Институт техничкиох наука САНУ

Кнез Михаилова 35/IV

Београд

Предмет: Захтев за покретање поступака за избор дипл. физикохемичара Ненада Филиповића, истраживача приправника у звање истраживач сарадник

НАУЧНОМ ВЕЋУ ИНСТИТУТА ТЕХНИЧКИХ НАУКА САНУ

Молим Вас да, у складу са Правилником о поступку и начину вредновања, и квантитативном исказивању научноистраживачких резултата истрживача (Сл. Гласник РС, бр. 38/08), и Правилником о стицању звања истраживач сарадник, Научно веће Института техничких наука САНУ покрене поступак за избор у звање истаживач сарадник.

За чланове комисије за припрему извештаја научном већу предлажем:

- др Магдалена Стевановић, виши научни сарадник Института техничких наука САНУ
- др Смиља Марковић, виши научни сарадник Института техничких наука САНУ
- др Драгана Југовић, научни сарадник Института техничких наука САНУ

У прилогу достављм:

1. биографију
2. библиографију са копијом рада
3. доказ о уписаним докторским студијама
4. диплому завршених основних студија

У Београду:
31.07. 2012.

Подносилац захтева:
Ненад Филиповић

Ненад Филиповић дипл. физикохемичар
истраживач приправник ИТН САНУ

Биографија Ненад Филиповић

Ненад Филиповић је рођен 25.11.1984. године у Нишу, држава Србија. Основне студије је уписао школске 2003/04. године на Факултету за физичку фемију, универзитета у Београду и завршио их 2011. са просечном оценом 8.23 и 10 на дипломском раду "Термичка стабилност и кристализација аморфне легуре $Fe_{89.8}Ni_{1.5}Si_{5.2}B_3C_{0.5}$". Исте године је уписао мастер студије и одбранио мастер рад са темом "Механизам првог кристализационог ступња аморфне легуре $Fe_{89.8}Ni_{1.5}Si_{5.2}B_3C_{0.5}$". Постдипломске докторске студије је уписао 2011. године, на истом факултету. Тренутно је на првој години студија.

У Институту техничких наука САНУ је запослен од новембра 2011. године као истраживач приправник. Ангажован је на пројекту интегралних и интердисциплинарних истраживања ИИИ 45004, „Молекуларно дизајнирање наночестица контролисаних морфолошких и физичкохемијских карактеристика и функционалних материјала на њиховој основи“.

Области интересовања су му: полимерни биоматеријали, њихова структура и својства, поли (епсилон-капролактон), контролисана достава медикамената и инкапсулација медикамената у полимерну матрицу.

Библиографија Ненад Филиповић

М 21 Рад у врхунском међународном часопису:

N. Filipović, M. Stevanović, A. Radulović, V. Pavlović, D. Uskoković, Facile synthesis of poly(ε-caprolactone) micro and nanospheres using different types of polyelectrolytes as stabilizers under ambient and elevated temperature, Composites: Part B: Engineering, (2012), DOI: http://dx.doi.org/10.1016/j.compositesb.2012.07.008
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М 34 Саопштење са међународног скупа штампано у изводу:

1. D.M. Minić, N. Filipović, V.A. Blagojević Kinetics of crystallization and phase transformation of Fe75Ni2Si8B13C2 amorphous alloy, Yucomat, September 5-9,2011 Herceg Novi, Montenegro, Abstract Book p.97
2. Nenad Filipović, Magdalena Stevanović, Vladimir Pavlović, Aleksandra Radulović, Zoran Stojanović, Dragan Uskoković, Synthesis and the effect of processing parameters on characteristics of poly-ε-caprolactone micro- and nanospheres**,** Tenth Young Researchers' Conference-Material Science and Engineering 2011, Belgrade, December 21-23, Abstract Book p.2

Учешће на међународном пројекту:

International research projects (active): COST (Chemistry and Molecular Sciences and Technologies (CMST))-*Theragnostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-Guided Drug Delivery*- MC Chair Prof. Silvio AIME (IT).
Participant from Serbia: Centre for Fine Particles Processing and Nanotechnologies, Institute of Technical Sciences of the Serbian Academy of Sciences and Arts MC Member: Dr Magdalena Stevanović
(http://www.cost.eu/domains_actions/cmst/Actions/TD1004?management).
Duration: 2011 - 2015 (48 months)

РЕПУБЛИКА СРБИЈА

УНИВЕРЗИТЕТ У БЕОГРАДУ

ФАКУЛТЕТ ЗА ФИЗИЧКУ ХЕМИЈУ

ДИПЛОМА

О СТЕЧЕНОМ ВИСОКОМ ОБРАЗОВАЊУ

Филиповић Радета Немања

РОЂЕН-А 25-XI-1984. ГОДИНЕ У НИШУ, НИШ
СРБИЈА , УПИСАН-А 2003/2004. ШКОЛСКЕ ГОДИНЕ,
А ДАНА 11. МАРТА 2011. ГОДИНЕ, ЗАВРШИО-ЛА ЈЕ СТУДИЈЕ НА

ФАКУЛТЕТУ ЗА ФИЗИЧКУ ХЕМИЈУ

СА ОПШТИМ УСПЕХОМ 8.23 (осам и 23/100) У ТОКУ СТУДИЈА И ОЦЕНОМ 10 (ДЕСЕТ) НА ДИПЛОМСКОМ ИСПИТУ.

НА ОСНОВУ ТОГА ИЗДАЈЕ МУ-ЈОЈ СЕ ОВА ДИПЛОМА О СТЕЧЕНОМ ВИСОКОМ ОБРАЗОВАЊУ И СТРУЧНОМ НАЗИВУ

ДИПЛОМИРАНИ ФИЗИКОХЕМИЧАР

РЕДНИ БРОЈ ИЗ ЕВИДЕНЦИЈЕ О ИЗДАТИМ ДИПЛОМАМА 252011

У БЕОГРАДУ, 15. МАРТА 2011. ГОДИНЕ

ДЕКАН

ПРОФ. ДР [illegible]

РЕКТОР

ПРОФ. ДР [illegible]

Универзитет у Београду
Факултет за физичку хемију
Број индекса: 2010/0238
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У В Е Р Е Њ Е

Ненад Филиповић

име једног родитеља Раде, ЈМБГ 2511984730022, рођен 25.11.1984. године, Ниш, Република Србија, уписан школске 2010/11. године, дана 28.09.2011. године завршио је мастер академске студије на студијском програму Физичка хемија, у трајању од једне године, обима 60 (шездесет) ЕСПБ бодова, са просечном оценом 9,00 (девет и 00/100).

