A STUDY OF PCL10 AT DIFFERENT OIL IN WATER VOLUME RATIO USING POLYVINYL ALCOHOL, SUGAR ESTER AS SURFACTANT

by

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DECLARATION

I declare that this thesis entitled "A Study of PCL10 at Different Oil In Water Volume Ratio Using Polyvinyl Alcohol, Sugar Ester as Surfactant" 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.

Signature : .....

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Date : 9 JANUARY 2017

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ABSTRACT

The preparation of PCL colloidosome at different oil in water ratio by using polyvinyl alcohol (PVA) and S1670 (based of sugar ester) as surfactants. The preparation of PCL colloidosome were carried out by using two steps of solvent evaporation method. The study were carried out in three different parameters. The first parameter is, different volume of oil phase and second is using, two different types of surfactant (PVA and S1670) were used while keep constant the value for their concentration at (1.2 wt%). The third parameters utilized for the same type of stablizer, S1670 but their concentration were diversified such as S1670 (1.2 wt%) and S1670 (0.1 wt%). Effects for all of three parameters such as particle stability, average size of particle, yield of particle and surface tension were determined. The higher volume of oil to water phase ratio, the larger size of particle formed. By using PVA as stablizer in colloidosome preparation, the size of colloidosomes formed are larger than used of S1670. When the colloidosomes from all of the three systems were compared together, their size are in various of microns and due to that, it resulted to some features such as rate of active agent release and degradation rate of colloidosomes which are related to the specific target application, either for short or long term recovery application. As this study aims toward an injectable scaffold as a microparticle carrier, the selection of colloidosome are depends on their target application for example, large sized of colloidosomes are suitable to apply in long term recovery application and contrast to the small particles sized.

ABSTRAK

Penyediaan PCL koloidosome pada nisbah minyak yang berbeza dalam nisbah air dengan menggunakan polyvinyl alkohol (PVA) dan S1670 (berdasarkan ester gula) sebagai surfaktan. Penyediaan PCL koloidosome telah dijalankan dengan menggunakan dua langkah kaedah penyejatan pelarut. Kajian ini telah dijalankan dalam tiga parameter yang berbeza. Parameter pertama adalah, isipadu fasa minyak yang berbeza dan kedua ialah menggunakan, dua jenis surfactant (PVA dan S1670) pada nilai kepekatan yang sama iaitu pada (1.2% berat). Parameter ketiga ialah menggunakan jenis surfaktan yang sama iaitu, S1670 tetapi nilai kepekatannya telah dipelbagaikan pada S1670 (1.2% berat) dan S1670 (0.1% berat). Kesan untuk kesemua tiga parameter seperti kestabilan zarah, saiz purata zarah, zarah yang terhasil dan ketegangan permukaan ditentukan. Semakin tinggi nisbah isipadu minyak di dalam fasa air, semakin besar saiz zarah yang terhasil. Dengan menggunakan PVA sebagai surfaktan dalam menyediakan koloidosome, saiz koloidosome yang terbentuk adalah lebih besar daripada yang menggunakan S1670. Apabila koloidosome daripada semua sistem dibandingkan bersama, pelbagai mikron saiz koloidosome yang terhasil dan dan kerana itu, ia telah memberi kesan terhadap beberap ciri seperti kadar pelepasan ejen aktif dan kadar degradasi koloidosome yang berkaitan dengan sasaran khusus aplikasi, sama ada untuk permohonan pemulihan jangka pendek atau panjang. Oleh kerana kajian ini adalah bertujuan ke arah suntikan kerangka yang bertindak sebagai pembawa zarah mikro, pemilihan koloidosome adalah bergantung kepada sasaran mereka sebagai contoh, bersaiz colloidosomes besar sesuai untuk digunakan dalam pemulihan jangka panjang yang berbeza dengan zarah bersaiz kecil.

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LIST OF ABBREVIATION

Abbreviation	Description
PCL	Polycaprolactone
PVA	Polyvinyl alcohol
CH_2Cl_2	Dichloromethane
SEM	Scanning Electron Microscope
FE-SEM	Field Emission Scanning Electron Microscope
OM	Optical Microscope
PM	Polarised Micrscope
O/W	Oil-in-water
W/O	Water-in-oil
SE	Sugar Ester

CHAPTER 1

INTRODUCTION

1.1 Background of study

Hollow particle was a solid material that contains interior hollow structure and commonly surrounded by a solid shell (Fuji et al., 2012) as the shell consisted of natural or synthetic polymer (Madhav and Kala, 2011). Colloidosomes are hollow particles which shells composed of colloid particles. It formation were most commonly based on the self-assembly of colloidal particles at the interface between two immiscible liquids, typically water and oil phases (Thompson et al., 2014). Colloidosomes have pore known as interstices. In this study, the hollow colloidosome were prepared.

Solvent evaporation method was a method to produce colloidosome since it able to isolate between volatile and non-volatile solvent. In case of preparing colloidosomes, polycaprolactone (PCL) and dimethylchloride (CH_2Cl_2) and were used as oil phase while polyvinyl alcohol (PVA) and sugar ester (SE) as surfactant and dioinized water were as water phase.

In case of choosing for polymers used in this study, there were some of criteria that should be meet which including the surface of polymer should be permitted for cell attachment and promoted cell growth while the polymer also should be biocompatible, biodegradable and should be not provoked inflammation or toxicity in vivo.

1.2 Problem Statement

The study of control size of colloidosome formation is importance since it may affect to the release rate of active agent from the colloidosome. It is because the rate flux of active agent out of colloidosome increased, as the size of colloidosome decreased (Kim and Pack, 2006).

The purpose of control the colloidosome size is to avoid the burst release of active agent from the colloidosome and able to achieve a constant release rate thereafter. The higher the rate of burst release, may cause to the low effective life time of colloidosome that trigger to the lack of recovery situation due to the high of degradation rate of colloidosome (Huang and Brazel, 2001).

In this study, the hollow colloidosome were prepared by controlling their size formation. Previous report have not study size control by using this method while in this study it was investigated by proposing three different parameters including the effect volume of oil-to-water phase ratio, effect of surfactant concentration and effect of surfactant type on the size of colloidosome.

There are various techniques to control the size of colloidosome formation including solvent evaporation, phase seperation and spray-drying method (Xu et al., 2009; Kim and Pack, 2006). In this study study, two steps of solvent evaporation method were used as it is a simple, scalable and very economical method (Hwisa et al., 2013; Shahidan et al., 2013).

1.3 Objectives

1. To prepare PCL10 colloidosome at different ratio of oil-to-water phase.
2. To prepare PCL hollow colloidosome using different surfactant type
3. To characterize the morphology and size of PCL colloidosome.

1.4 Expected Outcome

There are several expectation from this study, which included to get a larger size of colloidosome as volume of oil to water phase ratio increased, can get larger colloidosome size by using PVA as surfactant, compared to using S1670, sugar ester as surfactant and able to observe the birefrigence and morphology of colloidosome.

CHAPTER 2

LITERATURE REVIEW

2.1 Tissue Engineering

The concept of tissue engineering scaffold were emerged to address the transplant of a biofactor (cells, genes and/or proteins) (Hollister, 2005). Tissue engineering was a treatment for tissue defects and organ failure (Li et al., 2015) by incorporating cells from the body (O'Brien, 2011) with tissue construction or organ replacement (Mohanty et al., 2016) of portion or whole tissues (Woodruff and Hutmacher, 2010) that were damaged by disease or injury (Karp et al., 2003).

The replacement of failed function organs mechanically (dialysis and heart-lung bypass machine), or implantation of synthetic replacements (blood vessel and joint replacement) have been tried by clinicians to overcome the problems but these were often only temporary solutions and still not allow the patient to completely resume normal activities. Tissue engineering were needed since it able to regenerate the damaged tissue by involving the cells and natural substances (Mikos and Temenoff, 2000).

There were various techniques to fabricate tissue engineering scaffold included by using phase separation and freeze-drying, solvent evaporation, electrospinning technique, particle leaching and gas foaming (Mohanty et al., 2016; Karp et al., 2003; Chang et al., 2014).

In this study, the hollow colloidosome were produced by using solvent evaporation method as it is a most common and simple method (Freitas et al., 2005; Hyun, 2015) and able to produce hollow particle structure (Woodruff and Hutmacher, 2010) and purposed to promote the cell penetration, tissue formation, cell attachment and cell growth (Chang and Wang, 2011).

Natural based polymer and synthetic based polymer are materials used to produce tissue engineering scaffold. The synthetic based polymer such as PCL were used. In this study, biocompatability and biodegradability are properties that act as main features that have been considered while choosing the material to fabricate the hollow colloidosome since it might become swelling, pain, redness and warmth due to the rejection by the body (O'Brien, 2011).

