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Development and Evaluation of Surface Modified Poly (lactic acid) Microsphere via Irradiation Techniques for Drug Delivery System

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Abstract

Poly (lactic acid) (PLA) has been used in medical field because it is biocompatible, biodegradable and has good mechanical properties. However, its surface characteristic which is hydrophobic and chemically inert is not suitable as a carrier in drug delivery system. Purpose of this study is to modify and improve PLA microspheres surface by grafting hydrophilic monomers onto its surface via irradiation techniques. Two sets of PLA microspheres with and without hydrophilic monomers were irradiated using high energy irradiations which are electron beam and Gamma (γ) rays respectively. Although dose used in irradiating samples were same for both electron beam and γ-rays, but the dose rate were different where electron beam has the higher dose rate. Free radicals will be formed when a matter is irradiated with ionising radiation and these radicals will interact with monomers and initiate grafting. Three hydrophilic monomers were used in this study i.e. Acrylic Acid (AA), Acrylamide and Maleic Anhydride (MAH). Surface modified PLA microspheres were characterised using Fourier Transform Infrared (FTIR) and Scanning Electron Microscopy (SEM). PLA microspheres surface were successfully modified and grafted with selected monomers and confirmed by FTIR results. The degrees of grafting were found to be dependent on irradiation dose and dose rate of irradiation as well as monomer used in the grafting. SEM shows surface of PLA microsphere after surface modification is rougher compared to before surface modification.

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Keywords: Poly(lactic acid), Surface modification, Radiation technique

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1. Introduction

Drug delivery refers to approaches, formulation, technologies and systems for transporting a pharmaceutical compound in the body as needed in a safe manner to achieve its therapeutic effect[1].

The challenge has been on three fronts: finding the proper target for a particular disease state; finding a drug that effectively treats this disease; and finding a means of carrying the drug in a stable form to specific sites while avoiding the immunogenic and nonspecific interactions that efficiently clear foreign material from the body.

PLA has been used in medical field quite sometimes and has gained FDA approval because it is biocompatible, biodegradable and has good mechanical properties[2]. However, it surface characteristics which is hydrophobic and lack of readily reactable[3] side-chain groups is not suitable to be used in drug delivery system. Thus, pure PLA may cause a mild inflammatory response if it is implanted into human tissues[2]. Therefore, surface modification strategies will be developed to modified PLA surface chemical functionalities[3], hydrophilicity, roughness, surface energy and topography.

There are different techniques that are used to modify its surface chemical functionalities, hydrophilicity, roughness, surface energy and topography such as surface coating, entrapment, plasma treatment, laser, irradiation and chemical[4]. Amongst all techniques, irradiation is one of the least techniques that are being studied.

Radiation is often categorised as ionising or non-ionising depending on the energy of the irradiated particles. Ionising radiation is able to ionise atom and molecules and break chemical bonds while the energy of non-ionising radiation is not enough to ionise atom and molecules[4]. Radiation can initiate chemical reaction of any material without the use of initiator[5, 6] and catalysts[5]. The irradiation of polymeric materials with high energy radiation will caused the emergence of very reactive intermediates or radicals[7] which rearrangements and/or formation of new bonds may be occurred and eventually oxidized products, grafts, scission of main chains (degradation) or cross-linking will be formed[7]. This process is illustrated in Fig. 1. Radiation sources that are used in this study are electron beam and γ-rays from Cobalt-60. Although both radiations are in the same ionising radiation category, there are differences with regards to penetration and dose rate[8]. γ-rays will give deep penetration into a material but at slow rate and electron beam will give less penetration has higher dose rate[9].

