Comparison of solvent based and solvent-free Encapsulation of Hydrophobic Actives

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***Abstract*—Solvent-free encapsulation is a new approach. The conventional method of using large amounts of solvents is effective. However, when compared to the solvent-free method, the latter is far superior, in terms of loading capacity, encapsulation efficiency, and number of particles formed. Although the research was done with pharmaceutical actives, exploration of other actives and drug-delivery efficiency is being conducted.**

Keywords-component; Solvent-free encapsulation; Diblock copolymer; Hydrophobic actives; Hydrophilic medium

I. INTRODUCTION

Encapsulation is used to capture, protect and deliver actives. This prolongs the shelf-life which may increase the effectiveness of the active. Since many biologically active substances are sensitive to temperature, pH, light and oxidation, encapsulation helps to reduce the extent of degradation tremendously [1]. This emphasizes the importance of encapsulation in the pharmaceutical industry.

Encapsulation using polymers has conventionally been performed with the use of large amount of solvents, such as tetrahydrofuran. This has a harmful effect on the users' health and environment, as tetrahydrofuran may cause cough, dizziness, nausea and unconsciousness when inhaled continuously. When polymer nanoparticles are used in a hydrophilic medium, tetrahydrofuran may be left in trace amounts and be detrimental to the environment and health.

Polyethylene glycol-polycaprolactone (PEG-PCL) is a biodegradable, diblock copolymer. This means that the formation of particles is mainly due to the difference in polarities of the dispersed and continuous phase. In addition, the biodegradability of PEG-PCL ensures low toxicity and no bioaccumulation while possessing the ability to be removed from the system by natural metabolic pathways. This makes PEG-PCL a top choice in the encapsulation of pharmaceutical actives.

This solvent-free method was designed to counteract the problems that solvents may give rise to; together with the use of PEG-PCL, reducing the health and environmental impacts faced.

In the following sections, the solvent-free and solvent-based methods were reviewed and compared to evaluate the efficiency of encapsulation and other related parameters.

II. EXPERIMENTAL

A. Solvent-free Encapsulation

Solvent-free encapsulation was carried out using ICES proprietary method of described in the patent Organic Solvent Free Encapsulation Of Actives In Biodegradable Particles Using Low Melting Co-Polymers Thoniyot, P. Parijat Kanaujia, Alexander M. van Herk Singapore Application No: 10201702525X dated 28 March 2017.

B. Solvent Encapsulation

This experiment was performed to compare the encapsulation efficiency between solvent-free and solvent encapsulation.

35mg of ibuprofen (IBU), and 140mg of PEG-PCL were weighed in a bottle. 2.2mL of tetrahydrofuran was added to the mixture and stirred until fully dissolved. 2mL of the mixture was dripped slowly via a programmed syringe pump into a larger vial containing 10mL of poloxamer 407 0.1% w/w in PBS, with stirring at a rate of 10μL/min. The new mixture obtained was stirred overnight. The resultant solution was sent for dynamic light scattering (DLS) and high performance liquid chromatography (HPLC) analyses.

C. *Release Study of Solvent-free Encapsulated Particles*

Four release study beakers were prepared.

In beaker 1, 450mL of PBS buffer solution pH = 7.47, and 18.5mg of IBU, dissolved in 4mL of poloxamer 407 in PBS 0.1%w/w was injected into a dialysis cassette, in which the cassette was then added to the beaker.

In beaker 2, 450mL of PBS buffer solution pH = 7.47, and 2mL of previously prepared solvent-free encapsulated supernatant, topped up with 2mL of poloxamer 407 in PBS 0.1%w/w was injected into a dialysis cassette, in which the cassette was then added to the beaker.

In beaker 3, 450mL of ultrapure water, and 19.3mg of IBU, dissolved in 4mL of poloxamer 407 in PBS 0.1%w/w was injected into a dialysis cassette, in which the cassette was then added to the beaker.

In beaker 4, 450mL of ultrapure water, and 2mL of previously prepared solvent-free encapsulated supernatant, topped up with 2mL of poloxamer 407 in PBS 0.1%w/w was injected into a dialysis cassette, in which the cassette was then added to the beaker.

Once the cassettes have been placed into the beakers, 1mL of the solution was taken out for HPLC analysis. Samples were taken at every 15-minute interval for the first hour and every hour subsequently for 6 hours, and lastly at 24 hours.

D. *Release Study of Solvent Encapsulated Particles*

Two release study beakers were prepared. The experiment was conducted to compare the release of solvent encapsulated particles against solvent-free encapsulated particles.

