Optimization of biodegradable polymeric submicroparticles preparation STLOUKAL PETR², KOUTNY MAREK^{1,2}, SEDLARIK VLADIMIR¹, PAVEL KUCHARCZYK¹

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Abstract: The influence of various surfactants and their combinations on the particle diameter and particles size distribution during their preparation from biodegradable poly(lactide acid) by the method of emulsification and solvent evaporation at reduced pressure was investigated. The attention was focused on the possibility to control the size of resultant particles by hydrophilic-lipophilic balance parameter (HLB), which is a characteristic of nonionic surfactants. First, nonionic surfactants were combined to obtain various HLB values of the system and then combinations with the biodegradable surface active polymer Gelatin A were assayed. Gelatin A proved to be a suitable emulsion stabilizer in the process of PLA submicroparticles preparation and it was found that to achieve PLA submicroparticles with the lowest diameter and narrow distribution HLB of the system has to be set up under about 16. For combinations of gelatin A and another nonionic surfactant the reduction of the aggregate HLB value of the system could better be achieved with an addition of a surfactant having HLB closer to the optimum like Tween 20 or Tween 85.

Key-Words: - microparticles, poly(lactide acid), Oil-in-water (O/W) emulsion solvent evaporation method, biodegradable copolymer, nonionic surfactant, gelatin

1 Introduction

In the last three decades polymer microparticles have found number of applications in the pharmaceutical industry [1], [2], agriculture [3], [4], and environmental engineering. One of such promising applications is filling polvmer microparticles with a bioactive low molecular weight compound which is subsequently, under suitable conditions, released in a controlled way. This application has been extensively studied in recent years. The most important feature is the releasing rate of the active agent, which is influenced by various parameters, for example by particle diameter or particles size distribution (PSD) [5]. The size of particles and PSD depends on the applied method of their preparation and the process parameters and conditions.

Micro and submicroparticles can be prepared by several methods. One of them, readily feasible in laboratory without the need for specialized equipment, is oil-in-water evaporation technique, which was described in details in a number of research papers [6, 7]. Here the utilization of an appropriate surfactant in an adequate amount is necessary to obtain microparticles of required size [8].

In many research works [2, 3, 5, 7] which were

focused primarily on the release of bioactive compounds, the effects of some process parameters and conditions such as the concentration of stabilizer in suspension, stirring speed [5, 7] and type of solvent [7] were observed.

In the O/W emulsion solvent evaporation method the particle diameters, PSD and even the shape of particles could also be affected by the type and amount of surfactant [9, 10].

However, little could be found in the literature about the influence of various surfactants, especially in the case of nonionic surfactant, influence of hydrophilic-lipophilic balance parameter (HLB) on the particles diameter, PSD and shape of microparticles. The hydrophilic-lipophilic balance (HLB) measure the relative influence of hydrophilic and hydrophobic parts in the surfactant molecule. It is a value between 0-60 characterizing the affinity of a surfactant for water or oil [11].

In present work, the influence of three types of nonionic surfactants and their mixtures with different HLB values on particle diameters and PSD was observed. Furthermore, a similar investigation was realized with gelatine alone as a surfactant or with a mixture of gelatin with some other non-ionic surfactant and mentioned effects were evaluated. The gelatin is considered as potentially good stabilizer for environmental applications due to its low price and good biodegradability. Particles were prepared from low molecular biodegradable poly(lactide acid) (PLA).

2 Materials and methods

2.1 Material

The polymer used throughout the study was polylactide acid (PLA, $Mw = 67,400 \text{ g mol}^{-1}$, Mw/Mn = 2) prepared in our laboratory (Tomas Bata University, Zlin). PLA was synthesized through direct melt polycondensation of lactic acid. Firstly, 50 g of 80% L-lactic acid (optical purity 99.9%, purchased from Penta s.r.o) was put into two-neck flask connected with condenser. The mixture was stirred and heated for 4 hours at 160 °C under reduced pressure of 20 kPa. After 4 hours 0.5 wt. % of catalyst tin (II) octoate (Sn(Oct)2) was added. The pressure was decreased to 0.1 kPa and continued for next 24 reaction hours. Finally, product was cooled down, dissolved in acetone, precipitated into water: methanol (1:1) solution, filtrated and washed with water several times. Obtained white powder was dried at 50°C for 24 hours and subsequently analyzed by GPC [12].

The surfactant used in the oil-in-water evaporation technique was Gelatin from porcine skin type A, and non-ionic surfactants Pluronic F68, Tween 20, Tween 85 and Span 40 (Sigma Aldrich).

2.2 Preparation of particles

Micro- and submicro-particles were prepared by the oil-in-water emulsion solvent evaporation technique. This method was carried out in the following way.

Firstly, the 400 mg of polymer was dissolved in 4 ml of chloroform. The resulting concentration of polymer in chloroform (P/C concentration) was

100 mg ml⁻¹. Chloroform was used because it has higher boiling point in comparison with similar solvents like e.g. dichloromethane. The relatively high value of boiling point ensures that chloroform do not evaporate from the mixture prematurely, when the emulsion is prepared by vigorous stirring.

