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Control of shape and size of poly (lactic acid) microspheres based on surfactant and polymer concentration

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ABSTRACT:

Biodegradable, poly (lactic acid) (PLA) microspheres have been synthesized via solvent evaporation method using ultrasonic homogenizer as emulsifier. The effect of added surfactant, PVA [poly (vinyl alcohol)], and PLA concentration on the shape and size of the resultant PLA microspheres has been studied. The prime objective of this work was to identify an optimal PVA/PLA concentration to prepare PLA microsphere with a size distribution of 1 to 2 micrometers as in many literature it has been reported as ideal range of size for non-endocytosis-mediated cellular drug delivery. In this pursuit it was found that an optimal concentration of 2.5% w/v of PVA into water and 2.5% w/v PLA into DCM [Dichloromethane] is suitable to create PLA microspheres with a size distribution of 1 to 2 micrometers. The PLA microspheres were characterized by FESEM to assess their shape and size. FT-IR analysis (Supplementary data) was used to assess the functional groups present in the PLA microspheres, whilst zeta-potential measurements (Supplementary data) provided insight into the likely dispersion properties of the microspheres in aqueous media.

KEYWORDS:

Poly (lactic acid) (PLA), Microspheres, Probe-based Ultrasonication, FESEM, FT-IR Spectroscopy

1- INTRODUCTION:

Cellular-level drug delivery techniques offers efficient and safe drug disposition in the affected tissues and thus provide a more suitable way to cure disease by selective drug delivery approaches [1].Recently, polymer microsphere-based intercellular delivery, termed beadfection, has been shown to be a facile and highly promising cellular-level drug delivery platform [2]. The reason why microspheres form such an attractive delivery mechanism is principally because nano-sized drug delivery vehicles and labeling agents are known to induce size dependent toxicity [3] in part due to their high surface area to volume ratio. On the other hand a number of recent reports have shown that cells are capable of engulfing larger particles by a non-endocytosis mediated mechanism in a process termed as beadfection [4, 5]. This discovery makes it possible to use micron-sized particles to deliver drugs and/or bio-imaging agents into cells. These reports suggest that microspheres with the size range of 1 to 2 micrometers are optimally sized for non-endocytosis-mediated, cellular level drug delivery [6].

There are many techniques that have been developed to convert polymers into uniform polymeric microspheres. Out of those techniques the solvent evaporation method is arguably the most simple to employ and is also readily scalable. This method is based on efficient emulsification of two different phases using a homogenizer. Among various homogenizing techniques [7] [8], use of ultrasonic homogenizer is preferable as it is relatively simple and significantly reduces the time of microsphere synthesis. Despite this, to date there are no significance efforts have been seen on the size and shape modulation of the microspheres generated using this technique.

Herein we report a systematic study that evaluates the effect of polymer: surfactant ratio on the shape and size of the resultant polymer microspheres. Such a study is vital as uniformity in size and shape of the microspheres is critical for applications like cellular level drug delivery assay such as

beadfection, where relative amounts of delivered drug are clearly essential. This article describes the synthesis of poly (lactic acid) microspheres, produced using a variety of reaction conditions, the identification of the optimal reaction conditions to generate microspheres and characterization of the morphology of the resultant microspheres in aqueous media. Specifically FESEM analysis was used to assess particle size and shape, FT-IR spectroscopy (Fig. S1) provided insight into microsphere chemical functionality whilst zeta potential measurements (Table S1) gave an insight into solvent accessible surface charge and thus likely aqueous compatibility of the microspheres, key for cell-based applications.

2- EXPERIMENTAL SECTION:

2.1 - Materials

Poly (lactic acid) (PLA) (MW 150, 000) was purchased from Sigma Aldrich, Poly (vinyl alcohol) (PVA) (MW160, 000) and Dichloromethane (DCM) (MW 84.93) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Millipore water was taken from laboratory Elix water purification system.

2.2- Microsphere Preparation Using Emulsion Solvent Evaporation Technique

The principle behind this method is the emulsification of a polymeric solution in a continuous phase. Accordingly, two phases are required in this fabrication procedure (Fig.1). Firstly, an organic phase was prepared by dissolving various concentrations of PLA in DCM (10 mL) and stirred for about one hour. The second aqueous phase was prepared by dissolving different concentrations of PVA in water (20 mL); dissolution was achieved by stirring the mixture at 90^{o}C for 2 hours using a magnetic stirrer [9, 10].