In beaker 1, 450mL of ultrapure water, and 2mL of previously prepared solvent encapsulated supernatant, topped up with 2mL of poloxamer 407 in PBS 0.1%w/w was injected into a dialysis cassette, in which the cassette was then added to the beaker.

In beaker 2, 450mL of PBS buffer solution pH = 7.47, and 2mL of previously prepared solvent encapsulated supernatant, topped up with 2mL of poloxamer 407 in PBS 0.1%w/w was injected into a dialysis cassette, in which the cassette was then added to the beaker.

Once the cassettes have been placed into the beakers, 1mL of the solution was taken out for HPLC analysis. Samples were taken at every 15-minute interval for the first hour and every hour subsequently for 5 hours, and lastly at 24 hours.

III. Results and Discussion

The results of the experiments mentioned above will be discussed below.

Fig. 1 depicts the NMR spectrum for PEG-PCL used in the study.

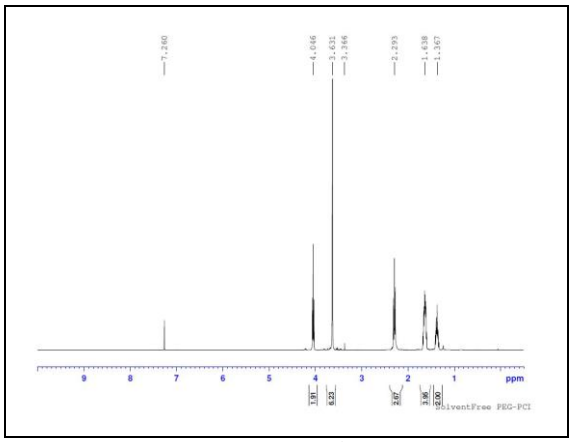

Figure 1. NMR spectrum of PEG-PCL used

A. *Solvent-free Encapsulation*

Figs. 2 and 3 show the particle sizes of solvent-free encapsulated particles by means of DLS.

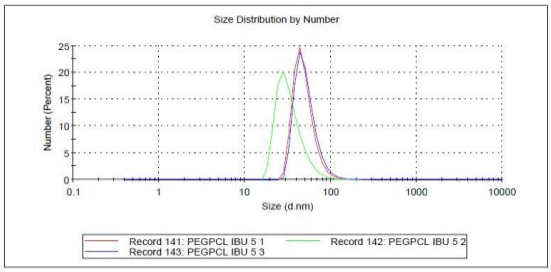

Figure 2. Intensity analysis of solvent-free encapsulated particles

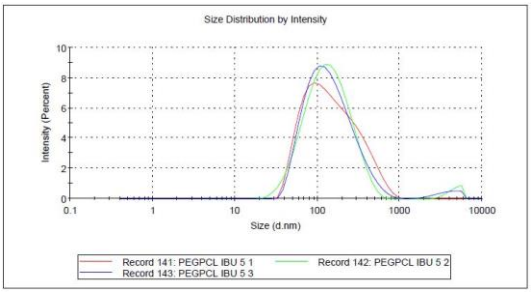

Figure 3. Number analysis of solvent-free encapsulated particles

The above reports (Figs. 2 and 3) show the intensity and number analyses using DLS analysis. It can be observed that IBU has been loaded into the PEG-PCL particles as evident in the increase in particle size from 30 d. nm to 70d. nm to 116.8 d. nm as shown by the Z-average depicted above.

Figs. 4, 5, and 6 show the chromatograms of the resultant samples obtained, using a reverse phase high performance liquid chromatography.

