A STUDY OF HOLLOW COLLOIDOSOMES USING POLYHYDROXYBUTYRATE (PHB) AS POLYMER VIA TWO STEPS SOLVENT EVAPORATION METHOD

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A Study of Hollow Colloidosomes Using Polyhydroxybutyrate (PHB) As Polymer Via Two Steps Solvent Evaporation Method

by

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A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Materials Technology) with Honours

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2016

DECLARATION

I declare that this thesis entitled "A Study of Hollow Colloidosomes Using Polyhydroxybutyrate (PHB) As Polymer Via Two Steps Solvent Evaporation Method" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Date : _____

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A Study of Hollow Colloidosomes Using Polyhydroxybutyrate (PHB) As Polymer Via Two Steps Solvent Evaporation Method

ABSTRACT

Colloidosomes is a hollow and composed of a closely-dense packed layer of colloidal particles whose permeability and elasticity can be tuned and highly controllable. Hollow colloidosomes offer a great potential in wide areas such as controlling the release rate of entrapped materials, cosmetics, food supplements and living cell, as protection of biologically active species and removal of pollutants. PHB hollow colloidosomes has attracted interest in research owing to their biocompatibility for the design of implantable drug delivery system and tissue engineering. Previous study established the two steps solvent evaporation method. Similar optimum conditions of 1.5 wt.% PCL/1.2 wt.% PVA were applied to prepare PHB/PVA hollow colloidosomes and the results showed PHB has the potential to be prepared as colloidosomes. However, the particle size is small and low in yield. By varying the concentrations and molecular weight of surfactant used, the optical images showed small particles with higher yield and larger particles with higher yield respectively. Morphology study shows that PHB colloidosomes are more porous compared to PCL hollow colloidosomes.

Satu Kajian Koloidosome Berongga Menggunakan PHB Sebagai Polimer Dengan Dua Langkah Penyediaan Penyejat Pelarut

ABSTRAK

Koloidosome adalah berongga dan mengandungi beberapa lapisan zarah koloid yang mana kebolehtelapan dan keanjalannya dikawal. Koloidosome berongga menawarkan potensi yang bagus dalam pelbagai bidang seperti mengawal kadar pembebasan bahan-bahan yang terperangkap, kosmetik, makanan tambahan, dan sel-sel hidup, sebagai perlindungan kepada spesies aktif biological dan penyingkiran bahan-bahan pencemar. PHB Koloidosome berongga telah menarik kepentingan dalam penyelidikan disebabkan bioserasi untuk reka bentuk penghantaran dadah implant system dan kejuruteraan tisu. Penyelidikan terdahulu telah menghasilkan kaedah dua langkah penyediaan penyejat pelarut '*solvent evaporation*'. Pendekatan situasi dan keadaan yang sama dari 1.5 wt.% PCL/1.2 wt.%PVA telah digunapakai dalam penyediaan PHB/PVA koloidosome dan keputusan menunjukkan PHB mempunyai potensi untuk dihasilkan sebagai koloidosome. Walau bagaimanapun, partikel saiz adalah kecil dan hasil akhir adalah rendah. Dengan membezakan kepekatan dan berat molekul surfaktan, gambar dari optikal menunjukkan partikel kecil dengan hasil akhir yang tinggi dan partikel yang besar dengan hasil akhir yang tinggi masing-masing. Morfologi pembelajaran menunjukkan PHB koloidosome adalah lebih berliang berbanding PCL koloidosome berongga.

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LIST OF ABBREVIATIONS

SEM	Scanning electron microscope
PVA	Polyvinyl alcohol
HLB	Hydrophilic-lipophilic balance
O/W	Oil-in-Water
rpm	Rotation per minute
ME	Microencapsulation
LbL	Layer by layer
PHB	Polyhydroxybutyrate
PCL	Polycaprolactone
PHA	Polyhydroxyalkanoates

LIST OF SYMBOLS

M_n	Number average molecular weight
w/v%	Weight/volume. Percentage of a solute is dissolved in a solvent
D_n	Number-averages diameters
%	Percentage
X	Magnification
°C	Temperature (degree Celcius)

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Hollow particles are the newly developing material, containing an interior hollow or void structure which is usually covered by a solid shell coupled with certain properties of low density, thermal insulation and distinct optical activity (Fuji *et al.*, 2007). Colloidal particles with hollow interiors play significant roles in microencapsulation: a process that has been used varies in applications such as controlled release of drugs, cosmetics and protection of biologically active species (Caruso, 2001; Langer, 1998; Wilcox, 1995). The use of biodegradable polymers produced from renewable resources has attracted considerable attentions as a potential in recent years. Polyhydroxyalkanoates (PHA) polymers are naturally produced by bacteria in general cultivated on agricultural raw materials (Bugnicourt, 2014). PHA is said to have a good potential to be used as tissue engineering biomaterials such as PHB, PHBV, and PHBHHx, where PHB becomes the most thoroughly researched members of the PHA family (Chang *et al.*, 2014). PHB particularly has attracted commercial and academic interests for its applications as carrier for drug delivery or scaffolds in tissue engineering owing to its advantages compared to other chemically produced polymers like polyglycolate, polylactate, and poly(lactide-co-glycolide) which include excellent biocompatibility, biodegradability and easy processibility which can be modulated by variations in processing and molecular weight of the polymer composition (Shrivastav *et al.*, 2013). Due to these favorable properties of

PHB, this study proposed of using PHB as polymer to prepare hollow colloidosomes. These biopolymers are available from certain agricultural activities which may possess the potential to be used for preparation of colloidosomes, as they represent unused resources, widely available and ecofriendly. Thus, this study hopes to wider the selection of biodegradable structural polymers to be prepared via two steps solvent evaporation method in association with green technology in the preparation of raw materials for varies applications in the future.

Preparation for microcapsules techniques usually takes several steps (Caruso, 1998) which are complex, time consuming and in need of binders (Dinsmore *et al.*, 2002). A study by Thompson *et al.* (2015), reported that latex colloidosomes prepared via thermal annealing is likely to be detrimental for the encapsulation of thermally-sensitive actives such as fragrances or drugs. Templating against hard (solid) templates such as layer-by-layer self-assembly of desired materials onto the pre-formed colloidal particles followed by the core removal, is one of the most common methods used for preparation of hollow structures (Caruso, 1998). This approach however, has several recognizable drawbacks due to the slow multistep synthetic processes which eventually make this technique tends to consume a lot of times (Mak *et al.*, 2008; Thompson *et al.*, 2015) . In addition, this method is also pretty challenging of refilling the hollow interior with functional species (Horecha *et al.*, 2009).

Hollow colloidosomes are indeed giving a lot of benefits for a wide variety of many applications and emerging area of technologies. For examples, the implantable drug deliver and pharmaceutical (Porta & Kros, 2013; Shah *et al.*, 2010), offer a great potential in controlling the release rate of entrapped materials, cosmetics, food supplements and living cell (Wang *et al.*, 2007), as protection of biologically active species and removal of pollutants (Im *et al.*, 2005).

1.2 Problem Statement

In this study, the method used is the simple and newly discovered solvent evaporation method (Shahidan *et al.*, 2013). This method is adapted from the most commonly route to colloidosomes based on the self-assembly of colloidal particles at the interface between two immiscible liquids (oil/water interface) in which has proven to minimize the time and cost dealing.

This study will attempt to prepare PHB-shell hollow colloidosomes via two steps solvent evaporation method. The biological microorganisms that have the abilities of producing PHB polymer will be used to widen the possibility for the future development research in fabricating the hollow colloidosomes with distinct and desired properties.

Due to these favorable PHB polymer properties together with an approach of quick and measurable standard laboratory technique, the preparation of hollow colloidosomes could be prepared. In addition, it can give wider selection of materials prepared using the established two step preparation method by Shahidan *et al.* (2013).

1.3 Objectives

This study has three major objectives:

a) To investigate the potential of PHB biopolymer as hollow colloidosomes.

b) To prepare PHB colloidosomes via two steps solvent evaporation preparation by using different concentrations and molecular weight of surfactant.

c) To characterize and analyze the size and morphology of the hollow colloidosomes prepared by using PHB based polymer.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, several key points of encapsulation, microcapsule, colloidosomes, biopolymer used (PHB) and the solvent evaporation method will be briefly discussed. The biopolymer and surfactants that will be used in this study are powdered form PHB and polyvinyl alcohol, respectively. Optical, polarized and scanning electron microscopes will be used for characterizations processes.

2.2 Microcapsule

Encapsulation is the process of forming a capsule around one substance within another substance. Meanwhile the microcapsule is a very small container-like capsule designed to release its contents when broken, melted or dissolved. Microencapsulation (ME) has been defined as the technology of packaging solid, liquid and gaseous materials (the core) in small capsules (the shell) aiming for the release of their contents at controllable rates over periods of time (Figure 2.1) (Champagne & Fustier, 2007; Tyagi *et al.*, 2011).

