PREPARATION OF WATER-IN-OIL-IN-WATER EMULSIONS BY REPEATED PREMIX CELLULOSE ACETATE MEMBRANE EMULSIFICATION

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Specially dedicated to my beloved and supporting family:

Chang Choo Man,

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Chang Cik Nam,

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ABSTRACT

The purpose of this study is to determine the potential of cellulose acetate membrane for preparation of formulated water-in-oil-in-water (W/O/W) emulsions by repeated premix membrane emulsification. The effects of selective membrane emulsification process parameters (concentration of the emulsifiers, number of passes of the emulsions through the membrane, and storage temperature) on the properties and stability of W/O/W emulsions were also investigated. The preparation of multiple W/O/W emulsions by repeated premix membrane emulsification (ME) using cellulose acetate membrane (0.8 μm mean pore size) has proposed in this study to reduce low stability problem of the emulsions. A coarse emulsion was passed through the same membrane five times at a constant pressure of 0.3 MPa to minimize the droplets size and to increase the uniformity of droplets size. About 7 wt. % polyglycerol polyricinoleate (PGPR) and 0.5-10 wt. % Tween 80 (Polyoxyethylene (20) sorbitan monooleate) were used as lipophilic and hydrophilic emulsifiers, respectively. In addition, 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (PTSA) was used as a hydrophilic model ingredient for the encapsulation of bioactive substances. By using 7 wt. % PGPR concentration and 0.5 wt. % Tween 80 concentrations, the most uniform particles with minimum mean size of oil drops (9.926 μm) were obtained after four passes through the membrane. The encapsulation efficiency greater than 70 % was achieved after emulsions storage at 4°C for seven days with amount of serum about 41.02%. It can be concluded that cellulose acetate membrane does not possess very high potential for preparing a stable W/O/W emulsion by repeated premix membrane emulsification. However, this membrane is still acceptable for preparing W/O/W emulsions with the optimum conditions (0.8 μm mean pore size and applying pressure of 0.3 MPa) obtained throughout this study since cellulose acetate membrane is low cost and relatively easy to handle.

ABSTRAK

Kajian ini bertujuan mengenalpasti potensi membran selulosa asetat bagi penyediaan emulsi air-dalam-minyak-dalam-air (W/O/W) yang diformulasi dan mengkaji kesan parameter membran pengemulsian yang terpilih (kepekatan pengemulsi, bilangan untuk emulsi melepasi membran dan suhu penyimpanan) terhadap ciri-ciri dan kestabilan emulsi W/O/W. Penyediaan emulsi berganda W/O/W dengan mengunakan kaedah pengulangan pra-campuran membran pengemulsian (ME) dan membran selulosa asetat (0.8μm purata saiz liang) telah dicadangkan dalam kajian ini untuk mengurangkan masalah kestabilan emulsi yang rendah. Emulsi kasar melepasi membran yang sama sebanyak lima kali pada tekanan tetap 0.3 MPa untuk meminimumkan saiz titisan dan meningkatkan keseragaman saiz titisan. Sebanyak 7 % jisim poligliserol poliricinoleat (PGPR) dan 0.5-10 % jisim Tween 80 (Polioksietilena (20) sorbitan monooleat) digunakan masing-masing sebagai pengemulsi lipofilik dan pengemulsi hidrofilik. Garam 1,3,6,8-pirenetetrasulfonik asid tetrasodium (PTSA) digunakan sebagai model bahan hidrofilik untuk pengkapsulan bahan bioaktif. Dengan kepekatan PGPR pada 7 % jisim dan Tween 80 pada 0.5 % jisim, saiz partikel yang paling seragam dengan purata saiz minimum titisan minyak (9.926 μm) telah diperoleh selepas empat kali emulsi melepasi membran. Kecekapan pengkapsulan lebih dari 70 % telah dicapai selepas penyimpanan emulsi pada 4°C selama tujuh hari dengan jumlah serum sebanyak 41.02 %. Dapat disimpulkan bahawa membran selulosa asetat tidak mempunyai potensi yang begitu tinggi untuk penyediaan emulsi W/O/W yang stabil dengan menggunakan kaedah pengulangan pra-campuran ME. Namun demikian, membran ini masih boleh diterima untuk penyediaan emulsi W/O/W dengan keadaan optimum (0.8 μm purata saiz liang dan menggunakan tekanan tetap 0.3 MPa) yang didapati melalui kajian ini, tambahan pula membran selulosa asetat adalah lebih murah dan senang digunakan.