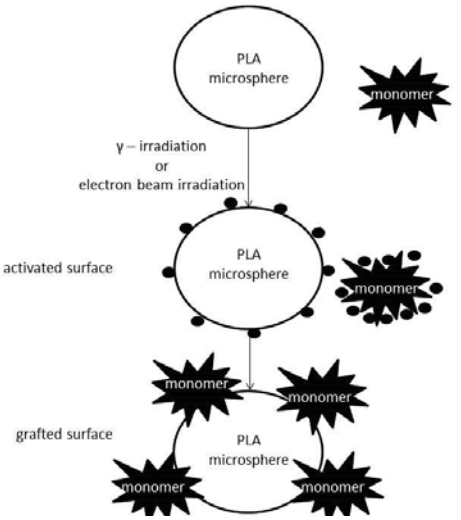

Fig. 1. General scheme proposed in surface modified PLA microsphere

2. Experimental

2.1. Materials

Materials were used in this work are PLA pallet from NatureWorks, Acrylic Acid (AA), Acrylamide (AAm) and Maleic Anhydride (MAH) and Poly(vinyl) Alcohol, all from Sigma-Aldrich GmbH, and Dichloromethane (DCM) from Merck Millipore.

2.2. *Sample preparation*

Two types of samples have been prepared in this study which is films and microspheres. Films were fabricated by hot pressed technique where PLA granules was compressed at 170 ^{o}C for 8 minutes, followed by cool pressed for 3 minutes. PLA sheets were then cut into small pieces.

Emulsion evaporation technique was used to fabricate microspheres. PLA pellets was dissolved in DCM and poured into dissolved PVA with ratio PLA to PVA is 1:3. Then, it was stirred at 1250 rpm at room temperature for a three minutes until stable. After that, the speed was decreased to 250 rpm and leaved overnight to allow DCM to be evaporated slowly. The PLA microspheres that were settled at the bottom of the flask was filtered, washed with distilled water to remove surfactant and finally dried overnight at room temperature.

PLA without monomer as a control and PLA with 3 hydrophilic monomers; 20 % (w/v) Acrylic Acid (AA), 20 % (w/v) Acrylamide (AAm) and 20 % (w/v) Maleic Anhydride (MAH) were placed into glass tubes covered and labeled accordingly.

2.3. PLA grafting

No initiator has been used in this procedure and both irradiations have been carried out at ambient temperature. The grafted samples were washed with distilled water, then centrifuged for 3 minutes at 5000 rpm with ethanol and dried overnight to remove residual or monomer that was not grafted.

2.3.1. Electron beam treatment

Electron beam irradiation was carried out by ALLURTRON unit at Nuclear Malaysia. Electron beam energy was 2.5 MeV and current used was 2.0 mA. Samples were irradiated at different dose which are 20, 40, 60, 80 and 100 kGy.

2.3.2. γ-rays *treatment*

Samples were irradiated at SINAGAMA, Nuclear Malaysia. Source of γ-rays is Cobalt-60 with energy 1.33 Mev and dose rate of 0.028 kGy/minute. Samples were irradiated at different dose which are 20, 40, 60, 80 and 100 kGy.

2.4. Characterization Methods

2.4.1. Fourier Transform Infrared Spectroscopy (FTIR)

The chemical characterization of PLA, PLA-g-AA, PLA-g-AAm and PLA-g-MAH were represented by FTIR on a Spectrum Perkin Elmer with wavelength ranging from 4000cm^{-1} to 500cm^{-1} and 16 individual scans at 4cm^{-1} resolution.

2.4.2. Scanning Electron Microscopy (SEM)

The surface morphology of samples was examined using Zeiss Supra 35Vp at accelerating voltage 3.00kV. Samples were coated with gold using Bio-Rad Laboratories SEM coating system prior to scanning to prevent scanning faults caused by electrostatic charge at the surface.

3. Results and Discussion

3.1. Surface Chemistry

The surface chemistries of neat and grafted PLA were examined by FTIR. Fig. 2 and 3 are FTIR spectrum of PLA grafted AAm via electron beam and γ-rays respectively. In the neat PLA spectrum, the peaks can be observed at 1747cm^{-1} corresponding to an ester C=O band[6, 10]. Other peaks can be observed are 1453cm^{-1} and 1081cm^{-1}, corresponding to the –C-O- stretching and –CH3 bending vibration[6] respectively Fig. 2 and 3.