2.2 Hollow Particle

Hollow particle is a hollow spherical particle structure with diameter of 60-70 nm, wall thickness of approximately 10 nm that contain of interior hollow structure. Its commonly sorrounded by a solid shell (Fuji et al., 2012). The polymer shell to encapsulate the active ingredient (Kumar et al., 2011) as illustrates in Figure 2.1. There are two types of materials which often to be as solid shell which are natural and synthetic polymers (Madhav and Kala, 2011). Albumin, collagen, chitosan and fibrin are some of examples for natural polymer while poly (lactic acid), polycaprolactone and poly (β-hydroxybutyric acid) were the examples for synthetic polymer (Tiwari and Verma, 2011)

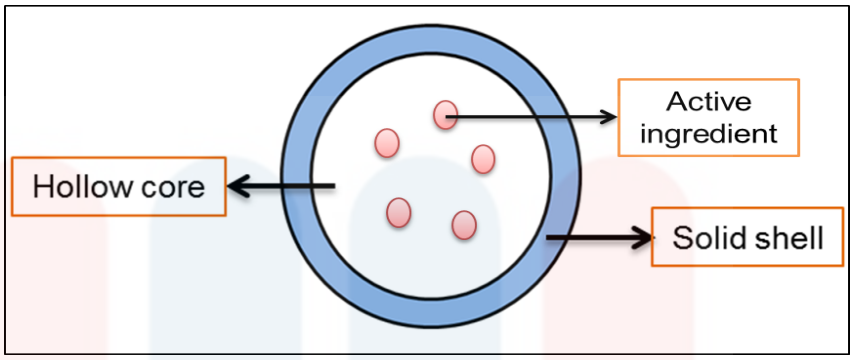


Figure 2.1 : Schematic diagram of hollow particle structure edited from (Fuji et al., 2012)

There are special properties of hollow particles including compatibility, flexibility and versatility (Fuji et al., 2012). Hollow particles are commonly used in biomedical application as a drug delivery system (Fuji et al., 2012; Kreuter, 1996; Madhav and Kala, 2011) since it able to control the drug release. The rate for drug to release from the hollow particles was depended on degradation of polymer that used to encapsulate it (Tiwari and Verma, 2011). In drug delivery system, the hollow particle function as a carrier to deliver the drug to the specific target.

2.3 Colloidosome

Colloidosomes are hollow particles structure that prepared from closed packed of colloidal particles (Dinsmore et al., 2002; Shahidan et al., 2013; Liu et al., 2010). When the colloidal particles were closely packed together, it caused interstices between the neighboring particles (Thompson et al., 2014).

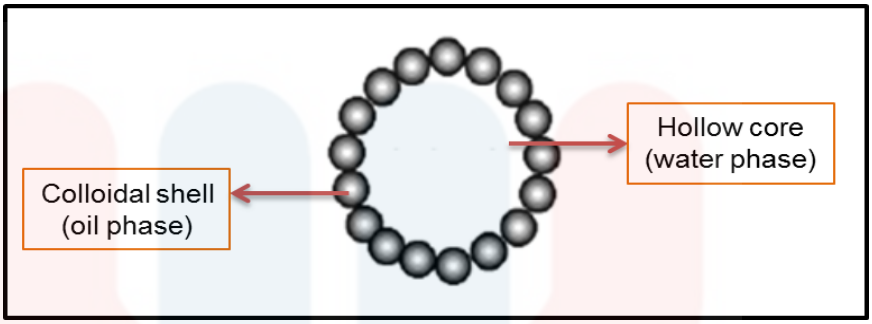


Figure 2.2 : Colloidosome structure edited from (Thompson et al., 2014)

The colloidal particles have particles size that are smaller than core material. There are some types of colloidal particles such as semiconductor colloidal particles, polymer colloidal particles, inorganic colloidal particles and magnetic colloidal particles (Parthibarajan et al., 2011). In this study, polymer colloidal particles were carried out.

Colloidosomes are widely used to encapsulate agent in biomedical industries due to the strong interest in encapsulation, control release and permeability (Dinsmore et al., 2002) of materials like proteins and vitamins as it used in biomedical, pharmaceutical and cosmetic industries application (Saraf et al., 2011). The colloidosomes were also used in application of drug delivery systems (Nan et al., 2014) since colloidal particles can further act as targeting agent, to direct the colloidosomes to a desired location (Parthibarajan et al., 2011).

Therefore, the size of colloidosome is an important issue to be highlighted as it can affect to the drug release rate from the carrier. As size of colloidosome decreased, surface area-to-volume ratio increased and it lead to the increasing rate flux of drug out of colloidosome (Kim and Pack, 2006).

2.4 Solvent Evaporation

Solvent evaporation is a method to produce hollow colloidosome. There are two types of emulsion preparation based colloidosome which including oil-in-water emulsion (o/w) and water-in-oil emulsion (w/o). In (o/w) emulsion, water phase as continous phase while the oil phase as dispersed phase. In contrast with (w/o) emulsion, the oil phase as continous phase (Parthibarajan et al., 2011).

In this study, oil-in-water (o/w) emulsion were used to produce colloidosome. Rotary evaporation were used to accelerate solvent evaporation and increase in dispersion stability (Shahidan et al., 2013).

In solvent evaporation preparation method, the oil phase and water phase were prepared first (see Figure 2.3(a) and (b) respectively) and added together which produced of two immiscible phases as present in Figure 2.3(c). The two immiscible phases were then homogenized by using the homogenizer as shown Figure 2.3(d). As a result, the oil-in-water (o/w) emulsion were produced.

In this technique, the internal phase of an emulsion were evaporated to form hollow colloidosome by agitation while the external phase became harden to form solid shell of colloidosome (Hwisa et al., 2013) as shown in Figure 2.3(e).

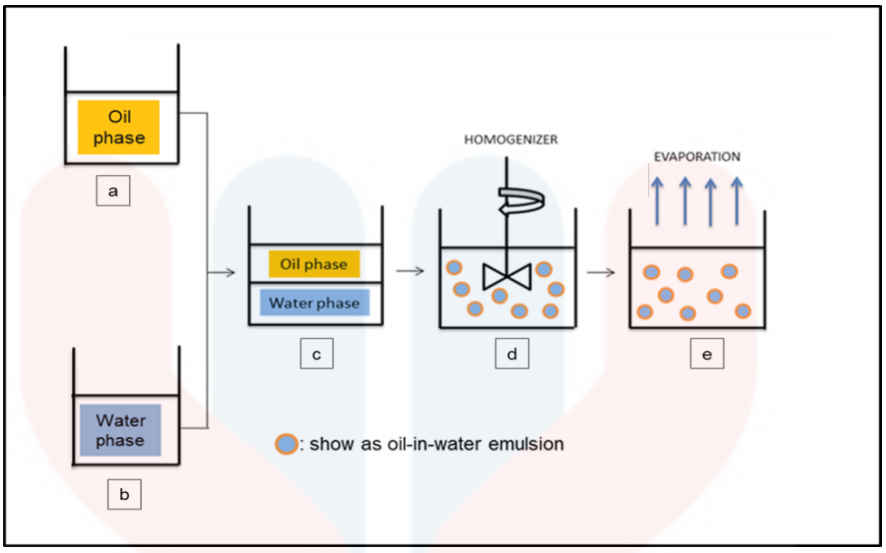


Figure 2.3 : Solvent evaporation method modified from (Madhav and Kala, 2011)

Solvent evaporation process was very economical and simple (Saralidze et al., 2010) to use since it were exposed to the room temperature without high temperature involvement during the process was carried out (Hwisa et al., 2013) while it also have become an useful method for preparing colloidosome because of it ability to control particle size in the nano to micrometer (Tiwari and Verma, 2011).

2.5 Polycaprolactone

There are two types of polymer materials that can be use in producing of hollow colloidosome for tissue engineering purpose which are synthetic polymer and naturally derived polymer. In this study, a synthetic polymer, polycaprolactone (PCL) were used. PCL is one of the polymer that have biodegradable properties and suitable for implantation towards the tissue that requires stability and strength (Gunatillake et al., 2003).

PCL is a polymer material that have been received increasing attention used in biomedical field since it easy to control of biodegradability and processability (Chen et al., 2002). PCL is a semicrystalline aliphatic polyester as presented in Figure 2.4 and was a great interest as it can be obtained by ROP of relatively cheap monomeric unit "Ɛ –caprolactone".

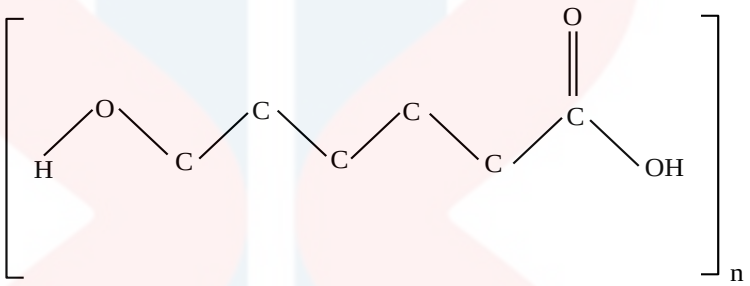


Figure 2.4 : Structural of polycaprolactone (PCL)

PCL some of its attractive qualities are it able to enhance solubility in organic solvent which in this study was dichloromethane (CH_2Cl_2) which have the ability to be processed at low temperatures and its non-toxic degradation byproducts (Sravanthi, 2009). The presence of crystalline PCL region may provide additional benefits for biomaterial application including providing micrometer-scale which is directional interactions with cells and tissue and also may result in enhanced elasticity of the colloidosome (Shahidan et al., 2013).

PCL is relatively inexpensive, highly elastic polyester that demonstrates a lack of toxicity and have good mechanical properties such as high permeability for many types of different drugs, low acidic environment during degradation and low degradation rate that suitable for long-term delivery regarded as safe materials to be use in the body (Patel et al., 2011; Woodruff and Hutmacher, 2010; Hyun, 2015).

According to their features, PCL are widely applied in biomedical application such as in tissue engineering scaffold,it was the mostly common polymers to be used due to the biodegradation products (glycolic acid and lactic acid) of that polymer were presented in human body and removed by natural metabolic pathways (Sachlos and Czernuszka 2003).

