Preparation of submicroparticles based on biodegradable co-polyester

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Abstract**—** The preparation of submicro particles from biodegradable copolyesther by the method of emulsification and solvent evaporation at reduced pressure was investigated. During the study the influence of various process parameters and conditions such as stirring speed, amplitude of sonication and concentration of polymer on the particle diameter and particles size distribution was observed. It was found that it is not possible to achieve a diameter lower than 1 μm without sonication at a relatively high concentration of copolyesther. Without sonication the typical mean diameter ranged from 1-10 μm. On the other hand, sonication enabled to prepare particles with diameter lower than 1 μm. A significant influence of the concentration of polymer on final diameter was found, too.

Keywords**—**microparticles, Oil-in-water (O/W) emulsion solvent evaporation method, biodegradable copolymer

I. INTRODUCTION

THE increasing interest in the area of polymer microparticles is due to a number of promising applications in pharmacology [1], [2], agriculture [3], [4], and environmental engineering. One of such applications is filling polymer microparticles with bioactive a low molecular weight compound which is subsequently, under suitable conditions, released in a controlled way. This application has been extensively studied in recent years. A central issue is the releasing rate of active agents, which is influenced by various parameters, for instance particle diameters or particles size distribution (PSD), as described in [5]. The size of particles and PSD depends on the chosen method of preparation and the process parameters and conditions.

Micro and submicroparicles can be prepared in several ways. However, in the literature, two preparation processes predominate. The first one is the Oil-in-water (O/W) emulsion solvent evaporation method [6], [7]. It is a popular way to get microparticles, which is described in a number of research papers in details and can also be readily realized in the laboratory without the need for specialized equipment [8]. On the other hand, the second method, which is based on the utilization of supercritical fluids [9], [10], requires more sophisticated laboratory equipment.

Regarding the availability of the O/W method and the lack of sufficient data taking into account the influence of process parameters and conditions on the resulting particles diameter/PSD, the present work is focused on investigation and evaluation of these effects.

In some of research works [2], [3], [5], [7] which are oriented primarily on the release of bioactive compounds, the influence of the concentration of stabilizer in suspension, stirring speed [5], [7] and solvent [7] were observed. However, after closer study of the O/W emulsion solvent evaporation method, it is possible to find that the particles diameter and PSD are influenced by other process parameters and conditions, such as concentration of polymer in solvent, solvent/water ratio in emulsion or type of stabilizer. Particles which were investigated in all of these studies comprised encapsulated bioactive compound. It is presumable that even such low molecular weight compounds can have a certain influence on the size of particles.

In some papers [11]-[13] ultrasonication, which can considerably help to achieve smaller particles in the resulting suspension, was used during preparation. Although it is an indispensable factor in the preparation by the O/W method, the impact of process parameters such as time and amplitude on the resulting diameter and PSD has not been satisfactorily investigated and described yet.

In this study the possibilities of preparation of micro and submiscroparticles from commercially available biodegradable copolyester were tested. The influence of stirring speed of the homogenizer and sonication amplitude on the particle diameters and PSD is evaluated and compared with the literature.

II. MATERIALS AND METHODS

A. *Materials*

Polymer used throughout the study was aromatic-aliphatic copolyesther Ecoflex purchased from BASF (Germany). Airvol 205 - polyvinylalcohol (JVP, Japan) was applied as an emulsion stabilizer in the form of 0.5 % aqueous solution.

B. *Preparation of particles*

Micro and submicroparticles were prepared by the

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oil-in-water emulsion solvent evaporation technique. This method was carried out in the following way.

Firstly, the polymer (2, 1.5, 1, 0.5, 0.25 g) was dissolved in 10 ml of chloroform. The resulting concentration of polymer in chloroform (P/C concentration) was 200, 150, 100, 50, 25 mg ml^{-1}. Chloroform was primarily used because it has the higher boiling point in comparison with similar solvents such as dichloromethane. The relatively high value of boiling point ensures that chloroform will not evaporate from the mixture when the emulsion is prepared by vigorous stirring.

Then, the polymer solution was emulsified into 0.5 % (w/v) water solution of PVA (emulsifying agent) and dispersed under the continuous stirring by the homogenizer Ultraturax (IKA T18, Germany) at stirring speeds of 6000, 10000, 14000, 18000, 22000 and 24000 rpm. The mixture was stirred for 10 min. and during this period the produced emulsion was cooled with ice. The ratio between water solution of PVA and chloroform solution of Ecoflex was the same in all experiments (4:1).