In PLA grafted with Acrylamide (PLA-g-AAm) by irradiating with electron beam (Fig. 2) shows new shoulder at band 1667cm^{-1} starting at dose 20k Gy and becomes new peak as dose increased. This new peak is correspond to C=O (amide) [10, 11]. FTIR results showed AAm began to grafting onto PLA surface at 20 kGy and grafting yield increased as dose increased. After 60 kGy, band C=O amide is getting wider while band 1747cm^{-1} less visible. This probably because C=O amide grafted is higher than C=O from PLA.

Fig 3 shows IR spectrum of PLA-g-AAm via γ-rays where shoulder can be observed at 1731 cm^{-1} starting dose 20 kGy. The shoulder however shows no much different as dose increased up to 100 kGy. This probably due to low dose rate of γ-rays which is 0.028 kGy/minute resulted in low grafting obtained (Fig. 3).

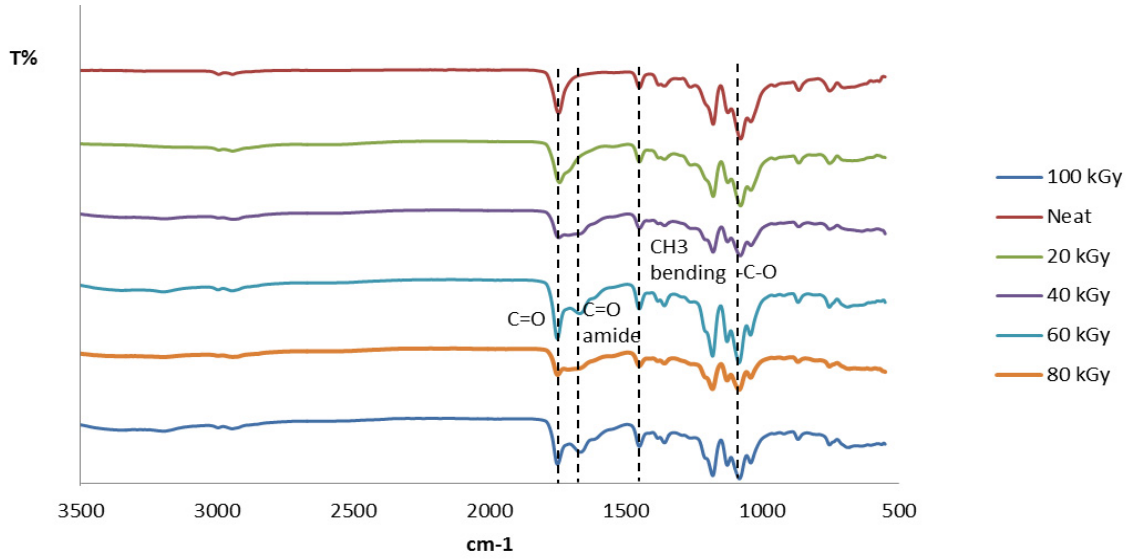

Fig. 2. PLA-g-AAM via electron beam irradiation at dose 20, 40, 60, 80, 100 and 120kGy.

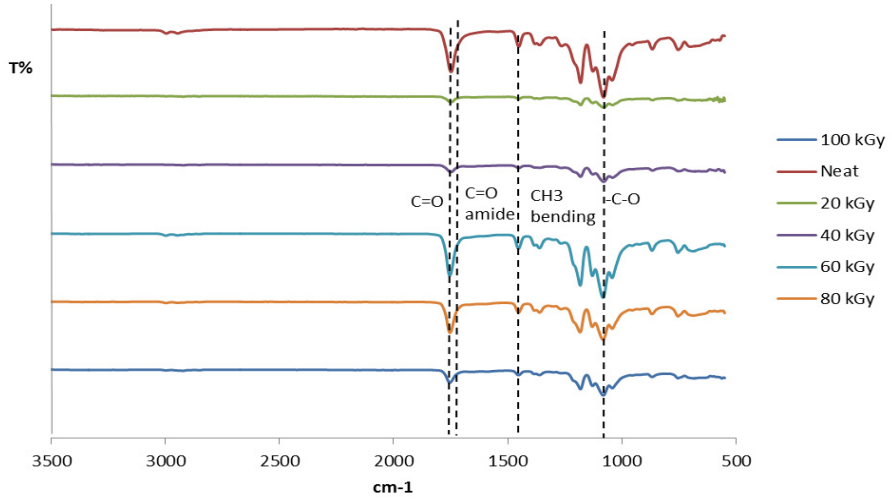

Fig. 3. PLA-g-AAM via Gamma γ- rays at dose 20, 40, 60, 80, 100kGy.