На основу наведеног издаје му се ово уверење о стеченом високом образовању и академском називу **мастер физикохемичар.**

Декан

Проф. др Шћепан Миљанић

РЕПУБЛИКА СРБИЈА

УНИВЕРЗИТЕТ У БЕОГРАДУ
ФАКУЛТЕТ ЗА ФИЗИЧКУ ХЕМИЈУ
Студентски трг 12-16
БЕОГРАД

(назив факултета - више школе)

(седиште)

БРОЈ 315/11

31. 10. 2011 године

УВЕРЕЊЕ

Дилитовић Раде Ненад (презиме, име једног од родитеља и име) рођен-а 25. 11. 1974-ће

у Нишу Република, односно држава Србија, уписао-ла је ПРВА ГОДИНА (словима)

годину студија, школске 2011/2012 године, као РЕДОВАН (редован-ванредан) студент

факултета - више школе у УНИВЕРЗИТЕТ У БЕОГРАДУ ФАКУЛТЕТ ЗА ФИЗИЧКУ ХЕМИЈУ

(одсек, смер, група)

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Facile synthesis of poly(ε-caprolactone) micro and nanospheres using different types of polyelectrolytes as stabilizers under ambient and elevated temperature

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ABSTRACT

Poly(ε-caprolactone) (PCL) particles were prepared by physicochemical method with solvent/non-solvent systems. The synthetic polymer polyvinylpyrrolidone (PVP) and natural polymer poly(α,γ,L-glutamic acid) (PGA), were used as stabilizers and their influence on size and morphology of the particles was examined. The results were compared with those obtained without the use of stabilizers. This study evaluated, for the first time, PGA as stabilizer of PCL particles and demonstrated that PGA is more efficient stabilizer of PCL particles than most commonly used PVP. The particles obtained by using PGA as stabilizer were spherical, with smooth surfaces and dimensions below 1 μm. The drying conditions were also varied in order to examine their influence on the formation of PCL particles. The samples were characterized by Fourier-transform infrared spectroscopy, scanning electron microscopy, particle size distribution (DLS) and zeta potential measurements.

1. Introduction

The past few decades have witnessed a growing interest in biodegradable and biocompatible polymeric materials, mostly because they enable the advancement of pharmaceuticals by providing better therapy through controlled drug release [1,2]. Biodegradable polymers can be natural, modified or synthetic. The most used and extensively investigated synthetic polymers are aliphatic polyesters, such as poly(ε-caprolactone) (PCL), poly(lactide acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA). All of them are FDA-approved and well known for their biodegradability, biocompatibility and non-toxic properties which makes them suitable as matrices for controlled drug release, especially when they are in form as microparticles and nanoparticles [2]. From the time it was first synthesized in the early 1930s, through the identification of its biodegradable properties in 1973, by now, PCL has still remained one of the most exploited synthetic polymer for medical applications [3–8]. One of the big advantages of PCL is that it can be easily blended with other polymers to improve stress crack resistance, dyeability and adhesion [5,9]. Moreover, its physical, chemical and mechanical properties can be also improved by copolymerization. The formation of such copolymers and polymer blends allows direct modulation of its degradation kinetics adjusting them for the desired application [10,11]. In comparison to polylactides and polyglycolides, poly(ε-caprolactone) has high permeability to small drug molecules and a slow degradation rate, which make it suitable for extended long-term drug delivery over a period of more than a year. While PLGA generates an acidic environment during degradation, which can lead to peptide/protein instability, the ability to avoid acidification has become one of the major advantages for selecting PCL as a drug carrier [2,6].

The main role of polymeric particles in drug delivery systems is to protect the active agent during its transport throughout the body, i.e. to control its release. In order to accomplish such task, some of the basic requirements are ideal spherical shape of the polymeric particles and narrow distribution of their sizes. The size and shape of the particles also play key role in their adhesion and interaction with the cells. PCL can be easily obtained in the form of micelles, films, fibers and hydrogels and as such be used for the controlled release of drugs [1–4]. All this, together with the notion that PCL is cytocompatible with a several body tissues, makes this polymer an ideal candidate for tissue engineering in various forms: scaffolds, fibers, foams, etc. In fact, this new field of biomedicine has played a major role in resurgence of the research on PCL making it the polymer of choice for controlled drug delivery and as a base for tissue growth [4].

Methods for the preparation of polymer particles can be generally divided into three groups: dispersion of pre-formed polymers,

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polymerization of monomers, and ionic gelation or coacervation [12]. Nevertheless, other methods such as particle replication in non-wetting templates, supercritical fluid technology, coaxial electrohydrodynamic atomization or method with tri-needle coaxial electrohydrodynamic device have also been described in the literature [13–17].

So far, many techniques have been developed for synthesis of PCL micro/nanoparticles (nanoparticles can be prepared by the same or similar methods as microparticles, except that manufacturing parameters are adjusted to obtain nanometer size droplets) and all have their advantages and disadvantages [6]. Thus, the ideal and universal method does not exist and the choice depends on the size of polymeric particles that is targeted, the solubility of drug that is to be encapsulates/incorporate, necessity for the modulation of the degradation rate, etc. [17]. Although, a stabilizer is not always required it has been proven that its addition helps to preserve the particle suspensions from agglomeration [18] as well as that its nature and concentration do affect the size of polymeric particles [19]. This strongly absorbed monolayer of usually organic molecules helps stabilization of particles by keeping them separated. The choice of the nature and the concentration of the stabilizers remains the main challenge. If considered that mentioned polymer particles are targeted for biomedical application, it would be favorable that all components used in their synthesis are biocompatible and less harmful in various concentrations. Also, a known problem addressed in the literature, which can occur with the particle stabilizer is its difficult removal from the system [20]. The remains of the stabilizer are often modifying surface characteristics of the particles, thus affecting the biocompatibility, distribution throughout the body, its presence can hinder biochemical reactions and reduce drug efficacy, etc. [20]. It is therefore necessary to accurately determine the concentration of stabilizers for a given method.

