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Research Article

Preparation and Characterization of Poly(D,L-Lactide-co-Glycolide) Nanoparticles Containing Ascorbic Acid

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This paper is covering new, simplistic method of obtaining the system for controlled delivery of the ascorbic acid. Copolymer poly (D,L-lactide-co-glycolide) (DLPLG) nanoparticles are produced using physical method with solvent/nonsolvent systems where obtained solutions were centrifuged. The encapsulation of the ascorbic acid in the polymer matrix is performed by homogenization of water and organic phases. Particles of the DLPLG with the different content of ascorbic acid have different morphological characteristics, that is, variable degree of uniformity, agglomeration, sizes, and spherical shaping. Mean sizes of nanoparticles, which contain DLPLG/ascorbic acid in the ratio 85/150%, were between 130 to 200 nm depending on which stereological parameters are considered (maximal diameters Dmax, feret X, or feret Y). By introducing up to 15% of ascorbic acid, the spherical shape, size, and uniformity of DLPLG particles are preserved. The samples were characterized by infrared spectroscopy, scanning electron microscopy, stereological analysis, and ultraviolet spectroscopy.

1. INTRODUCTION

The systems for controlled delivery of the medicaments in the body are causing real revolution in the medicine and pharmacy in the recent years, and all in favor of better medical treatments of the patients [1]. Using the system for the controlled and balanced release of medicaments, opposing to standard and conventional methods, constant and uniform concentration of medicament in the body is achieved throughout longer period of time. Copolymer poly(D,L-lactide-co-glycolide) is used for the controlled delivery of several classes of medicaments like anticancer agents, antihypertensive agents, immunomodulatory drugs, hormones, and macromolecules like nucleic acid, proteins, peptides, antibodies, DLPLG nanospheres are very efficient mean of transdermal transport of medicaments in the body, for example, ascorbic acid [2]. DLPLG polymer particles allow the encapsulation of the medicament within the polymer matrix, where the principle requirement for the controlled and balanced release of the medicament in the body is the particle's ideal spherical shape and narrow distribution of its size. The size and shape of the particles play key role in their adhesion and interaction with the cell. Dynamic of the release (pace and concentration) depends of the morphology, that is, structure of the copolymer. The chemical structures, molecular weight, composition, as well as the synthesis conditions, are parameters which influence the final morphology of the polymer. The direct relation between these parameters and morphology is inadequately examined thus making it a topic of many researches. Depending on the nature and matrix of the selected material, methods of obtaining polymer particles can be divided in general into dispersion of the polymer solution method, polymerization of the monomer method, and coacervation [3–6]. The PLGA spheres obtained with emulsion process are in range of 150–200 μm [7], 45 μm [8], 30 μm [9]. With modified emulsion method, the particle sizes are decreased to 10 μm [10]. Further modification of the process for synthesis of the particles, that is, emulsification solvent evaporation method, the obtained particles are in nanometer scale of 570–970 nm [11] and 244–260 nm

[12–14]. The latest researches in this field indicated the possibility of producing DLPLG spheres with average diameter under 100 nm [15]. Controlling the conditions of obtaining DLPLG by solvent/nonsolvent method, changing the parameters like aging time, after adding nonsolvent, time and velocity of centrifugal processing, it is possible to influence on morphology (size and shape) and uniformity of DLPLG polymer powder [16]. DLPLG powder with short aging time with nonsolvent and longest time and velocity of the centrifugal processing has smallest particles and highest uniformity. DLPLG copolymer has potential to be used for transport of ascorbic acid in the body, thus considerably increasing its efficiency. Ascorbic acid reduces free radicals, and in that way damages created by oxidative stress which is a root cause of, or at least associated with, many diseases are minimized. The aim of this research is obtaining the nanoparticles of copolymer poly(D,L-lactide-co-glycolide) in which ascorbic acid is encapsulated, as well as examining the influence of the synthesis method on morphological characteristics of poly(D,L-lactide-co-glycolide) particles with the different content of ascorbic acid.

2. MATERIALS AND METHODS

2.1. Materials

Poly(D,L-lactide-co-glycolide) (DLPLG) was obtained from Durect, Lactel, Adsorbable Polymers International and had a lactide to glycolide ratio of 50 : 50. Molecular weight of polymer was 40000–50000 g/mol. Time of complete resorption of this polymer is 4–8 weeks. Molecular weight of ascorbic acid was 176.13 g/mol. Polyvinyl alcohol (PVA) was used with a 98% hydrolization degree. All other chemicals and solvents were of reagent grade.

