Asian Journal of Chemistry; Vol. 27, No. 4 (2015), 1358-1360

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17983

Effects of Microemulsion Conditions on Drug Encapsulation Efficiency of Salicylic Acid in Poly(lactide-*co*-glycolide) Microparticles

ING HONG OOI* and SET HUI NG

Department of Pharmaceutical Chemistry, School of Pharmacy, International Medical University, 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia

*Corresponding author: Fax: +60 3 86567229; Tel: +60 3 86567228; E-mail: inghong_ooi@imu.edu.my, inghooi@gmail.com

Received: 10 May 2014; *Accepted*: 28 July 2014; *Published online*: 4 February 2015; AJC-16792

Various microemulsion generation conditions in emulsion solvent evaporation technique can affect the encapsulation efficiency of a drug. In this study, homogenization speed, homogenization temperature and organic-to-aqueous phase ratio were varied and the resulting encapsulation efficiency of a model hydrophobic drug *i.e.*, salicylic acid, in poly(lactide-*co*-glycolide) (PLGA) microparticles was determined using UV spectrophotometry. Results showed that the encapsulation efficiency of salicylic acid ranged from 8.5 to 17 % depending on the microemulsion conditions. Under the same temperature (15 °C) and homogenization speed (19000 rpm) conditions studied, a relatively high organic-to-aqueous phase ratio (1:5) provided salicylic acid loaded PLGA microparticles with significantly higher drug encapsulation efficiency. In addition, under all microemulsion conditions, PLGA microparticles obtained were spherical and aggregation between the particles was not observed. This indicates that PLGA microparticles with desirable amount of drug and with anticipated size and shape could be realized by controlling emulsification process conditions.

Keywords: Poly(lactide-*co*-glycolide), Emulsion solvent evaporation, Microparticles, Encapsulation efficiency, Salicylic acid.

INTRODUCTION

Emulsion solvent evaporation technique is widely used to prepare nano-/microparticles of many polymer material based drug carriers for medical and biomedical applications[1-3]. One of the most widely investigated synthetic polymers for controlled drug delivery is FDA-approved biocompatible, biodegradable poly(lactide-*co*-glycolide) copolymers (PLGAs). The PLGA nano-/microparticles containing drug, protein or nucleic acid were prepared using emulsion solvent evaporation technique[4]. One key indicator in drug loaded PLGA nano-/microparticles is the drug encapsulation efficiency. The higher the drug encapsulation efficiency, the better is in terms of therapeutic bioavailability and efficacy in particular for specific targeted delivery. Depending on the processing parameters of emulsion solvent evaporation technique, nano-/microparticles of PLGA with varying amount of encapsulated drugs could be prepared. The aim of this study was to investigate the effects of emulsification conditions, namely, homogenization speed, homogenization temperature and organic-to-aqueous phase ratio on drug encapsulation efficiency of a model drug, salicylic acid, in PLGA microparticles formed by emulsion solvent evaporation method. Salicylic acid, a classic non-steroidal anti-inflammatory drug, is of interest for studies related to its pharmacological effects[5,6]. In particular, decline in irritancy of salicylic acid has been achieved through encapsulation in controlled release systems such as liposome, hydrogel and solid lipid nanoparticle[7].

EXPERIMENTAL

Poly(DL-lactide-*co*-glycolide) (75:25) (PLGA) with an inherent viscosity of 0.55-0.75 dL/g was purchased from Birmingham polymer Inc, USA. Salicylic acid was purchased from Sigma-Aldrich. Sodium hydroxide, poly(vinyl alcohol) 87-89 % hydrolyzed and chloroform were purchased from Fisher Scientific and were used as received.

Preparation of poly(lactide-*co*-glycolide) (PLGA) microparticles: Typical salicylic acid loaded PLGA microparticles were prepared as follows: 0.4 g of PLGA was dissolved in 10 mL of chloroform to form a polymer solution. 80 mg of salicylic acid was then added into the polymer solution to produce an organic dispersed phase. The resulting organic layer was mixed with 100 mL of 0.5 % (w/v) poly(vinyl alcohol) aqueous phase. The mixture was homogenized at 11,000 rpm for 15 seconds using a homogeniser (IKA Ultra-Turrax T-25 Homogeniser) to obtain an off-white oil-in-water (O/W) emulsion. The resulting O/W emulsion was stirred with a magnetic stirrer (Daihan Labtech Co. Ltd., Korea) for 4 h at 25 °C to allow solvent evaporation and microparticles formation. The white particles were recovered by centrifugation using a

centrifuge (Sigma2-16KC, Ultracentrifuge) spun at 5000 rpm for 10 min at room temperature. The clear supernatant that contains dissolved substances such as poly(vinyl alcohol) and free salicylic acid was removed. The residue containing PLGA microparticles was washed using 100 mL of distilled water and centrifugation was repeated twice. Finally, the purified nanoparticles were transferred into a serum bottle for freeze-drying. Salicylic acid loaded PLGA microparticles with whitish appearance were thus obtained.