Polyelectrolytes have been widely used as stabilizers of the particles [21–23]. Depending on the charge carried by the polymer, polyelectrolytes are classified into anionic (negatively charged), cationic (positively charged) and non-ionic (no charge). In this study two different types of polyelectrolytes were used as stabilizers and they are non-ionic polyvinylpyrrolidone (PVP) and anionic poly(α,γ,L-glutamic acid) (PGA).

Polyvinilpyrrolidone (synthetic water-soluble polymer made from the monomer *N*-vinylpyrrolidone) has been extensively used in controlled release drug delivery due to its biocompatibility, chemical stability, and excellent aqueous solubility. It is mainly considered as a steric stabilizer and has been widely used in the chemical synthesis of many types of colloidal nanocrystals [24]. Poly(glutamic acid) (PGA) is a highly anionic polymer with a great range of applications [20] where repeating unit of glutamic acid are connected by peptide bond between α-amino and γ-carboxyl residues. PGA is a hydrophilic, biodegradable, non-toxic, biocompatible, and naturally available biopolymer usually produced by various strains of Bacillus [20,25]. As a result this biopolymer has been of interest in the past few years and has found widespread use in the preparation of various products: vaccine, drug carrier, skin care products, biological adhesives, etc. [20,25,26].

Although PCL is one of the extensively studied synthetic biodegradable polymers in various formulations for drug delivery and tissue engineering, still there is a need to develop new, and enhance already existing approaches to make its production rapid and economical (inexpensive reagents, short reaction time and relatively simple fabrication process). On the other hand, undesirable characteristics should be kept to minimum.

The objective of this study was to synthesize poly(ε-caprolactone) micro/nanospheres and investigate the influence of different processing parameters on size and morphology of obtained particles. Therefore different samples were prepared: (i) without the stabilizers; (ii) with the addition of PVP as most commonly used stabilizer and (iii) with the addition of PGA as a new stabilizer for PCL particles. The physico-chemical properties such as morphological characteristics of the particles, particle size distribution, zeta potential, i.e. particle stability in dispersion, were examined. For the sample with the best characteristics drying conditions were varied to investigate their influence on the particles formation.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) was purchased from Durect, Lactel (Birmingham, Alabama, USA). Polyvinylpyrrolidone (povidone, PVP) was obtained from Sigma Aldrich Chemie GmbH (k-25, Steinheim, Germany). Poly(α,γ,L-glutamic acid) (PGA) with M_w = 20–40 kDa (99.9% HPLC purity) was purchased from Guilin Peptide Technology Limited (China).

All reagents were of the analytic grade and were used as received without further purification.

2.2. Synthesis of PCL spheres

PCL particles were synthesized following slightly modified method, previously described [27]. This is physicochemical method with solvent/non-solvent systems (Fig. 1). Briefly, commercial granules of poly(ε-caprolactone) (200 mg) have been dissolved in the chloroform (3 mL) and, after approximately 1 h, ethanol (22 mL) has been rapidly added into the solvent mixture, followed by precipitation of PCL and the solution became whitish. The obtained suspension was homogenized on magnetic stirrer for less than 1 min at 500 rpm, and after that poured into a petri dish and left to dry at room temperature during the next 72 h.

2.3. Influence of different stabilizers on morphological characteristics of PCL micro and nanospheres

The performance of the samples is strongly affected by the nature and concentration of the compounds added to stabilize the dispersions. Polyelectrolytes are polymers whose repeating units bear an electrolyte group. These groups will dissociate in aqueous solutions, making the polymers charged. Polyelectrolyte properties are thus similar to both electrolytes (salts) and polymers. Like salts, their solutions are electrically conductive. Like polymers, their solutions are often viscous. Charged molecular chains play a fundamental role in determining structure, stability and the interactions of various molecular assemblies [21–23].

In order to investigate the influence of different types of the stabilizers on size and morphology of PCL particles during its formation, synthetic, non-ionic, polymer polyvinylpyrrolidone and natural, anionic, polymer poly(α,γ,L-glutamic acid) were added dropwise in homogenized suspension (Fig. 1). Concentration of PVP or PGA in water was 0.05%. The solution was stirred for over 20 min to ensure that the reaction had been completed.

2.4. Influence of drying temperature during the synthesis

The drying conditions were also varied in order to examine their influence on the formation of PCL particles. Instead of drying at room temperature samples prepared with addition of PGA were dried at reduced pressure (0.001 bar) and elevated temperature (~45 °C) for 72 h. PCL is a hydrophobic polymer; having a glass transition temperature of −60 °C and melting point ranging between 59 and 64 °C, dictated by the crystalline nature of PCL which enables easy formability at relatively low temperatures [4]. The

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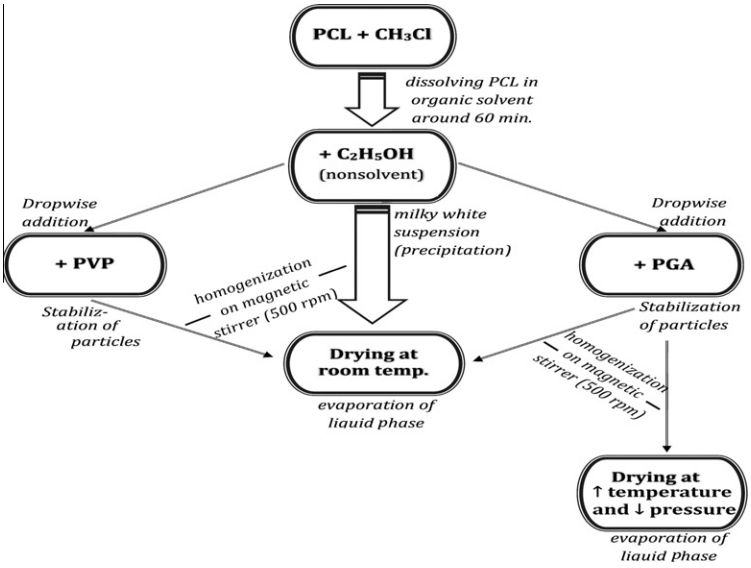