2.2. Preparation of nanoparticles

Copolymer powder DLPLG was obtained by means of physical methods from commercial granules using solvent/nonsolvent systems (Figure 1). Commercial granules poly(D,L-lactide-co-glycolide) (0.05 g) were dissolved in 1.5 mL of acetone and, after approximately two hours, 2 mL of methanol was added into solvent mixture. DLPLG precipitated by the addition of methanol and the solution became whitish. The polymeric solution thus obtained was very slowly poured into 20 mL of aqueous PVA solution (0.02% w/w) while continuous stirring at 1200 rpm by a stirrer. After that, the solution was centrifuged and decanted. Time and velocity of the centrifugal processing were 120 minutes and 4000 rpm. PVA is used as a stabilizer which creates negative charge of the DLPLG particles, that is, it creates negative zeta potential [17]. By creating specific zeta potential, PVA brings to reduction of agglomeration of the particles. All used solutions are nontoxic for environment. The ascorbic acid was encapsulated into the polymer matrix by means of homogenization of water and organic phases. The water solution with the variable ratio of the ascorbic acid was added to the polymer solution. This was followed by the precipitation using alcohol methanol. In the particles of DLPLG copolymer,

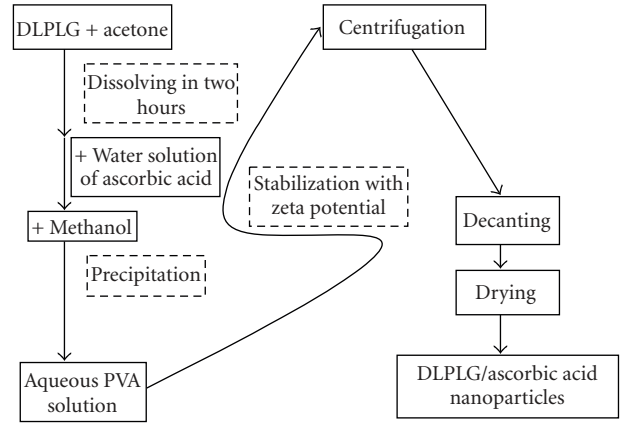

Figure 1: Schematics for obtaining of the DLPLG/ascorbic acid nanoparticles.

different concentration of ascorbic acid has been encapsulated with ratios 85% DLPLG to 15% ascorbic acid, 70% DLPLG to 30% ascorbic acid, 50% DLPLG to 50% ascorbic acid, and 30% DLPLG to 70% ascorbic acid.

2.3. Infrared (IR) spectroscopy measurements

The quality analysis of the samples was performed with IR spectroscopy. The IR measurements were performed on Perkin-Elmer 983G infrared spectrophotometer, using the KBr pellet technique, in the frequency interval of 400–4000 cm^{-1}.

2.4. Scanning electron microscope (SEM) observation

The morphology of obtained particles of DLPLG was examined by scanning electron microscope (SEM) JEOL JSM-646OLV. The powder samples for SEM analysis were coated with gold using the physical vapor deposition (PVD) process. Samples were covered with gold (SCD 005 sputter coater), using 30 mA current from the distance of 50 mm during 180 seconds.

2.5. Stereological analysis

The particle size and morphology were examined using the area analysis method [18, 19] by semiautomatic image analyzer (Videoplan, Kontron), connected with a scanning electron microscope (SEM). From 200 to 300 particles in the SEM were measured and the following parameters were determined: area section Aa, perimeter Lp, maximal diameter of the particle Dmax, feret x and feret y, and form factor (fL) (Figure 2).

2.6. Ultraviolet (UV) spectroscopy

Release of the ascorbic acid from DLPLG particles in vitro in physiological solution (0.9% sodium chloride in water) was studied with UV spectroscopy. The UV measurements were