Determination of encapsulation efficiency of salicylic acid in the poly(lactide-*co*-glycolide)

Microparticles: 30 mg of the PLGA microparticles were dissolved in 10 mL of chloroform. The encapsulated salicylic acid was extracted three times with 20 mL of 0.1 M NaOH solution using a separating funnel. The aqueous layer was separated and the concentration of sodium salt of salicylic acid was measured spectrophotometrically (Shimadzu 1240, UK) in a 1 cm cell at 296 nm. Based on the sodium salicylate standard calibration curve of UV absorbance *versus* its concentrations, the total amount of encapsulated salicylic acid was computed. The encapsulation efficiency (EE) of salicylic acid in the PLGA microparticles was calculated using the following equation:

$$EE(\%) = \frac{\text{Total salicylic acid encapsulated (g)}}{\text{Total salicylic acid used in formulation (g)}} \times 100$$

Characterization of drug-loaded PLGA microparticles: Dried PLGA microparticles were spread on a glass slide and visualized using a light microscope (Nikon Eclipse 80i and Leica) under 1000 × magnification. One drop of immersion oil was added to the slide to obtain a clear image. Morphology of the microparticles and formation of aggregates were examined and an image of each specimen was captured using a microscope digital camera.

Statistical analysis: The encapsulation efficiency data are expressed as mean ± standard deviations (s.d). The significant differences were assessed by one way ANOVA and considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The results of the effects of homogenization temperature, homogenization speed and organic-to-aqueous phase ratio on encapsulation efficiency of salicylic acid in PLGA microparticles are shown in Table-1 and Fig. 1. The results showed that a homogenization temperature of 15 °C gave PLGA microparticles that have lower encapsulation efficiency in comparison to those

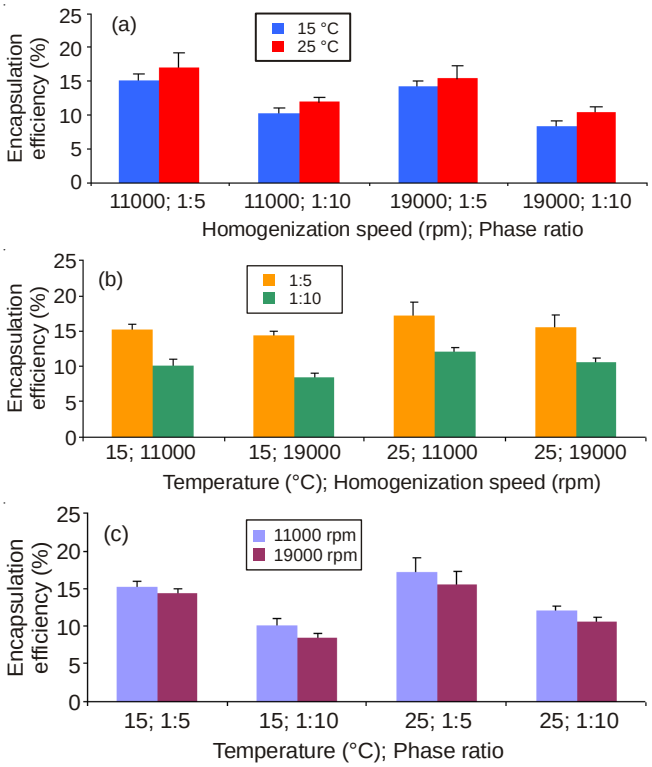

Fig. 1. (a-c) Effects of homogenization speed, homogenization temperature and phase ratio on encapsulation efficiency of salicylic acid in PLGA microparticles

produced at higher homogenization temperature of 25 °C under all conditions studied. This could be due to the fact that poly(vinyl alcohol) is at a lower kinetic energy state at lower temperature, which would delay the migration of poly(vinyl alcohol) to the oil/water interfaces, causing the poly(vinyl alcohol) effective concentration to be low. This thinner layer of surfactant on the surfaces of microemulsion would increase the probability for the drug to diffuse out from the organic phase into the external aqueous phase, thus resulting in lower encapsulation efficiency owing to greater drug loss. In addition, low effective poly(vinyl alcohol) concentration around microemulsion may have caused unstable emulsion that entrapped lesser drug. A previous study reported that a stable emulsion prevents the mass transfer of a drug to the external phase by which the drug is more evenly distributed in the matrix of PLGA microparticles[8].

