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RESEARCH PAPER

Synthesis Very Fine PLGA Nano Particles Using Double Micro Emulsion: Effect of Different Parameters

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ABSTRACT

In this work PLGA copolymers with different high molecular weight and up to 95 per cent efficiency are economically synthesised using a novel ring opening as the heart of process and then with micro double emulsion technique in which nanoparticles size are below 60 nanometer is obtained. Conditions under that we were successful to such synthesis are discussed later. At first, characterisation tests are accomplished for all polymers/copolymers to choice the best sample to be thermo-stable enough and also have more stability in the human body and with higher molecular weight. Double micro emulsion method with very polar solvent propylene carbonate and a co-solvent PVA and DMAB as the main surfactant are used to produce nanoparticles. Some critical parameters like homogeniser speed and ultrasonic device to look into nanoparticles size, as well as surfactant type are explored whilst homogeniser worked from 5000 to 30000 rpm, surfactant was DMAB and ultrasound worked with 8 min at 75% amplitude sonicator. It is found that copolymer concentration and surfactant type affected sharply on the nanoparticles size. Meanwhile, show that ultrasound operation is not suitable to give fine nanoparticles, likely, due to high momently temperature rise in/around/surface the emulsion. For this reason a powerful homogeniser is employed so that very fine nanoparticles are reached under low temperature. Characterisation tests such as DLS and SEM verify fine size and show a vast particle size distribution with an average 40-60 nanometer particles which is very valuable through others work because of its fine particle size. Former works have been reported large PLGA nanoparticles (up to 150 nm), perhaps to, usage of low molecular weight polymer/copolymer or sonication condition. As another novelty is that we did not buy PLGA form any company but we synthesise/purify high molecular weight (above 1000000) type of copolymers according to 10 year of our experience in this field. This was the key of our success. Our next work would be focus on the drug delivery system with PLGA nano carrier and smart nanoparticles that would be reported later.

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INTRODUCTION

Nowadays, PLGA copolymers are considered extensively in the world in different application. For instance in clinical application, drug delivery systems and so on but often researchers reached large size nanoparticles which is not appropriate for drug delivery in the human body[1]. Meanwhile, none of them can produce high molecular weight PLGA and they almost purchased PLGA samples from some company expensively because synthesis of samples is very difficult and time consumer [1-4]. This causes low molecular weight to be available for nanoparticles produce that results in a large and inconclusive size particles, for example 300-400 nanometer [4-6]. This size of particles cannot be injected into the body straightly, especially in the blood. Precipitation and agglomeration and vein/capillary block of these particles are dangerously observed several times. Then, a particle distribution below 100 nanometer and with high molecular weight (up to 100000) nanoparticles is necessary. Anyway, several researches about PLGA nanoparticle synthesis are reported in the past decades [3, 5 and 7]. All of them bought PLGA samples with low molecular weight which is unsuitable for drug delivery due to fast degradation in the body. Certainly, low molecular weight polymer/copolymer results in large nanoparticle size [8]. Several methods are used to synthesis nanoPLGA particles in which just double emulsion method results lower size [9-10]. Also, several method exist to synthesise PLGA inter alia ring-opening obtains the best molecular weight [10 and 11]. We use this method for synthesising and achieve high molecular weight random copolymer/polymer for deal with drug delivery or something else. Recently encapsulation of drug through PLGA has been interested for researcher [12]. Of course with a large size up to 100 nanometer. Finer PLGA particles, more efficient for drug release and more permanence are reported [13]. Much effort has been done to decrease particle size with different method [14-16]. It is clear that double emulsion evaporation method represents a significant finer particle but yet up to 100 nanometer (for instance up to 297 nm) [17]. Recently, PLGA has been used for dug carrier and cancer therapy, so for brain cancer the size of carrier must be below 100 nm to diffusion into cells [18]. They were able to reproduce PLGA drug loaded but by an average range of 138-180 nm. Dang et al. use PLGA capsules for cancer therapy whilst Nano capsules were larger than 100 nm [19]. Beside, several researchers try to encapsulate different drug into PLGA empty capsules once more with higher size than 100 nm [19-23]. Also coated PLGA nanoparticles with chitosan for cellular uptake evaluation and cytotoxity are reported in which cellular uptake was disappointing [26]. Effect of surfactant whether type or concentration as well as micelle formation are studied [27]. Several parameters are effective to obtain satisfactory results that in this article some of them are investigated [14-28].

We emphasise that this problem would be solvable by higher PLGA molecular weight and novelty in the micro emulsion method for example usage of additional ion/counter ion into the solvent or low temperature or good PH selection. In this work fine PLGA nanoparticles are produced via double emulsion method with cationic surfactant DMAB and additional ion. The main novelty of this research stands for rendering a new method of synthesising PLGA very fine nanoparticle (below 100 nm) to be suitable for drug delivery aspects and also investigating critical parameters affect on the size. For the first time, we report this process from zero until 100 per cent i.e. making proper native PLGA for tests, instead buy from companies.

MATERIALS AND METHODS

Propylene carbonate, PVA and toluene were all from Merck, Germany, Deionised water from Ripi. DMAB was from Sigma USA and Lactic acid and Glycolic acid purchased from Fluka. Antimony three oxide Sb_2O_3 was from Merck.

At first Lactic acid and glycolic acid with catalyst Antimony three oxide in the reactor made as set up dehydrates (physical water) at 100 °C and under nitrogen atmosphere and then chemical water are separated at high temperatures (200-280°C). Table 1 shows the conditions to create all samples. This table gives approximately the best samples with experimentally maximum molecular weight as well as mechanical resistance. The best sample is shadowed/intercalated in the table due to characterisation test results.

