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The Formulation Parameter Influence on PHB Hollow Colloidosomes Formation Prepared via Two Step Solvent Evaporation Method

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ABSTRACT. This study demonstrates polyhydroxybutyrate (PHB) as a potential polymer to produce colloidosomes trough two step solvent evaporation method. In this study, the surfactant concentrations, molecular weight and oil phase volume were varied which proven that a range of average diameter, D_{ave} and higher yield of PHB colloidosomes can be successfully achieved. The D_{ave} increased slightly by 0.03 µm when surfactant concentration decreases from 1.2 wt.% to 0.5 wt.%. However, D_{ave} increases significantly from 1.33 to 8.68µm when the average molecular weight, *Mw* of surfactant used increases from 13,000 to 130,000. In addition, the colloidosomes yield also increases as the surfactant average *Mw* increases. This study also displayed the simplicity of the method, where it is straightforward and used commercially available materials.

Keywords: *PHB, Colloidosomes, Encapsulation, Solvent evaporation method;*
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1. INTRODUCTION

Colloidosomes are hollow particles whose shells are composed of a closed-packed layer of colloidal particles [1]. The work on colloidosomes was first reported by Velev and colleagues in 1996 [2] although the term was introduced later by Dinsmore et al.[1].This hollow particle plays significant roles in microencapsulation: a process in microscopic formation of thin coatings of wall material around an active ingredient [3] This process has been used in varies applications such as controlled release of drugs [4], aroma [5], food industries [6] and protection of biologically active species [7]. Microcapsules preparation methods can takes several steps which might be complex, time consuming and in need of binders [1,8]. A study by Thompson et al. [9] reported that latex colloidosomes prepared via thermal annealing might be unfavorable to thermal sensitive active ingredient such as fragrances or drugs. Layer-by-layer (LbL) method usually uses solid templates. The desired materials are self-assembled layer-by-layer onto the pre-produced colloidal particles which will be removed at the end of the process to achieve the hollow structures [10]. However, LbL's drawback is the multistep processes which is time consuming [9]. Therefore, the two-step solvent evaporation method was chosen as preparation method because it is simple, straightforward and less time consuming.

Polyhydroxyalkanoates (PHA) polymers are natural polymers which are produced by bacteria and PHB is the most researched members of the PHA family [11]. PHB particularly has attracted considerable interests for its applications as delivery system or as scaffolds in tissue engineering. This is owing to its advantages of excellent biocompatibility, biodegradability and easy processability. Due to these favorable properties of PHB, this study proposed PHB as potential structural polymer to prepare hollow colloidosomes via two-step solvent evaporation method.

2. MATERIALS AND METHODS

2.1 Colloidosomes Preparation.

The terms PHB(*x*)/PVA*z*(*y*) was used to indicate the colloidosomes formulation. The terms PHB(*x*)/PVA(*y*) is referring to structural polymer/surfactant, where *x* and *y* are referring to the structural polymer and surfactant concentration (wt.%) respectively and *z* is referring to the average molecular weight, *Mw* of the surfactant used to prepare the sample. The mixture of the oil and water phase will form emulsions. Here, O/W emulsion preparation route was used. The oil phase consists of PHB in CH_2Cl_2 is fed to the water phase which consists of polyvinyl alcohol, PVA (98% hydrolysed) in deionized water whilst homogenized at 9000 rpm for 30 min using IKA T18 Digital Ultra Turrax homogenizer. The emulsion will be immediately rotary evaporated to trigger the colloidosomes formation. All polymers and surfactant were purchased from Sigma Aldrich and used as received.

2.2 Physical Measurements.

A Leica DM750 microscope was used to obtain optical images. For this work, the analyser was fixed at 90°. For a given sample,a drop of fully evaporated emulsion was placed on a microscope slide and viewed immediately. The objective lenses used had magnifications of ×10, ×40, and ×60. The average diameter, D_{ave}were determined by counting at least 100 microparticles. Zeiss Supra 55VP field emission scanning electron microscopy (FESEM) was used to study the morphology of the colloidosomes. The samples were dried at room temperature overnight prior to measurement.All samples for characterization are washed three timesby centrifugation at 3000 rpm.Table 1 shows the colloidosomes formulation of PHB/PVA and the resulting sizes.