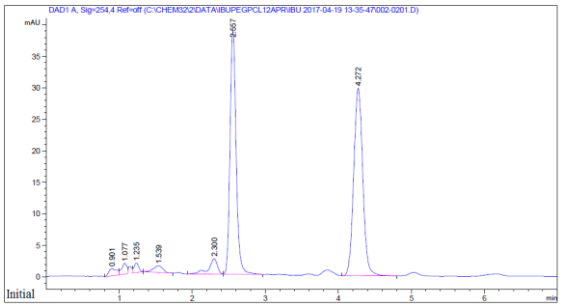

Figure 4. Chromatogram of initial sample

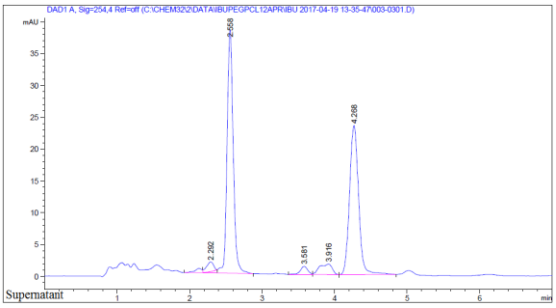

Figure 5. Chromatogram of supernatant sample

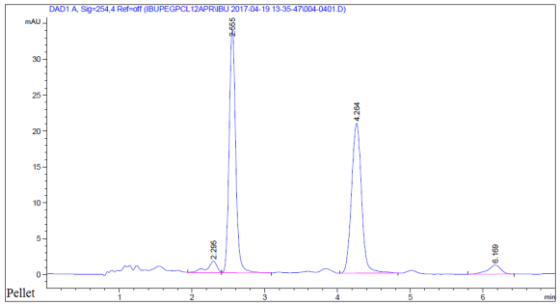

Figure 6. Chromatogram of pellet sample

As evident in the table below, 47.02mg of IBU was detected in the supernatant of a 50mg sample, which proves that 94.05% of the hydrophobic drug was encapsulated in the PEG-PCL particles.

B. Solvent Encapsulation

Figs. 7 and 8 show the particle sizes of solvent encapsulated IBU in PEG-PCL particles.

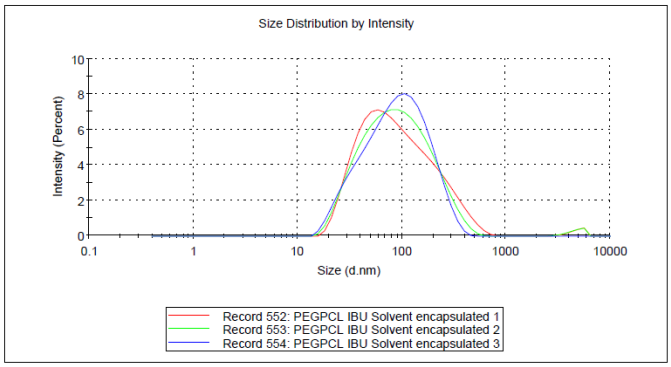

Figure 7. Intensity analysis of solvent encapsulated particles

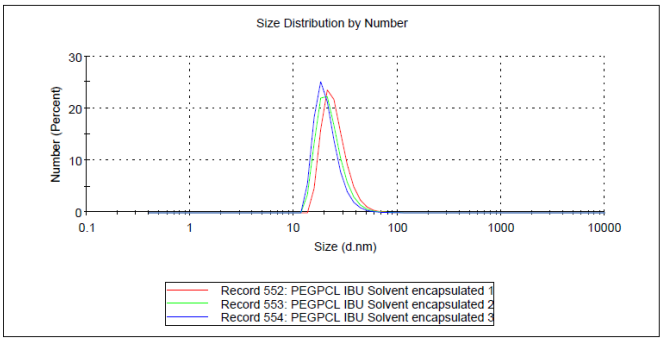

Figure 8. Number analysis of solvent encapsulated particles

TABLE I. TABLE OF HPLC DATA OBTAINED IN SOLVENT-FREE ENCAPSULATION

HPLC Data	**Results and Calculations**				
	Dilution Volume (µL)	**Concentration (µg/mL)**	**Final Concentration (µg/mL)**	**Total Amount (mg)**	**Percentage Encapsulation Efficiency (%)**
Initial	50	229.4334399	4588.668797	45.88668797	
Supernatant	50	235.1336496	4702.672991	47.02672991	94.05345982
Pellet	10	203.5789174	2035.789174	2.035789174	4.071578347
Total amount of IBU detected in a 50mg in 10mL sample				49.06251909	

As evident in the above reports, the solvent-based method yields a smaller particle size as compared to the solvent-free method. Table 2 below shows the comparison between the sizes of particles obtained from solvent-free and solvent-based method.

TABLE II. TABLE OF COMPARISON BETWEEN PARTICLE SIZES OF SOLVENT AND SOLVENT-FREE ENCAPSULATED PARTICLES

Particle Size	**Methods of Encapsulation**	
	Solvent-free encapsulated particles	**Solvent encapsulated particles**
Z-Ave (d. nm)	116.8	69.96

DLS measures the hydrodynamic size by the scattering of light. Hence, it is not an accurate representation of the particle sizes. More measurements need to be taken to ensure the accurate measurement of particle sizes.

C. Release Study of Solvent-free Encapsulated Particles

The amount of IBU released detected by HPLC was plotted against time as observed in Fig. 9.