Fig. 4 shows spectrum of electron beam and γ-rays at dose 100 kGy with different monomers. Similar pattern was observed for PLA microspheres irradiated without any monomers via electron beam and γ-rays. However, the intensity of peak 1747 cm^{-1} is higher for irradiated PLA microsphere without any monomer as compared to neat PLA.

Different peaks can be observed for each PLA grafted monomers spectrum via electron beam irradiation. See Fig 5. For PLA-g-AA spectrum, shoulder shown at 3600 cm^{-1} corresponding to OH and peak exist at 1707 cm^{-1} corresponding to band C=O[13]. For PLA-g-AAm, two small peaks of NH (amide) band detected at wavelength 3192 and 3075 cm^{-1} and 1667 cm^{-1} correspond to C=O (amide)[10,11]. For PLA-g-MAH, in the region of 1840-1720 cm^{-1}, two separated C=O bonds are expected to be found assuming that the anhydride functionality is grafted. However, only one C=O band is detected at 1707cm^{-1} as a shoulder and this may indicate that MAH was covalently bound to the surface of PLA microsphere since the absorption bands in the carbonyl frequency range of the anhydride (1650–1850 cm^{-1})[14]. It shows that AA were easier to be grafted on PLA microsphere compare to MAH using irradiation probably because MAH is less responsive to radiation than AA[15].

No new peaks can be observed when PLA grafted with any monomer via γ -rays at dose 100 kGy (Fig 5). Only shoulder can be seen at 1731 cm^{-1} although samples were irradiated at the same dose with electron beam shows new peaks. This might relate to different energy between these two radiations where more monomers were grafted via electron beam because it produces higher energy than γ -rays. AAm at different concentration which is 20 % (w/v) and 50 % (w/v) were grafted onto the PLA microsphere and compared with FTIR. Results demonstrate different intensity at peak 1747 cm^{-1} where AAm with 50 % (w/v) shows higher intensity. This indicates successful of grafting also depends on the concentration of monomers[16].

FTIR shows crosslink happened at lower dose for electron beam irradiation but only at higher dose for γ-rays. This could probably due to dose rate of γ -rays emitted by Cobalt-60 are low in comparison with the high energy electron beams[17, 18].

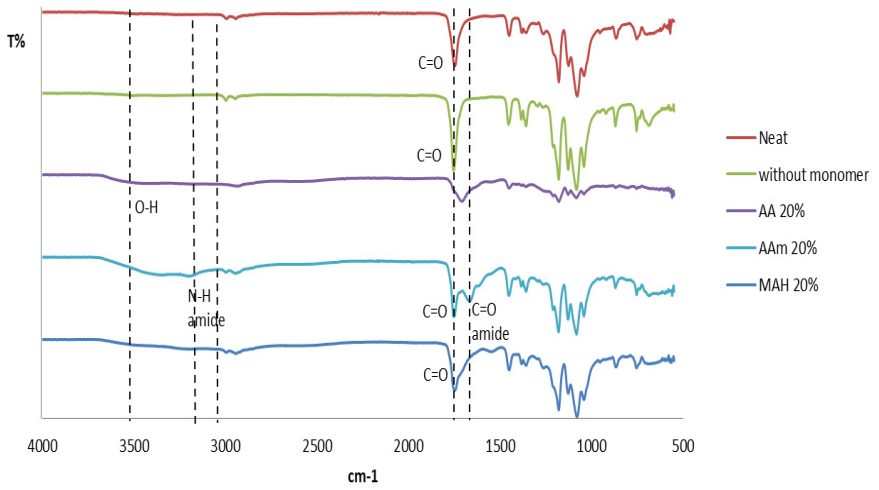

Fig. 4. Neat PLA, irradiated PLA without any monomers, PLA-g-AA, PLA-g-AAm and PLA-g-MAH at dose 100kGy via electron beam.