Fig. 1. Schematic illustration of preparation PCL micro/nanoparticles.

increased pressure enables dissolution of the polymer in a liquid that is a non-solvent of the polymer under atmospheric conditions [28,29]. The choice of the values for the elevated temperature and for the reduced pressure is made to avoid the melting of the PCL as well as the dissolution of the PCL in the non-solvent.

2.5. Fourier-transform infrared spectroscopy (FTIR)

The quality analysis of the samples was performed with FTIR spectroscopy. FTIR spectra of the samples were recorded in the range of 400–4000 cm^{-1} using a Carl Zeiss SPECORD 75 Spectrometer at 4 cm^{-1} resolution. FTIR measurements of the samples were carried out to identify the possible interactions between PCL and different stabilizers, PVP or PGA, which are responsible for stabilization, i.e. protection of the PCL particles from agglomeration.

2.6. Scanning electron microscopy (SEM)

The morphology of obtained nano and microparticles and porous samples was evaluated by scanning electron microscope (SEM) (JEOL JSM-639OLV). The samples for SEM analysis were coated with gold using the physical vapour deposition (PVD) process. Samples were covered with gold (Baltec SCD 005 sputter coater), using 30 mA current from the distance of 50 mm during 180 s.

2.7. Particle size distribution (DLS)

The particle size distribution of PCL particles obtained with PVP or with PGA as stabilizers was determined by the PSA Mastersizer2000 (Malvern Instruments Ltd., UK). For the particle size measurements the powder was deagglomerated in an ultrasonic bath (frequency 40 kHz and power 50 W) for 15 min.

2.8. Zeta potential measurements

Zeta potential was measured by Zetasizer (Nano ZS, Model ZEN3600, particles size range for zeta potential determination (5 nm to 10 μm), Malvern Instruments, Malvern, UK) using the principle of electrophoretic mobility under an electric field. Zeta potential is the function of dispersion/suspension pH, which determines particle stability in dispersion.

2.9. Water absorption

Water absorption of the PCL porous samples obtained at elevated temperature and reduced pressure was measured in the manner described in detail elsewhere [30]. Water absorption was measured by immersing the samples in distilled water for a predetermined time span, then the samples were taken out and dried by removing the free water on the surface with filter paper and weighed ($W1$). Then the samples were thoroughly vacuum-dried and weighed again ($W2$). The water absorption could be calculated as follows:

$$\text{Water absorption } (\%) = (W1 - W2)/W2 \times 100$$

3. Results and discussion

The synthetic method, used in this study, consists of precipitation of a pre-formed polymer from an organic solution and the diffusion of the organic solvent in the non-solvent medium (in this case ethanol) in the presence or absence of a stabilizer [18,27]. Polymer deposition on the interface between the ethanol and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a white suspension. Generally, this is a convenient, rapid and reproducible method, but in the way of its realization many difficulties might appear such as the right choice of solvent and non-solvent, determination of their corresponding molar fractions, the choice of stabilizer and the conduct of good drying method. Accordingly, all of these above factors will influence the morphology and particle size of the obtained powder. In this study two different stabilizers were used, one is natural, anionic and not well known while the other is synthetic, non-ionic and well known as stabilizer in drug delivery systems.

3.1. Fourier-transform infrared spectroscopy (FTIR)

The samples were first characterized with FTIR spectroscopy and results are shown in Fig. 2. Several characteristic bands are noticeable for all samples. Those are: distinct carbonyl (C=O) stretching band at 1720 cm^{-1}, symmetrical CH_3 and antisymmetrical CH_2 stretching band at 2930 and 2850 cm^{-1} respectively, and the ester COO stretching band at 1190 cm^{-1} and at 1240 cm^{-1} [31]. The CH_2 band vibrations of the polymer are also present at