A two-level homogenization speed was used in this study, namely at 11000 and 19000 rpm to investigate their effects on

TABLE-1
EFFECTS OF MICROEMULSION CONDITIONS ON ENCAPSULATION EFFICIENCY OF SALICYLIC ACID IN POLY(LACTIDE-CO-GLYCOLIDE) MICROPARTICLES

Homogenization Temperature (°C)	Homogenization Speed (rpm)	Organic-to-aqueous phase ratio (v/v)	Encapsulation efficiency % (w/w)			
			1	2	3	Mean ± standard deviation
25	11000	1:10	10.69	12.28	13.08	12.02 ± 1.22
15	11000	1:10	10.20	11.68	8.75	10.21 ± 1.47
25	19000	1:10	10.60	11.81	9.31	10.57 ± 1.25
15	19000	1:10	7.77	9.53	8.07	8.46 ± 0.94
25	11000	1:5	20.80	14.42	16.22	17.15 ± 3.29
15	11000	1:5	14.94	13.98	16.71	15.21 ± 1.38
25	19000	1:5	18.80	12.93	14.85	15.53 ± 2.99
15	19000	1:5	15.17	12.97	14.79	14.31 ± 1.18

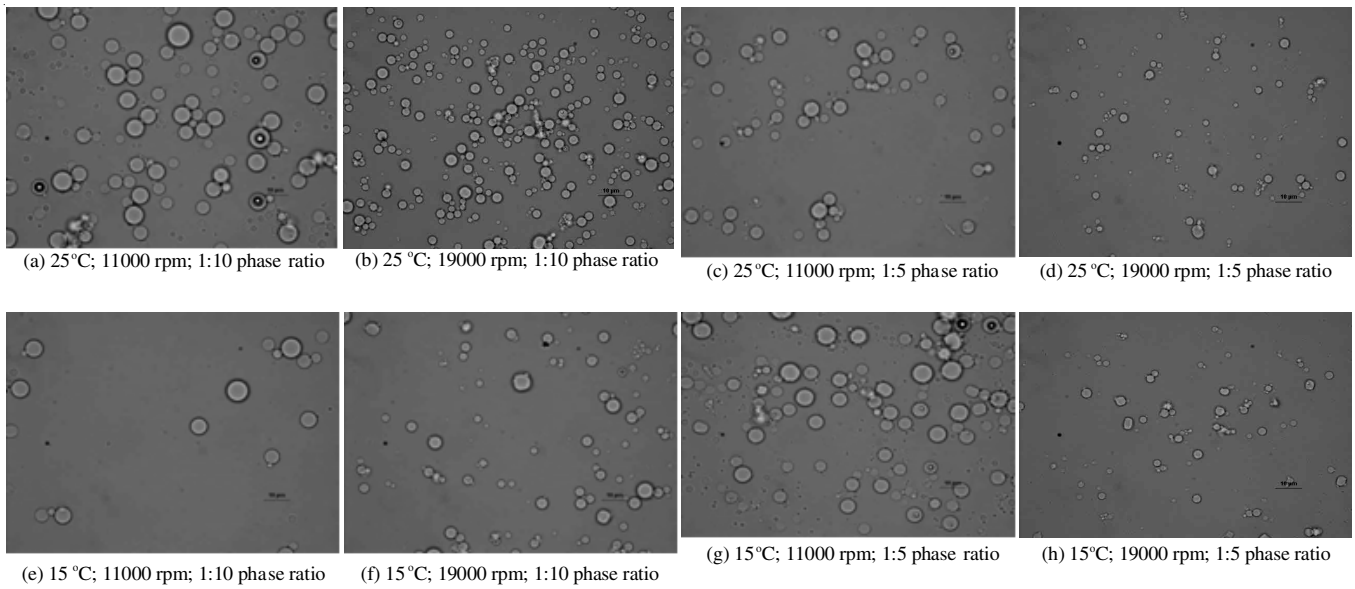

Fig. 2. Light microscope images of salicylic acid loaded PLGA micoparticles produced using emulsion solvent evaporation method under different emulsification conditions