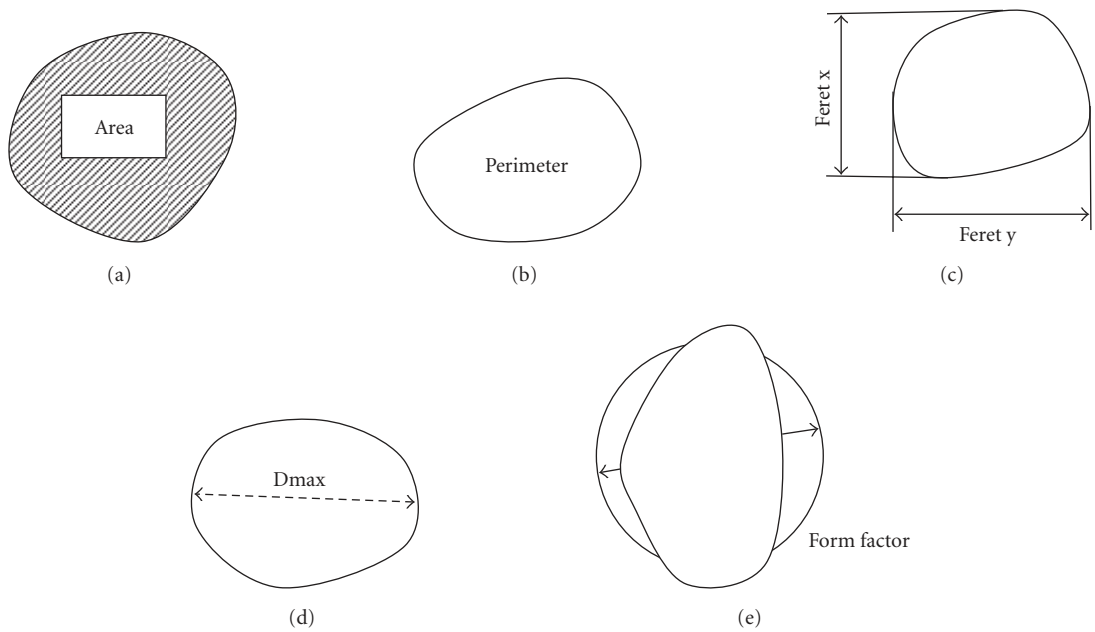

Figure 2: Shematics of the stereological parameters: (a) area (Aa); (b) perimeter (Lp); (c) feret x and feret y (d) maximal particle diameter (Dmax); (e) form factor (fL).

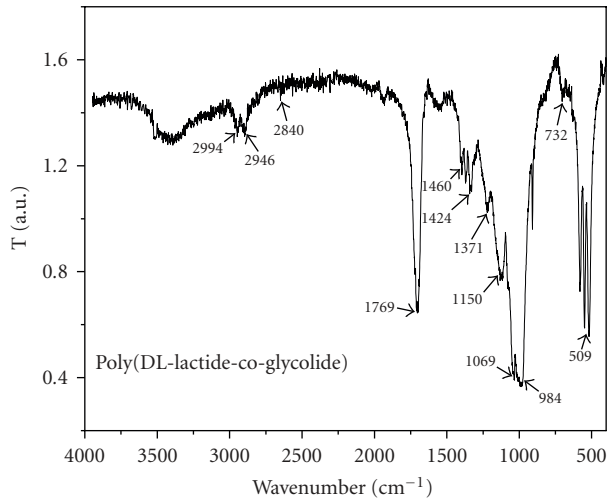

Figure 3: IR spectra of the DLPLG nanoparticles.

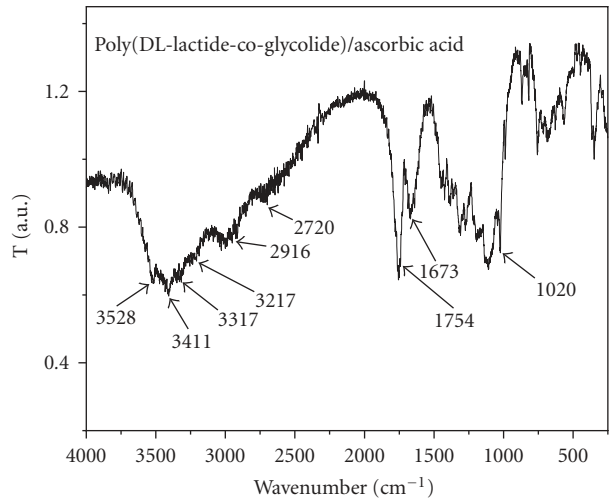

Figure 4: IR spectra of the DLPLG/ascorbic acid 85/15% nanoparticles.

performed on Perkin-Elmer Lambda 35 UV-V is spectrophotometer in the frequency interval of 200–400 nm.

3. RESULTS AND DISCUSSION

The IR spectra in Figure 3 illustrate all characteristic groups for copolymer poly(D,L-lactide-co-glycolide). The IR spectra of DLPLG show peaks at 2994, 2946, 2840 (CH bend), 1769 (C=O ester), 1460, 1424, 1371 (CH_3), 1150, 1069 984 (C−O stretch), 732 509 (CH-bend) cm^{-1} while the band on 3100–3600 cm^{-1} belongs to the OH group of the water molecule [20].