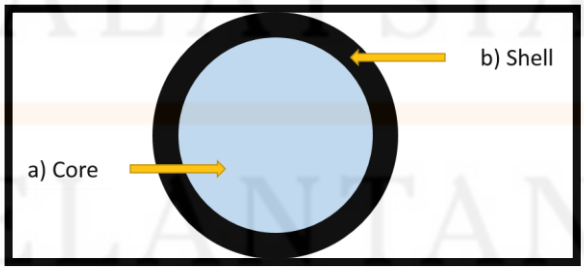

Figure 2.1: The structure of a microcapsule. (a) The material inside the microcapsule is referred as the core. (b) The wall is called as the shell. Retrieved and adapted from (Tyagi *et al.*, 2011)

Many microencapsulation processes have been introduced from the alteration of the three basic techniques: solvent extraction/evaporation, phase separation (coacervation) and spray-drying (Freitas *et al.*, 2005).

The microencapsulation process was discovered in the 1930s (Cosco, 2007) and since then, a lot of developments and discoveries have been introduced. Microencapsulation has also attracted significant attention in biotherapeutics and medical applications (Tomaro-Duchesneau *et al.*, 2012), biotechnology, food and beverage (Zandi & Mohebbei, 2014), and as useful tools to improve the delivery compounds intro particularly probiotics, minerals, vitamins and antioxidants (Champagne & Fustier, 2007).

2.3 Colloidosomes

Colloidosomes is a term introduced by Dinsmore *et al.* (2002). They are hollow composed of a closely-dense packed layer of colloidal particles (Figure 2.2) whose permeability and elasticity can be tuned and highly controllable. Although, the name was first introduced by Dinsmore *et al.* (2002), the first to study on the production of colloidosomes was prior reported by Velev *et al.* (1996).

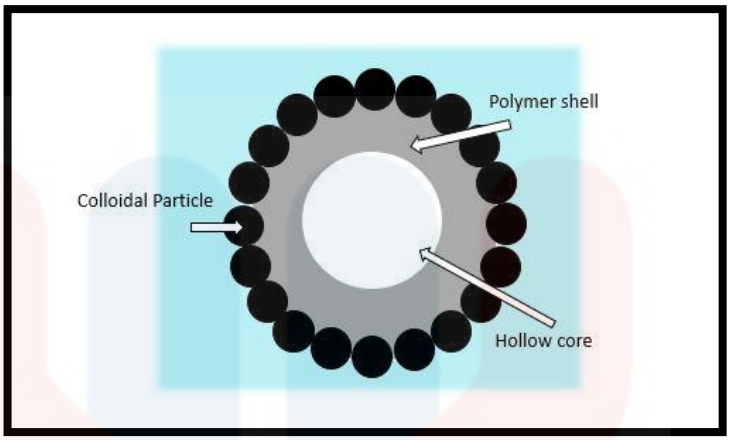

Figure 2.2: The hollow structure of colloidosomes which composed of a closely-packed layer of colloidal particles (Popadyuk *et al.*, 2015)

As a result of having such benefits and favorable properties of the hollow colloidosomes such as removal of pollutants, drug delivery agents and in controlling the release rate of entrapped particles (Im *et al.*, 2005; Porta & Kros, 2013; Shah *et al.*, 2010; Wang *et al.*, 2007), a variety of different techniques (coacervation, fluid extrusion as well as coating immiscible templates by electrostatic deposition) and scalable materials have been used to fabricate hollow colloidosomes (Dinsmore *et al.*, 2002).

2.4 Polymers

In this study, there were two types of polymers used; polyhydroxybutyrate (PHB) and polycaprolactone (PCL).

2.4.1 Poly (3-hydroxybutyrate)

Poly(3-hydroxybutyrate) (PHB), Figure 2.3, is a homopolymer of 3-hydroxybutyrate and is the most widespread and best characterized member of the polyhydroxy-alkanoate family (Bugnicourt, 2014). Poly-3-hydroxybutyrate (PHB) is a linear polyester of D (-)-3-hydroxybutyric acid which was first discovered in bacteria

by Lemoigne in 1925 (Belgacem & Gandini, 2011; Ebnesajjad, 2012). The PHB polymer is obtained as it is accumulated in intracellular granules by a wide variety of Gram-positive and Gram-negative organisms under conditions of a nutrient limitation other than the carbon source (Dawes & Senior, 1973).

This polymer possesses the important and required properties of thermoplasticity and biodegradability in compost and different environments comprising marine water, and therefore, has attracted considerable commercial interest (Bugnicourt, 2014). PHB also offers a promising in forming films as matrices for in vitro cell cultures (Shishatskaya & Volova, 2004), as scaffolds to be used in tissue engineering applications (Güven *et al.*, 2008) as well as microspheres that are proved to have excellent biocompatibility; they neither inhibited growth and metabolic activity of fibroblasts nor caused negative response in animals that were intramuscularly injected before (Shishatskaya *et al.*, 2008).

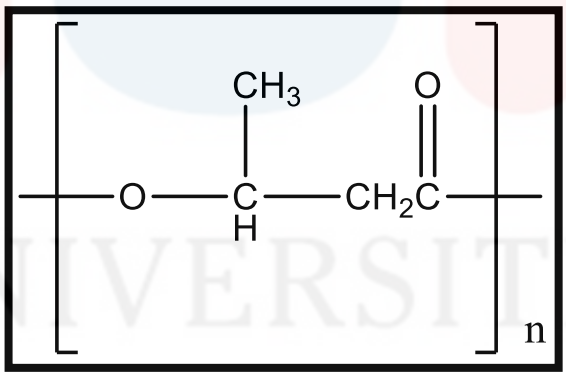

Figure 2.3: Chemical structure of PHB

2.4.2 Polycaprolactone

Polycaprolactone (PCL), Figure 2.4 is commercially emerged as a biomaterials due to its numerous advantages over the other biopolymers which include tailorable degradation kinetics and mechanical properties, ease of shaping and manufacture enabling appropriate pore sizes conducive to tissue in-growth, and the controlled

delivery of drugs contained within the matrix (Azevedo *et al.*, 2003; Woodruff & Hutmacher, 2010).

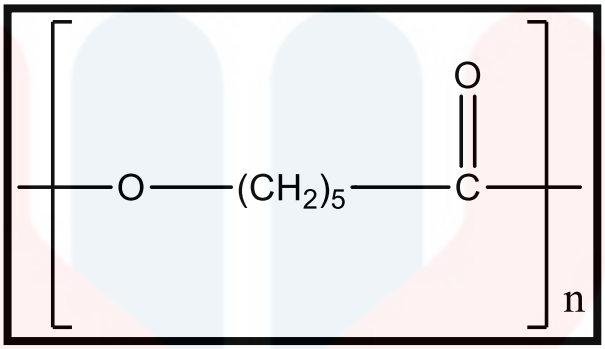

Figure 2.4: Chemical structure of PCL (Woodruff & Hutmacher, 2010)

It has received great attentions as commercially available biodegradable plastics favoring a good flexibility and biodegradability plus with its hydrophobic nature which is suitable for the drug delivery systems (Shahidan *et al.*, 2013; Wu, 2003).

2.5 Surfactant

A surfactant is defined as a substance that can greatly lessen the surface tension of water when used in very low concentrations (Myers, 2005). Such compounds, like short-chain fatty acids, are amphiphilic or amphipathic which giving the meaning of having one part that has an affinity for non-polar media and one part that has an affinity to polar media, are termed as surfactants (Schramm *et al.*, 2003). Furthermore, surfactant was originally registered as a trademark for selected surface-active products and later been released to the public domain (Schramm *et al.*, 2003). In general, surfactant molecule is made up of a "water loving (hydrophilic) and a "water repellent" (hydrophobic) components (Hansson & Lindman, 1996). Surfactant is important for stabilizing emulsion (Youan *et al.*, 2003). In this study, surfactants that will be used are polyvinyl alcohol (PVA) with low and high molecular weight.

2.5.1 Polyvinyl Alcohol

Polyvinyl alcohol (PVA) is a special synthetic polymer, in that it cannot be prepared by polymerization process of the corresponding monomers (Sakurada, 1985) instead, is essentially made from polyvinyl acetate through hydrolysis. PVA is an artificial polymer that has been used since the first half of the 20th century worldwide (Gaaz *et al.*, 2015). PVA has such good biological properties such as biocompatibility, high water solubility and chemical resistance (Baker *et al.*, 2012) that make it possible to be applied in industrial, commercial, medical, and food sectors (DeMerlis & Schoneker, 2003). The unique and similarity physical properties such as water soluble, available in film form, partially crystalline, has substantial tensile strength, more flexibility and hardness (Gaaz *et al.*, 2015), makes it compatible to be used in human tissue. PVA besides, has a special structure that is able to absorb protein molecules and engage with minimal cell adhesion and has no toxic effects (Yang *et al.*, 2004). In this study, about 88% to 98% hydrolyzed PVA will be used since PVA differs in chemical structures for both fully and partially hydrolyzed (Figure 2.5).