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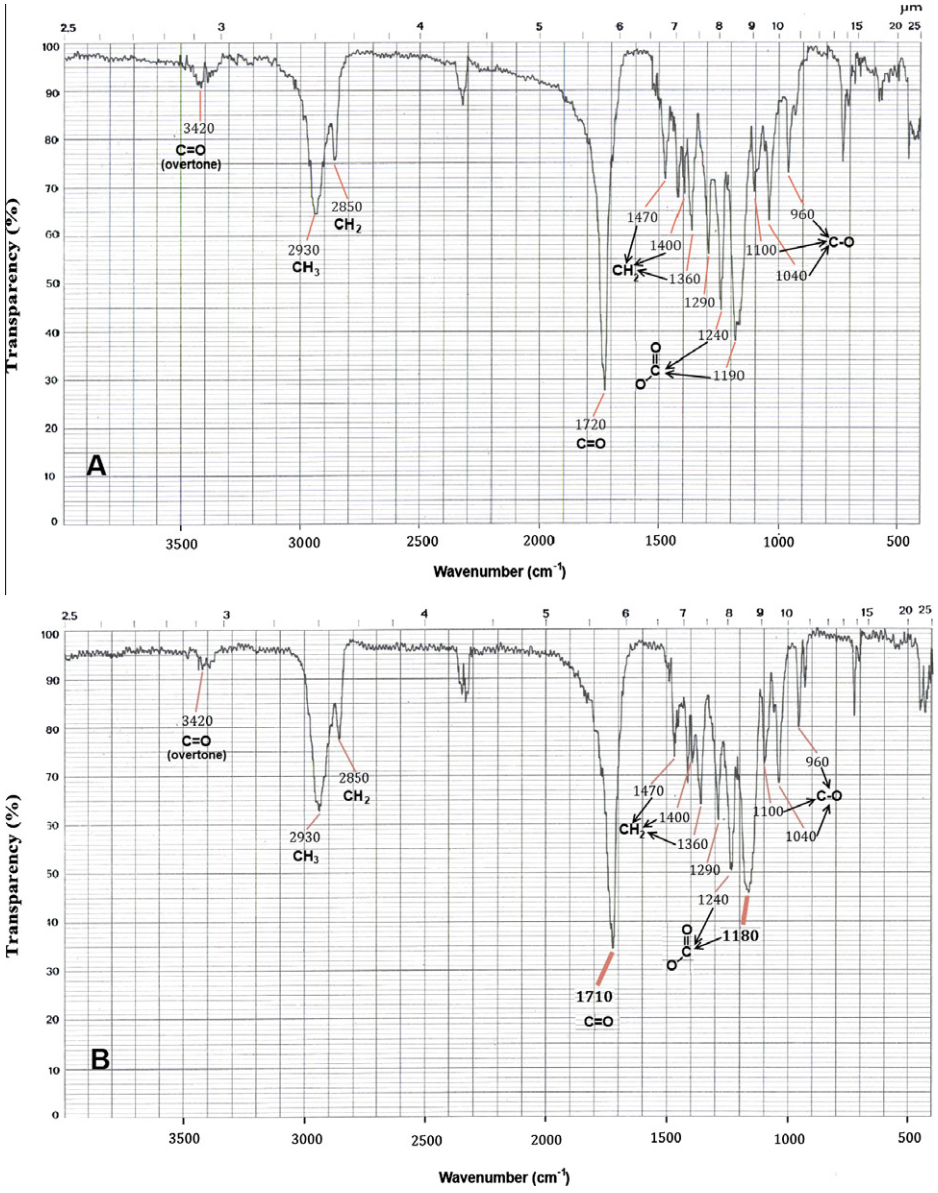

Fig. 2. FTIR spectra of (A) PCL obtained without stabilizer, (B) PCL obtained with polyvinyl pyrrolidone, and (C) PCL obtained with poly(α,γ,L-glutamic acid) as stabilizer.

1360, 1400, and 1470 cm^{-1} and the peaks at 1100, 1040, and 960 cm^{-1} are due to O—C vibrations [32]. The broad peaks centered at 3420 cm^{-1} is the overtone of the carbonyl stretching band. As Coleman and Zarian reported, the band at 1294 cm^{-1} (here at 1290) can be assigned to the backbone C—C and C—O stretching mode in the crystalline phase of PCL [33], while in amorphous phase this band should appear at 1157 cm^{-1}. Since no peak was noticed nearby it clearly indicates the high crystallinity degree of obtained PCL. In the spectrum of PCL particles prepared with addition of PVP there was no change in intensity, but peak of C=O was blue shifted to 1710 cm^{-1} as well as ester stretching band to 1180 cm^{-1}. This could be caused by conjugation with the R—C bond due to the binding of PVP on the surface of PCL. However, there is no resonance peak of carbonyl group (at approximately 1650 cm^{-1}), characteristic for PVP, to prove this theory. It is more likely that PVP was not able to strongly adsorb on the whole surface of the PCL particles, but only on small part of it or it might be that in the case of this sample the interference between the ester COO stretching band known at 1190 cm^{-1} and the amorphous phase band known at 1157 cm^{-1} occurs. Conversely, for the sample prepared using PGA, bands intensities have increased significantly. This is probably result of overlapping bands from PGA adsorbed on same functional groups, or on different functional group but with similar wave numbers. Identical shifting of carbonyl and ester bands, as it was in the sample with PVP, was noticed. The exceptions are CH_2 bands at 2850 and 1360 cm^{-1} that were shifted to 2860 and 1380 cm^{-1}, while new band was observed at 1300 cm^{-1}, which could be assigned to N—H band from PGA [20]. These results led us to conclusion that PGA managed to form a protective layer around PCL particles.

3.2. Morphological characteristics of PCL micro and nanoparticles obtained without and with different stabilizers

Effect of stabilizer on the morphology and particles size was examined by scanning electron microscopy (SEM). PCL particles prepared without stabilizer formed irregularly shaped and strongly agglomerated particles (Fig. 3). The coalescence and agglomeration occurs due to instability of suspended particles and the solvent evaporation. Evaporation of solvent led to an increase in viscosity

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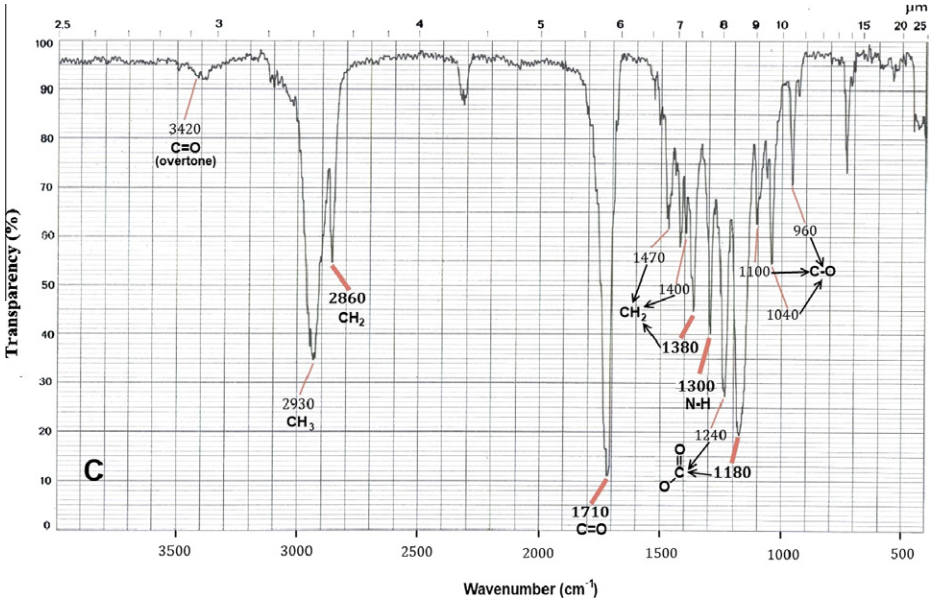