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LIST OF ABBREVIATIONS

XME	-	Cross flow membrane emulsification
DME	-	Direct membrane emulsification
DDS	-	Drug delivery system
EE	-	Encapsulation efficiency
h_t	-	Height of the opaque emulsion phase
H_S	-	Height of the serum layer
HLB	-	Hydrophilic-lipophilic balance
h_0	-	Initial emulsion height
LPC	-	Lysophosphatidylcholine
MCT	-	Medium-chain triglyceride
ME	-	Membrane emulsification
MC	-	Microchannel emulsification
C8TG	-	Octanoic acid triacylglycerol
O/W	-	Oil-in-water emulsions
O/W/O	-	Oil-in-water-in-oil emulsions
PIT	-	Phase-inversion temperature
PC	-	Phosphatidylcholine
PGPR	-	Polyglycerol polyricinoleate
Tween 80	-	Polyoxyethylene (20) sorbitan monooleate
Tween 20	-	Polyoxyethylene sorbitan monolaurate
PME	-	Premix membrane emulsification
PTSA	-	1,3,6,8- pyrenetetrasulfonic acid tetrasodium salt
RME	-	Rotating membrane emulsification
S	-	Sedimentation stability
SPG	-	Shirasu porous glass membrane
Span 80	-	Sorbitan monooleate
Span 83	-	Sorbitan Sesquioleate

SME	-	Stirring membrane emulsification
TGPR	-	Tetraglycerol polyricinoleate
H_E	-	Total height of the emulsions
φ_i	-	Volume percent of inner aqueous phase in W_1/O emulsion
φ_o	-	Volume percent of W_1/O emulsion drops in $W_1/O/W_2$ emulsion
W/O	-	Water-in-oil-emulsions
W/O/W	-	Water-in-oil-in-water emulsions

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CHAPTER 1

INTRODUCTION

1.1 Background of the problem

Emulsion is a mixture of at least two immiscible liquids (usually oil and water, but not always), with one of the liquids (the dispersed phase) being dispersed as small spherical droplets in the other (the continuous phase) (McClements *et al*., 2007). An interfacial layer between the two phases is adsorbed by some necessary surfactant (Charcosset, 2009).

Emulsions can be classified as simple and multiple emulsions. Simple emulsions consist of two main types: (a) oil-in-water (O/W) emulsions, and (b) water-in-oil (W/O) emulsions. The oil-in-water (O/W) emulsions comprise of oil droplets suspended in an aqueous continuous phase (Charcosset, 2009). On the other hand, the water-in-oil (W/O) emulsions consist of water droplets suspended in an oil continuous phase. Multiple emulsions are double emulsions, that is, "an emulsion in an emulsion". Multiple emulsions can be classified into two major types: water-in-oil-in-water (W/O/W) emulsions and oil-in-water-in-oil (O/W/O) emulsions. In the case of W/O/W emulsions, a W/O emulsion itself is dispersed as globules within an aqueous phase whereas the O/W/O emulsions consists of an O/W emulsion dispersed as globules within an oil phase (Graaf *et al*., 2005). Examples of different types of emulsions are shown schematically in Figure 1.1.