Individual series of samples were marked S, U, A, C. For samples S ultrasonication was not used in second step of the procedure.

For labelings U, A, C, the obtained emulsion was ultrasonicated by ultrasonic probe (Hielscher UP 400S, Germany) for 5 min, when the cycle of sonication was 0.5 and amplitude 20, 25, 30, 35, 50, 70 %.

At last, the prepared emulsion (for all labels) was placed into the erlenmayer flask and stirred. Subsequently the organic solvent was evaporated from the emulsion under reduced pressure and the suspension of microparticles was formed. Reduced pressure was created by a membrane pump. The samples were stored in freezer at -20 °C for analyses.

C. Particle characterization

Particle parameters were measured with the help of optical microscope. During the microscopic investigation, the samples were placed on a glass slide with a graduated grid.

Where the diameter of particles allowed (particles smaller than 5 μm), the particle distribution was measured with Zetasizer (Zetasizer Nano ZS, Malvern Instruments, UK).

D. Evaluation of images from the optical microscopy

The length of the edge of a square in graduated field was 50 μm. From each image, six squares were randomly chosen for evaluation. The number of particles was counted in each square for all predetermined size intervals.

III. RESULTS AND DISCUSSION

Table I shows the conditions used to prepare the micro and submicroparticles and the obtained mean diameter (weighted average relative to the intensity of scattering). As can be seen, except for samples of S1-S6, the mean diameter of particles below 1 μm was achieved due to sonication.

In comparison with literature [5]-[7], where preparation was

TABLE I
THE CONDITIONS USED TO PREPARE THE MICRO AND SUBMICROPARTICLES AND THE OBTAINED DIAMETER

Sample	Stirring speed (rpm)	Polymer concentration (mg ml^{-1})	Ultrasonication	Amplitude (%)	Mean diameter of particles (μm)
S1	6000	100	no	-	9.44
S2	10000	100	no	-	4.74
S3	14000	100	no	-	2.60
S4	18000	100	no	-	2.64
S5	22000	100	no	-	1.94
S6	24000	100	no	-	1.74
U1	6000	100	yes	35	not determined
U2	10000	100	yes	35	0.94
U3	14000	100	yes	35	0.79
U4	18000	100	yes	35	0.94
U5	22000	100	yes	35	0.87
U6	24000	100	yes	35	0.57
A1	24000	100	yes	20	0.88
A2	24000	100	yes	25	0.79
A3	24000	100	yes	30	0.89
A4	24000	100	yes	35	0.57
A5	24000	100	yes	50	0.59
A6	24000	100	yes	70	0.72
C1	24000	25	yes	35	0.40
C2	24000	50	yes	35	0.61
C3	24000	100	yes	35	0.57
C4	24000	150	yes	35	0.95

performed without sonication, the diameter of the obtained particles was always in order of micrometers, whereas in research papers [12]-[14], where sonication was used, particles smaller than 1 μm were achieved.

The results are discussed in details in further paragraphs. It is necessary to say that the comparison with the mentioned research papers has some limitations, which will be discussed, too.

A. Influence of stirring speed on particle distribution

The mean diameters of particles for samples S1-S6 (described in Table 1) are shown in Figure 1.

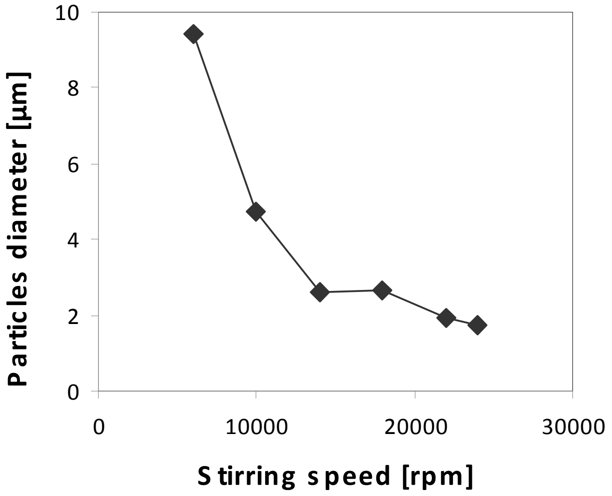

Fig. 1 Dependence of the mean particle size on stirring speed without ultrasonication

As can be seen, whereas in the interval from 6000 rpm to 14000 rpm the particle size is strongly affected by the stirring speed, for speeds over 14000 rpm the medium particle size remains almost constant. The application of higher stirring speed than 14000 rpm is not very efficient for the further reduction of mean diameter. As mentioned before, with stirring only we did not succeed to prepare particles under diameter 1 μm. In samples which were stirred at speed 14000 rpm and higher, the largest particles were reduced and mean diameter over 3 μm was achieved. However, the certain number of particles was bigger than 5 μm, therefore z-sizer could not be used to measure their diameters.