Comparing the obtained IR spectra for DLPLG and ascorbic acid (Figure 4) with the IR spectra charecteristics for ascorbic acid shown in the literature [21, 22], it is confirmed that obtained nanoparticles are composed of poly(D,L-lactide-co-glycolide) and ascorbic acid. Besides the characteristic groups for copolymer DLPLG, the four O−H bands of ascorbic acid could be assigned by means of infrared investigations at 3528, 3411, 3317, 3217 cm^{-1}. The spectra show bands that can be assigned to CH_3,

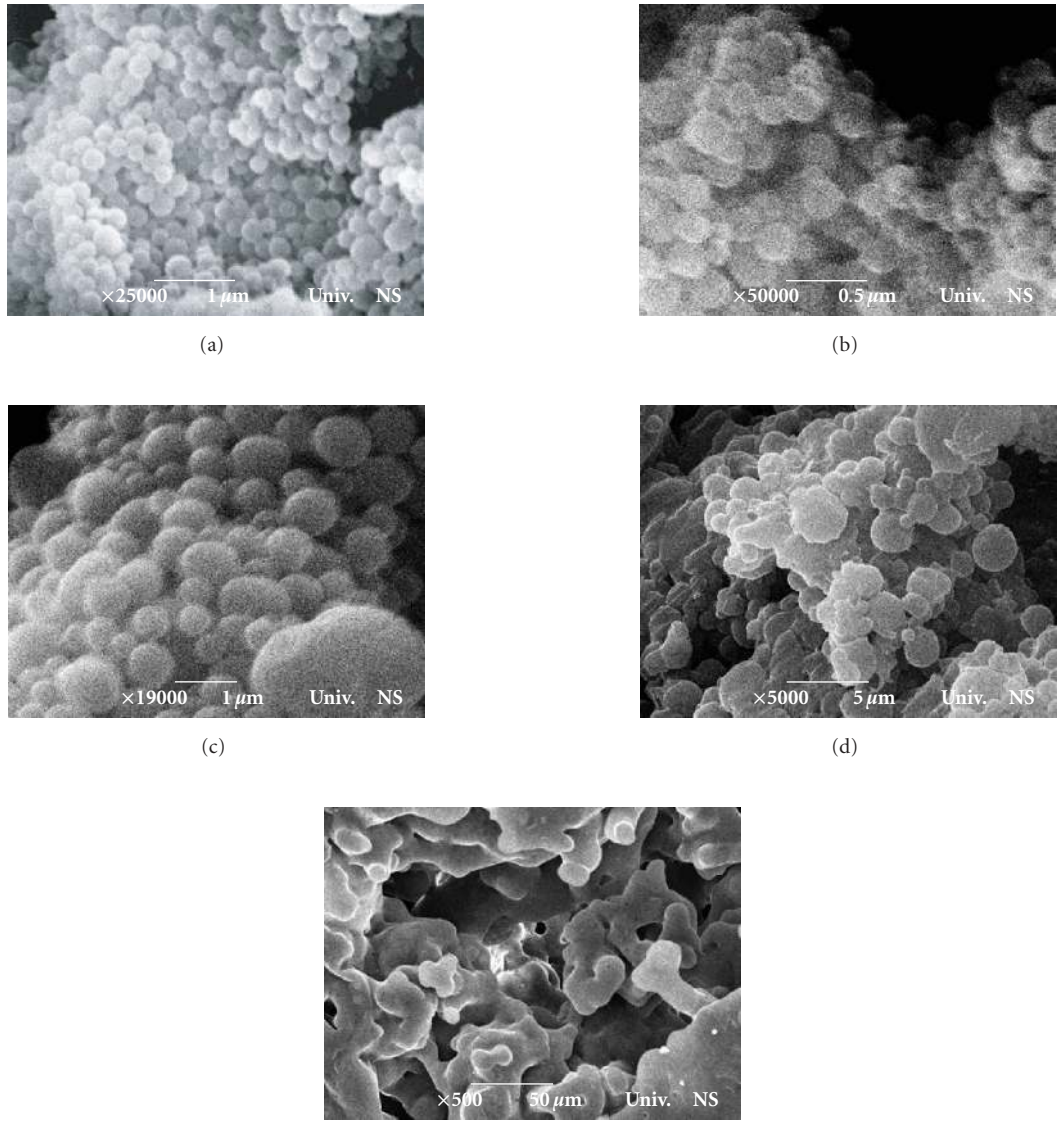

Figure 5: SEM images of (a) DLPLG nanoparticles; (b) DLPLG/ascorbic acid 85/15% nanospheres; (c) DLPLG/ascorbic acid 70/30%; (d) DLPLG/ascorbic acid 50/50%; (e) DLPLG/ascorbic acid 30/70%.