the drug encapsulation efficiency. The encapsulation efficiency of salicylic acid in PLGA microparticles was higher when the speed was reduced from 19000 to 11000 rpm. The same trend was observed in all experiments conducted under different homogenization temperature and phase ratio conditions. This higher encapsulation efficiency could most likely be due to an increase in apparent viscosity of the microemulsion at a lower homogenization speed. As viscosity of microemulsion increases, diffusion of drug to the continuous phase is hindered and, thus, minimising drug loss and, hence, improved encapsulation efficiency[9].

The effects of organic-to-aqueous phase ratios of 1:10 and 1:5 on the encapsulation efficiency of salicylic acid were investigated. The two phase ratios were obtained by mixing 100 and 50 mL of external aqueous phase, respectively, with 10 mL of organic phase. The results showed that an increase in the organic-to-aqueous phase ratio from 1:10 to 1:5 resulted in an increase in the encapsulation efficiency of the model drug in the PLGA microparticles prepared under all conditions studied. Similar observations are reported in a previous study[10]. This shows that PLGA microparticles prepared using relatively lower volume of aqueous phase effectively encapsulated salicylic acid. The possible explanation is that increasing the organic-to-aqueous phase ratio decreased the extent of contact of drug with water molecules in the external aqueous phase during emulsification process. Under this condition, more drugs remained in the microemulsion droplets to interact with PLGA molecules, thus, giving higher encapsulation efficiency. Fig. 2 shows images of salicylic acid-loaded PLGA microparticles prepared by emulsion solvent evaporation method. It is clearly observed that the particle size is generally smaller when homogenization speed is increased.

Statistically, it was found that only at 15 °C and at 11000 or 19000 rpm, respectively, the encapsulation efficiency of salicylic acid in PLGA microparticles was significantly higher when the organic-to-aqueous phase ratio was 1:5 as compared to those of ratio of 1:10. The large variability in experimental results as reflected in some standard deviations may have reduced the significant differences between results. Therefore, larger replication of experiments under each set of conditions will certainly provide a better test of significant differences in the efficiency of drug entrapment studied under different conditions.

Conclusion

Encapsulation efficiency of salicylic acid into PLGA microparticles ranged from 8.5 to 17 %. At 15 °C and homogenization speed of 19000 rpm, significantly higher drug encapsulation efficiency was obtained with an organic-to-aqueous phase ratio of 1:5 than ratio of 1:10.

ACKNOWLEDGEMENTS

The authors thank the International Medical University (IMU) for financial support of this work.

REFERENCES

1. M.J. Alonso, in eds.: ed. S. Cohen and H. Bernstein, Microparticulate Systems for the Delivery of Proteins and Vaccines, Vol. 77, Chap. 7, Marcel Dekker, New York, pp. 203-242 (1996).
2. Y. Inoue, S. Yoshimura, Y. Tozuka, K. Moribe, T. Kumamoto, T. Ishikawa and K. Yamamoto, *Int. J. Pharm.*, **331**, 38 (2007).
3. V.J. Mohanraj and Y. Chen, *Trop. J. Pharm. Res.*, **5**, 561 (2006).
4. S. Freitas, H.P. Merkle and B. Gander, *J. Control. Rel.*, **102**, 313 (2005).
5. S. Sakamoto, Y. Kabe, M. Hatakeyama, Y. Yamaguchi and H. Handa, *Chem. Rec.*, **9**, 66 (2009).
6. M.L. Johnson and K.E. Uhrich, *J. Biomed. Mater. Res. A*, **91**, 671 (2009).
7. A.A. Date, B. Naik and M.S. Nagarsenker, *Skin Pharmacol. Physiol.*, **19**, 2 (2006).
8. H. Hamishehkar, J. Emami, A.R. Najafabadi, K. Gilani, M. Minaiyan, H. Mahdavi and A. Nokhodchi, *Colloids Surf. B*, **74**, 340 (2009).
9. H. Zhao, J. Gagnon and U.O. Hafeli, *Biomagn. Res. Technol.*, **5**, 2 (2007).
10. W. Chaisri, W. Hennink and S. Okonogi, *Curr. Drug Deliv.*, **6**, 69 (2009).