means of a Malvern MasterSizer/E and the images were obtained by using a brightfield microscope.

3.4.1 Ultrasizer

The Malvern Ultrasizer (Malvern Instruments, UK) consists of two pairs of ultrasound transducers (a low frequency pair and a high frequency pair) manufactured from polarized PVDF. The transducers are mounted on an optical bench that permits automatic alignment under computer control. The path length and frequency ranges used during the measurement are controlled by proprietary software, which also corrects for beam divergence, electronic noise and intrinsic frequency responses of the transducers for accurate measurement of attenuations. The instrument is capable of precision measurement of attenuation over the frequency range 2-180 MHz (Povey *et al*, 1999).

Volumes (500 ml) of the homogenized w/o emulsion sample homogenized under varying conditions were analysed to obtain the mean particle diameter and particle-size distribution. Scattering matrices capable of measuring particles of size range 10nm - 1000µm were used. As a bimodal distribution was usually observed, a weighted mean diameter was calculated as follows;

$$d_{WMD} = \frac{(GM_{(1)} Prop_{(1)} + GM_{(2)} Prop_{(2)})}{Prop_{(1)} + Prop_{(2)}} \dots\dots\dots (3.4)$$

d_{WMD} = weighted mean diameter

$GM_{(1)}$ = geometric mean of the 1st peak (as given by the Ultrasizer output)

$GM_{(2)}$ = geometric mean of the 2nd peak (“)

$Prop_{(1)}$ = Proportion of the first peak. (“)

$Prop_{(2)}$ = Proportion of the second peak. (“)

A typical output of the Malvern Ultrasizer is shown in Figure 3.2. The plots obtained were bimodal in nature and hence the geometric means and the proportion of droplets belonging to each peak is taken into consideration and weightage is given for the samples containing greater proportion of the smaller droplets by multiplying the geometric means of each peak with the proportion of the same peak. The weighted mean diameter was taken only to have a representative value for droplet diameter in the case of determining the effect of various processing parameters on the droplet size of the w/o emulsion.

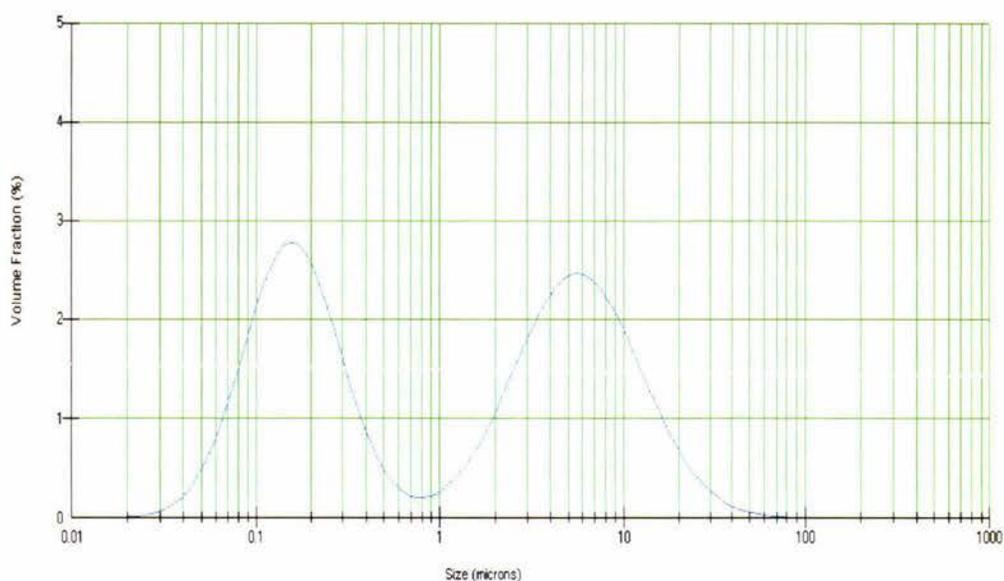


Figure 3.2 Droplet size distribution of a w/o emulsion produced with 25% aqueous phase, 10% PGPR in the oil phase and homogenised at 500 bar (2 passes) measured with a Malvern Ultrasizer.

3.4.2 Confocal Microscope

The emulsion images were taken using a Leica CLSM TCS 40 microscope with 100x magnification under oil immersion. The software used was Scanware and the laser was an Argon-Krypton mixed gas laser with an excitation wavelength of 488nm.

The oil phase of the primary w/o emulsion samples were stained with Nile Blue at 1 wt% of the oil phase before the pre-mixing step. The samples were then pre-mixed and homogenized. A drop of the sample was placed on a cavity slide and the cover slip placed on it. This was then viewed under the 100x objective and one or more images were obtained.

The secondary emulsions were stained after their formation. Nile Blue (5 wt%) was used for staining the w/o/w emulsions. The w/o/w emulsions samples containing the stain were agitated thoroughly. A drop of the sample was placed on a cavity slide and the cover slip placed on top. This was then viewed under the 100x objective (oil immersion) of the confocal microscope and the images were taken.

3.4.3 MasterSizer

A Malvern MasterSizer/E (Malvern Instruments Ltd, UK) was used for the droplet size determination of the w/o/w emulsions. The principle involved in the Mastersizer is laser ensemble light scattering. The presentation code is Custom 2NAD and model is polydisperse. This represents a relative refractive index (N) of 1.095, i.e. ratio of refractive index of emulsion particle (1.456) to that of the dispersion medium (1.33), and an absorbance value (A) of the emulsion particle of 0.001. The main parts of the MasterSizer include an optical measurement unit forming a basic particle size sensor and a computer, which manages the measurement and performs result analysis and presentation. The laser beam used is a 2mW He-Ne Laser with 633nm wavelength and with 18mm beam expansion. The laser is diffracted by the dilute dispersion of the emulsion and is captured over a range of scattering angles by a series of semi-circular photodiodes. The refractive index of the particle, the absorbance of the particle and the refractive index of the medium are the parameters required for particle size determination by the MasterSizer.

The calculation of the different mean diameters are as follows;

- $d_{(3,0)} = \text{Number, Volume Mean Diameter} = (\Sigma x^3 dN / \Sigma dN)^{1/3}$
- $d_{(3,2)} = \text{Surface, Volume Mean Diameter} = \Sigma x^3 dN / \Sigma x^2 dN$
- $d_{(4,3)} = \text{Weight, Moment Mean Diameter} = \Sigma x^4 dN / \Sigma x^3 dN$

where N is the number of particles having a diameter x . The equipment was first cleaned so that the laser power reading was above 66. A few drops of the w/o/w multiple emulsion samples were then dispersed until the obscuration was between 19% and 21%. The analysis was continued and the results obtained were printed out in a report format. The Mastersizer cannot measure droplets of size above 64.92 μm . The following parameters were recorded: $d_{(0,1)}$, $d_{(0,5)}$, $d_{(0,9)}$, $d_{(3,2)}$, $d_{(4,3)}$.

3.4.4 Brightfield Microscope

Images of the multiple w/o/w emulsions were taken using an Olympus BX51 microscope with 20x magnification. The camera used was an Optronic Magnifier and the software used for getting the images was Magnafire. The image analysis was done using Image Pro Plus software Version 4.5 of Media Cybernetics.

A drop of the w/o/w multiple emulsion samples was placed on a glass slide and observed under the 20x magnification. The cover glass was not placed on top of the slide as this may disrupt the droplets. Some images were taken under the 100x magnification oil immersion objective, in which case a cover slip was placed on the slide.

3.5 Yield Analysis

Five different concentrations of glucose solutions were selected and the reading of the glucose meter was monitored on an hourly basis. It was observed that the glucose readings changed with time and it took about 4 – 5 hours for obtaining a consistent reading. (Figure 3.3)

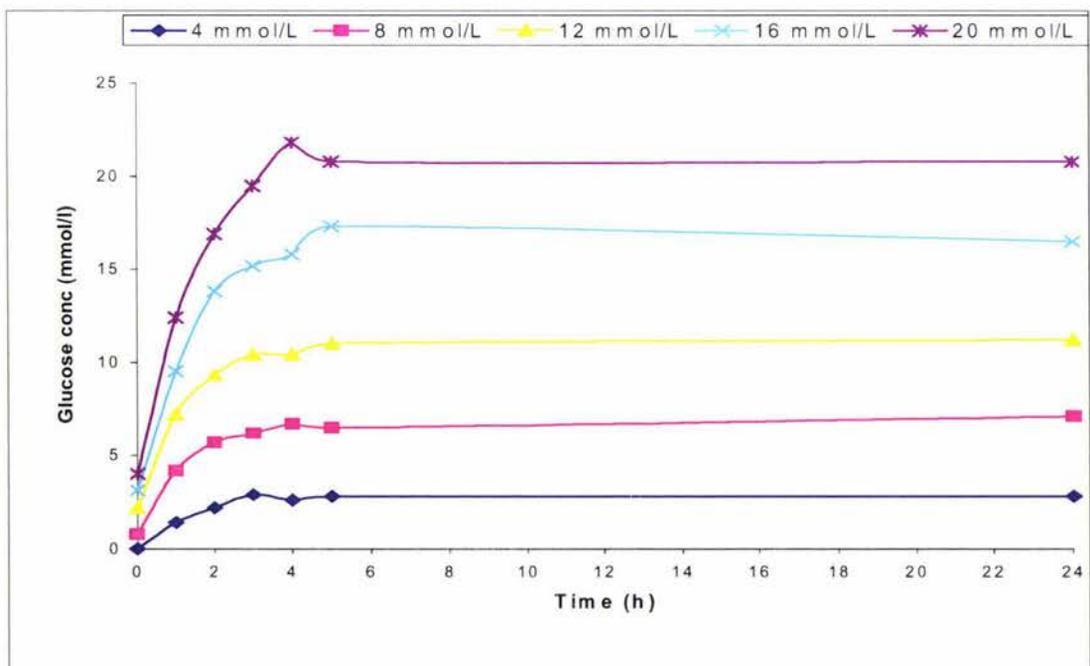


Figure 3.3 Change in glucose readings due to mutarotation. Graph showing the increase in the readings shown by the Advantage glucose meter with time with different concentrations of glucose.

The change in glucose readings was due to the phenomenon of mutarotation. The glucose used as the marker was α -glucose, but the Advantage glucose meter measures only the β -glucose present. As soon as the α -glucose is dissolved in water mutarotation occurs and the α form present in the sample gets converted to β form. This is a reversible reaction and takes place slowly with time until equilibrium is attained. The process is temperature dependant and an increase in temperature results in faster attainment of the equilibrium condition. Using the polarimeter the process was observed to about 4 hours at room temperature (20° C) (Figure 3.4). The specific rotation of α -D glucose starts at +112.2 and ends at +52.7 and that of β -D glucose starts at +17.5 and ends at +52.7 at 20° C (Weast, Astle, Beyer, 1988).

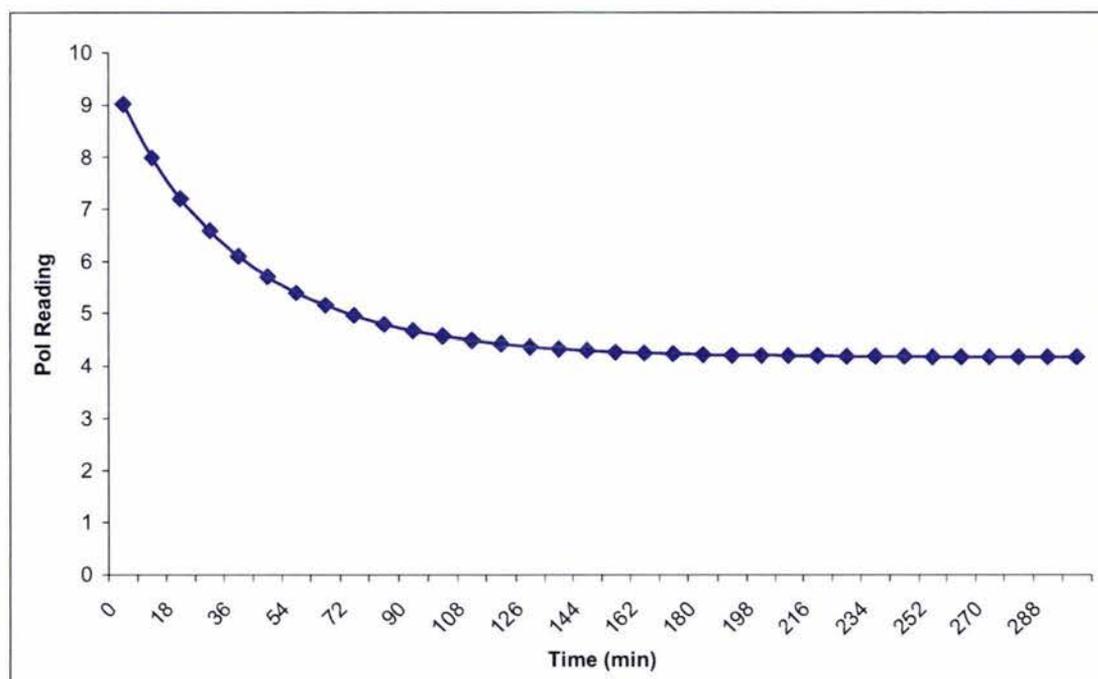


Figure 3.4 Decrease in the Pol readings as shown by the Polarimeter when a known concentration of glucose was inserted and the readings monitored over time.

The same samples were also tested with another equipment (Cobas Fara, Roche Diagnostics, New Zealand) to confirm the correctness of the Advantage Glucose meter. The results obtained were as follows (Table 3.2):

Glucose Concentration (g/68ml)	Advantage	Cobas Fara
0.05	2.8	3.8
0.1	7.1	7.7
0.15	11.2	11.6
0.2	16.1	16.2
0.25	21.4	20.1

Table 3.2 Table showing the readings obtained with Advantage Glucose meter and Cobas Fara for the same concentrations of glucose.

The results as shown in Table 3.2 indicate that the Advantage Glucose Meter is showing fairly consistent results when compared with that of the Cobas Fara. The slight difference in the readings should be due to the fact that mutarotation is an ongoing process and is not stopped even if equilibrium condition is attained.

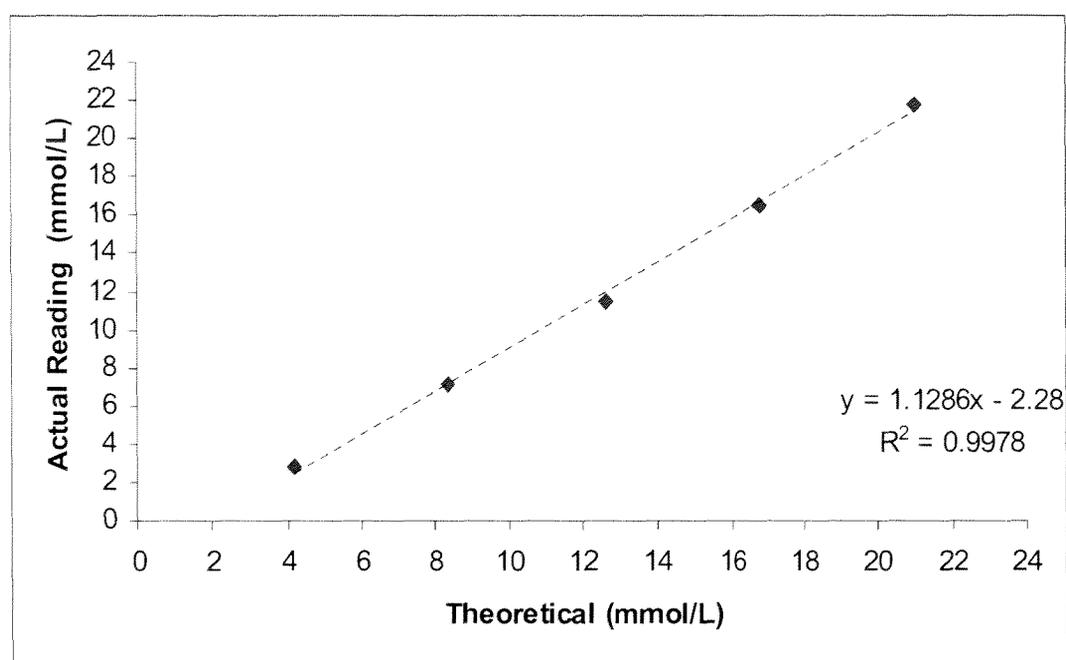


Figure 3.5 Graph showing the maximum readings obtained with the Advantage Glucose Meter for different concentrations of glucose.

Five different concentrations of glucose (0.074, 0.147, 0.22, 0.294, 0.368) were prepared and readings were taken with the glucose meter, which when plotted against the theoretical values gave a linear relationship (Figure 3.5). The straight line obtained in the Figure is a further indication of the consistency of the readings made with the Advantage Glucose Meter.

For yield measurements 21 mmol/L was taken as the standard. The amount of glucose added to the inner phase of the multiple emulsions was calculated in such a way so that a complete breakage of the emulsions indicating 0 yield should indicate 21 mmol/L in the outer phase.

In another set of experiments, attempts were made to break the w/o/w emulsions formed and to liberate the entire encapsulated glucose to confirm that there is no interference by the emulsion constituents. In the first experiment the emulsions formed was treated with 10% Triton x100 and in the second experiment the emulsion was stirred well with an Ultra-Turrax at 13500 rpm. In the first case the reading obtained from the glucose meter indicated 18.4mmol/L and in the second case a reading of 20.1mmol/L was obtained. These indicate an 88% and 96% liberation of glucose respectively and suggest that there was no or very limited interference of the multiple emulsion components with readings of the Advantage glucose meter.

3.6 Creaming Index

Multiple emulsions soon after preparation was poured into graduated centrifuge tubes and kept at room temperature. The height of the serum layer (H_s) and the height of the cream layer (H_c) were noted after 24 hours (1day) and 168 hours (1week). The stability index was calculated as:

$$S = \left(\frac{H_s}{H_s + H_c} \right) 100 \quad \dots\dots\dots (3.5)$$

4. Influence of Primary Emulsion Composition & Process Conditions on W/O and W/O/W Emulsion Properties

4.1 Introduction

Formation of a stable primary w/o emulsion is very important for the preparation of a high yielding w/o/w multiple emulsions with a long-term stability. The water-in-oil volume fraction [$\Phi_{(w/o)}$] should be as high as possible to facilitate the maximum loading of the ingredient (in this case WPC) into the w/o/w system and for this purpose a small and even droplet size distribution in the primary w/o emulsion is very important. The preparation of a monodispersed w/o emulsion is difficult compared to an o/w emulsion because the water droplets are difficult to stabilise by an electrical double layer repulsion force in an oil phase with a low dielectric constant (Kandori, 1995).

The formation of a very fine stable w/o emulsion with a reasonably narrow droplet size distribution typically requires a rather high lipophilic emulsifier concentration and vigorous homogenisation conditions (Dickinson & McClements, 1996). Kita *et al* (1978) showed that good w/o/w emulsions would be obtained only if the hydrophobic emulsifier to hydrophilic emulsifier concentration ratio was in the range 2 – 10. It was therefore common practice to use a 10-fold amount of hydrophobic emulsifier than the hydrophilic emulsifier for w/o/w preparation (Garti, 1997). Hasegawa *et al* (2001) reported that w/o/w emulsions can be produced at a lower emulsifier concentration, but if the focus is on the long-term stability of the emulsions then higher emulsifier concentrations are desirable. According to Abd-Elbary *et al* (1984), the chemical structure of the lipophilic non-ionic emulsifier is important for producing stable multiple emulsions and not the HLB value.

In this work, experiments were conducted with Span 80 and PGPR as the hydrophobic emulsifiers to determine the boundaries and the optimum conditions of composition and homogenisation pressure to prepare a stable w/o emulsion with a very small droplet size that in turn will give a w/o/w emulsion with high yield and stability.

4.1.1 W/O emulsions prepared with Span 80 as the hydrophobic emulsifier

Omotosho *et al* (1986) found that increasing the concentrations of Span 80 from 1wt% to 10wt% resulted in an increase in stability of w/o/w emulsions. Apenten & Zhu (1996), in their experiments to find out the effective minimum concentration (EMC) of Span 80 to produce stable water-in-kerosene-in-water emulsions found that the EMC for Span 80 is $\geq 2.5\%$ (w/w). But according to them, the migration of Span 80 monomers from the inner w/o interface to the outer o/w interface resulted in destabilisation of the emulsions, which can only be rectified by increasing the concentration of Span 80. Hence, in all the experiments in this work involving Span 80 as the hydrophobic emulsifier, a concentration of 10% (w/w) of the oil phase was fixed. Several methods of primary emulsification (Ultra-Turrax, homogenisation and ultra-sonification) were tried by varying the internal phase compositions to obtain the best yield in the w/o/w multiple emulsions formed. Results are discussed in detail in coming sections.

4.1.2 W/O emulsions prepared with PGPR as the hydrophobic emulsifier

Benichou *et al* (2001) concluded that soyabean oil and PGPR are capable of giving a very fine droplet size with the mean diameter of the droplets in the range of 1 μm . Mine *et al* (1996), through his experiments to produce stable w/o emulsions by membrane emulsification found that a stable w/o emulsion cannot be produced by using egg PC alone as surfactant, but a combination of egg PC with PGCR gave a fine emulsion with a sharp droplet size distribution. The fineness of the droplet size and the long-term stability of the primary w/o emulsions with 10% (w/w) PGPR in the oil phase gave w/o/w emulsions with a high yield. Hence in experiments where Span 80 was replaced with PGPR as the hydrophobic emulsifier at a concentration of 10wt% of the oil phase, a homogenisation pressure of 500 bar and a water-in-oil volume fraction ($\Phi_{(w/o)}$) of 25% of the primary w/o emulsion were fixed as the standard for preparing the secondary w/o/w emulsions unless specified otherwise.

4.1.3 Droplet size of primary W/O emulsions

The droplet size of the primary w/o emulsion is of utmost importance when the secondary w/o/w emulsions are produced by means of a membrane. The droplet size

of the primary w/o emulsions should be less than the pore-size of the membrane for the efficient formation of a high yielding w/o/w emulsion. If the droplet sizes are larger it will result in their disruption, resulting in reduced yield of the multiple emulsion. Hence investigations were conducted to find out the factors affecting the droplet size and stability of the primary w/o emulsions so that a standard formulation and set of conditions can be devised for the production of a base w/o emulsion for all future experiments.

4.2 Objectives

- To determine the effect of primary emulsification parameters like Span 80 or PGPR concentration, primary emulsification pressure and internal water phase concentration on the yield stability of w/o/w emulsions produced by Ultra-Turrax and membrane emulsification.
- To determine the effect of primary emulsification parameters (stated above) on the droplet size of the w/o emulsion produced.

4.3 Experimental

The emulsions were prepared with reverse osmosis water as the inner and outer aqueous phase. The oil phase used consisted of soyabean oil purchased from Davis Trading, Palmerston North, New Zealand. The inner aqueous phase consisted of glucose (6.4wt%) as the marker (Refer 3.1.5) and protein WPC (ALACEN 3423) as the encapsulated material (Refer 3.1.3).

Primary (W/O) Emulsion

The hydrophobic emulsifiers tried were Span 80 (S-6760), Sigma Chemical Company, USA and PGPR (Palsgaard 4125), Palsgaard A/S, Denmark, supplied by Hawkins Watt, New Zealand. The primary (w/o) emulsions were prepared with an Ultra-Turrax (Heidolph DiAx 600, Heidolph Elektro GmbH & Co, Germany) at 20,500 rpm as well as a valve homogeniser (APV 2000, Invensys APV Products, Denmark).

Secondary (W/O/W) Emulsion

The secondary (w/o/w) emulsions were prepared with the Ultra-Turrax at 8000 rpm or using a SPG membrane, SPG Technology Co. Ltd, Japan, of nominal pore size 2 μm . The hydrophilic emulsifier in the outer aqueous phase was Tween 80 (P-8074), Sigma Chemical Company, USA added at 1wt%.

The yield stability measurements were done using an Advantage glucose meter (Roche Diagnostics, New Zealand) as previously described in Section 3.2.7. The droplet size measurements were conducted using a Malvern Ultrasizer (Malvern Instruments, UK) (Section 3.4.1).

4.4 Results and discussion

The two factors which have a considerable effect on the formation and stability of the w/o emulsion are the composition of the emulsion and the mode of primary emulsification. Hence experiments were conducted to understand these effects by varying the composition of the primary emulsion and also by trying different primary emulsification methods and emulsification conditions.

4.4.1 Effect of hydrophobic emulsifier type and concentration

Previous research has proved that the hydrophobic emulsifier concentration has a very significant effect on the stability of both the primary and secondary emulsions. Emulsifiers are essential for stabilising the interfaces by reducing the interfacial tension and stabilise the droplets by diffusing to the interfaces (Bergenstahl, 1997). The faster the adsorption of the emulsifier molecules at newly formed interfaces the smaller the emulsion droplets formed (Joscelyne & Tragardh, 2000). For w/o emulsions Schroder *et al* (1998) showed that for the same time interval (10s), the decrease in interfacial tension is fastest and greatest for SDS stabilised o/w emulsions (4mN/m) than for Tween 20 (7mN/m) or Lacprodan 60 (a form of WPC) (22mN/m) stabilised o/w emulsions. As a result of this, the droplet size of SDS stabilised emulsions were lower [$d_{(3,2)} = 1 \mu\text{m}$] than that of Tween 20 stabilised emulsions [$d_{(3,2)} = 2 \mu\text{m}$] and Lacprodan 60 stabilised emulsions [$d_{(3,2)} = 10 \mu\text{m}$]. The droplet size of

the primary w/o emulsion is to a large extent dependent on the concentration of the hydrophobic emulsifier. For w/o emulsions the choice of the emulsifier is more limited, and as indicated above, only Span 80 and PGPR and mixtures of the two, were evaluated.

4.4.1.1 W/O/W Emulsions prepared with Ultra-Turrax

In the initial stages of the research, experiments were conducted by preparing both the primary (w/o) and the secondary (w/o/w) emulsions by an Ultra-Turrax to understand the behaviour of the emulsions in general. The primary emulsification was done at a higher speed (20,500 rpm) and longer time (15 minutes) to produce the maximum shear necessary for disrupting the water particles and to obtain droplets with minimum possible droplet size. The secondary emulsification was done at a comparatively lower speed (8,000 rpm) and shorter time (3 minutes) so that the shear produced was only enough for producing the multiple emulsion droplets and did not result in their disruption.

A comparative study of yield was conducted with the two types of hydrophobic emulsifiers (PGPR and Span 80) added in the same quantity (10 wt% of oil phase) in the two samples.

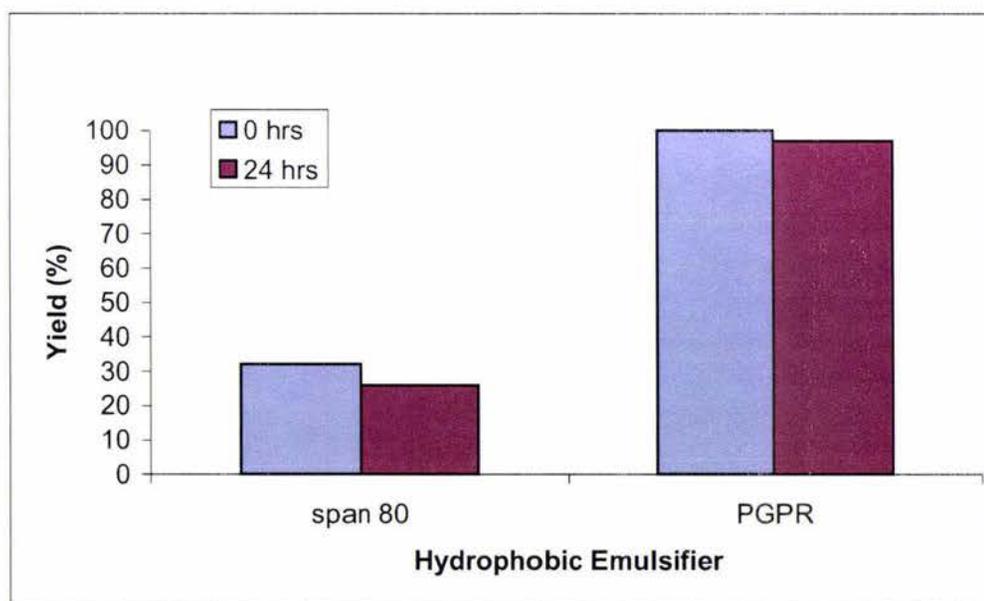


Figure 4.1 Variation in yield with change in hydrophobic emulsifier. W/O (primary) emulsions were prepared with 20% inner phase, 10% hydrophobic emulsifier and Ultra-Turraxed at 20500 rpm. W/O/W (secondary) emulsions prepared with 1% Tween 80 in the outer phase and 30% w/o emulsion Ultra-Turraxed at 8000 rpm.

Results obtained (Figure 4.1) showed that PGPR was clearly a better hydrophobic emulsifier than Span 80. The initial yields obtained with Span 80 as the hydrophobic emulsifier were as low as 30%, whereas the initial yield obtained with PGPR as the hydrophobic emulsifier was 100%. One reason for this behaviour would be the high elasticity and surface potential of PGPR (Kanouni *et al*, 2002); another possible explanation is provided below.

Investigations were conducted on the effectiveness of using a mixture of PGPR and Span 80 as the hydrophobic emulsifier. Accordingly experiments were conducted with PGPR and Span 80 ratios of 1:9, 3:7, 5:5, 7:3 and 9:1 as shown in Table 4.1. The total concentration of the hydrophobic emulsifier was fixed at 10 wt% of the oil phase.

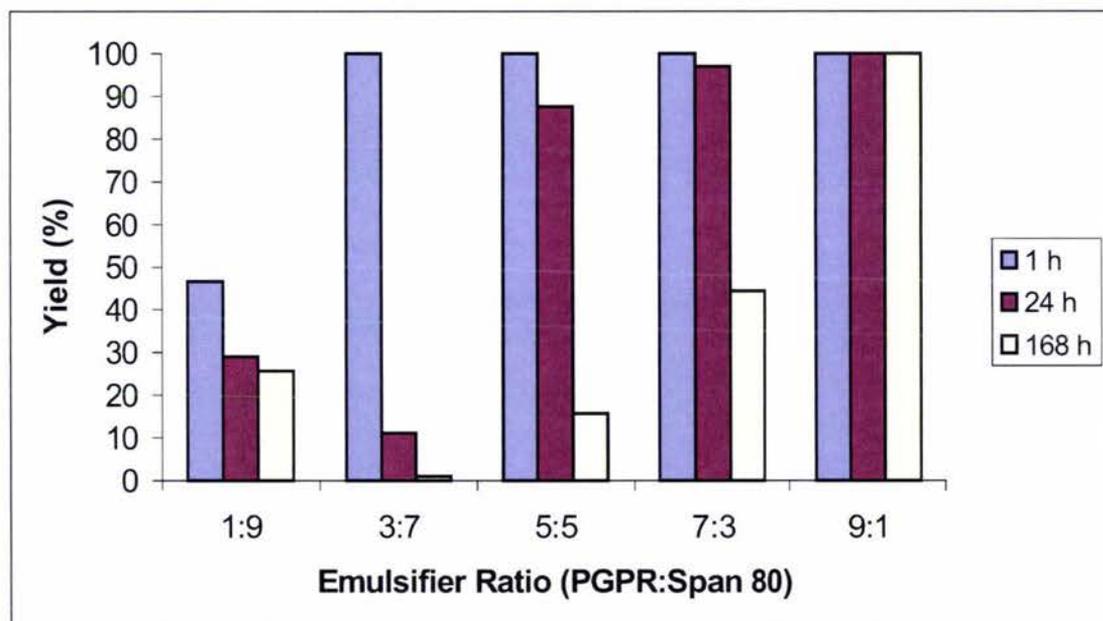


Figure 4.2 Variation in yield with change in lipophilic emulsifier ratio. W/O/W emulsions prepared with 1% Tween 80 in the outer phase and 20% w/o emulsion Ultra-Turraxed at 8000 rpm. W/O emulsions were prepared with 25% inner phase, 10% hydrophobic emulsifier (a combination of Span 80 and PGPR in the ratios 1:9, 3:7, 5:5, 7:3, 9:1) and Ultra-Turraxed at 20500 rpm.

It was observed (Figure 4.2) that as the concentration of PGPR increased the yield stability of the w/o/w multiple emulsions also increased. This again points to the better emulsifying abilities of PGPR. The HLB value for Span 80 is 4.3 and that for PGPR is 2. The lower the HLB value the higher the hydrophobicity of the emulsifier. Hence we can say that PGPR is more hydrophobic than Span 80. When combinations

of PGPR and Span 80 were used the weighted HLB values were 4.07, 3.61, 3.15, 2.69 and 2.23 respectively for PGPR:Span 80 ratios of 1:9, 3:7, 5:5, 7:3 and 9:1.

Another major observation was the increase in apparent (visual) viscosity and the percentage of cream phase of the w/o/w emulsions with increase in PGPR as shown in Fig 4.3 – 4.5. This appears to be due to the swelling of the droplets and may help to explain the yield stability. Further work needs to be done in the future to quantify the effects of viscosity in w/o/w emulsions observed in Fig 4.2. Immediately after the formation of the w/o/w emulsions the sample with the highest concentration of Span (1:9) showed a much reduced yield (Fig (4.2)) and a very low viscosity (Fig 4.3). The rest of the samples showed higher yields and the viscosity of these samples were on the increase at first. However the viscosity decreased with time (Fig 4.4), as did the yield stability (Fig 4.2). The viscosity and the yield of the 5:5 and 7:3 samples were high for the first 24 hours, but decreased significantly after a week (168 hours). The 9:1 sample showed the same yield and viscosity over this time (Figure 4.3, 4.4, 4.5).

An explanation for this effect is the osmotic effect generated by the marker (glucose) in the inner phase (Refer 5.4.3). Soon after emulsion preparation there would be osmotic movement of water from the outer phase to the inner phase due to the osmotic gradient created by the higher solute concentration (marker) in the inner phase. This would increase the size of the oil globules thereby increasing the viscosity. As mentioned earlier and unlike Span 80, PGPR has the capacity of forming highly stable and elastic monolayers (Kanouni *et al*, 2002) that may help the oil globules to withstand the pressure generated by influx of water from the outer phase and this would be more marked in samples with a higher concentration of PGPR.

In samples with a lower concentration of PGPR there may be a threshold above which any more influx of water from the outer phase may result in the breakage of the oil globules, thereby liberating the inner phase and reducing the yield as well as the viscosity. Span 80 appears not to be capable of withstanding such high pressures and so the sample (1:9) showed a very low viscosity and yield initially. This however promises scope for future work and ideally the experiments should be conducted by balancing the osmotic pressure with sorbitol (Figure 4.3, 4.4, 4.5). However the

behaviour of the mixtures is complex as the 1:9 sample shows a slower decrease in stability over the 1-week period than the 3:7 sample. The reason for this is unknown.

Sample Name	PGPR:Span 80	Time (hrs)	Emulsion Type
A	1:9	0	W/O
B	3:7	0	W/O
C	5:5	0	W/O
D	7:3	0	W/O
E	9:1	0	W/O
F	1:9	0	W/O/W
G	3:7	0	W/O/W
H	5:5	0	W/O/W
I	7:3	0	W/O/W
J	9:1	0	W/O/W
K	1:9	24	W/O/W
L	3:7	24	W/O/W
M	5:5	24	W/O/W
N	7:3	24	W/O/W
O	9:1	24	W/O/W

Table 4.1. Table showing the details of the primary and secondary emulsions prepared with the Ultra-Turrax with different concentrations of PGPR and Span 80.



A B C D E

Figure 4.3 Creaming stability of w/o emulsions prepared in an Ultra-Turrax with different concentrations of PGPR and Span 80 as specified in the Table 4.1.



F G H I J

Figure 4.4 Creaming stability and the apparent increase in visual viscosity of w/o/w emulsions prepared in an Ultra-Turrax with different concentrations of PGPR and Span 80 as specified in the Table 4.1.



K L M N O

Figure 4.5 Creaming stability and the apparent increase in visual viscosity of w/o/w emulsions prepared in an Ultra-Turrax with different concentrations of PGPR and Span 80 as specified in the Table 4.1.

Another set of experiments was conducted to find the optimum concentration of PGPR to be used as the standard in future experiments. Benichou *et al* (2001) suggests a concentration of 10 wt% of the oil phase as the best to get the smallest possible droplet size. So concentrations of 1 wt%, 2.5 wt%, 5 wt%, 7.5 wt% and 10 wt% were selected for the trials. Higher concentrations were not considered on economic grounds.

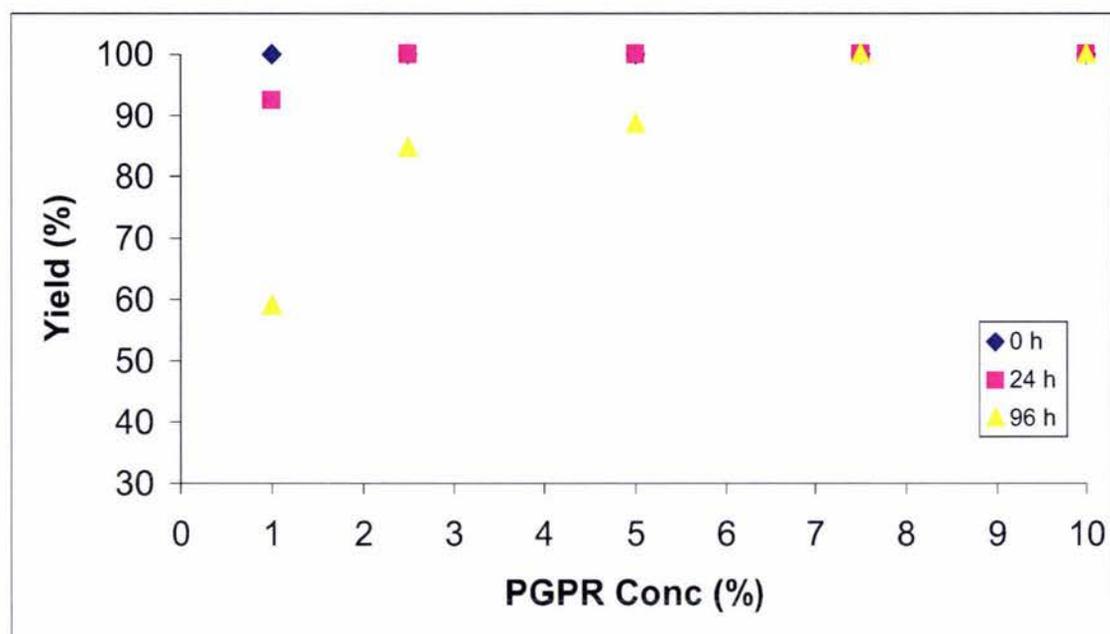


Figure 4.6 W/O/W emulsions prepared with 1% Tween 80 in the outer phase and 30% w/o emulsion Ultra-Turraxed at 8000 rpm. W/O emulsions were prepared with 20% inner phase, varying hydrophobic emulsifier concentrations (1%, 2.5%, 5%, 7.5%, 10%) and Ultra-Turraxed at 20500 rpm.

From Figure 4.6 it can be seen that all PGPR concentrations gave a very high yield initially. The sample with 1% PGPR showed reduced yields within the first 24 hours and after a week, only the samples with 7.5% and 10% PGPR showed no change in the yield stability. As long-term stability will be a key factor in a successful commercial product, a PGPR concentration of 10% of oil phase was taken as the standard. According to Yan *et al* (2003) the w/o interfacial membrane viscosity increases with increase in surfactant concentration which in turn helps in the long term stability of the w/o/w emulsions by preventing the release of solutes from the inner phase.

Csoka & Eros (2003) reported similar results with experiments conducted using Span 80 as the hydrophobic emulsifier. They found that the initial as well as the long-term

stability of w/o/w multiple emulsions were increased on increasing the Span 80 concentrations from 10% to 20% of the oil phase of the w/o emulsion. Hence it was decided that for all future experiments a PGPR and/or Span 80 concentration of 10% of oil phase would be used.

Also the results obtained with glucose as the marker can be considered only as an indicative yield as it may occur that the reduction in yield with time may be due to the diffusion of glucose through the oil layer and not due to the breakage of oil droplets. The relative diffusivity of different marker compounds like glucose, NaCl, dyes, proteins etc will likely vary through the surfactant film and hence further research needs to be done to determine optimum conditions for a given encapsulated compound.

4.4.1.2 W/O/W Emulsions prepared with the membrane

Experiments were conducted with different concentrations (2.5%, 5%, 7.5%, 10%) of PGPR to find the optimum concentration for high yield and a long-term stability with the secondary emulsification carried out with SPG membrane.

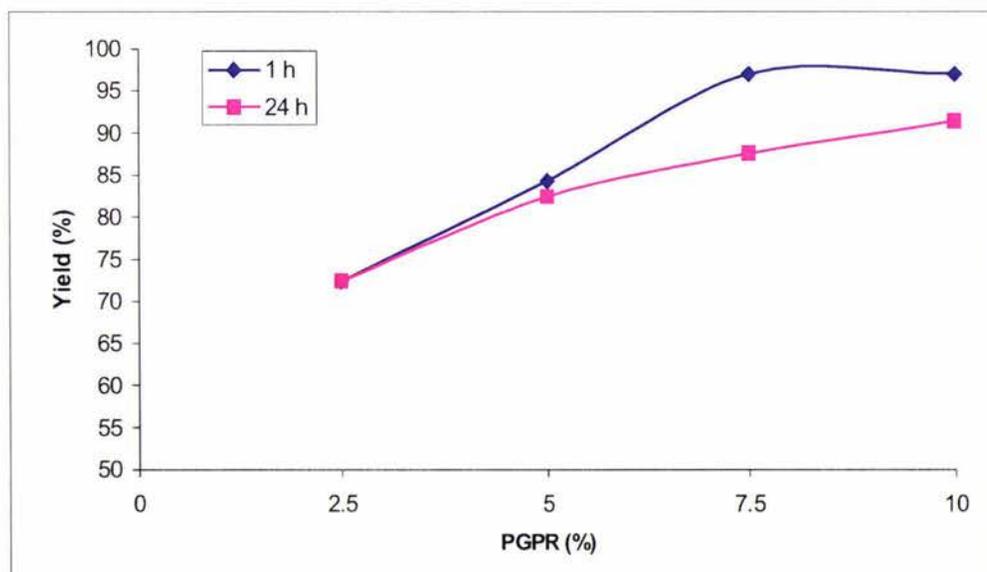


Figure 4.7 Variation in yield with concentration of hydrophobic emulsifier. Yield of w/o/w emulsions prepared with 25% inner phase of the w/o emulsion at a homogenisation pressure of 250 bars and with varying hydrophilic emulsifier concentration (PGPR – 2.5%, 5%, 7.5%, 10%) of oil phase. The secondary emulsification was performed with a 2 μm SPG membrane and 1% Tween 80 in the outer phase as the hydrophilic emulsifier. Readings are taken after 1 hr and 24 hrs.

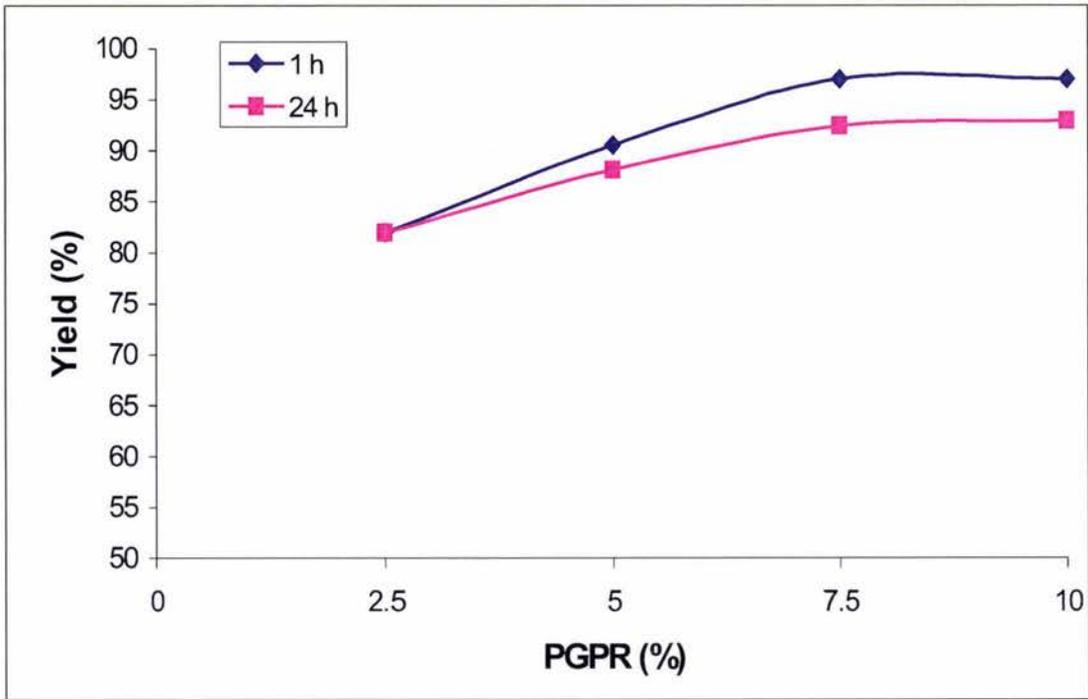


Figure 4.8 Variation in yield with concentration of hydrophobic emulsifier. Primary emulsification (homogenisation) pressure of 500 bars. Conditions as in Figure 4.7

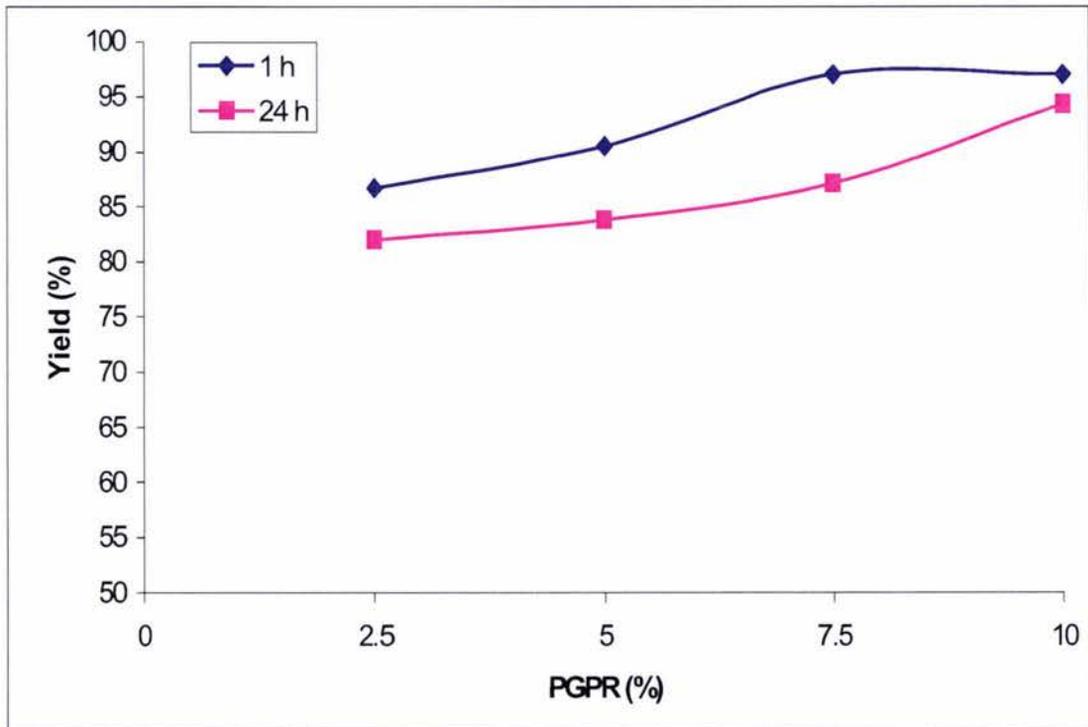


Figure 4.9 Variation in yield with concentration of hydrophobic emulsifier. Primary emulsification (homogenisation) pressure of 1000 bars. Conditions as in Figure 4.7

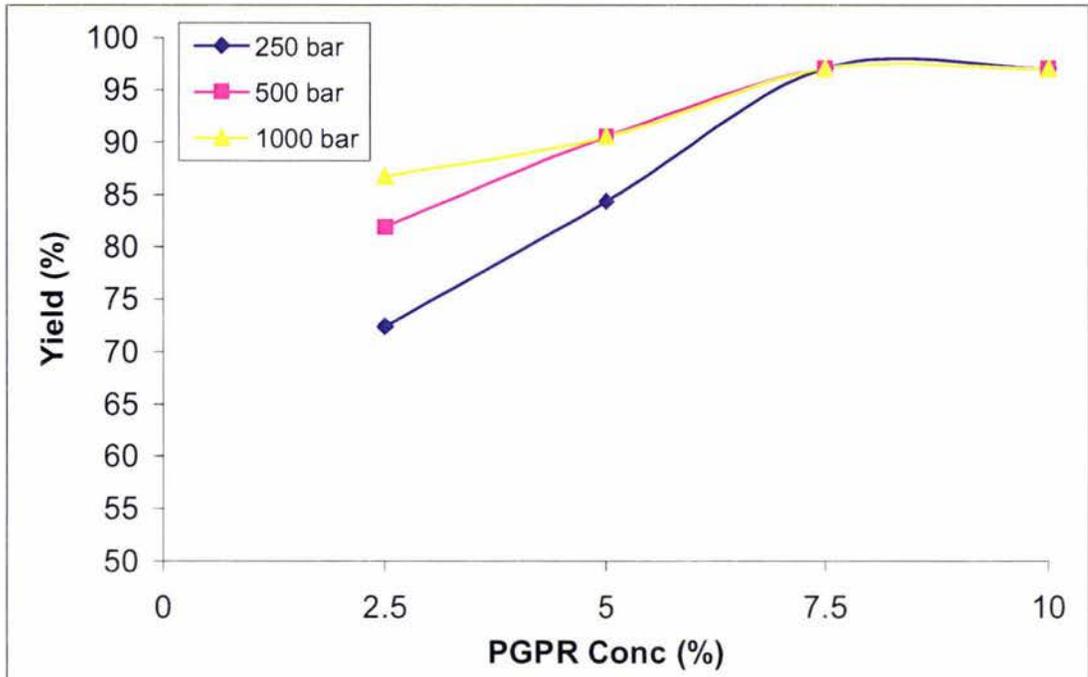


Figure 4.10 Variation in yield with PGPR concentration and homogenisation pressure. W/O/W emulsions prepared with 25% inner phase of the w/o emulsion at a homogenisation pressures of 250 bars, 500 bars, 1000 bars and with varying hydrophilic emulsifier concentration (PGPR – 2.5%, 5%, 7.5%, 10%) of oil phase. The secondary emulsification was performed with a 2 μ m SPG membrane and 1% Tween 80 in the outer phase. Readings are taken after 1hr.

Experiments were conducted with three different primary emulsification pressures (250bar, 500bar and 1000bar) and it was found that the behaviour of multiple emulsions with respect to yield was the same in all the three cases. It is clearly visible from Figure 4.7, 4.8 and 4.9 that a lower emulsifier concentration results in a lower yield and as the concentration is increased from 2.5wt% PGPR to 7.5wt% PGPR the yield also increased proportionally and remained constant at 10wt% PGPR. From Figure 4.10 it can be seen that with 2.5wt% and 5wt% PGPR, better yields were obtained by increasing the homogenisation pressure from 250 bars to 1000 bars. This may be due to the reduction in internal droplet size with increasing homogenisation pressure, which is quite consistent with the findings of Benichou *et al* (2001), but was not visible with higher concentrations of 7.5wt% and 10wt% PGPR. According to Yan *et al* (2003) the percentage break-up of the w/o/w emulsion droplets decreases with the increase in hydrophobic emulsifier concentration. They claim that the emulsion stability is influenced by the hydrophobic emulsifier concentration in two respects: 1) a decrease in surface tension 2) an increase in w/o membrane strength.

4.4.1.3 Droplet size distribution of w/o emulsions

The most important constituent in the successful formation of a w/o emulsion with a small droplet size is the type and concentration of the hydrophobic emulsifier. Two types of hydrophobic emulsifiers were tested – PGPR and Span 80. Measurement of yield of w/o/w emulsion and visual observation of the w/o emulsion with time proved beyond doubt that PGPR is a better emulsifier than Span 80. (Section 4.4.2.1). Benichou *et al* (2001) suggested an optimum concentration of 10% PGPR of the oil phase for obtaining the smallest droplet size in a w/o emulsion. But a concentration of 10% hydrophobic emulsifier is high for a food product (the regulatory limits of PGPR being 7.5mg/kg body weight per day) as it was thought that the lesser the emulsifier the better the product on safety and economic grounds. Attempts were made to obtain a small droplet size in w/o emulsion with a lower concentration of hydrophobic emulsifier and hence experiments were conducted with various concentrations (2.5wt%, 5wt%, 7.5wt%, 10wt%) of hydrophobic emulsifier (PGPR) in the oil phase.

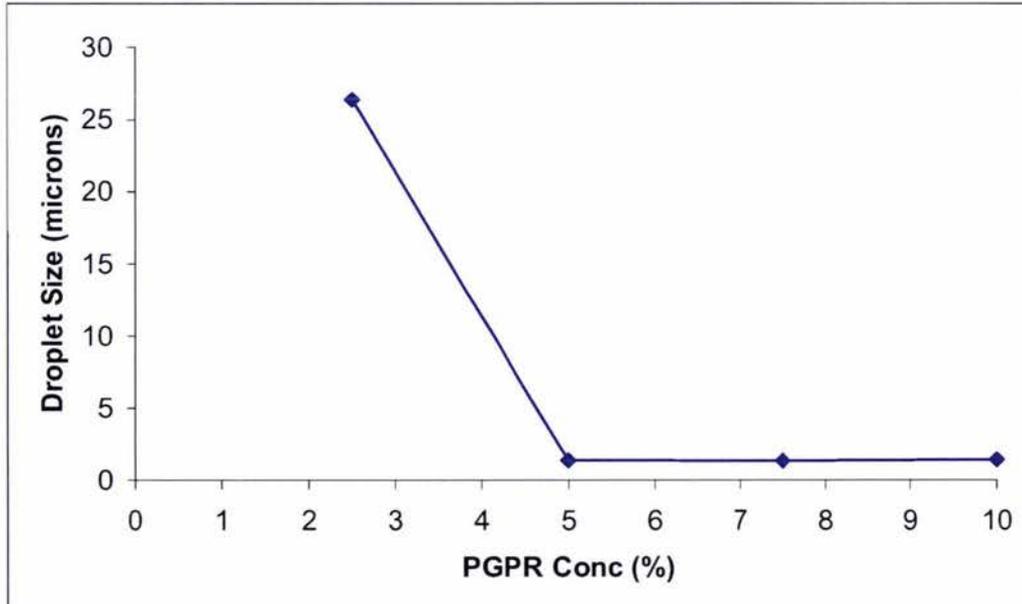


Figure 4.11 Variation in droplet size $d_{(WMD)}$ with PGPR concentration. W/O emulsions prepared with 25% water and varying concentration of hydrophobic emulsifier (2.5%, 5%, 7.5%, 10%) (PGPR) in the oil phase homogenised at 250 bars. Water phase contains 10% WPC.