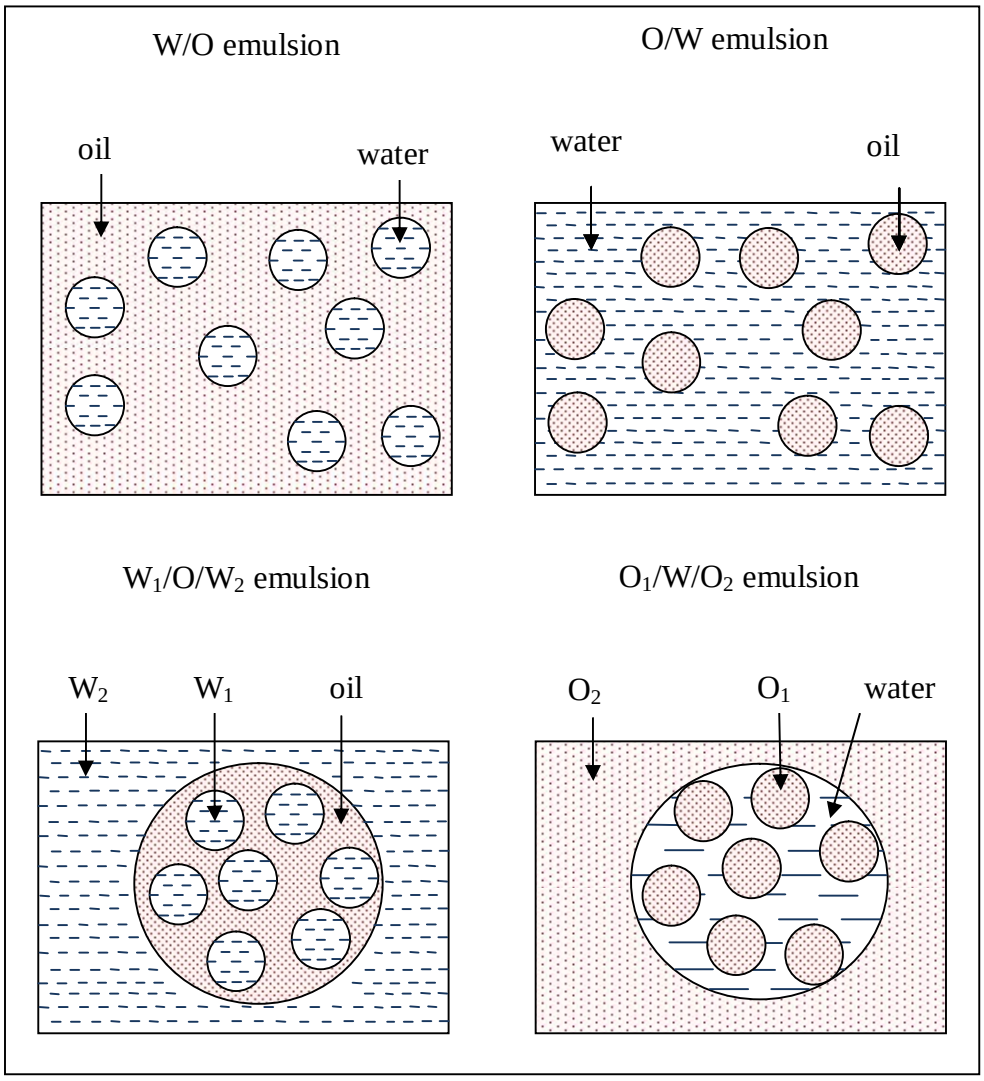

Figure 1.1 Different types of emulsions (Vladisavljevic and Williams, 2005)

Multiple emulsions have immense potential for applications in the food, cosmetic and pharmaceutical industries. In food industry, for example, multiple emulsions have been examined to produce low calorie foods; improved sensoric characteristic and taste-masking (Graaf *et al.*, 2005). In cosmetics products, easily spreadable creams with encapsulated ingredients in both water and oil phases has been reported (Graaf *et al.*, 2005). W/O/W emulsions have been studied intensively on the pharmaceutical applications as a means of drug delivery system (Graaf *et al.*, 2005). Nevertheless, the use of multiple emulsions has been restricted because of their inherent thermodynamic instability resulting in leakage of the encapsulant from the inner aqueous phase. In addition, flocculation of the droplets or phase separation

during processing and storage occurs as consequences of their intrinsic instability (Su *et al*., 2006).