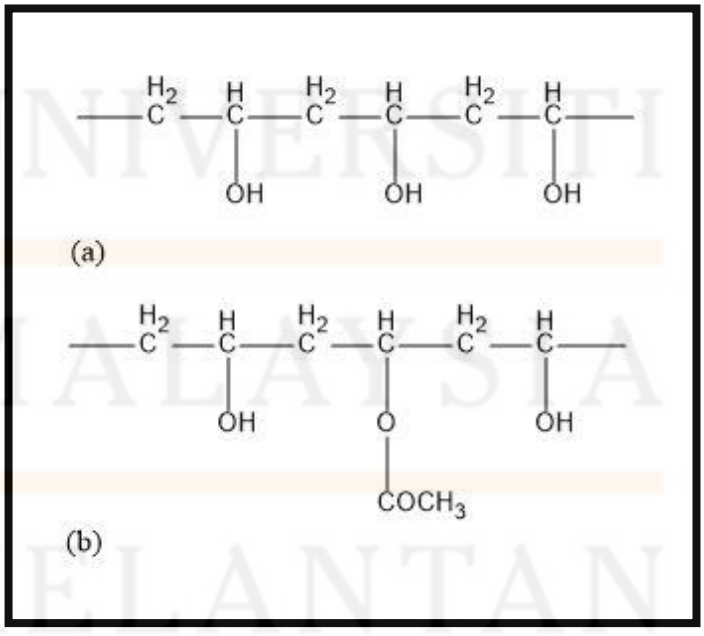

Figure 2.5: Chemical structures of PVA. (a) Fully hydrolyzed and (b) partially hydrolyzed PVA. (Fan, 2005)

2.6 Solvent Evaporation Method

In this study, the solvent evaporation technique is chosen in order to prepare hollow colloidosomes. Typical solvent evaporation method is commonly being used in microencapsulation processes (Freitas *et al.*, 2005; Tiwari *et al.*, 2011). A study by Tomaro-Duchesneau *et al.* (2012) reported that the methods used to prepare microcapsules can be divided into three categories which are chemical, physical and physicochemical methods, where the solvent evaporation is considered to be in chemical method of microencapsulation. The basic process of solvent evaporation method is shown in Figure 2.6. The oil and water phase are prepared (Figure 2.6 (a)) to be homogenized. The emulsification phase occurs. The extraction of the solvent from the emulsification by the continuous phase, accompanied by solvent evaporation, will transform the droplets of dispersed phase into solid particles (Figure 2.6 (b)). The recovery and drying in order to get the microparticles (Figure 2.6 (c)).

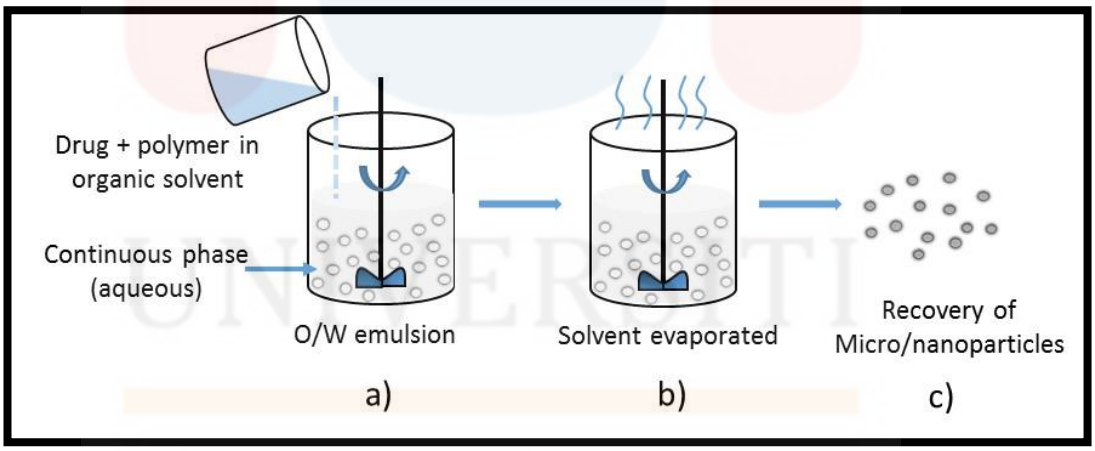

Figure 2.6: Basic steps of microencapsulation by solvent evaporation method (Li *et al.*, 2008)

In contrast to several other methods (Thompson *et al.*, 2015; Wang *et al.*, 2007), this solvent evaporation method is able to control the particles sizes in the nano to micrometer range. It also requires less dealing time, coating factor and operational skill as well as easy to understand and less cost (Freitas *et al.*, 2005; Shahidan *et al.*,

2013). Moreover, this method does not require preformed stabilizing particles (Dinsmore *et al.*, 2002) or the addition of binding species. The approaches of using polyvinyl alcohol (PVA) that is commercially available as the controlled surfactant indicates that this method might be a good candidate for future research in biomaterials applications (Shahidan *et al.*, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

In this study, the biopolymers used were PHB and PCL polymers. Polycaprolactone, PCL with M_n values of 10kg/mol (PCL10, M_w/M_n = 1.4). The solvents used in this research are Dichloromethane, (CH_2Cl_2) (98%) and Chloroform ($CHCl_3$) supplied from Merck brand. The surfactant polyvinyl alcohol, (PVA) (98% hydrolyzed, M_n 13-28 kg/mol) and PVA (88% hydrolyzed) with average M.W 88000 from Sigma Aldrich and Acros Organics respectively.

3.2 Instrumentations

In this study, the instruments used were divided into two categories; to prepare the hollow colloidosomes and for the characterizations part. Homogenizers, NE-1002X Syringe Pump and R-215 Rotavapor were used in hollow colloidosomes preparations while for characterizations the optical, polarized and scanning electron microscope were used.

3.2.1 Homogenizer

The homogenizer used was IKA® T18 Digital ULTRA TURRAX® (Figure 3.1).

Figure 3.1: Homogenizer used in hollow colloidosomes preparation

3.2.2 Syringe Pump

New Era Syringe Pump Systems (NE-100X Microfluidics Syringe Pump) was used in this study (Figure 3.2). It was used as to inject the oil phase (PHB or PCL solution) systems into water phase (PVA solution) with uniform feeding rate during the homogenizing process.

Figure 3.2: Syringe pump used in hollow colloidosomes preparation

3.2.3 Rotaevaporator

Rotaevaporator is known to be useful for accelerating the evaporation rate of the solvents used in controlling and stabilizing the size of the particles produced. It is an efficient yet simple system in preparation of hollow colloidosomes. R-215 Rotavapor (Figure 3.3) was used in this study.

Figure 3.3: The rotaevaporator used in hollow colloidosomes preparation

3.2.4 Optical Microscope

Optical Microscope is a type of light microscope aiming to magnify the image of small specimens by making use the visible light and a combination system of lenses. Resulting image from an optical microscope can be captured with the normal light-sensitive camera. Due to the modern developments in microscopy, the advanced digital microscopes are possible nowadays where they can be used to examine the samples and producing the image directly on a computer screen. Meiji MT800 Metallurgy

Microscope (Figure 3.4) was used in this study for particle sizing of the hollow colloidosomes.

Figure 3.4: An optical microscope used in hollow colloidosomes preparation

3.2.5 Polarized Microscope

Polarized microscope is a type of light microscopy technique that uses plane-polarized light to analyze structures that are birefringent; structures that have two different refractive indices at right angles to one another. It will be used specially to observe and investigate the optical properties of the colloidal samples. Plus, polarized light is a transverse wave light whose vibration possesses direction that gives to birefringent phenomenon, also known as double refraction (Sharma, 2006). DM500 Leica microscope (Figure 3.5) was used in this study.