It is clear from Figure 4.11 & Table 4.2 that at a 2.5% PGPR concentration the mean droplet size ($d_{(WMD)}$) (Refer 3.4.1) was as high as 26 μm , but this decreased to around 2 μm for 5 wt%, 7.5 wt% and 10 wt% concentrations. However the higher droplet size (26 μm) at 2.5% PGPR was not confirmed with the confocal microscope image whereas the droplet sizes estimated using the Ultrasizer for the 5 wt%, 7.5 wt% and 10 wt% PGPR showed acceptable agreement.

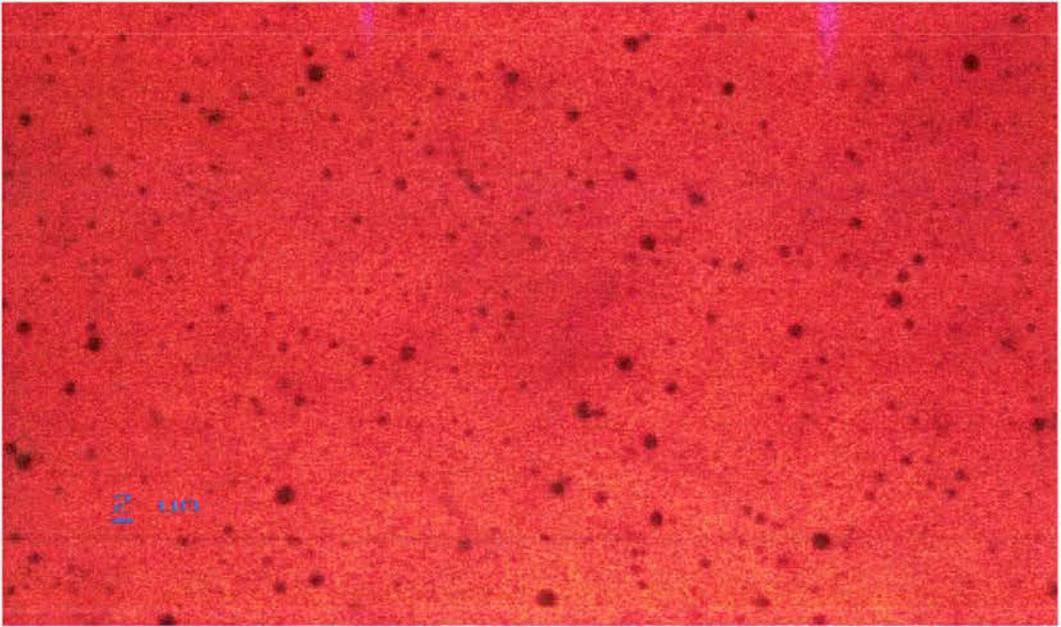


Figure 4.12 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 2.5% PGPR in the oil phase homogenised at 250 bar. The red background is the oil phase and the dark dots are the water droplets.

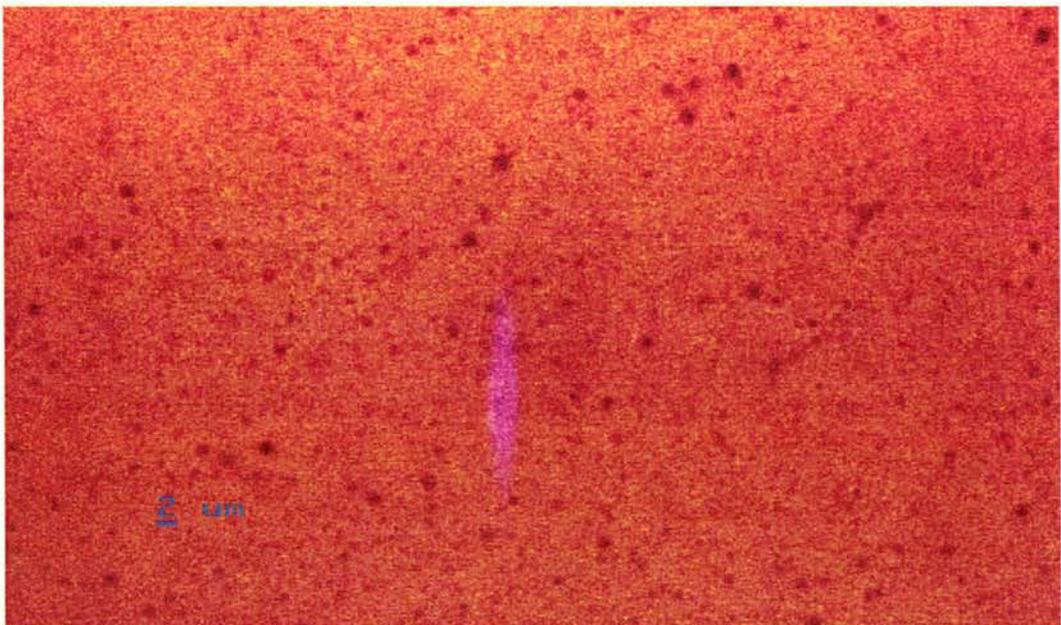


Figure 4.13 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 5% PGPR in the oil phase homogenised at 250 bar. The red background is the oil phase and the dark dots are the water droplets.

Calculations were done to find out the apparent thickness of the hydrophobic emulsifier (PGPR) at the primary w/o interface. The results are shown in the Table 4.2 below.

PGPR Conc.	d_{WMD} (weighted droplet diameter) (μm)	* $A = 3V/r$ (interfacial area) (m^2)	N (amt. of emulsifier) (Kg)	$\Gamma = N/A$ (mg/m^2)	ρ (density of PGPR) (Kg/m^3)	$\delta = \Gamma/\rho$ (interfacial thickness of PGPR) (nm)
2.5%	26.38	28.4×10^3	2×10^{-3}	0.07	1044.2	0.067
5%	1.39	539.6×10^3	3.55×10^{-3}	0.0065	1044.2	0.006
7.5%	1.34	560×10^3	5.25×10^{-3}	0.0093	1044.2	0.009
10%	1.4	536×10^3	7.5×10^{-3}	0.014	1044.2	0.013

Table 4.2. Table showing the apparent thickness of the PGPR layer at the w/o interface with different PGPR concentrations.

* V – Volume of the internal phase in the w/o emulsion and r is the droplet radius (i.e. $r = d_{WMD} / 2$).

It can be seen from the Table 4.2 that at the lowest PGPR concentration of 2.5wt% the apparent thickness of the emulsifier layer obtained at the w/o interface was 0.067 nm. This is a higher value than the values obtained at PGPR concentrations of >2.5wt% and is probably due to the higher droplet size at this lower concentration thereby decreasing the total interfacial area. Yield analysis showed a decrease in yield with decrease in PGPR concentrations. The argument that this effect is probably due to the lowering of the viscosity of the oil layer due to lowering of the PGPR concentration leading to an easier diffusion of the encapsulant as advanced by Yan et al (2003) may not apply in this case as an increase in apparent thickness of the surfactant layer was observed at a lower PGPR concentration. Rather it can be argued that the decrease in yield should be due to the apparent disruption of the larger droplets during their passage through the comparatively smaller pores of the SPG membrane. The lower dispersed phase flux experienced with a 2.5 wt% PGPR concentration can thus be explained as due to the higher droplet size (26 μm) of the primary w/o emulsion. (Section 6.4.4.2).

Even though a PGPR concentration of 5 wt% of the oil phase is found to give a good droplet size in this case, the w/o/w emulsions prepared exhibited better yield and long-term stability only in the case of 7.5 wt% and 10 wt% PGPR. This can be explained by the increase in membrane viscosity with increase in emulsifier concentration and decrease in release rates with increase in membrane viscosity (Section 4.4.2.1) as it is evident from Table 4.2 that the apparent thickness of the

surfactant layer at the w/o interface increases with increase in concentration. Hou and Papdpoulous (1996) reported that the adsorbed layers of surfactants at the o/w interface with effective thickness of 0.1-0.3 nm does not have a strong influence on interaction energy leading to an absence of obstacle for oil film rupture. But as the surfactant concentration increases the adsorbed layer grows thicker and when it reaches a critical thickness of 0.5 nm, the internal water droplets experiences a net repulsive van der Waals interaction with external aqueous phase. Again it should be noted that the calculations are based on the weighted mean diameter of droplets (d_{WMD}) and hence any change in this value may cause a variation in the calculated apparent thickness of the surfactant layer. Hence further research should be conducted for confirmation.

4.4.2 Effect of Primary Emulsification Pressure

According to Benichou *et al* (2001) the homogenisation pressure has a significant effect on the droplet size of primary emulsions stabilized by PGPR and they found a decreasing trend in the droplet size as the homogenisation pressure was increased from 700 atm to 2000 atm. Three homogenisation pressures (250 atm, 500 atm and 1000 atm) were tried and the droplet size distribution and yield stability of the w/o/w emulsions formed using these w/o emulsions were investigated. Figure 4.14 and 4.15 show the results of the yield stability analysis of the resultant w/o/w emulsions.

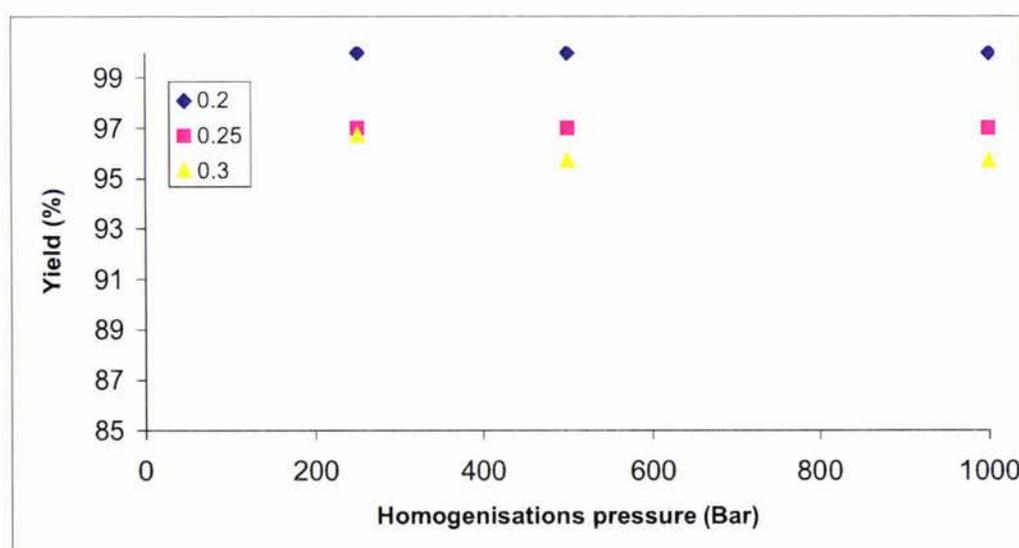


Figure 4.14 Variation in yield with different primary emulsification pressures. Conditions as in Figure 4.15. Glucose readings for yield determination taken after 1hr of preparation.

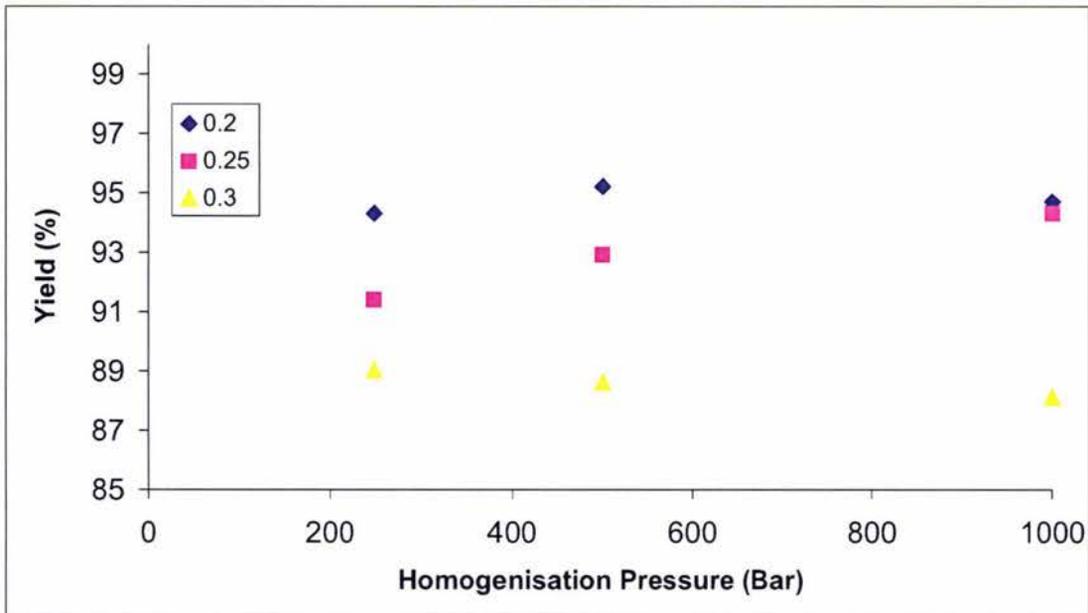


Figure 4.15 Variation in yield with different primary emulsification pressures. Yield of w/o/w emulsion prepared with w/o fraction – 0.2, 0.25, 0.3, 10% PGPR of the oil phase as hydrophobic emulsifier, at homogenisation pressures of 250 bar, 500 bar and 1000 bar in the primary emulsification stage and using a 2 μ m SPG membrane in the secondary emulsification and 1% Tween 80 as hydrophilic emulsifier in the outer phase. Glucose readings for yield determination taken 24 hrs after preparation.

It can be seen that there is not much effect of the variation in homogenisation pressure on the yield (encapsulation efficiency) of the multiple emulsions formed with relatively high yields of >95% in all three cases. The yields decreased to ~ 90% after 24 hours. Garti *et al* (2001) showed that at the lowest homogenisation pressure tried (700 atm) the droplet size was below 2 μ m. The membrane pore-size of 2 μ m in this research would not be an obstacle to the passage of the small sized water droplets. Also the research conducted by Kanouni *et al* (2002) has shown that the w/o surface properties of polyglycerol ester of ricinoleic acid (Grinstead PGR-90) is highly stable comprising compressible and reversibly expandable monolayers with a high elasticity and surface potential. These properties makes it highly resistant to shear and as a result water droplets which are slightly above the 2 μ m limit may still be able to squeeze through the membrane pore without much damage.

4.4.2.1 Droplet size distribution of W/O emulsions

Benichou *et al* (2001) in their investigations on the optimum homogenisation pressure for the preparation of a w/o emulsion (10% PGPR as the hydrophobic emulsifier, iso propyl myristate as the oil phase and 30% water) found that a homogenisation

pressure of 2000 atm was best for the production of water droplets with a mean size of 0.8 μm . A homogenisation pressure of 2000 atm was found to be practical only under laboratory conditions and not under actual industrial conditions. Hence attempts were made to reduce the homogenisation pressure and still obtain a droplet size below 2 μm . These experiments were conducted at 1st stage homogenisation pressures of 250 bars, 500 bars and 1000 bars; the homogenisation pressure in the 2nd stage was maintained at one-fifth of the 1st stage homogenisation pressure. Even though the Ultrasizer measurements and the computation of the mean droplet size with the formula (3.4) shown in section 3.4.1 gives a rough idea of droplet size of the w/o emulsion produced with variation in emulsions composition and processing conditions, the graphs shown cannot be considered as completely reliable. This is because the confocal microscope images of the samples prepared under the same conditions are not in agreement with the Ultrasizer results in all the cases (as noted above).

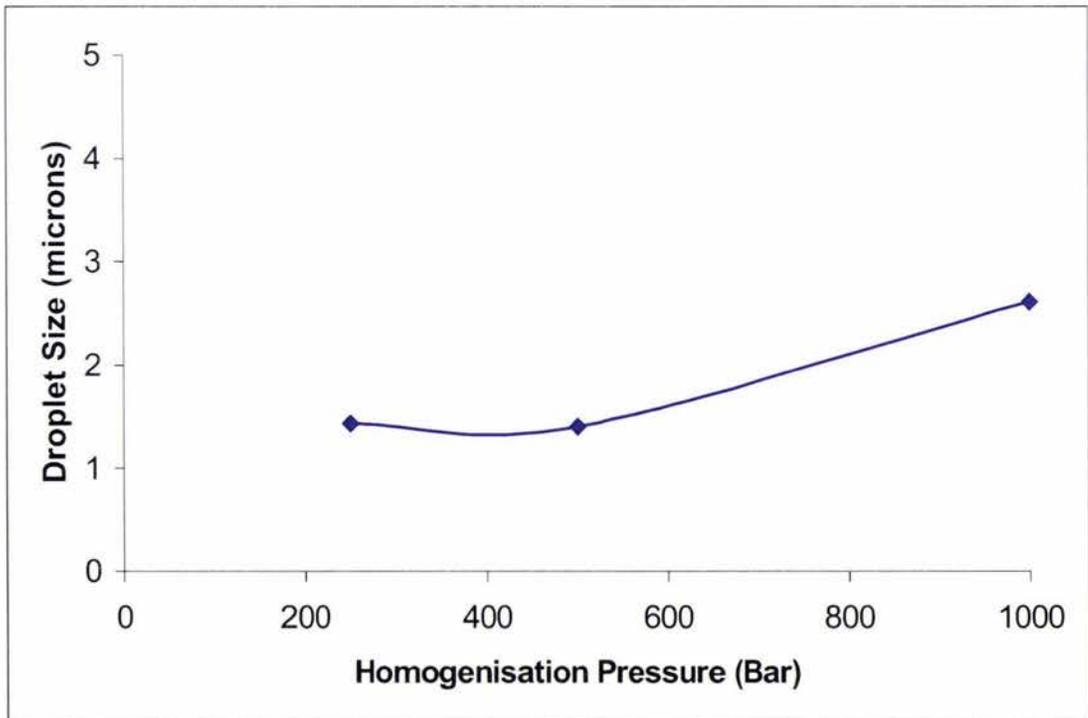


Figure 4.16 Variation in droplet size $d_{(WMD)}$ with homogenisation pressure. w/o emulsions prepared with 25% water and 10% hydrophobic emulsifier (PGPR) in the oil phase homogenised at various pressures (250 bars, 500 bars, 1000 bars). Water phase contains 10% WPC.

From Figure 4.16 it can be seen that the mean droplet size ($d_{(wMD)}$) of the water droplets produced at pressures of 250 bars and 500 bars were estimated to be very similar at $1.43\mu\text{m}$ and $1.4\mu\text{m}$ respectively, but when the pressure was increased to 1000 atm it was found that the mean droplet size increased to $2.61\mu\text{m}$. This result differed from that obtained by Benichou *et al* (2001). Also the yields of the w/o/w emulsions obtained did not show any decrease with increasing homogenisation pressures. In fact the yield of w/o/w with lower concentrations of PGPR showed an increasing trend with increase in homogenisation pressures (Section 4.2.1 & 4.2.2.2).

Overall, a homogenisation pressure of 500 bars was taken as the standard in all the future experiments. An advantage of this was that this pressure could be reproduced on the Rannie high pressure homogeniser, which is typical of much large-scale homogenisation equipment. A key issue in this work was the droplet size comparison made with the ultrasizer and the confocal microscope, which need to be addressed by further research. For example the increase in homogenisation pressure from 500 bars to 1000 bars showed the production of a much finer w/o emulsion by the confocal images (Figure 4.17 & 4.18), whereas the Ultrasizer results shows a slight increase in apparent droplet size (Figure 4.16). A possible explanation is provided in Section 4.4.3.2.

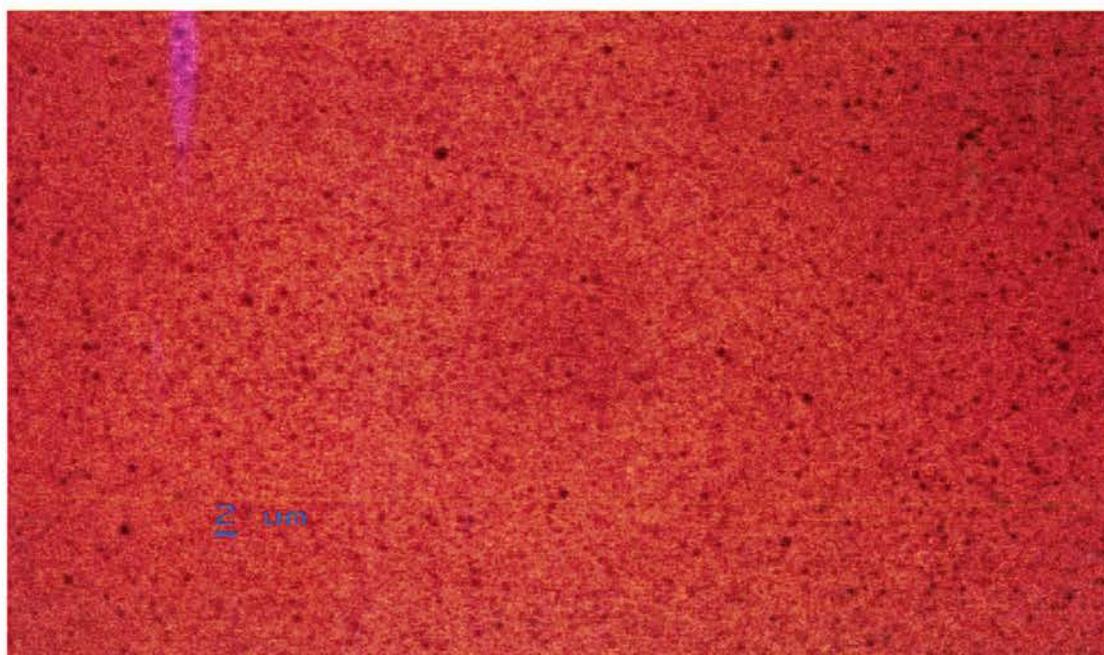


Figure 4.17 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 10% PGPR in the oil phase homogenised at 500 bar. The red background is the oil phase and the dark dots are the water droplets.

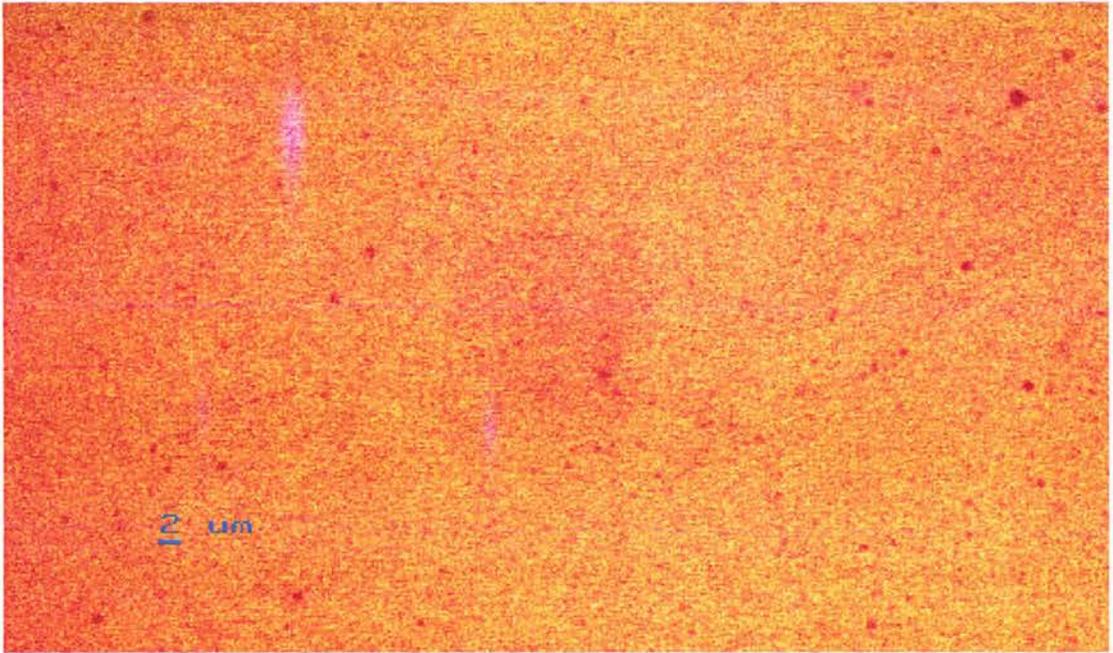


Figure 4.18 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 10% PGPR in the oil phase homogenised at 1000 bar. The red background is the oil phase and the dark dots are the water droplets.

Another set of experiments was conducted to investigate the effect of the number of passes of the w/o emulsion through the homogeniser (Figure 4.19). This was done to find out whether there is a role played by the number of passes in reducing the droplet size. One, two and three passes through the homogeniser were evaluated.

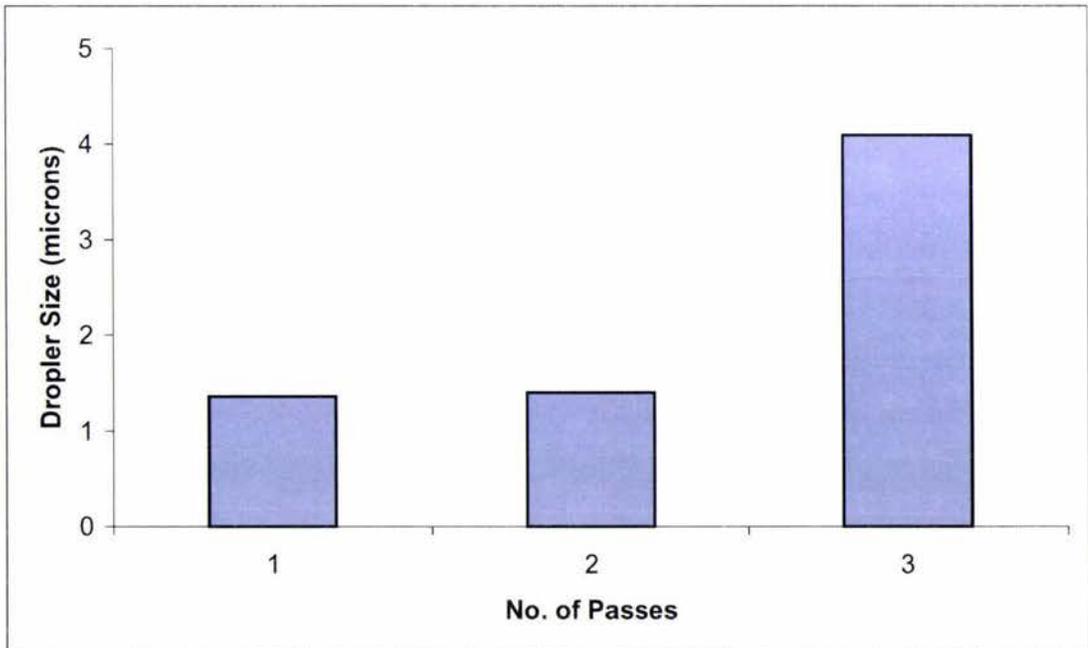


Figure 4.19 Droplet size $d_{(WMD)}$ of w/o emulsions prepared with 25% water and 10% hydrophobic emulsifier (PGPR) in the oil phase homogenised at 500 bars. The emulsion samples underwent 1, 2 and 3 passes. Water phase contains 10% WPC.

From Figure 4.19 it can be seen that there was not much change in the mean droplet size with the first and the second passes. But the droplet size increased considerably with the third pass indicating a negative effect. Hence in all the future experiments a total of two passes through the homogeniser at a particular pressure was taken as the standard.

4.4.3 Effect of Internal Phase Concentration

4.4.3.1 With PGPR as the hydrophobic emulsifier and primary emulsification by the homogeniser

Research conducted by Benichou *et al* (2001) proved that PGPR stabilized primary w/o emulsions can accommodate up to 30 wt% water without much effect on the stability on the emulsions. At 10 wt% water the average droplet size was 1.3 μm without much change until 30 wt% level is reached. But as the concentration is increased beyond 45 wt%, the droplet sizes grew to an average of 2.8 μm with a high viscosity. A similar behaviour was experienced in the experiments conducted in this study. Different concentrations of the internal phase (20 wt%, 25 wt%, 30 wt%, 40 wt% and 50 wt%) were tried but good results were obtained only with the first three (20 wt%, 25 wt% and 30 wt%).

The process of secondary emulsification was difficult with the latter two (40 wt% and 50 wt%) as a very low dispersed phase flux was experienced even at a very high transmembrane pressure. The emulsification process had to be stopped due to blockage of the membrane pores and tubing breakage due to excessive pressure. This is probably due to the increase in droplet size with the increase in inner phase concentration making it impossible for the droplets to pass through the narrow pores (2 μm) of the membrane. An increase in the percentage of water in the internal phase decreases the absolute amount of hydrophobic emulsifier and the oil phase for a fixed proportion of these, thus decreasing the surface coverage by the hydrophobic emulsifier of the water droplets and leading to formation of larger droplets. The excess amount of droplets formed at a given homogenisation pressure may result in coalescence due to incomplete surface coverage by the comparatively smaller amount of hydrophobic emulsifier. Figure 4.20, 4.21 and 4.22 show the variation in yield with internal phase volume.

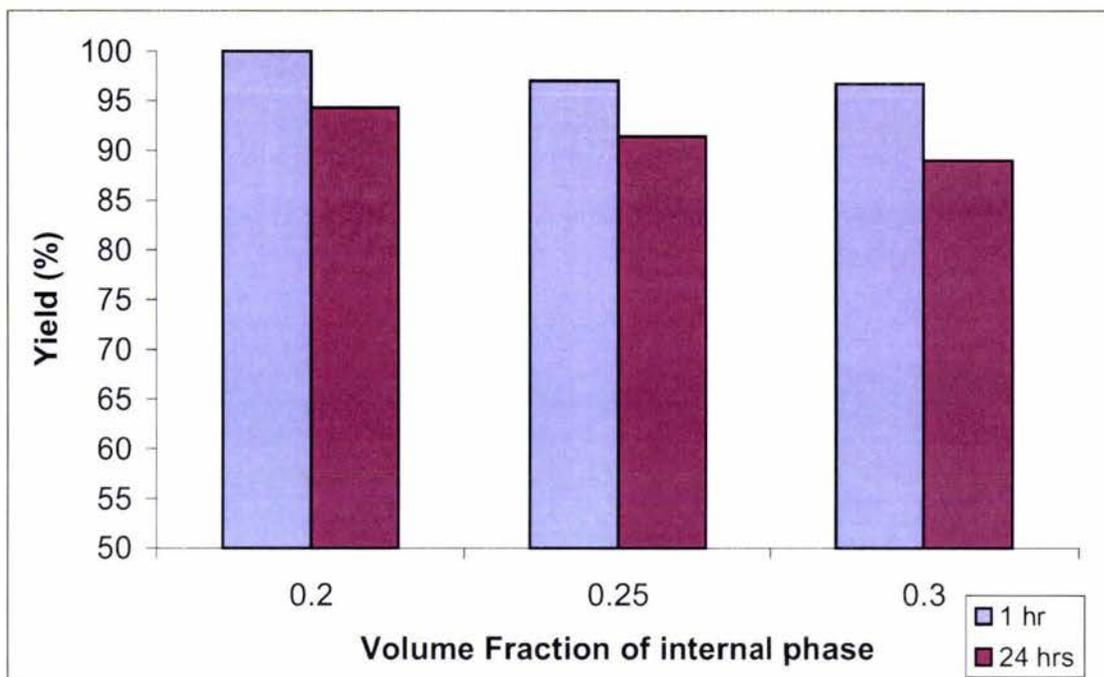


Figure 4.20 Variation in yield with internal phase volume. W/O/W emulsions prepared by varying the inner phase (20%, 25%, 30%) of the w/o emulsion at a homogenisation pressure of 250 bars and at a PGPR concentration of 10% of oil phase. The secondary emulsification was performed with a 2 μm SPG membrane and 1% Tween 80 in the outer phase as the hydrophilic emulsifier. Readings are taken after 1 hr and 24 hrs.

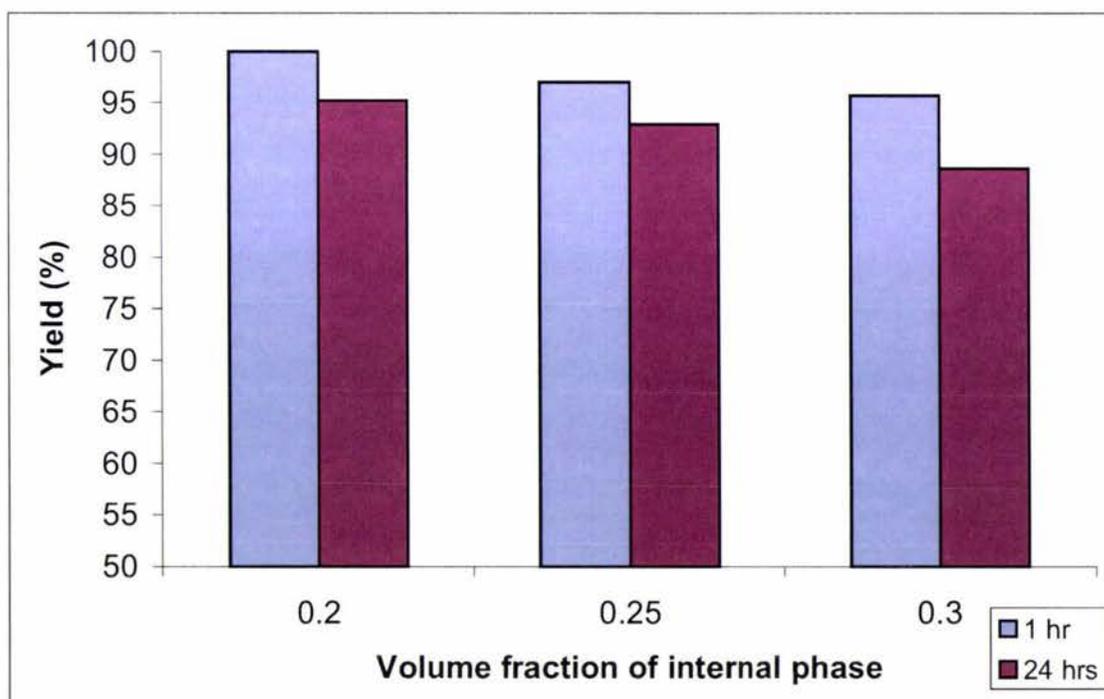


Figure 4.21 Variation in yield with internal phase volume. Primary emulsification (homogenisation) performed at 500 bars. Conditions as in Figure 4.20

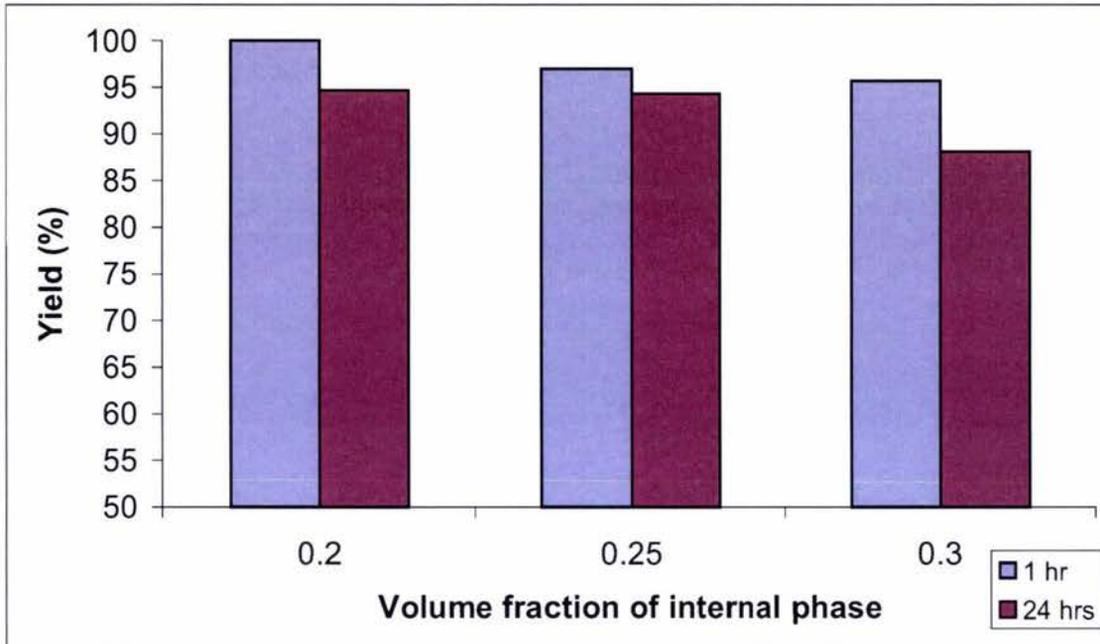


Figure 4.22 Variation in yield with internal phase volume. Primary emulsification (homogenisation) performed at 1000 bars. Conditions as in Figure 4.20

From Figure 4.20, 4.21 and 4.22 it can be seen that there is a noticeable effect of the internal phase volume on the encapsulation efficiency of the multiple emulsion. There is a consistent inverse proportionality between the internal water phase concentration and the yield of the multiple emulsions that did not change much with increasing homogenisation pressure. According to Yan *et al* (2003), increasing the internal phase concentration increases the chemical potential difference between the inner phase and the outer phase. Water permeation from the outer phase to the inner increases, which results in oil layer thinning, and in turn, droplets break-up resulting in reduced yield.

Also the increasing internal phase concentration reduced the thickness of the electric double layer at the interface of the droplets decreasing the stability of the emulsion. As the concentration of the internal phase increases, a greater amount of hydrophobic emulsifier is needed to cover the surface of the additional water droplets. Also the increase in internal phase concentration triggers the dual effect of increase in the number of water droplets formed and also a decrease in the effective amount of hydrophobic emulsifier available to stabilize the surface.

4.4.3.2 Droplet size distribution of W/O emulsions

According to Benichou *et al* (2001) the concentration of water in a w/o emulsion prepared at a particular concentration of hydrophobic emulsifier and a particular homogenisation pressure has a considerable influence on the droplet size of the water droplets formed in the final emulsion. The droplet size of the primary emulsion was of first concern in the preparation of w/o/w emulsions by membrane emulsification as it should be made sure that the water droplets formed as a result of primary emulsification were able to pass through the pores of the 2 μm SPG membrane (the standard membrane used for secondary emulsification) without disruption so that a high yielding w/o/w emulsion was formed. Hence experiments were conducted with varying concentrations of water (10%, 20%, 25%, 30%) in the w/o emulsion to determine the maximum possible concentration (for the best encapsulation) that would give a mean droplet size close to 2 μm .

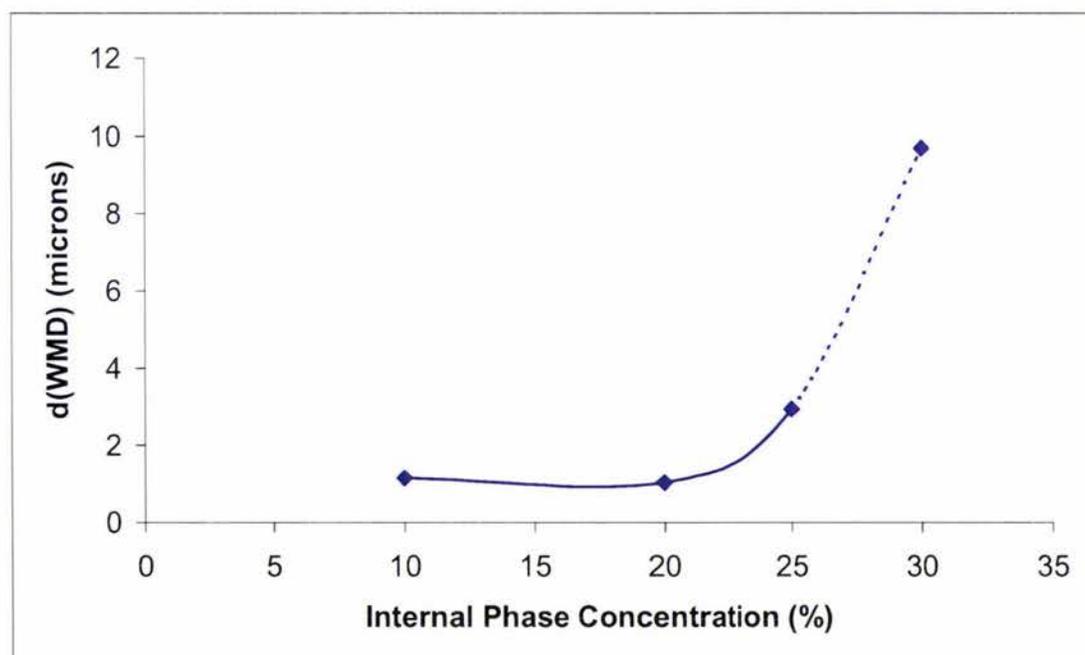


Figure 4.23 Variation in droplet size $d_{(WMD)}$ with internal phase volume. W/O emulsions prepared with various concentrations of water (10 wt%, 20 wt%, 25 wt%, 30 wt%) and 10 wt% PGPR as the hydrophobic emulsifier in the oil phase homogenised at 500 bars. Water phase contains 10% WPC.

From the Figure 4.23 it can be seen that an increase in the internal water phase concentration leads to an increase in the droplet size $d_{(WMD)}$ of water formed in the

w/o emulsion. A possible explanation is as follows: As the concentration of water increases a greater number of water droplets are formed during emulsification, hence the surface area to be covered by the hydrophobic emulsifier also increases. But as the concentration of water increases from 10% to 30% in a w/o emulsion the concentration of oil decreases from 90% to 70%. As the concentration of PGPR (hydrophobic emulsifier) is fixed at 10% in the oil phase, there is also a proportional decrease in the absolute amount of emulsifier, which may result in incomplete coverage of the water droplets formed, possibly leading to coalescence and increase in droplet size. Thus the decrease in yield of w/o/w multiple emulsions with increasing internal phase concentrations explained in section 4.2.3 may be due to the disruption of the higher diameter water droplets while passing through the pores of the 2 μm SPG membrane. Calculations were done to find the apparent thickness of the w/o interfacial layer of PGPR and the results are as shown in the table 4.3 below.

Internal phase concentration	d_{WMD} (weighted droplet diameter) (μm)	* $A = 3V/r$ (interfacial area) (m^2)	N (amt. of emulsifier) (Kg)	$\Gamma = N/A$ (mg/m^2)	ρ (density of PGPR) (Kg/m^3)	$\delta = \Gamma/\rho$ (interfacial thickness of PGPR) (nm)
20 wt%	1.04	577×10^3	8×10^{-3}	0.014	1044.2	0.034
25 wt%	2.94	255×10^3	7×10^{-3}	0.027	1044.2	0.013
30 wt%	9.69	93×10^3	6.4×10^{-3}	0.069	1044.2	0.026

Table 4.3 Table showing the apparent thickness of the PGPR layer at the w/o interface with different internal phase concentrations.

* V – Volume of the internal phase in the w/o emulsion and r is the droplet radius (i.e. $r = d_{WMD} / 2$).

It can be seen that with increase in internal phase concentration and the relative decrease in the amount of PGPR the apparent interfacial area decreased as expected due to the increase in the mean droplet diameter (d_{WMD}). This reduces the apparent thickness of PGPR at the interface due to comparatively lower surface excess. This may in turn contribute to the yield drop due to the easier diffusion offered by the oil layer resulting from lower viscosity.

However the confocal microscope images of w/o emulsions prepared with an inner aqueous phase of 30% and 25% do not show much difference (Figure 4.24 and 4.25).

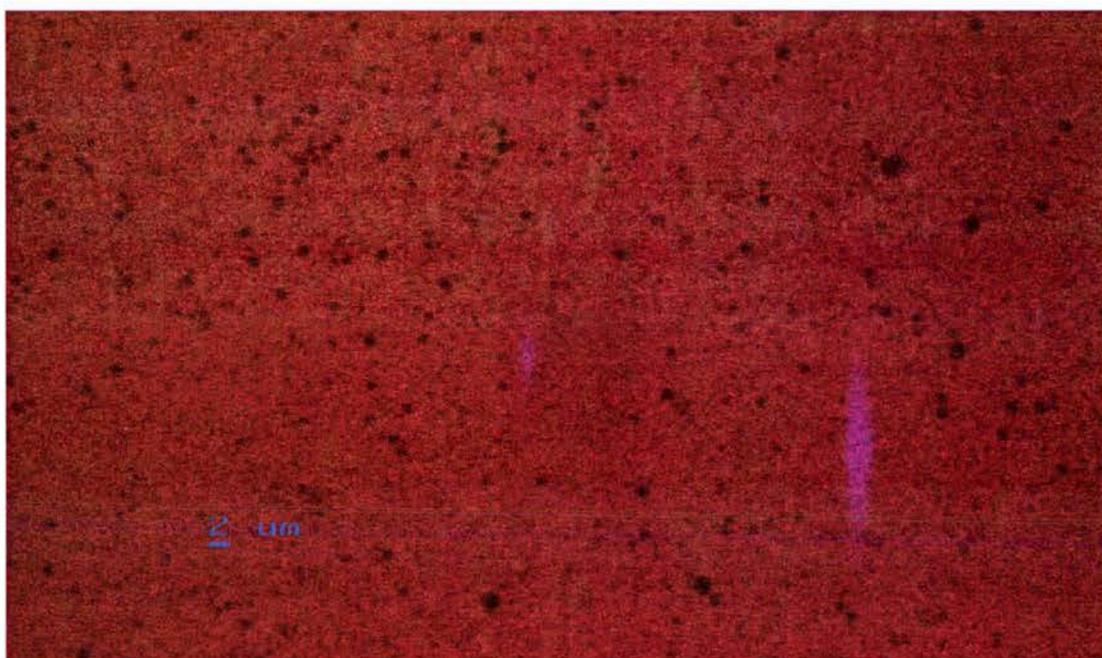


Figure 4.24 Confocal microscope image of a w/o emulsion produced with 30% inner phase and 10% PGPR in the oil phase. The red background is the oil phase and the dark dots are the water droplets.

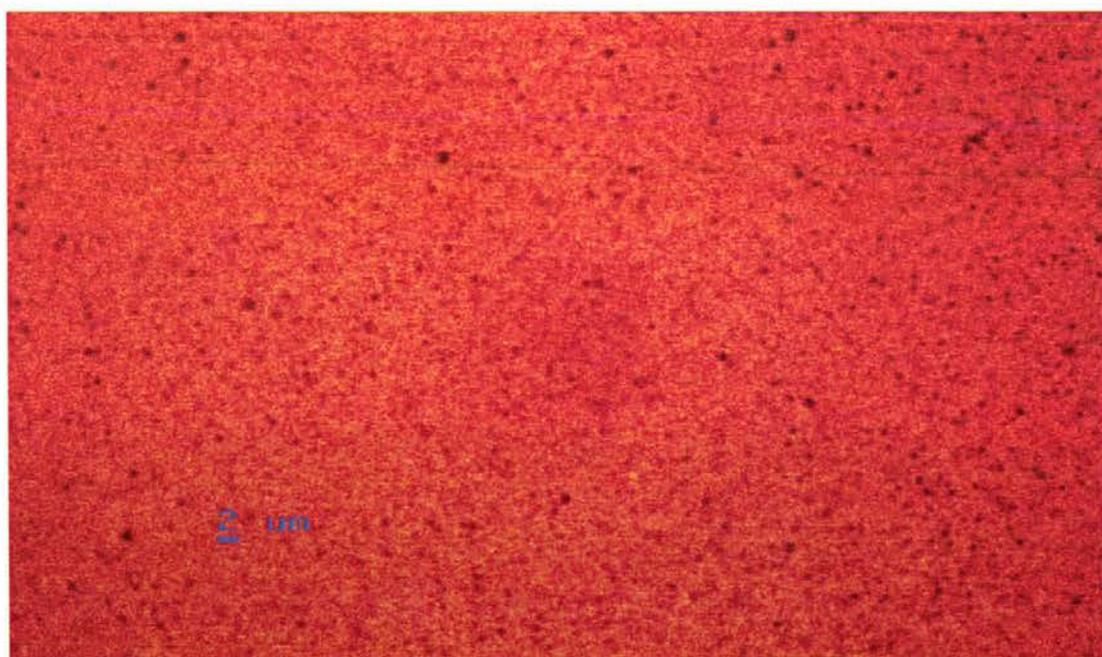


Figure 4.25 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 10% PGPR in the oil phase homogenised at 500 bar. The red background is the oil phase and the dark dots are the water droplets.

The images do exhibit a slight difference, as the droplets in the case of emulsions with 25% inner phase were smaller as expected. But the difference is certainly not as high as indicated by the Ultrasizer (Figure 4.23). This can be due to two reasons: 1)

the theory underpinning the Ultrasizer analysis works well only with lower concentrations of inner aqueous phase (<25%) and above which the quality of fit progressively declines as the multiple scattering effects becomes more important (Coupland & McClements, 2001) or 2) the confocal microscope is able to capture the image at a particular depth of the drop of the sample on the slide giving us an incomplete picture of the whole. As no earlier research has been conducted on the estimation of droplet size of w/o emulsions using the Ultrasizer it is difficult to draw a conclusion regarding these, hence further research need to be conducted in this direction to confirm the findings. A similar case is seen in the w/o emulsions prepared with different hydrophobic emulsifier concentration. The graph (Figure 4.17) shows a sharp decline in droplet size with increasing hydrophobic emulsifier concentration.

However apart from the three instances of poor agreement between the confocal images and the Ultrasizer measurements, there is considerable agreement between the two in all other cases. Hence further research is highly recommended to better understand the process.

Figure 4.26 shows a typical output as obtained from a Malvern Ultrasizer. The graph is bimodal in nature with two peaks. The $d_{(WMD)}$ was calculated by taking the weighted mean of the droplets under either peaks in the distribution (Refer 3.4.1).

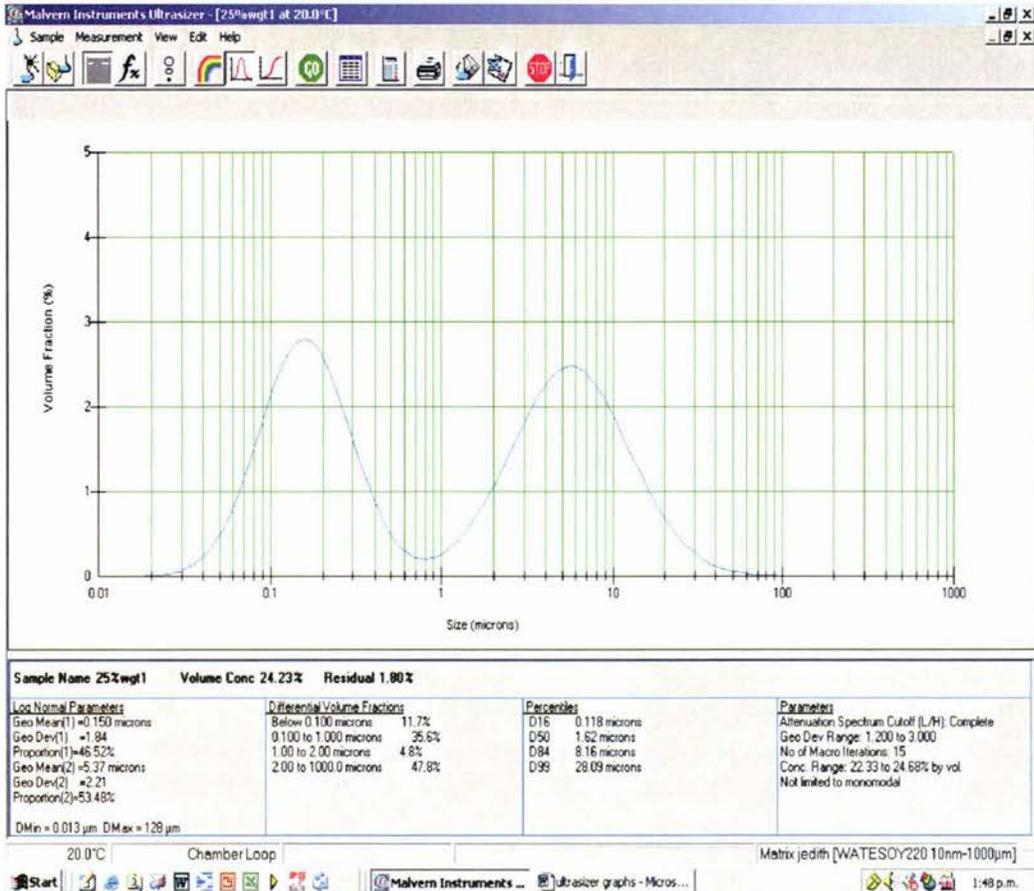


Figure 4.26 A typical output of the droplet size distribution of a w/o emulsion produced with 25% aqueous phase, 10% PGPR in the oil phase and homogenised at 500 bar (2 passes) measured with a Malvern Ultrazizer.

4.4.3.3 With Span 80 as the hydrophobic emulsifier and primary emulsification by the Ultra-Turrax

Experiments were conducted in the initial stages with Span 80 as the hydrophobic emulsifier and Ultra-Turrax as the primary emulsification step to identify the optimum internal phase concentration for the production of a high yielding stable w/o/w multiple emulsions. Hence the primary emulsions were prepared by Ultra-Turraxing the various concentrations of water phase (5%, 10%, 25%, 50%) at 20,500 rpm for 5 minutes with 10% (w/w) Span 80 in the oil phase.

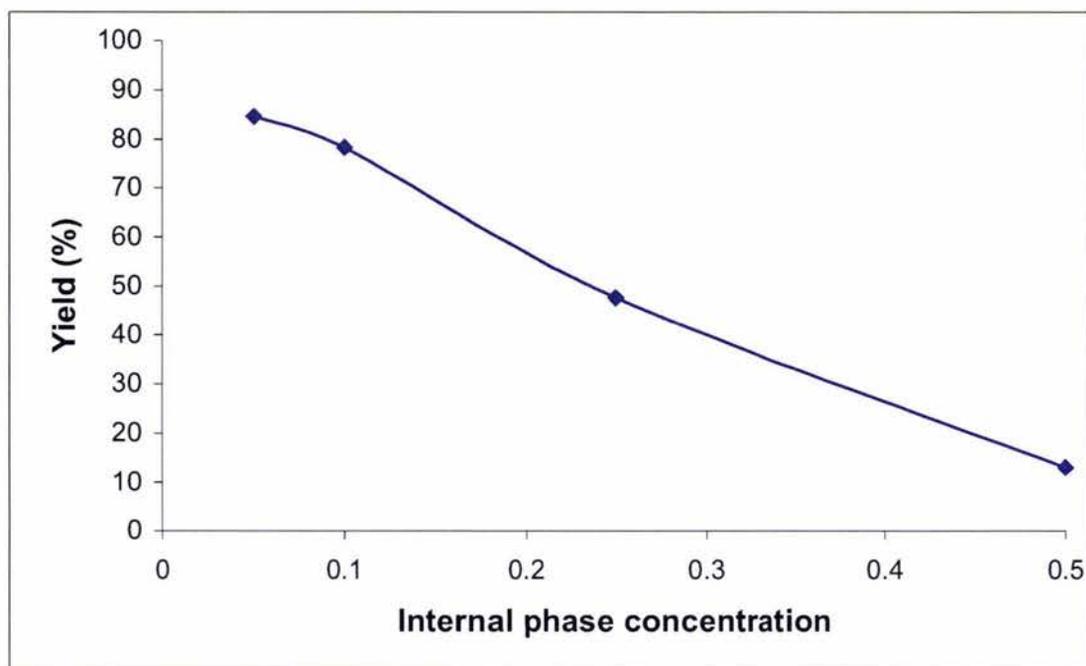


Figure 4.27 Variation in yield with internal phase concentration. W/O/W emulsions prepared by varying the inner phase (5%, 10%, 25%, 50%) of the w/o emulsion and at a Span 80 concentration of 10% of oil phase. The Primary emulsification is done at 20500 rpm with an Ultra-Turrax and the secondary emulsification was performed with a 2.1 μm Ceramic membrane and 1% Tween 80 in the outer phase as the hydrophilic emulsifier. Readings taken after 1 hr.