Fig. 1 show our set up for polycondensation and also for dimerisation processes. All steps incorporated in the Table 1 completely experimentally become tested by this set up. The set up, as one can see in Fig. 1, is designed multifunctional. At first for making polymer/copolymer with low molecular weight, secondly

for dimerisation process and tertiary for ring opening procedure and reaching high polymer/ copolymer molecular weight. Temperature inside reactor (Pyrex glass with 6 mm thickness and 600 °C resistance) become controlled in four separated zones and data logged at time interval below 10 second in which a control automatic close loop with a delay offset near ±2 second designed. A double pipe heat exchanger is used to cold samples or dimers. A mixer made in Germany hermetically seals up the reactor gap in the above with the help of high silicon grease and work with a speed of 60-120 rpm controlled manually. There is no consequential reason for selection of time and temperature. In fact, just these numbers become experimentally selected due to results on the best sample with higher mechanical resistance.

The separation process (dehydration) has efficiency higher than 95 per cent for all samples. The efficiency is calculated by:

$$\frac{(W_{theory} - W_{weighted})}{W_{theory}} * 100 \geq 95 \text{ for all cases}$$

Table 1. Steps to synthesis samples by polycondensation.

	Stage No.							
	1		2		3		4 (vacuum)	
Polymer/Copolymer	t (min)	T (°C)	t (min)	T (°C)	t (min)	T (°C)	t (min)	T (°C)
PGA	240	120	120	180	240	140	960	50
PLLA	120	150	300	175	90	190	960	50
25PGA-75PLLA	30	120	60	150	330	180	540	170
50PGA-50PLLA	60	120	300	170	30	190	600	170
75PGA-25PLLA	130	130	170	180	60	190	720	170

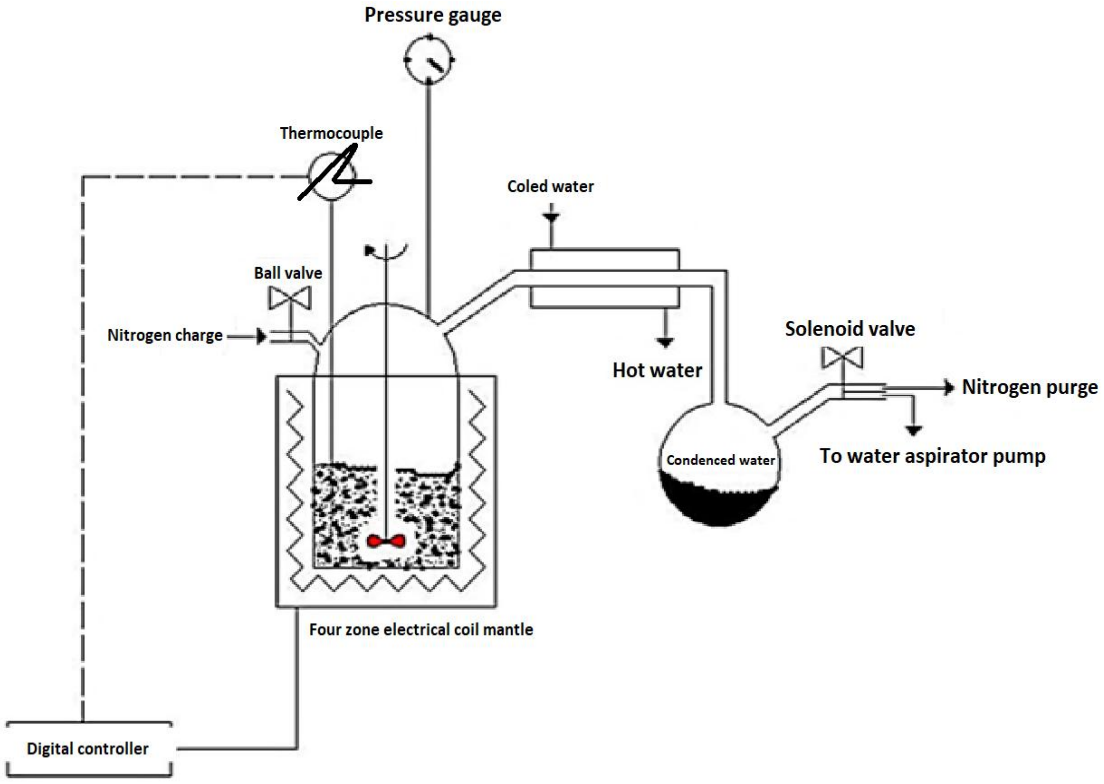

Fig. 1. Multipurpose set up.

Free water plus to chemical water give us w_{theory} and weighted water separated become $w_{weighted}$. Fig. 2 shows a schematically diagram for example for PGA sample.

Results for other either polymer or copolymer are similar except for times and temperature reported in Table 1. After sample preparation and purification in ethyl acetate or toluene (ethyl acetate purchased from an Iranian company), demerisation process in the set up get started by sample loading, i.e., PLA and PGA so far. Dimers of these two samples are purified via 7 steps with recrystallisation in toluene/ ethyl acetate step by step.

Dimers with different weight per cent are loaded into reactor under vacuum atmosphere plus 1 pill per 100 gr of Sn(II)-2-ethyl hexanoate as catalyst for ring opening process(was from Sigma USA), which is orally legal to eat according to FDA enouncement under adapted temperature