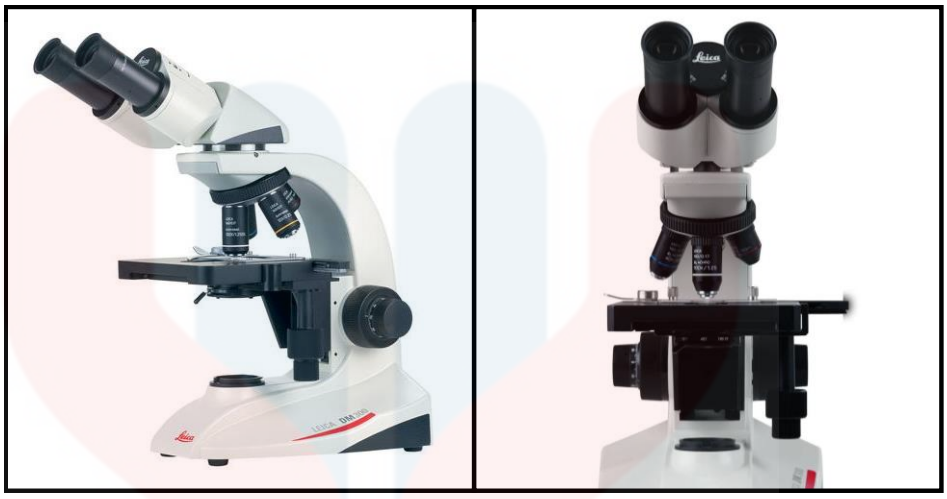

Figure 3.5: A polarized microscope used in hollow colloidosomes preparation

3.2.6 Scanning Electron Microscope

The scanning electron microscope (SEM) is used to gain the morphology of the hollow colloidosomes. While the optical microscope is using the visible light, the SEM does not require the visible light instead, SEM is using electron to scan the morphology of the sample. Due to the limitations in magnification range from optical microscope, SEM will be used to characterize and determine the morphology of the hollow colloidosomes. In this study, FESEM in Centre of Research and Instrumentation Management (CRIM) UKM was used.

3.3 Characterization

There were three (3) main instruments used in this study starting from optical and polarized microscope which basically used to observe and investigate the optical properties of the samples as well as for sizing analysis and birefringence phenomenon observation. The third one is the scanning electron microscope (SEM) which is basically used for morphology study.

3.4 Methods

There were three preparations used which are oil phase preparation, oil-in-water (O/W) emulsification and preparation of the hollow colloidosomes.

3.4.1 Hollow Colloidosomes Preparation

Throughout the study, the PHB and PCL were used for colloidosomes preparation as the main structural biopolymers. The terms PCL/PVA and PHB/PVA indicate the colloidosomes preparations where ((structural polymer)/(surfactant)). The two steps preparations of hollow colloidosomes by solvent evaporation method will include the formation of O/W emulsion. Combinations of the biopolymer and surfactant forms an emulsion. The Oil phase consisted of CH_2Cl_2 with PCL or $CHCl_3$ with PHB while for the water phase, it consisted of water, surfactants (PVA) (Figure 3.6 (a)).

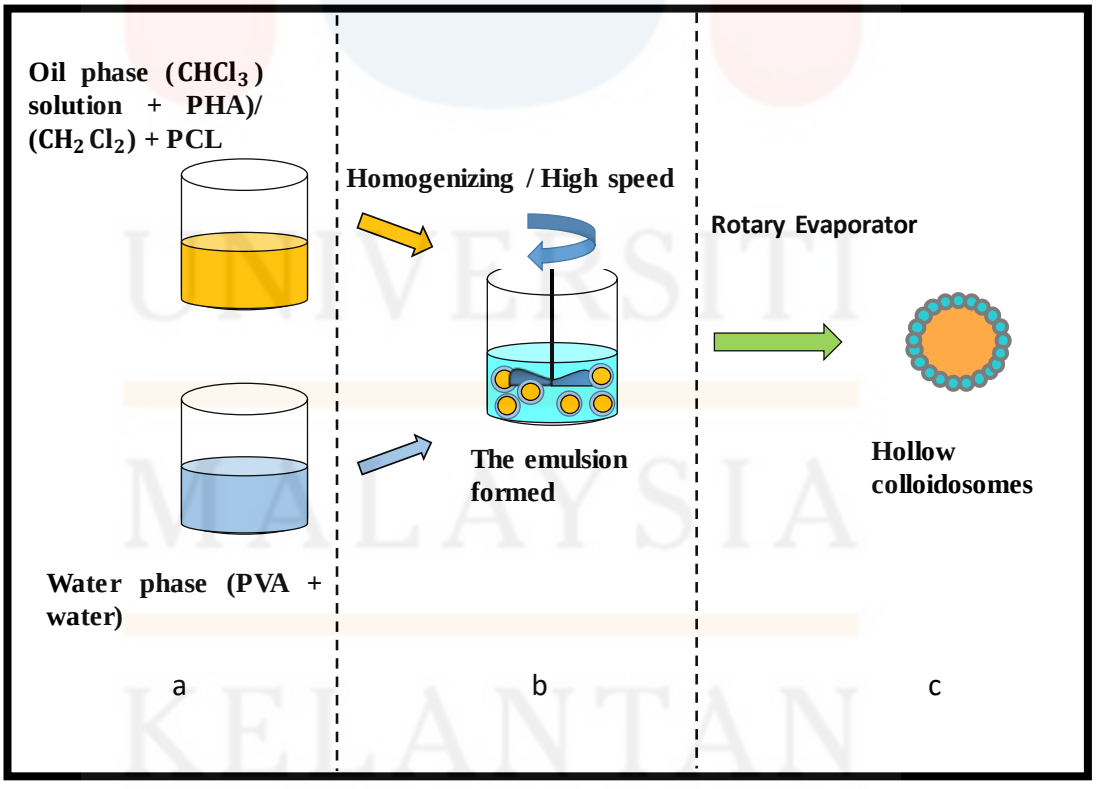

Figure 3.6: The preparation of hollow colloidosomes by solvent evaporation method

The O/W emulsion was prepared by using the homogenizer with high speed (9000 rpm) for 30 minutes (Figure 3.6 (b)). The emulsion was then immediately rotary evaporated at room temperature in order to remove CH_2Cl_2 and trigger the colloidosomes formation (Figure 3.6 (c)).

3.4.2 PCL/PVA Hollow Colloidosomes Preparation

The homogenizer used was IKA® T18 Digital ULTRA TURRAX®. A 75mL solution of 1.5 wt.% PCL polymer was dissolved in CH_2Cl_2 before being fed at a constant rate of 2.5mL/min by using a syringe pump into a beaker that contained 50mL of 1.2 wt.% PVA dissolved in deionized water. The mixture was homogenized at 9000 rpm. After the homogenization about as 30 minutes, the emulsion formed was immediately rotary-evaporated at room temperature of 25°C with a round bottom flask speed at 95 rpm for up to 10 minutes.

Observable optical images produced by using microscope were analysed. Several colloidosomes droplets were dispersed in a microscopic slide for the observation of the size and morphology. Optical microscopy images were obtained by using transmitted light source from optical and polarized microscopes with varies magnifications for examples: X4, X10, X40, X60 and X100. At least 100 colloidal particles were counted in order to determine the number-averages diameters, D_n. SEM was used for further characterizations and the analysis on the sizing and morphology of the colloidosomes formed.

3.4.3 PHB/PVA Hollow Colloidosomes Preparations

The exact same homogenizer was used with the same diameter. A 75mL solution of 1.5 wt.% PHB readily dissolved in $CHCl_3$ was fed at the same rate of 2.5

mL/min by using a syringe pump into a beaker containing 50mL of 1.2 wt.% PVA solution readily dissolved in deionized water, whilst being homogenized at 9000 rpm. After the homogenization, the emulsion formed was immediately rotary-evaporated at room temperature of 25°C. The speed used was the same as before with 95 rpm for about 5 to 10 minutes.

Observable optical images produced by using microscope were analysed. Several colloidosomes droplets were dispersed in a microscopic slide for the observation of the size and morphology. Optical microscopy images were obtained by using transmitted light source from optical and polarized microscopes with varies magnifications for examples: X4, X10, X40, X60 and X100. At least 100 colloidal particles were counted in order to determine the number-averages diameters, D_n. SEM was used for further characterizations and the analysis on the sizing and morphology of the colloidosomes formed.

3.4.4 Centrifugation

Centrifugation process was used in this study specially for the preparation PCL/PVA colloidosomes as it involves the separation of two immiscible substances by using centripetal for the sedimentation of heterogenous mixtures (Berk, 2008; Burtis *et al.*, 2012) The sample solution of PCL/PVA colloidosomes was centrifuged at 4000 rpm for 1 minute by using Eppendorf MiniSpin® plus Microcentrifuge at room temperature. The supernatant was removed for the washing system. This extraction procedure was repeated for 5 times. The addition of deionized water was done during the washing system to approximately increase the sample solution up to 1mL. Figure 3.7 shows the centrifuge used in this study.

Figure 3.7: The centrifuge used in PCL/PVA hollow colloidosomes preparation

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Hollow Colloidosomes

The research in this study is a continuation research by Shahidan *et al.* (2013). The very first stage of the laboratory work was to prepare PCL/PVA sample at fixed parameters and conditions of 9000 rpm, 25°C and 2.5 mL/min followed from previous study. Shahidan *et al.* (2013) reported that these parameters and conditions showed a good result on the formation of hollow colloidosomes where the best average size of the particles produced to be nearly 3.8μm. Thus, in this study this was a control step to ensure all the data and results produced from the current study will approximately have the same results as previous study. Figure 4.1 shows the comparison data and results between previous study and the new current study based on the constant optimized parameters and condition as mentioned before.