2.6 Polyvinyl alcohol

Polyvinyl alcohol (PVA) was prepared from polyvinyl acetate that hydrolyzed in ethanol with potassium hyroxide since vinyl acetate monomer was the main raw material used to manufacture the PVA (Saxena, 2004). Figure 2.5 shows the chemical structure of PVA.

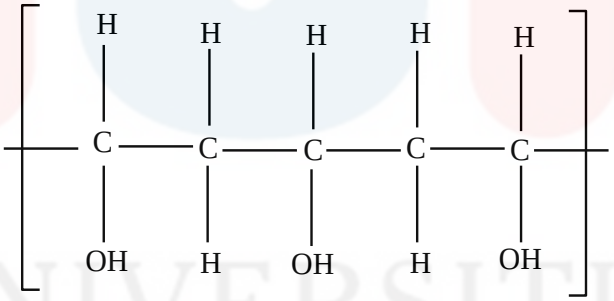


Figure 2.5 : Structural of poly(vinyl alcohol) (PVA)

PVA acts as a stabilizer that most commonly used to maintain the physicochemical for example solubility and stability state of a dispersion of two immiscible phases (Iqbal et al., 2015; Hyun, 2015) and it is soluble in water (Saxena, 2004).

There were some of advantages of PVA include non-toxic, non-carcinogenic, bioadhensive characteristic since it ease of processing (Hassan and Peppas, 2000), easily degrade by biological organism, harmless (Gaaz et al., 2015) and based on the advantages, it suitable to be uses in preparing of colloidosome.

2.7 Sugar Ester

Sugar ester is a natural surfactant which directly taken from natural sources of plants (Gonçalves and Fonseca, 2009) that produced by esterification reaction (Neta et al., 2012). Figure 2.6 shows the chemical structure of sugar ester.

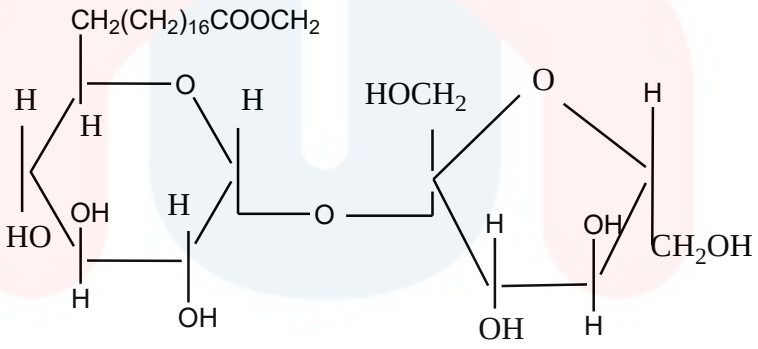


Figure 2.6 : Structural of sugar ester (SE)

Sugar ester are also known as a biosurfactant. Classification of sugar ester were included sucrose stearate, sucrose palmitate and sucrose laurate. In this study, sucrose stearate of sugar ester were used. There properties and advantages of suar ester which included biodegradable, non-toxic, non-allergenic, antiadhesive, antimicrobial and stablizing agent properties (Szuts and Szabó-Révész, 2012; Masoud et al., 2012).

Based on the features of sugar ester, it had been used in various field of application, such as in pharmaceutical application, food industry and cosmetic field (Szuts and Szabó-Révész, 2012).

2.8 Material Characterization

2.8.1 Scanning Electron Microscope

Scanning electron microscopy (SEM) were used to study the morphology of hollow colloidosome to present the image in three dimension (Tai et al., 2007; Hyun, 2015; Dinsmore et al., 2002; Shahidan et al., 2013).

The sample to be use in SEM must be in dry condition since the water able to vaporize in the SEM vacuum condition. The vacuum system uses as a purpose to remove air particles and dust inside the chamber that might bother the electrons reach towards the colloidosome. Sample were coated by conductive material as SEM uses electron to produce image (Shahidan, 2014).

SEM then started to work as shown in Figure 2.7 which that the electron beam were emitted by the electron gun next, the condenser lens then condensed the electron beam. Magnetic field were produced by scan coils functioned to deflect the electron beam then the electron beam were passed to the objective lens to focus and scan the colloidosome surface in a raster pattern. When the electron contact with the sample, it caused emissions of the secondary electron. The secondary electron were drawn to detector and image displayed on cathode ray tube (CRT) (Parry, 2000).

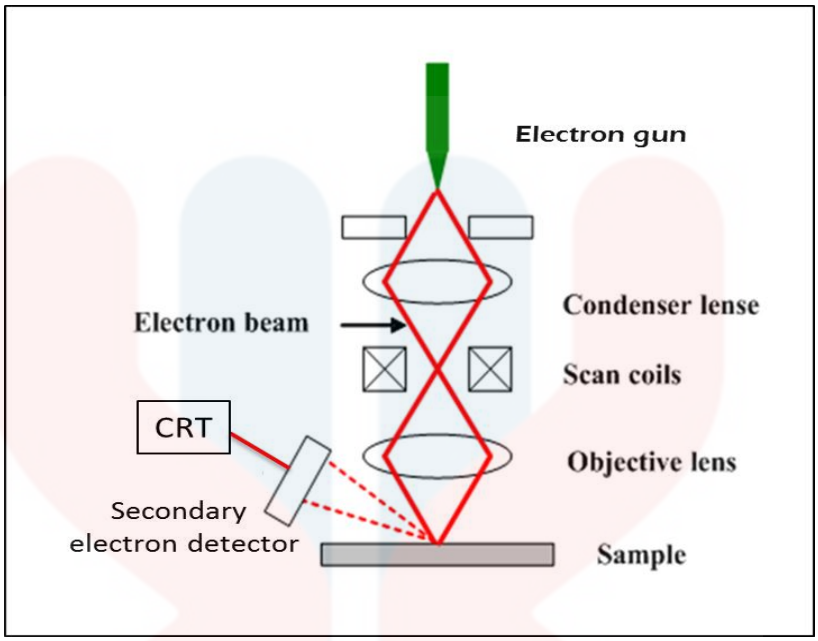


Figure 2.7: Imaging principle of scanning electron microscope

(Cheney, 2007)

The acceleration volt used to the SEM, able to affect to the image obtain due to that, the selection of accelerating voltage was significant to gain of excellent clear image. However, the level of volt were depend on the material tested either it metals, ceramics or polymers. As the colloidosome were fabricated from polymers material, it preffered to use the voltage less than 10kV (Cheney, 2007). SEM was one of major characterization technique that used routinely in materials science technology since it used to study topograh of solid samples.

Only the solid sample can be detect by SEM. It produced images by probing the specimen with a focused electron beam that was scanned across a rectangular area of the specimen. Scanning the electron probe over surface and collect the image signal. Image was develop point-by-point collecting signal generated by electron.

2.8.2 Optical Microscope

Optical microscope were used to get optical image and determine the size of colloidosome. OM is a microscope that present a magnified image of sample structure by using visible light. The advantages of OM included compact, light-weight and inexpensive microcope while it also a simple of working principle uses (Mudanyali et al., 2010).

In (Figure 2.8(a)), the illumination light were transmitted to the sample towards objective lens. The objective lens were funtioned to collect the light (Figure 2.8(b)) and transfer it to the eyepiece (Figure 2.8(c)) to be seen by the eyes of observer as shown in (Figure 2.8(d)).

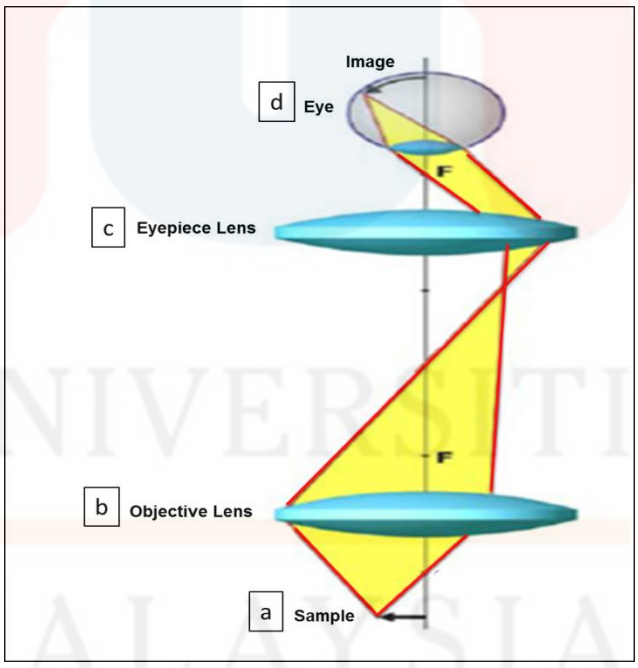


Figure 2.8 : Principles of imaging with an optical microscope about the ray diagram of the simplest two-lenses (Lee et al., 2011)

There were two combination lenses, one was an objective lens and another one was an eyepiece lens which acted to give the final image (Lee et al., 2011; Mudanyali et al., 2010). Based on the ability of OM properties in analyzation of biomedical structure materials, it was employed in this study to observe the colloidosome.

2.8.3 Polarized Microscope

A polarized light microscope were used to observe the birefrigence of the colloidosome (Lee et al., 2011). Birefrigence is a double refraction rays which a single refraction ray of unpolarized ligtht enters the anisotropic sample (material that have different of physical properties when measured in different axes). The polarized light microscope consist of two polarizer such as polarizer and analyzer that placed at the bottom and top of microscope respectively.