Fig. 2 (*continued*)

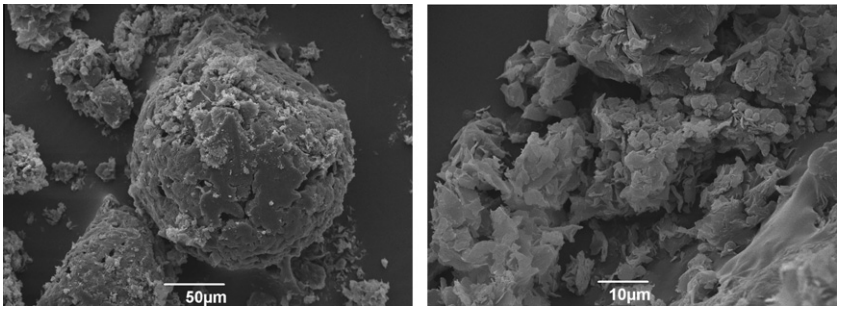

Fig. 3. Representative SEM images of PCL sample obtained without stabilizer. Different fields and magnifications are shown (bars 50 μm (left) and 10 μm (right)).

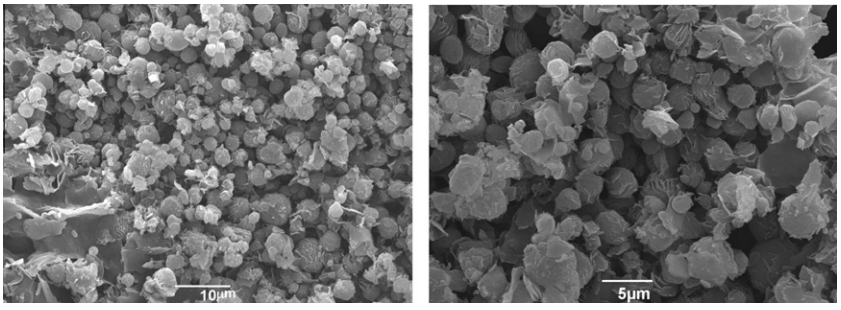

Fig. 4. Representative SEM images of PCL sample obtained with PVP as stabilizer. Different fields and magnifications are shown (bars 10 μm (left) and 5 μm (right)).

and a gradual decrease in the volume of suspended droplets [34]. Reduction of the coalescence and agglomerations was achieved by the addition of stabilizers. When PVP was used as stabilizer, particles were still agglomerated and irregular in shape, but the presence of particles with spherical shape was also observed (Fig. 4). However, the addition of PGA gave better results. The particles were spherical in shape, with smooth surface and size below 1 μm, as shown in Fig. 5. This can be explained by the formation of thin protective layer around the suspended PCL particles which prevents them to agglomerate during the early step of the solvent removal, and confirms the theory mentioned in the previous section. Particles have a smooth surface due to the complete removal of the organic solvents during the preparation process [35].

3.3. Particle size distribution (DLS)

The next step in the particles characterization was to determine its size distribution. Since the produced powders are mostly aggregates of primary nanoparticles, the accuracy of the measurement depends on the degree of the powder dispersion. After 15 min of dry powder deagglomeration with the aid of low-intensity ultrasound, the following results were obtained: in the case of PCL

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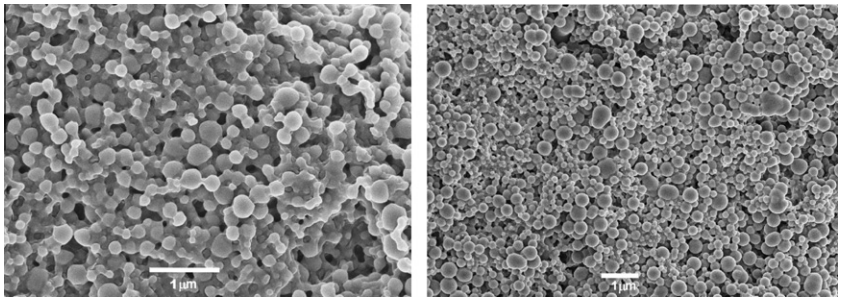

Fig. 5. Representative SEM images of PCL sample obtained with PGA as stabilizer. Different fields and magnifications are shown (bar 1 µm).

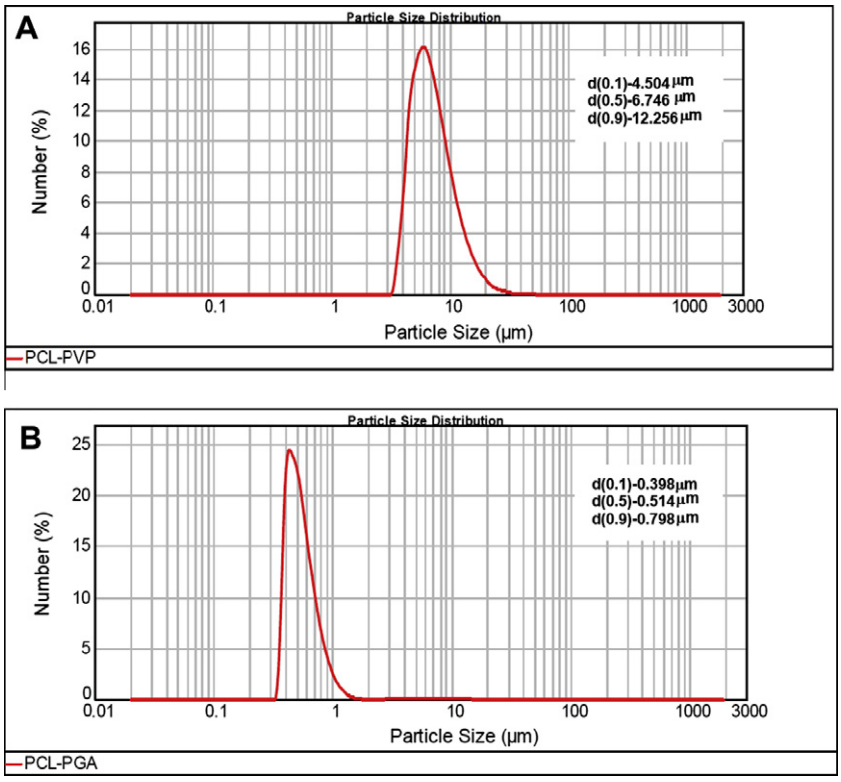

Fig. 6. Particle size distribution of PCL samples prepared with addition of PVP (A) or PGA (B).