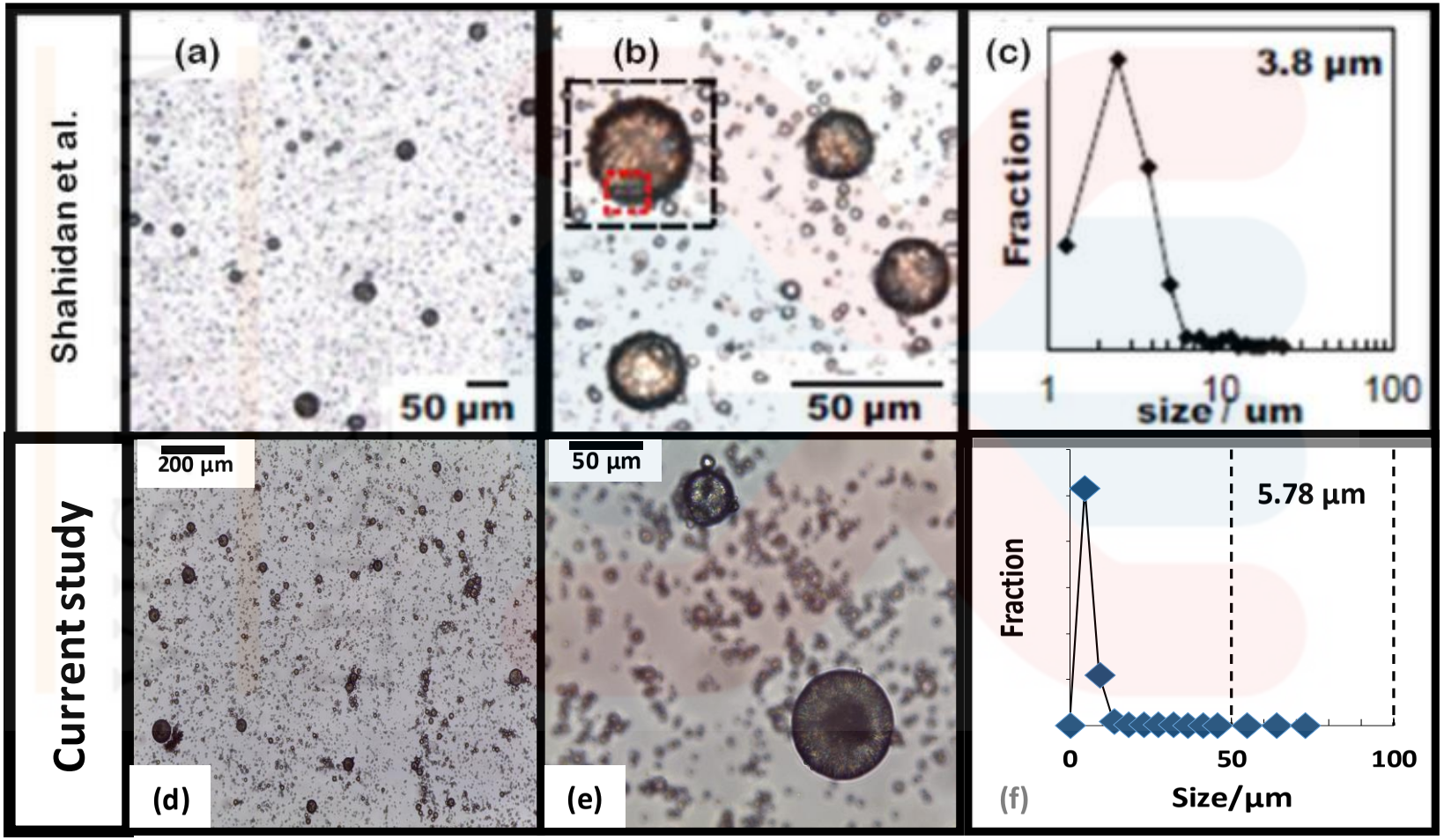

Figure 4.1: The results between previous study and the current study. (a - c) Current study. (b - d) Previous study. Both studies were using 9000rpm, 2.5mL/min and 25°C

Based on Figure 4.1, it shows that the number-average diameter of both studies was completely different. The average of 3.8μm particle size was not achieved in the current study as compared to the previous study. The differences may be due to the different type of homogenizers used. The research then was further continued by studying the effect of different parameters on the preparation of the hollow colloidosomes.

Table 4.1 shows the different parameters used to prepare the hollow colloidosomes and their size distributions.

Table.4.1: PCL/PVA and PHB/PVA hollow colloidosomes preparation condition with different parameters and size distributions. [a] surfactant concentration, [b] concentration of structural polymer and [c] number-average diameter

Entry	System	V_W [a]/mL	C_{Pol} [b]/w/v%	Parameters		D_n [c]/μm
				Average Molecular Weight of Polymer Surfactant (M_w), kg/mol	Ratio Oil phase to water phase	
*	PCL/PVA	1.5	1.2	13-28	1.5:1	3.80
1	PCL/PVA	1.5	1.2	13-28	1.5:1	5.78
2	PCL/PVA	1.5	0.5	13-28	1.5:1	5.83
3	PCL/PVA	1.5	0.25	13-28	1.5:1	6.33
4	PCL/PVA	1.5	0.1	13-28	1.5:1	12.47
5	PCL/PVA	1.5	1.2	13-28	2.5:1	9.73
6	PHB/PVA	1.5	1.2	13-28	1.5:1	1.33
7	PHB/PVA	1.5	0.5	13-28	1.5:1	1.37
8	PHB/PVA	1.5	0.5	88	1.5:1	8.57
9	PHB/PVA	1.5	0.5	88	2.5:1	-

There were two different parameters applied in order to prepare the hollow colloidosomes. The further research was to study the effect of different concentrations of surfactant used. The concentrations used were as such: 1.2 wt.%, and 0.5 wt.%. The 0.25 wt.% and 0.1 wt.% PVA surfactant concentration were also specially applied for PCL/PVA hollow colloidosomes preparation in order to investigate the effect of a significant low concentration surfactant towards the formation of hollow colloidosomes. The second one was to study the effect of different ratios of oil phase to water phase used. The ratios used were 1.5:1 and 2.5:1. The average size distributions of the particles were all calculated manually.

The same parameters and conditions were used for the preparations of PHB/PVA hollow colloidosomes. However, the centrifugation process was negligible in the PHB/PVA hollow colloidosomes preparation because of the lower yield of particles produced. In order to determine the number-average sizes, 100 or more colloidosomes particles were counted. All images obtained by using DM500 Leica microscope and Olympus BX53-P with magnifications of 4x, 10x, 20x, 60x, 100x and specially for PHB/PVA hollow colloidosomes prepared with a higher molecular weight of surfactant. Birefringence images literally obtained from polarizer Leica microscopes. As for morphology study, SEM was used to characterize the morphology of the hollow colloidosomes produced.

4.2 A Brief Study of PHB Hollow Colloidosomes

A brief study on PHB hollow colloidosomes was firstly to explore the PHB potential to be prepared as hollow colloidosomes. The first attempt on preparing PHB hollow colloidosomes was done following the same conditions and parameters as PCL

hollow colloidosomes preparation, PHB with oil to water phase ratio of 1.5 to 1 with 1.5 wt.% PHB and 1.2 wt.% PVA surfactant.

Based on the Figure 4.2 (a), the optical image indicates the potential of PHB to be used as polymer for hollow colloidosomes preparation. The PHB hollow colloidosomes were produced however they were low in yield and small size. This is because the conditions and parameters to prepare PHB hollow colloidosomes yet to be optimized due to the formation of flakes (red arrow in Figure 4.2 (a)).