CH_2, or CH groups in the ascorbic acid environment at 2720 cm^{-1} and the spectra also clearly show the band corresponding to C=O groups at 2916 cm^{-1}. The bands that correspond to the wave number 1754 cm^{-1} belong to C=C groups, 1673 cm^{-1} C−O−C, and 1020 cm^{-1} C−O, respectively.

The morphological characteristics of the obtained DLPLG particles, with and without encapsulated ascorbic acid, were examined with a scanning electron microscope. From the SEM recordings of DLPLG particles without ascorbic acid (Figure 5(a)), it is visible that the particles have spherical shape, smooth surface, low level of agglomeration, and high level of uniformity—higher than other samples. From the SEM recordings of the second sample (Figure 5(b)), where DLPLG copolymer has encapsulated ascorbic acid in ratio DLPLG/ascorbic acid 85/15%, it is visible that particles also have spherical shapes, that is, spherical shape of the initial DLPLG has not been compromised. DLPLG/ascorbic acid 85/15% nanoparticles are uniform with sizes from 130 to 200 nm depending on which stereological parameters are considered (Dmax, maximum diameters, feret X, or feret Y). The particles of the sample DLPLG/ascorbic acid 70/30% (Figure 5(c)) also have spherical shapes, but their sizes are increased. In case of the fourth sample, DLPLG/ascorbic acid 50/50% (Figure 5(d)) uniformity is perturbated, particles have both spherical and irregular shapes and they are much agglomerated. For the fifth sample, DLPLG/ascorbic acid 30/70% (Figure 5(e)), the particles were very much agglomerated, so stereological analyses could not be performed.

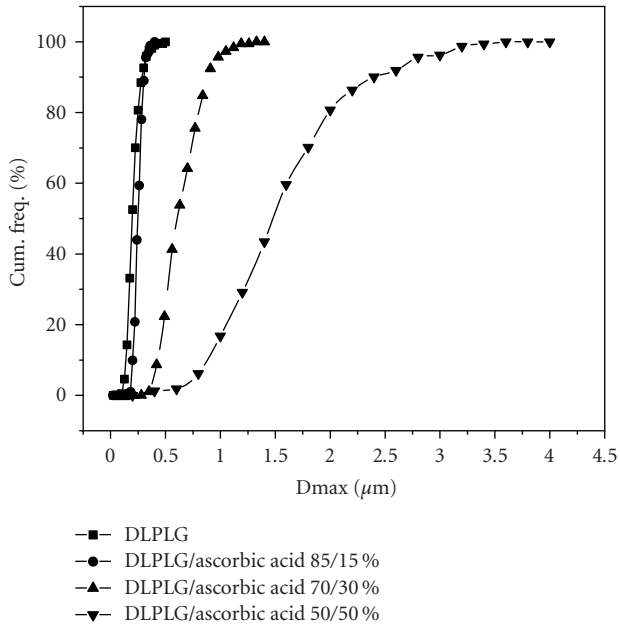

Figure 6: Comparative results of the stereological examining of DLPLG particles and particles with different ratio of DLPLG and ascorbic acid, DLPLG/ascorbic acid 85/15%, DLPLG/ascorbic acid 70/30%, DLPLG/ascorbic acid 50/50%, based on maximal diameter of the particle Dmax.