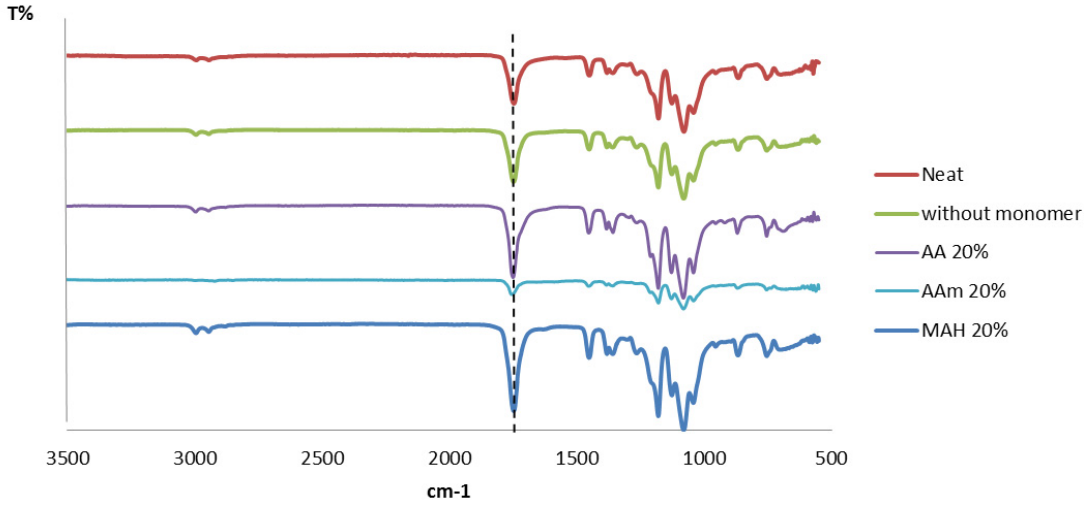

Fig. 5. Neat PLA, irradiated PLA without any monomers, PLA-g-AA, PLA-g-AAm and PLA-g-MAH at dose 100kGy via γ- rays.

3.2. Particle Surface Morphology

Morphology of PLA microsphere before and after grafting was observed under SEM at 100 times magnification to see how it affected the morphology. Surface of neat PLA is smooth[11, 12] as be seen in Fig. 6(a).

After irradiated with electron beam at 100 kGy, the PLA microsphere surface seems rough (Fig. 6(b)) while surface of microspheres after grafted with AAm using the same irradiation dose by electron beam seems improved and roughness is reduced (see Fig. 6(c)). These results corroborate FTIR results where new peaks are observed. Surface of microsphere irradiated with γ-rays at dose 100 kGy is similar to neat PLA resemble FTIR results where spectrum of PLA microsphere irradiated with γ-rays similar to neat PLA, although SEM image shows random small holes on the surface (Fig.6 (d)) this might results of air humidity during the fabrication.

There is a difference between PLA irradiated with and without monomer where the holes are no longer visible after irradiated with AAm indicating good surface coverage of AAm onto PLA microspheres. Not much difference can be observed between PLA-g-AAm using different irradiation source i.e electron beam and Gamma rays at 20 kGy (Fig. 6 (c) and (e)) as microspheres surface has been coated. SEM images Fig 6(f), 6(c) and 6(g) were compared where images PLA-g-AA, PLA-g-AAm and PLA-g-MAH respectively irradiated with electron beam at 100 kGy. All images shows PLA grafting with monomers still has same sphere shapes but with rough surface[11, 19] although the difference texture of grafted from one monomer to another cannot be distinguished.

SEM images support the FTIR results where all monomers are successfully grafted onto PLA microspheres surface. The topography could be further examined with Atomic Force Microscopy (AFM) to obtain an even more detailed analysis of the surface.