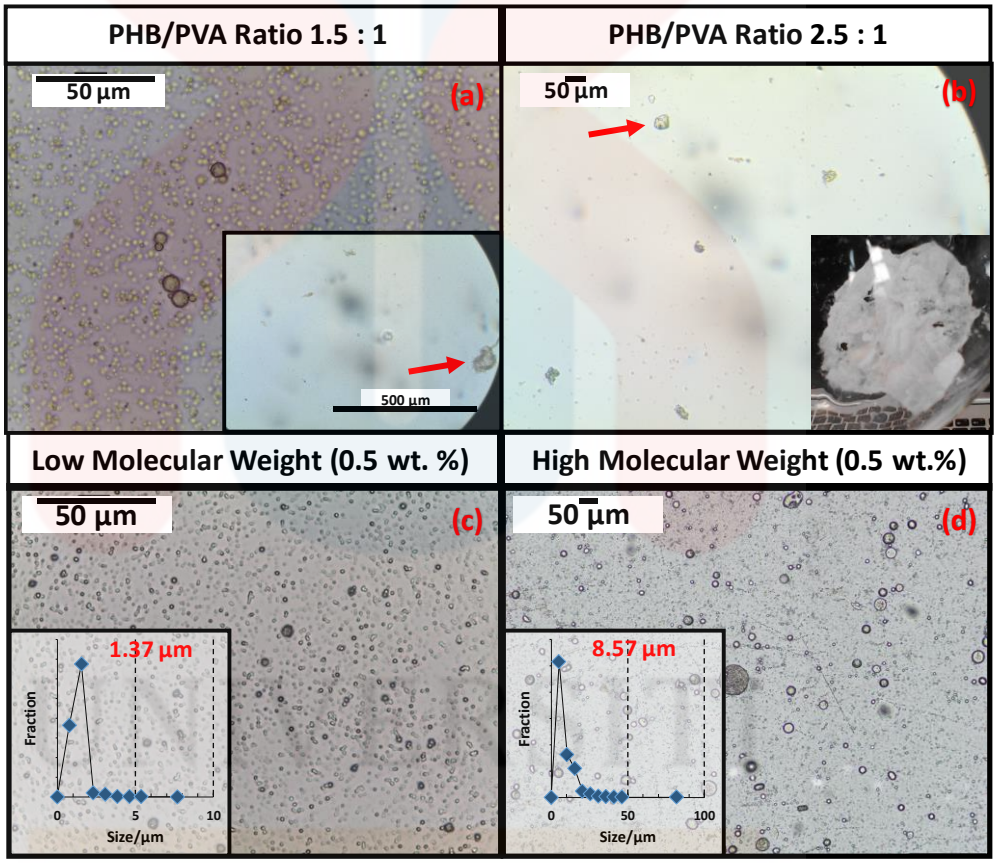

Figure 4.2: A brief study of PHB hollow colloidosomes

Knowing that the low yield and small size produced, we used the ratio to 2.5 to 1 of oil to water phase in order to produce larger particle size. However, larger flakes were produced as shown in Figure 4.2 (b). We proposed that this is due to steric repulsion of colloid when preparing PHB hollow colloidosomes. The low yield of PHB hollow colloidosomes formed might due to the formation of larger flakes in the

beginning (red arrow in Figure 4.2 (b)) of the preparation process. We suspect that the remaining PHB colloidosomes were too large, collapsed and flatten.

Next, different surfactant concentrations were examined to stabilize the emulsified systems. By varying the concentration of surfactant used (from 1.2 to 0.5 wt.%), the Figure 4.2 (c) shows a slightly larger size however, a low yield of PHB colloidosomes. The effect of concentration of surfactant used will be further studied in Section 4.3.

After the third trial, we have come to a new alternative of varying the molecular weight of PVA surfactant used in order to improve the stability of the colloidosomes formation. This introduction of changing to a higher molecular weight of PVA surfactant used was indeed a good alternative as shown earlier (entry 8, Table 4.1), the obvious increase in average particles size as compared to the previous used PVA surfactant. A better yield and distinct differences in size of the PHB hollow colloidosomes is shown in Figure 4.2 (d). The particle size produced was large which is approximately to 100μm. Yeo *et al.* (2001) reported the same trend during concentration modification from 4% to 8% the particle size increased linearly with the increase in polymer concentration or by in this case with a higher ratio. Higher ratio means higher concentration of polymer used.

4.3 Effect of Surfactant Concentration

In this case study of PCL/PVA hollow colloidosomes preparation, the particles size was increased with decreasing the concentration of surfactant (entry 1-3, Table 4.1). The result produced is theoretically supported by Shahidan *et al.* (2013). The study by Shahidan *et al.* (2013) showed that the lower the concentration of surfactant used, the larger the colloidosomes were produced. PVA concentration used in this

study were 1.2 wt.% which is the controlled parameter from previous study, 0.5 wt.% and 0.25 wt.%. Figure 4.2 shows that PCL/PVA hollow colloidosomes particle distribution when using different surfactant concentration. Based on the Figure 4.3, it is proved that in the sample preparation of PCL/PVA hollow colloidosomes, the size of particles is increasing as the polymer surfactant concentration is decreased.

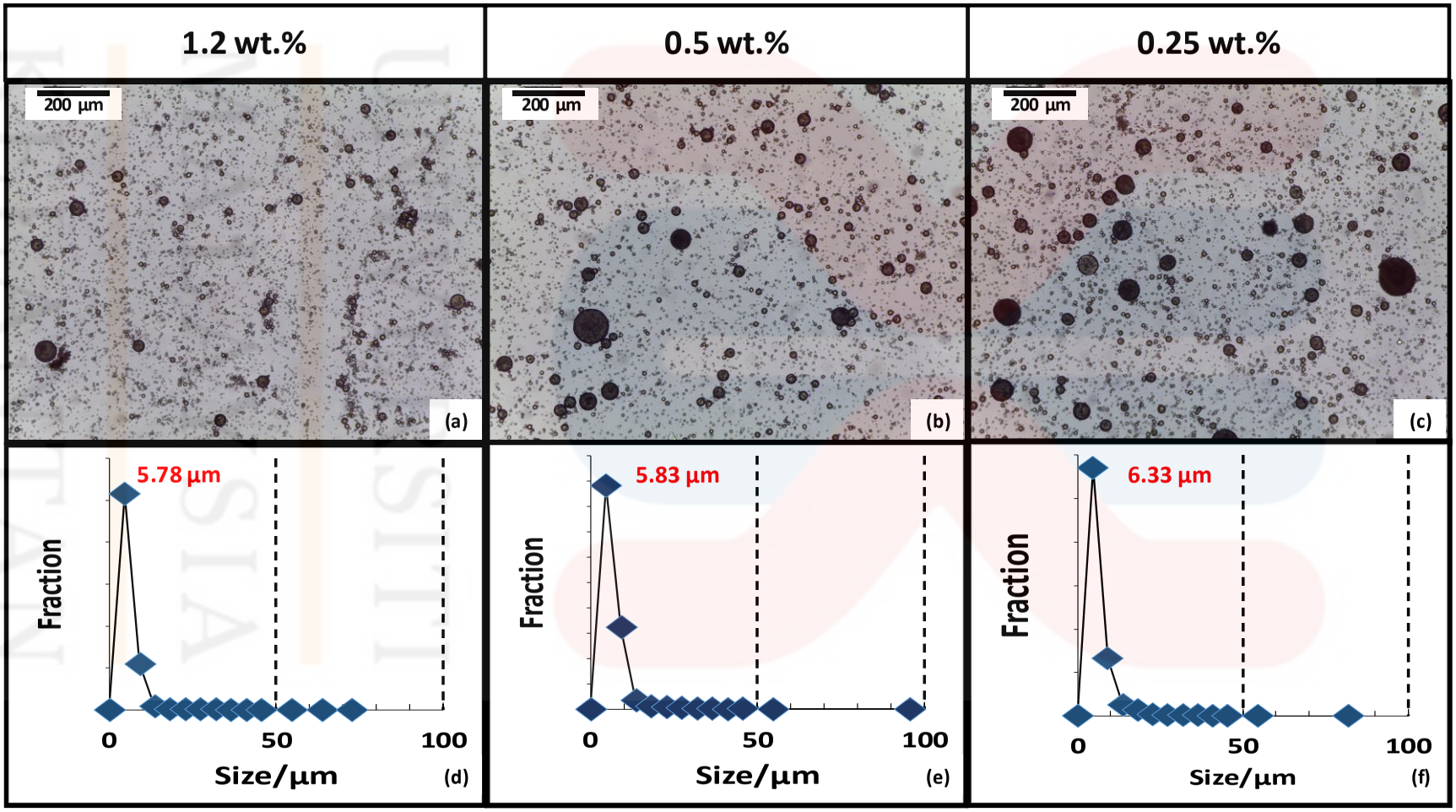

Figure 4.3: PCL/PVA hollow colloidosomes particles distributions when using different surfactant concentrations

As for the PHB/PVA hollow colloidosomes preparation, the work was done similar to the one from PCL/PVA colloidosomes preparations where the result showed the same trend as PCL/PVA samples. Based on the Table 4.1, the average size of particles produced was increasing (entry 6 – 7, Table 4.1) as the concentration of PVA surfactant was reduced. This exactly proved and followed the previous studies by (Neta *et al.*, 2012); Shahidan *et al.* (2013); (Taghipour *et al.*, 2014). As for PHB/PVA colloidosomes preparations, 0.25 wt.% of PVA surfactant was not use due to the time and materials limitation together with the condition of the PHB/PVA hollow colloidosomes produced with 0.5 wt.% PVA surfactant concentration. Ideally the particles size produced was better as compared to the PHB/PVA with 1.2 wt. % PVA surfactant concentration however, the yield of the colloidosomes produced was very low as they were hard enough to be counted for at least 100 or more colloidosomes particles. In related to this, the centrifugations step was skipped as the lower yield of colloidosomes produced means the lower number of smaller particles produced.

