

# Stereological analysis of the poly-(DL-lactide-co-glycolide) submicron sphere prepared by solvent/non-solvent chemical methods and centrifugal processing

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**Abstract** Fine particles made of poly(lactide-co-glycolide) (DLPLG) are excellent candidates for controlled release of delivering drugs and genes, because of their degradable nature. The preparation of DLPLG submicron spheres poses serious challenges that are not necessarily present when preparing macroparticles. In the present paper, DLPLG powder is produced with chemical method using solvent/non-solvent systems with subsequent centrifugation of the solution. The samples were characterized by infrared spectroscopy (IR), scanning electron microscopy (SEM) and stereological analysis. By changing the aging time with non-solvent and time and velocity of the centrifugal processing, it is possible to influence on the morphology and uniformity of the copolymer particles. Powder of the series with short aging time with non-solvent and longest time and velocity of the centrifugal processing has smallest particles and highest uniformity, where mean particles sizes were between 150 nm and 230 nm depending on which stereological parameters are considered ( $D_{\max}$ , maximal diameters, feret X or feret Y).

## Introduction

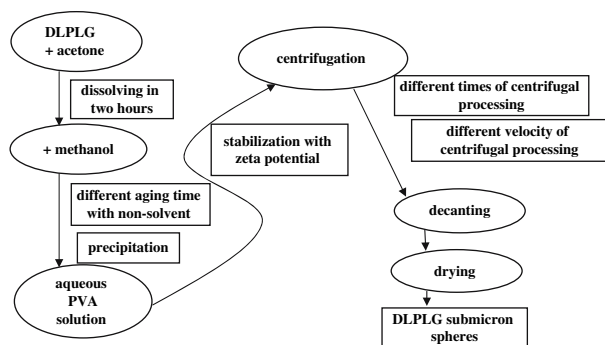
Biomaterials are under steady development, reaching its peaks in the last few years. The usage of biomaterials is becoming more and more significant. Biomaterials are used for replacement of a body part that has lost function due to disease or trauma, to assist in healing, to improve function, and to correct abnormalities [1]. Polymer biomaterials are synthetic polymers among which biodegradable polymers are particularly significant [2]. Biodegradable polymers with hydrolysable chemical bonds are being researched extensively for medical, pharmaceutical, agricultural, and packaging applications [3–5]. In order to be used in medical devices and controlled-drug-release applications, the biodegradable polymer must be biocompatible and meet other criteria to be qualified as a biomaterial-process able, sterilizable, and capable of controlled stability or degradation in response to biological conditions [4–6]. The degradation products often define the biocompatibility of a polymer, not necessarily the polymer itself.

Poly(esters) based on polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), and their copolymers have been extensively employed as biomaterials [4, 5]. Degradation of these materials yields the corresponding hydroxyl acids, making them safe for in vivo use. Biodegradable and biocompatible micro- and nanospheres made of poly(lactide-co-glycolide) are very potent drug and antigen delivery systems with inherent potential for drug and antigen targeting [6–8]. Until now a number of experiments for obtaining particles DLPLG have been performed using different approaches. There are several mostly used methods of preparing DLPLG and those are: a double

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**Fig. 1** Schematics for obtaining of the DLPLG submicron spheres

emulsion process [9], w/o/w emulsification solvent evaporation method [7, 10, 11], modified solvent evaporation/extraction technique (the single emulsion technique) [12], emulsion-diffusion-evaporation technique [13], extrusion [14], spray drying [15] and supercritical fluid extraction [16]. Emulsion process produced DLPLG spheres of 100–250  $\mu\text{m}$  [17], 45  $\mu\text{m}$  [18], 30  $\mu\text{m}$  [19] diameters. Modification of emulsion process led toward obtaining spheres with smaller diameters up to 10  $\mu\text{m}$  [20]. Further modifications of the process with additional evaporation produced spherical particles with diameters on submicron scale. First obtained submicron spherical particles were 570–970 nm [12] and 244–260 nm [21] in diameters. The latest researches in this field indicated the possibility of producing DLPLG spheres with average diameter under 100 nm [22, 23].

In this paper we show the results of the investigation of the influence of changes in experimental conditions (such as aging time with non-solvent, time and centrifugation velocity of the processing) on stereological characteristics of polymer poly-(DL-lactide-co-glycolide) (DLPLG) submicron powder. Bearing in mind that ideally spherical particles and narrow distribution of its sizes is a fundamental requirement for controlled and steady drug delivery, this research was focused on the stereological analysis of the obtained particles.