Table 1 The formulation used in this study to prepare the PHB/PVA colloidosomes

Entry	System	D_{ave} /µm
1	PHB(1.5)/PVA13k(1.2)	1.30
2*	PHB(1.5)/PVA13k(1.2)	-
3	PHB(1.5)/PVA13k(0.5)	1.33
4	PHB(1.5)/PVA30k(0.5)	1.37
5	PHB(1.5)/PVA130k(0.5)	8.68

* Only entry 2 use higher oil volume

3. RESULTS AND DISCUSSION

The formulation for PHB colloidosomes started from the optimized PCL(1.5)/PVA13k(1.2) system (Entry 1 in Table 1) reported in previous study by Shahidanet al. [8].Fig.1 shows that PHB has the potential to be prepared as colloidosomes. However, optimum condition for PHB/PVA13k system need to be investigated. The results shows that theaverage size, D_{ave} are small (D_{ave}=1.30µm) and the yield is low (Fig.1(a)) compared to PCL/PVA13k system(inserted images) under optical microscope. Interestingly, the PHB/PVA colloidosomes morphology shows that PHB colloidosomes are more porous (Fig. 1(b)) compared to PCL hollow colloidosomes (Fig. 1(c)). In addition to PHB's advantages stated previously, highly porous morphology is one of the important 3D scaffolds features for tissue engineering application [12].

In order to increase the colloidosomes yield, we increased the oil volume from 75ml to 125ml (Entry 2, Table 1). However, the increasing oil volume induced flakes formation which indicated that the surfactant might not be enough to stabilize the emulsion and resulting aggregation which lead to flakes formation (not shown). Therefore, we reduced the oil volume to 75ml and reduce surfactant concentration from 1.2wt.% to 0.5wt.% to increase the system D_{ave} (Entry 3, Table 1). Fig.2(b) shows colloidosomes prepared at lower PVA concentration. The result shows the D_{ave} for PVA concentration of 1.2wt.% and 0.5wt.% is not significantly

different (D_{ave} =1.30 and 1.33 µm respectively). However, the small flakes are no longer visible. The insignificant D_{ave} difference between entry1 and 3 shows that, in order to stabilize the emulsion, higher molecular weight might be needed to increase the stearic repulsion.

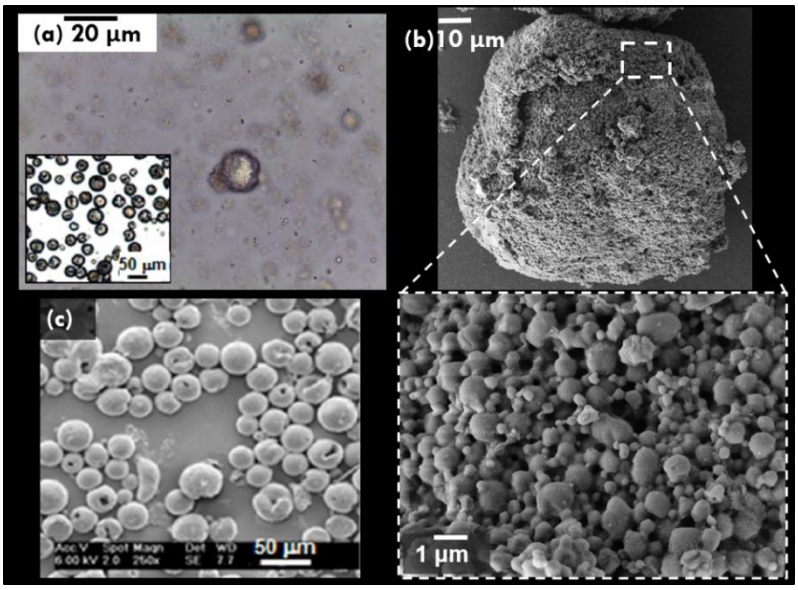

Fig. 1 Shows hollow colloidosomes prepared using PHB(1.5)/PVA13k(1.2) system and PCL(1.5)/PVA(1.2)13k system under (a) optical microscope (insert shows the PCL/PVA system), (b) and (c) FESEM for the system respectively. Images for PCL/PVA system