The imaging ray diagram of polarized microscope is show in Figure 2.9. The birefrigent sample were placed in the between of polarizer and analyzer . The light source from the lower part of microscope was passing through the polariser (Figure 2.9(a)).

The polarized light was passing through condenser (Figure 2.9(b)) to condensed the light and the light penentrate the colloidosome sample (Figure 2.9(c)) as it is an anisotropic material, then refracted into two different rays (ordinary and extraordinary rays) (Figure 2.9(e)) as enters to the objective lens (Figure 2.9(d)) due to the light vibration to each other.

As a result, the ordinary ray remain unchanged while the extraordinary ray became bent (Abramowitz and Davidson, 2015). The only light that have parallel axis to the analyser were allowed to pass through it (Figure 2.9(f)) then, the spectrum colour of colloidosome result were observed.

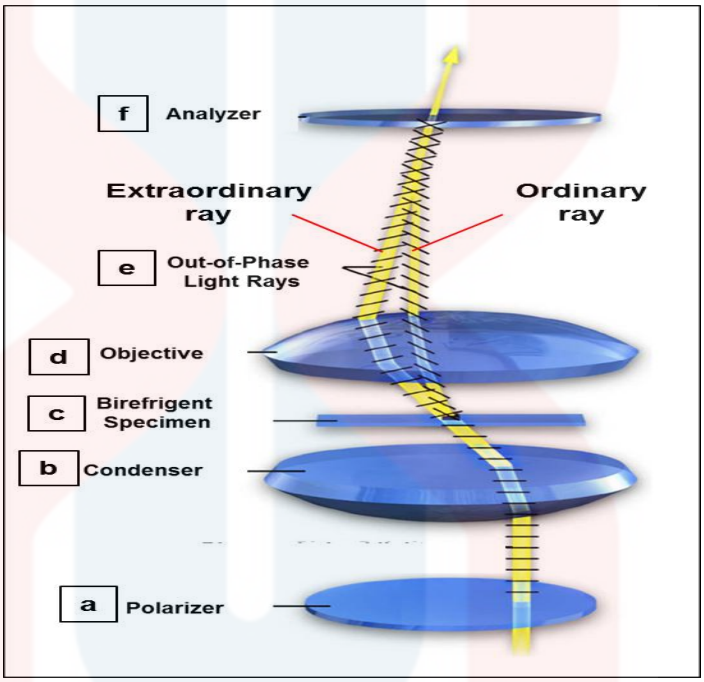


Figure 2.9 : Polarised light microscopy configuration (Shahidan, 2014)

The polarizer and analyser had been oriented at angle in range of 30-40 degree due to the maximum brightness for a birefrigence material to be showed (Shahidan et al., 2013; Abramowitz and Davidson, 2015).

CHAPTER 3

MATERIALS AND METHODS

3.1 Reagents

Dichloromethane, CH_2Cl_2 (98%) and Polycaprolactone with a number-average molecular weight (M_n) 10 kg/mol (PCL10, M_w/M_n= 1.5) were purchased from Aldrich and used as received. Dichloromethane, CH_2Cl_2 (98%). Polyvinylalcohol (PVA) (98% hydrolyzed, M_n= 13-28 kg/mol) was purchased from Aldrich and used as received. Sugar ester, S1670. Dioinized water.

3.2 Preparation Of Hollow Colloidosome

The oil phase and water phase were prepared to make hollow colloidosome preparation as presented in Figure 3.1. As preparing oil phase, polymer were weighed and dissolved in solvent for a certain time, while, surfactant were dissolved in dioinized water for 3 hours placed in water bath with shaker at 80 °C.

Polymer and solvent as oil phase were injected to the water phase that consist of surfactant and dioinized water by using 2.5 ml/min of feeding rate. IKA T-18 Ultra Turrax Digital Homogenizer were used to homogenize two immisible phases together.

The water phase beaker were fully covered by parafilm to avoid solution evaporation. At the same time, the rate of rotatio is about 9000 rotation per minute (rpm) then, the emulsion were produced. Immediately, the solution (emulsion) were

exposed to the accelerated evaporation operated by rotary evaporator approximately in 10 minutes to form hollow colloidosome.

The Meiji MT800 of optical microscope were utilized to observe the optical image of hollow colloidosome structure. There are three different parameters applied in this study, which included the parameter of different oil in water phase ratio, parameter of different surfactant and parameter of surfactant concentration.

The hollow colloidosome were prepared by using 3 different system parameters while the water phase volume were fixed as show in Table 3.1 :

Table 3.1 : Parameters used in colloidosome preparation

Entry	System	Polymer concentration (wt%)	Surfactant concentration (wt%)	Volume oil phase (ml)	Volume water phase (ml)	Ratio
1	PCL/PVA	1.5	1.2	25	50	0.5 : 1
2	PCL/PVA	1.5	1.2	50	50	1.0 : 1
3	PCL/PVA	1.5	1.2	75	50	1.5 : 1
4	PCL/PVA	1.5	1.2	100	50	2.0 : 1
5	PCL/PVA	1.5	1.2	125	50	2.5 : 1
6	PCL/S1670	1.5	1.2	25	50	0.5 : 1
7	PCL/S1670	1.5	1.2	75	50	1.5 : 1
8	PCL/S1670	1.5	1.2	125	50	2.5 : 1
9	PCL/S1670	1.5	0.1	25	50	0.5 : 1
10	PCL/S1670	1.5	0.1	75	50	1.5 : 1
11	PCL/S1670	1.5	0.1	125	50	2.5 : 1

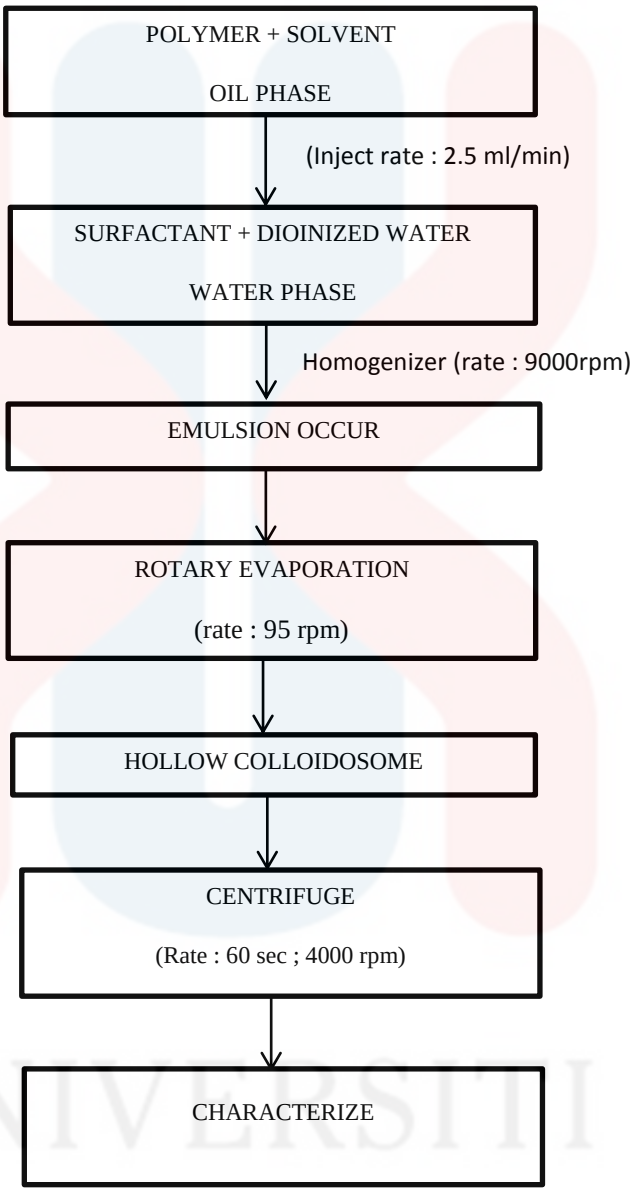


Figure 3.1: Research flow chart

3.3 Characterization

The hollow colloidosome were analyzed by using three types of different microscope towards the different purpose of characterization which include of scanning electron microscope (SEM), optical microscope (OM) and polarised microscope (PM).

The Supra 55 variable pressure field emission scaning electron microscope (VP FE-SEM) were used to study the morphology of hollow structure of colloidosome. As preparation for doing this, a little amount of sample were dropped onto a slide and dried at room temperature for overnight to remove all the water from colloidosome. A thin layer of carbon were applied towards the colloidosome to coat it. As a purpose to use SEM, the colloidosome sample were characterized at University Kebangsaan Malaysia.

A Meiji MT800 microscope was used to get optical images and identify the size of colloidosome. Sample preparation for this measurement was started as a small amount of sample solution were dropped onto a glass slide by using a disposable pippete. Sample dropplet then shear around the surface of glass slide and measurement begun. The objective lens used at the magnification of 5x, 10x and 20x. The work was carried out in University Malaysia Kelantan.

A Leica DM500 microscope were used to study the birefrigence image of colloidosome. A typical preparation for this characterization method was started by placing the birefringent specimen in between polarizer and analyzer of microscope. Then sample were deposited onto a microscope slide and observed immediately. There theree different magnification of objective lens utilized which are at 10x, 40x and 60x. The test were executed at University Kebangsaan Malaysia.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Parameters applied in colloidosome preparation

In this study, there are eleven samples of colloidosome were prepared by using different ratio of volume oil phase to water phase are fixed. Two types of surfactant were used such as polyvinyl alcohol (PVA) and sugar ester (SE). Concentration of surfactant were diversified while concentration of polymer are constant. All the sample prepared is shown in Table 4.1 and further discussed in the next section.