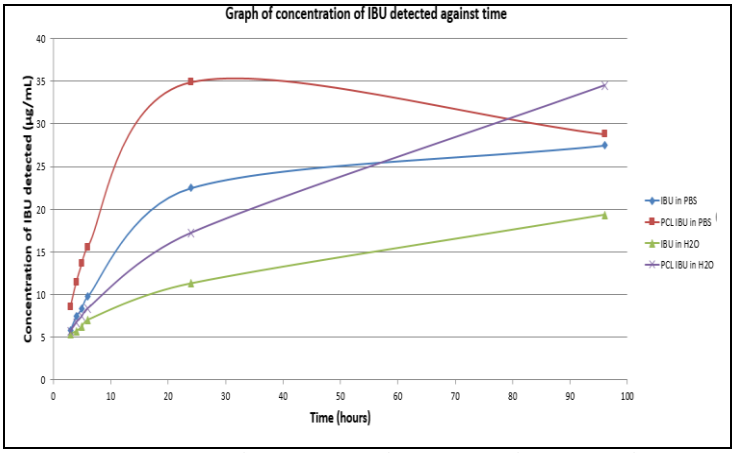

Figure 9. Graph of concentration of IBU released from solvent-free encapsulated particles against time

TABLE III. TABLE OF HPLC DATA OBTAINED IN SOLVENT ENCAPSULATION

HPLC Data	**Results and Calculations**				
	Dilution Volume (µL)	**Concentration (µg/mL)**	**Final Concentration (µg/mL)**	**Total Amount (mg)**	**Percentage Encapsulation Efficiency (%)**
Initial	100	304.1469	3041.469	30.4146	
Supernatant	100	294.3751	2943.751	29.4375	83.629
Pellet	100	28.0628	286.028	0.2860	0.8125
Total amount of IBU detected in a 35mg in 10mL sample				29.7235	

Comparing the encapsulation efficiencies of Solvent-free and Solvent-based encapsulation (from Table III), as seen in Table IV below, the solvent-free method has a significantly higher efficiency than the particles encapsulated via the solvent method. This proves that the solvent-free method encapsulates hydrophobic actives more readily and has thus increased the efficiency by approximately 10%.

TABLE IV. TABLE OF COMPARISON BETWEEN ENCAPSULATION EFFICIENCIES OF SOLVENT AND SOLVENT-FREE ENCAPSULATED PARTICLES

Encapsulation Efficiency	**Methods of Encapsulation**	
	Solvent-free encapsulated particles	**Solvent encapsulated particles**
Percentage Encapsulation Efficiency (%)	94.053	83.629

From the graph in Fig. 9, the release of IBU in PBS was evidently slower with less IBU being detected by the end of 96 hours than the amount of IBU detected in water.

The dissolution of IBU in beakers 1 and 3 served as a control of non-encapsulated active.

D. Release Study of Solvent Encapsulated Particles

Fig. 10 depicts the graph with respect to the concentration of IBU released against time for the solvent encapsulated particles.

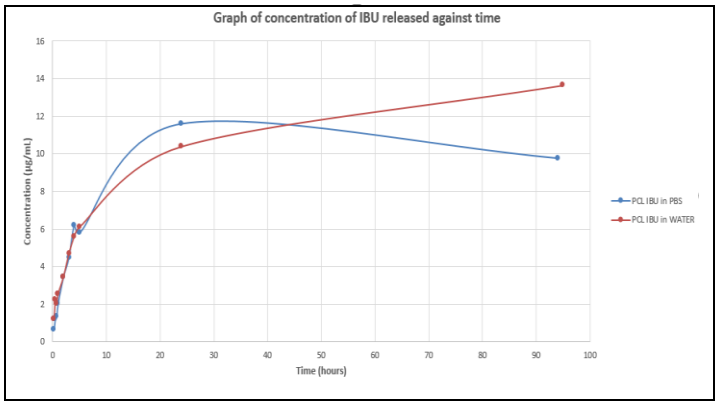

Figure 10. Graph of concentration of IBU released from solvent encapsulated particles against time

The results of the release solvent encapsulated particles were observed to be significantly slower than that of the solvent-free encapsulated particles. However, if the actives were released too quickly or too slowly, it may hinder the overall drug effectiveness. Hence, an adequate speed of release is desired.

IV. Conclusion

In conclusion, the research conducted has proven much about efficiency of the solvent-free method as compared to that of the solvent method. Based on results obtained, there has been a significant increase in the efficiency of encapsulation, by 10%, the loading capacity, and the quality of particles when using the solvent-free method as compared to the conventional solvent method. However, the research on the compatibility of more hydrophobic actives and experiments is currently being conducted, together with the study of drug delivery efficiency between solvent-free and solvent-based encapsulation methods.

Acknowledgment

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