The results obtained were similar to those with PGPR as the hydrophobic emulsifier. It was found that the yield consistently decreased from 84.5% to 13% on increasing the volume fraction of water in the internal phase from 0.05 to 0.5. The reason for this type of behavior is explained in detail above.

4.4.4 Effect of temperature on droplet size distribution of W/O emulsion

Several researchers (e.g. Joscelyne & Tragardh (2000), Katoh *et al* (1997)) have reported on the positive effects of elevated temperatures during emulsification. The former attributed this to the fluidisation of the oil phase and the dissolution of the emulsifier whereas the latter suggested the reduced viscosity of the continuous phase may lead to the water droplets formed being smaller in size. Hence experiments were conducted at two different temperatures to investigate the effect of temperature on the droplet size of the w/o emulsions formed. W/O emulsion samples were prepared at

room temperature (22°C) and at an elevated temperature of 50°C, which is commonly employed in other dairy homogenisation applications.

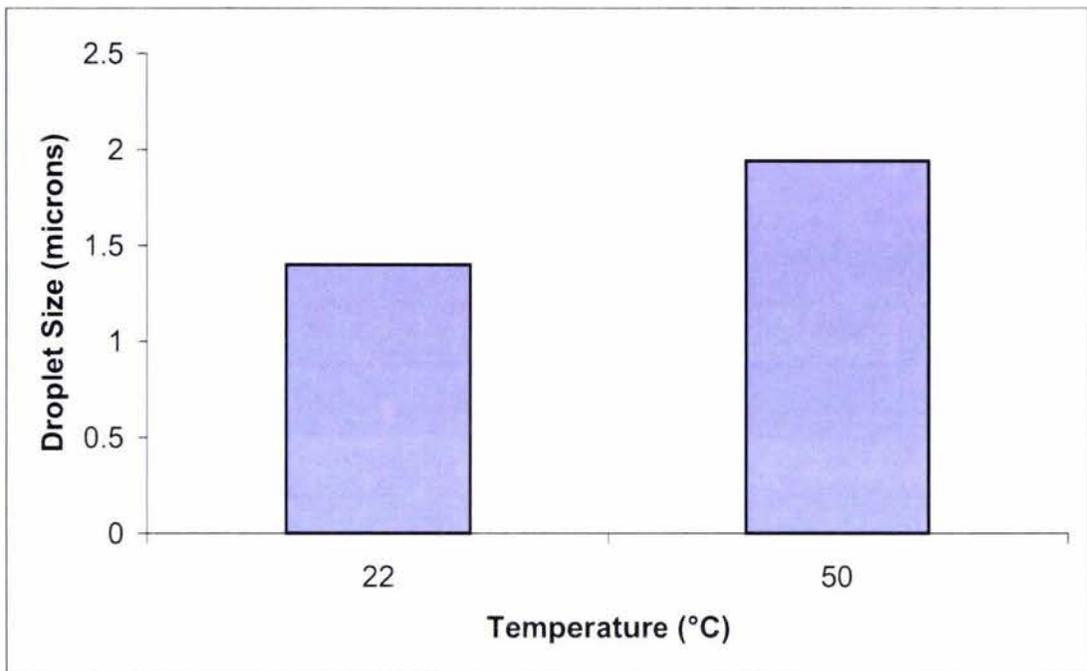


Figure 4.28 Variation in droplet size with temperature of formation. W/O emulsions prepared at 22°C and 50°C with 25% water and 10% hydrophobic emulsifier (PGPR) in the oil phase homogenised at 500 bars. Water phase contains 10% WPC.

The results obtained were contradictory to the results of the other researchers. It was found that the w/o emulsion produced at room temperature possessed a smaller mean diameter than the w/o emulsion produced at 50° C. Confocal microscope images also gives a similar result (Figure 4.29 and 4.30).

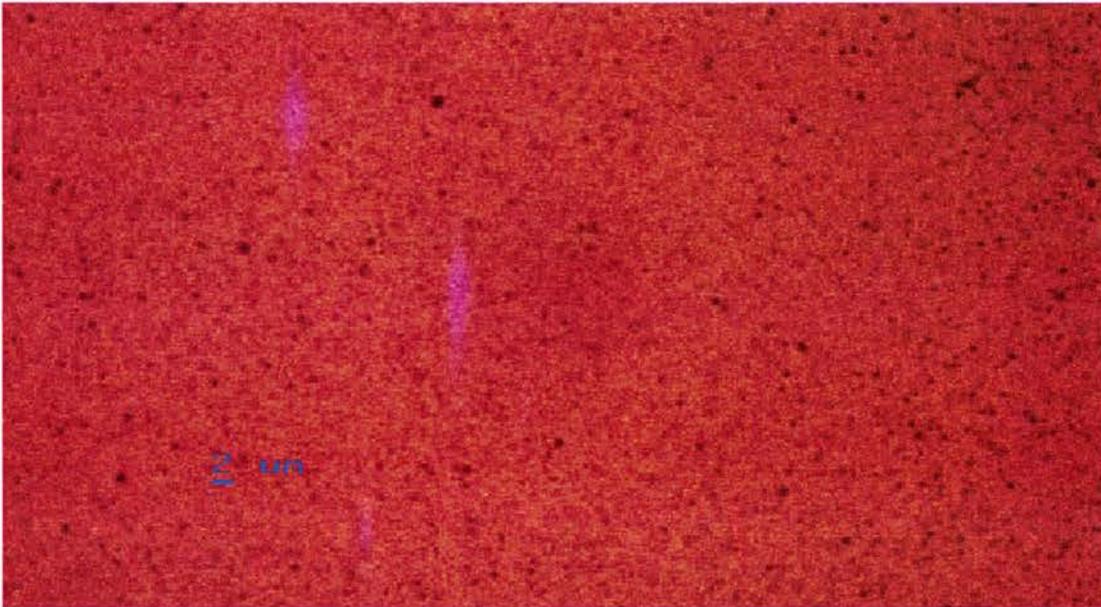


Figure 4.29 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 10% PGPR in the oil phase homogenised at 500 bar and 22°C. The red background is the oil phase and the dark dots are the water droplets.

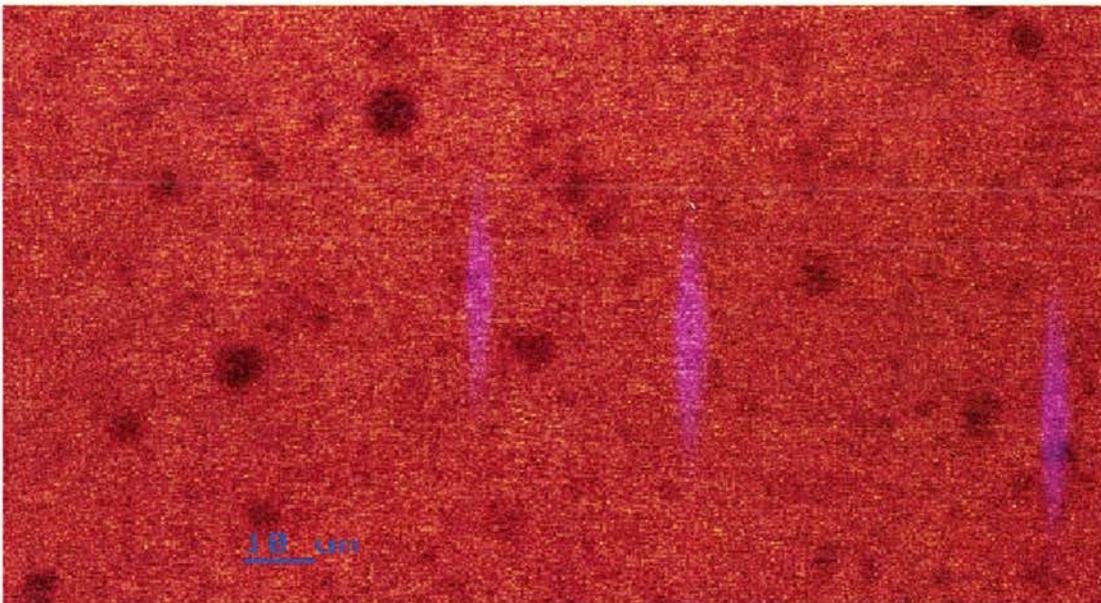


Figure 4.30 Confocal microscope image of a w/o emulsion produced with 25% inner phase and 10% PGPR in the oil phase homogenised at 500 bar and 50°C. The red background is the oil phase and the dark dots are the water droplets.

Further research need to be done to find out the exact explanation for this behaviour and to determine if an optimum temperature exists for the formation of primary w/o emulsion using PGPR.

4.5 Conclusion

The chapter mainly deals with the effect of primary emulsification conditions on the yield stability of w/o/w emulsions and also on the droplet size of w/o emulsions. Investigations were conducted on the effects of primary emulsification pressure, hydrophobic emulsifier type and concentration, internal phase concentration and also on the temperature of processing during primary emulsification by high pressure (valve homogenisation).

Primary emulsification pressure variations (250/50 bar, 500/100 bar, 1000/200 bar) did not show much effect on the yield stability of w/o/w emulsions. The initial yield stabilities exhibited by all the samples were similar and high (>95%), but decreased on storage. However when the hydrophobic emulsifier was reduced to 2.5% of the oil phase it was found that the initial yield stabilities increased with primary emulsification pressure. The droplet size estimation of w/o emulsions with the Ultrasizer as well as the confocal microscope showed a considerable agreement in the first two cases (250 bar, 500 bar), but at a 1000 bar primary emulsification pressure the Ultrasizer results showed an increase in mean droplet size whereas the confocal results showed a finer distribution of droplets.

Hydrophobic emulsifier concentration experiments conducted proved that PGPR at a concentration of 10 wt% of the oil phase is a better emulsifier than Span 80 at the same concentration. Further experiments conducted with mixtures of PGPR and Span 80 confirmed the better emulsifying capacities of PGPR as it was found that increasing the concentration of PGPR in the mixture increased the long-term stability of the w/o/w emulsions. Studies on the various concentrations of hydrophobic emulsifier proved that decreasing the hydrophobic emulsifier concentration from 10% to 2.5% decreased the yield stabilities of the w/o/w emulsion. The droplet size estimation of w/o emulsion with the Ultrasizer showed a decreasing trend in droplet size with increase in PGPR concentration. The confocal images also showed a similar trend with fine droplet size distribution in the case of emulsions produced at all PGPR concentrations. Thus there was a slight disagreement in the comparative results of Ultrasizer and confocal microscope images of w/o emulsions produced with 2.5%

PGPR concentrations. Viscosity changes in w/o/w emulsions prepared with PGPR need to be investigated in detail in future for further insight into the effect of viscosity on the yield stability and droplet size determination.

Experiments were conducted by varying the internal aqueous phase concentrations and it was found increasing the internal phase concentration decreased the yield stability of the w/o/w emulsions. The loading capacity or the amount of WPC to be encapsulated can be maximised only if the internal phase concentration is maximum. Droplet size estimation with the Ultrasizer proved that the droplet size of w/o emulsion decreased with decreasing internal aqueous phase concentration. There was a considerable increase in the mean droplet size of w/o emulsion produced with 30% inner phase over the one produced with 25% inner phase. But the difference was not so pronounced in the case of confocal images.

The droplet size estimation of w/o emulsions produced at different temperatures (22°C and 50°C) during primary emulsification with the Ultrasizer was in agreement with that of the confocal images. It was found that the droplet size of w/o emulsions was smaller and more optimal when the emulsion was produced at room temperature rather than at elevated temperatures.

In conclusion, the primary emulsification conditions selected as the optimum were 25% inner phase, 10 wt% PGPR in the oil phase and homogenisation pressure of 500 bar/100 bar at 22°C. The estimation of w/o droplet size with the Ultrasizer, as stated earlier, is a relatively new technique and should be further researched for confirmation. However efforts were made in the current research to provide the best possible results by comparing the Ultrasizer results with the confocal microscope images.

5. Influence of Secondary Emulsion Composition and Process Conditions on W/O/W Emulsion Properties

5.1 Introduction

According to Garti (1997), the hydrophilic emulsifier has an influence on the flux of water from the inner phase to the outer in a w/o/w emulsion: a higher emulsifier concentration solubilises more water and its transport in and out, controlled by the osmotic pressure, is enhanced. Matsumoto (1986) reported that the main factor affecting the formation of w/o/w emulsions by the two-stage process is the ratio of the hydrophobic to the hydrophilic emulsifier. Matsumoto (1986) and Wen & Papadopoulos (2000) reported that an excess of hydrophilic emulsifier (>1wt%) could cause greater solubilisation of the hydrophobic emulsifier leading to the breakage of the oil layer leading to release of encapsulated contents. According to research conducted by Magdassi *et al* (1985), higher yields were obtained with lower concentrations of hydrophilic emulsifiers (preferably 1 wt%) and emulsifiers with a lower HLB were preferred to those with a higher HLB. It has also been reported by Omotosho and co-workers (1986) that there was a correlation between the nature of oil and the hydrophobic emulsifier used in the formation of the w/o emulsion and that the selection of the oil should be done according to the size and stability of the internal droplets. However the research conducted by Benichou *et al* (2001) proved that soybean oil in combination with PGPR as the hydrophobic emulsifier is capable of producing very fine emulsions with an extremely small droplet size that were stable for months after preparation. Also in the preparation of a stable w/o/w emulsion the selection of the pore-size of the membrane and the water droplet concentration of the inner w/o emulsion is very important (Mine *et al*, 1996).

Clearly, several factors affects the production of w/o/w emulsions and the yield and droplet size of the emulsions so formed depend to a greater extend on the optimisation of these factors. The major focus of the research reported in this chapter was to investigate several of these factors for the production of multiple emulsions with membrane emulsification as the secondary emulsification step.

5.2 Objectives

- To study the effect of temperature of processing, hydrophilic emulsifier type and osmotic pressure gradients between the inner and outer aqueous phases on the yield stability, droplet size and physical stability or creaming stability of the w/o/w emulsion.
- To study the maximum loading capacity of the inner phase, i.e. the maximum amount of WPC that can be encapsulated in the inner aqueous phase of the w/o/w emulsion, and its effect on the yield stability and droplet size of the emulsions produced.

5.3 Experimental

The emulsions were prepared with reverse osmosis water as the inner and outer aqueous phase. The oil phase used consisted of soybean oil purchased from Davis Trading, Palmerston North, New Zealand. The inner aqueous phase consisted of Glucose (6.4wt%) as the marker and WPC (ALACEN 3423) as the encapsulated material.

Primary (W/O) Emulsion

The hydrophobic emulsifier used was PGPR (Palsgaard 4125), Palsgaard A/S, Denmark at a standard concentration of 10% of oil phase unless specified otherwise. The primary (w/o) emulsions were prepared with valve homogenisers (APV 2000, Invensys APV Products, Denmark and Rannie homogeniser (2-stage), Rannie A/S, Denmark) and homogenisation was performed at 500 bar/100 bar standard pressure.

Secondary (W/O/W) Emulsion

The secondary (w/o/w) emulsions were prepared with SPG membranes (SPG Technology Co. Ltd, Japan) of standard pore size 2 μm . However SPG membranes with pore-sizes of 1.4 μm and 3.8 μm were also used. The hydrophilic emulsifier tried in the outer aqueous phase were Tween 80 (P-8074; Sigma Chemical Company, USA), whey protein isolate (ALACEN 895) and sodium caseinate (ALANATE 180; New Zealand Milk Products, New Zealand), soy protein isolate (FP 940; Columbit

NZ Ltd, New Zealand). The w/o/w emulsions were prepared at different temperatures by circulating hot water through the jacket during the entire production process as shown in section 3.2.1.

The yield stability measurements were done using an Advantage glucose meter (Roche Diagnostics, New Zealand). The droplet size measurements were conducted using a Malvern Mastersizer (Malvern Instruments, UK). The physical stability tests were conducted as specified in section 3.5.

5.4 Results and Discussion

5.4.1 Effect of temperature

Viscosity is directly related to the temperature of a system. The inverse proportionality of viscosity with temperature is one factor that can be conveniently employed for improving the flow conditions during emulsification. According to Joscelyne & Tragardh (2000), w/o emulsions are usually prepared at higher temperatures (65° C) so that the oil phase is made more fluid and dissolution of the emulsifier is made easier. Studies conducted by Katoh *et al* (1997) found that increasing the viscosity of the continuous phase in w/o emulsion formation resulted in an increase in the droplet size and reduction in the dispersed phase flux.

Experiments were conducted to find out the effect of temperature on the yield and stability of w/o as well as w/o/w emulsions (Table 5.1 and Figure 5.1).

Sample Name	Primary Emulsification Temperature (°C)	Secondary Emulsification Temperature (°C)
Sample A	22	22
Sample B	50	22
Sample C	22	35
Sample D	22	50
Sample E	50	50

Table 5.1 Table showing the details of samples in the Figure 5.1

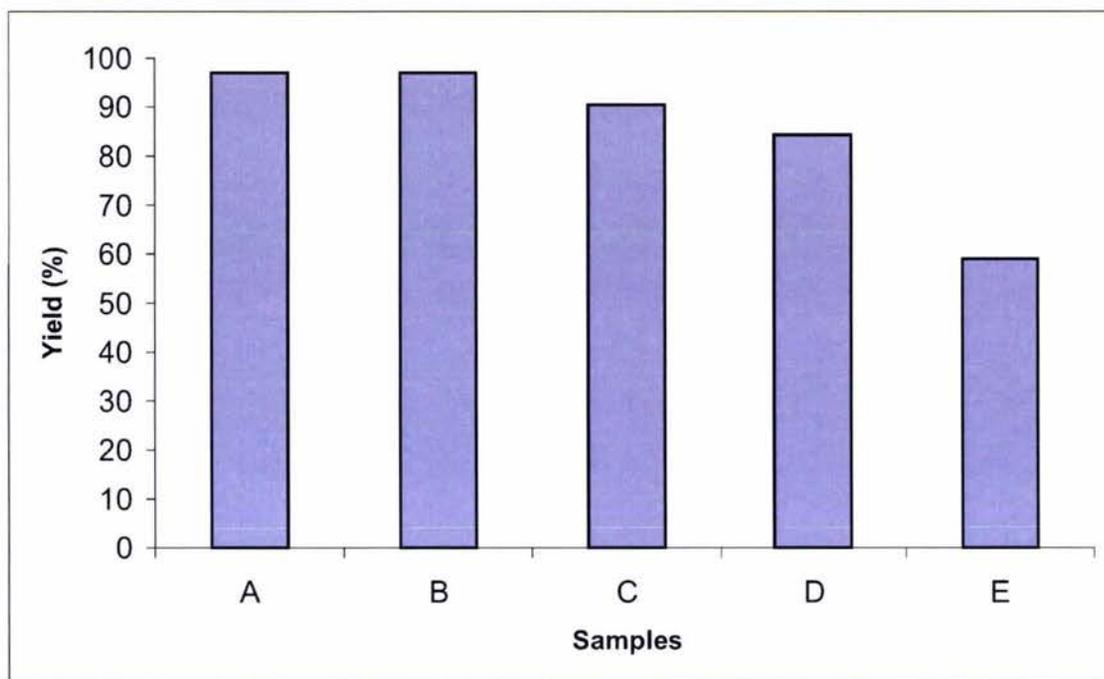


Figure 5.1 Variation in Yield with temperature of formation. W/O/W emulsions produced with 25% inner phase and 10% PGPR, homogenised at 500bar (primary emulsion). Secondary emulsions are produced with 1.4 μ m of SPG membranes. 1% Tween 80 was used as the hydrophilic emulsifier in the external phase. Sample details are specified in Table 5.1.

It is evident from the Figure 5.1 that an increase in temperature, especially in the secondary emulsification step, has a marked negative effect on the yield of the multiple emulsions. Dickinson *et al* (1993) reported a decrease in yield of w/o/w multiple emulsions with increase in storage temperature of both w/o primary emulsions as well as w/o/w multiple emulsions. The decrease in yield with increasing temperature may also be due to the lowering of the HLB value of Tween 80 due to the rise in temperature, favouring the formation of a w/o emulsion rather than o/w emulsion (Dickinson *et al*, 1993).

5.4.1.1 Droplet Size Distribution

As noted, researchers such as Joscelyne & Tragardh (2000) have advocated the preparation of emulsions at a higher temperature. Hence experiments were conducted to find out the effect of temperature on the yield stability and droplet size of the w/o/w multiple emulsions. Accordingly both primary w/o emulsions and secondary

w/o/w emulsions were produced at different temperature and analysed for droplet size (Section 4.4.4. Also see Table 5.1 and Figure 5.2).

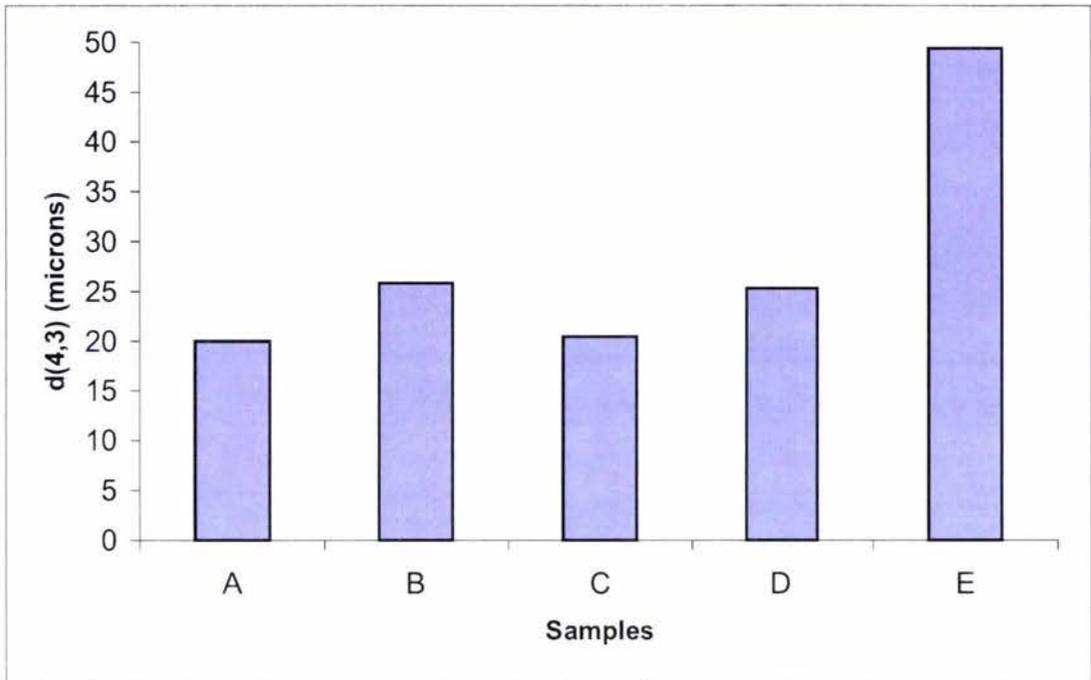


Figure 5.2 Variation in droplet size $d_{(4,3)}$ with temperature of formation. W/O/W multiple emulsion prepared with 25% inner phase and 10% PGPR in the primary w/o emulsion homogenised at 500 bar (2 passes) and hydrophilic emulsifier (1% Tween 80) in the outer phase for secondary emulsification with a membrane of pore size 2 μm . The temperatures of preparation are as described in Table 5.2.

Figure 5.2 indicates that the largest droplets were formed when both the primary (w/o) and the secondary (w/o/w) emulsions were produced at 50° C. This explains the lowest yield exhibited by these emulsions. Considering samples A, C and D we can see that there is a gradual increase in droplet size ($d_{(4,3)}$) from 20 μm to 25 μm , which shows that the increase in temperature during the secondary emulsification step leads to an increase in the droplet size. Considering samples B and E, it can be concluded that the temperature increase in the primary emulsification step has a major influence in determining the droplet size of the final emulsions. This however demands further work for optimisation.

5.4.2 Effect of Hydrophilic Emulsifiers

Even though the majority of the experiments in the study were conducted with Tween 80 as the hydrophilic emulsifier, attempts were made to evaluate natural emulsifiers as alternatives. Accordingly three types of proteins were selected - whey protein isolate (WPI), soy protein isolate (SPI) and sodium caseinate. The emulsification capacities of the three proteins were compared with that of Tween 80. All the proteins were used at a concentration of 0.5wt% in the outer water phase to avoid depletion flocculation (Srinivasan *et al*, 2000) for the experiment and Tween 80 in the usual 1wt% concentration. The w/o/w emulsions so formed were stored both at room temperature and at 5 °C for determination of yield with time.

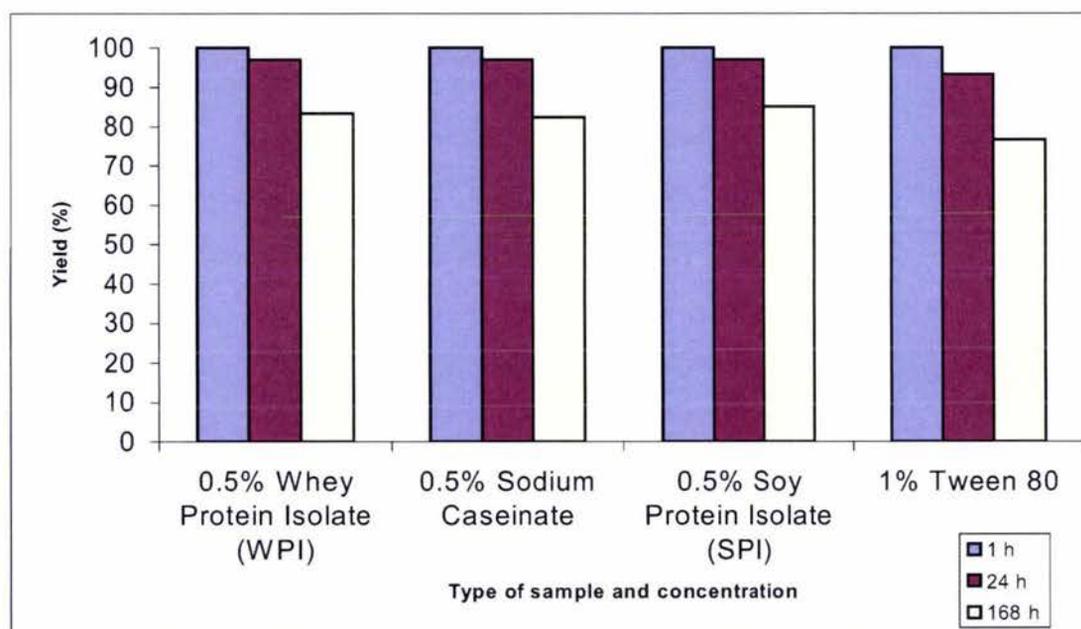


Figure 5.3 Variation in yield with different hydrophilic emulsifier concentration. W/O/W multiple emulsion prepared with 25wt % inner phase and 10wt% PGPR in the primary w/o emulsion homogenised at 500 bar (2 passes) and different hydrophilic emulsifiers (0.5wt% WPI, 0.5wt% sodium caseinate, 0.5wt% SPI, 1wt% Tween 80) in the outer phase for secondary emulsification with a membrane of pore size 2 μm and stored at 5°C.

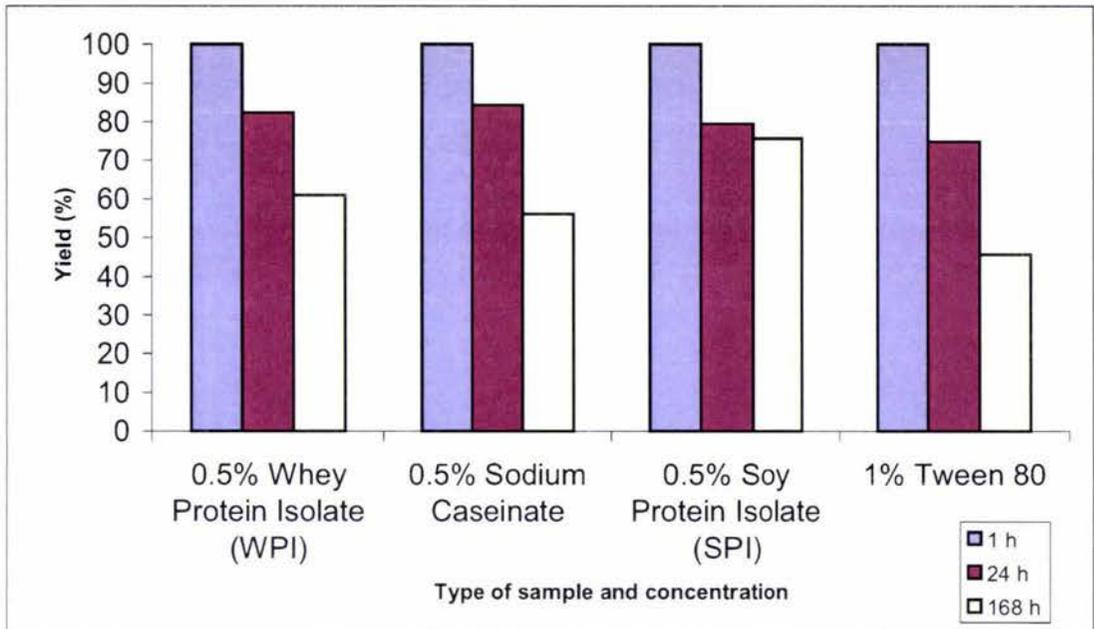


Figure 5.4 Variation in yield with different hydrophilic emulsifier concentration stored at room temperature (22°C). Conditions as in Figure 5.3.

From Figures 5.3 and 5.4 it can be seen that there was not much difference in the yield of the emulsions stored at 5 °C. However on long-term storage, it can be seen that all the protein-stabilized emulsions behaved similarly, but the Tween 80 stabilized emulsions showed a lower yield on storage. This effect was more pronounced in the case of Tween 80 stabilized emulsions stored at room temperature and suggests that for food applications, a natural protein may be preferred. The sample with SPI in the outer phase exhibits a better yield in terms of long term storage. This may be due to the better association of soy proteins with the soybean oil used as the oil phase. However a proper explanation cannot be provided at this point and further research should be done for confirmation.

5.4.2.1 Droplet Size Distribution

Experiments were conducted with Tween 80 (1wt%), sodium caseinate (0.5wt%), SPI (0.5wt%) and WPI (0.5wt%) as hydrophilic emulsifiers to evaluate the difference in droplet sizes as a result of replacing Tween 80 with proteins as emulsifiers. Figure 5.5 shows the variation in droplet size with different hydrophilic emulsifiers and this suggest that Tween 80 may have an advantage in this respect.

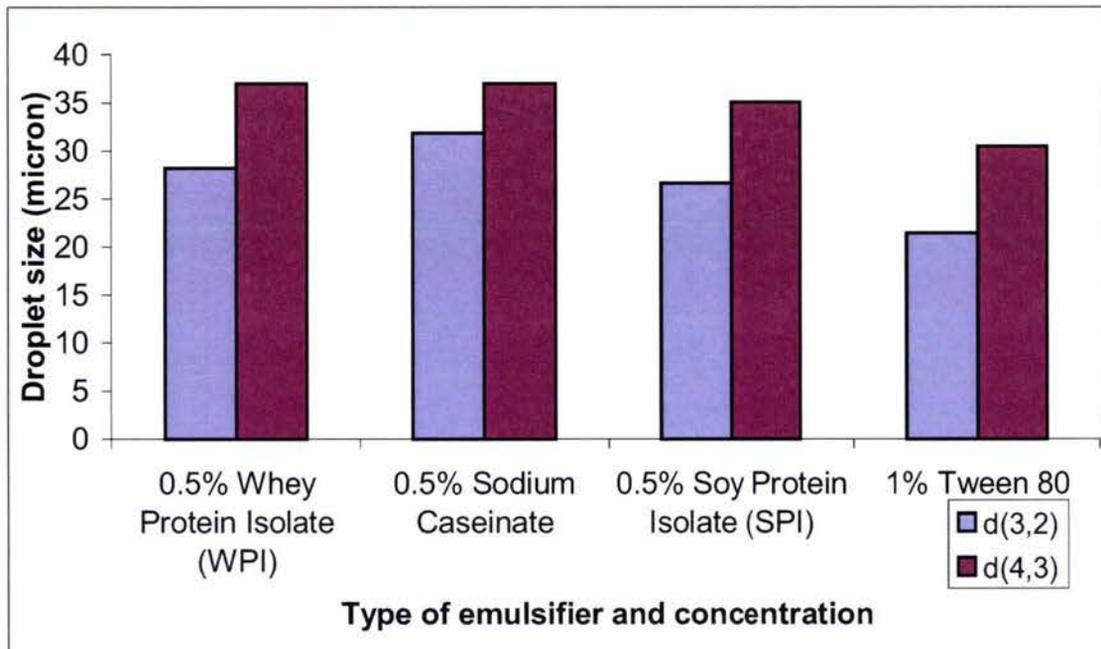


Figure 5.5 Variation in droplet size with different hydrophilic emulsifiers. W/O/W multiple emulsion prepared with 25 % inner phase and 10% PGPR in the primary w/o emulsion homogenised at 500 bar (2 passes) and different hydrophilic emulsifiers (0.5% WPI, 0.5% sodium caseinate, 0.5% SPI, 1% Tween 80) in the outer phase for secondary emulsification with a membrane of pore size 2 μm .

Considering the stability with respect to creaming, Tween 80 stabilised emulsions were more stable than the protein stabilised emulsions (results not shown). This may be due to the fact that Tween 80 adsorbs more quickly to an oil-water interface than most proteins, in turn reducing the chances of droplet flocculation immediately after formation. Similar results were reported by Einhorn-Stoll *et al* (2002). Even though the cream layers formed for the four types of emulsions have the same height, the serum layer formed for Tween 80 stabilised emulsions are more opaque than the serum layers formed for the protein stabilised emulsions (data not shown).

The opacity of the Tween 80 stabilised emulsions can be explained by the comparatively smaller droplet size of the Tween 80 stabilised emulsions than the protein-stabilised emulsions. The smaller $d_{(3,2)}$ and $d_{(4,3)}$ values of the Tween 80 emulsions were also probably due to the faster adsorption effect of the emulsifier. The faster an emulsifier adsorbs at the interface the smaller the droplet size and also smaller are the chances of droplet coalescence especially during droplet formation (Schroder *et al*, 1998). The slight increase in the droplet size of sodium caseinate stabilised emulsions when compared with WPI and SPI stabilised emulsions may be

due to the lower protein content in sodium caseinate than WPI and SPI. This may lead to lesser surface coverage and in turn larger droplet size. The Mastersizer plots obtained had residual values in the range of 3-4%.

5.4.3 Effect of Osmotic Pressure

One of the most striking features of w/o/w emulsions is the susceptibility of the droplets towards rapid shrinking or swelling in response to an osmotic gradient (Dickinson & McClements, 1996). If the osmotic pressure is higher in the internal aqueous phase, water will pass into this phase resulting in swelling and eventual rupture of the internal droplets with consequent release of their contents into the external phase. Conversely, if the osmotic pressure in the internal aqueous phase is lower then water will be transferred from the internal to the external phase causing shrinkage of the internal droplets and destabilization of the system (Jiao *et al*, 2002). The globule size and size distributions of w/o/w emulsions may change during globule size analysis when an isotonic condition does not exist between the two droplets (Matsumoto, 1986). Research conducted by Omotosho *et al* (1986) proved the existence of the osmotic gradient as they observed the bulging of oil droplets and the thinning of the oil membrane by the influx of water from the outer aqueous phase due to the osmolarity difference created by the entrapped NaCl (used as a yield marker), but the w/o/w emulsions were stable for long periods of time. The diffusion rates can be altered by changing the nature of the oil, increasing its viscosity and adding various carriers (Garti, 1997). Usually there are two mechanisms by which water permeates through the oil layer: reverse micellar transport and diffusion across a very thin lamella of the surfactant formed where the oil layer is very thin (Garti, 1997).

5.4.3.1 W/O/W Emulsions prepared with Ultra-Turrax

The primary emulsions were prepared with 30wt% water in the inner phase and 10wt% PGPR as the hydrophobic emulsifier. The emulsification was carried out by means of an Ultra-Turrax (Heidolph Diax 600) at 20500 rpm for 20 minutes. The secondary emulsions were prepared with 20wt% primary emulsion and 1wt% Tween

80 as the hydrophilic emulsifier in the external phase. The emulsification was done at 8000 rpm for 3 minutes.

There was a clear separation of the two phases within an hour after the preparation of the multiple emulsions. But when kept overnight at room temperature it was observed that the emulsion turned white and viscous with no separation. This may be due to the movement of water from the outer phase to the inner phase through the oil membrane according to the concentration gradient, i.e. the oil layer may be acting as a semi-permeable membrane allowing the transport of water across it. This in turn increases the size of the oil droplets making them occupy a greater volume fraction of the w/o/w multiple emulsions thus increasing the viscosity of the whole emulsion. Research conducted by Kanouni *et al* (2002) proved that PGPR (hydrophobic emulsifier) possesses a high elasticity and surface potential when bound to the oil-water interface and forms stable, compressible and reversibly expandable monolayers. Also the surfactant molecules lie flat on the o/w interface resulting in the high degree of elasticity of the interfacial film making it highly resilient under stress. This capacity of the PGPR molecules to withstand stress could contribute to the expansion of the oil globules due to osmotic influx of water without its breakage thereby increasing the apparent oil phase volume concentration in the w/o/w emulsion. This in turn affects the emulsion rheology by increasing the viscosity of the emulsion. However in all cases the emulsions showed very high yield of 100% for the first few days. This may be again due to the high resilience of the interfacial film, which does not break under stress and so contributes stability to the emulsion. It was also observed that the rapidity of the water transport across the oil layer depended on the concentration of the glucose (marker) used in the inner phase. This was similar to the findings of Matsumoto & Kang (1989) and can be explained by to the increased osmotic pressure exerted when an excess amount of glucose was used.

To check the osmotic effect on the w/o/w emulsions two samples of w/o/w multiple emulsions were prepared: - one with no marker (glucose) in the inner phase and the second with sorbitol in the outer phase. The water activity of inner phase and outer phase was noted to check the osmotic gradient and was found to be equal. The results are shown in Table 5.2.

Compound	Amount	Water Activity	Temp
Glucose	*1.6g in 25gwater	0.993	24.6
Sorbitol	† 22g in 400g water	0.993	25.6
Sodium Chloride	† 3.6g in 400g water	0.992	26.3

Table 5.2 Table showing the water activities of different solutes at different concentrations measured with a water activity meter.

* corresponds to internal water phase of the w/o/w emulsion

† corresponds to external water phase of the w/o/w emulsion

The precision of the water activity meter (Decagon) is + or – 0.003. Hence the readings were quite consistent.

Omotosho *et al* (1986) in their experiments balanced osmotic pressure using the equation 5.48% Sorbitol = 0.9% NaCl. The equivalent amount of glucose, sorbitol and NaCl to adjust the osmotic pressure on either sides of the oil membrane was also calculated from the equation

$$\Pi = \beta n R T \quad \dots\dots\dots (5.1)$$

where Π is the osmotic pressure, β is the number of hydrolysable ions, n is the molarity, R is the universal gas constant and T is the temperature.

It was observed that there was no increase in viscosity of the w/o/w multiple emulsions in both cases. On storage the emulsions appeared as two distinct cream and water layers and remained the same throughout. This confirms that an osmotic gradient existed and there was a transfer of water from outer phase to the inner phase through the oil layer.

The decrease in yield with time in the case of w/o/w emulsions produced with the Ultra-Turrax is shown in Figure 5.6.

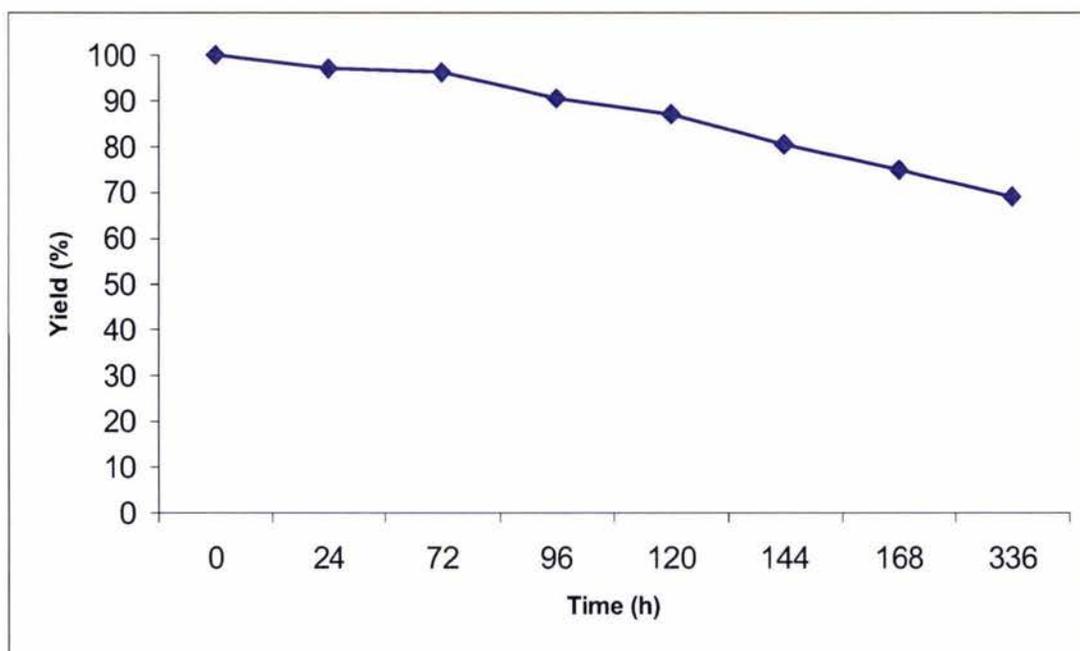


Figure 5.6 Yield decrease with time in w/o/w emulsions produced with Ultra-Turrax. W/O/W emulsions are produced with 30wt% water in inner phase, 10wt% PGPR, Ultra-Turraxed at 20,500 rpm for primary emulsification and then at 8,000 rpm for secondary emulsification. Readings taken at 0,24,72,96,120,144,168 and 336 hours after emulsion preparation.

5.4.3.2 W/O/W Emulsions prepared with membrane

Initially it was thought that there was little osmotic effect when the w/o/w emulsions were prepared with the membrane. This was because the effect was not as pronounced as in the case of w/o/w emulsions prepared with the Ultra-Turrax. Unlike the latter, the emulsions prepared with the membrane showed a distinct cream layer and there was no thickening of the emulsion on storage. On further reflection it was postulated that this did not show the absence of the osmotic effect, but rather a decreased effect when compared with the emulsions prepared with the Ultra-Turrax, possibly due to the smaller droplet size of the emulsions prepared with the membrane.

Experiments were therefore conducted to study the effect of osmotic pressure on the droplet size and yield of the w/o/w emulsions. The primary emulsions were prepared with 25wt% inner phase containing 6.4wt% glucose as the marker and 10wt% PGPR in the oil phase. The primary emulsions were pre-emulsified and homogenized at 500/100 bars. The secondary emulsions were prepared with a 2 μ m SPG membrane

and the external water phase consisted of 1wt% Tween 80 as the hydrophilic emulsifier. A range of concentrations of sorbitol was added to the outer phase to adjust the osmotic pressure so as to obtain a minimum droplet size (Figure 5.9) and maximum yield stability (Figure 5.7). The final w/o/w emulsions consisted of 20wt% primary emulsion.

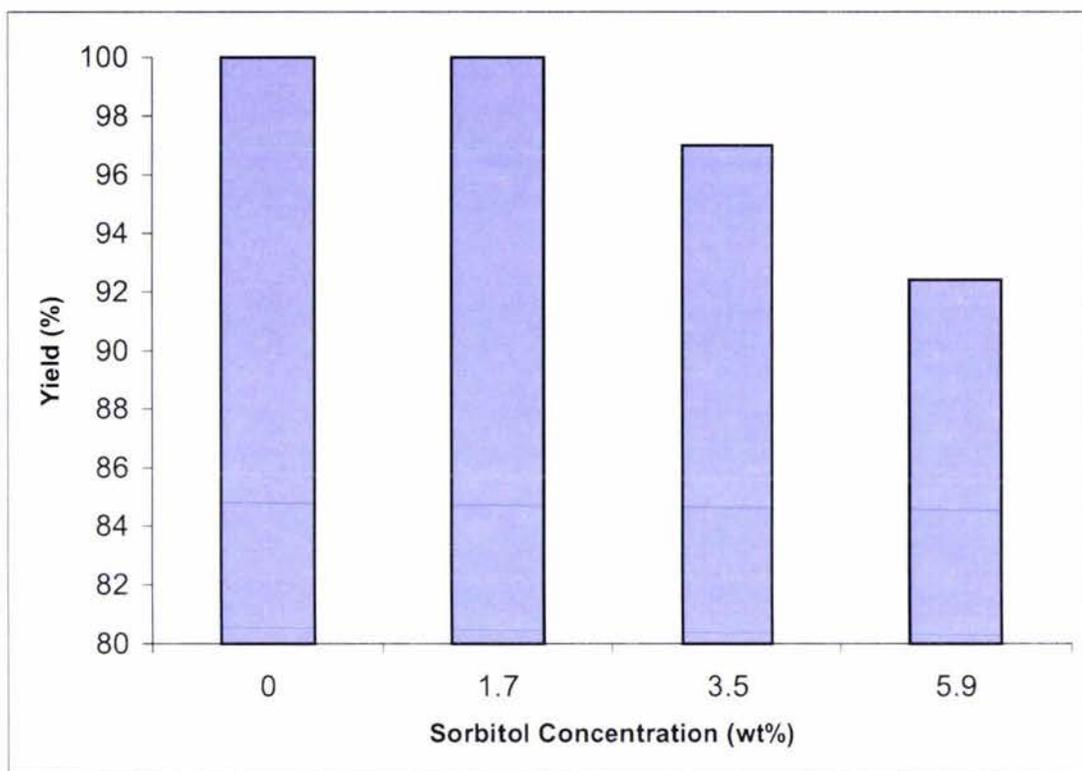


Figure 5.7 Variation in yield with change in sorbitol concentration in the outer phase. W/O/W emulsions prepared with 2 μm SPG membrane containing 1wt% Tween 80 in the outer phase and the appropriate amount of sorbitol to balance the osmotic pressure. The primary w/o emulsion consisted of 25% inner phase and the oil phase consisted of 10% PGPR. Yield measured immediately after preparation of the emulsion.

From Figure 5.7 it can be seen that an increase in the concentration of sorbitol in the outer water phase of the w/o/w multiple emulsion results in a decrease in yield. This may be due to the reverse transport of water from the inner phase to the outer phase according to the concentration gradient. The water activity meter readings showed that 1.6g glucose in 25g water was equivalent to 22g sorbitol in 400g water or 3.6g NaCl in 400g water. This was also consistent with the findings of Omotosho *et al* (1986) that 5.48% Sorbitol = 0.9% NaCl. However in actual practice it was seen that a concentration below the 5.5% Sorbitol level gave the highest yield.

Another experiment was conducted to find the extent of out flow of water from the inner to the outer phase when the osmotic pressure on the outside was greater than on the inside. The decrease in yield was observed over one week as shown in Figure 5.8.

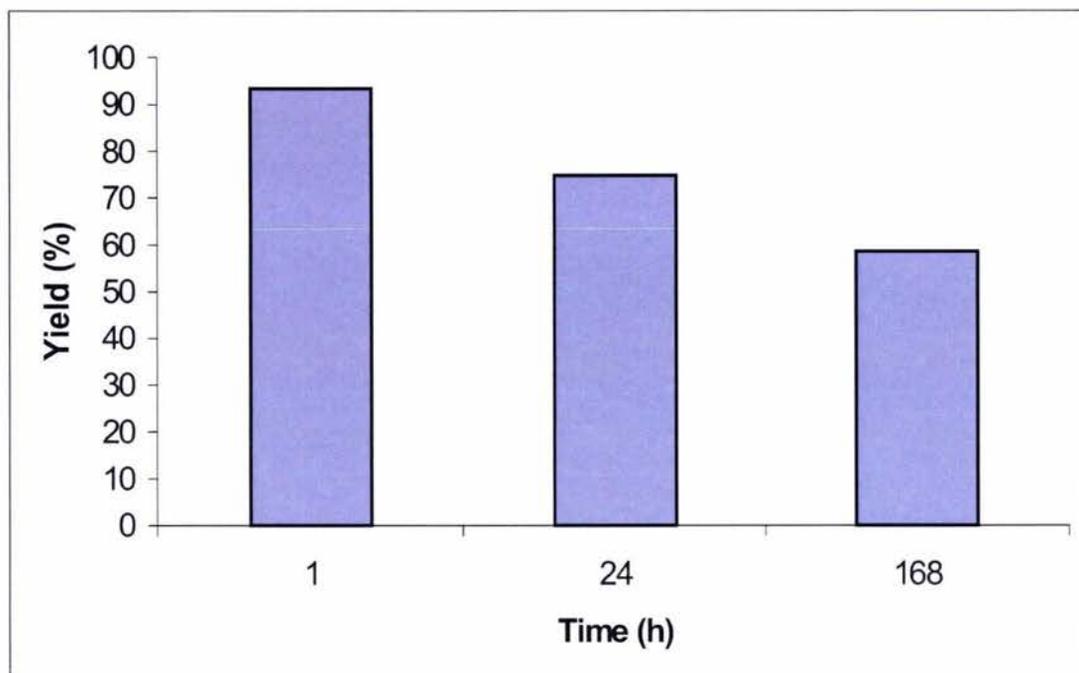


Figure 5.8 Variation in yield with time in w/o/w emulsions prepared with 2 μm SPG membrane containing 1wt% Tween 80 in the outer phase and 7.2% sorbitol to balance the osmotic pressure. The primary w/o emulsion consisted of 25% inner phase and the oil phase consisted of 10% PGPR.

5.4.3.3 Droplet Size Distribution

Following from these demonstrations of the effect of osmotic pressure ($\Delta\Pi$) on yield and the effect of $\Delta\Pi$ on droplet size was investigated (Section 5.2.2.2). The variation in droplet size with $\Delta\Pi$ can be seen in Figure 5.9.

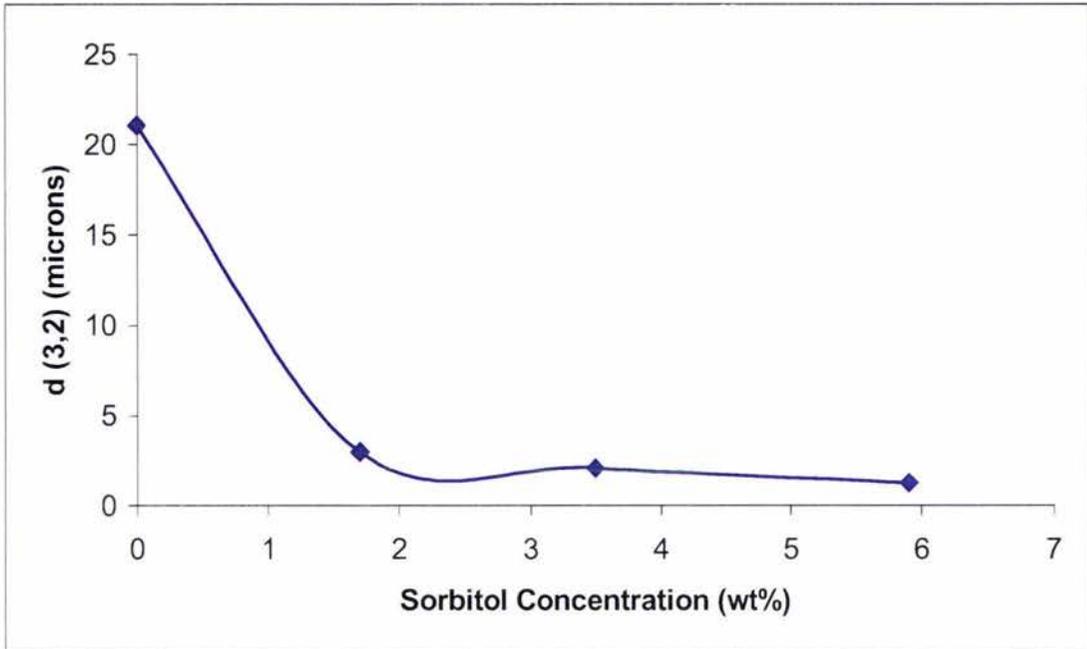


Figure 5.9 Variation in droplet size with sorbitol concentration. W/O/W emulsions prepared with 2 μm SPG membrane containing 1% Tween 80 in the outer phase and the appropriate amount of sorbitol to balance the osmotic pressure. The primary w/o emulsion consisted of 25% inner phase and the oil phase consisted of 10% PGPR homogenised at 500 bar.