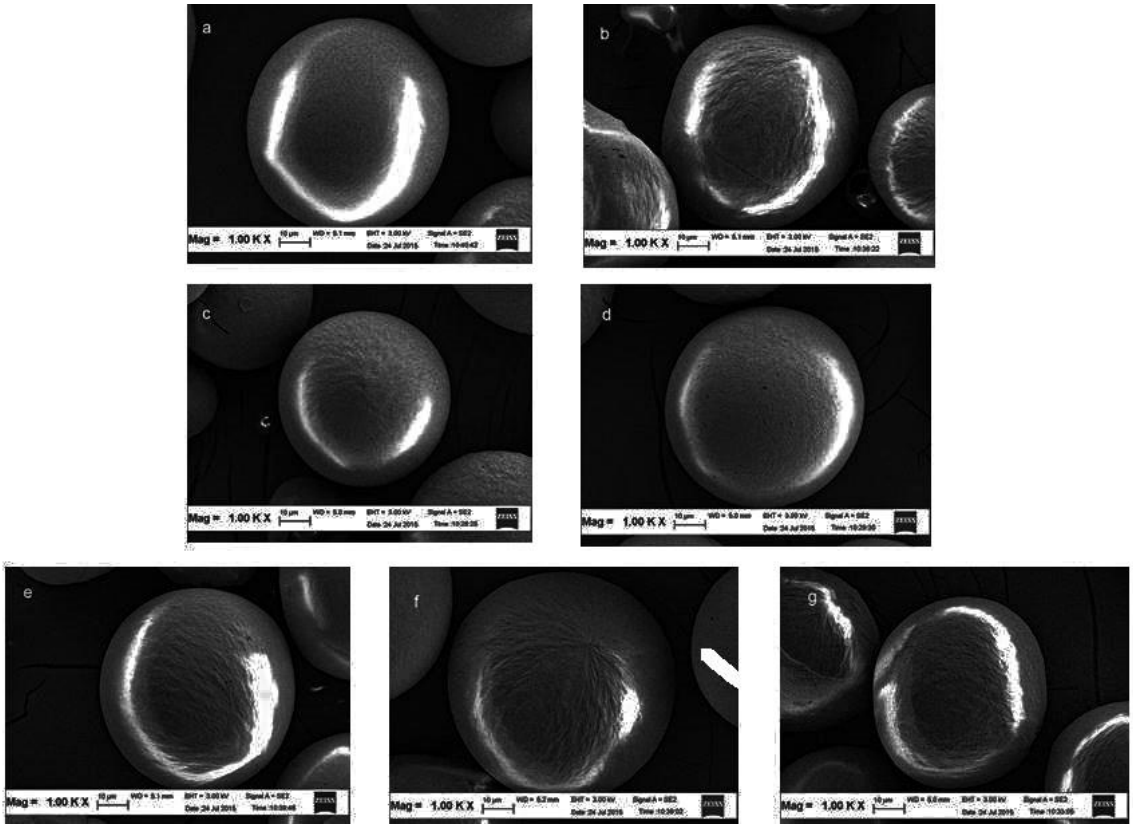

Fig. 6 . Morphology of irradiated and unirradiated PLA microspheres using SEM. (a) Neat PLA ;(b) PLA irradiated via electron beam at dose 100kGy ;(c) PLA-g-AAm irradiated via electron beam at dose 100kGy ;(d) PLA irradiated via γ-rays at dose 100kGy ;(e) PLA-g-AAm via γ-rays at dose 100kGy ;(f) PLA-g-AA PLA irradiated via electron beam at dose 100kGy ;(g) PLA-g-MAH PLA irradiated via electron beam at dose 100kGy.

4. Conclusion

FTIR and SEM results showed hydrophilic monomers was successfully grafted on the PLA samples. Surface modification via irradiation depends on dose and dose rate of the radiation. Degree of monomer grafted increased as dose increased and electron beam shows higher grafting compare to γ-ray because it possess higher dose rate.

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