The Figure 4.4 shows the images of both 1.2 and 0.5 wt.% of PVA concentrations used in the preparation of PHB/PVA hollow colloidosomes.

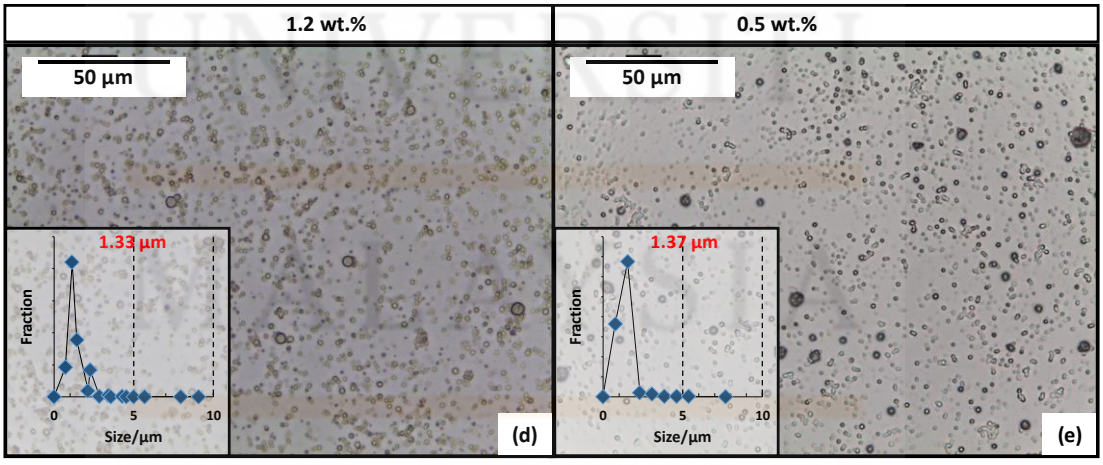

Figure 4.4: PHB/PVA hollow colloidosomes particles distributions when using different surfactant concentrations

4.4 Effect of Different Ratios of Oil phase to Water Phase

Another different parameter used was by altering the ratio of oil phase to water phase. The optimized ratio of 1.5 to 1 studied from Shahidan *et al.* (2013) was followed as it showed a bigger in size of colloidosomes particles. In this case, the ratio of 2.5 to 1 was approached as to increase the average diameter size of PCL/PVA hollow colloidosomes.

Previous study reported by Singh *et al.* (2011) showed that as the ratio of oil phase to water phase increase, the larger the average diameter size of the particles produced. This is in agreement with this study as the PCL/PVA hollow colloidosomes produced were bigger when compared to the optimized, controlled parameters of ratio 1.5 to 1. Similar trend was also reported by Neta *et al.* (2012) and Yeo *et al.* (2001).

Figure 4.5 shows the particles distribution and their size when the different ratios of oil phase to water phase was approached.

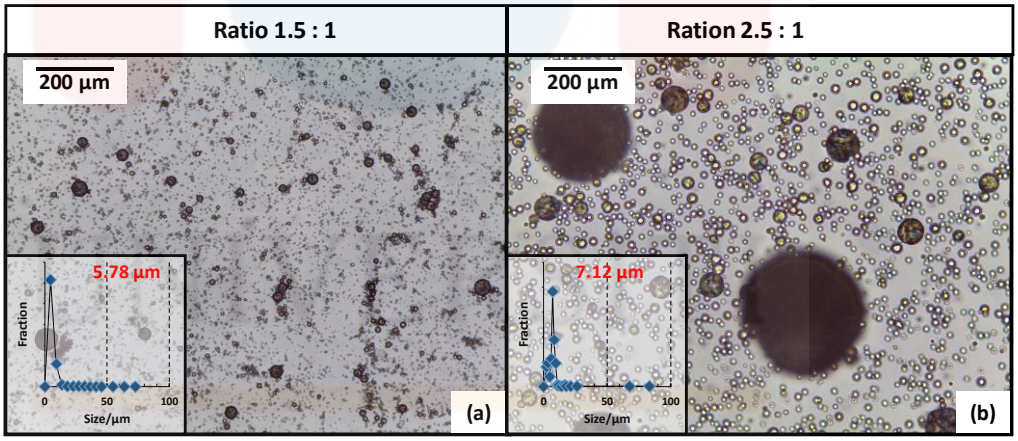

Figure 4.5: The effect of different ratios used on the size distributions and yield of PCL/PVA hollow colloidosomes preparation

Effect of ratio of oil phase to water phase was done once in the preparation of PHB/PVA hollow colloidosomes in order to investigate the average particles size as well as the yield produced. The result was expected to follow the same as the PCL/PVA hollow colloidosomes but rather the sample produced was completely in

different trend. The small flakes were immediately formed right away during the rotary evaporation process. The particles formed were almost flatten and any sign of good shape hollow colloidosomes cannot be detected.

4.5 The Birefringence Study

The birefringence phenomenon was previously discussed in Section 3.2.5. Figure 4.6 shows the hollow particles when being characterized and magnified under polarized microscope in order to clearly demonstrate the birefringence effect. The yellow dotted box was used as the subject of magnification for a better view. The birefringence phenomenon needs to be identified as to confirm that the particle produced is the hollow colloidosomes particle.

Based on the Figure 4.6, we also proposed that the PHB colloidosomes shell is thinner compared to PCL colloidosomes shell prepared using the same conditions. This is due to the fact that the particle produced was hardly showing the distinct effects of birefringence phenomenon. The shell produced slightly pale blueish colour (Figure 4.6 (a – b)) instead of showing a variety of different colours (Figure 4.6 (c – d)) as PCL colloidosomes. We proposed that this is due to the different in thickness of the shells. Thickness of the shells plays a significant parameter on examining the birefringence effect as proved from Michel Levy Chart. Birefringence effect literally affected by a family of lines that emanate radially from the origin, each with a different measured value of birefringence corresponding to thickness and interference colours (Jakli & Saupe, 2006). This is therefore indicating that PHB colloidosomes is thinner and highly porous compared to PCL colloidosomes.

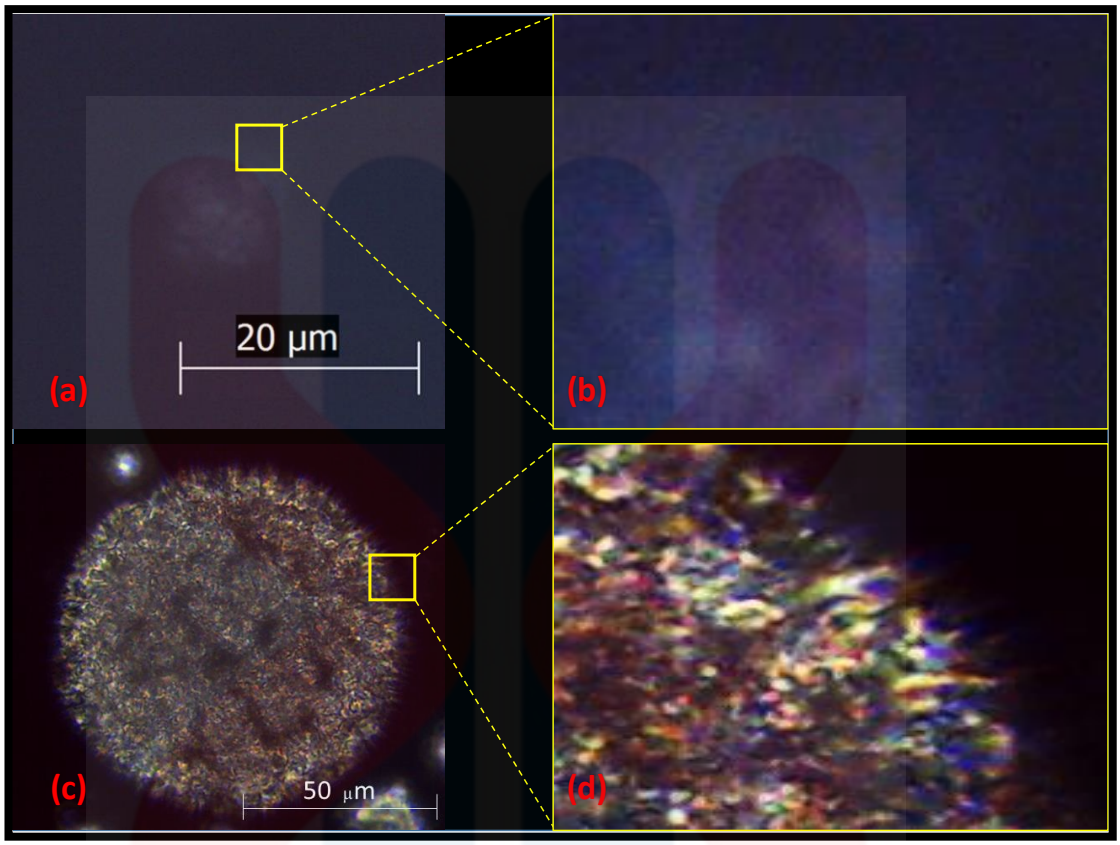

Figure 4.6: The birefringence phenomenon of PHB/PVA (a – b) and PCL/PVA (c – d) hollow colloidosomes