An emulsion was then formed in a two-step process. In the first step, an aliquot of the PLA-containing solution (1 mL) was added slowly to an aliquot of the PVA-containing solution (2 mL). The PVA-containing solution was mixed continuously at room temperature using a vortex mixer

during the addition of the PLA-containing solution. Due to high speed vortex mixing an emulsion started to form, the vortex mixing (SPINIX vortex mixer) was continued for a further 10 minutes. Subsequently, to reduce the size of the PLA particles, the emulsion was immediately transferred to an ultrasonic homogenizing unit (SONICS vibra cell- 20% amplitude with 3 second pulses).After which the emulsion was stirred at room temperature for a further 3 hours using a magnetic stirrer for self assembly and evaporation of residualorganic solvent. The microspheres were then isolated by centrifugation (Eppendorf Centrifuge 5810 R-6000 rpm for 15 minutes) and decantation and then washed three times with distilled water to remove the PVA surfactant .The final product was stored in aqueous solution in refrigerator at 4°C for further characterization.

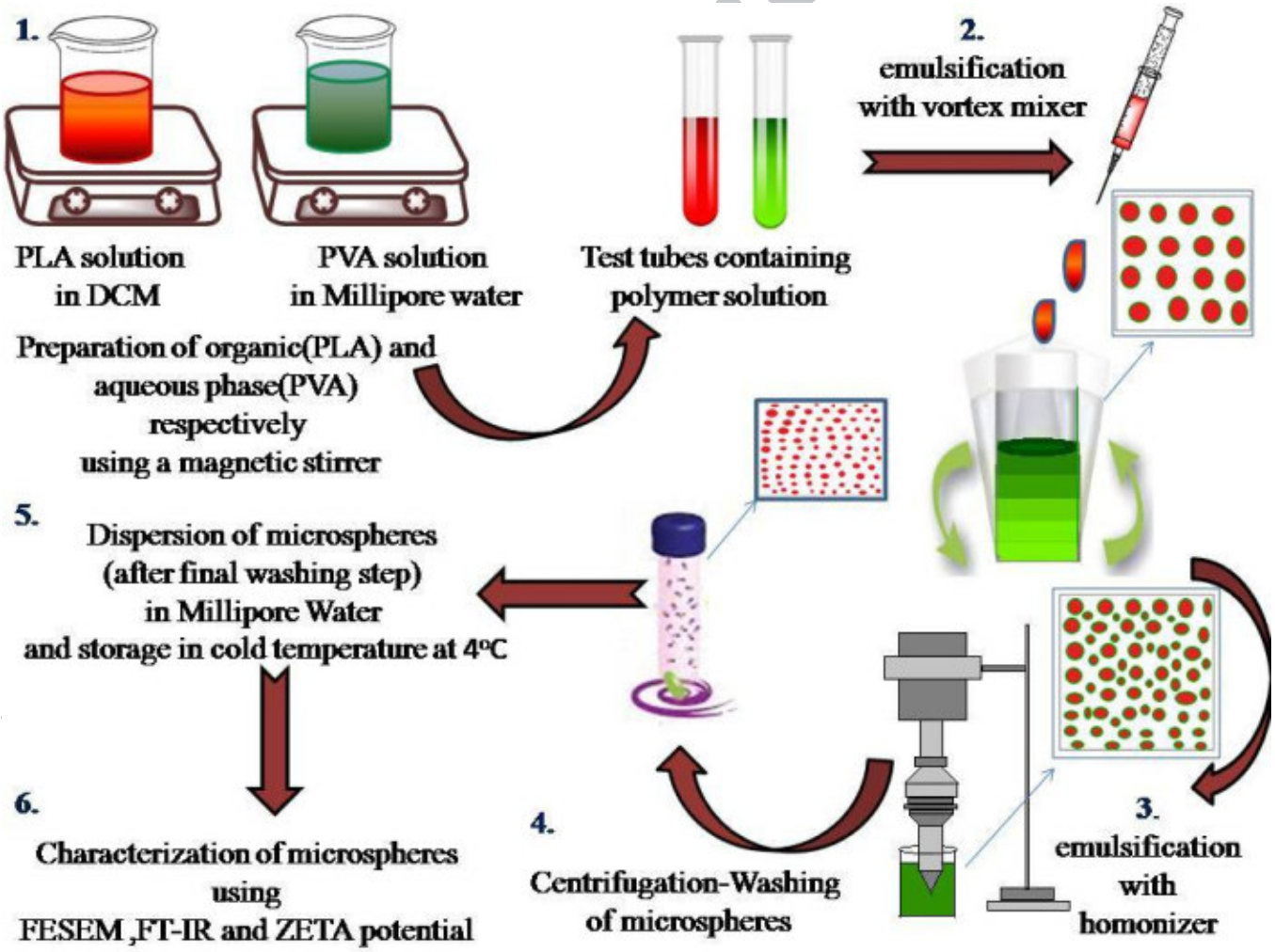

Fig. 1. Schematic representation of fabrication process of PLA microspheres

3- RESULTS AND DISCUSSION:

In preliminary experiments different combinations of PLA and PVA concentrations, ranging from 1% to 2.5% (w/v), were evaluated.After each synthesis the resultant microspheres were characterized in terms of their shape and size by FESEM. This study enabled selection of the ten best concentration combinations. To evaluate reproducibility of the method the procedure was repeated with just those ten concentration combinations and again the microspheres were evaluated by FESEM. In this way, four concentration combinations, that showed the best results in terms of shape and size and reproducibility, were identified. These four optimum concentration combinations are shown below in Table 1.

Table.1. PLA and PVA relative concentration chart

Sample no.	PLA conc. (w /v %)	PVA conc. (w /v %)
1	1.0	1.0
2	2.5	2.5
3	1.5	0.5
4	1.5	1.0

3.1- FESEM analysis

FESEM analysis is an ideal technique to analyze the shape, size variation of the microspheres produced by the emulsion solvent evaporation technique. Accordingly, FESEM was used to assess

the effects of PLA/PVA concentrations on microsphere formation. FESEM images of all the samples listed in Table 1 are shown in Fig. 2. The microspheres in sample 1, produced with PLA: PVA concentrations of (1w/v %): (1 w/v %), are relatively smooth and almost spherical but they are quite large (Fig. 2a-c). Indeed the size distribution histogram (Fig. 3b) shows that the majorities of the microspheres have diameters in the range of 3.5 micrometers and are thus too big for beadfection applications. Microspheres in sample 2, produced with PLA/PVA concentrations of (2.5w/v %) :(2.5w/v %) also possess smooth outer surfaces and are almost spherical in shape (Fig. 2d-f). Importantly, for our intended applications the size distribution histogram analysis (Fig. 3d), revealed that the sizes of most of the microsphere are in the range of 1 to 2.5 micrometers. FESEM analysis of sample 3(Fig. 2g-i), revealed that the microspheres made with PLA: PVA concentrations of (1.5w/v %): (0.5w/v %), had rough surface morphologies with poroussurfaces than the microspheres in samples 1 and 2. Moreover they also deviated from the ideal spherical shape and their overall size distribution was rather small for desired beadfection applications. Indeed, the size distribution histogram analysis (Fig. 3(f)) showed the diameters of the microspheres were mostly in the nanometer range with an average diameter of about 850 nm. It may be suggested that the surface and size properties of the microspheres in sample 3 result from the unequal concentrations of PLA and PVA used in the fabrication process. Sample 4 where the concentrations again differ but this time not so greatly, PLA: PVA concentrations of (1.5w/v %): (1.0 w/v %) supports this possibility. The microspheres in sample 4(Fig. 2j-i), are a little larger than those in sample 3 and have an average diameter of roughly 750 nm (Fig. 3h). In terms of their morphology, again like the microspheres in sample 3, the microspheres in sample 4 have porous surfaces than the microspheres in samples 1 and 2. However they have fewer pores than the microspheres in sample 3. In other words the same trend in terms of properties is observed for sample 4 as it is for sample 3 but the extent of the deviations is less pronounced perhaps reflecting the fact that the difference in PLA: PVA concentration is less in the case of sample 4, (1.5w/v %): (1.0 w/v %), than it is in sample 3, (1.5w/v %): (0.5 w/v %). In Fig. 3, histograms error bars

represent the variability in the mean data of size distribution taken into consideration in three successive iterations.