From the Figure 5.9 it can be clearly seen that as the concentration of sorbitol in the outer water phase decreases the droplet size increases until the $d_{(3,2)}$ reached the maximum at about 20 μm for the w/o/w emulsion with no sorbitol in the outer phase. This increase was due to the osmotic influx of water into the droplet after formation increasing its size considerably. According to Mine *et al* (1996), the size of the inner w/o emulsion increased with increasing difference in the osmotic pressure between the inner water droplets (30% glucose) and the outer continuous water phase (1% glucose). Initially as the droplet was discharged from the pore of the membrane into the continuous phase the size of the droplet may be the same as that when w/o/w emulsion is osmotically balanced. But as the droplet is dispersed in the continuous phase the osmotic effect comes into play, which results in the movement of water in or out of the droplet according to the concentration gradient, causing an increase in volume of the oil droplets thereby increasing the droplet size. But in the case of w/o/w emulsions, where the osmotic gradient is balanced this effect was not seen. However the effect is visible to a much lesser extent as shown by the decrease in droplet size and in turn the decrease in cream layer formed with increase in sorbitol

concentration in the outer phase. The residual values for the Mastersizer plots were in the range of 1%. In most of the w/o/w samples in which the osmotic pressure is not balanced, the plots obtained were broken probably showing that a good number of droplets were beyond the measurable limit of the Mastersizer. (See Appendix B)

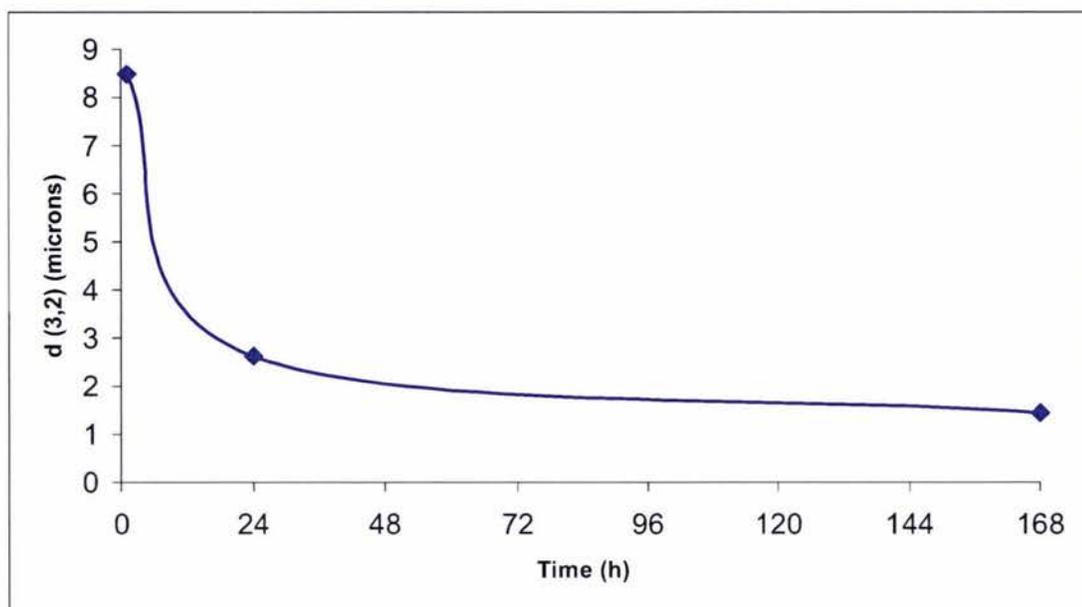


Figure 5.10. W /O/W emulsions prepared with 2 micron SPG membrane containing 1wt% Tween 80 in the outer phase and 7.2wt% sorbitol in the outer phase to adjust the osmotic pressure. The primary w/o emulsion consisted of 25wt% inner phase and the oil phase consisted of 10wt% PGPR.

Figure 5.10 makes it clear that there was a decrease in droplet size with time as would be expected for osmotic outflow of water from the inner phase to the outer. This outflow of water also decreased yield. This effect was the reverse of the initial effect seen where the droplet size increased with time due to the influx of water from the outer phase. This however emphasizes the fact that osmotic pressure has a considerable influence on the stability of a w/o/w emulsion and that the balancing of the osmotic pressure on both the water phases is a matter of importance for optimal stability of a w/o/w emulsion.

Calculations were done to find out the apparent thickness of the hydrophilic emulsifier (Tween 80) at 1wt% concentration at the primary w/o interface. The results are shown in Table 5.3.

Sorbitol Conc. in the outer phase	$d_{(3,2)}$ (μm)	* $A = 3V/r$ (interfacial area) (m^2)	N (amt. of emulsifier) (kg)	$\Gamma = N/A$ (mg/m^2)	ρ (density of Tween 80) (kg/m^3)	$t = \Gamma/\rho$ (interfacial thickness of Tween 80) (μm)
0	21.05	21.37	3×10^{-3}	140.4	1040.2	0.135
1.7	2.96	152.02	3×10^{-3}	19.7	1040.2	0.019
3.5	2.07	216.35	3×10^{-3}	13.9	1040.2	0.013
5.9	1.26	357.14	3×10^{-3}	8.4	1040.2	0.008

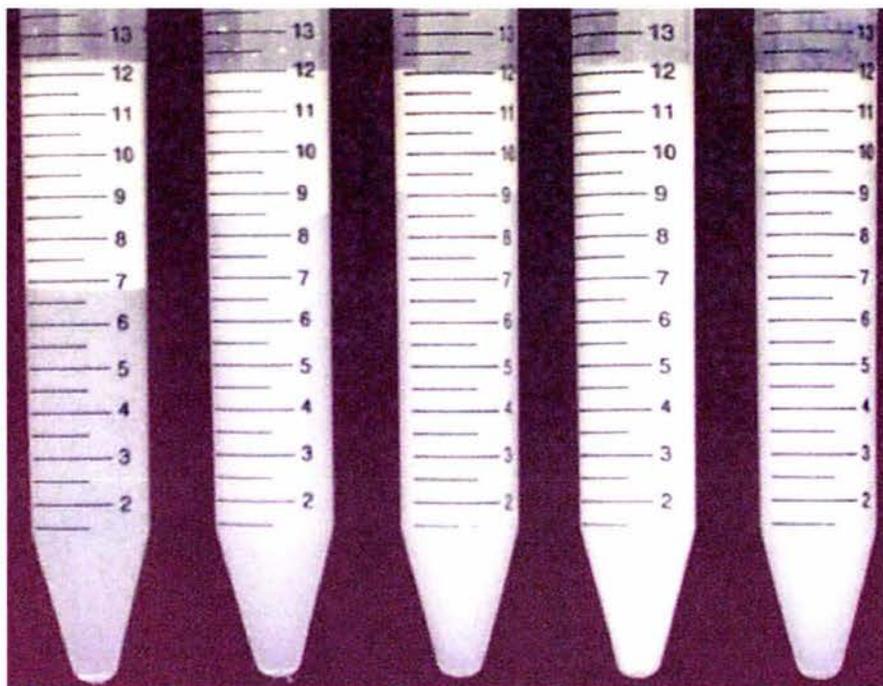
Table 5.3 Table showing the apparent thickness of the Tween 80 layer at the w/o/w interface.

* V – Volume of the w/o emulsion in the w/o/w emulsion and r is the droplet radius (i.e. $r = d_{(3,2)} / 2$).

The results listed in Table 5.3 clearly show a decrease in the apparent thickness of the Tween 80 layer at the w/o/w interface with increase in sorbitol concentration in the outer phase. This is due to the decrease in droplet size of the w/o/w emulsions formed thereby leading to the formation of larger number of smaller droplets and hence an increase in the interfacial area. At the given Tween 80 concentration this effect leads to a lower availability of the surfactant to cover the w/o/w interface leading the apparent thinning with respect to interfacial coverage. Lukham (1994) reports that increasing the wall thickness of the microcapsule will decrease the release of the core material in microencapsulation. The decrease in surface loading observed above can also be a reason for the reduction in yield with increasing sorbitol concentration in the outer phase.

5.4.3.4 Physical stability of W/O/W emulsions

As described earlier in Section 5.4.3, the osmotic pressure gradient created by the marker (glucose) in the inner phase causes the movement of water from the external phase to the internal phase. There can also be a reverse transport if the osmotic gradient was reversed by addition of sorbitol in the outer phase. Experiments were conducted to find out the optimum concentration of sorbitol required to give a high yielding fine w/o/w emulsion with a greater stability with respect to creaming.



A B C D E

Figure 5.11 w/o/w emulsions prepared with 2 μ m SPG membrane containing 1% Tween 80 in the outer phase and the appropriate amount of sorbitol to balance the osmotic pressure. The primary w/o emulsion consisted of 25% inner phase and the oil phase consisted of 10% PGPR and homogenised at 500 bars. The samples with their sorbitol concentrations are A-0%; B-1.7%; C-3.5%; D-5.9% and E-7.2%.

Figures 5.11 and 5.12 shows the decrease in cream layer with increasing concentration of sorbitol in the outer phase. This is in exact agreement with the droplet size results. The greater the concentration of sorbitol in the outer phase the smaller is the droplet size and hence the smaller the cream layer formed. The swelling of the w/o droplets causes an increase in the droplet size and since the velocity of creaming is directly proportional to droplet size according to Stoke's law, this results in a faster creaming rate for w/o/w emulsions without sorbitol in the outer phase. Also the increase in size of the w/o droplets due to the influx of water from the outer phase may also lead to the increase in the height of the cream layer. This can be seen in Figure 5.12. The physical stability can further be improved by the addition of gums in the outer phase. Experiments conducted by Huang *et al* (2001) with 14 different hydrocolloid gums proved that fenugreek gum could stabilize the o/w emulsions for longer periods of time. The droplets produced were small (with 70% < 1 μ m), possessing a very low interfacial tension. Xanthan gum was also reported to have

produced good results. Further research could be done in this direction for attaining a longer stability with respect to creaming. Figure 5.13 shows the typical Malvern Mastersizer outputs for samples with different osmotic pressure concentrations.

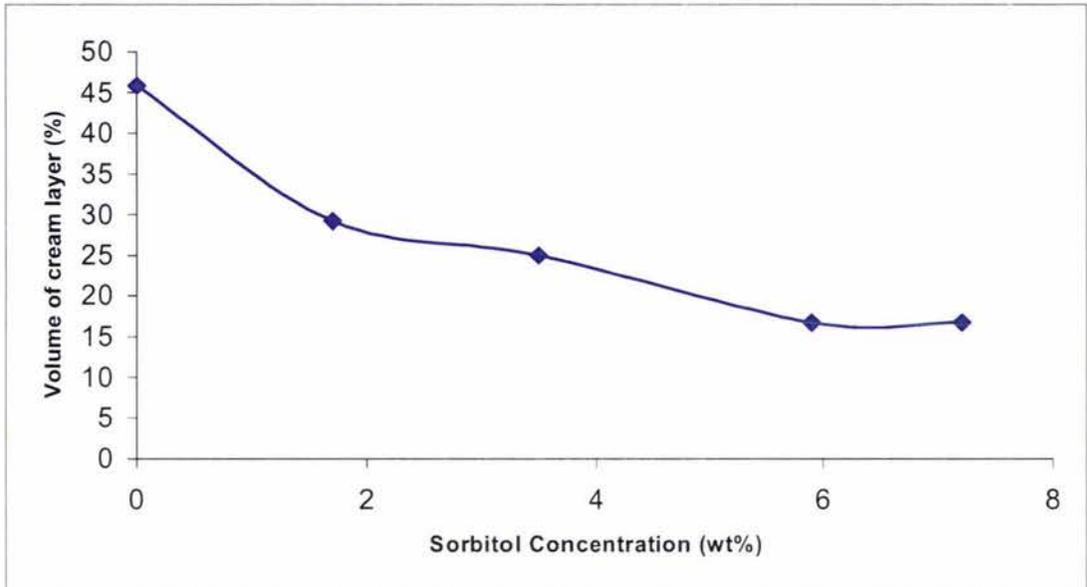


Figure 5.12 w/o/w emulsions prepared with 2 μm SPG membrane containing 1% Tween 80 in the outer phase and the appropriate amount of sorbitol to balance the osmotic pressure. The primary w/o emulsion consisted of 25% inner phase and the oil phase consisted of 10% PGPR and homogenised at 500 bars.

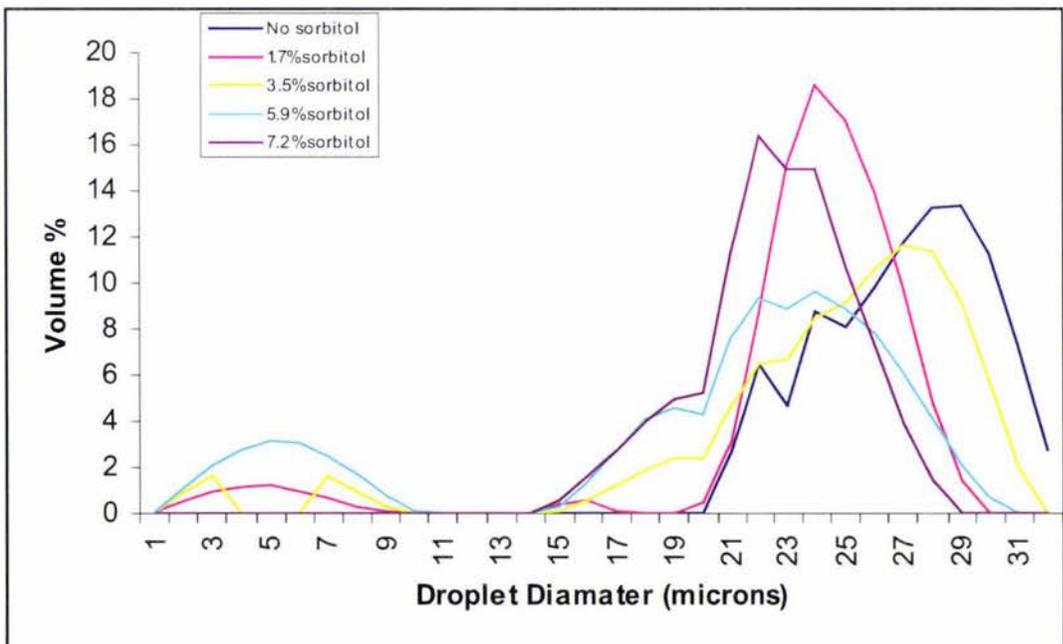


Figure 5.13 Typical output from Malvern Mastersizer reproduced in a spreadsheet. The osmotic pressure is balanced by different concentrations of sorbitol in the outer phase. Conditions as in Figure 5.12

5.4.3.5 Storage stability of w/o/w emulsions

A major challenge faced is the long term stability of w/o/w multiple emulsions. It was observed previously that the storage stability can be enhanced and the yield drop can be controlled to a considerable extent by storing the emulsions under the refrigerated conditions at about 5° C. Hence the storage stability of the emulsions prepared at different sorbitol concentrations was observed at this low temperature for different time intervals.

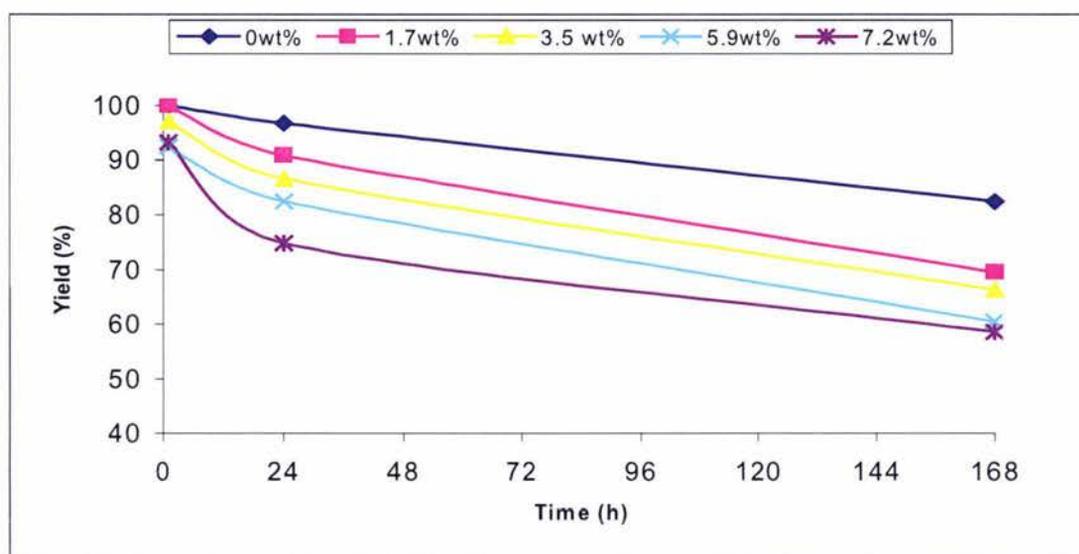


Figure 5.14 W/O/W emulsions prepared with 2 μm SPG membrane containing 1wt% Tween 80 in the outer phase and the appropriate amount of sorbitol to balance the osmotic pressure. The primary w/o emulsion consisted of 25wt% inner phase and the oil phase consisted of 10wt% PGPR homogenised at 500 bars.

Figure 5.14 shows that the storage stability was decreased for all sorbitol concentrations in the outer phase and the yield decline was more significant for w/o/w emulsions containing sorbitol in the outer phase. This may be due to the slow diffusion of the solute from the inner phase to the outer due to the osmotic pressure gradient. In the emulsion without sorbitol the flow of water is towards the inner phase, which may prevent the outflow of the solute. Even though the water droplets swell, this does not immediately increase their breakage as previously noted, thus capturing all the water and the marker within those intact globules. However the oil layer becomes thinner due to bulging of the droplet and this may lead to greater mass transfer of the marker through the oil layer. In contrast, emulsions containing sorbitol in the outer phase, the osmotic pressure is more balanced and so there is less

movement of water in and out of the droplets. This may result in slow diffusion of the marker with time resulting in the yield drop. This can be explained by the diffusion controlled release mechanism of reverse micellar transport (Garti, 1997). Also in emulsions with higher concentration of sorbitol, the increase in the osmotic pressure in the outer phase causes the outflow of the inner water along with the marker, resulting in drop in yield.

Studies conducted by Magdassi *et al* (1985) also showed that transport of electrolytes in the presence of monomeric emulsifiers takes place even if the droplets were stable to coalescence and even when the osmotic gradient of the two phases are equilibrated. Research conducted by Ueda and Matsumoto (1991) came to the conclusion that the osmotic movement of water across the oil layer is not only a function of molecular weight but also the number of equatorial hydroxyl groups present in the sugar molecule.

5.4.4 Effect of WPC Concentration

As mentioned earlier, the focus of the research was on the microencapsulation of WPC in the internal phase of the w/o/w emulsion. In the experiments conducted to date, 10 wt% WPC was taken as the standard. The maximum amount of WPC that can be encapsulated in the inner phase without adversely affecting the stability and yield of the w/o/w emulsions is of commercial and scientific interest. The higher the amount of protein in the encapsulated form the better the product in terms of nutritive value. Hence experiments were conducted to investigate the maximum amount of WPC that can be successfully encapsulated without unduly affecting the stability of the emulsions.

Various concentrations (1, 10, 20, 30, 40 and 50 wt%) were tried. However a 40 wt% concentration of WPC proved to be a thick paste and was not able to undergo primary emulsification under the usual procedure. The 50 wt% concentration of WPC was insoluble. Hence both 40 wt% and 50 wt% WPC samples were discarded and the experiments were limited to a maximum of 30 wt% WPC concentration.

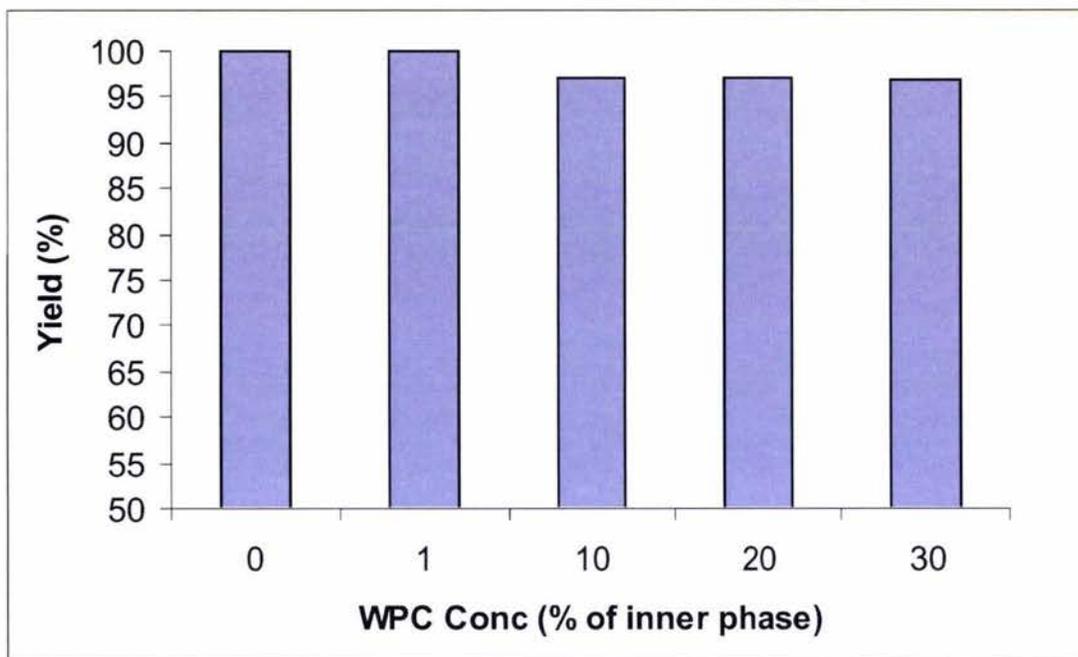


Figure 5.15 Variation in yield with WPC concentration. W/O/W emulsions prepared with a 2 μm SPG membrane and containing 1wt% Tween 80 in the outer phase. W/O emulsion consists of 25% inner phase with varying concentrations of WPC and 10% (of oil phase) PGPR homogenised at 500 bars. Readings were taken after 1hour of emulsion preparation.

From Figure 5.15 it can be seen that the WPC concentration in the inner phase does not greatly affect the yield stability of the w/o/w emulsion. The initial yields were all above 95%, even though there was a slight decrease in the yield of the w/o/w emulsions containing 10%, 20% and 30% WPC in the inner phase. The behaviour of the emulsions after 24 hours (results not shown) was also similar with respect to yield and was above the 85% level.

5.4.4.1 Droplet Size Distribution

The WPC concentration, even though is a constituent of the inner phase, was not expected to have a direct effect on the droplet size of the final w/o/w emulsion. Measurements of the droplet sizes of the w/o/w emulsions were taken to confirm this for the various WPC concentrations tested (Figure 5.16).

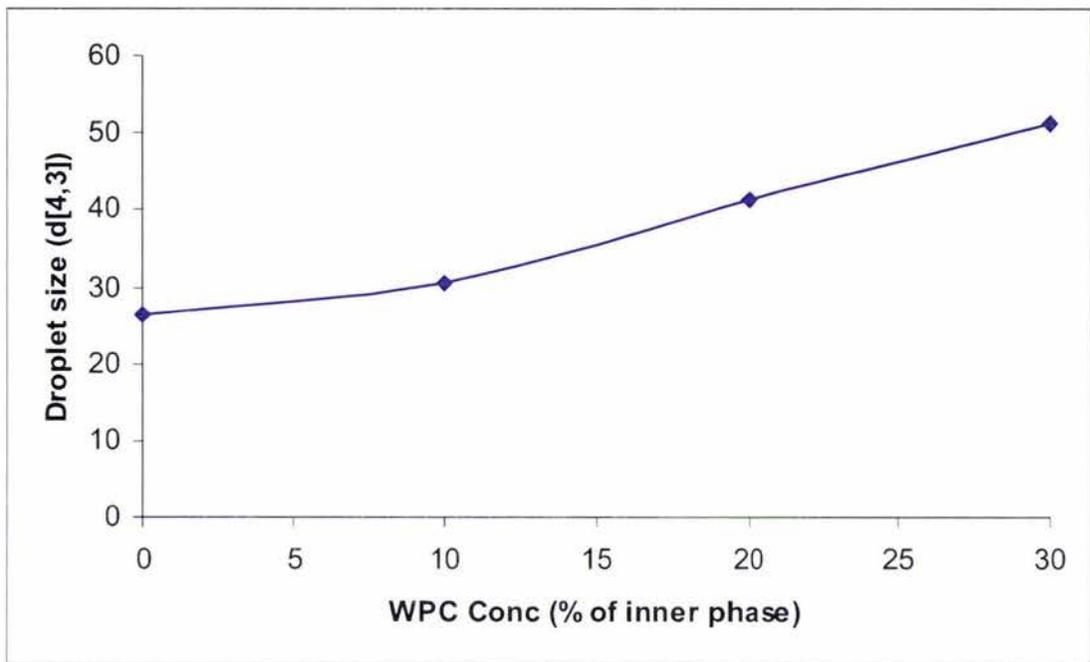


Figure 5.16 W/O/W emulsions prepared with a 2 μm SPG membrane and containing 1% Tween 80 in the outer phase. W/O emulsion consists of 25% inner phase with varying concentrations of WPC and 10% (of oil phase) PGPR homogenised at 500 bars.

The increase in droplet size of the w/o/w emulsion with increase in WPC concentration could be due to the osmotic pressure gradient created by the excess amount of solute (WPC) in the inner phase. As the osmotic pressure was not balanced by sorbitol in the outer phase in these experiments, the osmotic gradient may result in the influx of water from the outer phase to the inner phase increasing the droplet size of the w/o/w emulsion. The greater the amount of the solute (WPC) in the inner phase, the greater the osmotic gradient, the greater the influx of water from the outer phase and the greater the size of the droplets formed.

The possibilities of changes due to interactions between PGPR and WPC could also not be discounted. Reports by Garti *et al* (1999) suggest several effects of the mixed peptide and synthetic emulsifiers like proteins and Tweens. Competitive adsorption is very important among these and is highly researched. However it is also claimed that lower concentrations of biopolymers can produce a positive effect on emulsification in combination with non-ionic surfactants (McClements, 1999). There appears to be no previous work done specifically on the competitive, complimentary or antagonistic effects of PGPR with biopolymers and so further specific comments regarding the

possible effects in this case cannot be made. However there is scope for further research on the possible behavioural effects of PGPR in combination with WPC.

Figure 5.17 shows the confocal microscope image of a w/o/w emulsion. The inner water droplets can be seen as dark dots on the inside of the brighter oil droplets.

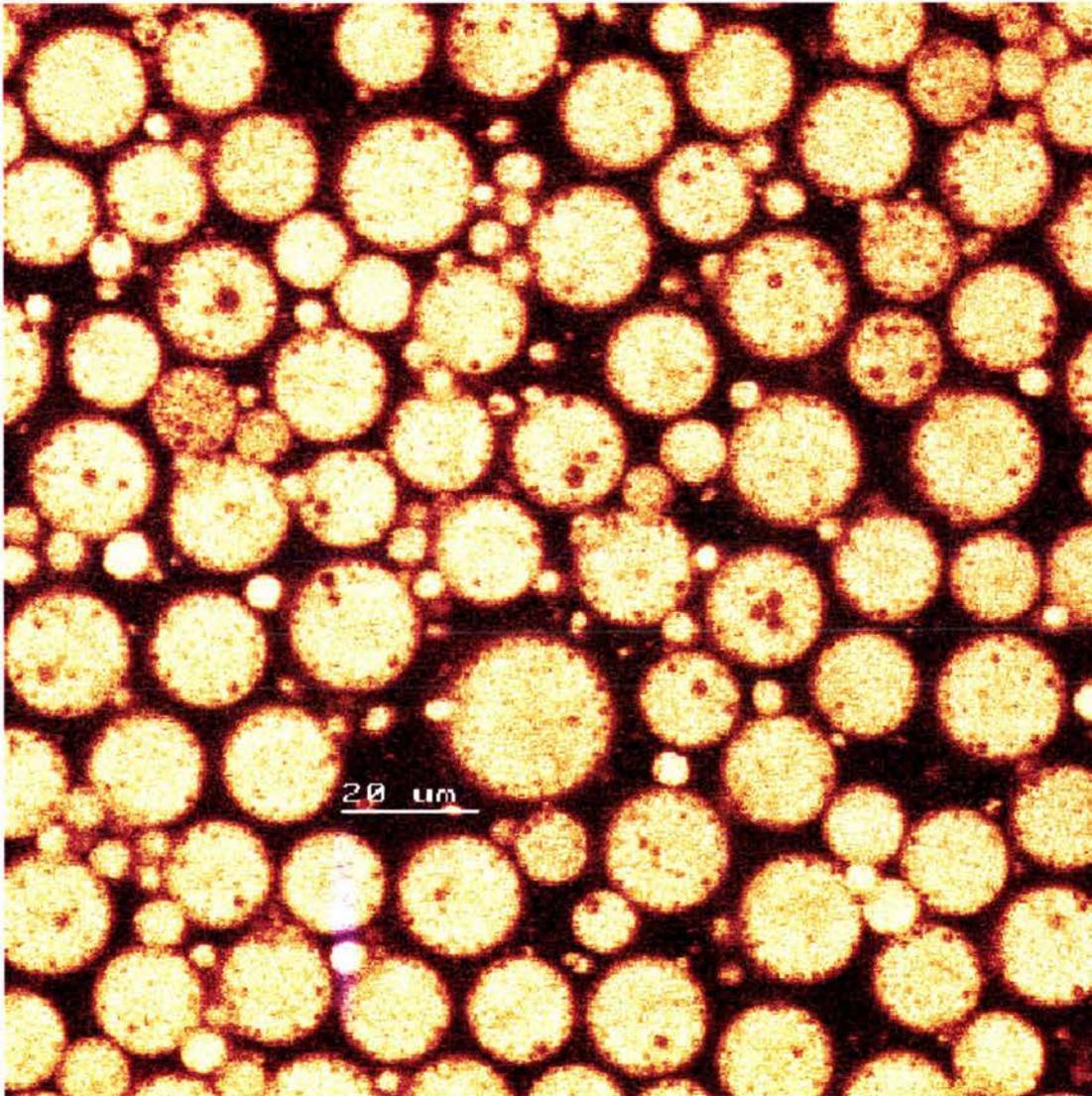


Figure 5.17. A confocal microscope image of a multiple emulsion. The smaller dark droplets within the bigger droplets are the internal water droplets.

5.5 Conclusion:

This chapter mainly deals with the effects of secondary emulsification conditions on the yield stability, droplet size distribution and physical stability / creaming stability of w/o/w emulsions. The major processing parameters studied included the effect of processing temperature, hydrophilic emulsifiers, osmotic pressure and WPC concentration.

Experiments conducted to study the effect of temperature showed that an increase in temperature during secondary emulsification decreases the yield stability thus indicating a negative effect. The droplet size [$d_{(4,3)}$] increases with increase in temperature.

Hydrophilic emulsifiers namely whey protein isolate (WPI), soy protein isolate (SPI), sodium caseinate and Tween 80 were tried in the outer phase of the w/o/w emulsions. It was found that all protein stabilised emulsions behaved similarly and exhibited higher yield stabilities. But Tween 80 exhibited slightly lower yield especially in the case of room temperature stored samples. The droplet size estimation of w/o/w emulsions proved that all the protein stabilised emulsions exhibited a similar $d_{(3,2)}$ and $d_{(4,3)}$, but the Tween 80 stabilised emulsions showed a much smaller $d_{(3,2)}$ and $d_{(4,3)}$, the effect being confirmed by the greater opacity of the serum layer of Tween 80 stabilised emulsions. The cream layer was the same in all the four cases.

Research conducted with PGPR as hydrophobic emulsifier and Ultra-Turrax as the method of primary and secondary emulsification proved that the presence of solute (glucose as marker and WPC as the core material) results in the creation of osmotic gradient across the oil layer between the inner and outer aqueous phases resulting in the transport of water into the inner phase thereby increasing the oil droplet size and hence the viscosity of w/o/w emulsion. This phenomenon was visible on storage of the emulsion. Efforts were made to reduce the osmotic effect to the minimum by the addition of sorbitol to the outer phase at a concentration equivalent to glucose in the inner phase. But this resulted in an outflow of water from the inner phase to the outer phase even at isotonic concentration resulting in low initial stabilities and storage

stabilities. However, lower concentrations of sorbitol in the outer phase resulted in w/o/w emulsions with low viscosity and high yield. The bulging of oil droplets due to osmotic influx of water resulted in erratic droplet size measurements. Osmotic balancing by sorbitol lead to the reduction of $d_{(3,2)}$ from $\sim 20\mu\text{m}$ to $< 2\mu\text{m}$. The residual values of the Mastersizer plots also decreased. Further evidence of this effect was provided by the lower cream layer exhibited by the w/o/w emulsions prepared with sorbitol in the outer phase.

A range of WPC concentration (0-30% of the inner phase) was investigated and it was found that yield stability was not affected much as all samples exhibited high yields of $> 95\%$. Droplet size distribution of w/o/w showed an increase with increase in WPC concentration. But this could be a result of osmotic gradient and not a direct effect of WPC concentration as the osmotic pressure was not balanced in these experiments. Further research needs to be done for confirmation.

6. Optimisation of Membrane Operating Parameters

6.1 Introduction

The production of a stable emulsion requires a small droplet size and narrow droplet size distribution. In membrane emulsification, these are influenced by the membrane pore-size, transmembrane pressure, continuous phase velocity and dispersed phase flux. Hence optimisation of these variables is a very important step. This chapter reports the results of experiments that were conducted to study the variation of yield, droplet size and physical stability of w/o/w emulsions with changing membrane emulsification conditions.

6.2 Objectives

- To examine the effect of membrane processing parameters in membrane emulsification (membrane pore-size, transmembrane pressure, continuous phase velocity and dispersed phase flux) by determining the variation in yield stability, droplet size and physical (creaming) stability of the w/o/w emulsions produced.
- To identify the key factors affecting the dispersed phase flux in membrane emulsification.

6.3 Experimental

The emulsions were prepared as described in Section 5.3. The secondary (w/o/w) emulsions were prepared with SPG membranes (SPG Technology Co. Ltd, Japan) of standard pore size 2 μm . However SPG membranes with pore-sizes of 1.4 μm and 3.8 μm were also used. The droplet size measurements were conducted using a Malvern Mastersizer (Malvern Instruments, UK). The physical stability tests were conducted as specified in section 3.5. The work is reported in four sets. In the first, the effect of membrane pore size on emulsion properties was examined. In the second and the third sections, the effects of transmembrane pressure and continuous phase velocity

were investigated respectively for a 2 μm membrane. The fourth section reports an analysis of flux and fouling behaviour.

6.4 Results and Discussion

6.4.1 Influence of Membrane Pore Size

6.4.1.1 Yield

The droplet size of the emulsion is generally related to the pore-size of the SPG membrane by a linear equation (Equation 2.8). So according to Mine *et al* (1996), the selection of the appropriate membrane pore size is one of the most important steps for the formation of a stable w/o/w emulsion. They recommended a hydrophilic membrane for the secondary emulsification step should possess a pore-size greater than or equal to twice the diameter of the water in the w/o primary emulsion; if the pore-size is equal to or smaller than the diameter of the water particles in the primary w/o emulsion, they will be rejected by the membrane making the formation of double emulsion impossible. Experiments were conducted with SPG membranes of three different pore sizes (1.4, 2 and 3.8 μm) (Figure 6.1). These were much greater than the apparent water droplet size indicated by for example, Figure 4.25

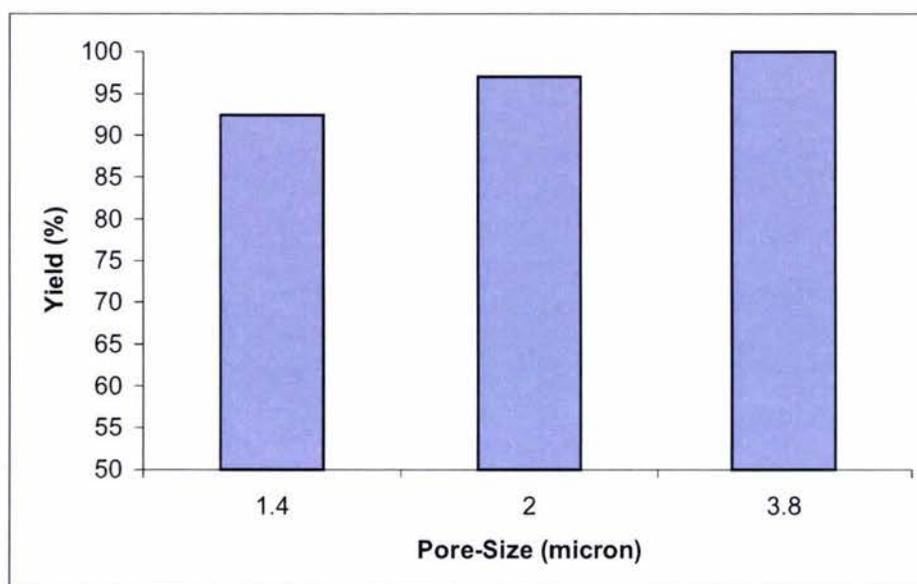


Figure 6.1 Variation in Yield with pore-size. Yield of w/o/w multiple emulsion prepared with 25% water in the inner phase, 10% PGPR of the oil phase, 500 bars homogenisation pressure in the primary emulsification step and using various (1.4, 2 and 3.8 μm) SPG membranes in the secondary emulsification step. 1% Tween 80 was the hydrophobic emulsifier in the external phase. Readings were taken after 1 hr.

From Figure 6.1 it can be clearly seen that the yield of the w/o/w multiple emulsions produced increases with increase in the pore-size. The lower yield (92%) with the 1.4 μm membrane may be due to the break up of a few larger droplets present in the w/o primary emulsion which are unable to squeeze through the small membrane pores. The transmembrane pressure and dispersed phase flux of emulsification with membranes of different pore-sizes is given in the Table 6.1.

Pore-Size (μm)	ΔP (kPa)	J ($\text{lm}^{-2}\text{h}^{-1}$)
1.4	220	23
2.0	150	127
3.8	125	164

Table 6.1 Table showing the transmembrane pressure and dispersed phase flux of secondary emulsification with membranes of different pore-sizes

The comparative increase in transmembrane pressure and the comparative decrease in dispersed phase flux as seen in Table 6.1 with respect to the 2 μm and 3.8 μm membranes is a clear indication of the difficulty in permeation of the w/o emulsion through the small pores of the 1.4 μm membrane.

6.4.1.2 Droplet Size Distribution

A lot of work has been done in the area of the relationship between pore-size of the membrane and the resulting droplet size of the emulsion. Almost all the previous researchers (Sotoyama *et al* 1999, Katoh *et al* 1996, Mine *et al* 1996, Kandori 1995, Schroder & Schubert 1999, Williams *et al* 1998 and Shiomori *et al* 1995) have observed a linear relationship between the droplet size and pore-size with the constant of proportionality varying between 2 and 12 (Refer 2.4.6.1.3). Droplet size increases with increasing pore-size (Schroder & Schubert, 1999) mainly due to the fact that the volume and the diameter of the droplets at the beginning of the formation are larger due to the larger pore diameter. Also the interfacial tension force is higher for a higher mean pore diameter of the membrane increasing the flow resistance force and the dynamic lift force, which in turn increases the droplet size.

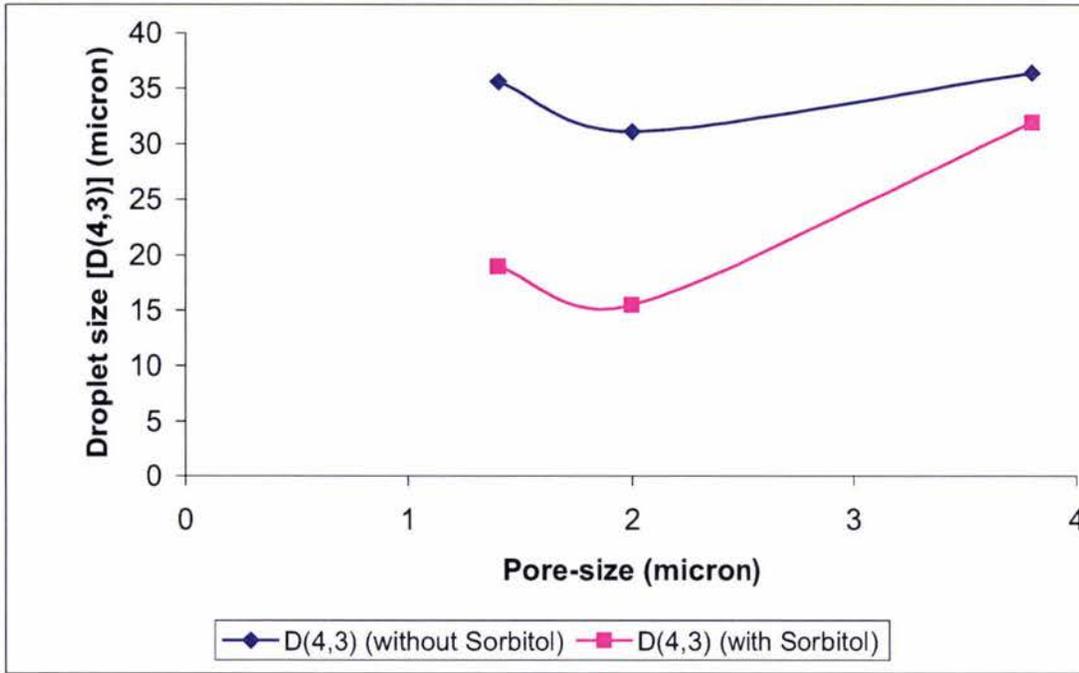


Figure 6.2 Variation in droplet size with pore size. Conditions as in Figure 6.1. Osmotic pressure gradient balanced with 1.7% sorbitol in the outer phase.

Figure 6.2 shows somewhat different results to other researchers in that the droplets produced with 2 μm membranes are smaller than the ones produced with a 1.4 μm membrane and a 3.8 μm membrane. A possible explanation for this effect may be that the effective transmembrane pressure also influences the droplet size of the w/o/w emulsion; the larger the effective transmembrane pressure the larger the droplets (Schroder & Schubert, 1999).

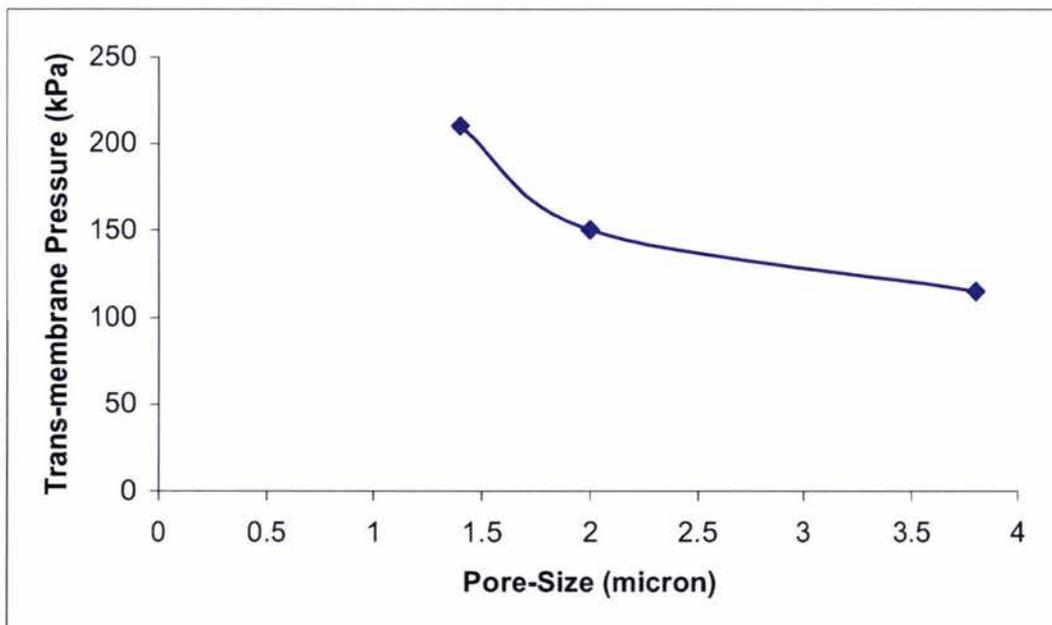


Figure 6.3 Variation in transmembrane pressure with pore-size. Conditions as in Figure 6.1

From Figure 6.3 it is clear that the transmembrane pressure increases with decreasing pore-size. Joscelyne & Tragardh (1999) reported that the initial critical pressure decreases with increasing pore-size at a particular emulsifier concentration. The higher transmembrane pressure for the 1.4 μm membrane (Table 6.1) when compared with that for the 2 μm and 3.8 μm membranes might therefore have resulted in the greater droplet size for the w/o/w emulsions produced. A reduced transmembrane pressure was not practical as 220 kPa was the minimum for the 1.4 μm membrane, which gave a dispersed phase flux of 23 $\text{l.m}^{-2}.\text{h}^{-1}$. It was not possible to estimate the critical pressure (ΔP_c) for the process and the values used are minimum that gave a reasonable performance and stability. Further research by running all the membranes at a constant (high) transmembrane pressure might be necessary to clarify this point.

6.4.2 Influence of Transmembrane Pressure

6.4.2.1 Yield

Optimising transmembrane pressure is vital to establish a steady production rate of the multiple emulsions. If the pressure is too low, it might not be sufficient to drive the dispersed phase through the pores of the membrane. If the pressure is too high, it may cause the dispersed phase to jet out of the pores as a stream resulting in higher droplet size and in turn low yield and stability. Usually pressures 2-10 times higher than the minimum transmembrane pressure can be applied under practical circumstances (Williams *et al*, 1998).

Experiments were conducted by varying the transmembrane pressures (100kPa, 150kPa, 200kPa and 250kPa) to evaluate the change in yield stability, droplet size and physical stability of w/o/w emulsions produced. The yield stability results showed that the multiple emulsions exhibit similar behaviour with different transmembrane pressures. The yield drop with time is almost the same irrespective of the variation in the transmembrane pressure. This suggests that transmembrane pressure has no significant influence on the yield of multiple emulsions, at least for the 2 μm membrane. This is made clear from Figures 6.4 and 6.5.

However another important observation is the reduction in the yield with time in those w/o/w multiple emulsions stored at 5 °C. There is a significant difference of almost 20% in yield between the emulsions at room temperature and the emulsions stored at 5 °C after a week of storage.

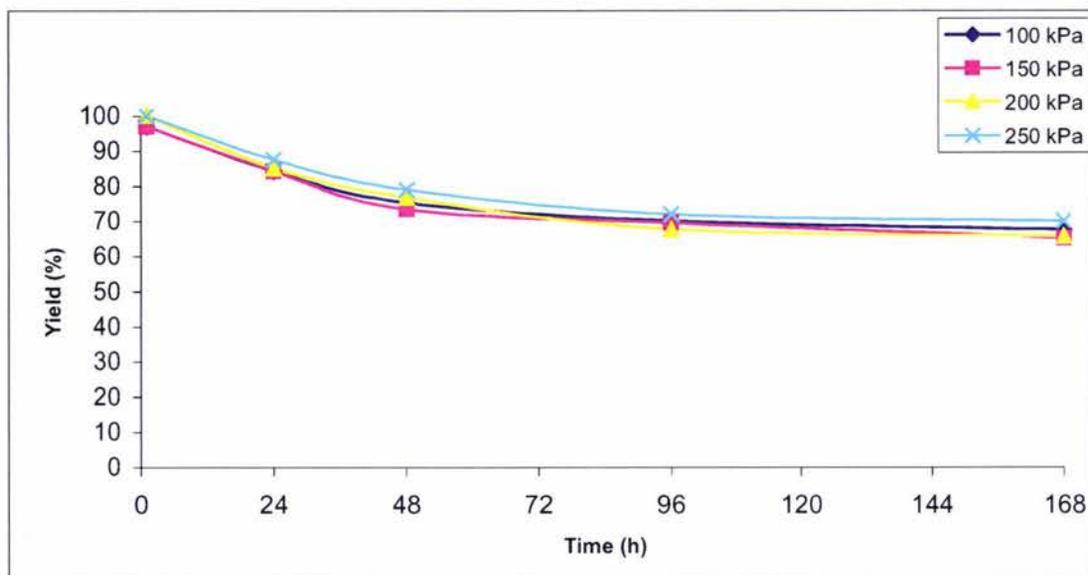


Figure 6.4 Variation in yield with transmembrane pressure. W/O/W emulsions prepared with 25% inner phase, 10% PGPR and primary emulsification done at 500 bar pressure and stored at 20°C. Secondary emulsification was done with a 2µm SPG membrane at different transmembrane pressures of 100kPa, 150kPa, 200kPa, and 250kPa. 1% Tween 80 was used as the hydrophilic emulsifier in the external phase.

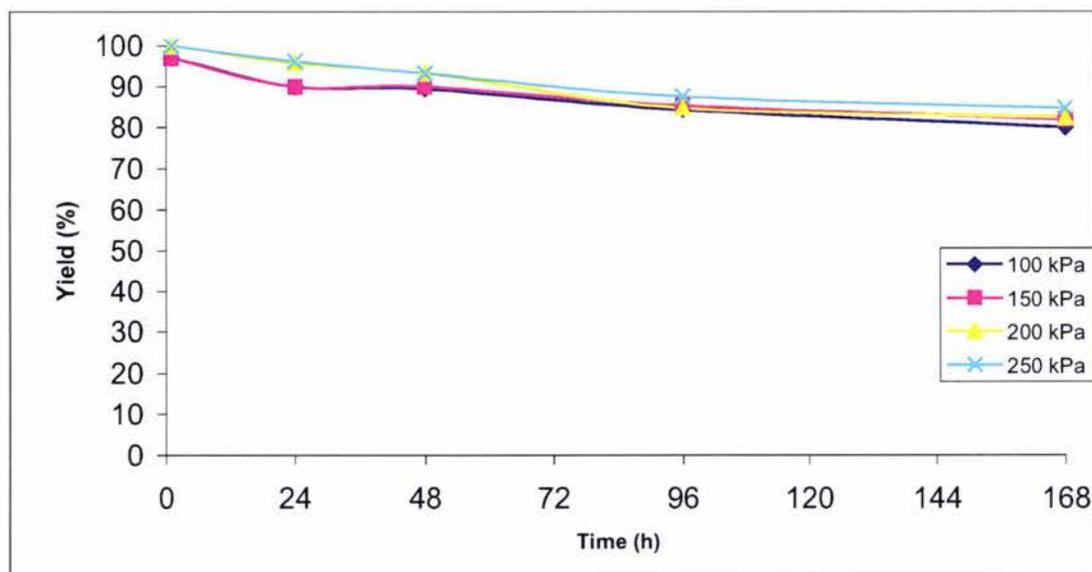


Figure 6.5 Variation in Yield with transmembrane pressure. W/O/W emulsions stored at 5°C. Conditions as in Figure 6.4.

6.4.2.2 Droplet Size Distribution

According to Peng and Williams (1998), the droplet size of emulsions does not depend much on the transmembrane pressure for a given continuous phase velocity. Also the droplet size increases slightly with transmembrane pressure especially when the cross-flow velocity is low (Section 2.5.4).

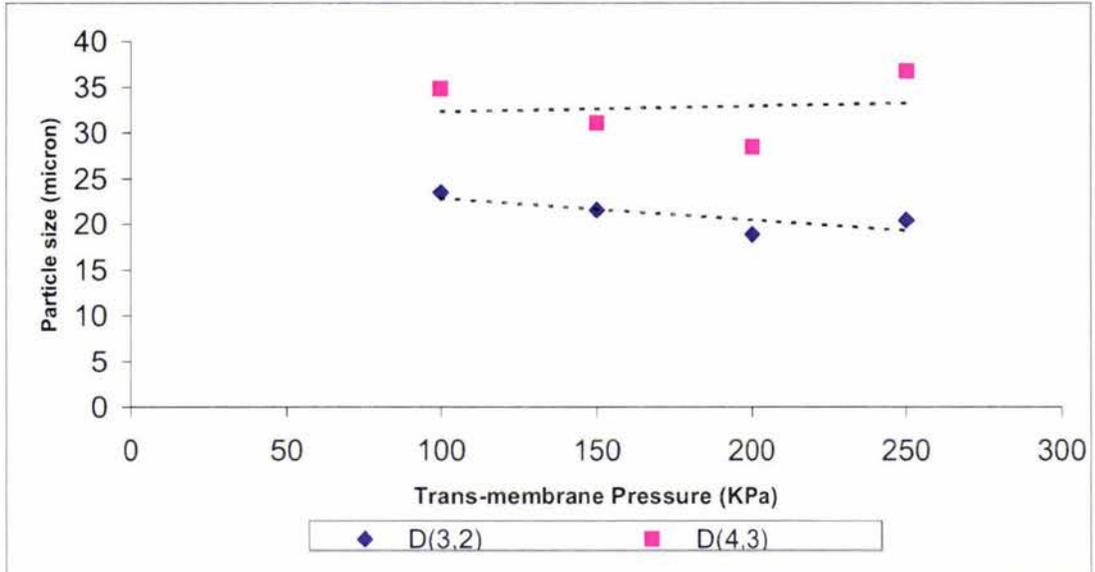


Figure 6.6 Droplet sizes of w/o/w emulsions produced with SPG membrane of pore-size $2\mu\text{m}$ at varying transmembrane pressures (100kPa, 150kPa, 200kPa, 250kPa). 1% Tween 80 is the hydrophilic emulsifier used. Primary w/o emulsion consisted of 25% inner phase, 10% PGPR and homogenised at 500 bars.

Figure 6.6 shows that there was not much effect of transmembrane pressure on the droplet size. According to Schroder *et al* (1998) the interfacial tension dynamics has no effect on droplet size if the droplet formation time is long compared to the time of decreasing interfacial tension and that the droplet size remains unchanged with increasing flux (Joscelyne & Tragardh, 2000). The droplet size $d_{(3,2)}$ remained almost within a range of 20-25 μm when the transmembrane pressure was varied from 100 kPa to 250 kPa, although there is a slight decrease in size, it is probably not significant. The $d_{(4,3)}$ exhibited a similar behaviour with the droplet size lying in the range of 30-35 μm . The increase in $d_{(4,3)}$ with the increase in transmembrane pressure to 250KPa was due to formation of a higher number of larger droplets as the $d_{(4,3)}$ index emphasises the sizes of the larger droplets (Dickinson *et al*, 1993). All the plots obtained as a result of analysis had a residual of around 3%.

6.4.2.3 Physical Stability of W/O/W emulsions

Transmembrane pressure is directly proportional to the dispersed phase flux for a given pore-size. An increased flux may lead to the production of larger droplets, which in turn may lead to a higher creaming rate. Hence experiments were conducted to find out the effect of transmembrane pressure on the creaming rate.