particles obtained with PVP as stabilizer, 10% of particles ($d0.1$) have diameter of about 4.5 µm, 50% of particles ($d0.5$) possess diameter of 6.75 µm, while 90% of particles ($d0.9$) are smaller than 13 µm (Fig. 6A).

In the case of PCL particles obtained with PGA as stabilizer, 10% of particles ($d0.1$) have diameter smaller than 400 nm, 50% of particles ($d0.5$) possess diameter of 514 nm, while 90% of particles ($d0.9$) are smaller than 800 nm (Fig. 6B). These results are in the agreement with the results obtained by SEM.

3.4. Zeta potential measurements

The results of zeta potential measurements for PCL samples obtained without and with different stabilizers are shown in Fig. 7. Zeta potential was reported as an average and standard deviation of measurements, with five readings taken per sample and was measured using the principle of electrophoretic mobility under an electric field. Zeta potential is the function of dispersion/suspension pH which determines particle stability in dispersion. Theoretically, zeta potential stabilizes suspensions when its value is either positive or negative. Stabilizer reduces the agglomeration because the particles of the same charge are not attracted to each other. From Fig. 7 it can be seen that an increase in the absolute values of zeta potential with the use of stabilizers was observed, whereas the highest values were measured for the sample prepared with the PGA. Hence, agglomeration of particles was lowest for this sample.

In aqueous suspensions, polyelectrolytes are thought to be effective in their role as a dispersant and stabilizer because of their propensity to adsorb onto the particle surface [36]. In this particular case, it can be assumed that the anionic PGA was more adsorbed at the PCL-water interface as a result of electrostatic attraction at pH 4.31–4.38 in comparison with the non-ionic PVP. Stabilizer adsorption depends on several factors such as chemical potential of the stabilizer molecules (monomers) in solution, the nature of the solid, solvent, presence of secondary competing-cooperative species, temperature and even the mode of mixing. Nature of the adsorbed layer determines the surface modification achieved and this, in turn, depends on the adsorption mechanisms and the conditions prevailing during and after adsorption.

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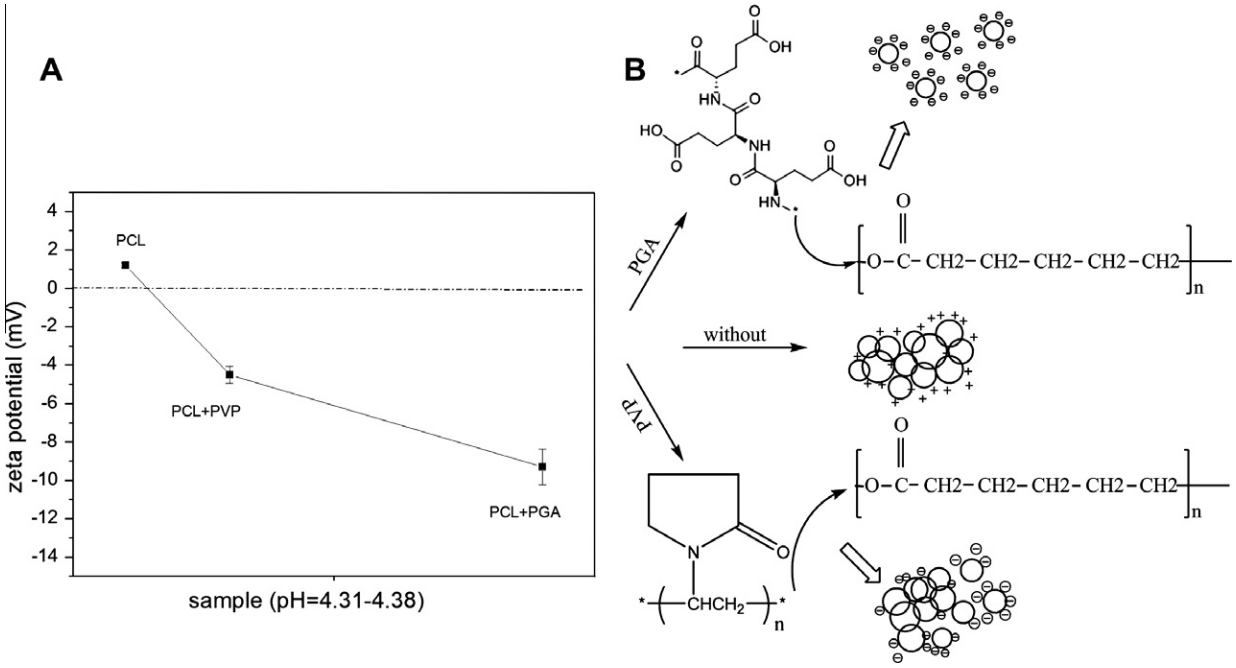

Fig. 7. (A) Zeta potential of PCL dispersion without and with PVP or PGA as stabilizers and (B) schematic illustration of obtaining PCL micro/nanoparticles without and with different polyelectrolyte (PVP or PGA) as stabilizer.