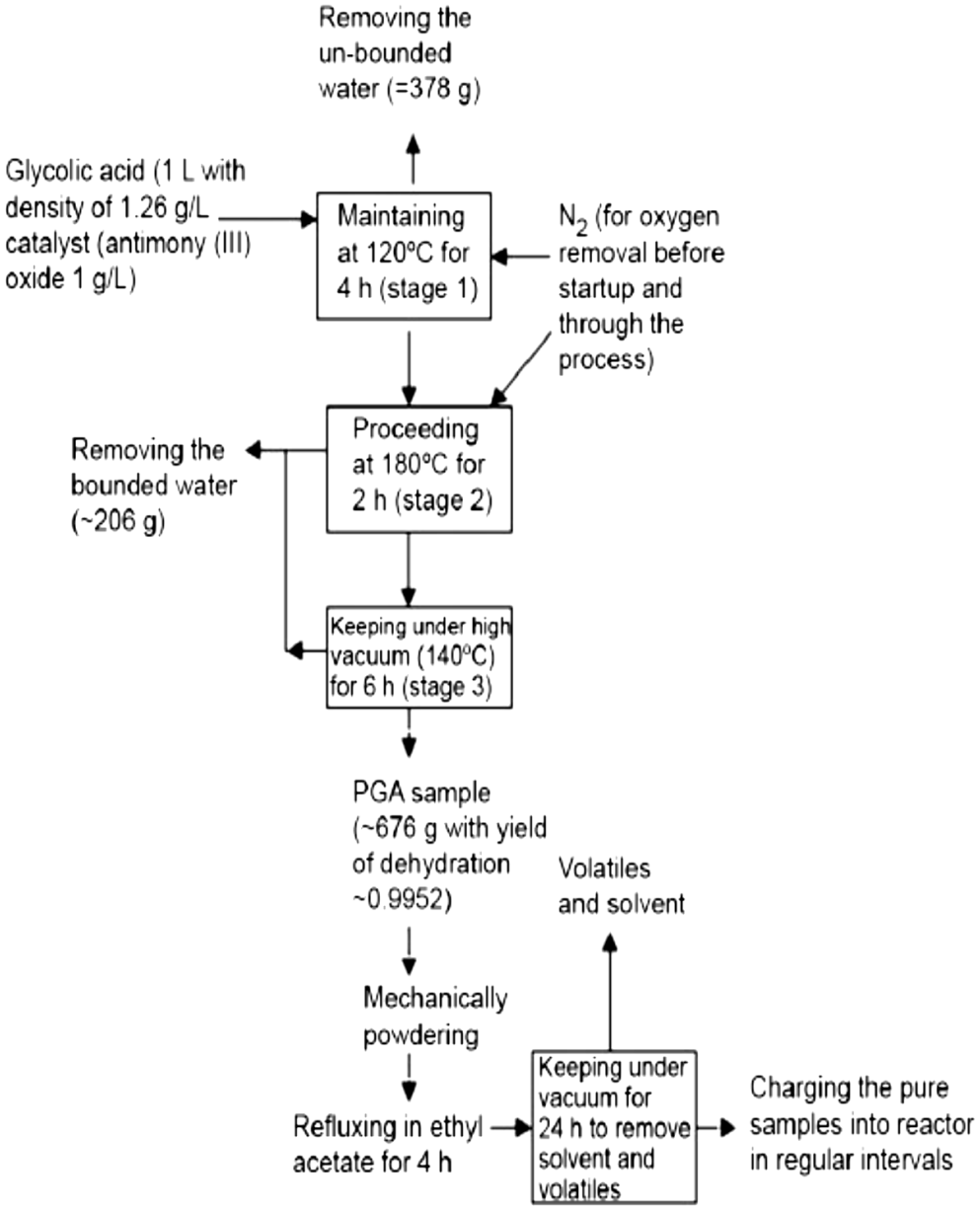

Fig. 2. Schematic diagram for PGA polycondensation.

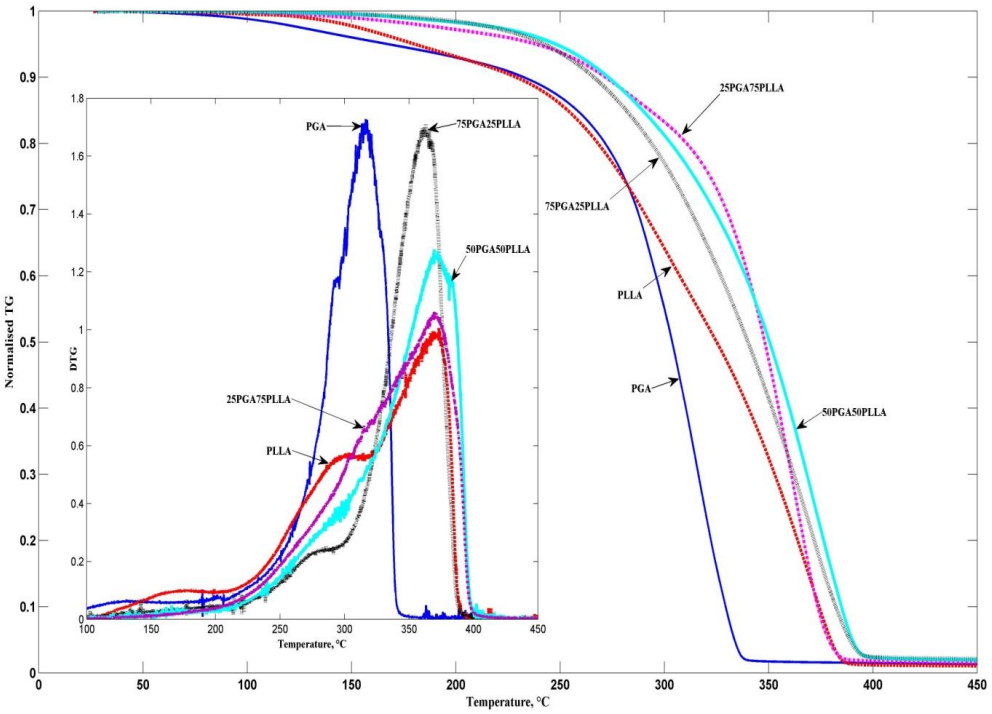

Fig.3. TGA and DTG analysis.