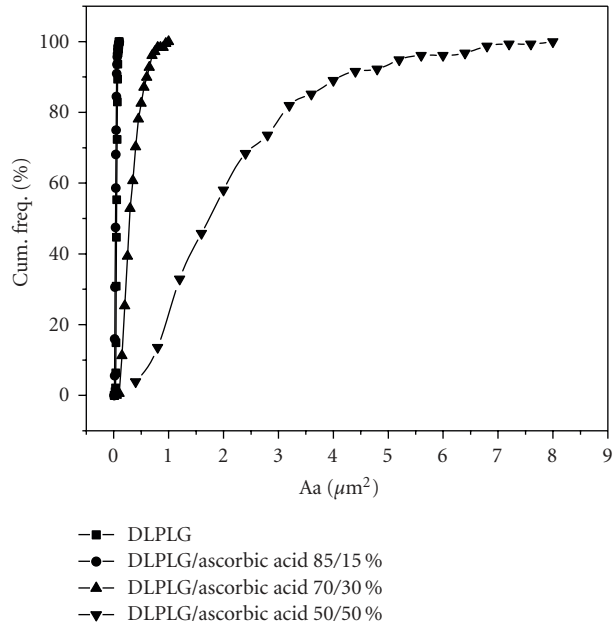

Figure 7: Comparative results of the stereological examining of DLPLG particles and particles with different ratio of DLPLG and ascorbic acid, DLPLG/ascorbic acid 85/15%, DLPLG/ascorbic acid 70/30%, DLPLG/ascorbic acid 50/50%, based on area section Aa.

The stereological analysis is giving us the parameters which are characterizing the particle sizes (area section—Aa, perimeter—Lp, maximal diameter—Dmax, and feret's diameters) and parameter which is characterizing the particle shape (perimeter form factor—fL). For all parameters, minimum, maximum, and mean values were recorded and presented in Table 1.

Based on the obtained results of the stereological analysis of DLPLG particles, it is visible that they are uniform, their average mean size varies from 0.15 to 0.23 μm depending on the stereological parameter taken in consideration (Dmax, feret X, or feret Y) (Table 1). Dmax values range from 0.09 to 0.39 μm with particle's mean size 0.23 μm (Figure 6). Figures 7 and 8 present comparative results of DLPLG particles with and without ascorbic acid based on their area section and perimeter form factor.

From the comparative results of the stereological analysis of the area section (Aa) of DLPLG with and without encapsulated ascorbic acid (Figure 7) as well as comparative results of the perimeter form factor (fL), (Figure 8) we can see that DLPLG particles without ascorbic acid have the smallest area section (minimum value for Aa is 0.02 μm^{2}and maximum is 0.08 μm^2) and the highest mean value of perimeter form factor which is 0.89. Nanoparticles DLPLG/ascorbic acid 85/15% have minimum Dmax of 0.09 μm and maximum Dmax of 0.49 μm, where their mean size is 0.20 μm (Figure 6). The mean value of the area section is 0.03 μm^2 (Figure 7) and of the perimeter form factor is 0.87 (Figure 8). For particles DLPLG/ascorbic acid 70/30%, minimum Dmax is 0.30 μm and maximum Dmax is 2.59 μm, where their mean size is 0.67 μm (Figure 6), which indicates that the uniformity is decreased and size is increased. The mean value of the area section is 0.41 μm^2 (Figure 7) and of the perimeter form factor is 0.77 (Figure 8). For particles DLPLG/ascorbic acid 50/50%, minimum Dmax is 0.28 μm and maximum Dmax is 4.51 μm, where their mean size is 1.60 μm (Figure 6). The mean size of the area section is 2.30 μm^2 (Figure 7) and of the perimeter form factor is 0.74 (Figure 8). In case of DLPLG/ascorbic acid 30/70%, the stereological analysis could not be performed.

The release amount of the ascorbic acid from the polymer particles was determined periodically during the eight weeks with UV spectroscopy. A calibration curve of the ascorbic acid in physiological solution at different concentrations has been prepared using the specific absorbance peak of the ascorbic acid at 264 nm.

Figure 9 shows the dependence of the maximum absorption from the degradation time in cases of DLPLG without ascorbic acid, DLPLG/ascorbic acid 85/15%, DLPLG/ascorbic acid 70/30%, and DLPLG/ascorbic acid 50/50%. This absorbance is correlated with the calibration curve and amount of ascorbic acid is determined in percentages. Figure 10 gives cumulative curves of the release of the ascorbic acid in percentages over the period of time of the degradation. Figure 10 also shows the relative review in percentages of the ascorbic acid release in periods of up to two days, 2–11, 11–17, 17–24, 24–31, 31–39, 39–46, and 46–55 days. In the first 24 days of the degradation, for all samples, less than 10% of the encapsulated ascorbic acid have been released. For all

Table 1: Results of the stereological analysis of DLPLG and DLPLG/ascorbic acid particles.