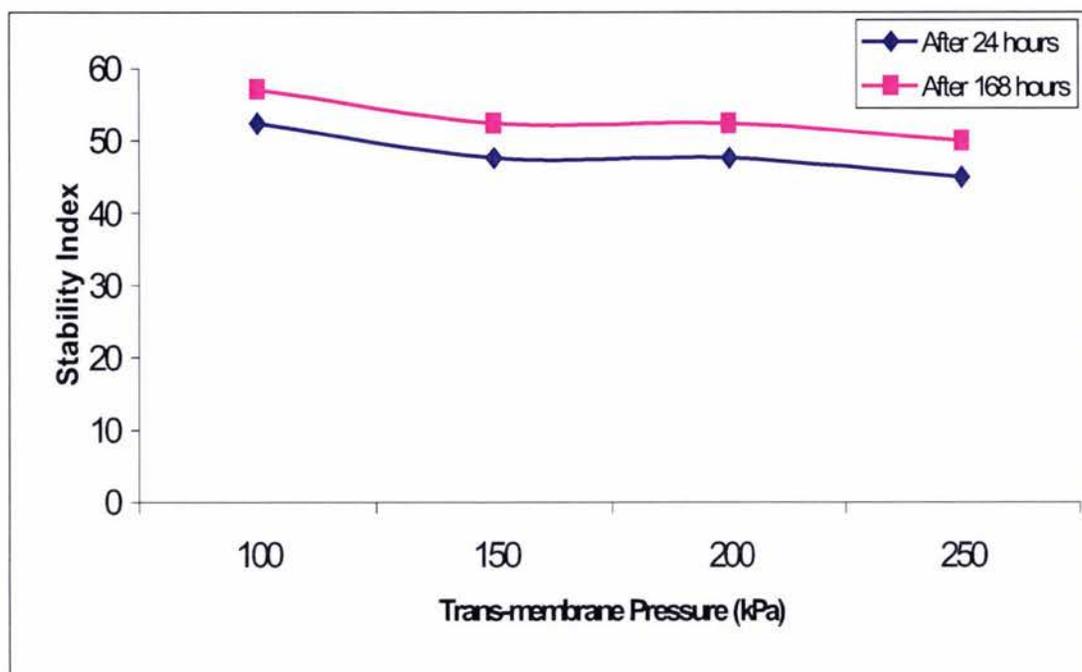


Figure 6.7 W/O/W emulsion prepared with 25% inner water phase, 10% PGPR pre-emulsified at 9500 rpm and homogenised at 500 bar pressure. The w/o/w multiple emulsion is made with 20% primary emulsion and 1% Tween80 in the external water phase with a 2 μm pore-size membrane. Osmotic pressure was not balanced.

The stability index was calculated as per the Equation 3.5. Figure 6.7 show that there was no considerable difference in the behaviour of multiple emulsions subjected to different transmembrane pressures. The emulsions creamed consistently with a slight increasing trend with increase in transmembrane pressure. The separation due to gravity was comparatively rapid in the first 24 hours and then it weakened with time until after a week. Figure 6.8 shows the separation in w/o/w emulsions prepared at different transmembrane pressures after 1 week of storage at 20°C.

6.4.3 Influence of Continuous Phase Velocity

6.4.3.1 Yield

Continuous phase velocity is another important factor determining the droplet size of a multiple emulsion. The influence of continuous phase velocity or crossflow velocity is expressed in terms of wall shear stress (Joscelyne & Tragardh, 2000) (Section 2.5.4). The droplet size of a multiple emulsion is inversely proportional to the crossflow velocity and the droplet size decreases as the crossflow velocity increases asymptotically. (Joscelyne & Tragardh, 2000; Peng & Williams, 1998). Schubert and Schroder (1997) found that smaller droplets are produced with a small membrane pore size and in this case a smaller wall shear stress is need for attaining a given size. The smaller droplets formed as a result of increasing continuous phase velocity may result the formation of w/o/w emulsions with a higher yield.

Experiments were conducted to investigate the effect of continuous phase velocity on the yield of multiple emulsions. For this 5 continuous phase velocities, 0.5, 0.75, 1, 1.25, 1.5 m/s, were selected. Figures 6.9 and 6.10 show that the yield behaviour over a period of 1 week (168h) remains essentially the same for all velocities. As noted before the considerable effect of storage temperature on the yield is evident.

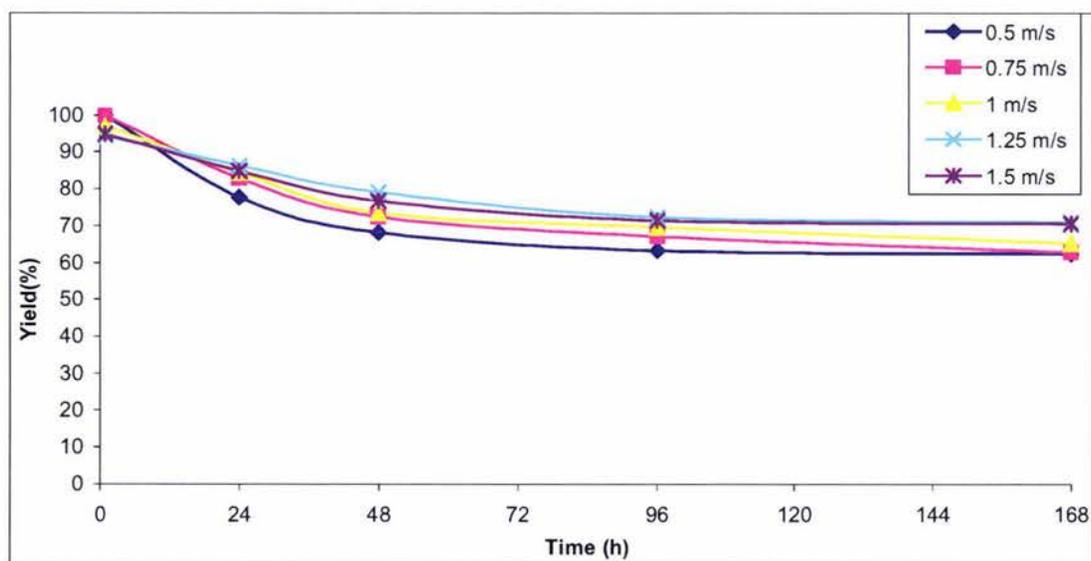


Figure 6.9 Variation in yield with continuous phase velocity. W/O/W emulsions prepared with 25% inner phase, 10% PGPR and primary emulsification at 500 bar. Secondary emulsification with a 2 μ m SPG membrane at varying continuous phase velocities of 0.5m/s, 0.75m/s, 1m/s, 1.25m/s, 1.5 m/s. 1% Tween 80 was the hydrophilic emulsifier. Emulsions were stored at room temperature (22°C)

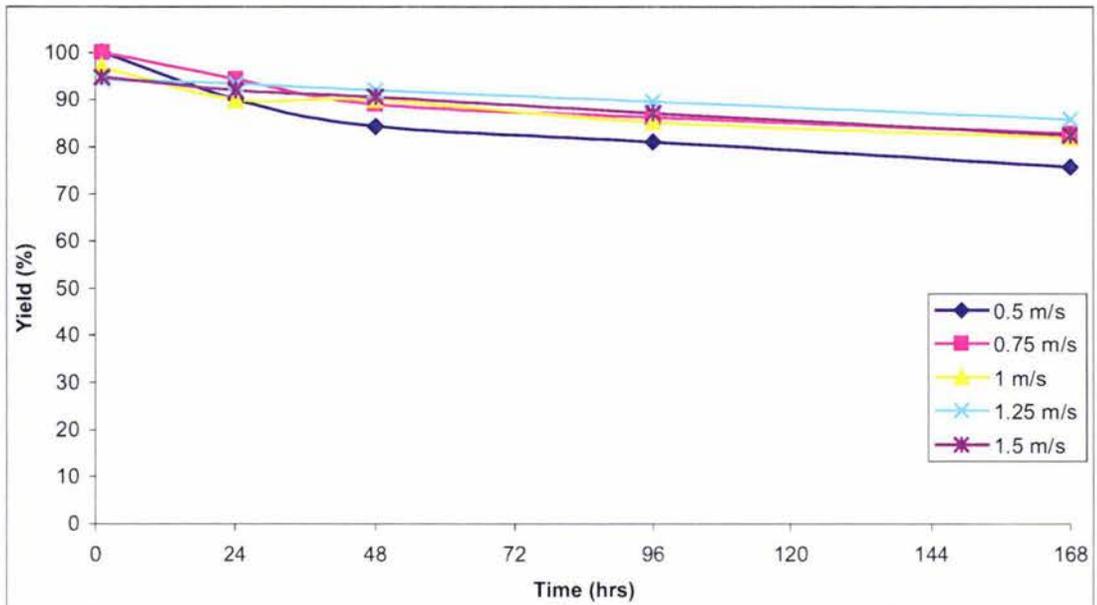


Figure 6.10 Variation in yield with continuous phase velocity. Conditions as in Figure 6.10. Emulsions are stored at cold storage temperature (5°C).

Overall, it can be seen that there is a slight improvement in the yield of the multiple emulsion as the velocity climbs from 0.5 m/s to 1.5 m/s. This may be due to the smaller droplet size of the w/o/w emulsions formed.

6.4.3.2 Droplet Size Distribution

As mentioned before continuous phase velocity has a direct influence on the particle size of the emulsions formed. For a given transmembrane pressure the particle size decreases as the continuous phase velocity increases (Peng & Williams, 1998) and this happens due to the reduction in the detachment time of the droplet from the membrane surface. Williams *et al* (1998) conducted research involving 4 continuous phase velocities from 1.12 m/s to 5.09 m/s and they found that the droplet size decreased with increase in velocity.

Experiments were conducted with 5 different continuous phase velocities (0.5, 0.75, 1, 1.25, 1.5 m/s) and the droplet sizes of the multiple emulsions were measured using a Malvern Mastersizer E.

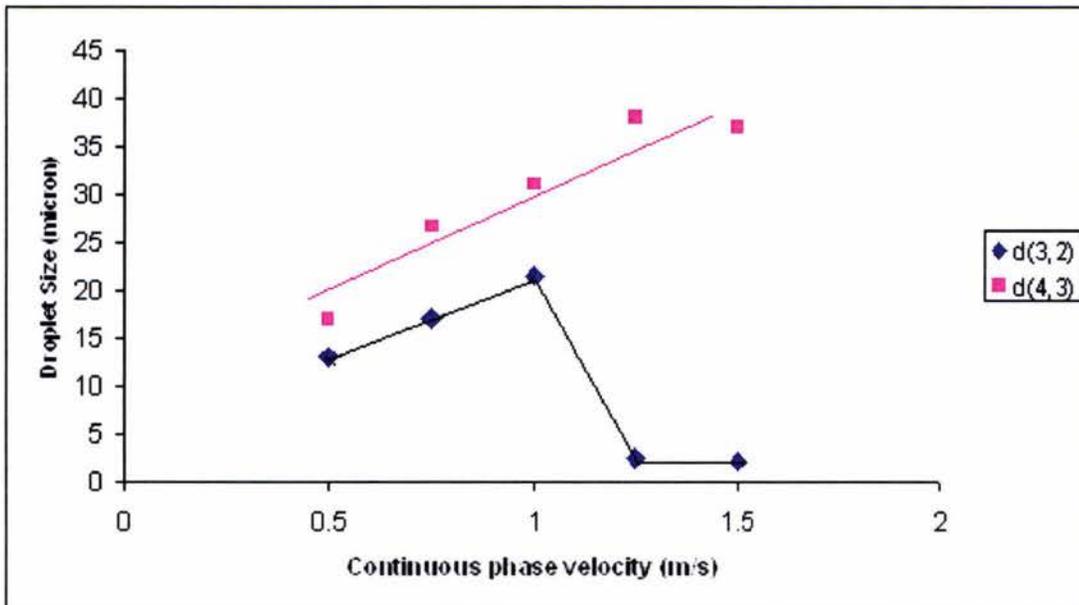


Figure 6.11 Droplet sizes of w/o/w emulsions produced with SPG membrane of pore-size $2\mu\text{m}$ at varying continuous phase velocities (0.5m/s, 0.75m/s, 1m/s, 1.25m/s, 1.5m/s). 1% Tween 80 is the hydrophilic emulsifier used. Primary w/o emulsion consisted of 25% inner phase, 10% PGPR and homogenised at 500 bars.

From Figure 6.11 it can be seen that there is small increase in $d_{(3,2)}$ from 0.5 m/s to 1 m/s and then there is a drastic decrease for $d_{(3,2)}$ with a further increase in continuous phase velocity. However, $d_{(4,3)}$ showed a different behaviour with a consistent increase in droplet size with increase in continuous phase velocity. This can probably be explained by a very important observation made during droplet size estimation.

The w/o/w emulsions produced at a continuous phase velocity of 1.25m/s and 1.5m/s was found to form lumps when diluted with water for droplet size estimation. In contrast, emulsions produced at a velocity of 1m/s or lower dispersed almost instantly when diluted with water, the reason for which is unknown. . The formation of lumps would reduce the specific surface area due to the apparent lesser number of droplets. This can lead to smaller $d_{(3,2)}$ values as the $d_{(3,2)}$ or the surface, volume mean diameter is a measure of the mean diameter with respect to surface area of the droplets. However, $d_{(4,3)}$ is a measure of the mean diameter with respect to weight of the droplets and as a result of this the higher values of $d_{(4,3)}$ may be due to the apparent formation of larger and heavier droplets due to lumping. As stated earlier, the $d_{(4,3)}$ index emphasises the sizes of the larger droplets formed (Dickinson *et al*, 1993). The $d_{(0,9)}$ values also exhibit a similar behaviour to $d_{(4,3)}$ with the $d_{(0,9)}$ values increasing consistently from 32 μm at a velocity of 0.5 m/s (residual $\sim 1.8\%$) to 70 μm at a velocity of 1.5 m/s (residual $\sim 4.4\%$). Also the plots obtained show that an increasing

number of droplet going out of the measurable range of 65 μm of the Mastersizer with increase in the continuous phase velocity with the effect more for velocities of 1.25 m/s and 1.5 m/s. Thus the insufficient amount of droplets available for droplet size determination may also be the reason for the drop in $d_{(3,2)}$ values for 1.25 m/s and 1.5 m/s samples (Appendix B).

However, in later experiments, when the osmotic gradient was balanced by adding sorbitol in the outer phase there was no such phenomenon as lumping. The droplets formed were much smaller in size under similar conditions (Figure 5.9). The lumping can be due the increase in droplet size due to the osmotic influx of water into the internal w/o droplets from the outer phase thereby increasing the size of the w/o/w droplets. All the plots obtained as a result of droplet-size estimation with the Malvern Mastersizer had a residual value around 4%.

6.4.3.3 Physical Stability of W/O/W emulsions

Even though the continuous phase velocity has a direct influence on the droplet size of w/o/w multiple emulsions formed, the stability of the emulsions with respect to creaming did not show much improvement. Tests were conducted as mentioned earlier and it was seen that the multiple emulsion formed with a continuous phase velocity of 0.5 m/s showed a distinct cream layer with a clear water portion beneath. In contrast all the other emulsions showed a dense white cream layer at the top and a less dense serum layer at the bottom.

Rather surprisingly, the formation of lumps did not have much influence on the creaming stability of the w/o/w emulsions produced. The cream layers formed were almost the same for all the w/o/w emulsions, although the one produced with a continuous phase velocity of 0.5m/s, the serum layers were much opaque for those produced at higher velocities of 1.25m/s and 1.5m/s, indicating the reduction in droplet size with increase in continuous phase velocity.

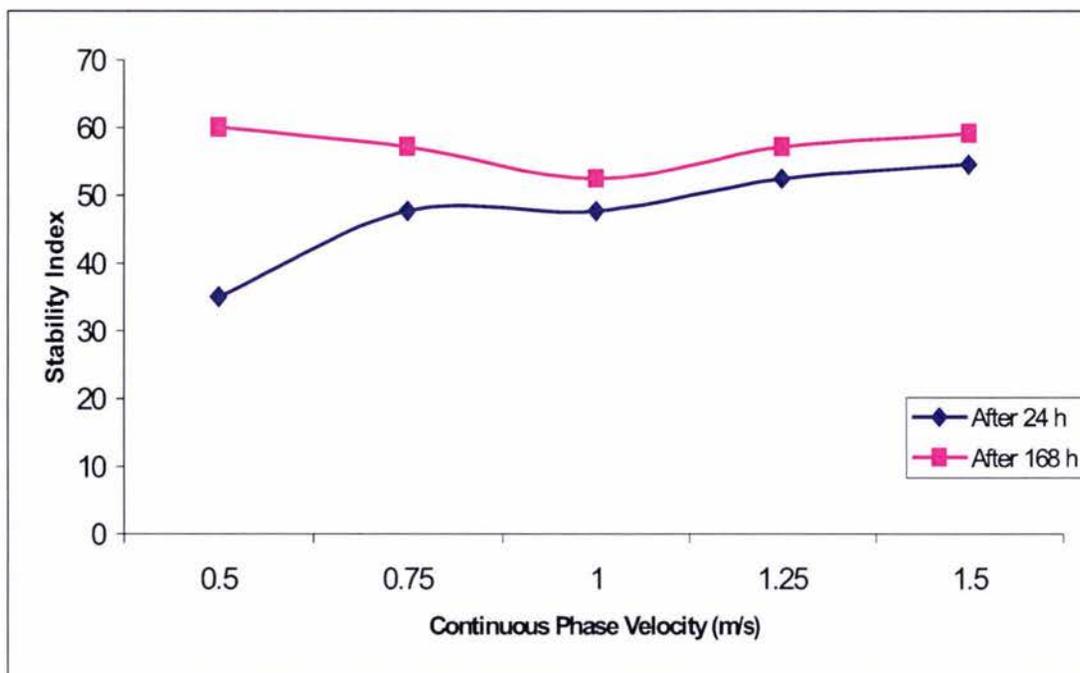


Figure 6.12 W/O/W emulsion prepared with 25% inner water phase, 10% PGPR pre-emulsified at 9500 rpm and homogenised at 500 bar pressure. The w/o/w multiple emulsions were made with 20% primary emulsion and 1% Tween80 in the external water phase with a 2 μm pore-size membrane. Osmotic pressure was not balanced.

From Figure 6.12, it can be seen that there is a very large drop in the stability of the multiple emulsion formed at a continuous phase velocity of 0.5 m/s when kept for a period of 1 week (168h). The extent of drop in yield was lower at 0.75 m/s velocity and was absent at 1 m/s and above. Another interesting point to note is that, the intensity in the opacity of the serum layer increased with increasing continuous phase velocity thereby indicating the production of greater number of smaller droplets with increase in velocity.

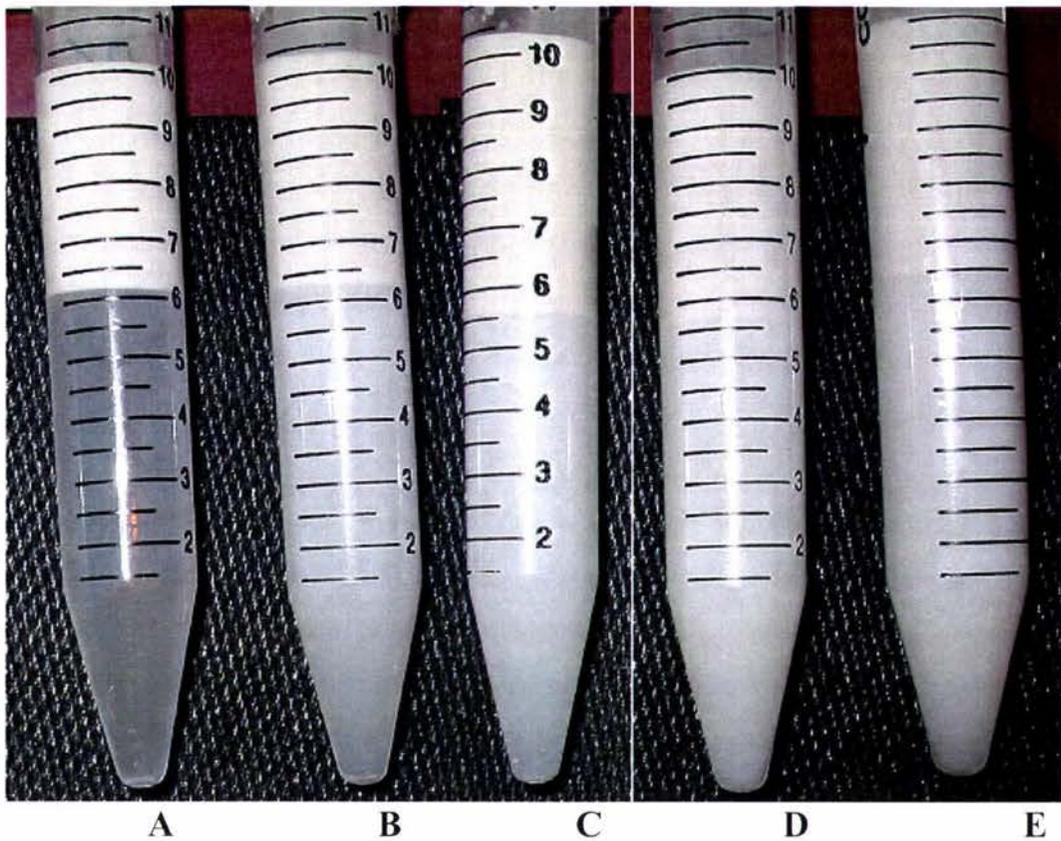


Figure 6.13 The continuous phase velocities are: A – 0.5m/s; B – 0.75m/s; C – 1m/s; D – 1.25m/s; E – 1.5m/s. Conditions as in Figure 6.13.

6.4.4 Analysis of dispersed phase flux and fouling behaviour

A high dispersed phase flux is very important for the commercial production of a product utilising the technology of membrane emulsification. Too low a flux will result in an uneconomical production (due to the increase in area required for a given throughput) and too high a flux will result in emulsions with a very low stability. Hence an optimum dispersed phase flux needs to be determined so that a stable, uniformly dispersed, high yielding w/o/w emulsion is formed at the highest rate. Of the various experiments conducted during the course of the research it was found that the transmembrane pressure, hydrophobic emulsifier concentration and membrane pore-size are the main factors affecting the flux of the dispersed phase into the continuous phase.

6.4.4.1 Effect of transmembrane pressure

During membrane emulsification the continuous phase velocity and pressure is typically constant throughout the process. Transmembrane pressure is the difference

between the dispersed phase pressure and the continuous phase pressure. The dispersed phase flux is directly proportional to the transmembrane pressure (Peng & Williams, 1998). According to Abrahamse *et al* (2003), the percentage of active pores increases linearly with increase in the transmembrane pressure. Ultrafiltration experiments conducted by Cheryan and Kuo (1984) on acidified cottage cheese whey and Cheryan and Nichols (1981) on defatted soy flour extracts prove the existence of a straight line relationship (direct proportionality) between transmembrane pressure and permeate flux.

In some experiments a reduced flux was observed even with a high transmembrane pressure. This could be due to the blocking of the pores by the constituents (WPC) of the w/o emulsion. The reduction in flux was thought to be due to membrane fouling as this occurs even when all the operating parameters like transmembrane pressure, temperature and concentration of dispersed phase are kept constant. As the feed stream's viscosity and density increases the flux should be lower than water purely from hydrodynamic considerations (Cheryan, 1998).

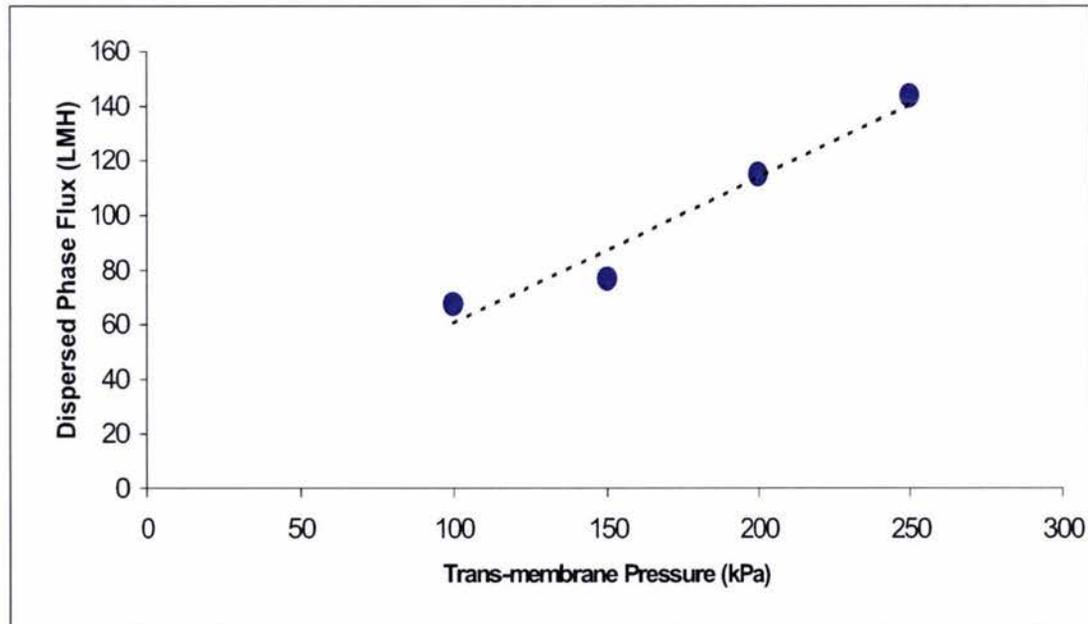


Figure 6.14 Variation in dispersed phase flux with transmembrane pressure. W/O/W emulsions produced with 25% inner phase, 10% PGPR, homogenised at 500bar (primary emulsion). Secondary emulsions are produced with a 2 μm SPG membrane. 1% Tween 80 was used as the hydrophilic emulsifier in the external phase. Figure shows the variation in dispersed phase flux with change in transmembrane pressure at a constant continuous phase velocity (1m/s). (LMH – $\text{L.m}^{-2}.\text{h}^{-1}$)

Figure 6.14 shows that the dispersed phase flux depends on transmembrane pressure. Darcy's law states that the dispersed phase flux (J) is directly proportional to the transmembrane pressure (ΔP) and inversely proportional to the membrane resistance (R_m) and coefficient of viscosity (η), i.e.

$$J = \frac{\Delta P}{R_m \eta} \dots\dots\dots (6.1)$$

The resistance (R) is an additive effect of membrane resistance (R_m) and fouling resistance (R_f). i.e. $R_{tot} = R_m + R_f$. Here R_m would remain a constant and the difference in R would only be brought about by the difference in R_f . Hence at a constant R_f an increase in transmembrane pressure causes an increase in dispersed phase flux.

Another possible explanation by Schroder and Schubert (1999) is,

$$J_d = \frac{V_d}{A} \dots\dots\dots (6.2)$$

where J_d is the dispersed phase flux, V_d is the volume flow rate and A is the area of the membrane surface, the dispersed phase flux depends on the volume flow rate which in turn depends on the transmembrane pressure and the membrane pore-size. The permeability of a membrane increases with increasing transmembrane pressure, which in turn results in the increased dispersed phase flux. Joscelyne & Tragardh (1999) reported that the Hagen-Poiseuille law could explain the increase in flux with the increasing effective transmembrane pressure.

$$J = \frac{\epsilon r^2 \Delta P}{8 \eta \tau \Delta x} \dots\dots\dots (6.3)$$

where ΔP is the pressure difference across a membrane of thickness Δx , ϵ is the surface porosity, r is the pore radius, η is the viscosity and τ is the pore tortuosity. According to Schroder *et al* (1999) the number of active pores taking part in the droplet formation for a transmembrane pressure of 100kPa is approximately 10% of the total number of pores in the membrane and the number increases with increasing transmembrane pressure.

Attempts were made to calculate the fouling resistance of the 2 μm SPG membrane at various transmembrane pressures (ΔP) and also the membrane resistance of various SPG membranes with different pore-sizes. Combining Equations 6.1 and 6.3 we get:

$$R_m = \frac{32 \delta_m \tau}{\varepsilon d_p^2} \dots\dots\dots (6.4)$$

where R_m is the membrane resistance, δ_m is the membrane thickness (usually 7mm), τ the membrane tortuosity (1.5), ε the membrane porosity (60%) (Vladislavljevic & Schubert, 2002) and d_p the pore diameter. Substituting the respective values, the Equation 6.4 can be simplified as:

$$R_m = \frac{0.056}{d_p^2} \dots\dots\dots (6.5)$$

Also Equation 6.1 can be rewritten as:

$$R_m = \frac{\Delta P}{J_w \eta_w} \dots\dots\dots (6.6)$$

where J_w and η_w are the water flux and coefficient of viscosity of water respectively. Using the Equations 6.5 and 6.6 the theoretical and actual R_m values respectively for different pore-size membranes was calculated and is shown in Table 6.2.

Pore-size (μm)	Dispersed ph flux (l.m⁻².h⁻¹)	R_m (x10¹¹)(m⁻¹) (theoretical)	R_m (x10¹¹)(m⁻¹) (actual)
0.9	--	0.691	0.83
1.4	21.42	0.286	0.49
2	101.74	0.14	0.23
3.8	163.71	0.039	0.06

Table 6.2 Table showing the relationship of pore-size with dispersed phase flux and membrane resistance.

It can be seen that the actual membrane resistance is more than the theoretical membrane resistance (Figure 6.15). Equation 6.4 suggests that the most likely factor that could account for this difference is variation in the porosity of the membrane

used, i.e. the estimated porosity of the membrane in use is less than that suggested by the manufacturer resulting in higher actual R_m . This is shown in Table 6.3.

Membrane Pore-Size (μm)	Porosity (stated)	Porosity (estimated)
0.9	60%	50%
1.4	60%	35%
2	60%	29%
3.8	60%	39%

Table 6.3 Table showing the stated and estimated porosities of the SPG membranes used.

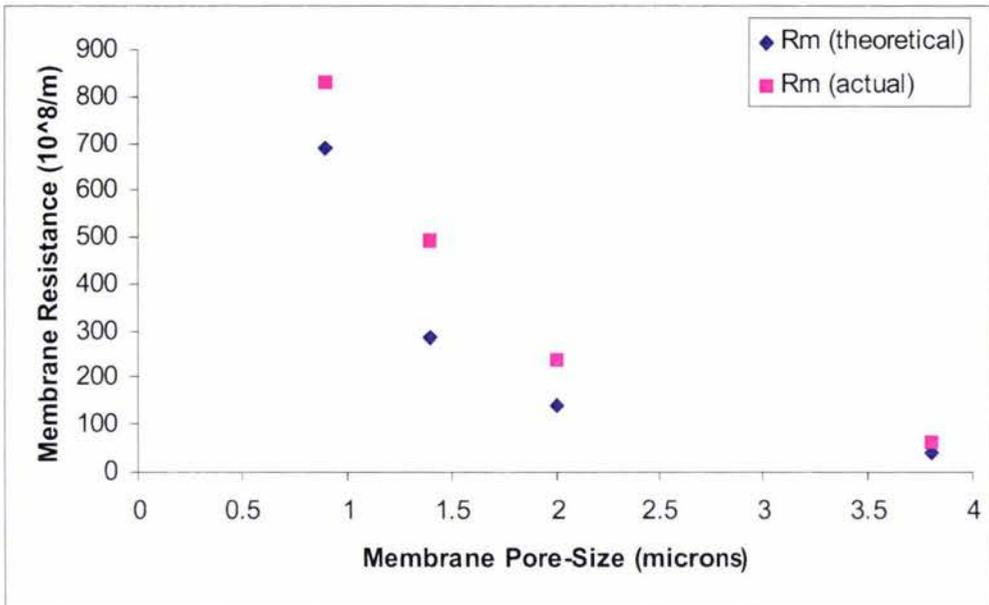


Figure 6.15 Theoretical and the actual membrane resistances for membranes of different pore-sizes (0.9, 1.4, 2 and 3.8 μm).

It can be seen that with increasing pore-sizes the R_m values (theoretical and actual) are closer to each other probably due to the fact that at higher pore-sizes the higher is the water flux through the membrane at the same transmembrane pressure (Darcy’s law). The fraction of pores that were active while measuring the R_m for different membranes at a particular ΔP of 100 kPa can be calculated (Vladisavljevic & Schubert, 2002):

$$k = \frac{J_w \eta_w R_m}{\Delta P} \dots\dots\dots (6.7)$$

The calculated values are given in Table 6.4.

Pore-Size (μm)	R_m (actual) ($\times 10^{11}$)	ΔP (kPa)	Fraction of active pores
0.9	0.93	100	1
1.4	0.49	100	0.99
2	0.238	100	1.02
3.8	0.06	100	0.99

Table 6.4 Table showing the fraction of active pores during water flux through the membranes at a ΔP of 100 kPa.

The direct proportionality of dispersed phase flux and the inverse proportionality of the membrane resistance with the membrane pore-size are shown in Figure 6.16.

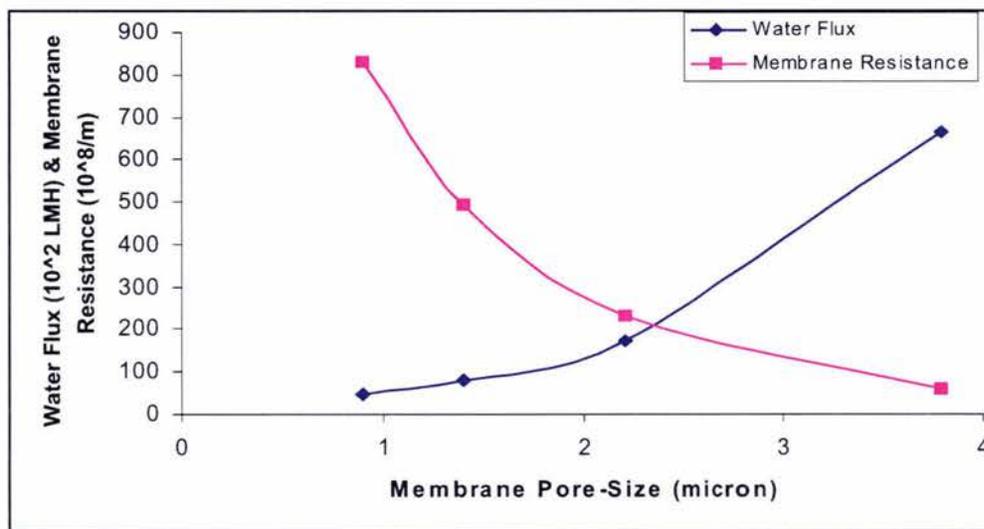


Figure 6.16 Graph showing the relationship of water flux and actual hydraulic membrane resistance with membrane pore size.

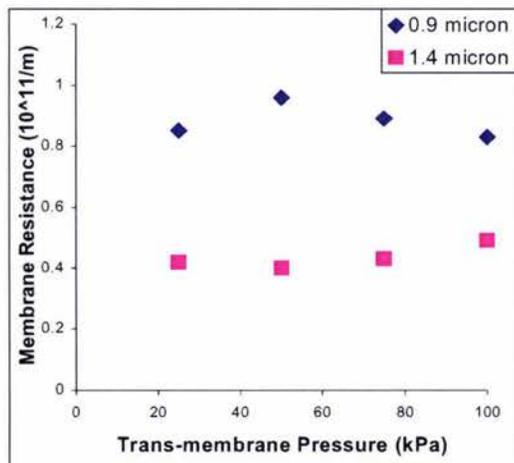


Figure 6.17 Graph showing the R_m of 0.9 and 1.4 μm membranes at different ΔP , (25, 50, 75 and 100 kPa).

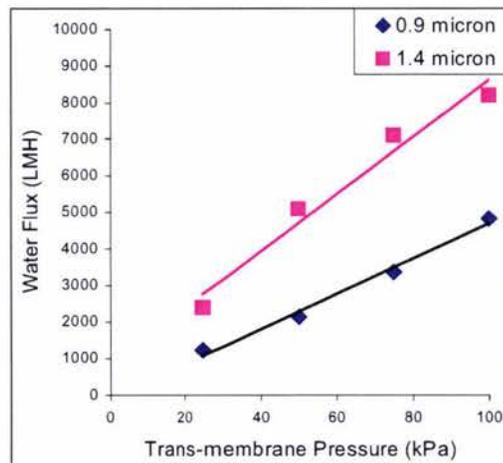


Figure 6.18 Graph showing the water flux through the 0.9 and 1.4 μm membranes at different ΔP (25, 50, 75 and 100 kPa).

From Figure 6.17 it can be seen that the R_m values for the 0.9 and 1.4 μm membranes are almost the same for different ΔP indicating the consistency in resistance offered

by membranes at higher water fluxes and transmembrane pressures as shown in Figure 6.18. Calculations done to estimate the fouling resistance (R_f) for the 2 μm membrane at different ΔP using Equation 6.1 gave results as shown in Table 6.5.

ΔP (kPa)	Dispersed phase flux (LMH)	R_m ($\times 10^{11}$) (m^{-1})	R_{tot} ($\times 10^{11}$) (m^{-1})	$R_f = R_{\text{tot}} - R_m$ ($\times 10^{11}$) (m^{-1})
100	67.32	0.23	1.07	0.84
150	76.5	0.23	1.41	1.18
200	114.8	0.23	1.25	1.02
250	143.8	0.23	1.25	1.02

Table 6.5 Estimated fouling resistances of a 2 μm membrane at different operating transmembrane pressures.

The data in Table 6.5 have been calculated using a viscosity of 50 m.Pa.s for the emulsions. The viscosity was not measured and this will be an important parameter to characterise in further work. From Table 6.5 it can be seen that the fouling resistance was similar at all transmembrane pressures for this data set. When the membrane pores are clear (as in the case of a new membrane) the dispersed phase passes through more freely and hence a high flux ($140 \text{ l m}^{-2} \text{ h}^{-1}$) can be attained with a low transmembrane pressure of 100-125 kPa and a membrane of pore size 2 μm . When the membrane pores gets blocked due to factors like fouling, higher internal water droplet size, higher concentration of WPC in the internal phase, improper cleaning etc, the transmembrane pressure builds up as either a reduced amount of membrane pores take part in the emulsification process or the effective pore-diameter is reduced. This leads to a condition where the dispersed phase flux may be only $20 \text{ l m}^{-2} \cdot \text{h}^{-1}$ even at a transmembrane pressure of 250 kPa. However, under optimal conditions, when the membrane resistance is lower, a dispersed phase flux of $145 \text{ l m}^{-2} \cdot \text{h}^{-1}$ could be attainable at 250 kPa. Thus, at a given ΔP of 250 kPa a high R_f means a reduced flux and a low R_f means an increased flux, as shown in the Table 6.6.

Dispersed phase flux ($\text{l m}^{-2} \text{ h}^{-1}$)	Fouling Resistance (m^{-1})
143.8	1.02×10^{11}
26	6.69×10^{11}
19	9.24×10^{11}

Table 6.6 Relationship of fouling resistance with dispersed phase flux for a 2 μm membrane at 250 kPa.

6.4.4.2 Effect of Hydrophobic Emulsifier Concentration

It was observed that there was a direct relationship between the concentration of hydrophobic emulsifier and the dispersed phase flux. According to Yuyama *et al* (2000) the rate of emulsification increases with an increase in hydrophobicity of the dispersion phase at a constant transmembrane pressure. Katoh *et al* (1996), through his experiments with the production of w/o emulsions with membrane emulsification proved that a suitable concentration and a suitable type of emulsifier could increase the dispersed phase flux up to 10 times.

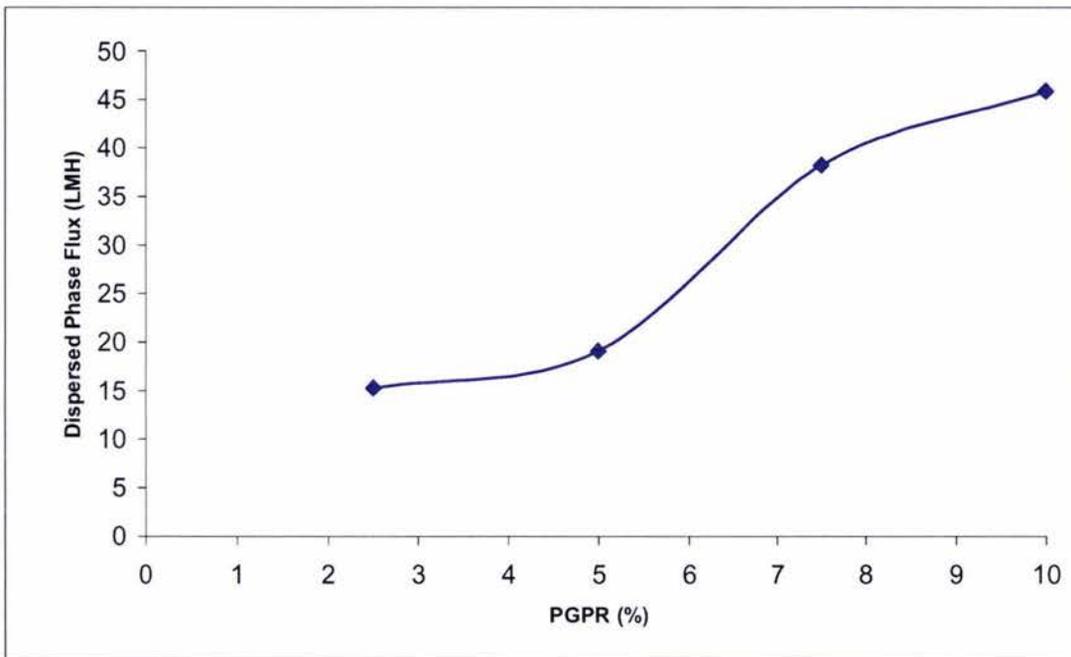


Figure 6.19 Variation in dispersed phase flux with hydrophobic emulsifier concentration. W/O/W emulsions produced with 25% inner phase and varying emulsifier concentrations of 2.5%, 5%, 7.5% and 10% PGPR, homogenised at 250 bar (primary emulsion). Secondary emulsions were produced with a 2 μm SPG membrane. 1% Tween 80 was used as the hydrophilic emulsifier in the external phase. Figure shows the variation in dispersed phase flux with change in hydrophobic emulsifier concentration.

From Figure 6.19 it can be seen that as the hydrophobic emulsifier concentration was increased from 2.5 wt% to 10 wt% the dispersed phase flux increased from $15 \text{ l.m}^{-2}.\text{h}^{-1}$ to $45 \text{ l.m}^{-2}.\text{h}^{-1}$. A similar behaviour was reported by Joscelyne & Tragardh (2000). Schroder *et al* (1998) reported that the mean flux depends on the emulsifier used. The above behaviour may be due to the decrease in droplet size with increase in the emulsifier concentration (Section 4.4.2.4 and Figure 4.11) leading to reduced resistance to the flow of the primary emulsion through the narrow pores (2 μm) of the

SPG membrane. Joscelyne & Tragardh (1999) reported that the critical pressure decreases on increasing the emulsifier concentration due to the reduction in oil-water interfacial tension. This can also increase the dispersed phase flux as the flux increases at a low critical pressure and the same transmembrane pressure. In this study, however, it was unable to determine the exact pressure at which permeation starts through the membrane and so the determination of critical pressure was impossible.

6.4.4.3 Effect of Membrane Pore-size

Dispersed phase flux depends to a very large extent on the pore-size of the membrane. Since the droplet size of the primary emulsion remains the same, the dispersed phase flux decreases with decreasing membrane pore size. This is because as the pore-size decreases it becomes more and more difficult for the primary w/o emulsion droplets to pass through.

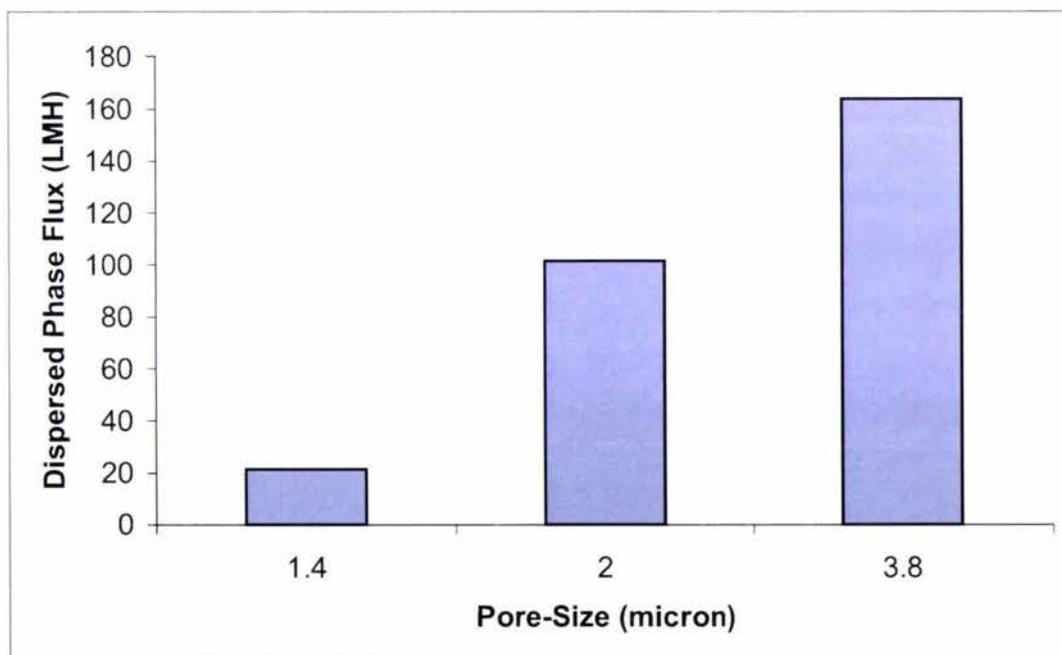


Figure 6.20 Variation in dispersed phase flux with pore size. W/O/W emulsions produced with 25% inner phase and 10% PGPR, homogenised at 500bar (primary emulsion). Secondary emulsions are produced with varying pore-sizes of SPG membranes (1.4 μ m, 2 μ m, 3.8 μ m). 1% Tween 80 was used as the hydrophilic emulsifier in the external phase. Figure shows the variation in dispersed phase flux with change in membrane pore-sizes.

In Figure 6.20 it is indicated that the dispersed phase flux depends not only on the transmembrane pressure but also on the pore-size of the membrane. Accordingly the Darcy's law is modified as (Schroder & Schubert, 1999),

$$J_d = \frac{B_0}{\eta_d D_p^2 P_e/L} \dots\dots\dots (6.8)$$

where J_d is the dispersed phase flux, B_0 is the modified permeability as determined by the membrane porosity and the number of active pores at which droplets form, η_d is the viscosity of the dispersed phase, D_p is the mean pore size and P_e/L is the pressure gradient. According to Joscelyne & Tragardh (1999) the increase in dispersed phase flux with increasing membrane pore-size can be explained by the Hagen-Poiseuille law since the flux through the capillary is super-proportional to the pore-size in the capillary. An increase in the pore resistance means a decrease in pore diameter due to fouling resulting in a lower droplet formation rate (Abrahamse *et al*, 2003).

6.4.4.4 Effect of WPC Concentration

In the experiments conducted with the aim to determine the effect of the increasing WPC concentrations on the stability of the w/o/w emulsions (Section 5.4.4) observations were also made on the influence of the WPC concentrations on the dispersed phase flux. Figure 6.21 shows the variation in dispersed phase flux with variation in WPC concentration in the inner phase.

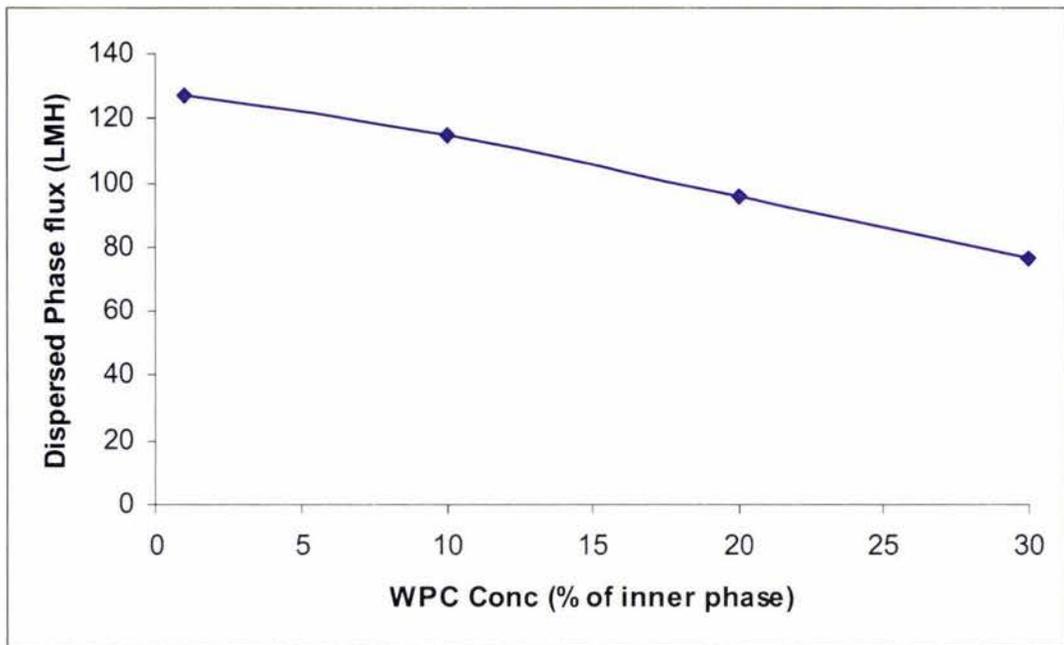


Figure 6.21 Variation in dispersed phase flux with WPC concentration. W/O/W emulsions prepared with a 2 μm SPG membrane and containing 1% Tween 80 in the outer phase. W/O emulsion consists of 25% inner phase with varying concentrations of WPC and 10% (of oil phase) PGPR homogenised at 500 bars.

It was observed that the dispersed phase flux decreases with increasing WPC concentrations in the inner phase of the w/o/w emulsions (Figure 6.21). One possible explanation for this kind of behaviour could be the decrease in permeation of the dispersed phase through the pores of the membrane. The increase in WPC concentrations means the increase in the amount of dissolved proteins in the inner phase. A higher concentration of the proteins may block the pores of the membrane and resulting in a decrease in the number of pores taking part in the emulsification process. This in turn may cause the decrease in the flux of the dispersed phase. The increase in the transmembrane pressure with time at a smaller dispersed phase flux was also observed, which also suggests that there is a decrease in permeation with increase in WPC concentrations. This effect can be explained by the fouling phenomenon (Section 6.4.4.1). But further research need to be done to confirm whether the effect is only restricted to microfiltration or if the same principle could be extended to explain the effects of membrane emulsification.

6.5 Conclusion

This chapter deals with the membrane process parameters, which include the membrane pore-size, transmembrane pressure, continuous phase velocity and dispersed phase flux and their effect on the yield stability and droplet size of w/o/w emulsions produced.

With membranes of different pore-sizes there was a slight difference in the initial yield of w/o/w emulsions produced as the pore-size increase results in a slight increase in yield. Droplet size of w/o/w emulsions increases linearly with increase in pore-size in the case of 2 μm and 3.8 μm membranes. However, the 1.4 μm membrane produced droplets of greater size than the 2 μm membrane, which was attributed to the higher transmembrane pressure required in the secondary emulsification with a 1.4 μm membrane.

No major effect of transmembrane pressure was observed on the yield stability of w/o/w emulsions produced. However the long-term stability was enhanced on storage of samples at 5 °C. Droplet sizes [$d_{(3,2)}$ and $d_{(4,3)}$] exhibited similar behaviour and no considerable differences were observed. This was confirmed with the physical stability results as all the samples showed the same amount of cream layer. Osmotic pressure was not balanced for these experiments as the effect of osmotic pressure were not investigated during the early stages of the project given its positive impact on droplet size distribution. Further research should be conducted to get a clearer picture of the effect of transmembrane pressure on droplet size of w/o/w emulsions produced under different osmotic gradients.

Continuous phase velocity did not show much influence on the yield stabilities of w/o/w emulsions produced. Again samples stored at 5°C were observed to show a greater long-term stability than samples stored at 20 °C. In the case of droplet sizes of emulsions, $d_{(3,2)}$ exhibited a downward trend and $d_{(4,3)}$ an upward trend with increase in velocity. The behaviour of $d_{(4,3)}$ was probably due to lumping as a result of osmotic effects. The physical characteristics supported the $d_{(3,2)}$ behaviour as it was found that w/o/w emulsions produced at higher velocities had more opaque serum layers than the ones produced at lower velocities consistent with a smaller droplet diameter.

With membranes of different pore-sizes there was a slight difference in the initial yield of w/o/w emulsions produced as the pore-size increase results in a slight increase in yield. Droplet size of w/o/w emulsions increases linearly with increase in pore-size in the case of 2 μ m and 3.8 μ m membranes. However, the 1.4 μ m membrane produced droplets of greater size than the 2 μ m membrane, which was attributed to the higher transmembrane pressure involved in the secondary emulsification with a 1.4 μ m membrane.

Experiments were also conducted on the effect of various processing parameters on the dispersed phase flux. It was observed that an increase in transmembrane pressure, hydrophobic emulsifier concentration and membrane pore-size increased the dispersed phase flux whereas an increase in the WPC concentration decreased the dispersed phase flux. Membrane fouling was significant and a strong influence on flux was observed. Further research to understand the causes of fouling and impact on cleaning would be helpful.

7. Overall Discussion, Recommendations and Conclusion

7.1 Overall Discussion

Membrane emulsification is a relatively new technique and is commonly accomplished using SPG (Shirazu Porous Glass) membrane. The method is ideal for the production of multiple emulsions owing to its advantages in comparison with the conventional emulsification methods. These major advantages include:

- low shear
- low energy consumption
- higher encapsulation efficiency
- uniform droplet size distribution and
- better control over the droplet size of the emulsion formed.

Multiple emulsions formed by membrane emulsification can be utilized for the microencapsulation of active ingredients within the inner phase of the emulsion. Microencapsulation is the process by which small particles of solid, liquid or gas are packaged within a secondary material to form a microcapsule (Augustin *et al*, 2001). The technology is well established in the fields of pharmaceuticals, cosmetics, chemical and printing. For the food industry, in recent times more applications are being discovered, especially in the area of flavour encapsulation as well as in controlled release of flavours. For example Augustin *et al* (2001) proposed that:

- minerals having a very bad taste can be conveniently masked
- desirable flavours can be encapsulated in chewing gums and frozen desserts

Several types of food ingredients have so far been microencapsulated and several methods of encapsulation have so far been tried. The focus of this research was on the microencapsulation of dairy proteins or protein products in the inner water phase of the water-in-oil-in-water multiple emulsions by membrane emulsification.

The technology of multiple emulsions and membrane emulsification, even though it has potential for industrialization, poses a few challenges. The first and foremost of these is the stability of the primary emulsion. As discussed in Section 4.4.2, the type and concentration of the hydrophobic emulsifier is of utmost importance for attaining a stable primary emulsion. Many previous studies (Section 2.1.5 and 4.1.1) discuss the use of Span 80 at a concentration of 10 wt% or higher. However this research did not show Span to be a suitable emulsifier. W/O emulsions stabilized by Span 80 not only formed an unstable primary emulsion but also a low yield w/o/w emulsion. In contrast, using PGPR as the hydrophobic emulsifier at a concentration of 10% gave excellent results with a primary emulsion that was stable for more than 6 months and a w/o/w emulsion that initially gave approximately 100% yield. This was similar to the results obtained for researchers like Benichou *et al* (2001). Thus PGPR satisfies a major requirement of an acceptable commercial product (high yield and high stability), but does have a disadvantage attached to it. The ADI (acceptable daily intake) of PGPR is only 7.5 mg/kg body wt. So, for instance, if the average body weight is taken to be 75 kg, the person can consume only 0.5625g of PGPR per day. This means that at a 10% (of oil) level the maximum daily intake of a product containing 15% oil should be restricted to ~ 100ml. This may be acceptable for a 'tonic' type of product, but not for a mainstream beverage or nutritional formulation.