**Table 1** Experimental conditions for preparation of the DLPLG spheres

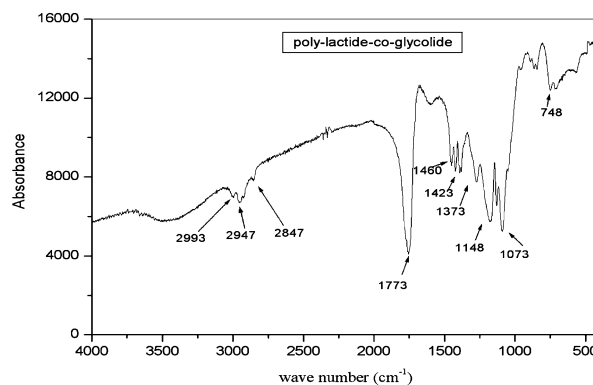
Powder DLPLG (series)	Aging time with non-solvent (min)	Time of the centrifugal processing (min)	Velocity of the centrifugal processing (rpm)
1	10	15	1500
2	30	30	3000
3	90	60	4000
4	5	60	4000
5	5	120	4000

## Experimental

Copolymer powder DLPLG was obtained by means of chemical methods from commercial granules (DLPLG, lactide/glicolide 50/50, SIGMA-Aldrich, Germany), using solvent/non-solvent systems. Molecular weight of polymer was 40,000–50,000 g/mol. Time of complete resorption of this polymer is 4–8 weeks. Commercial granules 50/50 poly-(DL-lactide-co-glycolide) (0.05 g) were dissolved in 1.5 mL of the acetone and, after approximately 2 h, 2 mL of methanol was added into solvent mixture. DLPLG precipitated by the addition of methanol and the solution became whitish. Series of the solvent were created with different aging time with non-solvent, which had been progressively changed (Fig. 1).

In the first three series, aging time was 10, 30 and 90 min, while fourth and fifth was about 5 min (Table 1). The polymeric solutions thus obtained were very slowly poured into 20 mL of aqueous PVA solutions (0.02% w/w) while continuously stirring at 1,200 rpm by a stirrer. After that the solutions were centrifuged and decanted (Fig. 1). Time and velocity of the centrifugal processing were varying, in the first series it was 15 min on 1,500 rpm, in the second 30 min 3000 rpm, in the third and fourth 60 min on 4,000 rpm, and in the fifth 120 min on 4,000 rpm (Table 1).

All used solutions are nontoxic for environment. The IR measurements were performed on Perkin-Elmer 983G Infrared Spectrophotometer, using the KBr pellet technique, in the frequency interval of 400–4,000  $\text{cm}^{-1}$ . 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 PVD process. Samples were covered with gold (SCD 005 sputter coater), using 30 mA current from the distance of 50 mm during 180 s. The particle size and