Table 4.1: Colloidosome prepared and their parameters

Entry	System	Polymer concentration (wt%)	Surfactant concentration (wt%)	Ratio	Volume oil phase (ml)	Volume water phase (ml)	Particle size (μm)
1	PCL/PVA	1.5	1.2	0.5 : 1	25	50	3.39
2	PCL/PVA	1.5	1.2	1.0 : 1	50	50	3.48
3	PCL/PVA	1.5	1.2	1.5 : 1	75	50	5.49
4	PCL/PVA	1.5	1.2	2.0 : 1	100	50	8.68
5	PCL/PVA	1.5	1.2	2.5 : 1	125	50	10.01
6	PCL/S1670	1.5	1.2	0.5 : 1	25	50	1.61
7	PCL/S1670	1.5	1.2	1.5 : 1	75	50	4.87
8	PCL/S1670	1.5	1.2	2.5 : 1	125	50	7.39
9	PCL/S1670	1.5	0.1	0.5 : 1	25	50	3.09
10	PCL/S1670	1.5	0.1	1.5 : 1	75	50	5.15
11	PCL/S1670	1.5	0.1	2.5 : 1	125	50	9.21

4.2 Effect of different volume oil-to-water phase ratio

(Figure 4.1(a)) shows the smallest size of particle were produced when the volume of oil phase is lower than volume of oil phase see (Entry 1, Table 4.1) while the largest particle presented in (Figure 4.1(e)) as the volume of oil phase used is higher than volume of water phase as stated in (Entry 5, table 4.1). (Figure 4.1(f)) shows the number of particle size in increasing order from lowest volume of oil phase to the highest volume of oil phase used while the volume of water phase are fixed.

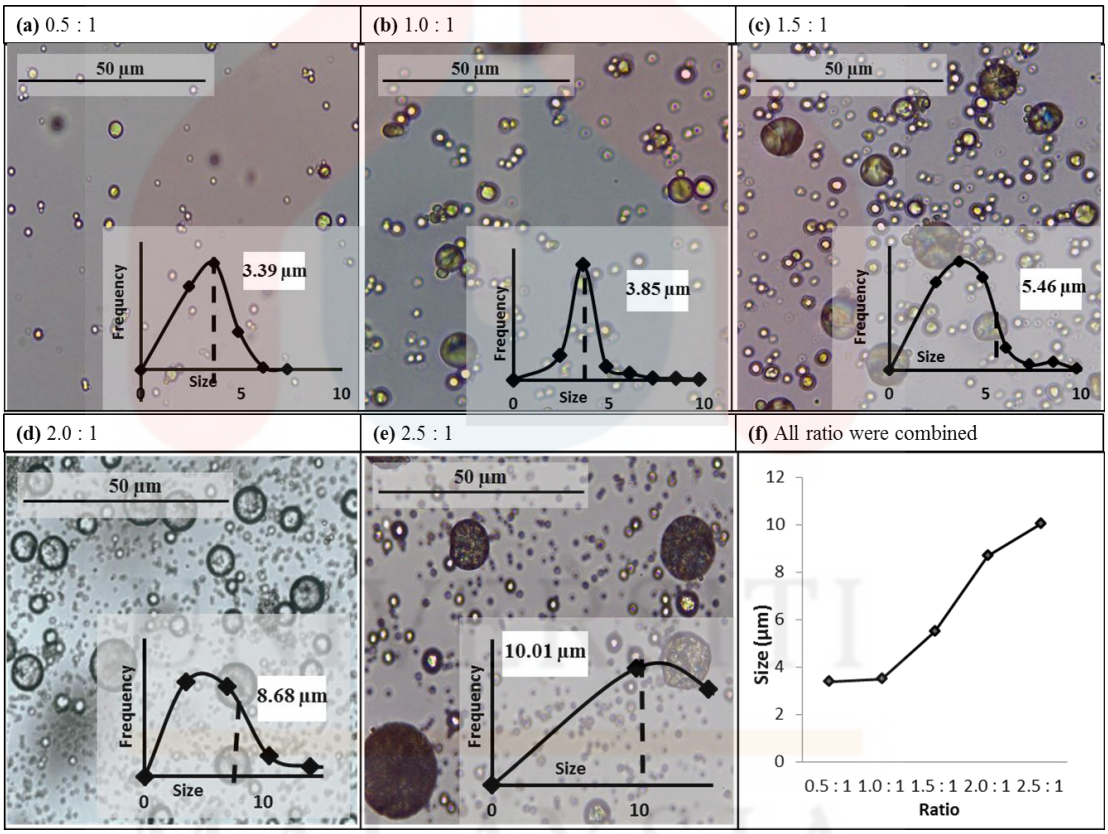


Figure 4.1 : Effect at different volume of oil to water ratio

The result clearly showed that the higher volume of oil to water phase lead to the larger size of colloidosome formed. Low volume of water phase, resulted to the

increased of particle size unable to prevent droplet coalescence between oil-to-oil droplet since it was not enough of surfactant to act as repelling force (Jelvehgari et al., 2010) to form particle as depicted in Figure 4.2 :

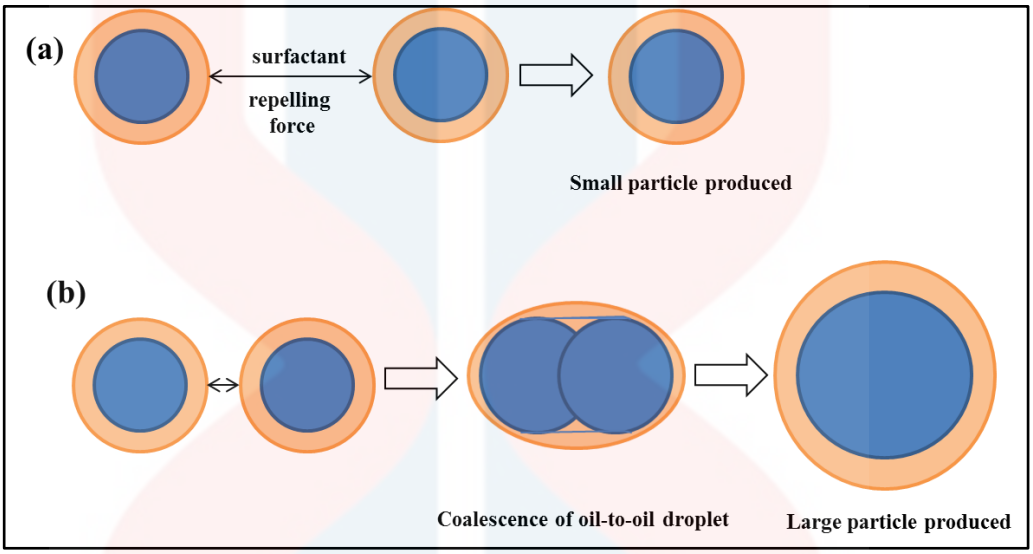


Figure 4.2 : Effect volume of oil to water phase ratio due to the surfactant content. The insets for (a) show effect of higher surfactant content while (b) show effect of lower surfactant content in colloidosome production

Based on the observation in (Figure 4.1 (a)), high yield of colloidosome were formed compared to the sample in (Figure 4.1(e)). It is because of the amount of surfactant used, as the higher of surfactant, the increase entrapment efficiency of surfactant to form emulsion (Jelvehgari et al., 2010; Shahidan et al., 2013).

4.3 The birefringence and morphology study of PCL/PVA hollow colloidosomes

4.3.1 Study birefringence of colloidosomes

The colloidosome sample were characterized under optical microscope to study the hollow structure of colloidisomes. In this study, there are three samples of different ratio for PCL/PVA system were investigated birefringent effect of colloidosome images as show in Figure 4.3. Interestingly, all the sample tested even at the different ratio, it presented the colored view of particles as demontrates in (Figure 4.3 a-c) :

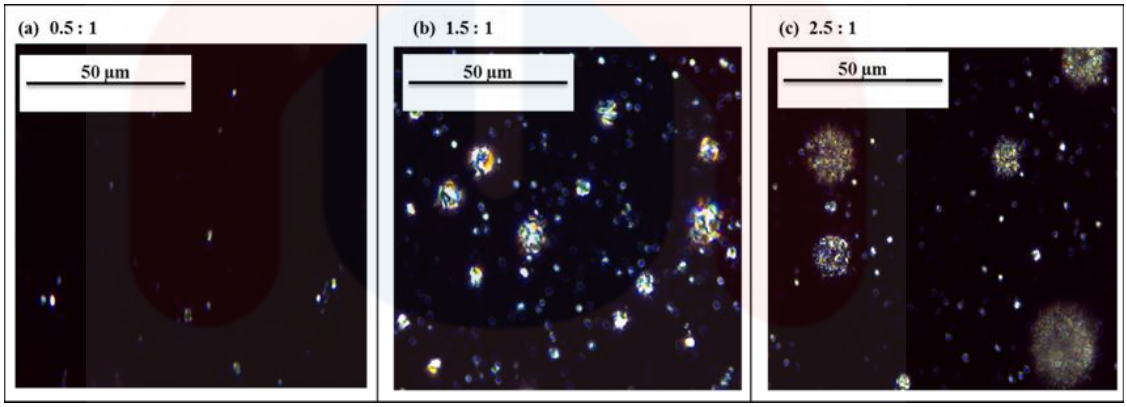


Figure 4.3 : Birefringence image for different ratio of colloidosomes

The sample are able to present birefringence image since PCL is well-know as a semi-crystalline or amorphous polymers that able to allow the light passing through it (Shahidan et al., 2013). Therefore , for smaller size particle, it show non-colloidosome but still present the small birefringence particle, see (Figure 4.3 (a)) as compared to the larger particles in (Figure 4.3 (b) and (c)) show of various colored that represent for the different thickness of shell.