Kothapalli et al. have been hypothesized that anionic polyelectrolytes can absorb by anion exchange with excess of OH^- groups present in the particle [37]. Adsorbed charged polymers lead to repulsion of neighboring particles by a combination of electrostatic and entropic repulsion. In addition, they can reduce particle agglomeration during shearing due to their ability to increase depletion-layer (osmotic) and hydrodynamic repulsion forces between the particles. The adsorption of non-ionic surfactants differs largely from that of ionic surfactants because of the absence of electrostatic interactions. Since hydrogen bonding is relatively weak in comparison to electrostatic and chemical bonding, the nature of the water structure at the solid–liquid interface is of particular importance for the adsorption of non-ionics. The weaker adsorption in the case of non-ionic stabilizer is due to the fact that the stabilizer molecules are less able to disrupt the rigid water layer surrounding the particle [38].

3.5. The influence of drying temperature

As the nanospheres were obtained only with addition of PGA as a stabilizer, the influence of drying parameters (temperature and pressure) were further examined on this sample by scanning electron microscopy. In order to increase evaporation rate, sample of PCL + PGA was dried at reduced pressure (1 mbar) and elevated temperature (~45 °C). The values for pressure and temperature at which the samples were dried were chosen to avoid the melting of the PCL as well as the dissolution of the PCL in the non-solvent. Images of resulting morphology were compared with those obtained at room temperature. In contrast to room temperature condition when spherical particles were obtained (Fig. 2C), at elevated temperature and reduced pressure resulting morphology was quite different (Fig. 8). The reason for forming such a porous structure could be that it is caused by rapid evaporation and rapid shrinkage of the whole system including the droplets of suspended polymer. Therefore there is a growing tendency of particles to agglomerate despite the repulsive effects of stabilizers. At some point this tendency of particles to reduce surface tension by agglomeration overcomes the repulsive effect of stabilizers, and at the end the balance is established by coalescence of spherical particles on a surface contact, leaving pores of different sizes in all directions. However Fig. 8 also strongly suggests the possibility that phase separation have taken place during this drying condition. This is because the degree of pressure and temperature change in this work significantly influences the solubility of the polymer, in addition to the changes of solvent evaporation behavior [28,29,39]. Phase separation leads to polymer-rich regions forming the "walls" and solvent-rich regions forming the pores after evaporation. It should be also noted that temperature and pressure change also might affect polymer crystallization during drying [40–42]. For polymeric materials crystallization is a general phenomenon and a very important process because it controls the polymer's structural formation and thereby strongly influences the final products' properties [40–42].

3.6. Water absorption

In the literature, it has been reported that wetting of a polymer scaffold is essential for homogeneous and sufficient cell seeding throughout the porous scaffold [43]. To evaluate hydrophilicity of PCL porous samples water absorption was measured. The way in which materials absorb water depends upon many factors, such as temperature, sample volume fraction, sample orientation, area of exposed surfaces, interfacial bonding, diffusivity, reaction between water and matrix, surface protection [44,45]. The water absorption of the samples changed especially with their porosity. PCL + PGA porous samples obtained at elevated temperature and reduced pressure exhibited very high water absorption of about 62 ± 9%. This water absorption was leveled off within 24 h and did not show an increase after the samples being longer immersed in distilled water for 2 or 7 days.

4. Conclusion

In this paper we presented a facile, rapid and reproducible method for obtaining PCL nanoparticles using the physicochemical method with solvent/non-solvent systems. It was found that addi-

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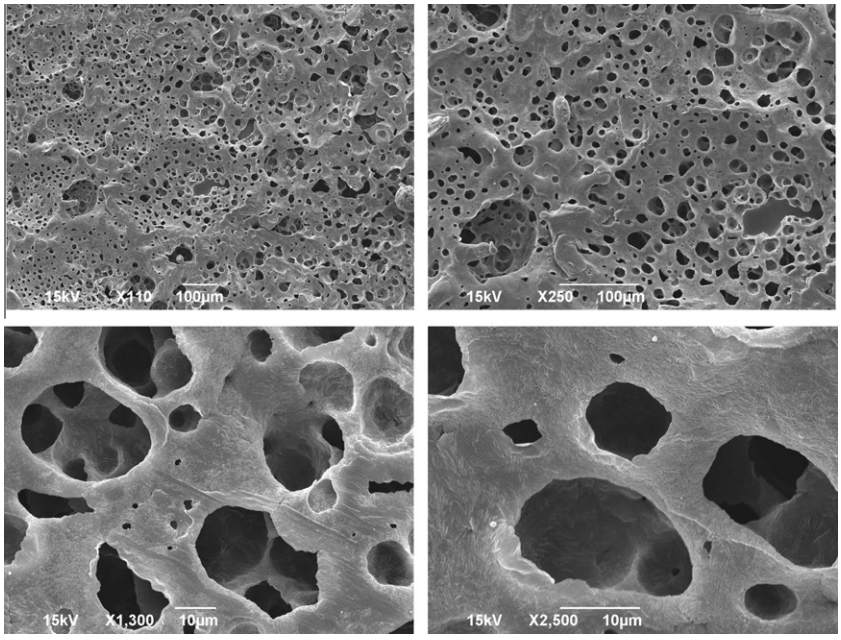

Fig. 8. Representative SEM images of PCL obtained with PGA when dried at reduced pressure and elevated temperature.

tion of PGA as stabilizer gives better results than PVP, although PVP has been used more often for stabilizing polymeric particles. The particles obtained using PGA as stabilizer were spherical, with smooth surfaces and dimensions below 1 μm (400–800 nm). The facts that the PGA is natural polymer that is already widely used in medicine, pharmacy and food industry, as well as that so far it has not been used to obtain micro- and nanoparticles of PCL, are additional reasons for its use as a stabilizer which gives this method a great potential in synthesizing carriers for drug delivery systems.

The effects of the drying temperature and pressure on the system in which PGA was used resulted in an interesting observation that the structure was highly porous and it deserves further investigation, as it represents a potential method for obtaining scaffold materials.

This new approach also opens up the exciting possibility of surface modification of PCLs with amino acids where binding with the PCLs surface may be accomplished through the amine functionality.

This research, though very preliminary, provides helpful insights to the development of poly(ε-caprolactone) nano and microspheres for controlled drug delivery or scaffold material for tissue engineering.

Acknowledgements

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