Conventional methods to prepare emulsions involve the use of colloid mills, rotor-stator systems and high pressure homogenizers (Joscelyne and Tragardh, 2000). These methods utilize turbulent shear stress to break-up droplet. However, coalescence of the dispersed phase occurs due to the strong shearing stress of these methods (Charcosset, 2009). A technique for producing emulsion known as 'membrane emulsification' (ME) has received growing attention in the late 1980s (Charcosset, 2009). The technique has received much attention due to its simplicity, low energy consumption, lower amounts of surfactant being utilized and enabling the production of droplet-size with narrow distributions (Trentin *et al*., 2011). This is an innovative method to prepare emulsions with a homogeneous droplets size (Kim and Schroen, 2008).

Membrane emulsification can be carried out in two different ways: direct ME and premix ME (Trentin *et al*., 2011). In direct ME, a low pressure is applied to force the dispersed phase to permeate through a membrane into the continuous phase. In addition, membrane with uniform pore-size distribution is employed. The difference between direct ME and conventional methods is that in direct ME, the resulting droplet size is controlled predominantly by the choice of membrane and not by the formation of turbulent flow to cause droplets break-up (Trentin *et al*., 2011). In premix ME, a coarse emulsion is forced through the membrane (Zhou *et al*., 2009). It is carried out in a two-step process: (i) the two liquids that cannot mix are mixed together first using a conventional stirrer mixer to form a preliminary emulsified coarse emulsion, which is then ii) passed through the membrane (Trentin *et al*., 2011).

Preparation of multiple emulsions involves two-step emulsification methods. A simple emulsion (W/O or O/W) is produced, and then re-emulsified to form W/O/W and O/W/O emulsions, respectively (Morais *et al*., 2009). Typically, the primary emulsion is formed by exerting a high shear homogenization process to produce internal droplet as fine as possible. The second emulsification step is carried

out in mild conditions in order to prevent rupture of the double emulsion droplets (Vladisavljevic and Williams, 2005). If the secondary emulsification is performed by conventional methods; the harsh mechanical stresses may disrupt the primary emulsion droplets, result in reduction of the yield of multiple emulsions (Surh *et al*., 2007). In order to avoid disruption of primary emulsion droplets, a low-shear mixer for the secondary emulsification step is favoured in previous works (Surh *et al*., 2007). As a consequence, highly polydisperse and coarse W/O/W emulsion droplets are often produced resulting in production of emulsions with poor creaming stability. This is due to the relatively large mean droplet size (Surh *et al*., 2007). Therefore, membrane emulsification may be a method of choice for the preparation of multiple emulsions owing to the low shear rates (Charcosset, 2009). This method not only allows the production of emulsions with external droplets that exhibit a narrow size distribution, but also enables it to maintain a high encapsulation yield of the internal droplets (Vladisavljevic and Williams, 2005).

A vast numbers of formulation for double emulsions can be found in literature with various types of oil; different fractions of phases and different kinds of surfactants in varying concentrations. In a nutshell, formulation of double emulsions has great impact on the stability and droplet size of emulsions. In addition, the choice of the preparation method should be taken into account at the same time (Graaf *et al*., 2005).

Table 1.1: Some examples of successful formulations used for the production of double emulsions ($W_1/O/W_2$) with membranes (Graaf *et al.*, 2005).