Birefringent images of this study, see (Figure 4.4 (a) and (b)) were compared to the image of Shahidan et al. (2013) as show in (Figure 4.4 (c) and (d)). Comparison are based on the PCL/ PVA system. The lower magnification were used to gain the distribution of colloidosome that consist in sample solution see (Figure 4.4 (a) and (c)) while the higher magnification then used to display image of hollow colloidosome structure in a more clear and closed view (see Figure 4.4(b) and (c)).

Hollow structure of colloidosome were proven as the image of particle presented colored effect in the particles. The image of particle were then enlarged to confirm the shell properties of colloidosome see (yellow dotted box and yellow box in Figure 4.4 (b) and (d)) that made from arrangement of colloidal particle that packed together among the hollow colloidosome shell.

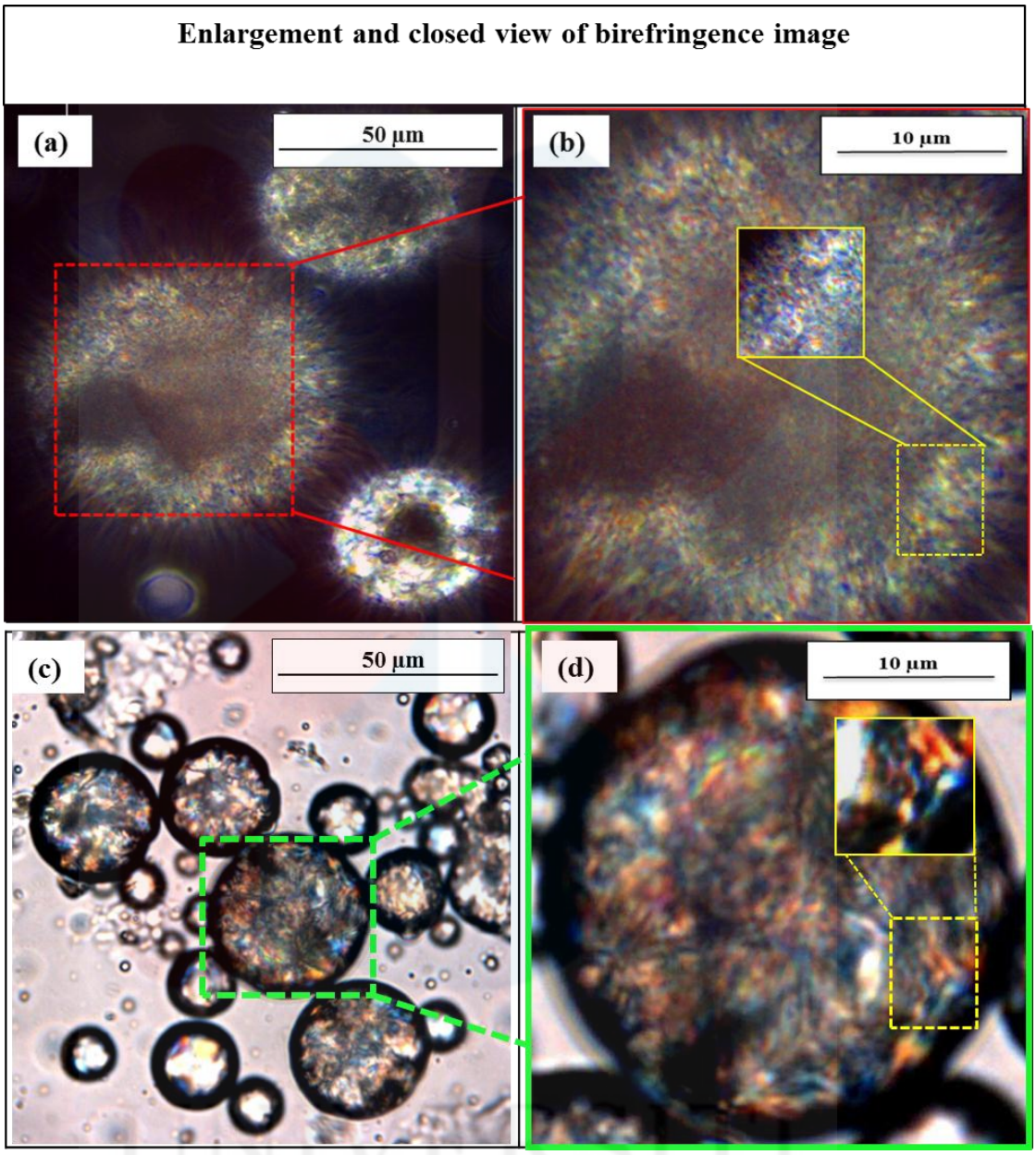


Figure 4.4 : Enlargement and closed view of birefringence images. The colloidosome prepared using PCL/PVA system. For (a and b), are the images that carried out of this study while, (c and d) are the images from reported by Shahidan et al. (2013) as a comparision

4.3.2 Study the morphology of colloidosomes

The particle was further characterized using field emission scanning electron microscope (FE-SEM) to study the morphology of hollow colloidosome prepared by PCL/PVA system.

In (Figure 4.5 a-e) showed the images that have been carried out from this study, see (Entry 5,Table 4.1) while (Figure 4.5(f) and (g)) are the image from Shahidan et al. (2013) as a purposed to compare with the result of this study.

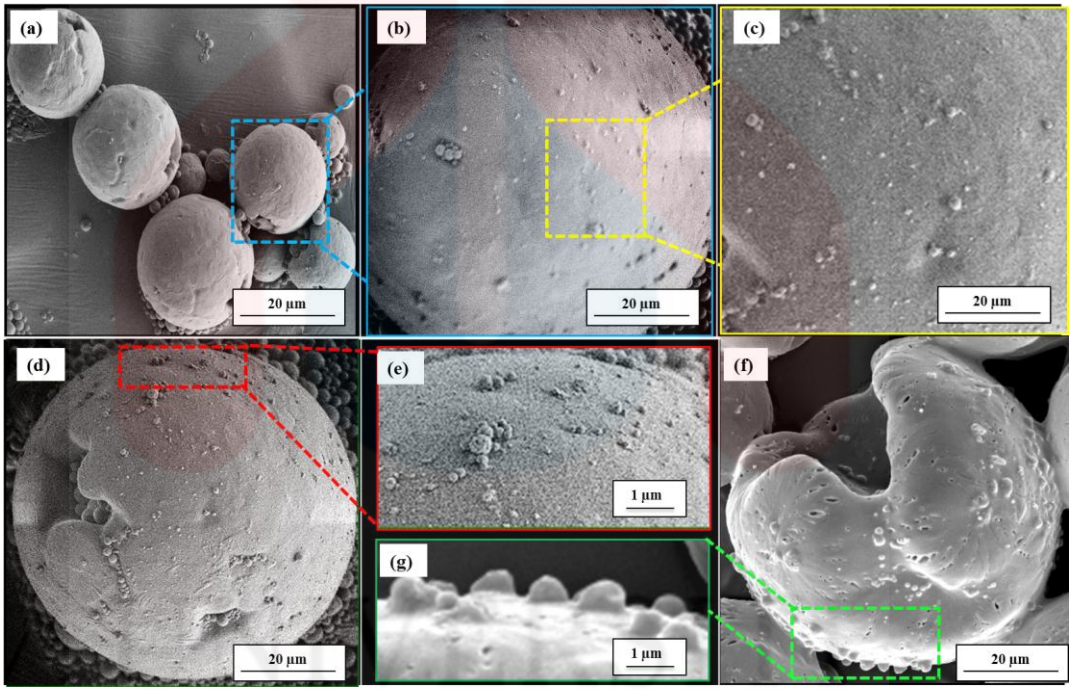


Figure 4.5 : Field Emission Scanning electron microscope images showing morphology of PCL10/PVA colloidosomes

The significance of shell features were discovered from the FE-SEM images in purposed to show packed arrangement of small particles among the shell. As in (Figure 4.5(a)) show the distribution of hollow colloidosomes while the closed up image of sample were then show in (Figure 4.5(b)). The arrangement of small particles among the hollow colloidosome shell were clearly proven in (Figure 4.5(c)).

The colloidosomes shell of this study, sometimes show for embedded small particles such in (Figure 4.5(d) and (e)) and due to that, the result of this study were compared with Shahidan et al. (2013) image, see (Figure 4.5 (f) and (g)) which are commited for the same ideas.

Packed arrangement of small particles among the shell, may cause to the intertices present between them to give an excellent properties of encapsulation efficiency of the colloidosome system for diffusion and permeability of various agents (Thompson et al., 2014). Meanwhile, the rate of diffusion are depends on the size of particles arranged among the shell that resulted to the diamter of intertices present.

4.4 Effect to the colloidosome structure based on different type of surfactant

As mention earlier in Section 4.1, two types of surfactant were used (e.g: PVA and S1670 based SE) while fixed their concentration at 1.2 wt%. The result for size of colloidosome prepared using PVA as stablizer were showed in (Figure 4.6 a-c) while result for using S1670 surfactant were displayed in (Figure 4.6 d-f).