Further research should therefore be conducted to overcome this problem. Preliminary attempts in this regard were made in two ways commencing with the use of emulsifiers with higher ADI values in combination with PGPR. Initial studies with combinations of Span 80 and PGPR did not show good stability (Section 4.4.2.1) but other combinations of emulsifiers may be more effective. A second key focus was to load the maximum possible amount of protein into the inner phase hence the maximum possible internal phase volume and the maximum possible WPC concentration were always sought after. Increasing the internal phase volume ($\Phi_{w/o}$) beyond 30 wt% led to a low yielding w/o/w emulsion (Section 4.4.3) and an increase in WPC concentration above 10% of the inner phase led to problems in membrane performance such as reduced flux (Section 6.4.4.4). Several other factors like transmembrane pressure, membrane pore-size and hydrophobic emulsifier concentration also affected membrane performance. Adequate flux and run times are highly significant on an industrial scale, when large volumes of the product need to

be continuously manufactured. Another major factor that needs mention here is that the inner phase of the w/o/w emulsion should only consist of materials that are completely soluble so that it may not block the membrane pores thereby increasing fouling and reducing the dispersed phase flux.

Yield measurements of the w/o/w multiple emulsions were done using an Advantage Glucose Meter. The technique is relatively new and there is not much reference to this method by the previous researchers. However the method proved to be very useful as sufficiently accurate glucose measurements can be taken conveniently from the sample without any sample preparation (Section 3.2.7 and 3.5) and so it would be appropriate for in-plant quality control. Obviously this only measures the stability with respect to glucose and the actual accuracy of the method, as an estimate of stability with respect to the encapsulated ingredient, will depend on their relative ratios of transport through oil. The glucose (marker) diffuses through the oil layer more easily than other solutes like proteins, presumably owing to the fact that glucose is a smaller molecule than protein. There is little data on the diffusivities of components in soybean or other oils and hence further investigations would be helpful to better understand the stability measurements. There is a visual proof of the influence of hydrophobic emulsifier (PGPR) on the diffusivity of glucose through the oil membrane (Section 4.4.2), so knowledge of the effect of different emulsifiers and their concentration on solute transport would also be helpful.

Previous research showed a considerable effect of process parameters on the w/o/w emulsions formed (Section 2.5). For instance, Joscelyne & Tragardh (2000) reported that the droplet size decreases considerably with increasing wall shear stress or decreasing transmembrane pressure. Overall, for the variables investigated in this study, the process parameters like transmembrane pressure and continuous phase velocity on the yield stability or droplet size did not show any particular marked effects. This may in past be due to the effect of osmotic pressure (Section 5.4.3) as many of the experiments conducted were under osmotically unstable conditions. However it appears that there is a suitable operating envelope between 100 kPa – 250 kPa (transmembrane pressure) and 1 m/s – 1.5 m/s (continuous phase velocity) for a 2 μ m pore-size membrane. A further increase in both parameters has not been tried and should be investigated prior progressing further towards commercialisation. As the

dispersed phase flux increases with transmembrane pressure, it may be possible to increase the production rate by this means without much change in the properties of the w/o/w emulsions formed. Membrane pore-size is also very important in the industrial scale production of the multiple emulsions. Results obtained in this study confirmed the literature as it was seen that there is a considerable influence of the membrane pore-size on the emulsion properties (Sections 2.5.1.3 and 6.4.1). A higher pore-size would be preferred for an increased productivity, but this in turn results in increased droplet size causing the physical instability (creaming) of the product. Of the three pore-size membranes used (1.4, 2 and 3.8 μm) the 2 μm membrane appeared to give the best results. Literature suggests the droplets formed to be 2 – 10 times bigger than the pore-size (Section 2.5.1.3). Thus the creaming instability can only be reduced and not eradicated by this method, as the droplets formed are usually in the range of 10 – 20 μm ($d_{(3,2)}$).

Droplet size can also be controlled by stabilizing the osmotic gradient between the inner and outer aqueous phases of the w/o/w emulsion using a suitable solute in the outer phase, e.g. sorbitol. As explained in Section 5.4.3 the osmotic inflow and outflow of water from the oil droplet results in bulging and shrinkage of the droplets, respectively, causing considerable variation in droplet sizes. Further research could be conducted into these effects as there is scope for exploiting this phenomenon. Increasing the osmotic gradients by increasing the solute concentration in the outer phase will lead to the shrinkage of droplets, which may in turn result in a more uniform emulsion with a much reduced cream layer. The main aim in rectifying the creaming instability is to give the product a uniform appearance and hence increase its consumer appeal. Coalescence of droplets in the cream layer did not appear to cause instability, at least with the PGPR / Tween system employed. Such approaches will not completely solve the problem of creaming, but related studies (data not shown) suggest the inclusion of gums like xanthan at suitable concentrations (0.2%) in the outer phase will assist in further alleviating the problem. However further research into the best combination of osmotic control and added stabilisers should be conducted as, for example, the viscosity of the final emulsion increases on addition of xanthan.

The droplet size of the primary emulsions was obtained using the Malvern Ultrasizer employing ultrasound as the measurement system. This is a relatively new method, and results depend on the accuracy of the system parameter values which the user enters. As values are not known for oil-emulsifier systems only oil data were used and hence the results obtained could only be expressed as an apparent value. As a consequence, w/o samples treated at the same conditions were observed under the confocal microscope for confirmation of the estimated droplet size. For most of the cases, the two methods agreed, but there were a few cases of disagreement. The reason for this could lie either with the microscopic image, as the confocal microscope provides a very narrow picture of one slice of a few micrometer thickness, or with the Ultrasizer, as the equipment, even though gives a broader picture, may experience technical difficulties when dealing with samples with a higher dispersed phase concentration ($> 25\%$ of the w/o) and the results were sensitive to the selected parameter values. Further research is needed as the Malvern Ultrasizer method might prove to be a very efficient method for droplet size determination of w/o emulsions.

Calculations were done to estimate the surface excess concentration of both the hydrophobic (PGPR) (Section 4.4.2.4) and hydrophilic (Tween 80) (Section 5.4.3.3) emulsifiers used and also to determine the surface loading of these emulsifiers. The results obtained showed an expected behaviour, but could only be considered as apparent values as the calculations were based on droplet size measurements with the Ultrasizer. Further research in this direction might prove to be helpful to understand, more clearly, the behaviour of emulsifiers at the w/o and o/w interfaces.

The effect of various hydrophilic emulsifiers were also investigated and it was found that there was not much difference on the yield with whey protein isolates, soy protein isolates, sodium caseinates and Tween 80 (Section 5.4.2). This is a very positive sign as it gives an indication that synthetic emulsifiers can be conveniently replaced by natural emulsifiers without affecting the emulsion properties. This will increase the customer appeal, make the product healthier, and provide some flexibility to product developers.

Thermal stability of the w/o/w emulsions formed was an important parameter investigated in order to indicate the acceptable range of processing conditions for potential commercial products. It was found that a higher temperature during production inversely affected the yield stability and droplet size of the emulsions formed (Section 5.4.1). This may present a challenge, as heat treatment of the product is essential for its sterilization. A possible alternative would be the sterilization of the ingredients before emulsification and final emulsification under aseptic conditions followed by aseptic packaging, but this may be uneconomical from an industrial point of view. Hence the effect of conventional heat treatment methods like retorting or UHT after emulsion formation must be evaluated.

The next important feature studied was the storage stability of the formed emulsions (Section 5.4.1 and 5.4.3.5). Optimum conditions were determined to make the product as shelf stable as possible. The main factor affecting the storage stability of the w/o/w emulsion was the temperature, as the yield decreased more rapidly with increase in temperature and osmotic gradient across the oil layer. This might indicate extra costs associated with the storage of the product as storage at 5°C was found to give the best results. Also, an osmotic gradient with the solute concentration slightly higher on the inner phase gave the maximum yield and minimum droplet size. Further research should be conducted to determine possible difference between the behaviour of the actual encapsulant and the marker (glucose). As indicated earlier in this chapter, the diffusion rates of different solutes through the oil layer are likely to be different and the additional expenditure involved with the refrigerated storage of the product might not be required if the yield decreases more slowly as a consequence.

7.2 Recommendations

- 1) In general the osmotic gradient between the inner and outer phase of the w/o/w emulsion should be balanced as this generally improves the yield stability, droplet size measurements and viscosity of the final emulsion formed. However, if required, the droplet size of the final emulsion can be manipulated by increasing the outer phase osmotic pressure by the addition of suitable solutes.

- 2) Emulsifier mixtures giving low HLB values, high ADI values and high tensile strength and flexibility at interfaces are recommended in the primary w/o emulsion. PGPR satisfies the first and last of these criteria, but has a very low ADI value of 7.5 mg/kg body weight.
- 3) There is a benefit of having a finer droplet size distribution for the w/o emulsions. Trials should be conducted with PGPR or suitable emulsifier mixtures as the hydrophobic emulsifier using the microfluidiser for primary emulsification.
- 4) The accuracy and reproducibility of Malvern Ultrasizer measurements of w/o emulsions should be further researched, perhaps using microspheres formed via membrane emulsification, as if successful, it provides a very convenient method of droplet size estimation.
- 5) Further investigation should be conducted to understand the effect of variation in hydrophilic emulsifier concentration and also the primary w/o emulsion volume fraction on the yield stability and droplet size of w/o/w emulsions formed. These should also include measurements of the surface coverage of emulsifiers (both hydrophobic and hydrophilic), as this would give an indication regarding the EMC (effective minimum concentration) of emulsifiers to be used.
- 6) More work is recommended on the characterization of SPG membrane performance as the dispersed phase flux is affected as a result of fouling in some cases. Devising a standard rapid cleaning procedure and water flux measurements before and after emulsification would be helpful.
- 7) Use of stabilizers in the outer phase to reduce the creaming effect is recommended to produce an emulsion with a uniform appearance and high consumer appeal. Xanthan gum at the rate of 0.2 wt% in the outer phase may solve this problem to a greater extent, but other stabilisers might be more suitable in different food applications.

- 8) Viscometer measurements of w/o/w emulsions should be conducted in future research in order to quantify the viscosity changes during storage of w/o/w emulsions. This may prove to be helpful in understanding the interfacial properties of PGPR or other emulsifiers or emulsifier blends.

7.3 Conclusion

Overall this study has demonstrated that a stable multiple emulsion can be prepared using SPG membrane containing 10% dairy protein in the inner phase, 25 wt% inner phase in the primary w/o emulsion, PGPR concentration of 10 wt% in the oil phase with the primary emulsification conducted in a homogeniser at 500 / 100 bars pressure, 20 wt% of primary w/o emulsion in the w/o/w emulsion and 1% Tween 80 in the outer phase. For a 2 μm membrane $\Delta P = 100 - 150$ kPa and $J = 140$ lm^2h^{-1} , droplets of 10 – 20 μm were formed and were stable with initial yield stability of 90 – 100% and > 70% yield stability on storage at 5°C for a week. This offers the potential to develop commercial products.

Glossary

P	-	Laplace pressure
γ	-	Interfacial tension
R_1 and R_2	-	Principal radii of curvature
R	-	Radius of spherical droplet
W_e	-	Weber number
η_c	-	Viscosity of the continuous phase
G	-	Velocity gradient
r	-	Droplet radius (μm), Membrane pore radius (μm)
V	-	Stoke's velocity
D_s and D_m	-	Densities of the dispersed phase and dispersion medium
η	-	Coefficient of viscosity of the dispersion medium
V_0	-	Stoke's velocity for simple droplets
x	-	Volume fraction of the dispersed phase
Y	-	Yield of w/o/w emulsion or encapsulation efficiency
M_i	-	Amount of dye encapsulated in the internal phase
M_o	-	Amount of dye in the outer phase
D_d	-	Diameter of the droplet (μm)
D_p	-	Diameter of the pore (μm)
ΔP	-	Transmembrane pressure (kPa)
P_d	-	Pressure of the dispersed phase outside the membrane
$P_{c,1}$ and $P_{c,2}$	-	Pressures at both ends of the membrane module
P_c	-	Critical pressure
J_d	-	Dispersed phase flux ($\text{l m}^{-2} \text{h}^{-1}$)
B_o	-	Modified permeability
η_d	-	Viscosity of dispersed phase
p_e/L	-	Pressure gradient
E_v	-	Energy density
P_v	-	Power density
P	-	Power input
V, V_d	-	Volume flow rate
t	-	Mean residence time of the droplets in the dispersing zone
d_{WMD}	-	Weighted mean diameter

$GM_{(1)}$	-	Geometric mean of the 1 st peak (Ultrazizer)
$GM_{(2)}$	-	Geometric mean of the 2 nd peak (“)
$Prop_{(1)}$	-	Proportion of the 1 st peak. (“)
$Prop_{(2)}$	-	Proportion of the 2 nd peak. (“)
$d_{(3,0)}$	-	Number, Volume Mean Diameter
$d_{(3,2)}$	-	Surface, Volume Mean Diameter
$d_{(4,3)}$	-	Weight, Moment Mean Diameter
S	-	Creaming stability index
H _s	-	Height of the serum layer
H _c	-	Height of the cream layer
A	-	Interfacial area (m ²), Membrane surface area (m ²)
V	-	Volume of the internal phase in the w/o emulsion (m ³)
N	-	Amount of emulsifier (Kg)
ρ	-	Density of emulsifier (Kg/m ³)
δ	-	Interfacial thickness of emulsifier (nm)
Γ	-	Surface loading of the emulsifier (mg/m ²)
Π	-	Osmotic pressure
β	-	Number of hydrolysable ions
n	-	Molarity
R	-	Universal gas constant
T	-	Temperature
R_{tot}	-	Total resistance to dispersed phase flux (m ⁻¹)
R_m	-	Membrane resistance (m ⁻¹)
R_f	-	Fouling resistance (m ⁻¹)
$\Delta x, \delta_m$	-	Membrane thickness
ε	-	Surface porosity of the membrane
τ	-	Membrane pore tortuosity.
d_p	-	Membrane pore diameter
J_w	-	Water flux
η_w	-	Coefficient of viscosity of water
k	-	Fraction of active pores on the membrane
B_0	-	Modified permeability
P/L	-	Pressure gradient

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Appendix A

(W/O emulsions)

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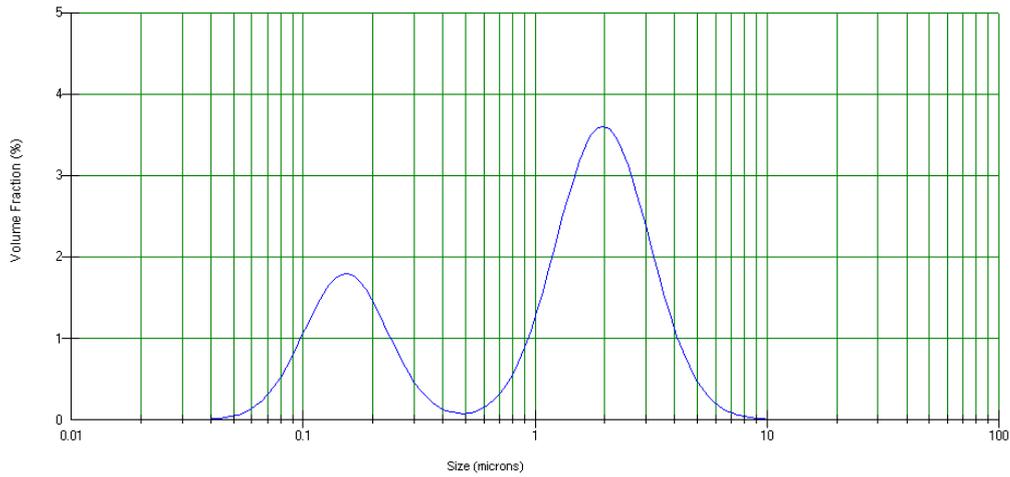


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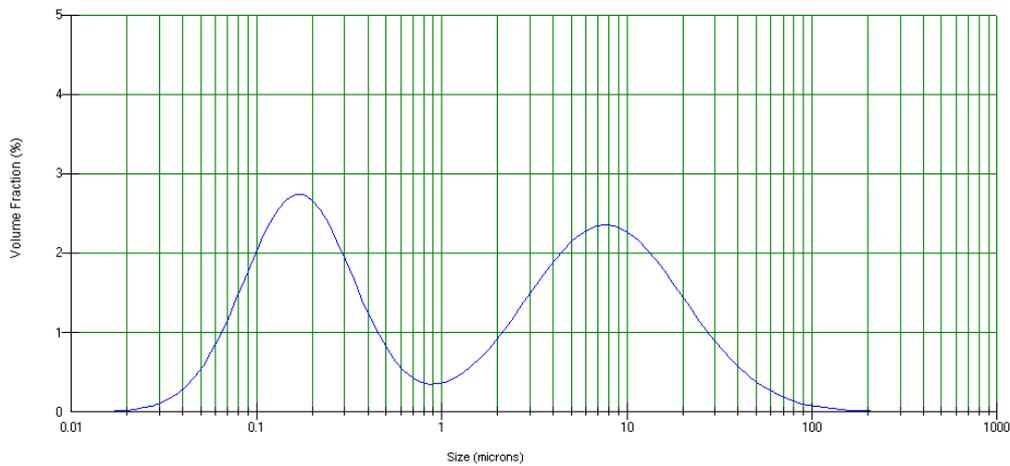


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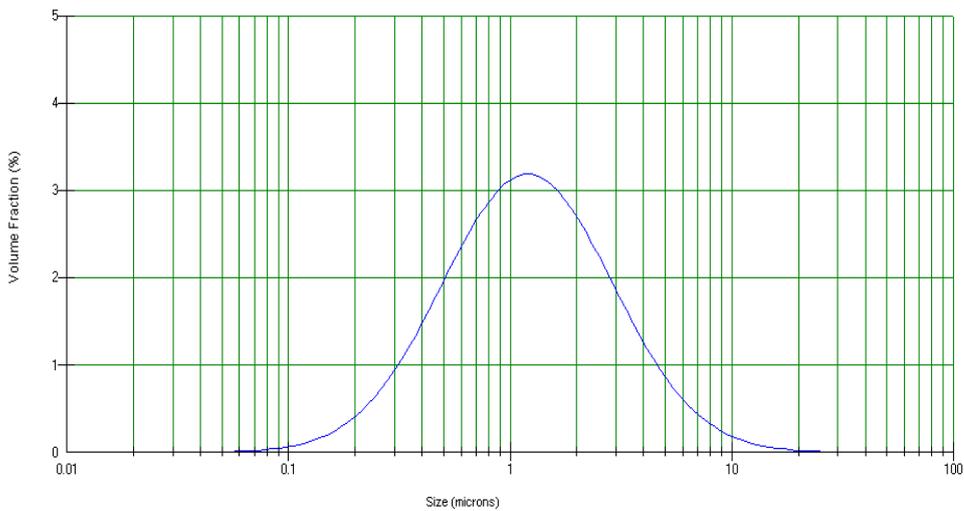


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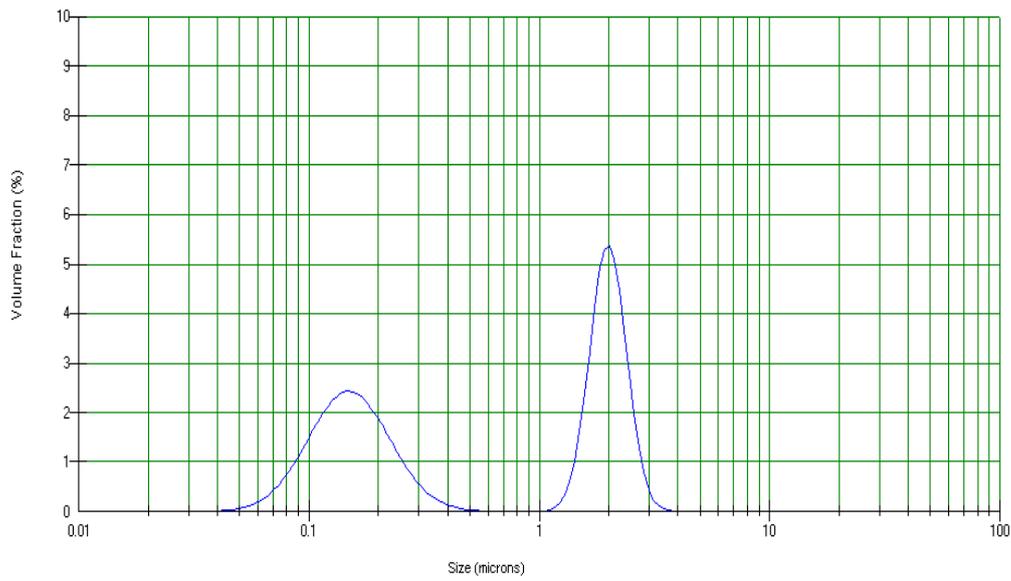


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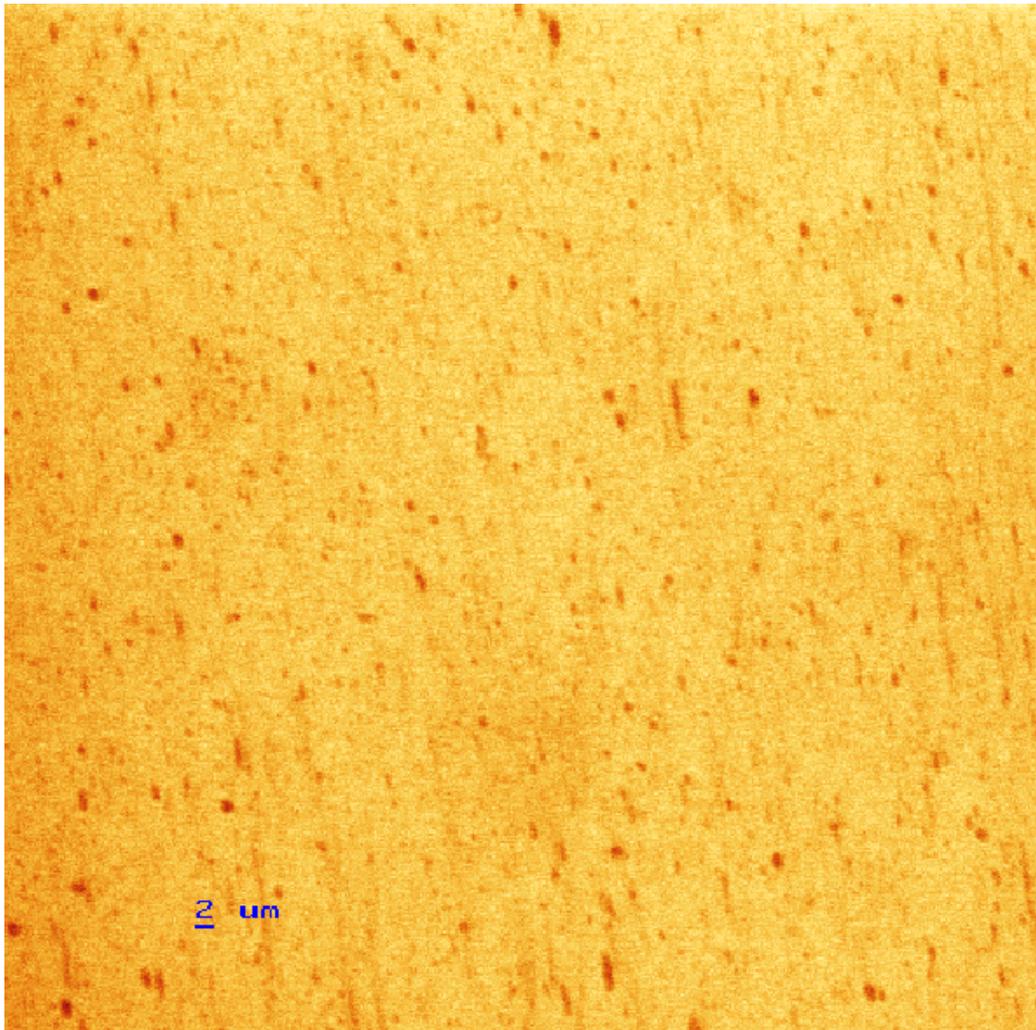


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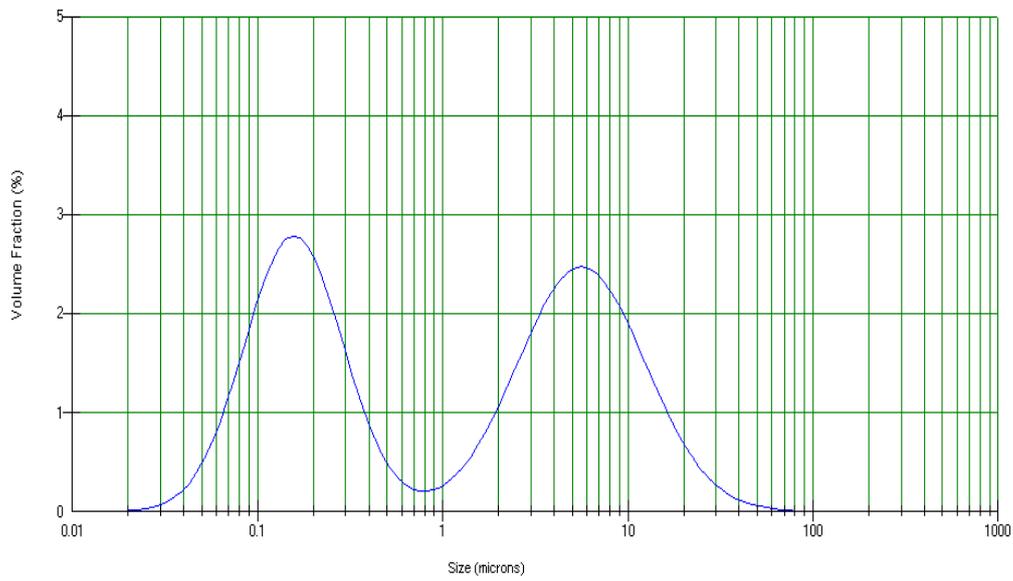


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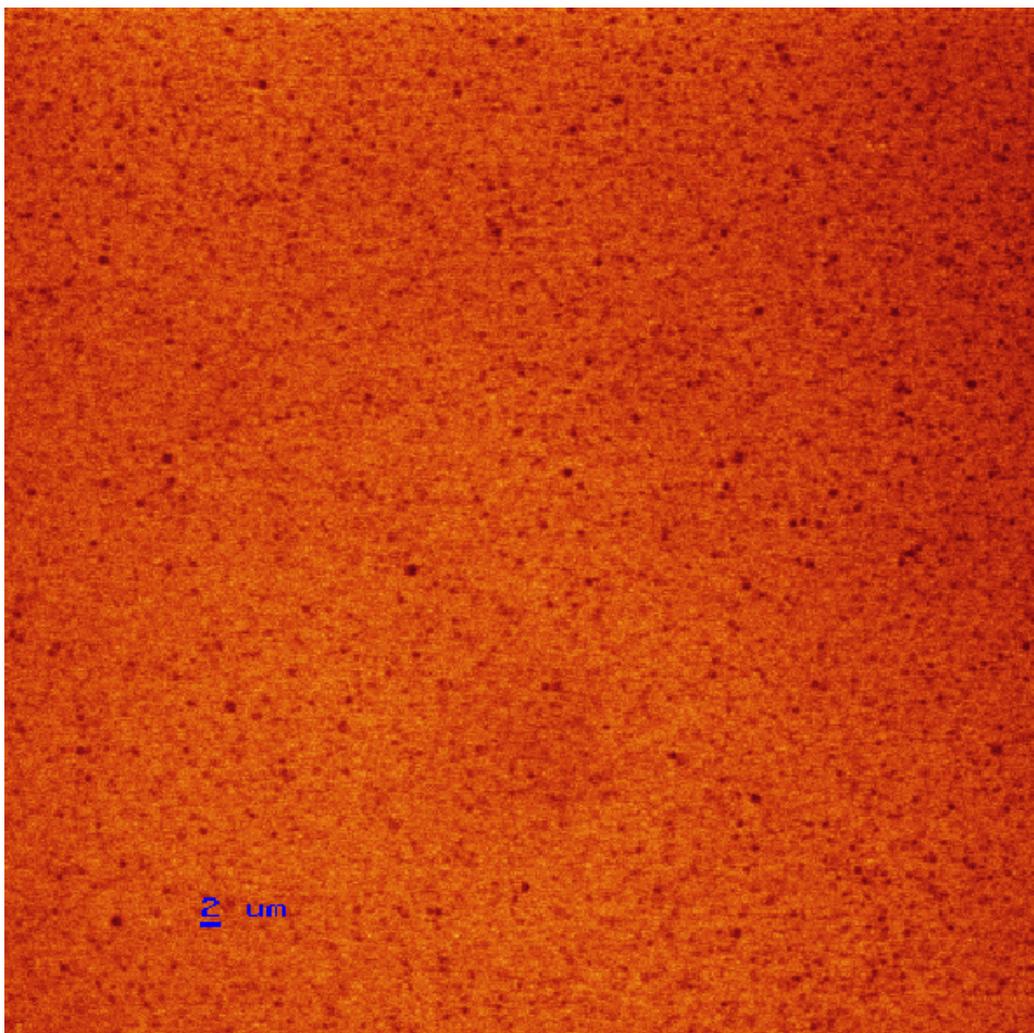


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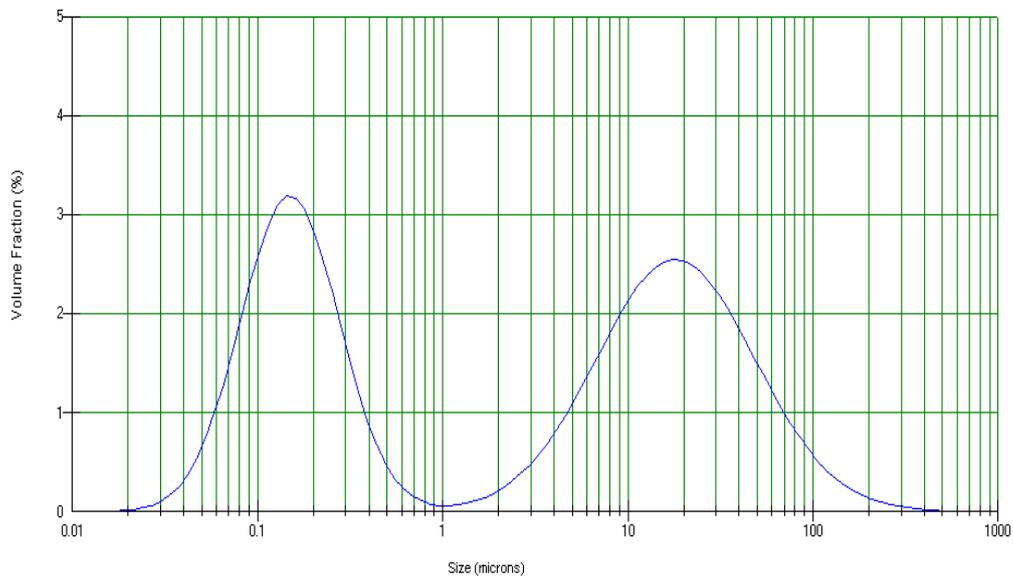


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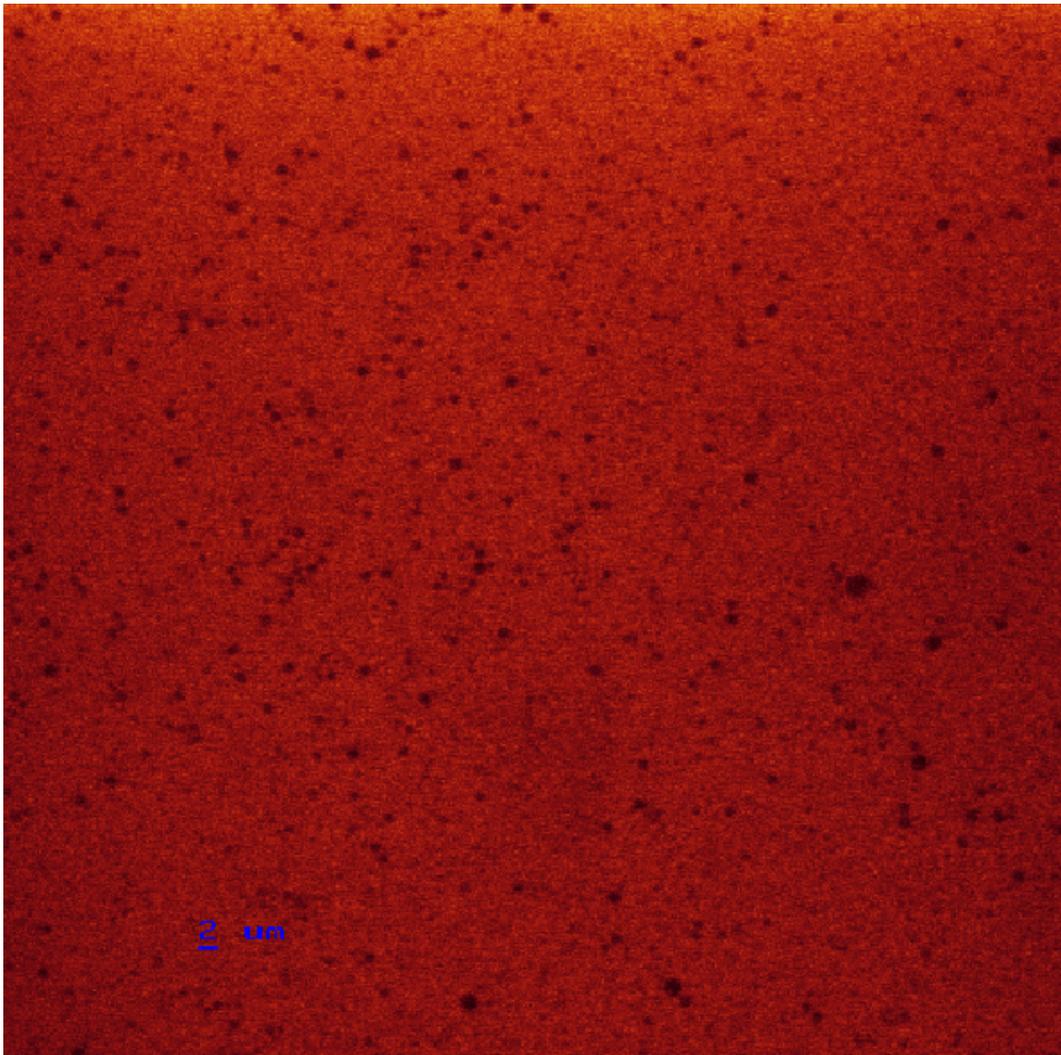


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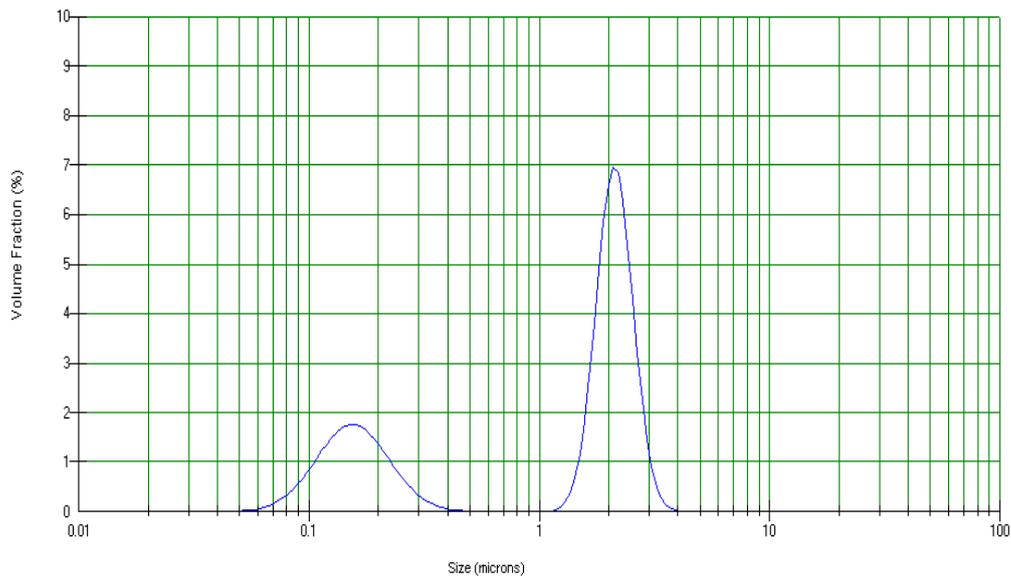


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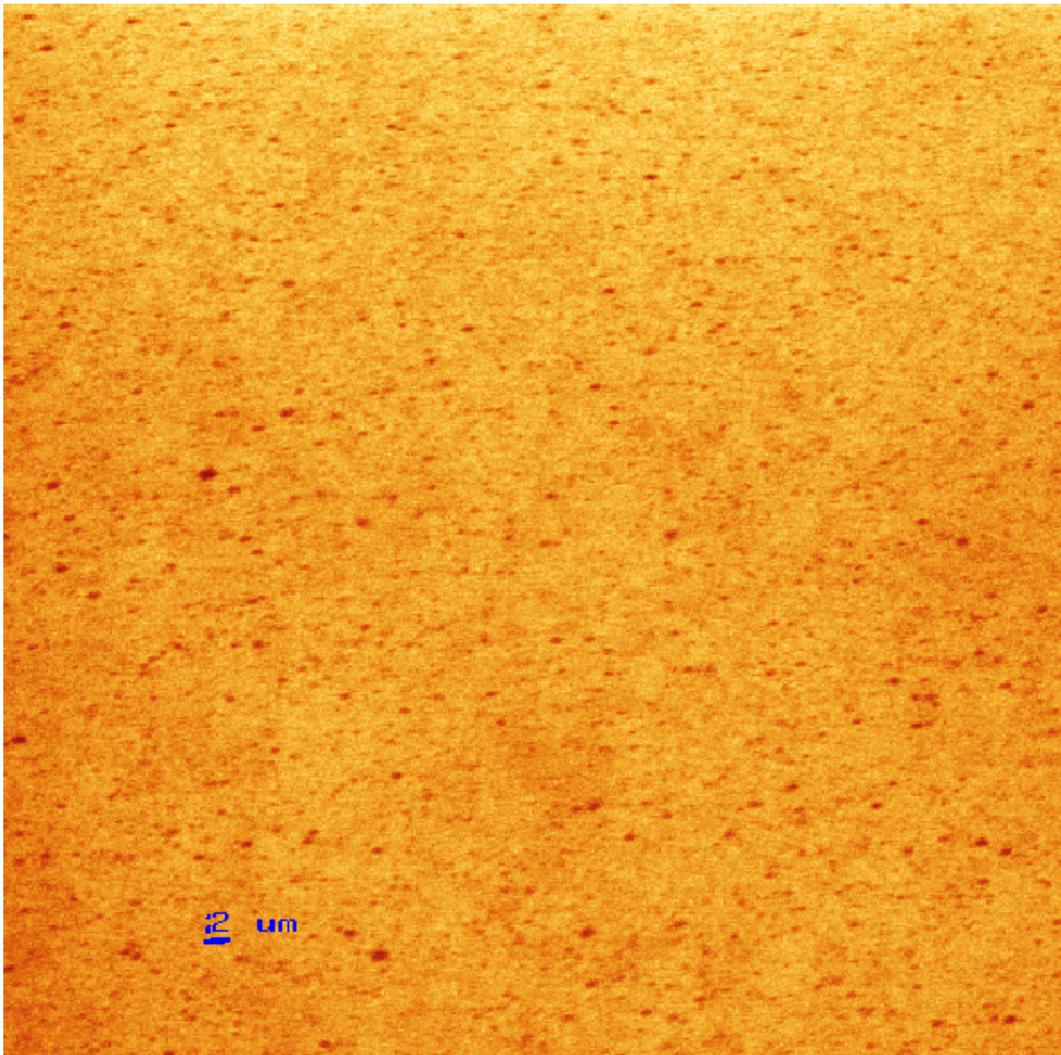


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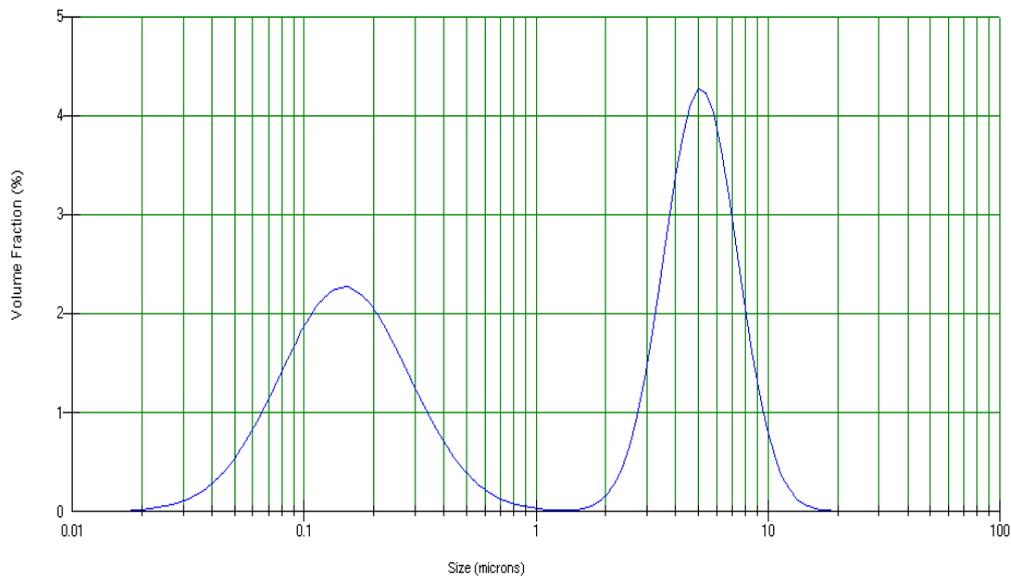


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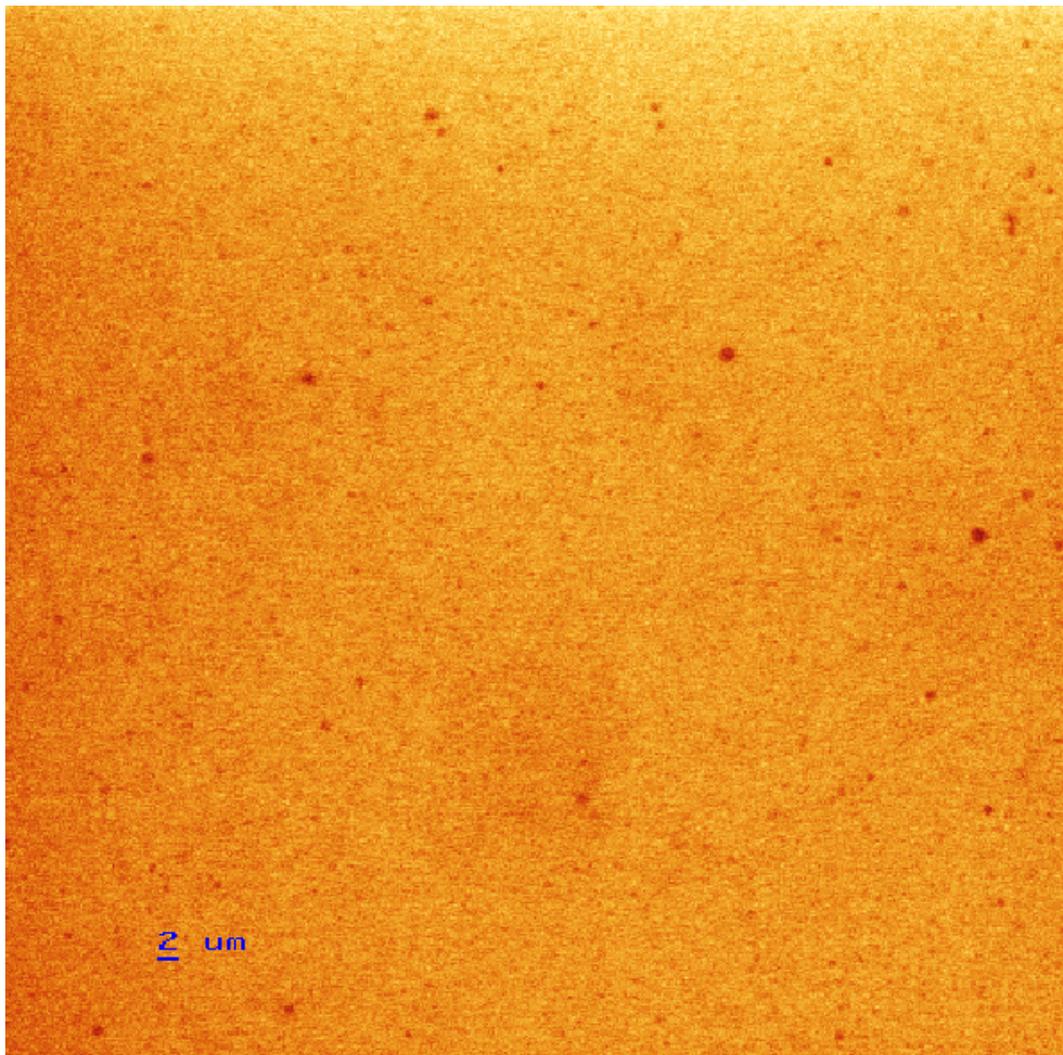


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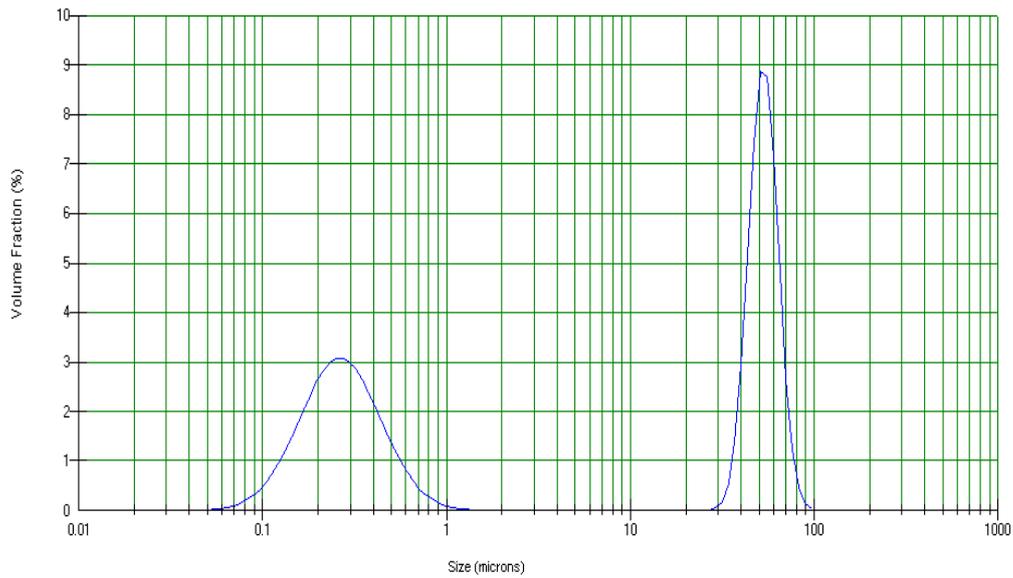


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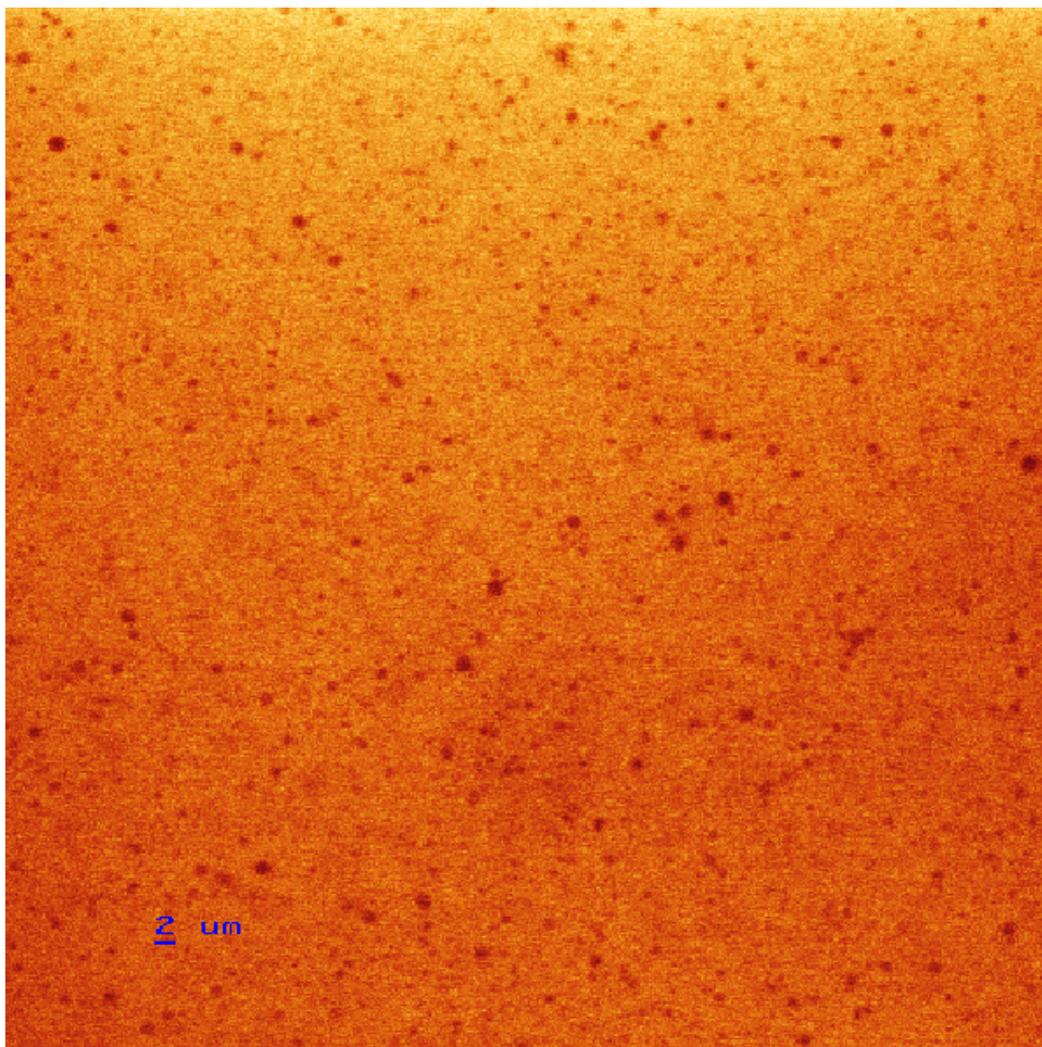


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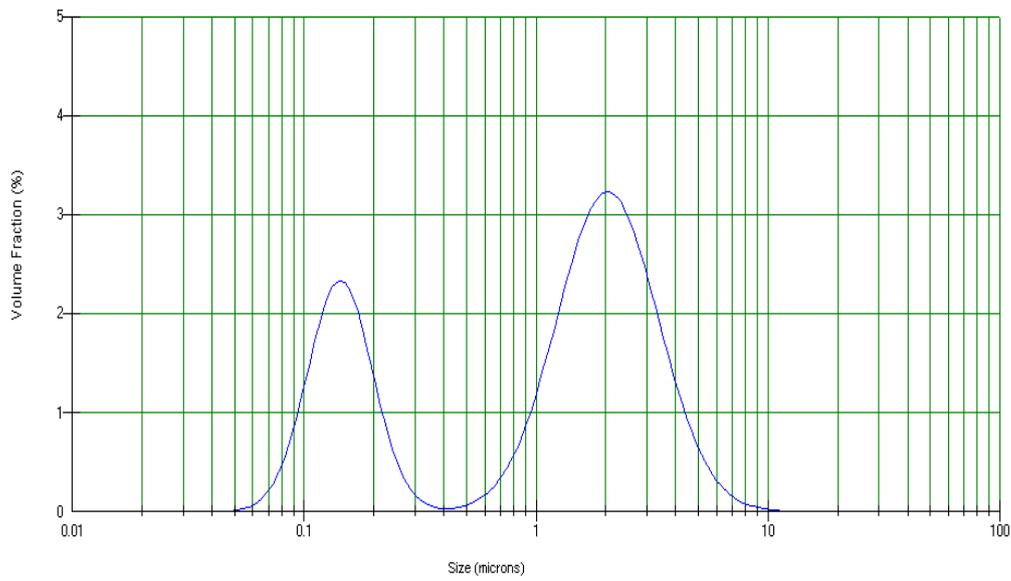


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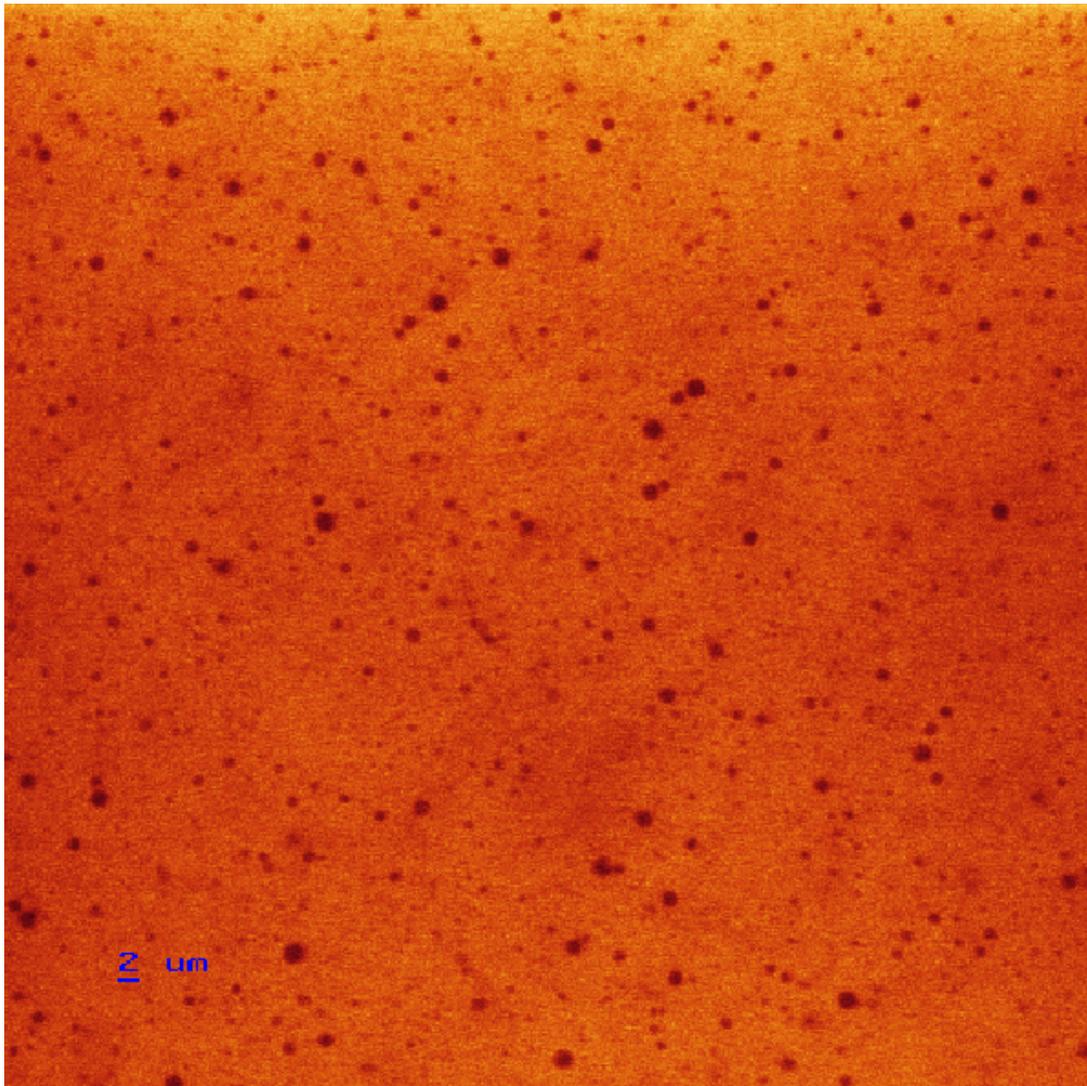