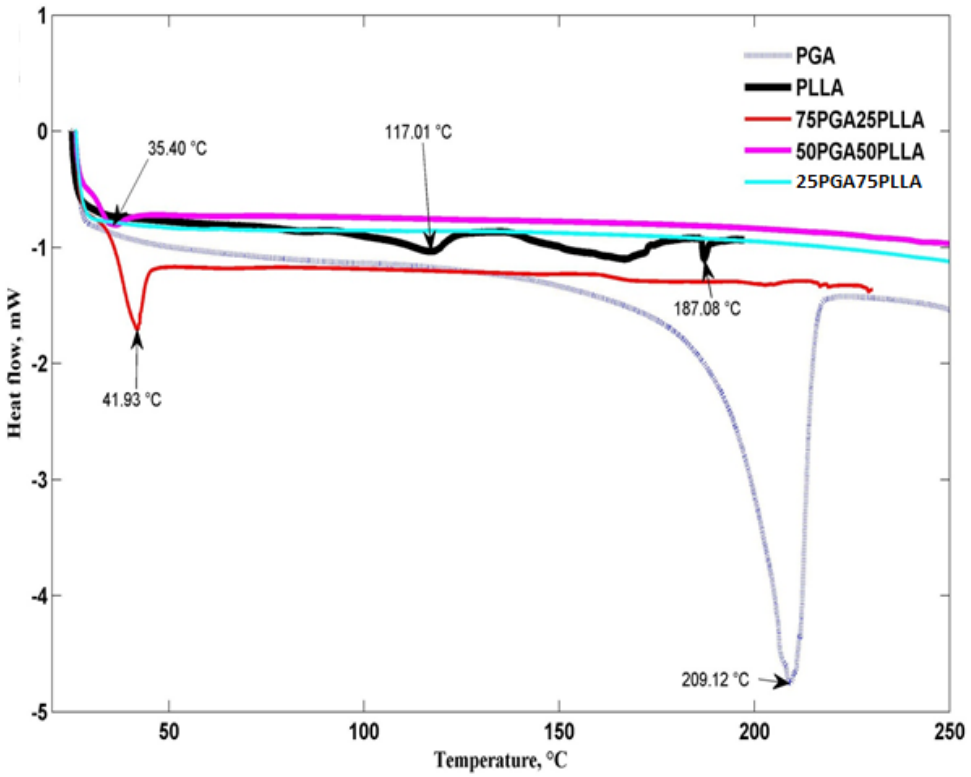

Fig. 4. DSC test for all samples.

at 150 ˚C. Copolymers/polymers obtained are characterised by TGA and DSC tests.

RESULTS AND DISCUSSION

Fig. 3 showing TGA and DTG characterisation analyses for all samples showing resistance against temperature. Temperature range was between 60-400 ˚C under helium atmosphere.

According to Fig. 3 it is transparent that 25:75 PLGA copolymer is resisted against temperature rise more than other samples. In this manner DSC results is very important because the sample candidate for nano sphere production has not to show crystal temperature about body

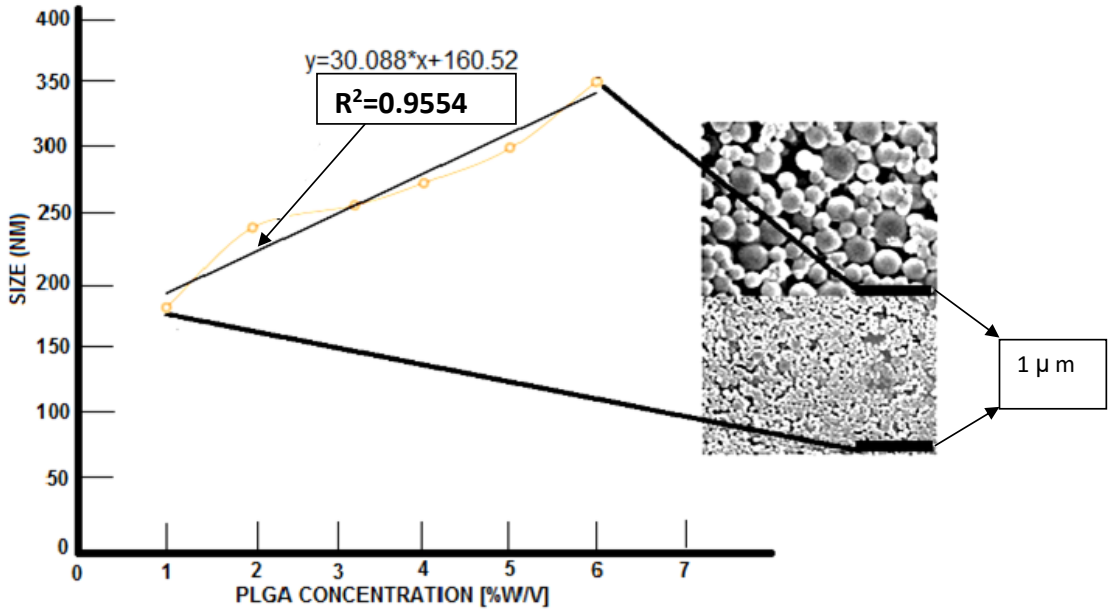

Fig. 5. PLGA concentration versus average size (left). SEM (up 1 per cent per lit, bottom 6 percent per liter), scale bar: 1 micro meter (right).

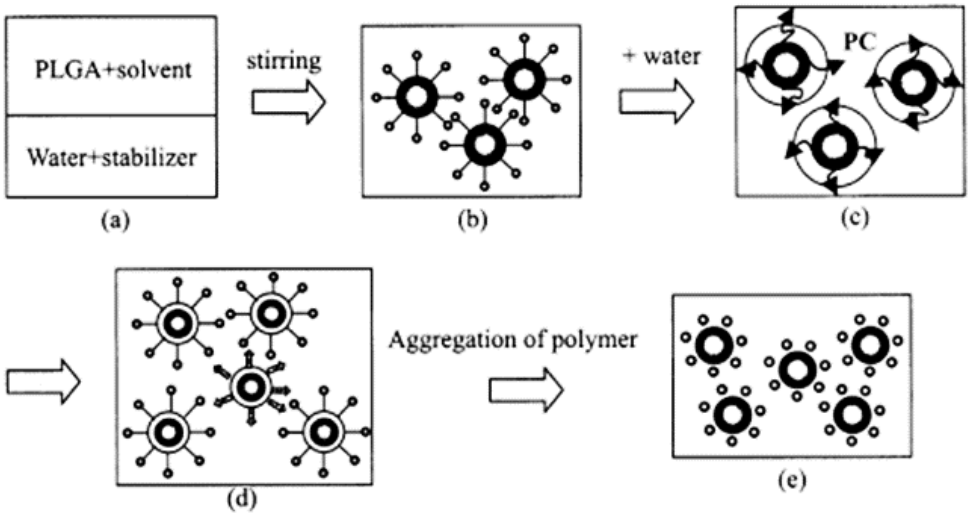

Fig. 6. Micelle of surfactant and nano sphere for polymer precipitation schematically.