In the research work [6], where the same method and similar process conditions were used, the diameter of particles in the order of micrometer was also obtained. The authors observed polymer with the molecular weight higher than 100,000, which corresponds with ours (polymer molecular weight higher than 100,000).

It is presumable that the achievement of the diameter under 1 μm, when the similar polymer (molecular weigh higher than 100,000) and process conditions are used, is impossible without sonication. However, the particles with the diameter lower than 1 μm could be successfully obtained if some process parameters, such as initial concentration of polymer in the organic solvent, are changed. Unfortunately, there is a lack of information from the literature, to prove our presumptions.

The particles diameter obtained with each stirring speed was calculated from the data in Figure 2. In this figure, the comparison of particles size distributions for different stirring speeds is presented. The overall shift and the decrease of particle size distributions with the increasing stirring can be observed. The larger particles disappear gradually and ,practically, only smaller ones remain. The shift of PSD to the lower values of particle diameters can be probably achieved by using longer time of stirring. However, the mentioned assumption should be verified by further investigation.

Figure 3 shows the significant difference between particles prepared by the lowest stirring speed (6000 rpm) and the highest speed (24000 rpm). The diameter of all particles was approximately determined with the help of calibrated grid, which is perceptible in the figure.

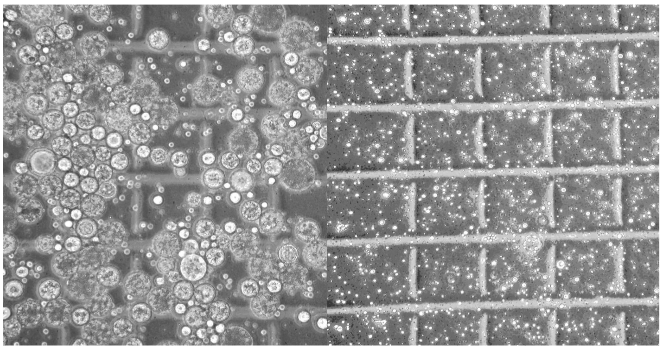

Fig. 3. Particles prepared at 6000 rpm (left) and 24000 rpm (right) the size of calibration chamber squares is 50 μm

As can be seen, the number of particles increased and size decreased with higher of stirring speed. This trend results in the inaccuracies of the measurement of the particle diameter and subsequent calculations for higher stirring speeds.

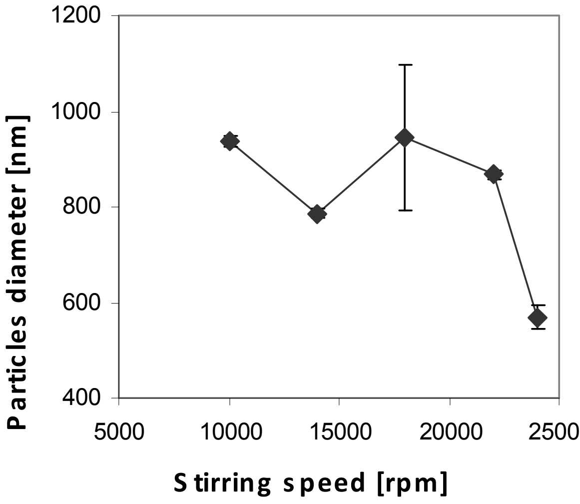

Fig. 4 Dependence of the mean particle size on stirring speed with ultrasonication, points are averages from 3 measurements. Error bars represent twice standard deviations.