Ratio DLPLG/ ascorbic acid	Lp (μm)			Aa (μm)^2			Dmax (μm)			Feret x (μm)			Feret y (μm)			fL		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
100% DLPLG	0.19	1.12	0.81	0.02	0.08	0.03	0.09	0.39	0.23	0.09	0.25	0.15	0.09	0.28	0.19	0.49	0.91	0.89
85/15%	0.37	1.39	0.70	0.01	0.14	0.03	0.09	0.49	0.20	0.05	0.43	0.15	0.03	0.26	0.13	0.57	1.00	0.87
70/30%	0.95	8.92	2.40	0.06	4.63	0.41	0.30	2.59	0.67	0.17	2.19	0.46	0.17	1.65	0.48	0.48	0.92	0.77
50/50%	1.62	14.23	5.86	0.20	13.25	2.30	0.28	4.51	1.60	0.21	4.13	1.13	0.15	3.10	1.09	0.35	0.97	0.74
30/70%	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

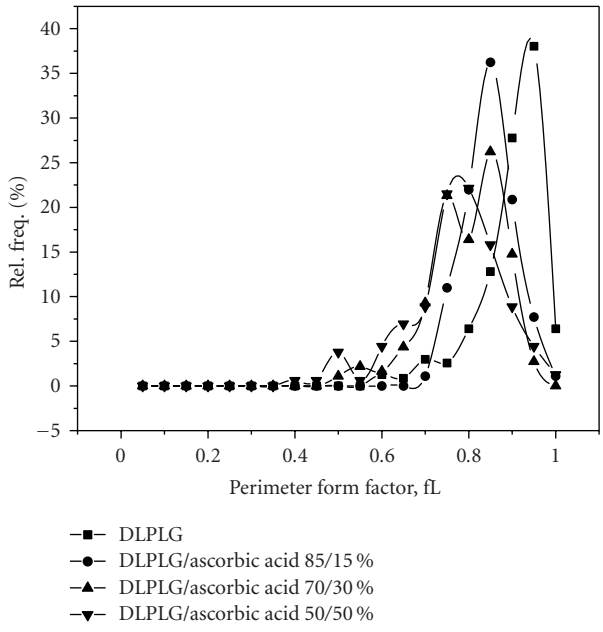

Figure 8: Comparative results of the stereological examining of DLPLG particles and particles with different ratio of DLPLG and ascorbic acid, DLPLG/ascorbic acid 85/15%, DLPLG/ascorbic acid 70/30%, DLPLG/ascorbic acid 50/50%, based on perimeter form factor fL.

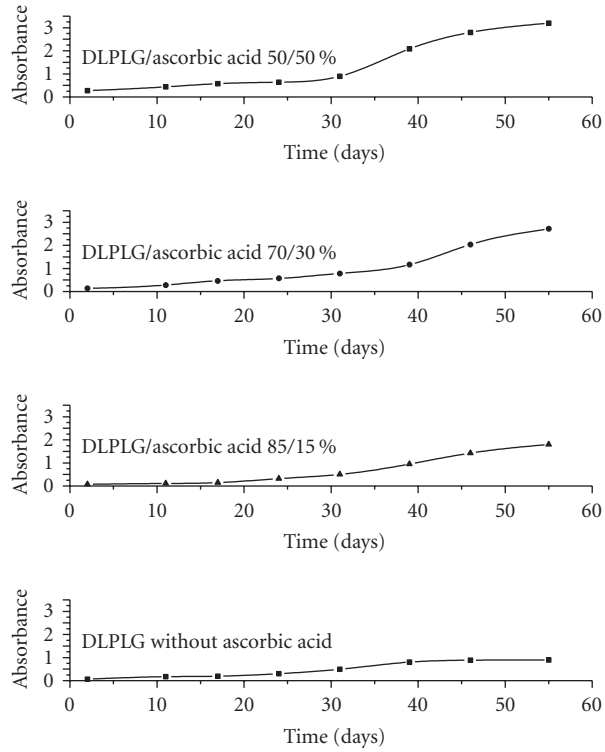

Figure 9: Comparative curves for the dependence of the maximum absorbance from the time of the degradation for the DLPLG with and without ascorbic acid.

DLPLG/ascorbic acid samples, the overall quantities of the encapsulated ascorbic acid have been released in 8 weeks of the degradation.

4. CONCLUSIONS

The particles obtained with solvent/nonsolvent physical method and technique of the centrifugal processing have potential use in transdermal systems for controlled delivery of ascorbic acid. It is possible to encapsulate ascorbic acid into DLPLG particles in various concentrations thus producing particles with different morphological characteristics. The nanoparticles of DLPLG/ascorbic acid with lesser ratio of ascorbic acid have higher uniformity, lower level of agglomeration, and smaller sizes. The nanoparticles of DLPLG/ascorbic acid 85/15% have spherical shapes and their sizes are from 130 to 200 nm.