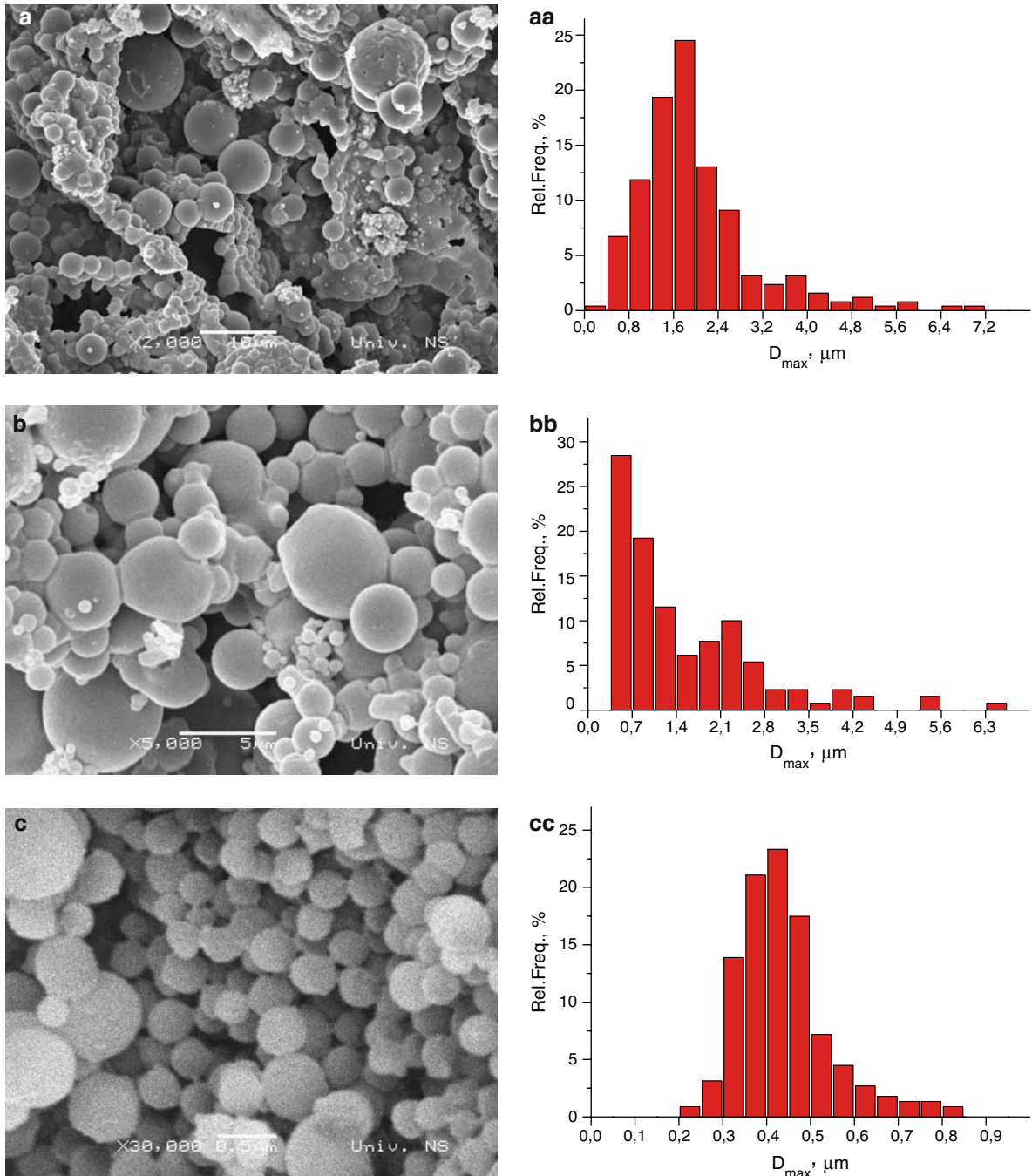


**Fig. 2** IR spectra of the DLPLG powder

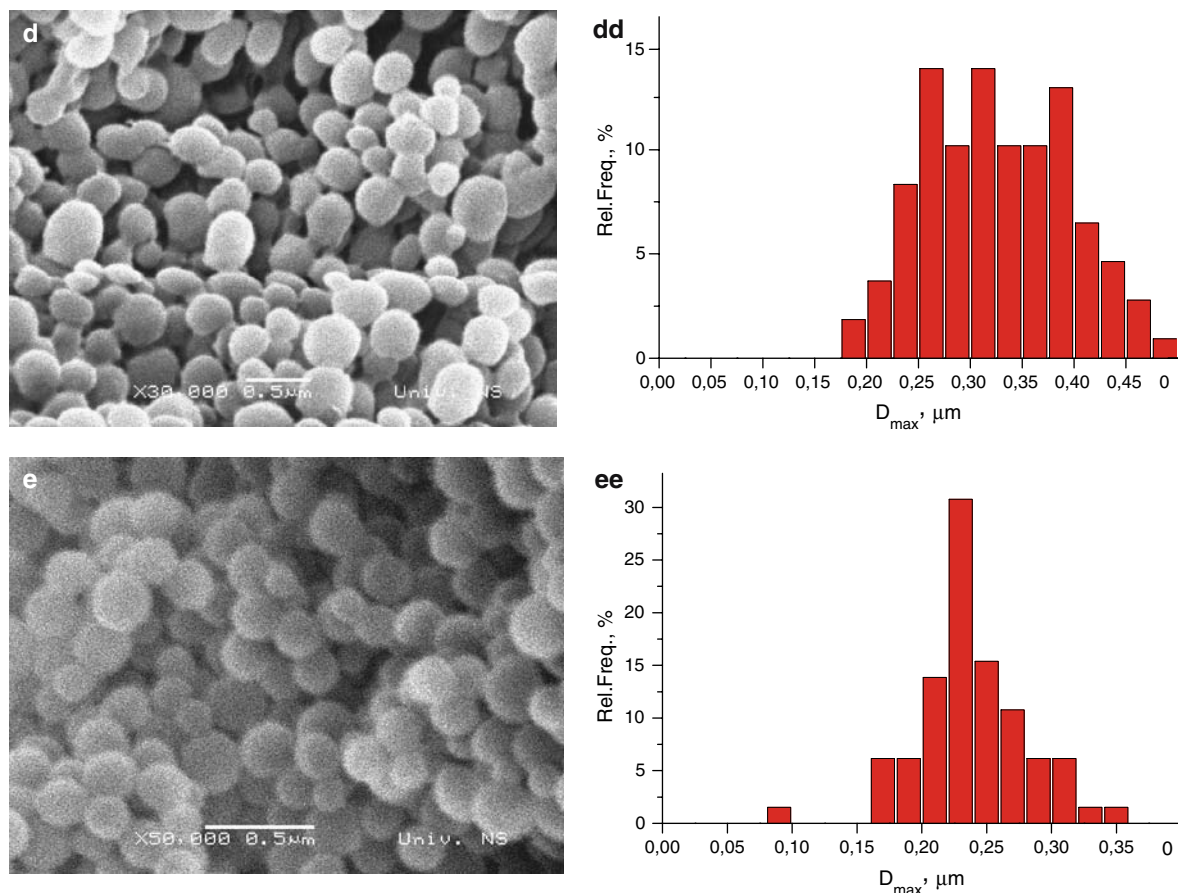
morphology were examined using the areal analysis method [24, 25] by semi-automatic image analyzer (Videoplan, Kontron), connected with a SEM. From 200–300 particles in the SEM were measured and the following parameters were determined: area section  $A_A$ , perimeter  $L_P$ , diameter of the largest particle,  $D_{max}$ , feret X and feret Y, and form factor ( $f_L$ ).

**Results and discussion**

The IR spectra in Fig. 2 illustrate all characteristic groups for copolymer poly(D,L-lactide-co-glycolide). The IR spectra of DLPLG show absorbance peaks at 2993, 2947, 2847 (CH bend), 1773 (C=O ester), 1460, 1423, 1373 (CH<sub>3</sub>), 1148, 1073 (C–O stretch), 748



**Fig. 3** SEM images of DLPLG powder and results of the stereological examining of DLPLG powder for diameter of largest particle: (a), aa, 1st series; (b), bb, 2nd series; (c), cc, 3rd series; (d), dd, 4th series; (e), ee, 5th series