temperature. Result of DSC test (Fig. 4) show this sample has not crystal temperature.

Copolymer 25:75 PLGA sample has not crystal temperature by chance and for this reasons it is the main candidate for nano sphere synthesis procedure as also reported in [14] although the samples are similar to [14] but they are different from them by repetition process.

Double emulsion techniques are used for producing 25:75 PLGA nanoparticles as presented completely in the following. The size desirable for us remains below 100 nm nanoparticles although higher sizes nano PLGA in literature have being reported [15-17]. As a merit, in this work nano particles below 100 nanometers will be reached to be suitable for encapsulation in the future and next works. In the first step we make two non-solvable phases by adding copolymer to propylene carbonate. Then surfactant DMAB and co-solvent PVA conducted with little amount of water for making double emulsion are injected into emulsion under a powerful homogeniser (5000 to 30000 rpm mesonyx USA) for a few minutes. In the following, some water is added into the double emulsion. This causes solvent (propylene carbonate) by a good diffusion becomes displaced due to higher solubility in water. So, here we have many amount of PLGA precipitating with time over. Nano spheres are separated with the help of a centrifuge (4000 rpm) for 8 hours, rinse with deionised water and preserve at -22 °C to protect from hydrolysation/oxidation and thermal degradation and so forth [14]. Some characterisation tests are accomplished to verify nano sphere size and other significant parameters. First of All, effect of copolymer loading on the size of nano spheres is depicted in Fig. 5, showing in low concentration of PLGA, the curve is linear with an R^2 of 0.95. Also it seems clearly that, the more polymer usage, the more particle size. For decreasing the size we used high homogeniser speed (5000-30000 rpm). It is found that this parameter is more effective than others to control on the nanoparticle size.

Surfactant concentration after CMC point is very vital because nano reactor be continuing toward

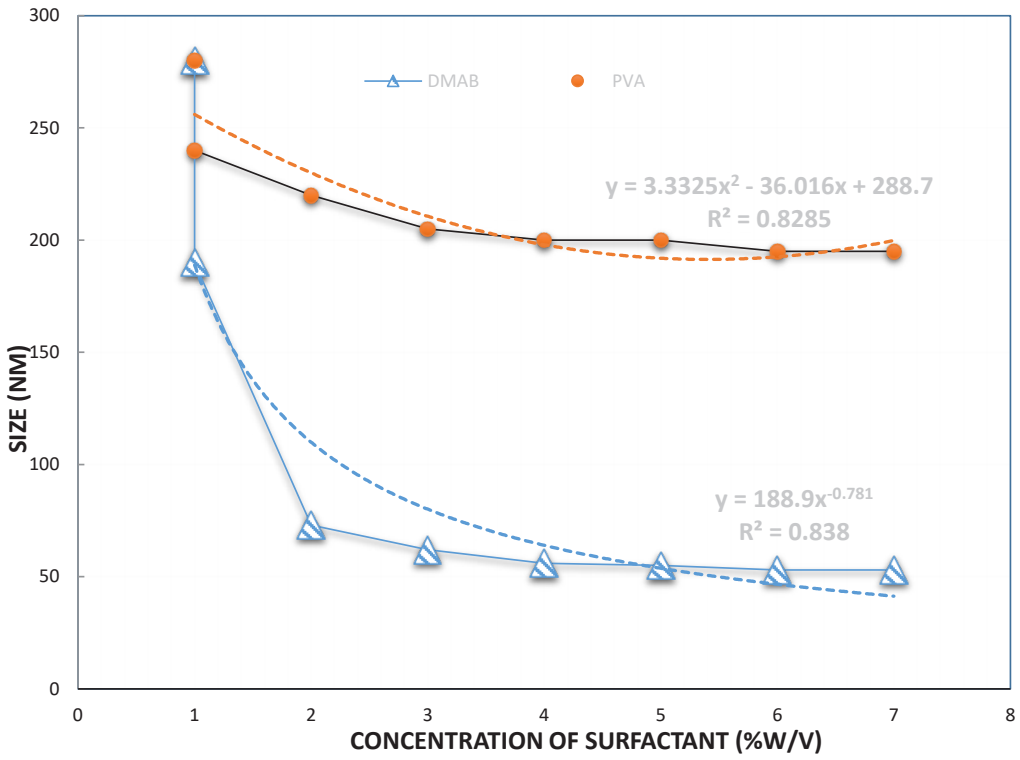

Fig. 7. Effects of type and concentration of surfactants versus average size nano spheres.