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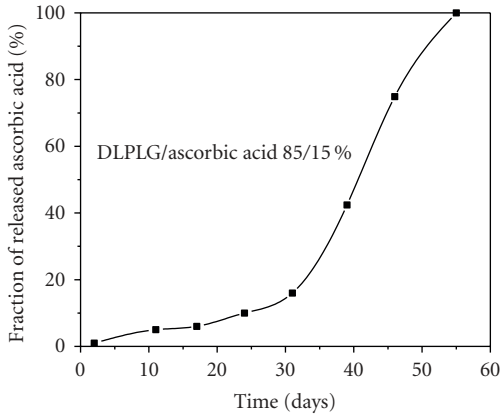
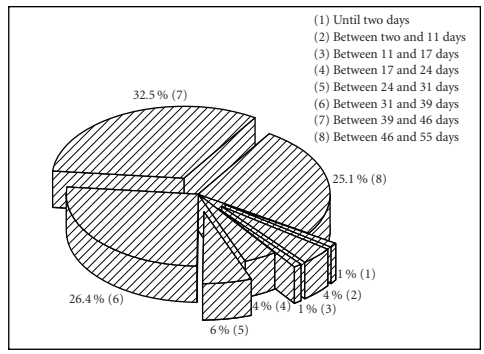
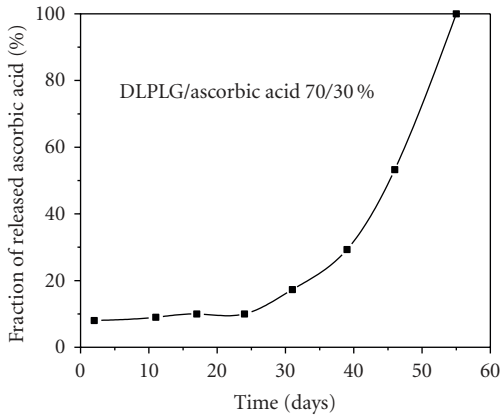


(a)



(b)



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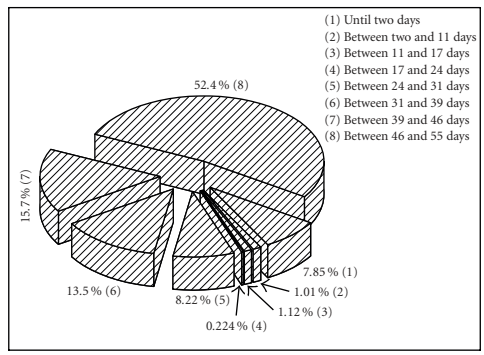

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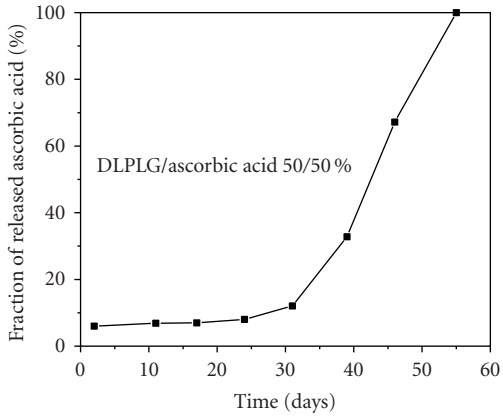

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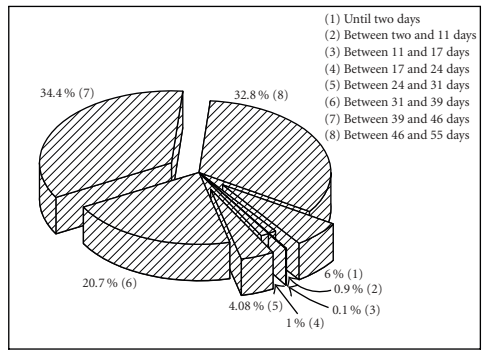

(f)

Figure 10: Release of the ascorbic acid in percentages over the period of time of the degradation: cumulative curves and relative review for (a)-(b) DLPLG/ascorbic acid 85/15%; (c)-(d) DLPLG/ascorbic acid 70/30% and (e)-(f) DLPLG/ascorbic acid 50/50% (relative review on figures (b)-(d)-(f): until two days (1), between two and 11 days (2), between 11 and 17 days (3), between 17 and 24 days (4), between 24 and 31 days (5), between 31 and 39 days (6), between 39 and 46 days (7), between 46 and 55 days (8)).

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