**Fig. 3** continued

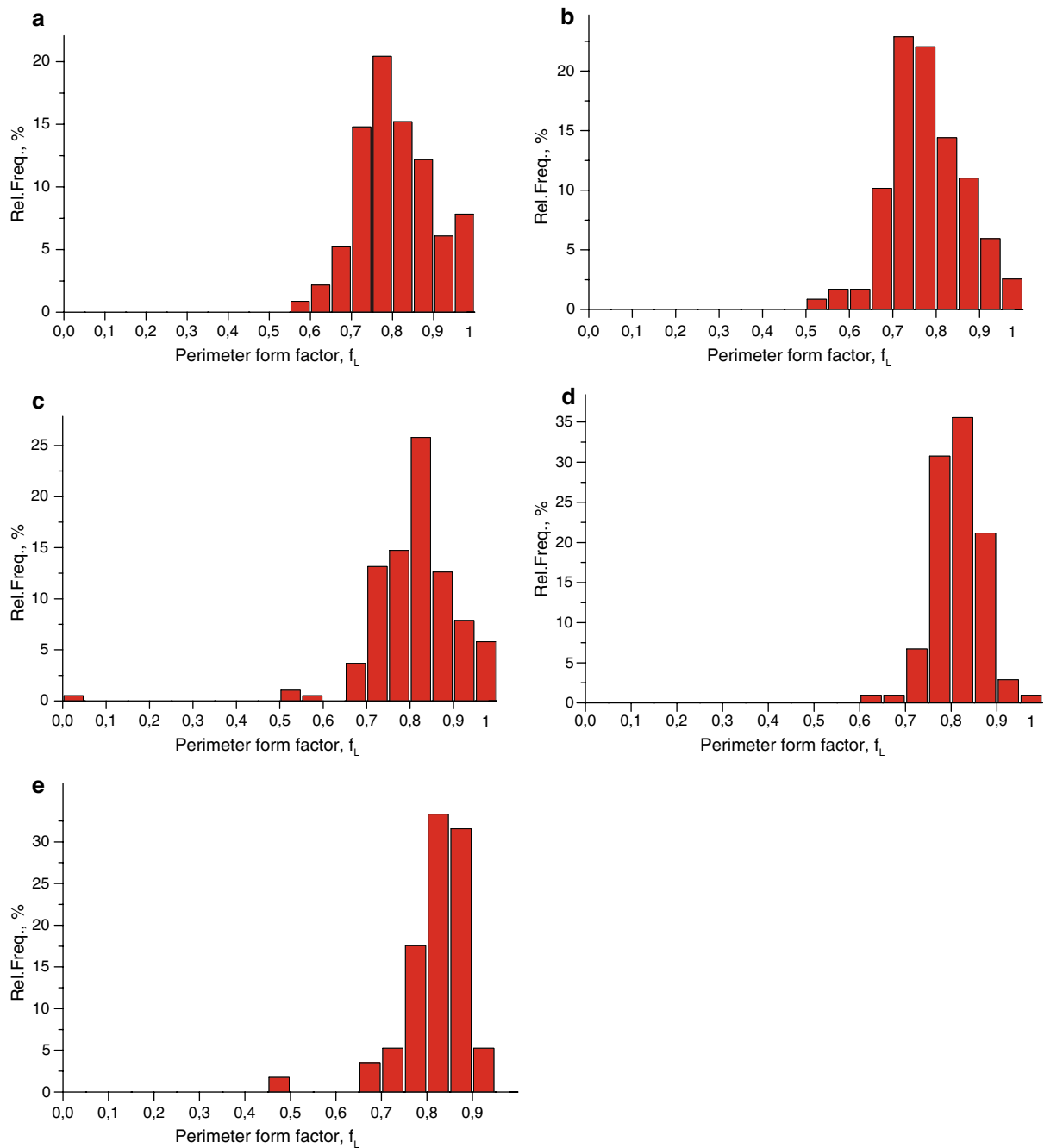
(CH-bend) $\text{cm}^{-1}$  while the band on  $3,100\text{--}3,600\text{ cm}^{-1}$  belongs to the  $\text{OH}^-$  group of the water molecule [26].

From SEM recordings of the first DLPLG powder sample (Fig. 3a), where aging time with non-solvent was 10 min and time and velocity of the centrifugal processing were shortest—15 min on 1500 rpm, we can see the presence of the spherical particles of different sizes in the wide specter where particles with larger dimensions are dominant. With the powder of the second series (Fig. 3b), where aging time with non-solvent was 15 min and time and velocity of the centrifugal processing were 30 min on 3,000 rpm, it is visible that the sizes of the

particles are in the wide spectra as with the first series and the larger particles are more dominant. From the SEM recordings of the third sample (Fig. 3c) we can see that uniformity is significantly increased and smaller particles are now more dominant. The particles of DLPLG powder of the fourth series (Fig. 3d) are smaller than previous series and uniformity is even more expressed. From the SEM recordings of DLPLG powder of the fifth series (Fig. 3e) we can see that increase in time of the centrifugal processing is increasing the uniformity, while at the same time reducing the size of the particles, where mean particles sizes are

**Table 2** Results of the stereological analysis of DLPLG powder

Powder DLPLG (series)	Lp ( $\mu\text{m}$ )			$A_A$ ( $\mu\text{m}$ ) <sup>2</sup>			$D_{\text{max}}$ ( $\mu\text{m}$ )			Feret X ( $\mu\text{m}$ )			Feret Y ( $\mu\text{m}$ )			Perimeter form factor ( $\mu\text{m}$ )		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean
1	1.18	40.5	6.48	0.15	91.3	4.17	0.4	10.6	2.08	0.28	7.98	1.46	0.18	8.44	1.45	0.57	2.3	0.89
2	1.02	19.9	4.65	0.08	27.2	2.1	0.36	6.62	1.53	0.22	5.39	1.02	0.25	4.58	1.12	0.53	1.23	0.8
3	0.1	3.67	1.59	0.1	0.76	0.18	0.23	1	0.44	0.16	0.69	0.32	0.11	0.71	0.29	0.1	1.28	0.85
4	0.64	1.66	1.11	0.03	0.18	0.08	0.19	0.48	0.32	0.12	0.33	0.2	0.15	0.4	0.25	0.64	0.95	0.81
5	0.19	1.12	0.81	0.02	0.08	0.04	0.09	0.34	0.23	0.09	0.25	0.15	0.09	0.28	0.19	0.49	0.91	0.82