4.6 The Morphology Study

Both PCL/PVA and PHB/PVA hollow particles were further characterized by using FESEM for morphology studies. Figure 4.7 (a – d) is showing a PHB/PVA with different concentration of surfactant as compared to the PCL/PVA particle (Figure 4.7 (e – f)). Based on the Figure 4.7, the PHB/PVA hollow particle produced from a higher concentration of surfactant is showing the highest porosity. The larger interstices contribute to the high porosity which is highly desirable for certain biomedical applications. In related to this, the Figure 4.7 (b) specifically, is indicating that the particle is a hollow colloidosome due to the fact that the shell was made of small particles. This practically proved that many small particles were partially fused within the shell of hollow colloidosomes.

As for Figure 4.7 (c – d), the particle was obtained from a slightly lower concentration of surfactant. The image shows the particle produced was more in spherical shape. From the particle shell, it obviously showed the porous structures of the PHB/PVA colloidosomes as compared to PCL/PVA particle. The Figure 4.7 (e – f) indicates the PCL/PVA particle is having less porosity with more layer of shells.

In a nutshell, we proposed that PHB/PVA hollow colloidosomes exhibit porous structures. Colloidosomes from a higher concentration of surfactant exhibits the highest porosity. The PCL/PVA hollow colloidosomes are less porous than PHB/PVA hollow colloidosomes.

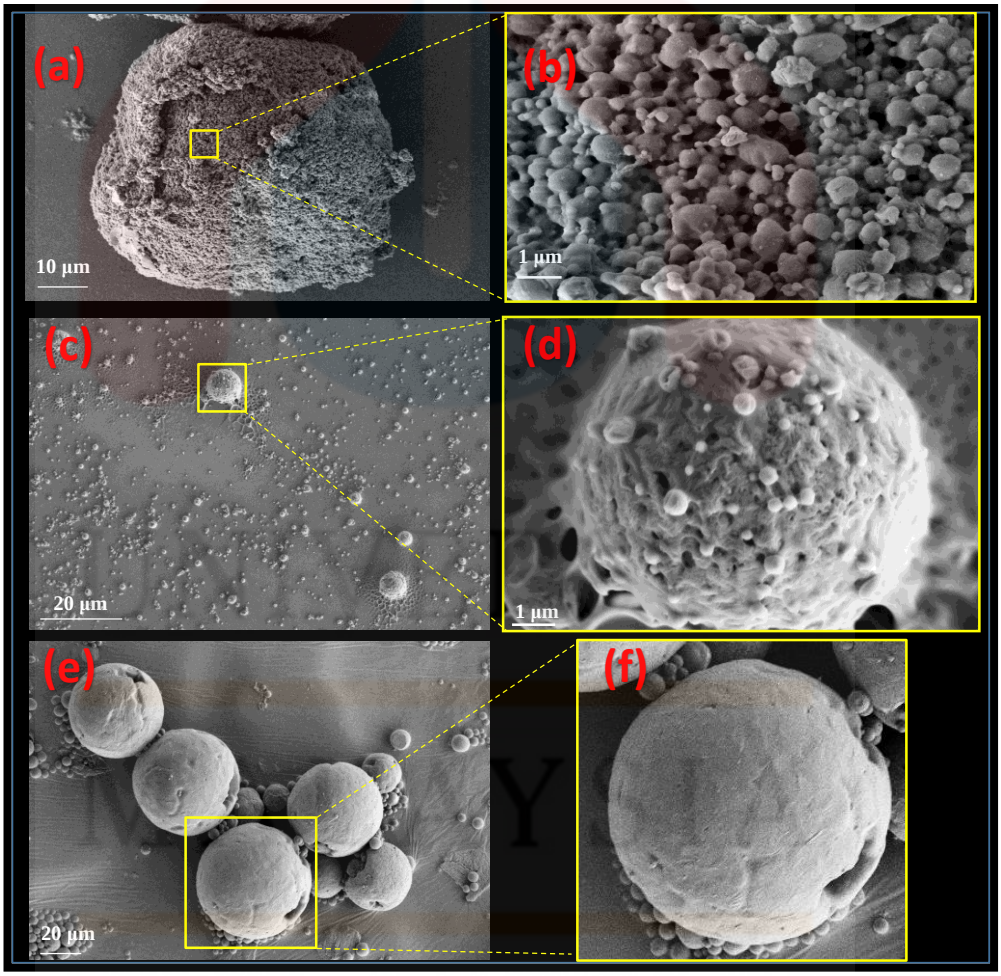

Figure 4.7: The morphology study of PCL/PVA and PHB/PVA hollow colloidosomes. PHB/PVA hollow colloidosome with a higher (a – b) and lower (c – d) concentration of surfactant. (e – f) PCL/PVA hollow colloidosomes

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, the project has succeeded in achieving its objectives. In this study, the PHB-shell hollow colloidosomes were produced, indicating that PHB polymer has the potential to be used as hollow colloidosomes. PHB is used in this study in order to widen the biomaterial selection of preparation of colloidosomes as well as to investigate the behavior of hollow colloidosomes formed. In fact, PHB is noticeably arises as promising biocompatible material in producing microcapsules for specific applications.

The PHB hollow colloidosomes were prepared by a simple and easy to understand method- the two steps solvent evaporation method. O/W emulsion was used where commercially available materials such as PVA as water phase and structural polymers of PCL and PHB as the oil phases which are known to giving harmless effect to the human body. This two steps method is indeed a simple and cost effective method to be used.

The characterization and analysis of both size and morphology of PHB hollow colloidosomes were also well proved. Several attempts were done to optimize the size and yield of PHB hollow colloidosomes. The first two trials were indicating the unstable conditions of PHB colloidosomes preparation. In this study, it is concluded that PHB can be used as a biocompatible material for preparation of hollow

colloidosomes by controlling the average molecular weight of surfactant used. A controlled condition was achieved by using a higher molecular weight of surfactant, leading to higher steric repulsion. Therefore, a stable condition as well as a better yield and large size of PHB hollow colloidosomes were formed. A lower concentration of surfactant together with varied ratio of oil to water phase also influence the particle size and colloidosomes yield.

Further characterizations were done by studying the birefringence effect and morphology studies. The PHB hollow colloidosomes showed pale blueish colour when observed under polarized optical microscope for birefringence effect study. It is proposed that the PHB colloidosomes shells are much thinner compared to PCL colloidosomes prepared using the same condition. It is proved that the colloidosomes formed were consisted of smaller particles of PHB, forming as the shell for hollow colloidosomes. From morphological study, PHB colloidosomes were producing a unique property, featuring larger interstices which means a high porosity material which is highly desired for certain biomedical applications such as scaffolding.

5.2 Recommendation

Based on the findings from this study, some further investigations can be undertaken in the future knowing a lot of possibilities could be achieved during the research study. Firstly, as mentioned earlier in Chapter 4, an attempt actually was made in order to find out the possibilities of the PHB/PVA hollow colloidosomes produced by varying the ratio of oil to water phase. It was however showing a different trend as PCL/PVA hollow colloidosomes. It is good to have future studies to work on finding a stable and controllable of oil to water phase preparation as it may contribute to more specific applications.

Next, the alternative of using a higher molecular weight of surfactant. This approach shows a great potential to fully understand the behavior of PHB hollow colloidosomes in term of sizing and yield. Further studies need to be done for better control and stability of PHB hollow colloidosomes preparation. Despite the good results produced from sample preparations by using a lower molecular weight of surfactant, it would be interesting to study more on PHB hollow colloidosomes preparation by using a higher molecular weight of surfactant. This could be resulted in better understanding of displacement of the particles as well as the particles sizing and yield. This is important for demanding researches on biomedical applications.

The third one is, the freeze-drying method at which the quantitative approach can be used to calculate the actual value of the colloidosomes yield. Last but not least, the freeze fracture can be used in future studies. This freeze fracture approach is critical for the conformation of hollow particles. The colloidosomes formed can be proved of having the hollow core by using this technique.

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