Surface Roughness, Hydrophilicity and Encapsulation Efficiency of Gentamicin Loaded Surface Engineered PLA Microspheres

Tran Thi Thu Trang 1, Mariatti Jaafar 1, Badrul Hisham Yahaya 1, Masakazu Kawashita 2, Nguyen Xuan Thanh Tram 3 and Zuratul Ain Abdul Hamid 1*

1Biomaterials Research Niche Group, School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia, Tel. +604 5996153, Fax. +604 5996907.

2Graduate School of Biomedical Engineering, Tohoku University, 6-6-12, Aramaki Aza Aoba Aoba-ku, Sendai, Miyagi 980-8579, Japan, Tel. +81 22 795 7491.

3Department of Silicate Materials, Faculty of Materials Technology, Ho Chi Minh City University of Technology, VNU-HCM, 268 Ly Thuong Kiet St, District 10, HCM city, Vietnam, Tel.+ 848 38 647 256.

Corresponding author: *srzuratulain@usm.my

Abstract. Polylactic acid (PLA) is one of the common biodegradable polymers utilised as therapeutic drug vehicles in drug delivery system (DDS). PLA has several desired properties for drug delivery including biocompatible, biodegradable and good mechanical properties. However, the poor hydrophilicity of PLA leads to low cell adhesion, disruption in the human body and causing inflammatory to the biological environment. Hence, the modification of PLA bulk and surface properties has become crucial to increase its potential. This research investigated the effect of surface modification with sodium hydroxide (NaOH) at different concentrations on the PLA microspheres properties which loaded with gentamicin at different loadings (0.25 ml, 0.5 ml, and 1 ml). The average size of the microspheres obtained was in the range of 1 μm to 50 μm; within the range of acceptable size in the parenteral injection. Confirmation of increased hydrophilicity has been evaluated using Fourier transform infrared (FTIR) and contact angle. Gentamicin was encapsulated within PLA microspheres and the PLA microspheres obtained the highest encapsulation efficiency (42%) for the highest volume drug loaded. Morphological changes of modified PLA microspheres have been confirmed via scanning electron microscopy (SEM) imaging.
1. Introduction

Biodegradable polymers are highly desirable in the field of drug delivery because they degrade in the body to biologically inert and compatible molecules. Besides that, by incorporating drugs in biodegradable polymers, this allow for no post-surgery since the remaining polymer will degrade and get cleared by the body. Thus, biodegradable polymers offer a versatile approach for developing sustained release drug delivery systems that are simple and convenient to the patient. One of the most commonly used for drug delivery system is poly(lactic acid) (PLA) since PLA comes from renewable resources [1]. In fact, PLA has outstanding advantages over other polymers such as biodegradable, biocompatible, non-toxic and good mechanical properties [2, 3]. It is being used a potential candidate for biomedical applications [4] and has been approved by the Food and Drug Administration (FDA) for certain clinical applications including drug delivery systems [5]. The solvent evaporation method is the most frequently used technique to prepare polymeric microspheres for drug delivery. The double emulsion method has been used by several researchers to entrap highly hydrophilic drugs such as gentamicin within biodegradable hydrophobic microspheres [6, 7]. Gentamicin, a highly water-soluble drug, is an aminoglycoside that has been used against a wide range of Gram-positive and Gram-negative bacteria [7]. The most disadvantage of PLA is poor its hydrophilicity due to it’s unstable property in wet condition, which can undergo chain disruption in the human body, lead to low cell adhesion inflammatory, etc [3]. Thus, PLA needs to be surface modified in order to introduce hydrophilicity so that the biocompatibility property will be enhanced and therefore it is suitable to be used as drug delivery vehicles. There are several surface modification methods such as physical method, including surface coating, entrapment, plasma treatment, and chemical methods [3]. Among all methods, the conventional alkaline hydrolysis technique using sodium hydroxide (NaOH) is chosen to modify the PLA microspheres because they are simple, cheap and promising in improving the hydrophilicity property of the microspheres. A low concentration of NaOH could be applied to avoid severe bulk degradation, and residual alkali was easy to remove. After surface hydrolysis, hydrophilic carboxyl and hydroxyl can be produced by cleaving the ester bond [8]. In this paper, PLA microspheres containing gentamicin was confirmed through UV-Vis and microspheres size was performed by particle size analysis. And the surface structure of PLA microspheres was also examined by using FTIR to determine the presence of hydroxyl groups. While for the surface morphology was investigated by using SEM. Other than that, dynamic water contact angle was done to assess the hydrophilicity of the microspheres after the modification.