**Fig. 4** Results of the stereological examining of DLPLG powder for perimeter form factor ( $f_L$ ): (a) 1st series, (b) 2nd series, (c) 3rd series, (d) 4th series and (e) 5th series

between 150 nm and 230 nm depending on which stereological parameters are considered ( $D_{max}$ , maximal diameters, feret X or feret Y).

Based on the stereological examining, we have selected parameters which characterize the particle size (area section— $A_A$ , perimeter— $L_p$ , diameter of largest particle— $D_{max}$  and feret’s diameters) and parameters, which characterize the particle shape (perimeter form factor— $f_L$ ). All parameters have

minimum, maximum and mean values determined. The obtained results are shown in Table 2.

From the results obtained from the stereological examining we can see that particles from the first series are not uniform and its sizes vary from 0.4  $\mu\text{m}$  to 10.6  $\mu\text{m}$  for  $D_{max}$ , where mean value of  $D_{max}$  is 2.08  $\mu\text{m}$  (Fig. 3a). Perimeter form factor characterize the shape of the particles and its mean value in case of the particles powder of the first series is 0.89 (Fig. 4a).

The powder of the second series is not uniformed either, but the obtained results show the particles size is considerably reduced with  $D_{\max}$  from 0.36  $\mu\text{m}$  to 6.62  $\mu\text{m}$ , where mean value of  $D_{\max}$  is 1.53  $\mu\text{m}$  (Fig. 3b). Perimeter form factor mean value of the particles from the second series is 0.80 (Fig. 4b). In case of the particles powder of the third series, the size is considerably reduced as it is shown through  $D_{\max}$ , which vary from 0.23  $\mu\text{m}$  to 1  $\mu\text{m}$  with mean value of 0.44  $\mu\text{m}$  (Fig. 3c). Mean value of the parameter form factor of the third series is 0.85 (Fig. 4c). For the fourth series minimum value of  $D_{\max}$  is 0.19  $\mu\text{m}$ , maximum value is 0.48  $\mu\text{m}$ , and mean value is 0.33  $\mu\text{m}$  (Fig. 3d). The particles are spherical and their mean parameter form factor is 0.81 (Fig. 4d). The most uniform particles with the smallest sizes and spherical shapes are present in the particles powder of the fifth series. The obtained particles have minimum  $D_{\max}$  0.09  $\mu\text{m}$ , maximum  $D_{\max}$  0.34  $\mu\text{m}$  and mean  $D_{\max}$  0.23  $\mu\text{m}$  (Fig. 3e). Mean parameter form factor is 0.82 (Fig. 4e).

The centrifugal processing is very powerful method in general performing separation and analysis of cells, biological macromolecules etc. The size and spherical shape of the particles are extremely important characteristics for the particles used for drug deliveries in the organism. The particles of various sizes and uniformities were obtained employing chemical method using solvent/non-solvent system and different conditions of the centrifugation. An influence of the centrifugation process on the particle sizes is supported by the fact that coefficient of friction for smaller and more compact spherical particles is lesser than of those with irregular shapes and same mass, which makes spherical particles sediment faster.

## Conclusion

Copolymer poly(lactide-co-glycolide) (DLPLG) is exceptionally important, from the medical but also from the pharmaceutical point of view, thus making it a subject of many researches. This research is covering a simple method for obtaining the particles of the huge uniformity with sizes on the submicron scale. Controlling the conditions of obtaining DLPLG; changing the parameters like aging time, after adding non-solvent, time and velocity of centrifugal processing; it is possible to influence on morphology (size and shape) and uniformity of DLPLG polymer powder. DLPLG powder with short aging time with non-solvent and longest time and velocity of the centrifugal processing has smallest particles and highest uniformity, where particles sizes are between 150 nm and 230 nm.

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