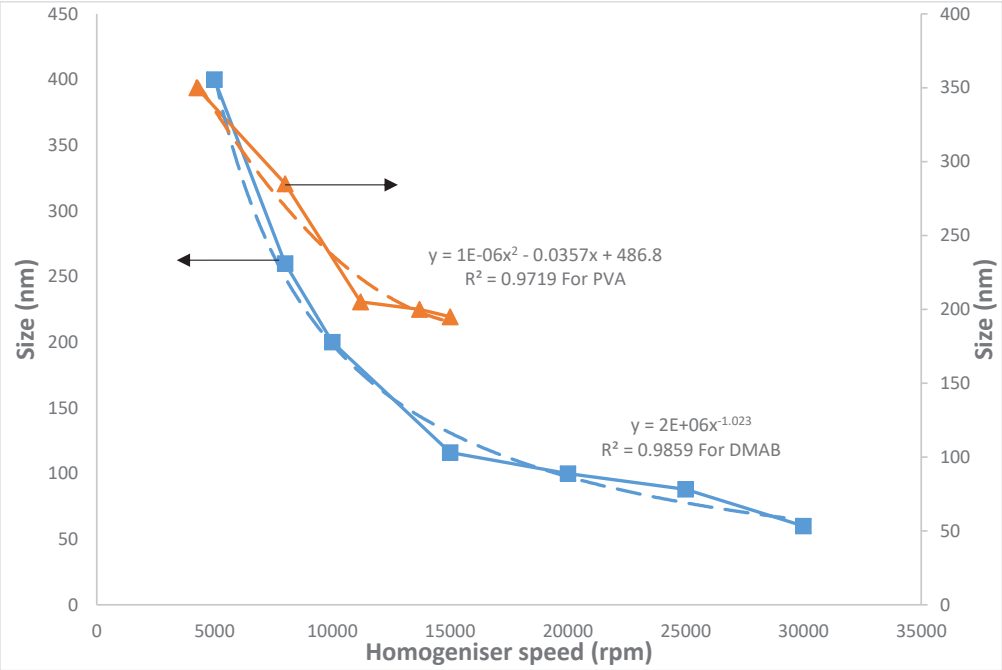

Fig. 8. Effects of surfactant type and speed of homogeniser on the nano spheres size.

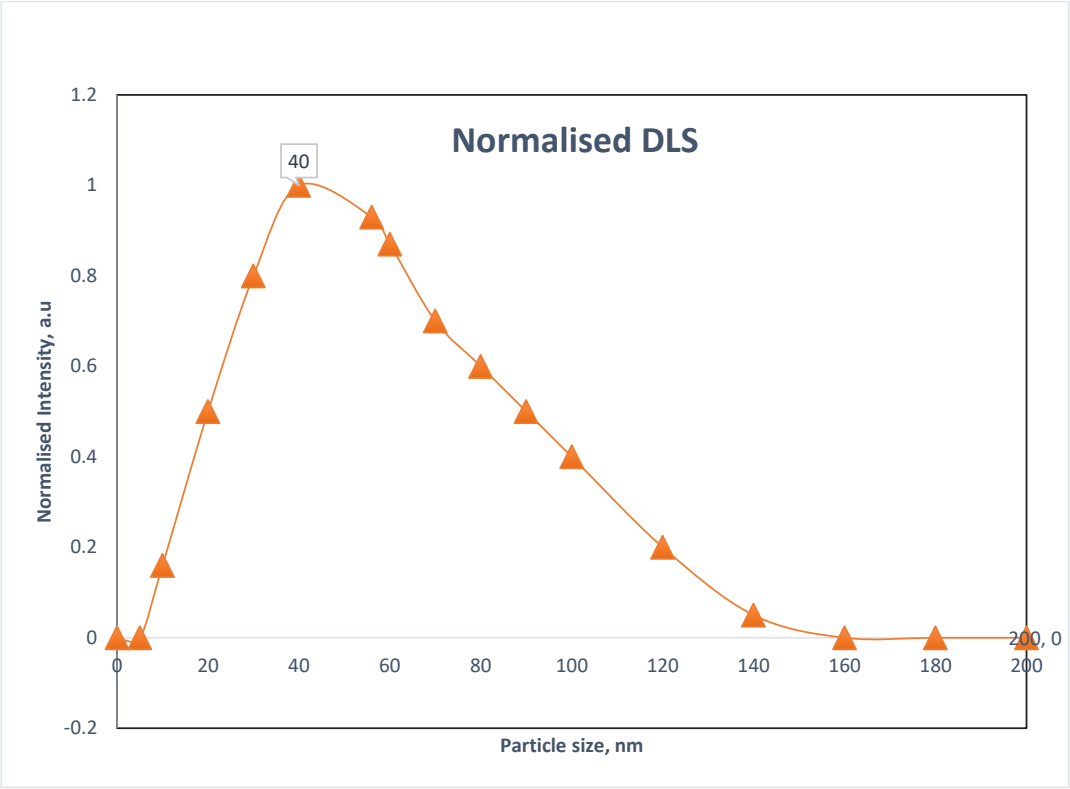

Fig. 9. DLS size distribution.

nano sphere reactor (micelle), as seen in Fig. 6. It must be said that essentially much surfactant amount does not results in more nano spheres.

In fact, always one has to work and add surfactant in the range of nano spheres, on the other hand, while surfactant adding much more, there may be lamellar, hexagonal and etc. structures would not be out of expectation. Anyway, more sphere micelle number, more nanoparticle production.

Fig. 7 has good agreement with others works [29-30]. Effect of two different surfactants is investigated as one can see in Fig. 7. Active DMAB with head group charge works superior to natural PVA. Adding PVA into emulsion has not important role except in the vicinity of 1%W/V and gives us nano spheres up to 200 nanometers, as Fig. 7 reveals. Instead, for more cationic effective DMAB concentration, we are success to obtain near the 40 nanometer spheres as others reported [31]. Ultrasound technique also is used but by rendering up to 200 nanometer nanoparticles. More encouraging parameters were the speed of homogeniser which is well-marked in Fig. 8, where for DMAB (left axes) and PVA (right axis) are prepared. It is clear that the average sizes of particles from 400 nanometers at 5000 rpm downs sharply to below 40 nano meters at 30000 rpm for DMAB surfactant, where for PVA although effective but ultimately obtain 200 nanometer particles. This can likely be related to head group charge effects for DMAB in comparison with PVA neutral head group.