Inner water phase (W1)	% Inner water phase	Oil phase (O)	Outer water phase (W2)	Reference
No additives	30 vol.%	Soybean oil;0.5% PC;0.5% PGPR	1 % LPC; 5% glucose	Mine *et al*. (1996)
No additives	10vol.%	Oleic acid; 3wt.% TGPR	3 wt.% Tween 20	Kawakatsu *et al*. (2001)
No additives	5 and 10 wt.%	Rape seed oil; 10wt.% PGPR	1-3 wt.% Tween 80	Lambrich *et al*. (2004)
Tris-HCl buffer	10 vol.%	Decane, ethyl oleate, MCT; 5 % Cr-310	Tris-HCl buffer (1% PGML)	Sugiura *et al*. (2004)
5wt.% D(+) glucose	10-30 vol.%	Soybean oil; 5wt.% PGPR	0.5wt.% Tween 80; 5 wt % D(+) glucose; sodium alginate	VladIsavljevic *et al*. (2004)

1.2 Statement of the problems

Water-in-oil-in-water (W/O/W) emulsions can be produced using membrane emulsification (ME). The technique is highly attractive since it consumes lower energy; enable better control of droplets size and droplets size distribution and especially the mild conditions that is involved (Graaf *et al.*, 2005). There are two distinct ways to perform membrane emulsification. One method is known as direct ME, while the other is known as premix ME (Trentin *et al.*, 2011). In premix ME, droplets of a coarse pre-emulsion are pressed through the membrane (Lambrich and Schubert, 2005).

Shirasu Porous Glass (SPG) membranes are studied extensively for premix ME. The membranes were reported to have several advantages such as (i) interconnected micro pores, (ii) a wide spectrum of available mean pore sizes (0.05-30 μm) with narrow size distribution, and (iii) a high porosity (50-60 %). However, SPG membranes are expensive and not easily available. Cellulose acetate (CA) membranes are easily available and cheaper. In addition, the study of this membrane for the production of W/O/W emulsions is rare and it had been studied for passing the emulsions one time (Shima *et al.*, 2004a) but not repeated times.

In this research, a method known as repeated premix ME was applied to produce a formulated multiple water-in-oil-in-water (W/O/W) emulsions. The use of cellulose acetate (CA) membrane had been proposed in order to study its potential for the production of W/O/W emulsions. The properties of the emulsions were also examined. The fine emulsion was repeatedly forced through the same cellulose acetate membrane several times in order to achieve further diminution of the droplets size and to increase the formation of uniformly sized droplets (Vladisavljevic and Williams, 2005). The effect of concentration of the emulsifier, (i.e. lipophilic emulsifier, polyglycerol polyricinoleate (PGPR) and hydrophilic emulsifier, Tween 80 (Polyoxyethylene (20) sorbitan monooleate), number of emulsification cycles and storage temperature on droplets size, particle size distribution, encapsulation efficiency and the stability were also investigated.

1.3 Objectives of the study

To study the potential of a cellulose acetate membrane for preparation of formulated water-in-oil-in-water (W/O/W) emulsions by repeated premix ME, and the effect of selective membrane emulsification process parameters (concentration of the emulsifiers, number of passes through membrane and storage temperature) on the properties and stability of W/O/W emulsions.

1.4 Scope of the study

1.) To prepare primary (W/O) emulsion using optimized concentration of lipophilic emulsifier, polyglycerol polyricinoleate (PGPR), and storage temperature that able to produce W/O emulsion with the smallest water droplets and highest stability.

2.) To prepare coarse and fine water-in-oil-in-water (W/O/W) emulsions using different concentration of hydrophilic emulsifier, Tween 80 (Polyoxyethylene (20) sorbitan monooleate), and the properties and stability of prepared emulsions were later studied.

3.) To study the effect of number of W/O/W emulsions passes through membrane on the properties and stability of fine W/O/W emulsions.

1.5 Significance of the study

This research is in significance of studying the potential of a cellulose acetate membrane in repeated premix membrane emulsification method in order to produce a formulated multiple water-in-soybean-oil-in-water (W/O/W) emulsions with small droplets size, narrow size distribution and improve stability in order to enhance their potential applications in various industries especially food industry. Therefore, production of multiple emulsions with repeated premix cellulose acetate membrane emulsification (ME) technique and understanding of the properties of multiple emulsions under different conditions are important in developing multiple emulsions with desirable properties. This will lead to increase application opportunities of multiple emulsions in various industries.

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