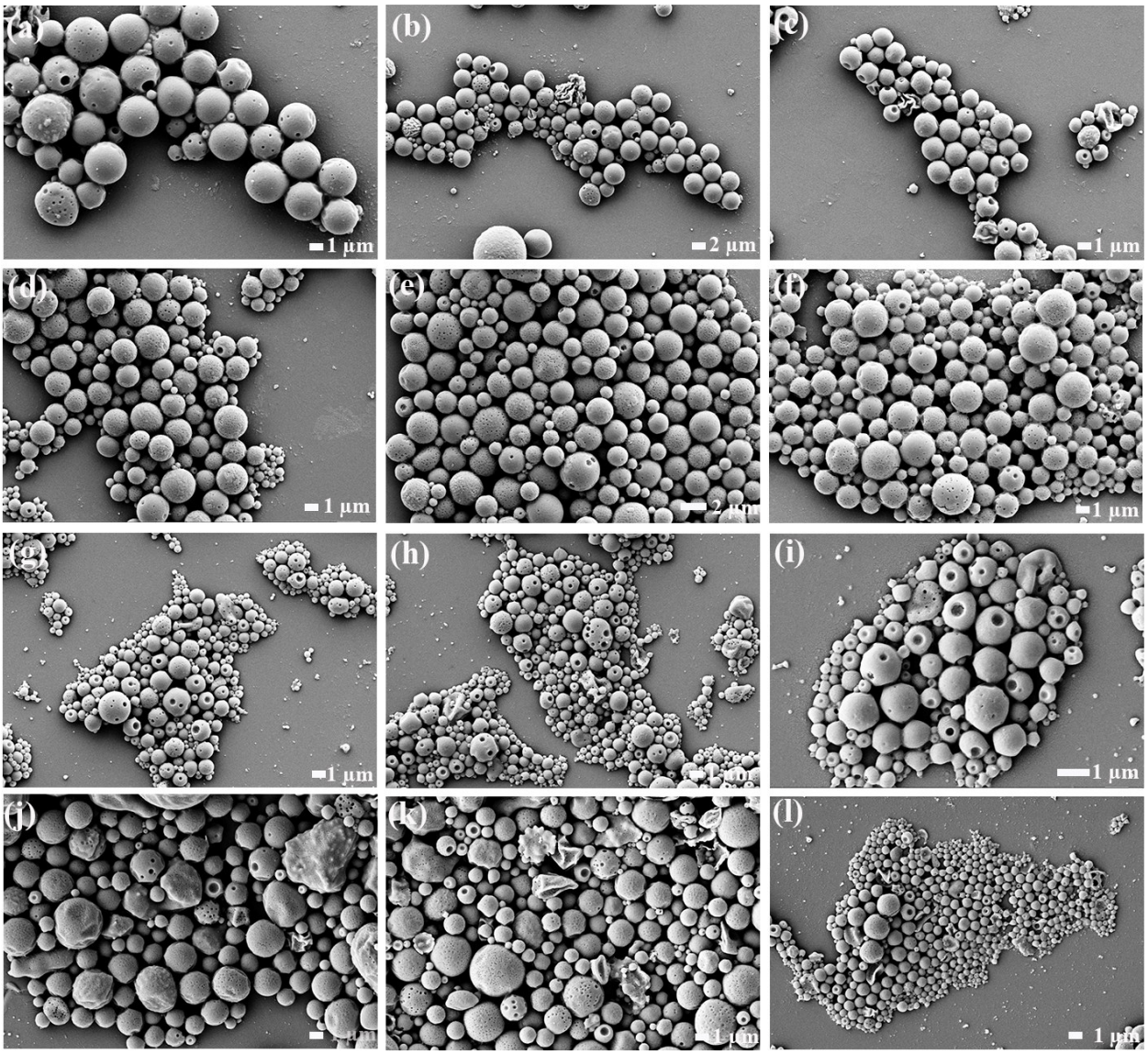

Fig.2. FESEM micrographs of PLA microspheres prepared from PLA/PVA solutions with various concentrations. PLA: PVA concentration (w/v%: w/v %); (a-c) 1.0: 1.0, (d-f) 2.5: 2.5, (g-i) 1.5: 0.5 and (j-l) 1.5: 1.0.