We add 7 weight percent DMAB as surfactant because the best results are reached for particles size. The SEM and DLS will verify our claim for nanoparticles size. DLS of zeta sizer device shows the average size of nano spheres stands for 40 nanometers which is very desirable for drug delivery systems. This size is rarely reported so far.

DLS test results are shown in figure showing

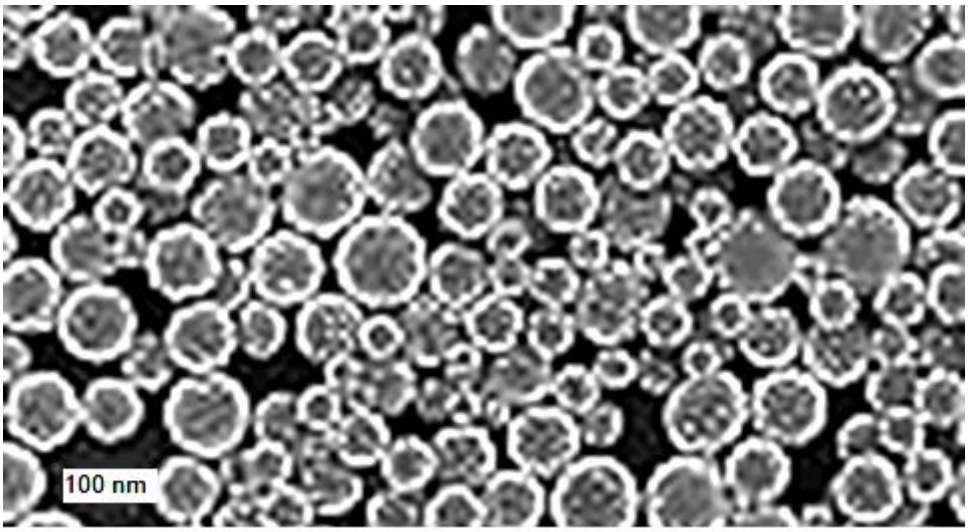

Fig. 10. SEM of obtained fine PLGA nanoparticles. Scale bar shows 100 nm.

Table 2. Average size distribution

References	Size (nm)
This work	40
[34]	150
[35]	266
[7]	100
[11]	100-300
[16]	160
[17]	305-489
[18]	138-180

a wide range of particles size distribution but with an average of 40 nanometers, totally in fact from 12 nanometers to160 nanometers. Large nanoparticles precipitated can be separated by centrifuge in lower times.

The SEM picture for gold coated nanoparticles exactly verifies DLS results. In Fig. 10 this picture is depicted. Our results satisfy our purposes and agree with others work [31]. Table 2 shows results comparing to others works.

CONCLUSION

In this work at first poly lactic acid and poly glycolic acid are synthesised using polycondensation and demerised for ring opening polymerisation processes. Several copolymer are made and it is specified that the best case remains for 25:75 PLGA with the help of TGA, DTG and DSC characterisations. Nano particles of 25:75 PLGA are generated by double emulsion evaporisation method which fine size of sample are obtained with a large variation of homogeniser speed and also surfactant DMAB concentration. Results show that the average size of nanoparticles is 40 nm (12 nanometer to160) via DLS and SEM tests. Therefore one of the best methods for PLGA fine nano sphere is reported here so that the results are with good agreement with others [32-33]. In the future, encapsulation of drug for purposes of drug delivery and clinical applications will be accomplished and we would focus on the smart drug delivery.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCE

1. Gupta AP, Kumar V. New emerging trends in synthetic biodegradable polymers – Polylactide: A critique. Eur Polym J. 2007;43(10):4053-4074.
2. Kricheldorf HR. Syntheses and application of polylactides. Chemosphere. 2001;43(1):49-54.
3. Cheng Y, Deng S, Chen P, Ruan R. Polylactic acid (PLA) synthesis and modifications: a review. Frontiers of Chemistry in China. 2009;4(3):259-264.
4. Singh V, Tiwari M. Structure-Processing-Property Relationship of Poly(Glycolic Acid) for Drug Delivery Systems 1: Synthesis and Catalysis. International Journal of Polymer Science. 2010;2010:1-23.
5. Jalabert M, Fraschini C, Prud'homme RE. Synthesis and characterization of poly(L-lactide)s and poly(D-lactide)s of controlled molecular weight. J Polym Sci, Part A: Polym Chem. 2007;45(10):1944-1955.
6. Ghorbani Chaboki M, Mohammadi-Rovshandeh J, Hemmati F. Poly(lactic acid)/thermoplasticized rice straw biocomposites: effects of benzylated lignocellulosic filler and nanoclay. Iranian Polymer Journal. 2019;28(9):777-788.
7. Lü J-M, Wang X, Marin-Muller C, Wang H, Lin PH, Yao Q, et al. Current advances in research and clinical applications of PLGA-based nanotechnology. Expert Rev Mol Diagn. 2009;9(4):325-341.
8. Jain R, Shah NH, Malick AW, Rhodes CT. Controlled Drug Delivery by Biodegradable Poly(Ester) Devices: Different Preparative Approaches. Drug Development and Industrial Pharmacy. 1998;24(8):703-727.
9. Prasad S, Cody V, Hanlon D, Edelson RL, Saltzman M, Sasaki CT, et al. Biopolymer nanoparticles as antigen delivery vehicles for immunotherapy of head and neck squamous cell carcinoma (HNSCC). Clin Otolaryngol. 2008;33(3):304-304.
10. Amecke B, Bendix D, Entenmann G. Reaorbable polyesters: Composition, properties, applications. Clin Mater. 1992;10(1-2):47-50.
11. Modified Magnesium Hydroxide Nanoparticles Inhibit the Inflammatory Response to Biodegradable Poly(lactide-co-glycolide) Implants. American Chemical Society (ACS).
12. Lee J-H, Gustin JP, Chen T, Payne GF, Raghavan SR. Vesicle−Biopolymer Gels: Networks of Surfactant Vesicles Connected by Associating Biopolymers. Langmuir. 2004;21(1):26-33.
13. Marslin G, Sheeba CJ, Kalaichelvan VK, Manavalan R, Neelakanta Reddy P, Franklin G. Poly(D,L-lactic-co-glycolic acid) Nanoencapsulation Reduces Erlotinib-Induced Subacute Toxicity in Rat. J Biomed Nanotechnol. 2009;5(5):464-471.
14. Feng P, Shen S, Shuai Y, Peng S, Shuai C, Chen S. PLLA grafting draws GO from PGA phase to the interface in PLLA/PGA bone scaffold owing enhanced interfacial interaction. Sustainable Materials and Technologies. 2023;35:e00566.
15. Han FY, Thurecht KJ, Whittaker AK, Smith MT. Bioerodable PLGA-Based Microparticles for Producing Sustained-Release Drug Formulations and Strategies for Improving Drug Loading. Front Pharmacol. 2016;7:185-185.
16. Rezvantalab S, Drude NI, Moraveji MK, Güvener N, Koons EK, Shi Y, et al. PLGA-Based Nanoparticles in Cancer Treatment. Front Pharmacol. 2018;9:1260-1260.
17. Cruz KP, Patricio BFC, Pires VC, Amorim MF, Pinho AGSF, Quadros HC, et al. Development and Characterization of PLGA Nanoparticles Containing 17-DMAG, an Hsp90 Inhibitor. Frontiers in chemistry. 2021;9:644827-644827.
18. Garms BC, Poli H, Baggley D, Han FY, Whittaker AK, A A, et al. Evaluating the effect of synthesis, isolation, and characterisation variables on reported particle size and dispersity of drug loaded PLGA nanoparticles. Materials Advances. 2021;2(17):5657-5671.
19. Dang Y, Guan J. Nanoparticle-based drug delivery systems for cancer therapy. Smart materials in medicine. 2020;1:10-19.
20. Pan X, Liu X, Zhuang X, Liu Y, Li S. Co-delivery of dexamethasone and melatonin by drugs laden PLGA nanoparticles for the treatment of glaucoma. J Drug Deliv Sci Technol. 2020;60:102086.
21. Phạm TL, Kim DW. Poly(Lactic-Co-Glycolic Acid) Nanomaterial-Based Treatment Options for Pain Management: A Review. Nanomedicine. 2020;15(19):1897-