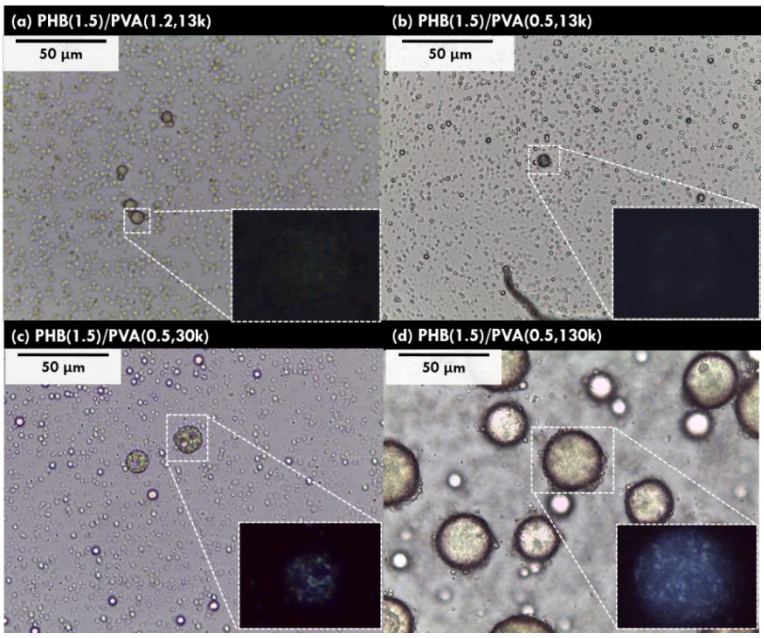

Fig. 2(a-d) shows PHB/PVA system formulation in entry 1,3 to 5, respectively. The insert in each figure shows crossed polarised colloidosomes Entry 4 and 5 (Fig.2(c) and (d)) shows colloidosomes prepared using higher molecular weight surfactant. The D_{ave} increased from 1.33 to 1.37 µm when the surfactant average *Mw* =13k increased to 30k. The D_{ave} increased significantly to 8.67 µm when surfactant with average *Mw* =130k was used in the preparation. The increased average *Mw* reduced the number of surfactant molecules. This could be seen that the D_{ave} increases as average *Mw* increases. However, they are more effective to

stabilize the emulsion for particle/colloidosomes formation. Their images under polarized microscope are more visible as the increasing average *Mw* increases D_{ave}(insert Fig. 2 (a-d)).