2. Experimental study

Poly(lactic acid) (PLA, Nature Work), dichloromethane (DCM, ACS Grade, Merck), poly(vinyl alcohol) (PVA, 80% hydrolyzed, Mw 9-10K, Aldrich Chemistry), sodium hydroxide (NaOH, M=40 g/mol, Merck), hydrochloric acid (HCl, 37%, fuming acid, Merck) and gentamicin (reagent solution, Gibco). All chemicals were used as received. PLA microspheres prepared through emulsion and solvent evaporation (ESE) technique. The primary emulsion was obtained by dispersing at the different volume of gentamicin in the solution of PLA pellets were dissolved in DCM by magnetic stirring for 3 minutes. Then the mixture was immediately with PVA solution to form secondary emulsion at 250 ± 5 rpm continuously stirring for 24 hours. PLA microspheres were washed, filtered and dried in a desiccator (Table 1).

### Table 1. The formula used for the synthesis of PLA microspheres

| Sample | PLA Concentration (%, g/ml) | PVA Concentration (%, g/ml) | Volume of gentamicin at 10000ppm (ml) |
|--------|-----------------------------|----------------------------|-----------------------------------|
| G0     | 7.5                         | 3                          | 0                                 |
| G1     | 7.5                         | 3                          | 0.25                              |
| G2     | 7.5                         | 3                          | 0.5                               |
| G3     | 7.5                         | 3                          | 1                                 |
After preparation of PLA microspheres was done, surface hydrolysis treatment was performed to modify surface and introduced functionally on PLA. PLA microspheres were immersed in both NaOH and HCl solutions adopted from previous studies [9]. The encapsulation efficiency was obtained using following equation 1: % EE = [(Total drug – Free drug in supernatant)/Total drug]*100 %. The obtained PLA microspheres were characterized by Malvern Mastersizer particle size analyzer (Worcestershire, UK), the transmission Fourier-Transform Infrared spectroscopy (FTIR) spectra, Dynamic Contact Angle Meter and Tensiometer (DCAT 11, Dataphysic), Cary 50-UV-Vis spectrometer (Varian Inc., Agilent Technology) and Zeiss Supra Gemini 35 VP field emission scanning electron microscope (SEM).

3. Results and Discussion

3.1 Particle size distribution

The particle size distribution curve of PLA microspheres is presented in Figure 1. It was found that size range of the measured particles was around 9-35 µm, where 90 % of the measured particles had a mean particle size ranging from 22-32 µm and below 50 µm. The homogeneity was determined based on the span values for each of formulations. The span value was calculated based on the formulation as described as follow: Span value = (D90 – D10)/ D50. Whereby D90, D10, and D50 are the particle distribution at 90 %, 10 %, and 50 % respectively. The span lower than 1 indicates that the size distribution can be considered as homogeneously dispersed [10]. Based on the results, the particle distribution of microspheres within the range of acceptable size in the parenteral injection.

![Figure 1. The particle size distribution of PLA microspheres](image)

3.2 FTIR Analysis

Figure 2 (a-c) show the FTIR spectra of series of surface modified PLA microspheres at difference concentration of NaOH. Alkaline hydrolysis will produce hydroxyl (-OH) through cleaving the ester bond in PLA. The appearance of (-OH) groups theoretically was observed at wavelength 3300 to 3610 cm⁻¹ and intensity of -OH peak increased as the concentration of NaOH used increases [9]. Besides that, the FTIR also showed that the PLA microspheres treated with 0.5 M NaOH showed the highest intensity because higher concentration NaOH was having more ability to cleave the ester bond. This confirmed the cleavage of ester bond when surfaces microspheres treated with NaOH has successfully occurred. Figure 2 d) shows the FTIR spectra of neat gentamicin, neat PLA (G0) and PLA loaded with 0.25 ml (G1), 0.5 ml (G2) and 1ml (G3) of gentamicin. It is known that amine group exist in gentamicin can be represented by peak in the range of 3100 - 3600 cm⁻¹ [11]. From Figure 2 d), it was found that significant peak appearance within this range. Therefore, it can be concluded that the gentamicin was loaded within the PLA microspheres after ESE process. Based on these results, the encapsulation of gentamicin within PLA
microspheres was achieved and hydrolysis treatment has resulted in increased of hydrophilicity property of surface engineered PLA microspheres.