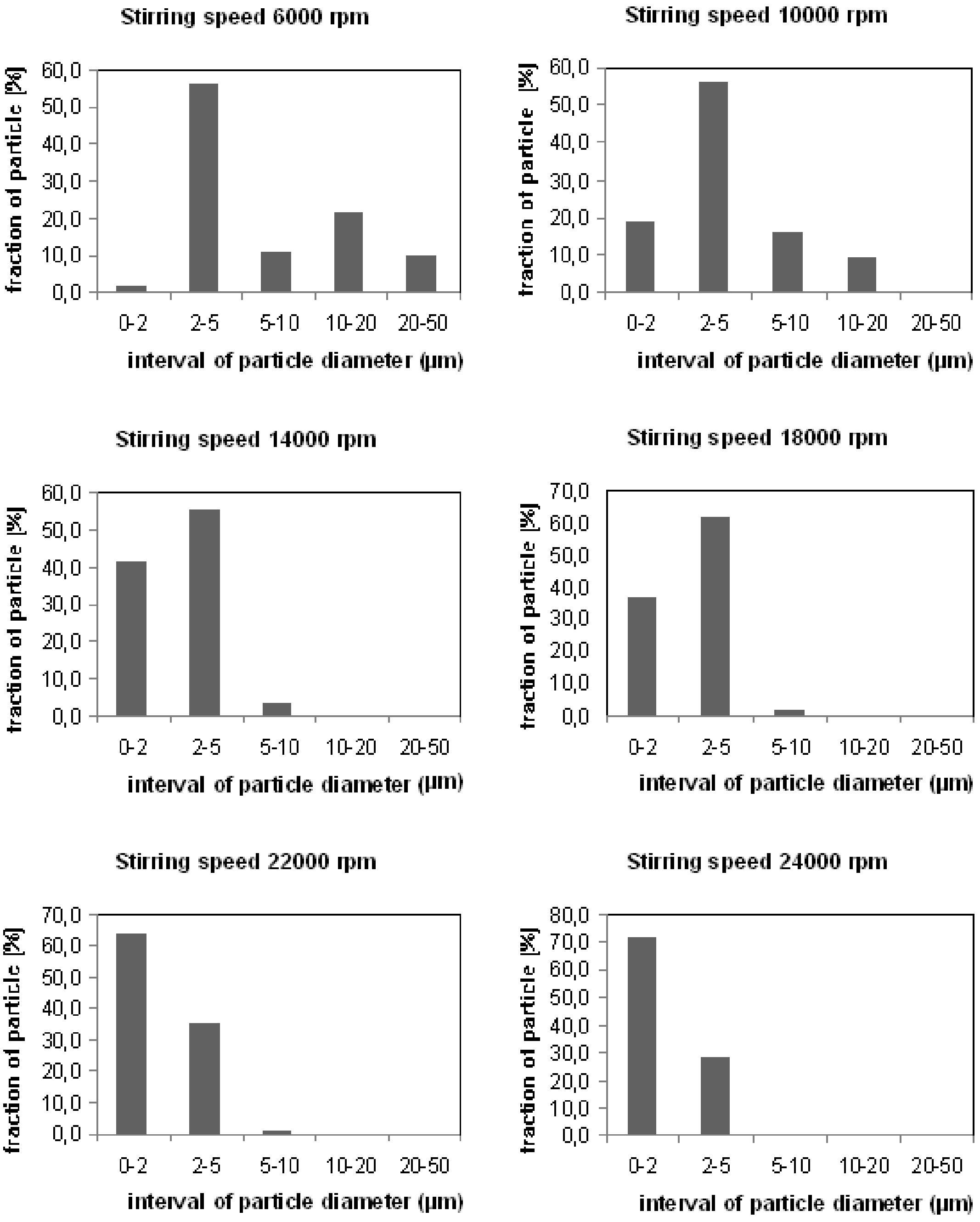

Fig. 2 Comparison of particles size distributions for different stirring speeds.

Figure 4 shows the dependence of particles diameters on stirring speeds. In this case ultrasonication was used during the preparation. The process parameters for samples U1- U6 are described in Table 1.

The mean particle diameter was reduced under 1 µm. After ultrasonication, PSD were shifted significantly toward lower diameters, which made possible to use z-sizer for their characterization. As can be seen in figure 4, the measured values of particles diameter for stirring speed 6000 rpm were not used because they seem to be unreliable. The wider standard deviation of particle diameter for stirring speed 18000 rpm is probably caused by non-homogeneity of the sample. It is evident that the particle diameter after ultrasonication is not so strongly dependent on the stirring speed. For almost all stirring speeds the particles diameters are very similar. However, for the highest homogenization stirring speed (24000 rpm) significantly smaller particles were obtained.

In literature [12]-[14], much smaller particle diameter was achieved. Main reason seems to be the use of considerably lower concentrations of polymers, which had, together with amount of stabilizer in suspension, important influence on the final mean diameters and PSD.

In [13] lower particle diameter was achieved even with electromagnetic stirring only (stirring speed 500 rpm). It is possible that ultrasonication has bigger influences on particles diameter and PSD than stirring speed. However, in all of these research papers the polymers with the molecular weight lower than 100,000 were used in contrast with Ecoflex which has the molecular weight higher than 100,000.