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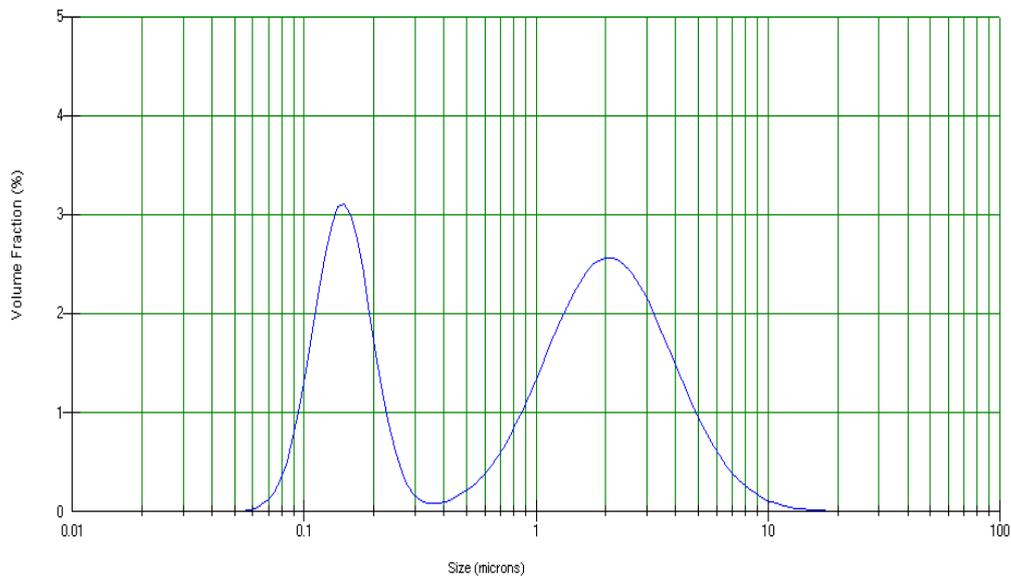


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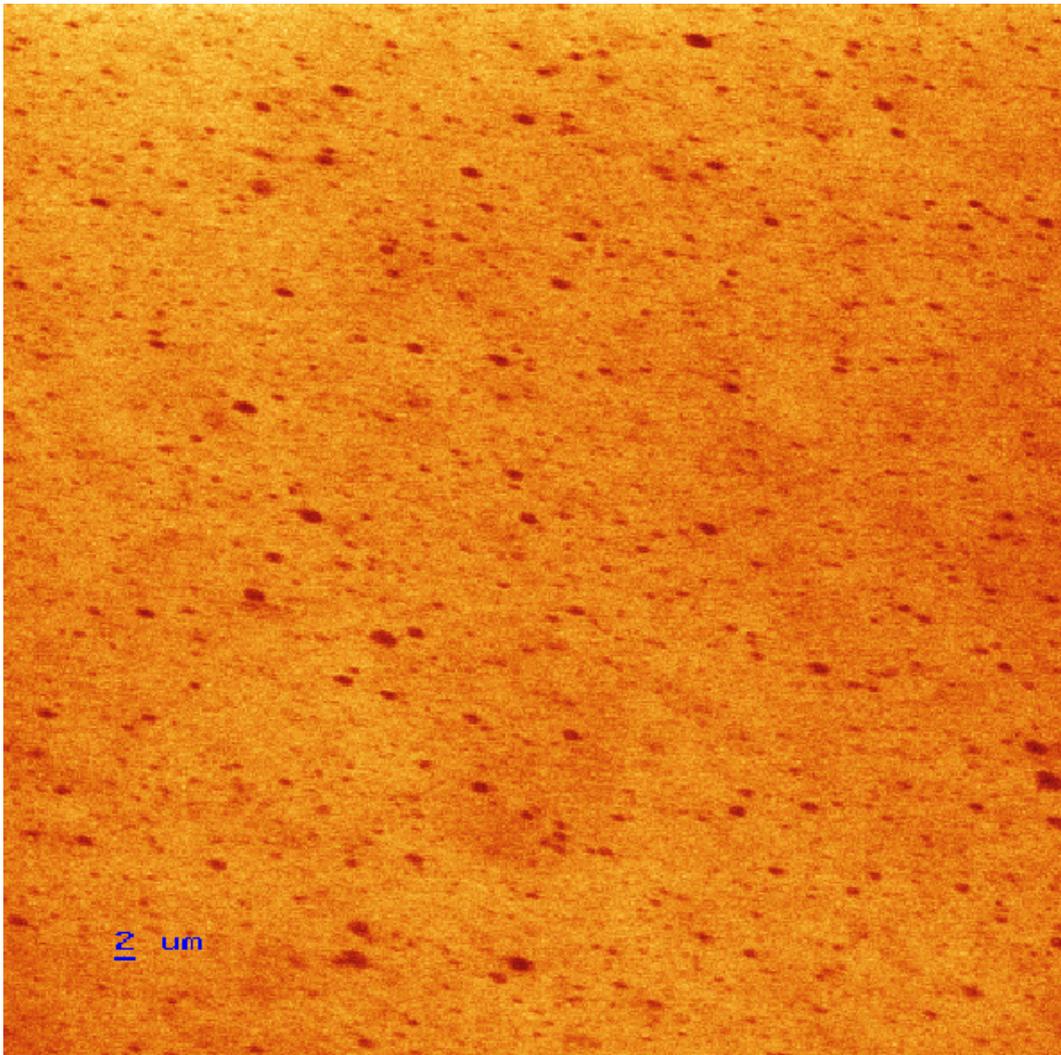


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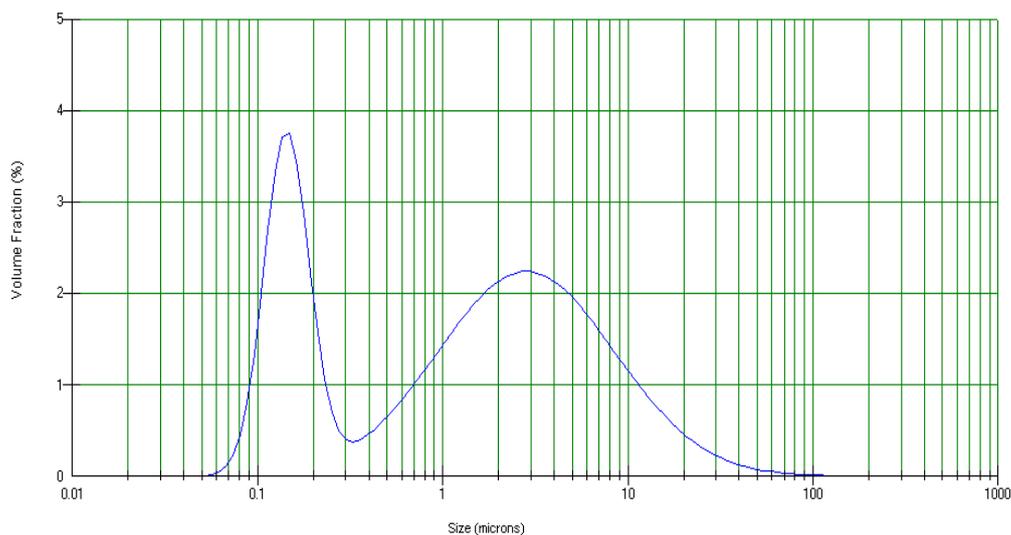


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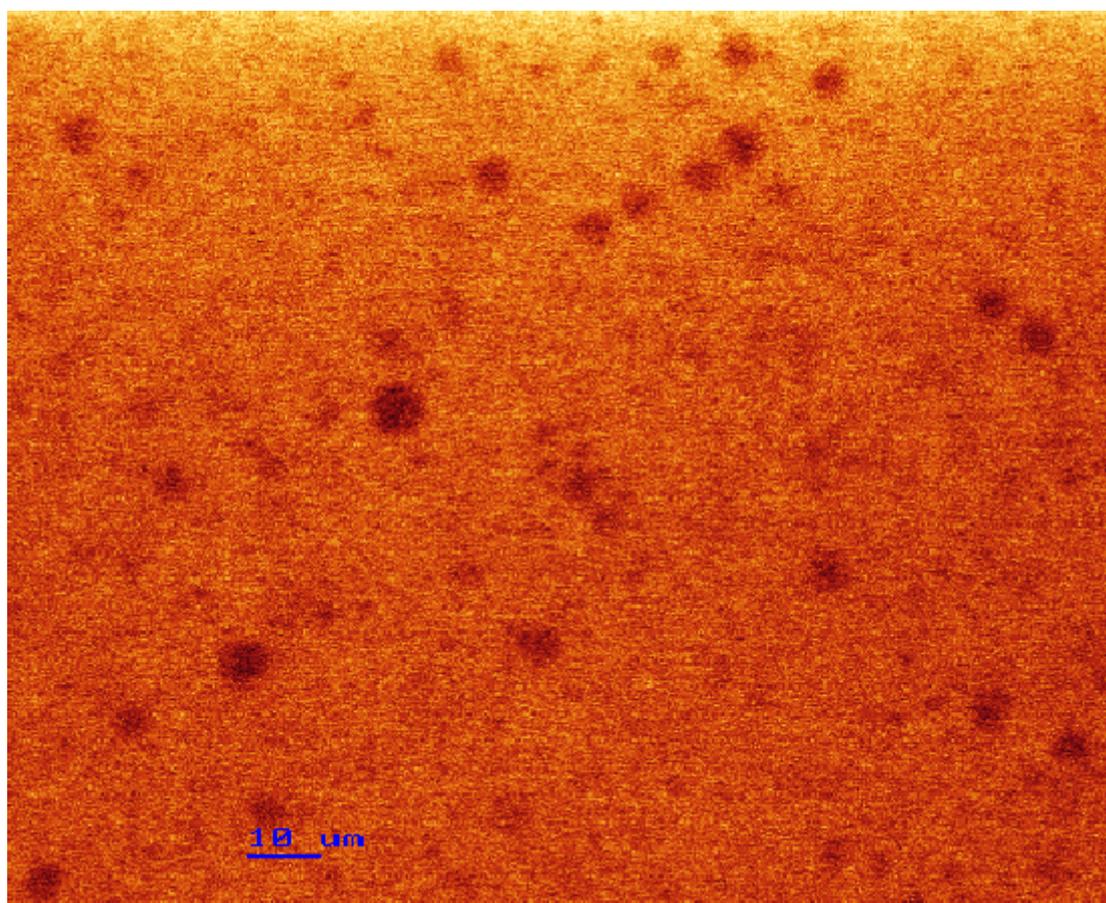


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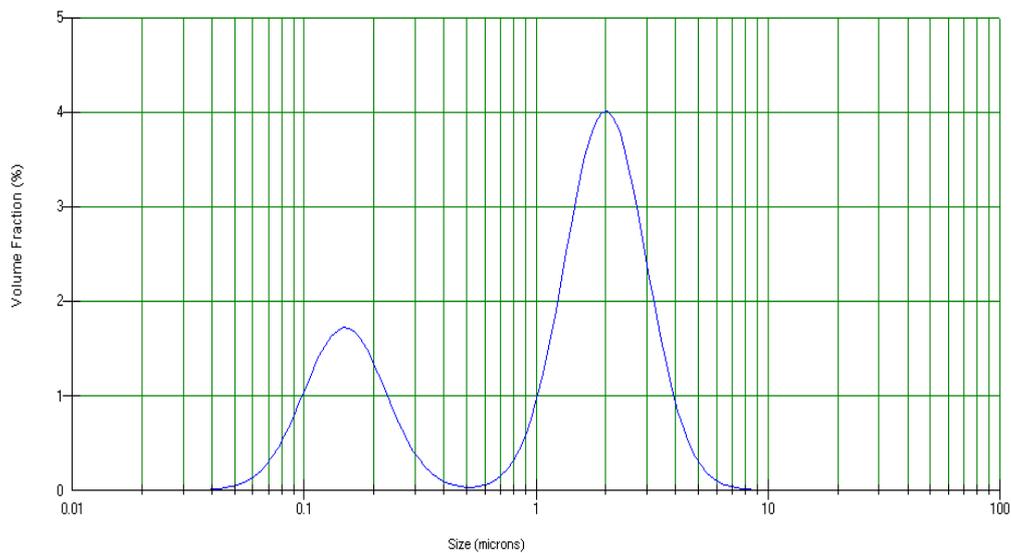


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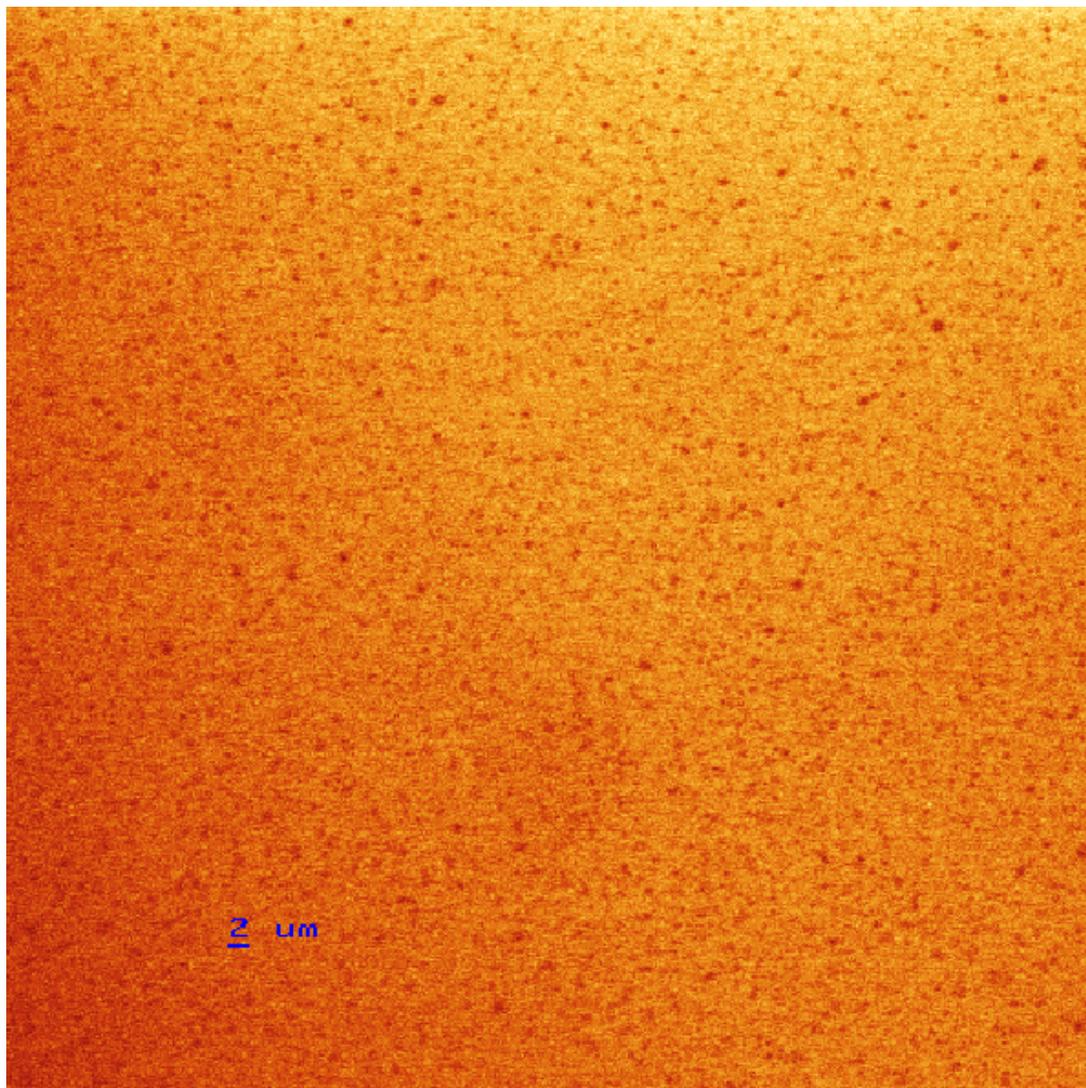


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(W/O/W emulsions)

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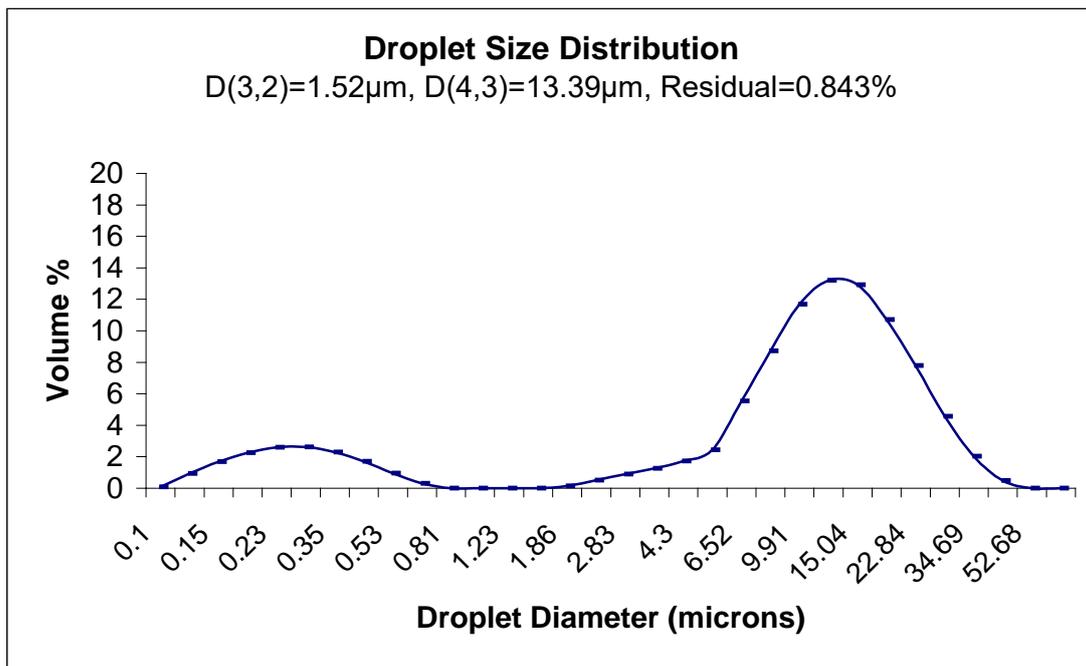


Figure 1 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ balanced by 6.4% glucose in the outer phase.

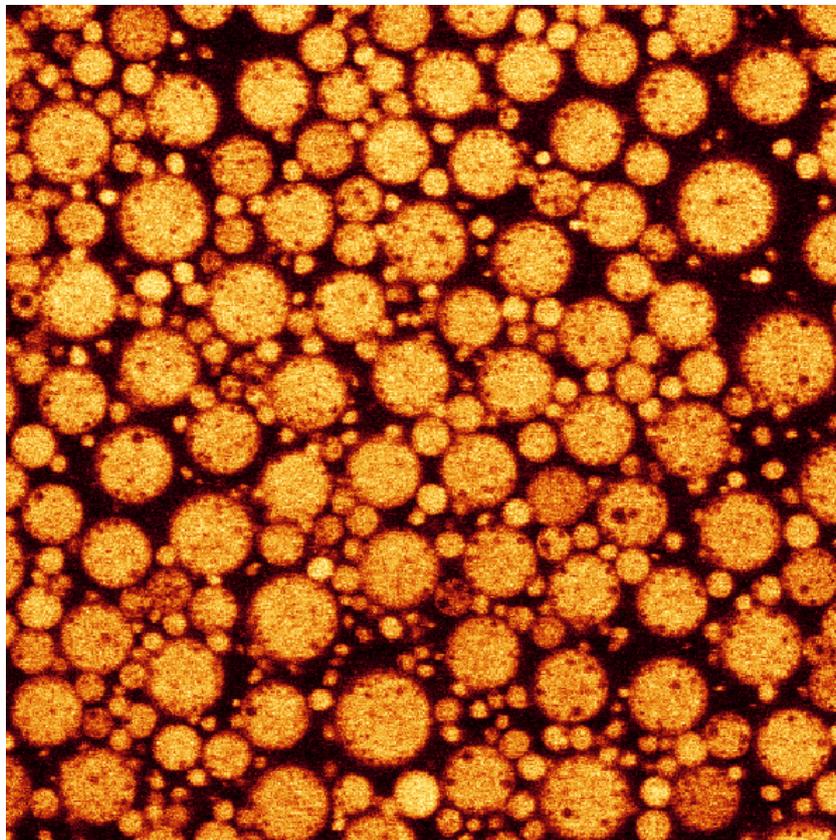


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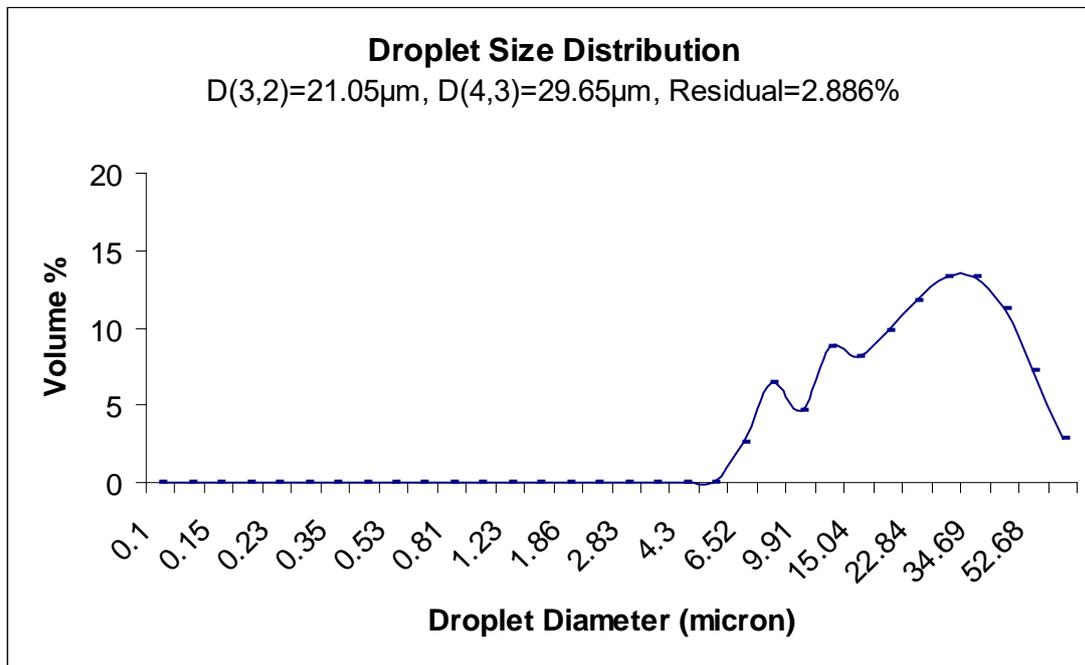


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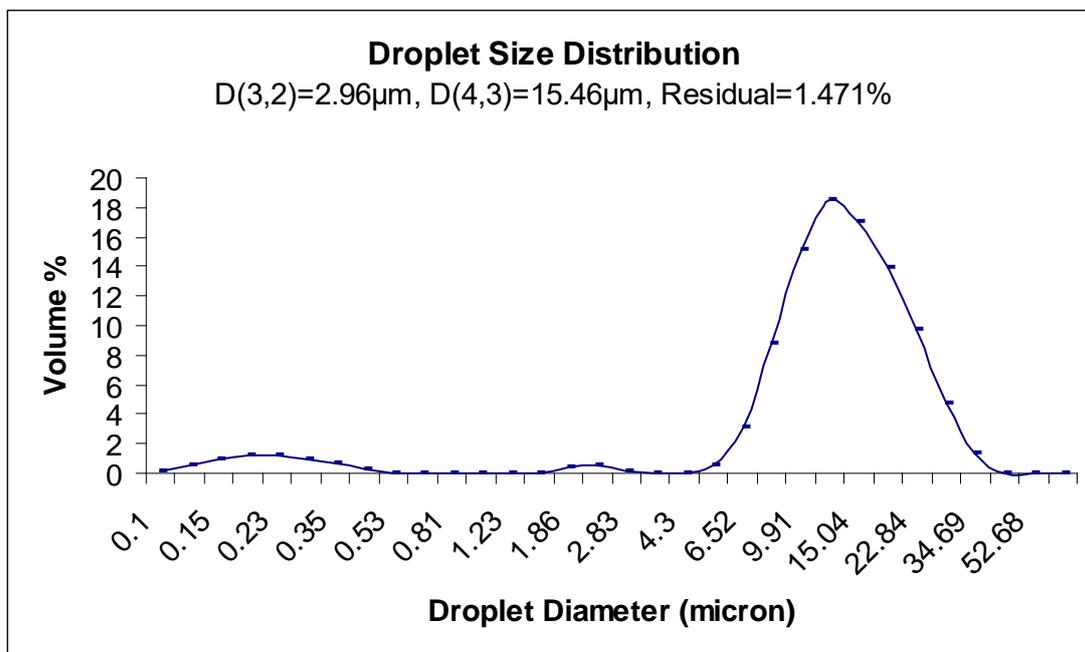


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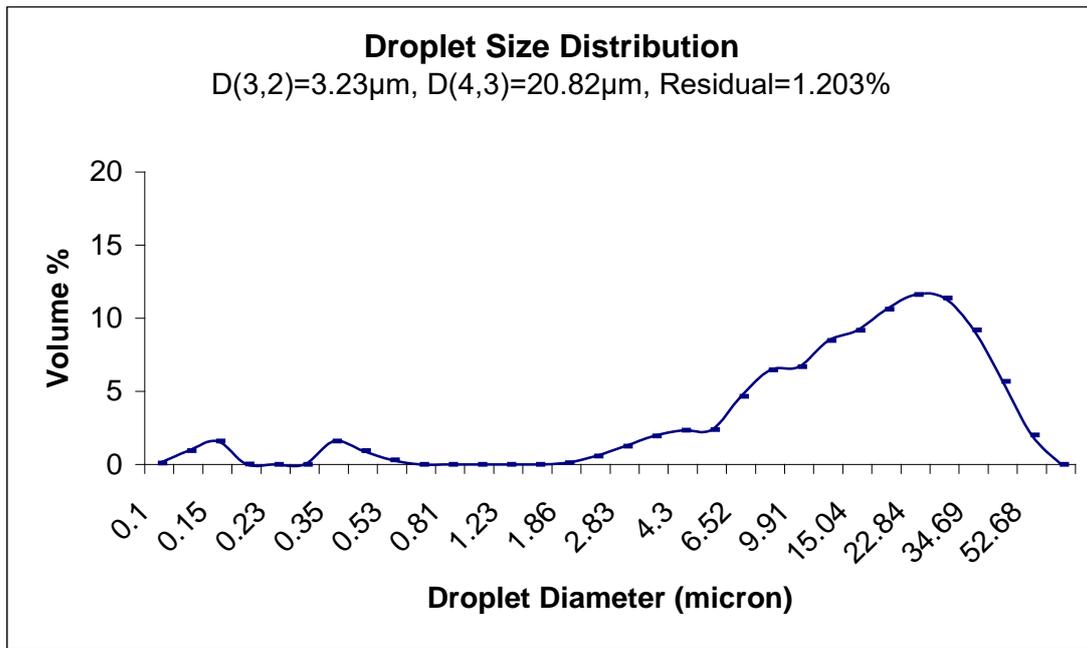


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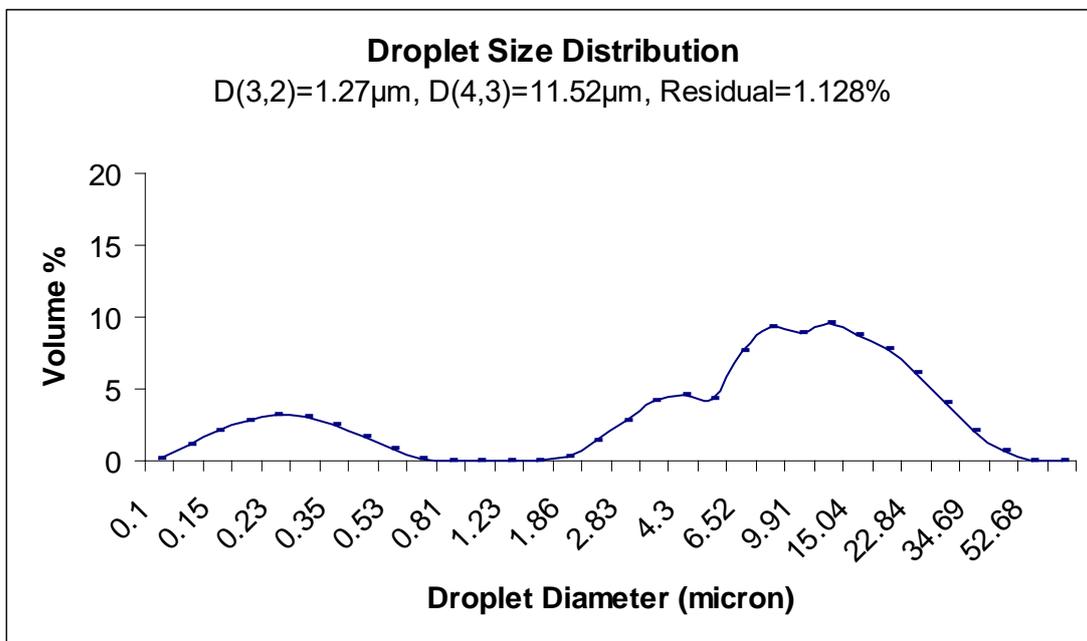


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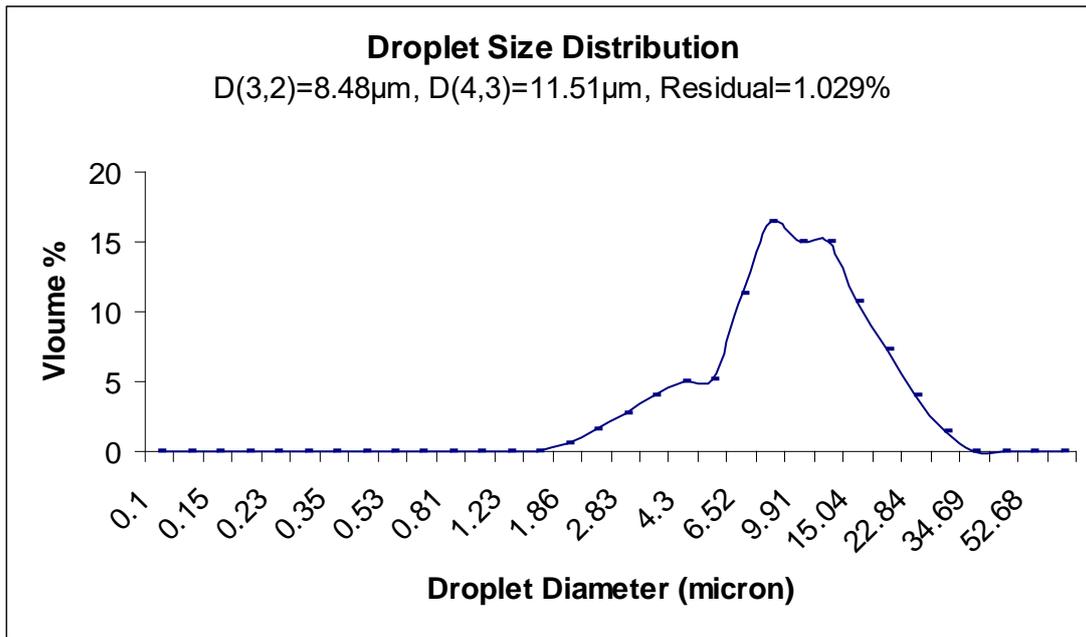


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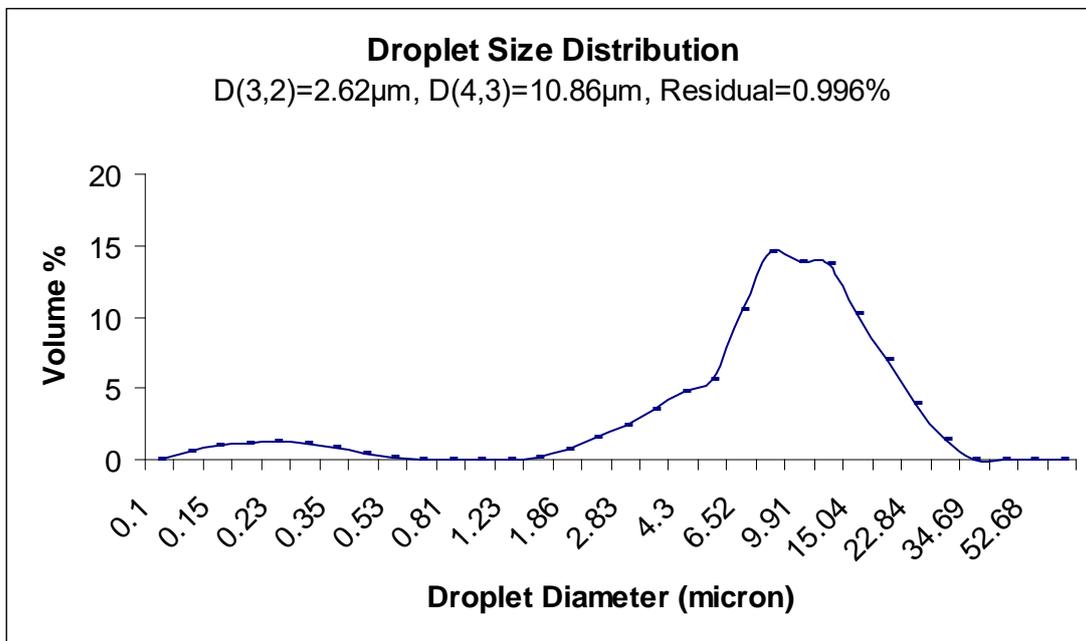


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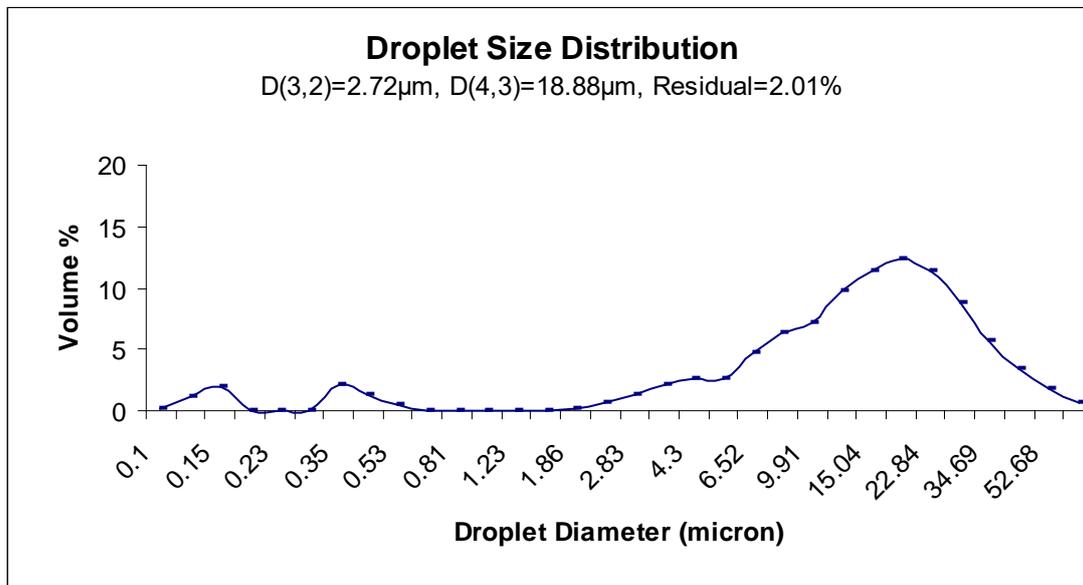


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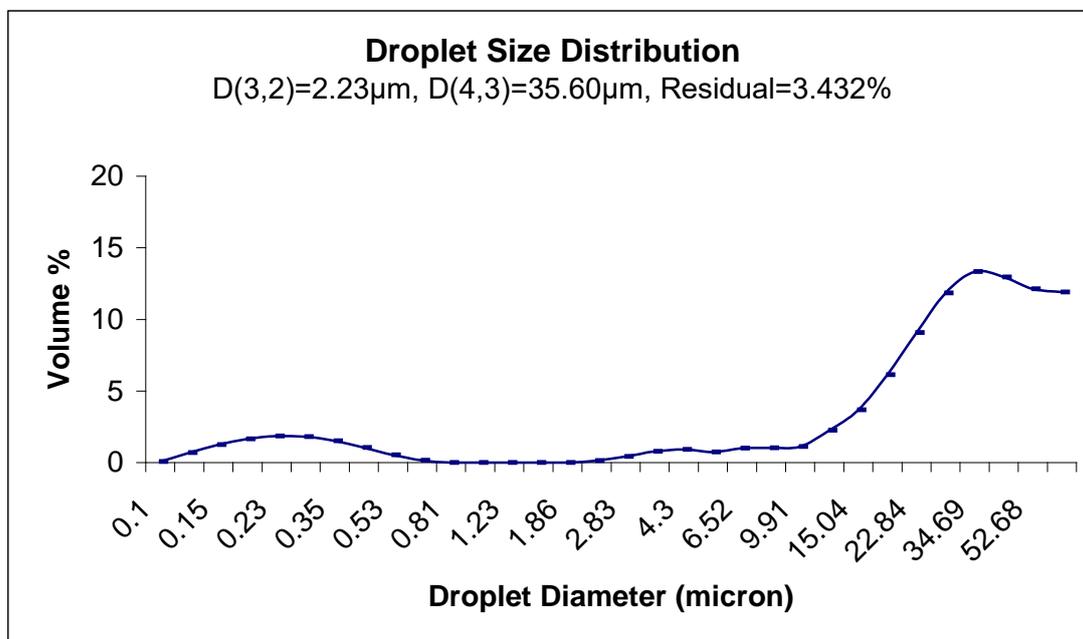


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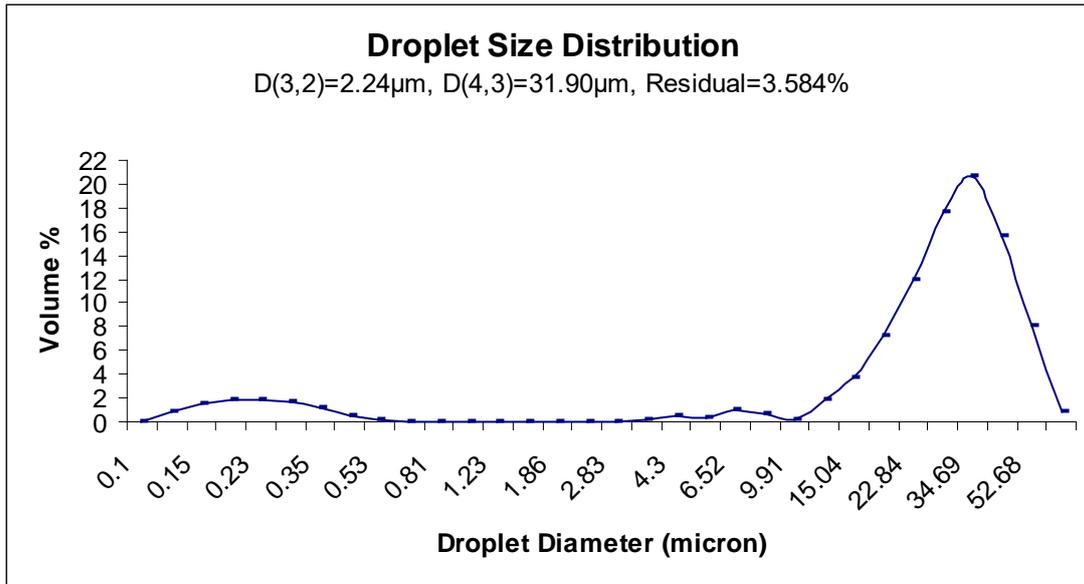


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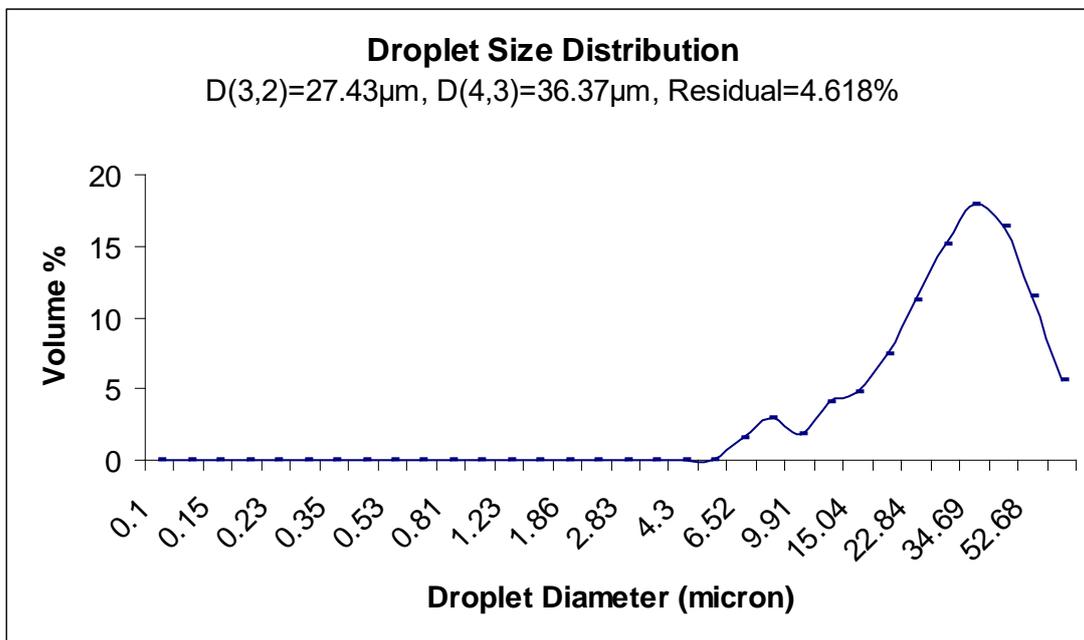


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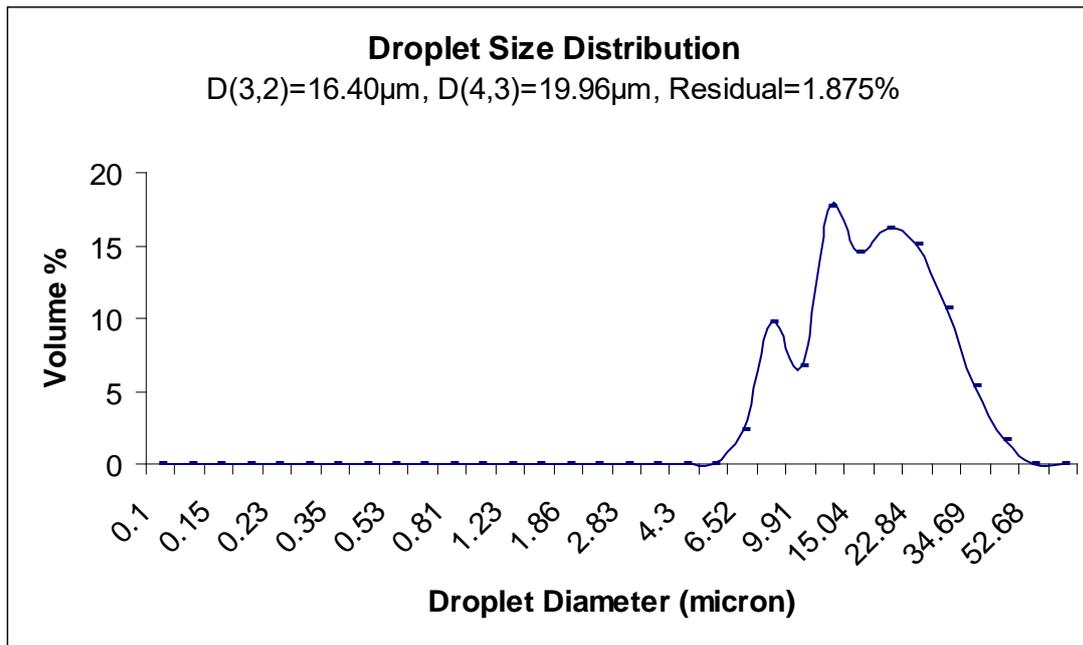


Figure 13 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced. Both w/o and w/o/w emulsions prepared at 22°C

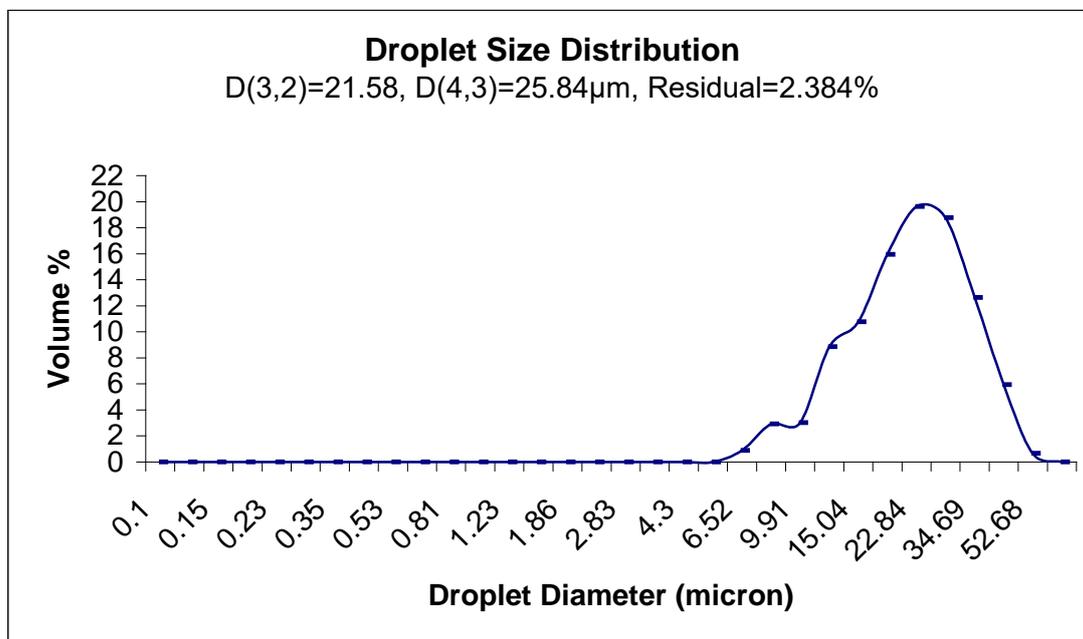


Figure 14 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced. w/o prepared at 50°C and w/o/w at 22°C.

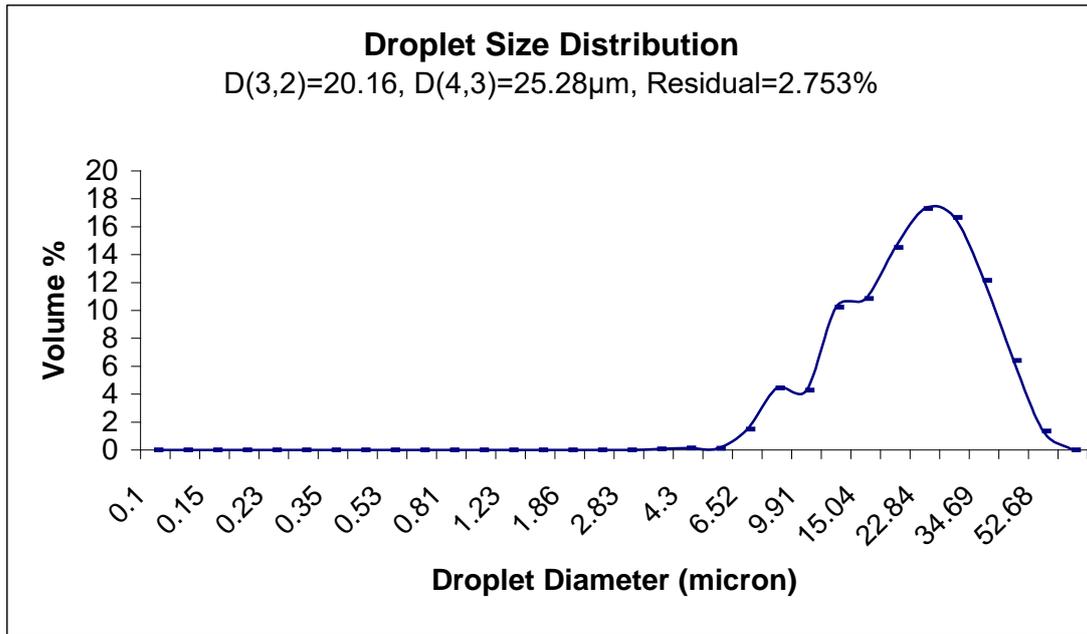


Figure 15 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced. w/o prepared at 22°C and w/o/w at 50°C.

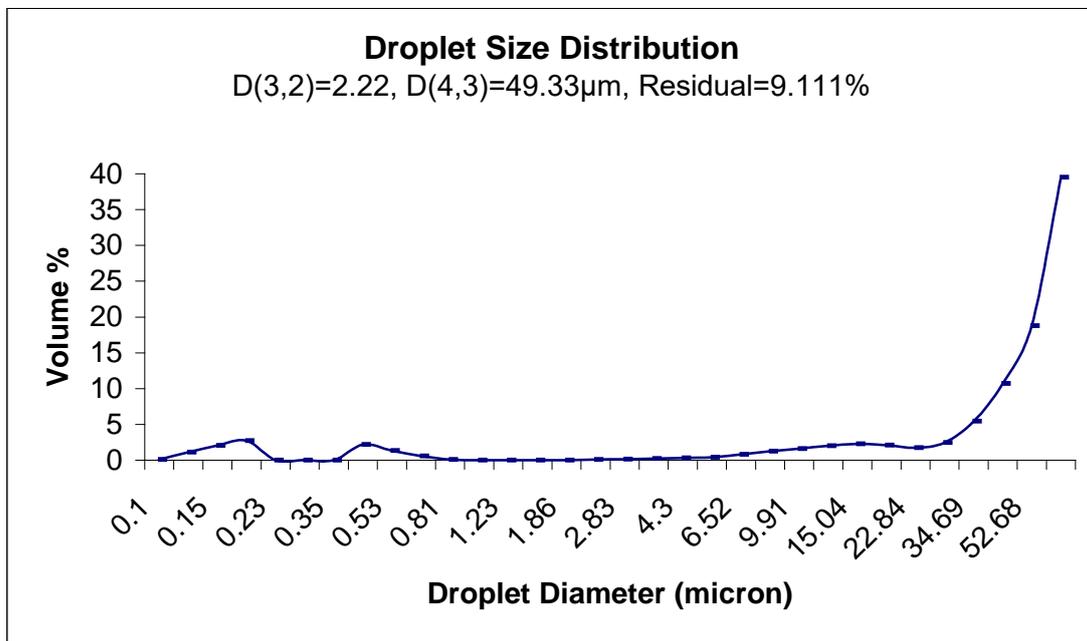


Figure 16 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced. Both w/o and w/o/w emulsions prepared at 50°C.

Figures showing the droplet size distribution of w/o/w produced with different hydrolysate concentrations in the inner phase: (Fig 17 to 19)

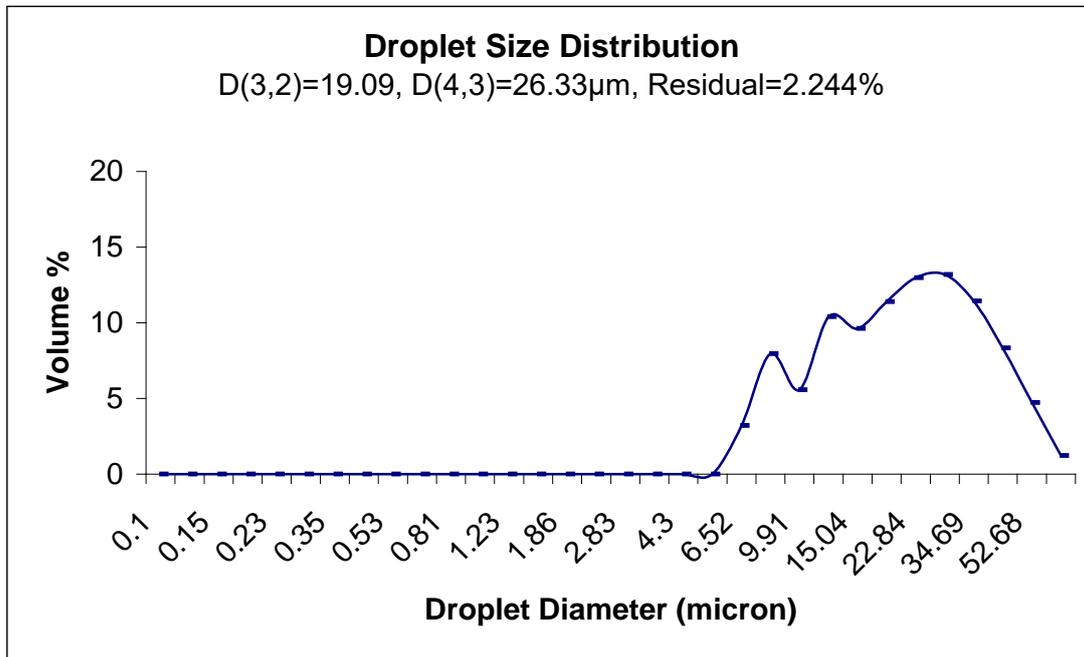


Figure 17 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced.