Figure 2. Comparison on chemical bonding Neat PLA (G0) with:
(a) PLA loaded 0.25 ml (G1) treated with 0.05 M, 0.1 M, 0.3 M and 0.5 M NaOH
(b) PLA loaded 0.5 ml (G2) treated with 0.05 M, 0.1 M, 0.3 M and 0.5 M NaOH
(c) PLA loaded 1 ml (G3) treated with 0.05 M, 0.1 M, 0.3 M and 0.5 M NaOH
(d) PLA loaded 0.25 ml (G1), 0.5 ml (G2) and 1ml (G3).

3.3 Hydrophilicity Testing

Figure 3 shows that the neat PLA (G0) has the highest hydrophobic behavior because the contact angle value obtained was more than 90° (103.91°). While PLA loaded gentamicin (G1, G2, and G3) undergo changes into hydrophilic behavior which was below 90°. This is due to the existence of loaded gentamicin within PLA microspheres. Furthermore, the contact angle values were lower when a higher concentration of NaOH. For PLA loaded 0.25ml (G1) was decreased from 72.8° – 58.59°, for (G2) also was decreased from 68.98° – 51.54°. The lowest degree of contact angle was observed for PLA loaded 1ml gentamicin (G3) from 57.78° – 46.33°. From the result, higher the concentration of NaOH used for surface treatment, the more hydrophilic the PLA microspheres. Therefore, PLA treatment with NaOH at difference concentration has successfully modified the surface hydrophilicity property of PLA microspheres.

Figure 3. The contact angle of PLA microspheres before and after treated with NaOH.

3.4 Encapsulation Efficiency

The absorbance of gentamicin was measured using UV-Vis at 250 nm. The standard curve of different concentration gentamicin was plotted, as illustrated in Figure 4. The concentration of free drug in
supernatant was determined from this standard curve and encapsulation efficiency was calculated based on the equation 1.

![Standard curve](image)

**Figure 4.** Standard curve obtained from absorbance intensity of Gentamicin at different concentration in 3% PVA solution.

The results of the encapsulated efficiency (EE) indicate that drug loading obtained was increased from 8.88 ± 2.05 % (G1), 18.36 ± 4.45 % (G2) to 42 ± 1.40 % (G3) when the volume of drug increased. This is due to the increased volume of drug in the formulation has resulted in higher encapsulation efficiency obtained [12, 13].

### 3.5 Morphology Characterization via SEM

From Figure 5, the surface structure of PLA microspheres was observed at the same magnification at 1800x. In Figure 5 (a) the neat PLA (G0), (b), (c), and (d) PLA loaded gentamicin (G1, G2 and G3), the surface of microspheres is smooth. But Figure 5 (e), (i) and (m) treated with 0.05 M NaOH, showed the change on the surface as it becomes rough compared with PLA microspheres before treatment. This is due to the hydrolysed surfaces which resulted in the change from smooth to rough surfaces. When the concentration of NaOH increased (Figure 5 (f), (j) and (n) of 0.1 M; (g), (k) and (o) of 0.3 M; (h), (l) and (p) of 0.5 M), the surface morphology showed that PLA microspheres become rougher and the introduction of porous structure was observed. This is due to higher concentration of NaOH used for the surface hydrolysis causes more breakage ester bonds occurred. Therefore, more −OH groups being introduced into PLA although the different volume of the drug has been used, the roughness of each sample almost showed the same pattern due to the same concentration of NaOH has been used.

![PLA microspheres surface morphology](image)

**Figure 5.** PLA microspheres surface morphology of (a) Neat PLA (G0) in comparison with: (b) PLA loaded 0.25 ml (G1) treated with (e) 0.05, (f) 0.1, (g) 0.3 and (h) 0.5 M NaOH (c) PLA loaded 0.5 ml (G2) treated with (i) 0.05, (j) 0.1, (k) 0.3 and (l) 0.5 M NaOH (d) PLA loaded 1 ml (G3) treated with (m) 0.05, (n) 0.1, (o) 0.3 and (p) 0.5 M NaOH
4. Conclusion
The surface engineered PLA microspheres loaded with gentamicin was successfully developed. An optimized encapsulation efficiency of gentamicin at 42% was achieved. The hydrolysis treatment of PLA microspheres with NaOH has resulted in the improvement of hydrophilicity property. This has been achieved as shown in FTIR, SEM and contact angle. Hydrophilic functional groups (-OH) that being introduced during surface treatment enhanced hydrophilic behavior at 0.5M NaOH. This hydrophilicity would be expected to improve the cell-biomaterials interaction during drug delivery. The gentamicin loaded surface engineered PLA microspheres has great potential carriers for drug delivery particularly for bone regeneration application.

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