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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 2μm or 3.8μm 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 μm to 3.8 μm. 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 19^{th} century with the production of "margarine" in 1870. Since then the 20^{th} 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.