Colloidosome prepared using PVA system produced larger size of particles compared to the sample prepared using S1670 as showed in (Figure 4.6 (a) and (d)) which are (3.39µm and 1.61µm), respectively. The comparison were did at various ratio (Figure 4.6 (b) and (e)) and (Figure 4.6 (c) and (f)), and still demonstated for the same trend as colloidosome prepared using PVA system is larger than S1670 system.

The phenomenon happened due to the sugar ester (eg: S1670) that have ability to reduce surface tension among the colloidosome surface (between the colloidal

particles) (Zirak and Pezeshki, 2015; Neta et al., 2012) while PVA are able to provide a stable condition among the colloidal particles when it strongly attach to colloidosome surface. According to PVA stability, it also able to form a thick layer of colloidosome surface (Abdelwahed et al., 2006).

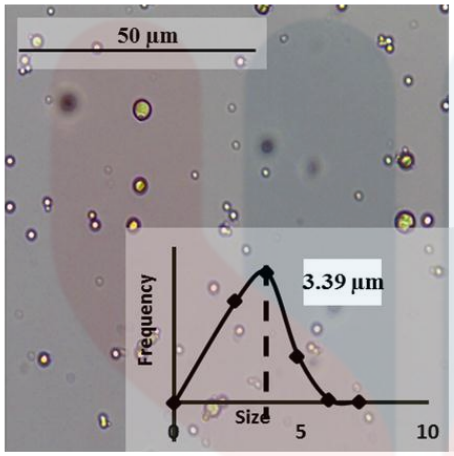
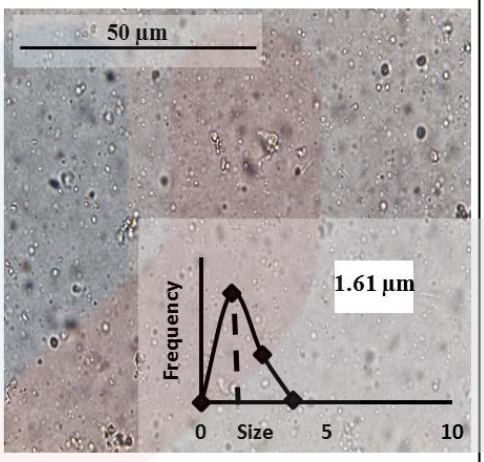
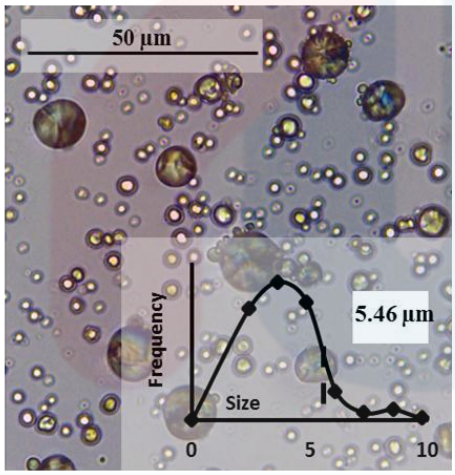
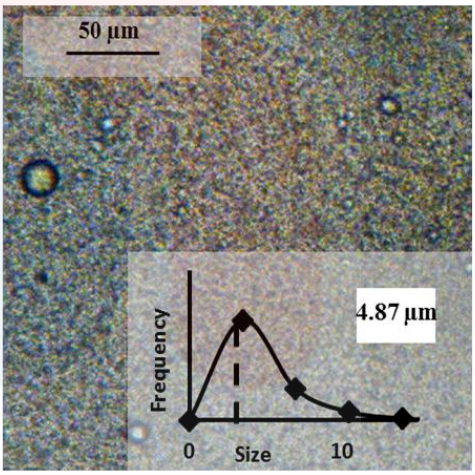
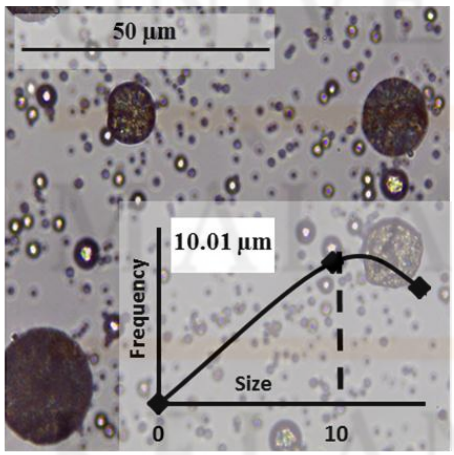
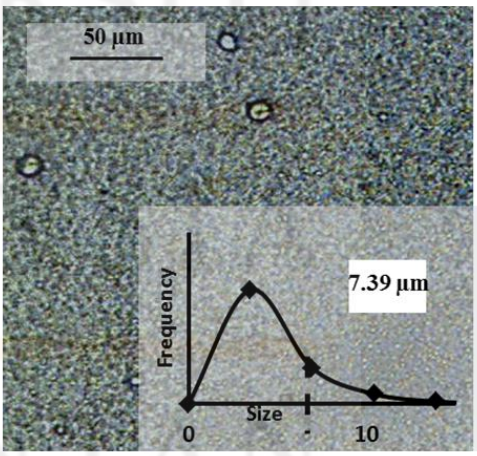
PCL / PVA(1.2 wt%)	PCL/ S1670 (1.2 wt%)
(a) 0.5 : 1	(d) 0.5 : 1
	
(b) 1.5 : 1	(e) 1.5 : 1
	
(c) 2.5 : 1	(f) 2.5 : 1
	

Figure 4.6 : Effect different types of surfactant

4.5 Effect to the colloidosome structure based on different concentration of surfactant

For the investigation of this parameter, the same type of surfactant were used (eg: S1670) at two different value of surfactant concentration which are at (1.2 wt%) and (0.1 wt%). Result for the sample that used high surfactant concentration were presented (see Figure 4.7 a-c) while for lower concentration were showed in (Figure 4.7 d-f).

Based on the result in (Figure 4.7 (a) and (d)) it shown that size of colloidosome produced by the lower surfactant concentration are larger than the higher of surfactant concentration which are 1.61µm and 3.09 µm, respectively.

The phenomenon were followed by the other ratio, as (Figure 4.7 (b) and (e) and (Figure (c) and (f)) were also displayed the larger size of colloidosome produced by as using the lower surfactant concentration since surfactant with low concentration are lack of energy for repelling force (Shahidan et al., 2013; Zirak and Pezeshki, 2015) between particles which have been discovered in that the previous section see (Section 4.2; Figure 4.2) which led to the coalescence of two different particles and resulted to the large particle size formed.

However, for colloidosome prepared by the lower concentration of stablizer were not in spheres shape as (Figure 4.7 (b) and (e)) clearly proved it since low of surfactant concentration trigger to the instability among the water phase to entrapp the oil phase towards producing colloidosome (Neta et al., 2012) due to that the particles were easily to exposed to the aggregation as evidenced (in Figure 4.7 d-f).

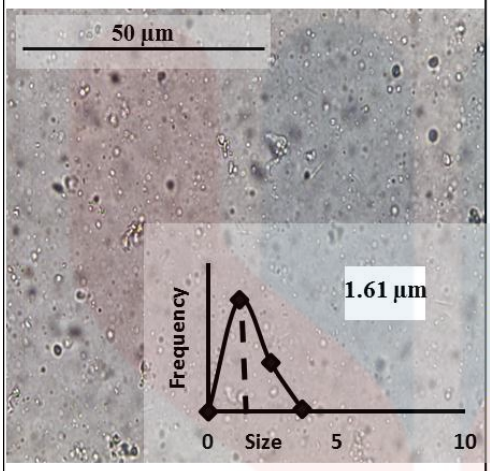
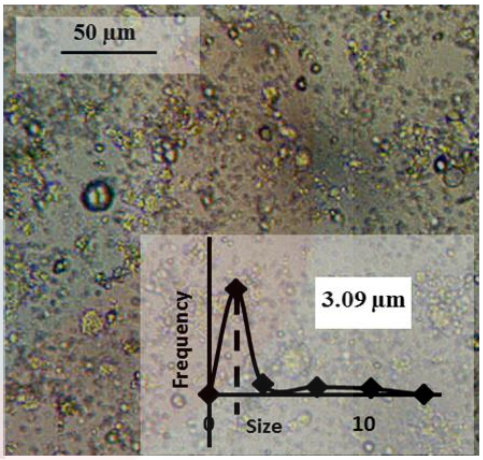
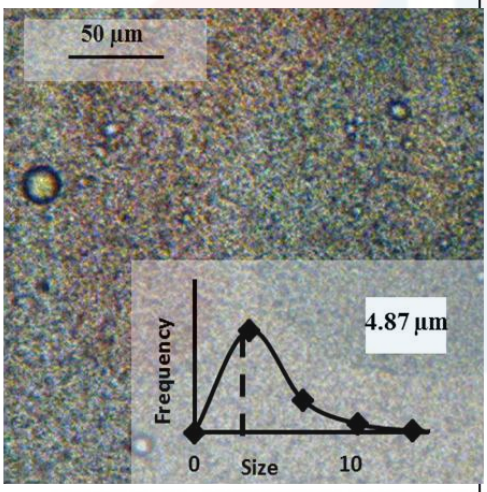
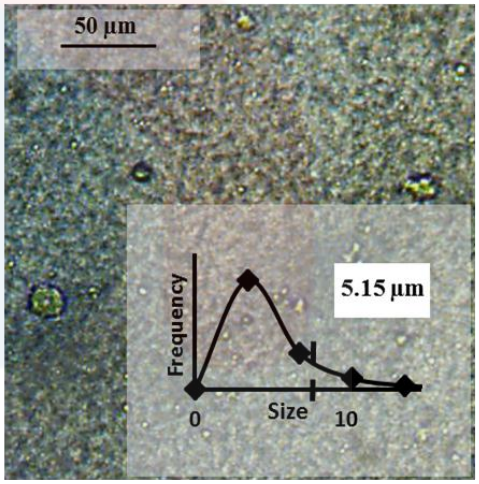
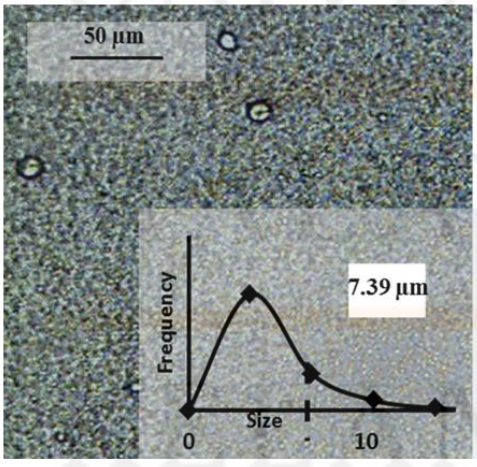
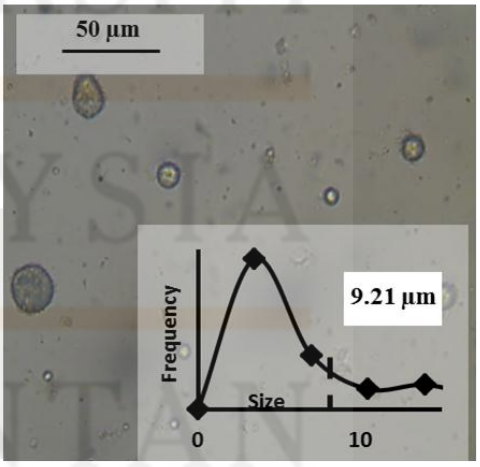
PCL / S1670 (1.2 wt%)	PCL/ S1670 (0.1 wt%)
(a) 0.5 : 1	(d) 0.5 : 1
	