1913.
22. Hu F, Zhang R, Guo W, Yan T, He X, Hu F, et al. PEGylated-PLGA Nanoparticles Coated with pH-Responsive Tannic Acid–Fe(III) Complexes for Reduced Premature Doxorubicin Release and Enhanced Targeting in Breast Cancer. Mol Pharm. 2020;18(6):2161-2173.
23. Zhang X-y, Zhang P-y. Polymersomes in Nanomedicine - A Review. Current Nanoscience. 2017;13(2):124-129.
24. Van Hees S, Elbrink K, De Schryver M, Delputte PL, Kiekens F. Improving Cellular Uptake and Cytotoxicity of Chitosan-Coated Poly(Lactic-Co-Glycolic Acid) Nanoparticles in Macrophages. Nanomedicine. 2020;15(27):2671-2688.
25. Ding D, Zhu Q. Recent advances of PLGA micro/nanoparticles for the delivery of biomacromolecular therapeutics. Materials Science and Engineering: C. 2018;92:1041-1060.
26. Isely C, Hendley MA, Murphy KP, Kader S, Annamalai P, Jabbari E, et al. Development of microparticles for controlled release of resveratrol to adipose tissue and the impact of drug loading on particle morphology and drug release. Int J Pharm. 2019;568:118469-118469.
27. Sharma N, Madan P, Lin S. Effect of process and formulation variables on the preparation of parenteral paclitaxel-loaded biodegradable polymeric nanoparticles: A co-surfactant study. Asian Journal of Pharmaceutical Sciences. 2016;11(3):404-416.
28. Hernández-Giottonini KY, Rodríguez-Córdova RJ, Gutiérrez-Valenzuela CA, Peñuñuri-Miranda O, Zavala-Rivera P, Guerrero-Germán P, et al. PLGA nanoparticle preparations by emulsification and nanoprecipitation techniques: effects of formulation parameters. RSC advances. 2020;10(8):4218-4231.
29. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Del Rev. 2003;55(3):329-347.
30. Wei Y, Wang Y-X, Wang W, Ho SV, Wei W, Ma G-H. mPEG-PLA microspheres with narrow size distribution increase the controlled release effect of recombinant human growth hormone. J Mater Chem. 2011;21(34):12691.
31. Abdullaeva Z. Eukaryotic Synthesis of Nanomaterials. Synthesis of Nanoparticles and Nanomaterials: Springer International Publishing; 2017. p. 55-78.
32. López-Royo T, Sebastián V, Moreno-Martínez L, Uson L, Yus C, Alejo T, et al. Encapsulation of Large-Size Plasmids in PLGA Nanoparticles for Gene Editing: Comparison of Three Different Synthesis Methods. Nanomaterials (Basel, Switzerland). 2021;11(10):2723.
33. Zhu C, Lee J-H, Raghavan SR, Payne GF. Bioinspired Vesicle Restraint and Mobilization Using a Biopolymer Scaffold. Langmuir. 2006;22(7):2951-2955.
34. Huang W, Zhang C. Tuning the Size of Poly(lactic-co-glycolic Acid) (PLGA) Nanoparticles Fabricated by Nanoprecipitation. Biotechnol J. 2018;13(1):10.1002/biot.201700203.
35. Mohan LJ, McDonald L, Daly JS, Ramtoola Z. Optimising PLGA-PEG Nanoparticle Size and Distribution for Enhanced Drug Targeting to the Inflamed Intestinal Barrier. Pharmaceutics. 2020;12(11):1114.