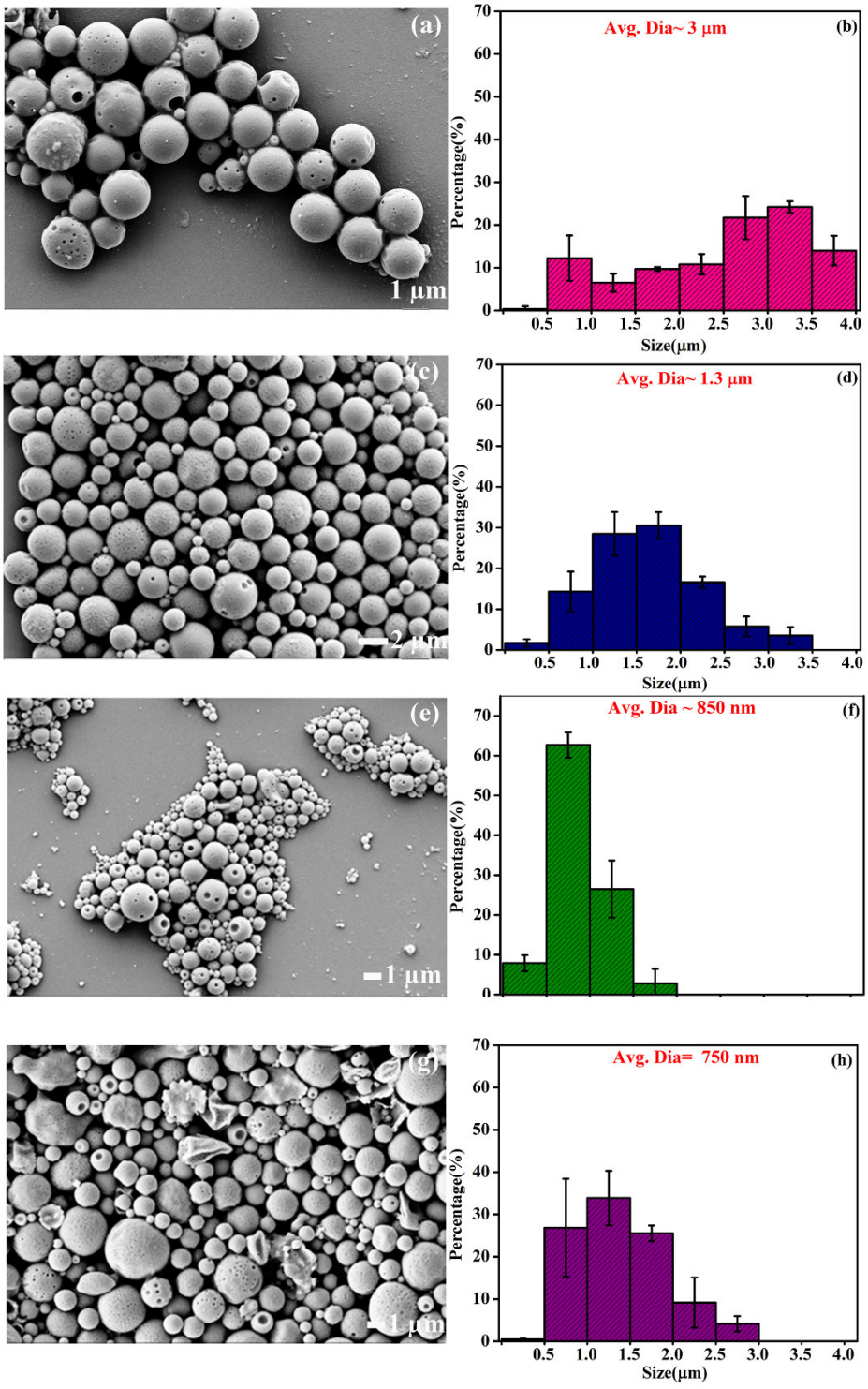

Fig.3. FESEM micrographs and histogram representations of the size distribution of microspheres fabricated with PLA: PVA concentrations (w/v %: w/v %); (a-b) 1.0: 1.0, (c-d) 2.5: 2.5, (e-f) 1.5: 0.5, and (g-h) 1.5: 1.0

4- CONCLUSION:

As for non-endocytosis-mediated drug delivery methods microspheres with diameters of 1-2 µm were found suitable, we have successfully demonstrated that 2.5 w/v % concentrations of both PLA and PVA, in DCM and water respectively, are ideal for preparing PLA microspheres of desired size range using emulsion solvent evaporation technique in conjunction with an ultrasonic homogenizer to generate spherical PLA microspheres. The particles were negatively charged and showed good dispersibility in aqueous solution.Moreover the protocol is readily reproducible giving virtually identical results a number of times. Currently, we are assessing the utility of PLA microspheres, generated using the same optimized ratio of PVA and PLA concentration, in loading with drug molecules and labeling agents for subsequent beadfection procedures.

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HIGHLIGHTS:

- PLA microspheres have been synthesized using ultrasonic homogenizer as emulsifier.
- The effect of surfactant on the shape and size of the microspheres has been studied.
- Optimal PVA/PLA concentration is used for desired size distribution of microspheres.
- FESEM revealed the successful formation of relatively uniform-sized microspheres.
- Zeta potentials showed good dispersion of synthesized microspheres in aqueous media.