(b) 1.5 : 1	(e) 1.5 : 1
	
(c) 2.5 : 1	(f) 2.5 : 1
	

Figure 4.7 : Effect different surfactant concentration

4.6 Particle size comparison between all of three systems

The size of colloidosomes formed for each system were compared to identify for their specific application. Figure 4.8 show the size of eleven hollow colloidosomes samples that carried out from this study, by using different of oil to water phase ratio for different systems which are including PCL/PVA(1.2 wt%), PCL/S1670(1.2 wt%) and PCL/S1670(0.1 wt%).

Based on the comparison in Figure 4.8, the largest size of colloidosomes were produced by using PCL/PVA(1.2 wt%) system while the smallest size of hollow colloidosome formed by using PCL/S1670(1.2 wt%) system and the hollow colloidosomes formed from PCL/S1670(0.1 wt%) are in intermidiate range as between the size of colloidosomes produced by PCL/PVA(1.2 wt%) and PCL/S1670(1.2 wt%) systems.

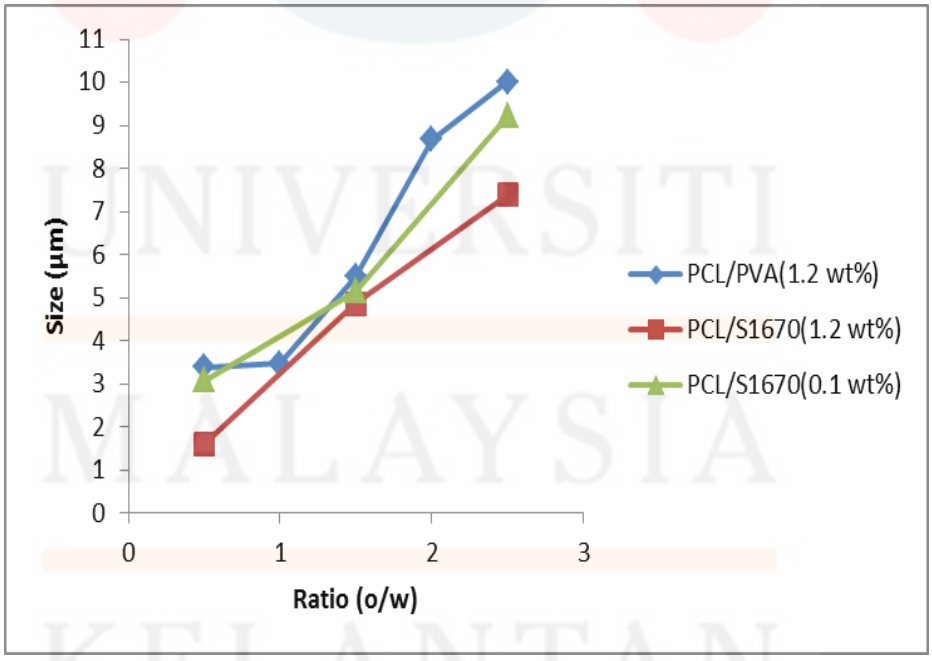


Figure 4.8 : Comparison in size between ratio among the three parameters involved

As started earlier in Section 1.2, the smaller the size of collidosome formed,it led to the higher rate of active agent release due to the large surface area per active agent of colloidosome (Huang and Brazel, 2001). When the active agent from the colloidosome release in high rate, it caused to the fast in degradation time and due to that, it suitable to apply in short-term recovery application. In contrast with larger size of hollow colloidosome properties and application as it able to load the active agent release which are match to be use in long-term recovery application (Obayemi et al., 2016).

According to all the systems applied in this study, there are no selection issues of colloidosomes since it depends on their target application.

CHAPTER 5

5.1 CONCLUSION

In this thesis, PCL colloidosome in range of average size 1-10 µm were formed as various parameters were conducted and the result for morphology, particle size and distribution were determined. The colloidosome preparation were carried out in three different parameters considered.

Based on the investigation of this study, there are three observation had been discovered. First, the higher the volume of oil to water phase, produced larger size particles. Secondly, by using PVA as surfactant, particle size have the tendency to produce larger particles in comparison to the particle preparation used S1670 as the surfactant. Third, high concentration surfactant resulted in smaller particle size production.

The colloidosome shell were consist of small particles fused together and had been viewed and proven by using FE-SEM. Polarised microscopy were used to study that PCL colloidosome optical properties: birefringent as it supposed to be, not only due to their semicrystalline chain within the particles but also the amorphous structure of the shell and its thickness.

The selection of hollow colloidosomes are depends on their specific target for condisideration of several issues such as type of cell and rate of cell growth since it may affect to the degradation rate of hollow colloidosome.

5.2 RECOMMENDATION

Future of this study, should be done by combining solvent evaporation method with other method such as salt leaching method towards the purpose of obtaining porous structure among the colloidosome shell. It was a common method that used, since the pore size could be control by controlling the amount, size and shape of particle added. By using salt leaching method, the preparation of porous hollow colloidosome are quick and inexpensive.

By having a porous structured toward the hollow colloidosome, there are several advantages included obtaining more seed cells quickly and able to provide appropriate conditions for cell growth. Having porous material structured also offer the flow of nutrients transport and allows the cell diffusion and tissue ingrowth among the particles.

In next to come of this study, thehigher molecular weight of polymer may be test since it will be resulted to the thickness of colloidosome shell. It is because of the higher the shell thickness, the longer the rate of colloidosome degradation. In the other hand, the thickness of shell are also related to the cells/tissues attachment among the colloidosomes.

Another new parameter that should be placed in this study is the paramater of different concentration of PVA surfactant. As the PVA at 1.2 wt% already tested in this study, but another concentration at 0.1 wt% of PVA surfactant should be investigate too in future to study the size of particle formed since S1670 (1.2 wt%) and S1670 (0.1 wt%) were determined their ability.

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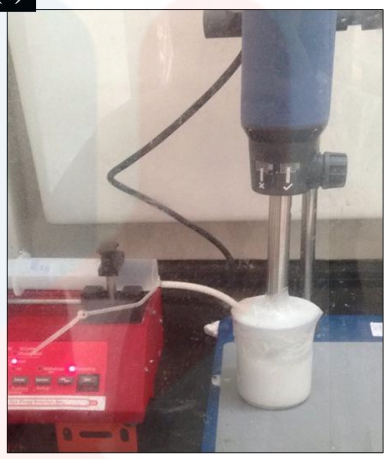
APPENDIX

(a)

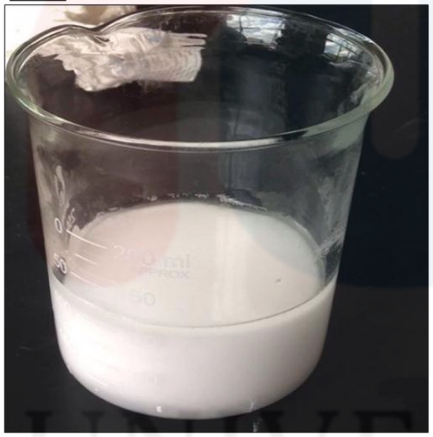
S1670 surfactant solution in deionized water

(b)



Water phase injected to oil phase and homogenize together

(c)



Solution after the homogenization process (before solvent evaporation)

(d)

Final result for colloidosome sample (after solvent evaporation)