B. Optimization of ultrasonication procedure

Figure 5 presents the dependence of particle size on the amplitude of ultrasonication for the series of samples A1 – A6. As can be seen, the amplitude was in the range of 20 % to 70 %. A sharp critical value close to 35% amplitude is apparent from the figure. It is very interesting that larger particles are formed for the amplitude 70 % than for the 35 %.

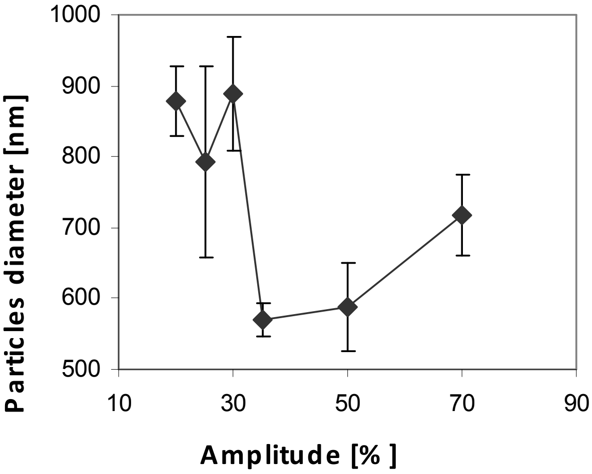

Fig. 5 Dependence of mean particle size on the amplitude of ultrasonication parameter. Points are averages from 3 measurements. Error bars represent twice standard deviations.

It could be explained by the partial agglomeration of particles instead of their reduction, which is caused by the great amount of energy delivered into the emulsion and possibly the local increase of temperature using higher amplitudes.

C. Influence of the polymer concentration

Finally, the measurement was performed for the series of samples C1-C5. The influence of polymer concentration was investigated.

As can be seen from Figure 6, the particle diameter increased rising the concentrations. The trend of increasing particle diameters with rising concentrations was also detected in [12] and [14] and could be explained in various ways, for example too small volume of the water phase, too short time of stirring and ultrasonication for higher concentrations of the polymer. The concentration of emulsion stabilizer (PVA) in emulsions has probably a crucial influence. For a large quantity of Ecoflex in the emulsion, the amount of stabilizer is unable to cover the surface of all of the small particles formed.

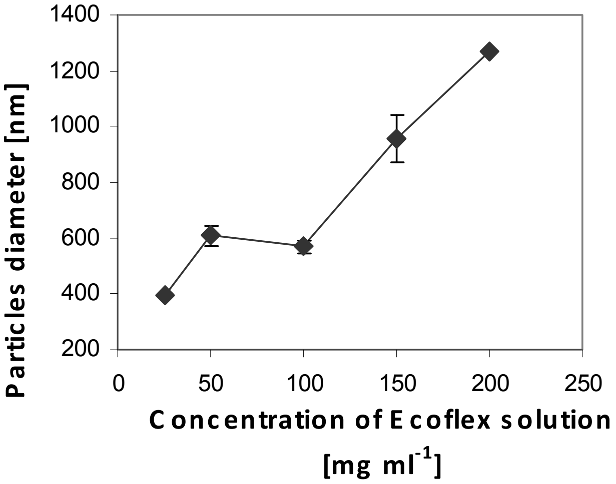

Fig. 6 Dependence of the mean particle size on the concentration of Ecoflex solution. Points are means from 3 measurements. Error bars represents twice standard deviations.

In the paper [14] authors presumed that the enlargement of nanoparticles is probably caused by the increasing viscosity of dispersed phase (more concentrated polymer solution), resulting of a poorer dispersability of the polymer solution into the aqueous phase caused by a high viscous resistance against the shear forces during emulsification. From which it follows that it is possible to obtain smaller particles from more concentrated polymer solutions by using either longer time of stirring or ultrasonication or both.

In our case, the linear dependence of the particle diameter on the concentration of Ecoflex solution can be observed. In [14], where three different but rather low concentrations were investigated the values of particles diameters for the two lowest concentrations were almost identical. It is possible, that lower diameters are not achieved by further reduction of the concentration under some limit.

IV. CONCLUSION

The study has proved that it is possible to prepare micro and submicro polymer particles in the procedure comprising emulsification and organic solvent evaporation at reduced pressure. Among the investigated processing parameters, sonication was found to be the crucial step in particle preparation that makes possible to prepare particles of diameters under 1 μm.

Further research should be focused on the investigation of the influence of other process parameters, namely the nature of the emulsifying agent and concentrations of all components used throughout the procedure and their influence on the particle size.

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