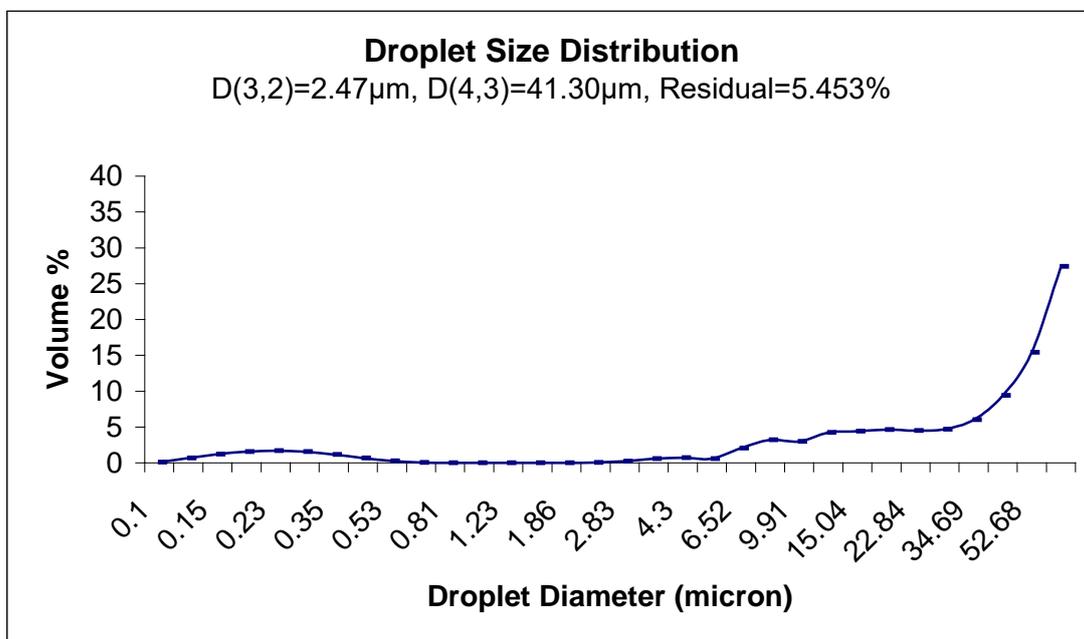


Figure 18 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 20% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced.

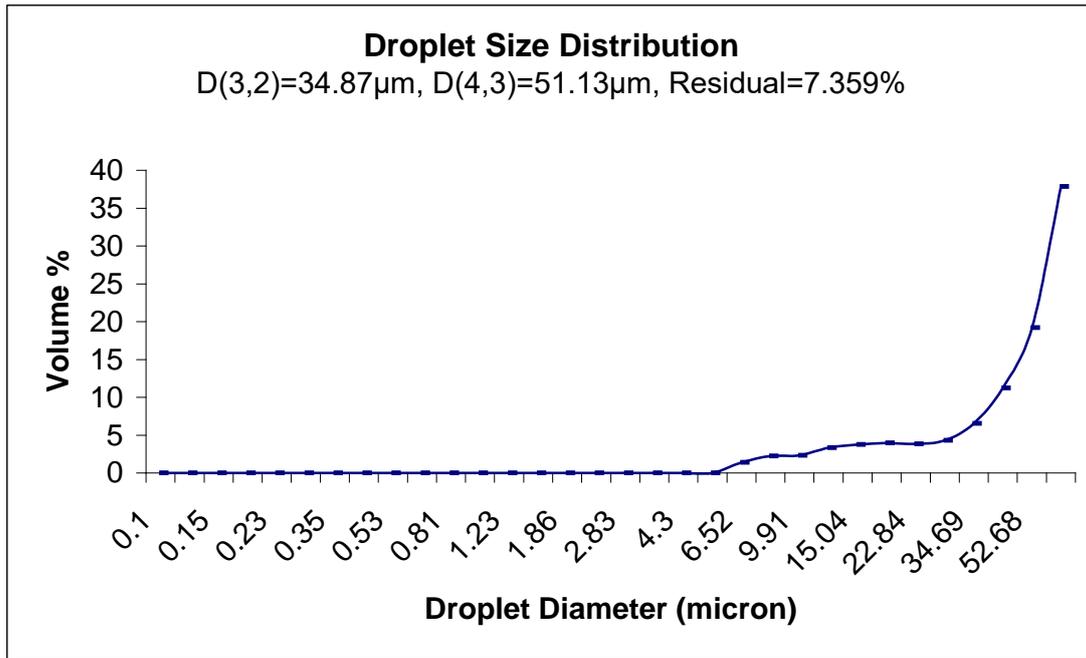


Figure 19 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 30% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced.

Figure showing the droplet size distribution of w/o/w produced with Ultra-Turrax:

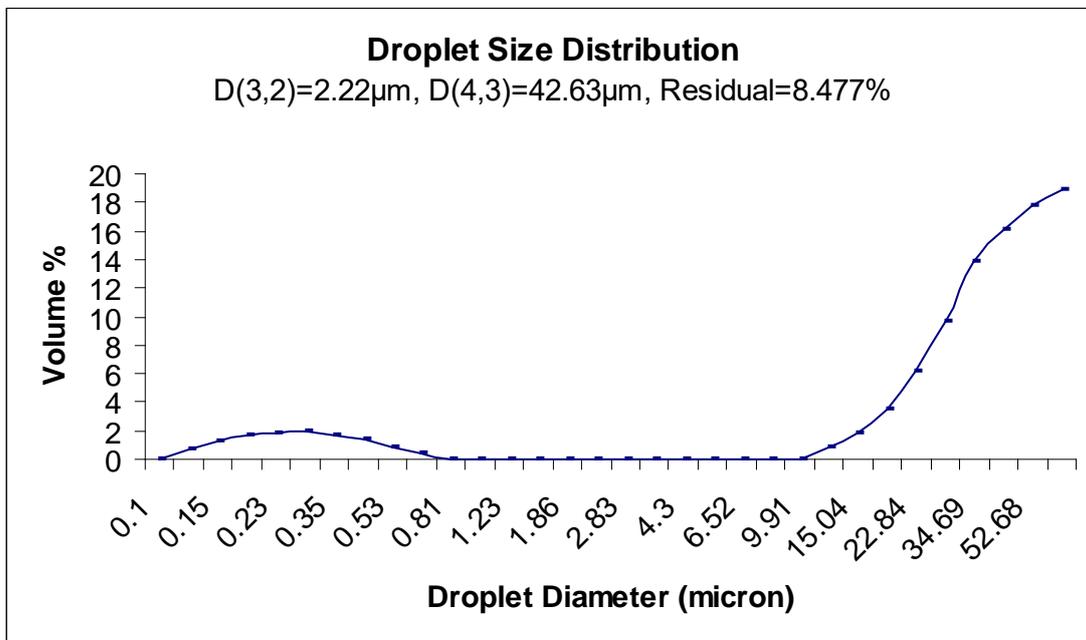


Figure 20 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $\Delta\Pi$ is not balanced. Both w/o (9500 rpm) and w/o/w (8000 rpm) emulsions prepared by ultra-turrax.

Figures showing the droplet size distribution of w/o/w produced with different hydrophilic emulsifiers in the outer phase: (Fig 21 to 24)

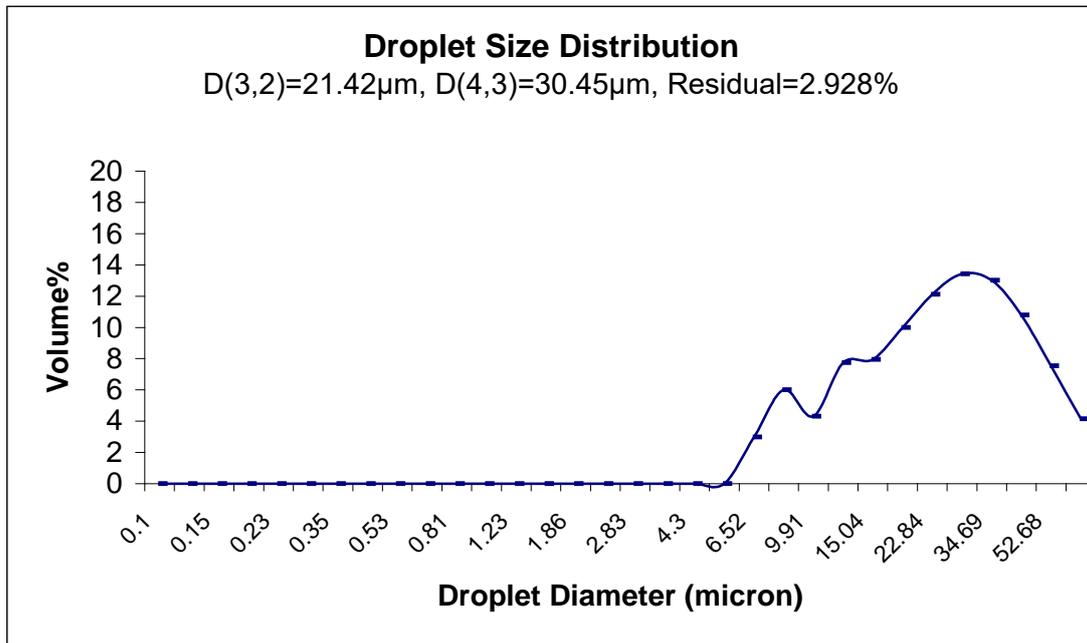


Figure 21 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced.

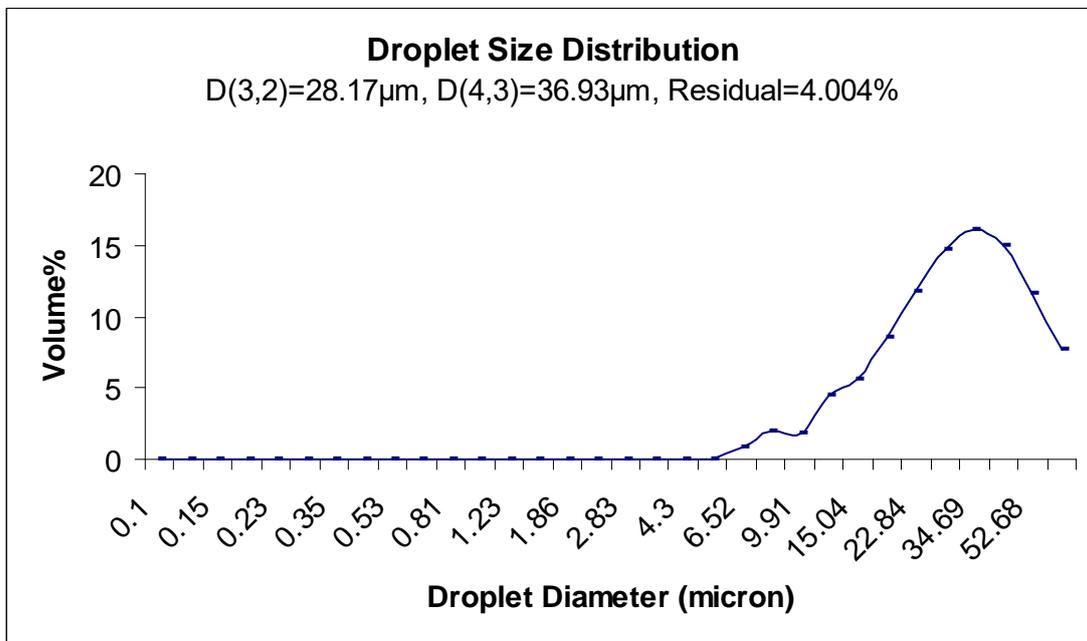


Figure 22 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 0.5% whey protein isolate in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced.

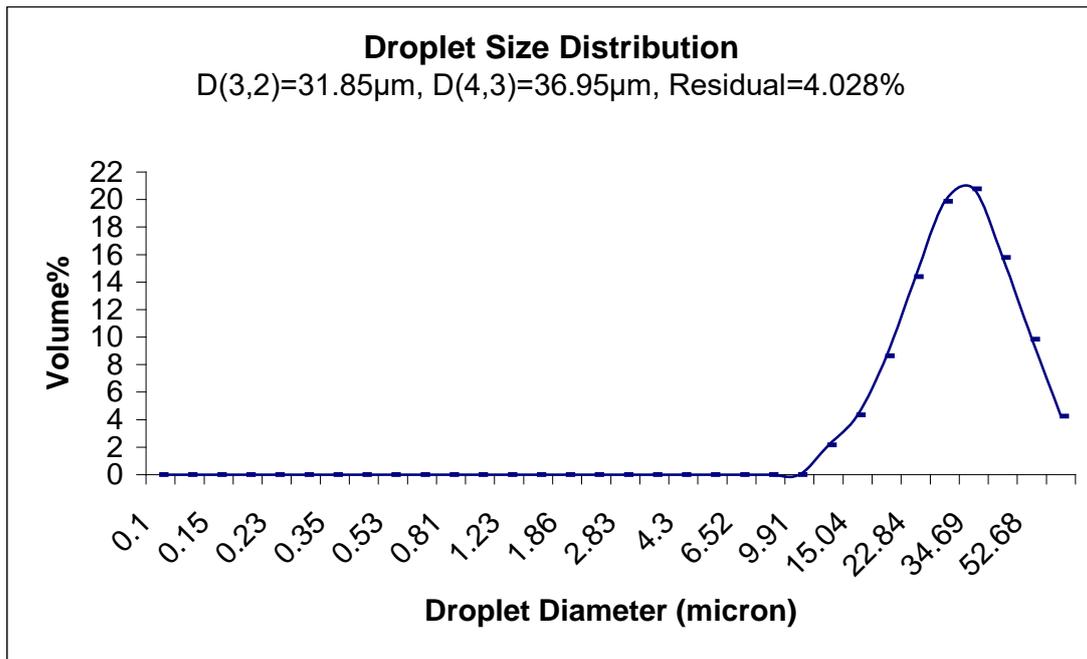


Figure 23 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 0.5% sodium caseinate in the outer phase, $D_p = 2\mu$ m, $\Delta\Pi$ is not balanced.

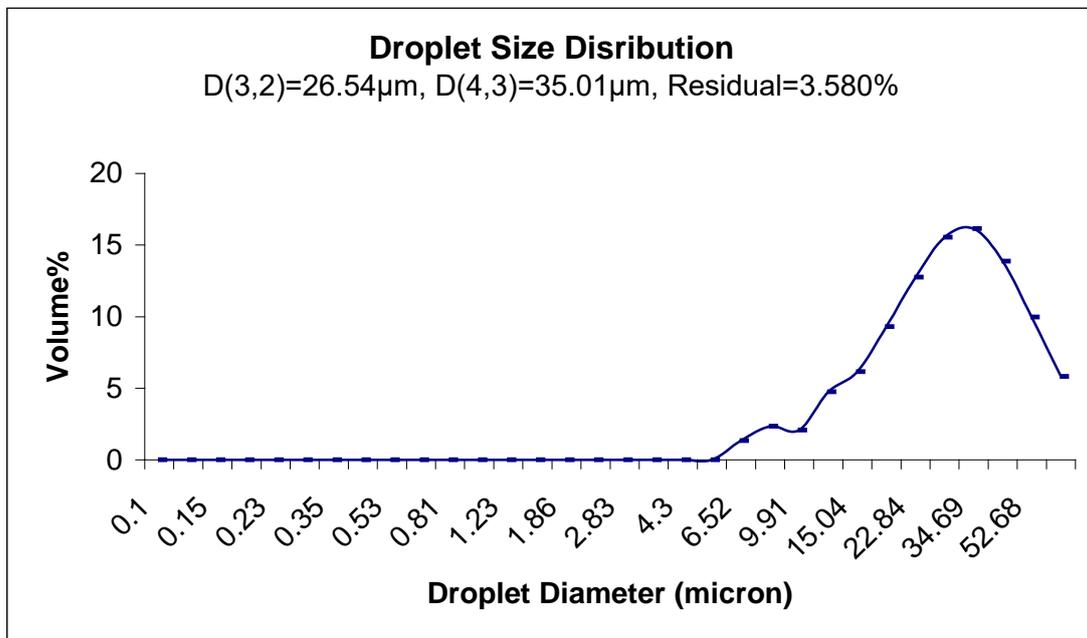


Figure 24 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 0.5% soy protein isolate in the outer phase, $D_p = 2\mu$ m, $\Delta\Pi$ is not balanced.

Figures showing the droplet size distribution of w/o/w produced with different continuous phase velocities: (Fig 25 to 28)

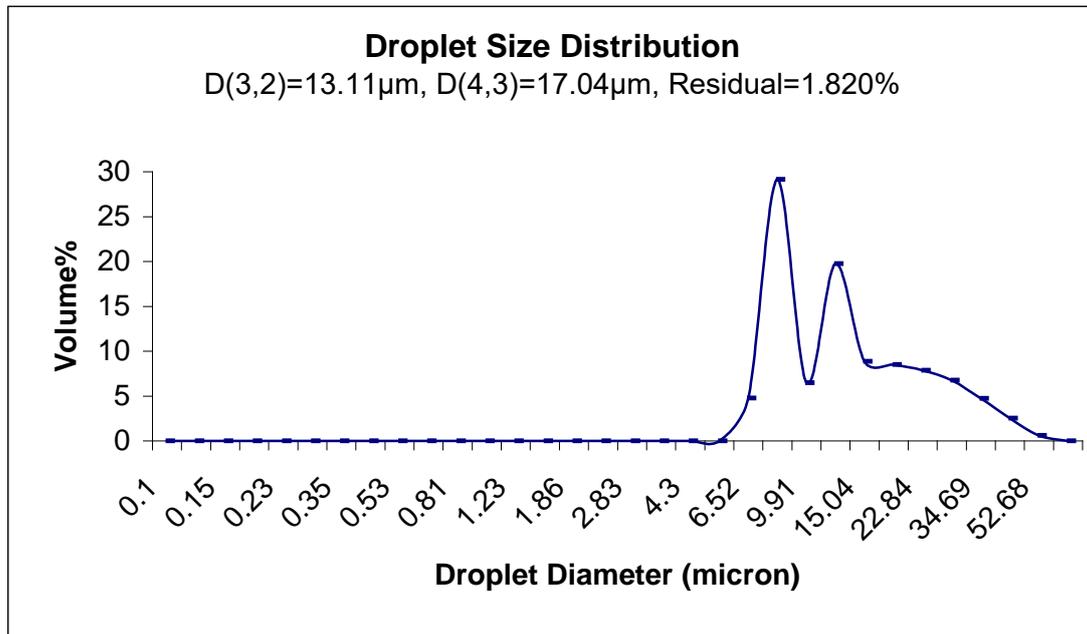


Figure 25. Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced, $v = 0.5\text{m/s}$.

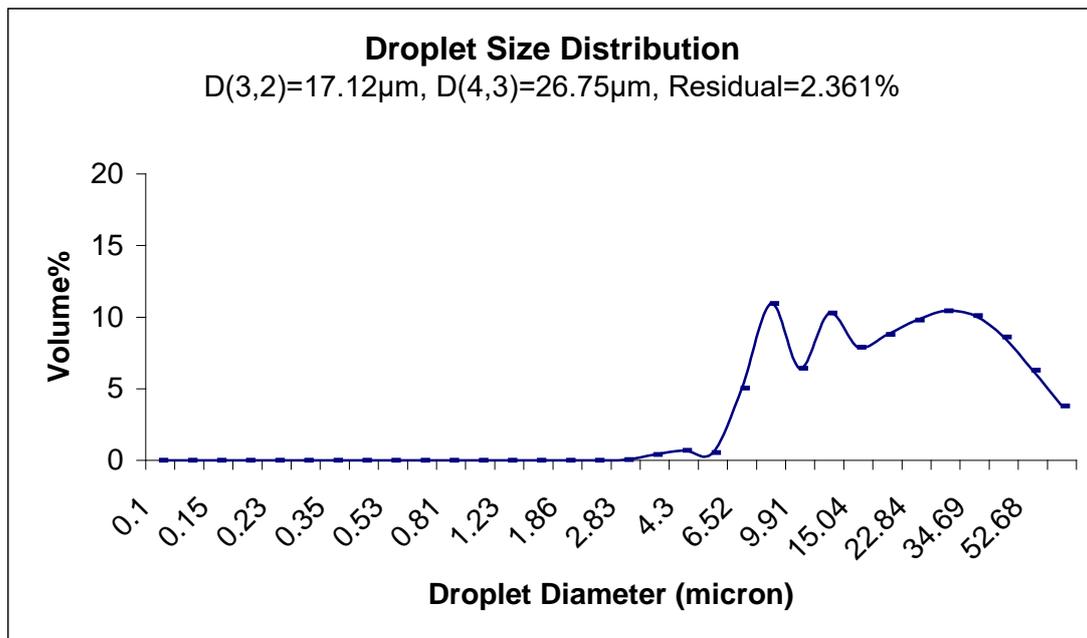


Figure 26 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced, $v = 0.75\text{m/s}$.

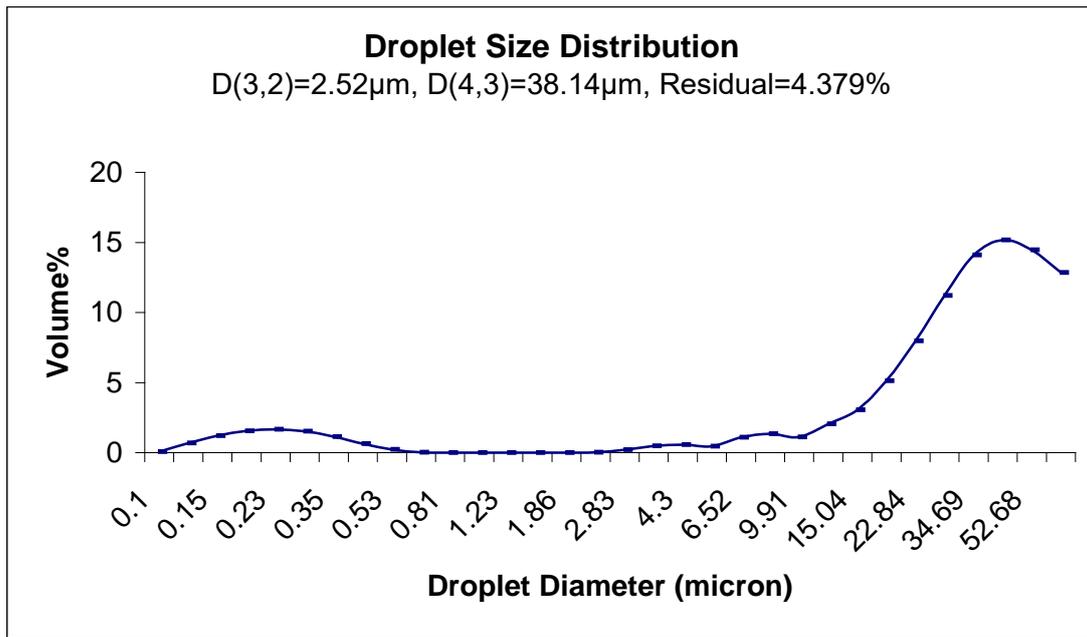


Figure 27. Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced, $v = 1.25\text{m/s}$.

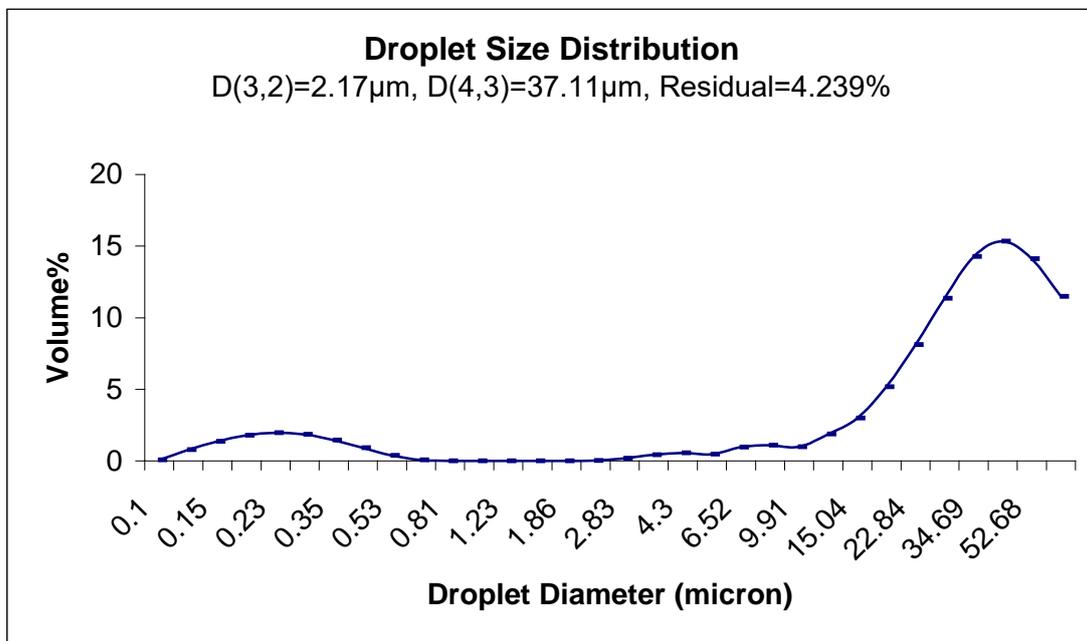


Figure 28 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced, $v = 1.5\text{m/s}$.

Figures showing the droplet size distribution of w/o/w produced with different trans-membrane pressures: (Fig 29 to 32)

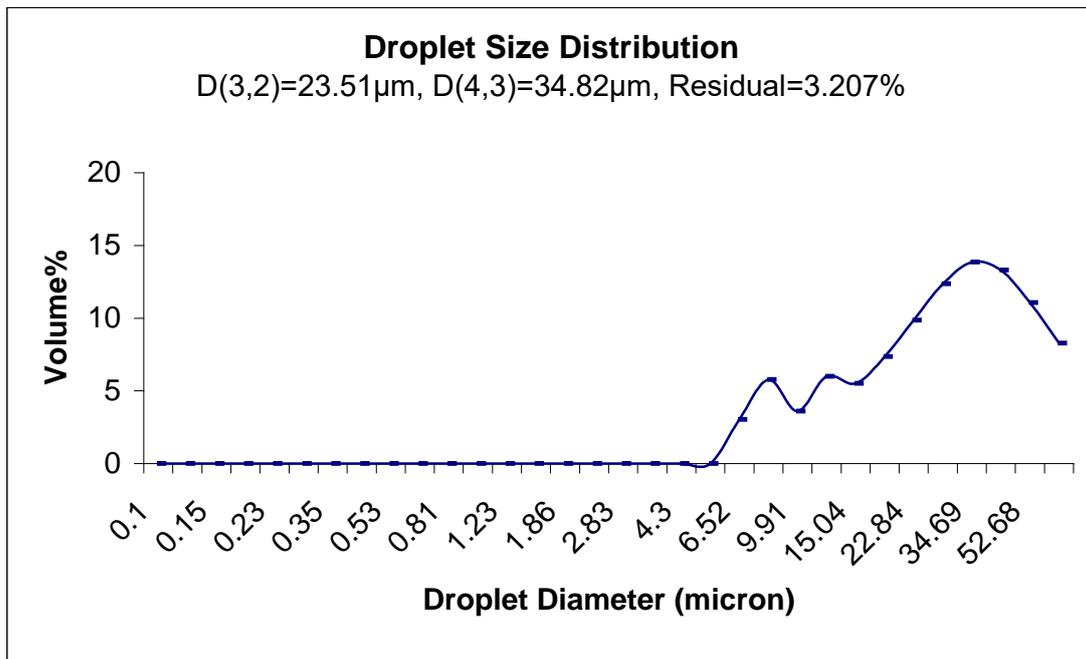


Figure 29 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced, $v = 1\text{m/s}$, $\Delta P = 100\text{KPa}$.

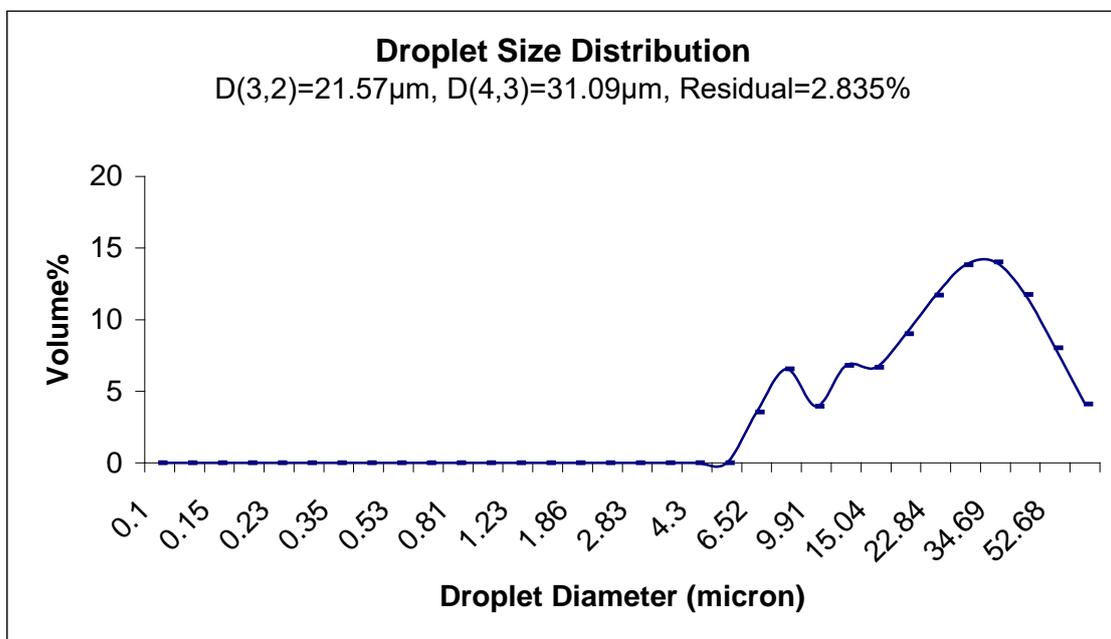


Figure 30 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu\text{m}$, $\Delta\Pi$ is not balanced, $v = 1\text{m/s}$, $\Delta P = 150\text{KPa}$.

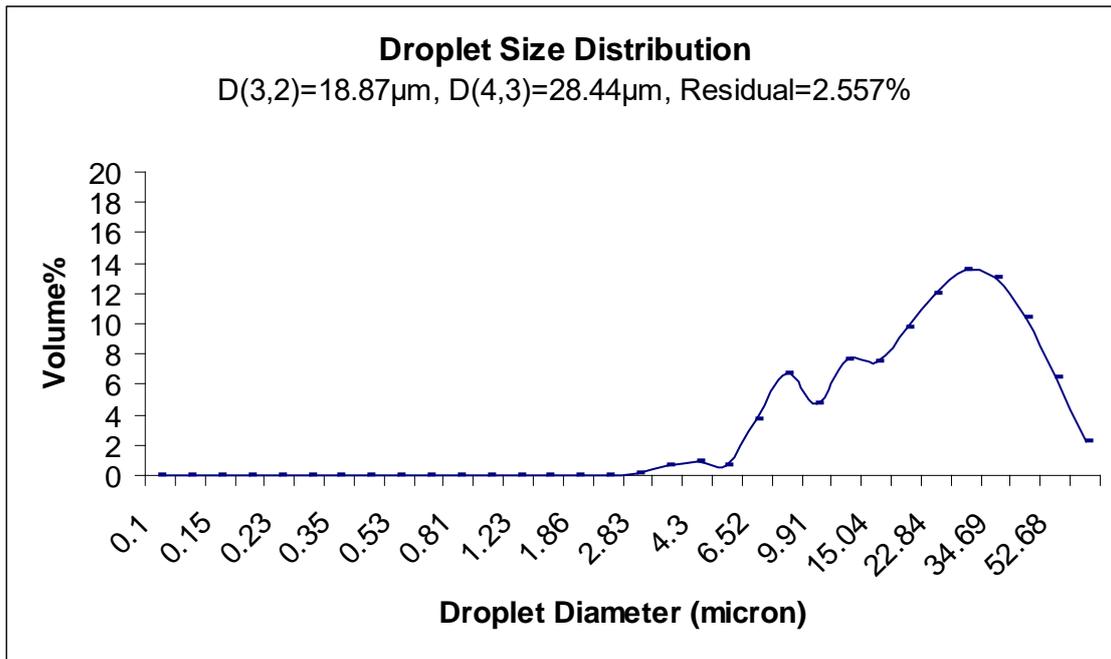


Figure 31 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu$ m, $\Delta\Pi$ is not balanced, $v = 1m/s$, $\Delta P = 200KPa$.

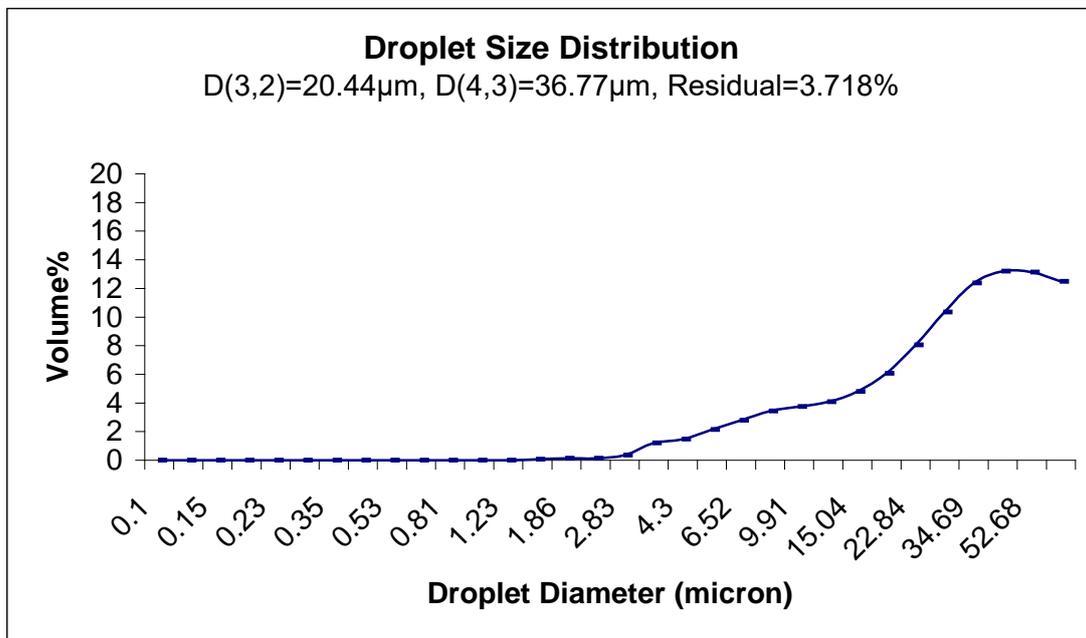


Figure 32 Droplet size distribution of w/o/w emulsion measured with a Malvern Mastersizer $\Phi(w/o) = 0.25$ containing 6.4% glucose (marker) and 10% hydrolysate (core material), 10% PGPR in the oil phase, w/o emulsion prepared by homogenization at 500 bars, $\Phi(w/o/w) = 0.2$, 1% tween 80 in the outer phase, $D_p = 2\mu$ m, $\Delta\Pi$ is not balanced, $v = 1m/s$, $\Delta P = 250KPa$.

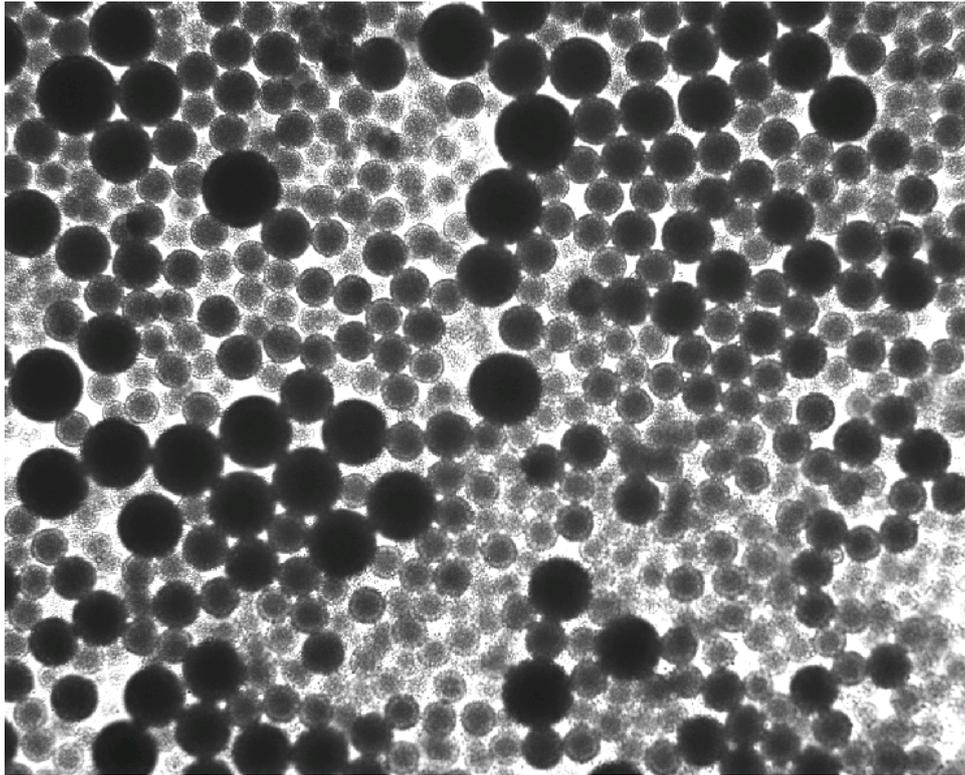


Figure 33 Brightfield microscope image (20x magnification) of w/o/w emulsion produced with an ultra-turrax. Photograph taken immediately after emulsification.

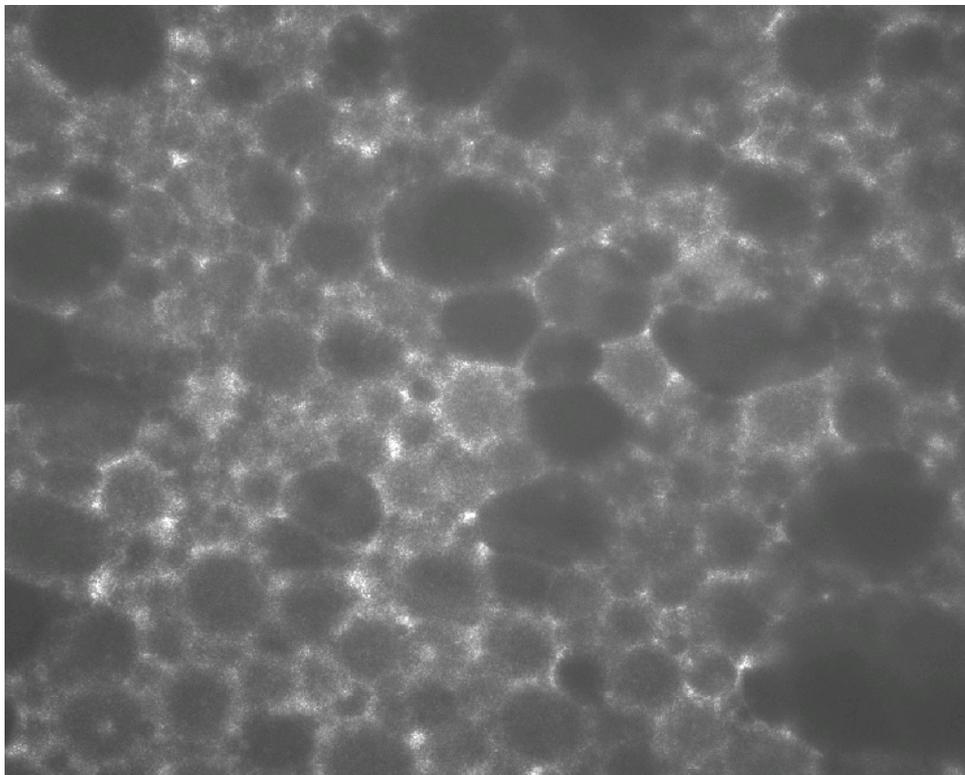


Figure 34 Brightfield microscope image (20x magnification) of w/o/w emulsion produced with an ultra-turrax. Photograph taken 24 hours after emulsification.

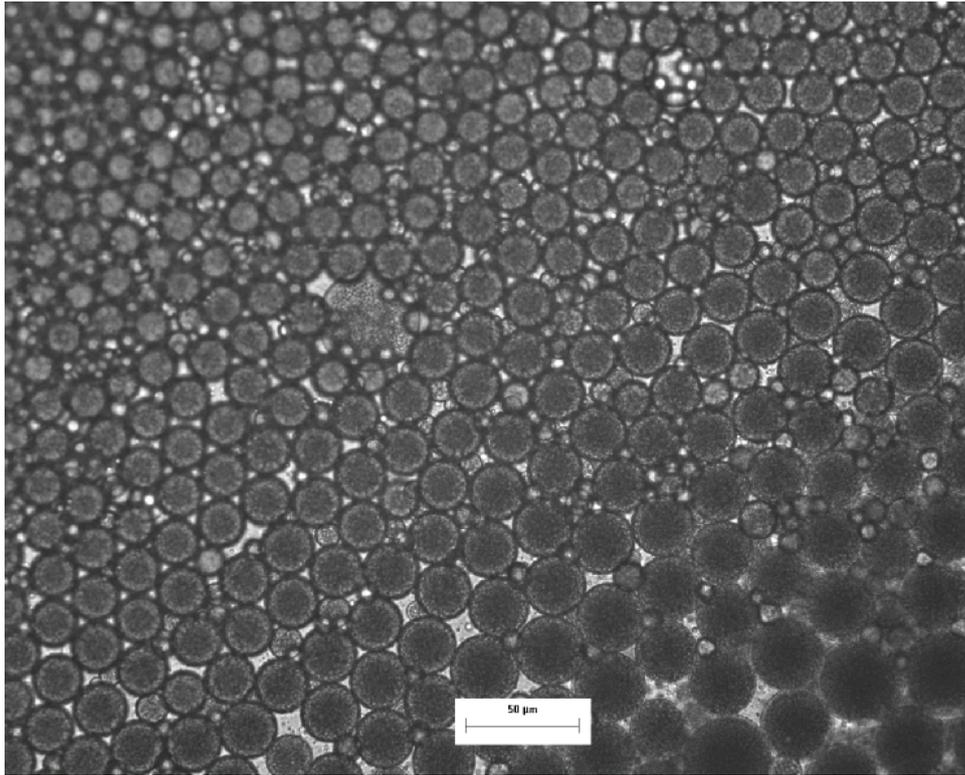


Figure 35 Brightfield microscope image (40x magnification) of a w/o/w emulsion prepared with a 2µm SPG membrane with the osmotic pressure exerted by the glucose (6.4%) and hydrolysate (10%) in the inner phase balanced by 1.7% Sorbitol in the outer phase. Photograph taken immediately after emulsification.

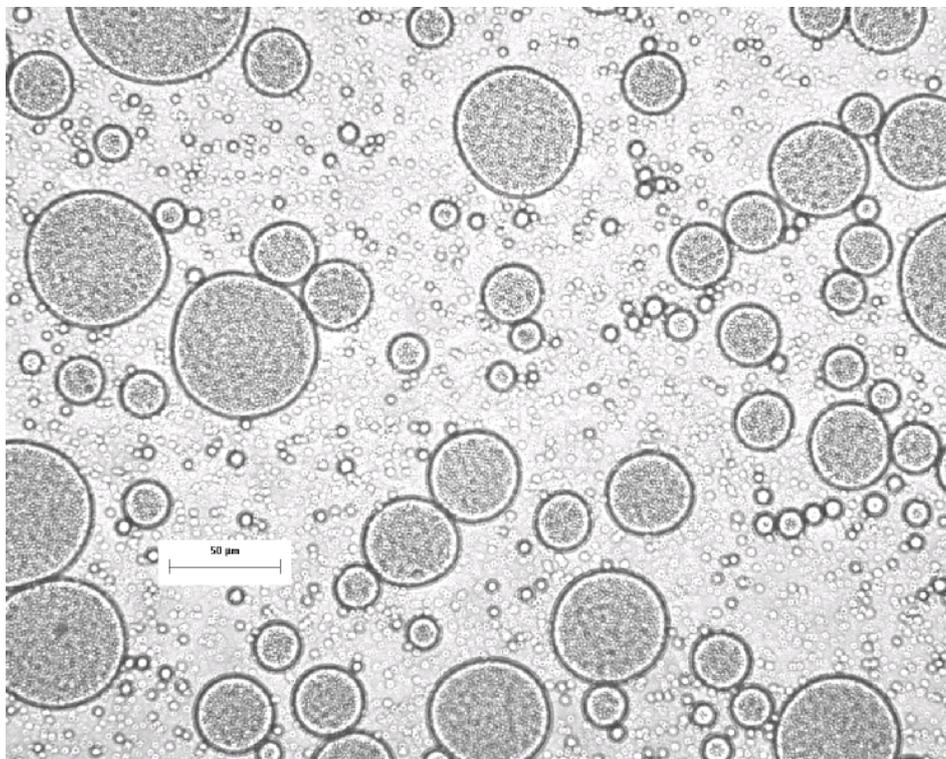


Figure 36 Brightfield microscope image (100x magnification) of a w/o/w emulsion prepared with a 2µm SPG membrane with the osmotic pressure exerted by the glucose (6.4%) and hydrolysate (10%) in the inner phase balanced by 1.7% Sorbitol in the outer phase. Photograph taken 1 week after emulsification.

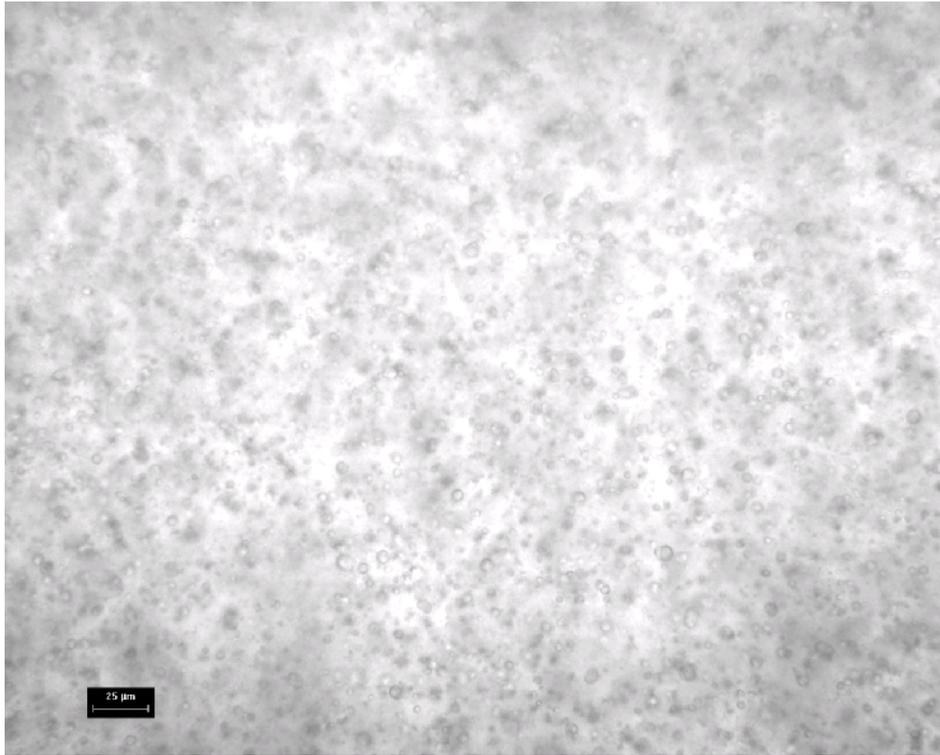


Figure 37 Brightfield microscope image of a w/o emulsion with 5% inner phase and 10% span 80 as the hydrophobic emulsifier prepared with a sonicator.

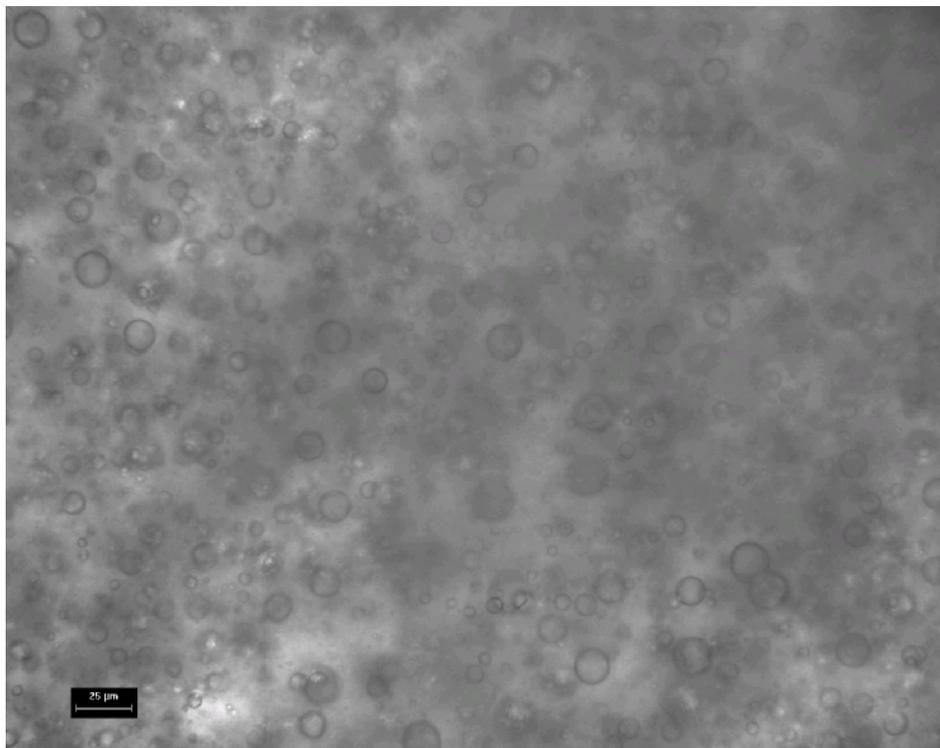


Figure 38 Brightfield microscope image of a w/o emulsion with 25% inner phase and 10% span 80 as the hydrophobic emulsifier prepared with an ultra-turrax.

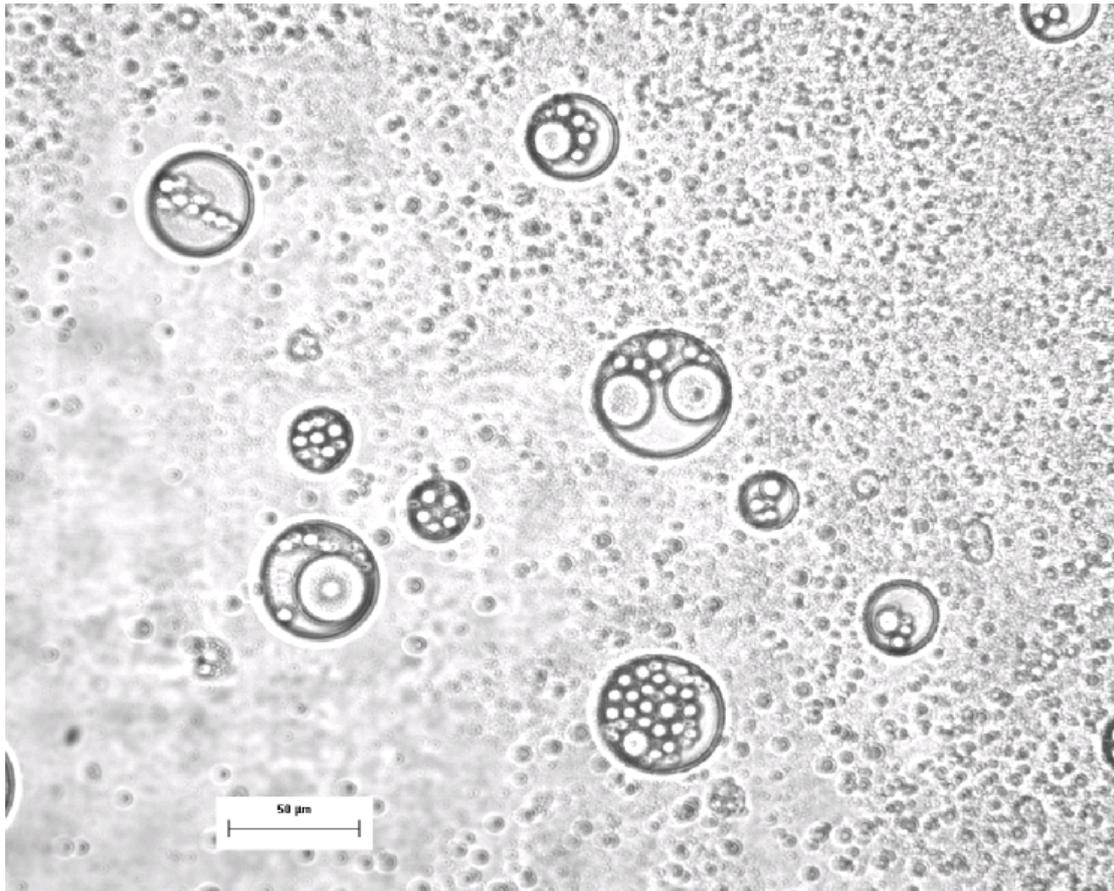


Figure 39 Brightfield microscope image of a w/o/w emulsion showing the internal water droplets.

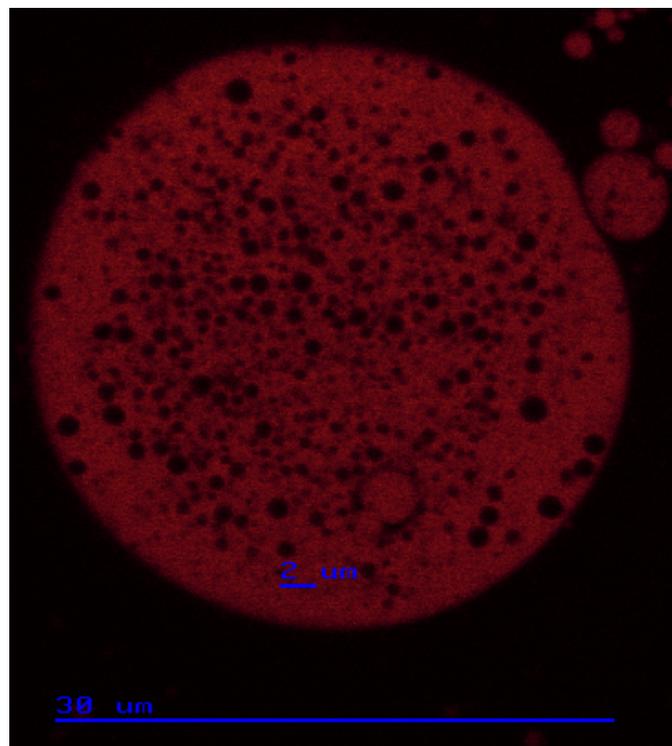


Figure 40 Confocal microscope image of a w/o/w emulsion showing the oil droplet ($\sim 30 \mu\text{m}$) and the internal water droplets ($\leq 2 \mu\text{m}$).