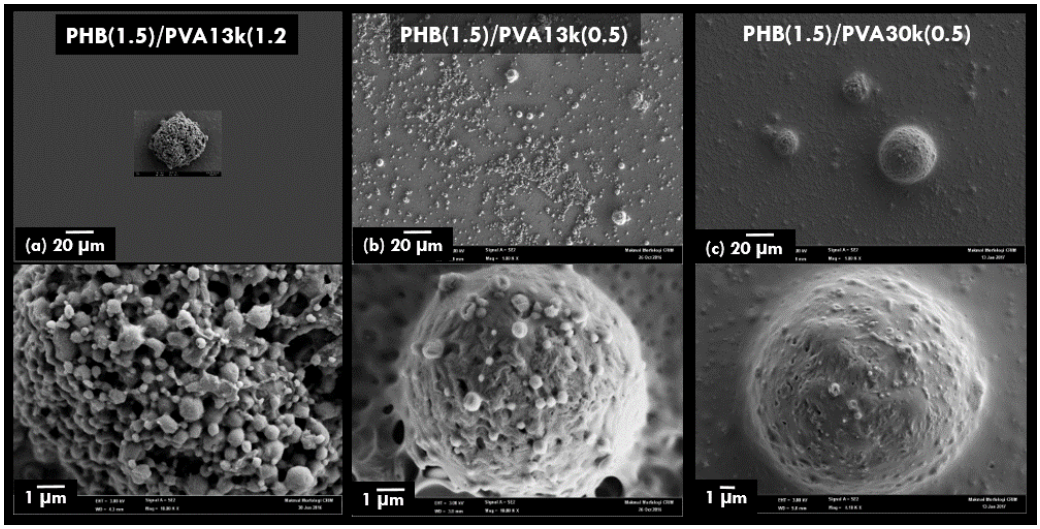

Fig. 3 (a-c) shows the colloidosomes morphology prepared using formulation in entry 1,3 and 4 respectively. The FESEM images below each system show an enlarged surface morphology

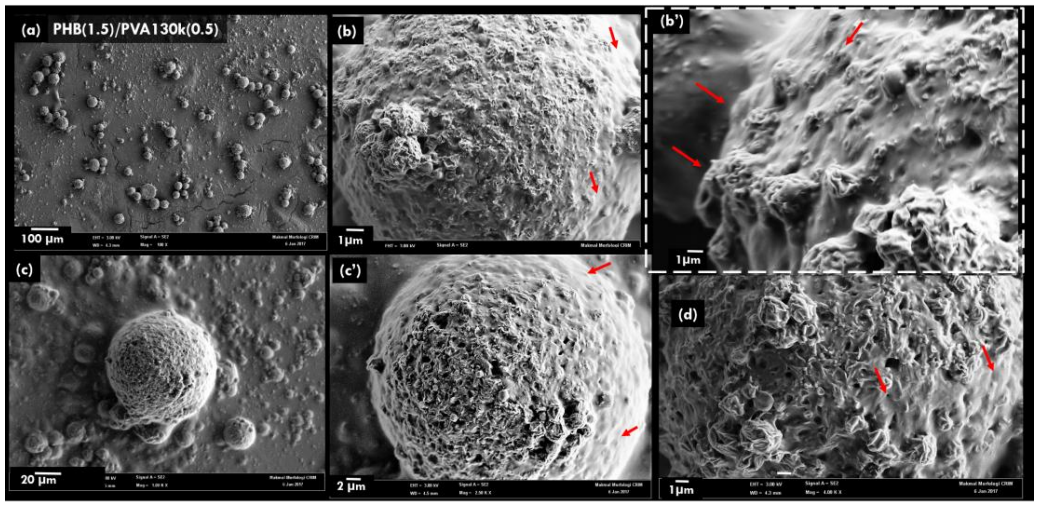

Fig.4(a-d) shows the colloidosomes morphology prepared using formulation in entry 5. Fig.4(b') and (c') are the enlarged images of (b) and (c) respectively. Red arrows show the smoothen area due to higher average *Mw* surfactant used.

The colloisodomes morphology prepared at different polymer concentration and average *Mw*of the surfactant is shown in Fig 3. The PHB(1.5)/PVA13k(1.2) system show highly porous morphology. When PHB(1.5)/PVA(0.5) system was used, the morphology are less porous and further smoothed (Fig. 3 (b) and (c) respectively). It is proposed that PHB(1.5)/PVA130k(0.5)system are porous. However, theremaining higher average *Mw*of the surfactant used might covered the porous surfaceas evidence of the smoothen colloidosomes surface (red arrows in Fig.4)) which might also be the case for PHB(1.5)/PVA30k(0.5) compared to PHB(1.5)/PVA13k(0.5) system.

4. SUMMARY

Is is proven that PHB could be the potential polymer structure to prepare hollow colloidosomes via two-step solvent evaporation method. This study also shown that the formulation control of surfactant concentration and molecular weight and oil phase volume could be altered to producehigher yield of hollow colloidosomes and varies their sizes.

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