Secondly, the polymer solution was emulsified in water solution of various surfactant systems (emulsifying agent) under continuous stirring with homogenizer DI 18 basic (Yellow line by IKA, Belgium) at stirring speed of 24000 rpm. The mixture was stirred for 5 min. and during this period the forming emulsion was cooled with ice. The ratio between water solution of surfactant and chloroform solution of PLA was identical in all experiments (4:1).

Subsequently, the obtained emulsion was ultrasonicated by ultrasonic probe (Hielscher UP 400S, Germany) for 5 min. (cycle 0.5; amplitude 35%).

At last, the prepared emulsion was transferred into the erlenmeyer flask, stirred, and the organic solvent was evaporated from the emulsion under reduced pressure which finally leads to the formation of microparticle suspension. The samples were stored in freezer at -20 °C for SEM.

2.3 Particles characterization

The particle size distribution was measured with Zetasizer (Zetasizer Nano ZS, Malvern Instruments, UK). The principle of method allows to measure the particles up to 5 μ m. Larger particles were judged with the help of optical microscopy only.

2.4 Scanning electron microscopy

Instrument VEGA-II (Tescan) was used to visualize the size and shape of particles in selected samples. Particles were washed with distilled water three times in order to remove soluble substances. Then samples were coated with gold-platinum layer under reduced pressure 2-2.1 kPa.

3 Results and discussion

3.1 Particle preparation with nonionic surfactants

Table 1. lists surfactants and binary combinations of surfactants which were used in the experiment as emulsion stabilizers. Furthermore, concentrations of surfactants in water solutions, HLB values which were calculated as weighted averages in the case of surfactant combinations and the obtained z-averages of the resulting particles are given. Z-average is defined as the intensity weighted mean of hydrodynamic diameters of the ensemble collection of particles measured [13]. In the case of using the pure Pluronic F68 and Tween 85 microparticles were not obtained because the collapse of emulsion and PLA precipitation occurred in the phase of solvent evaporation, in contrast with the literature [10] where the use of Pluronic was described. It could be explained by lower ratio of PLA and the surfactant in the mixture.

% concentration of						
Surfactants	surfactant (w/v)	HLB	Z-average of particles, nm			
Pluronic F68	1	29.0	Precipitate			
Pluronic F68/Tween 20	0.75/0.25	25.9	508.5			
Pluronic F68/Tween 20	0.5/0.5	22.9	393.8			
Tween 20	1	16.7	315.7			
Tween 85/Tween 20	0.5/0.5	13.9	358.7			
Tween 85/Tween 20	0.75/0.25	12.4	332.1			
Tween85	1	11.0	Precipitate			

 TABLE 1.

 PREPARATION OF SUBMICROPARTICLES WITH DIFFERENT SURFACTANTS AND THEIR COMBINATIONS

Precipitate, coarse PLA precipitate occurred in the phase of solvent evaporation

Fig. 1. presents the dependence of particle zaverages on HLB. As can be seen, the smallest particles were obtained for HLB = 16.7, when Tween 20 was used. Whereas, for higher HLB values prepared by mixing Pluronic F68 and Tween 20 the particle size was strongly affected by HLB increase in lower HLB interval achieved by Tween 20 and Tween 85 combinations z-averages did not changed significantly. However, lowering HLB to 11 lead to PLA precipitation and particles were not obtained as was already mentioned. It could be presumed that Tween 20 presence has crucial influence on the emulsion stability and the resulting particle size.



Fig. 1. Dependence of z-average on concentration of nonionic surfactants. Error bars correspond to twice standard deviation (n=3)

3.1 Particle preparations in gelatin A presence

Table 2. shows z-averages of particles prepared with 0.5% Gelatin A and one additional nonionic surfactant in selected concentrations.

First preparation of particles was made with 0.5% water solution of gelatin A only to compare and evaluate the influence nonionic surfactant addition on the resulting particle size. Z-averages resulting from different particle preparations were plotted in Figure 2. It could be presumed that gelatin has hypothetically rather high HLB value due to its high hydrophilicity. As it was found previously the smallest particles was formed for HLB value of about 16 thus it could be suppose that the size of particles could be reduced by addition of nonionic surfactants surfactant and HLB lowering.

As could be seen, z-average of particles is nearly always reduced by addition of nonionic surfactant. Except for the combination with Pluronic F68, some upper limit of the surfactant concentration, when rather coarse suspension of particles was obtained, could always be observed. Under such conditions the z-average could be measured further by the employed technique. As can be seen in Table 2., this limit decreases with decreasing HLB of nonionic surfactants.

The dependence of particle z-averages on surfactant concentrations in combination with 0.5% water solution of gelatin A are shown in Figure 2. Smaller particles were obtained using Tween 20 even for the lowest concentration applied (0.1 %). Particle z-averages were reduced considerably in comparison with particles prepared with gelatin as the only surfactant. However, the size of the particles was not reduced significantly by further addition of this surfactant. On the contrary, at

	Z-average of particles, nm				
0.5 % of Gelatin A	Pluronic F68	Tween 20	Tween 85	Span 40	
Surfactant concentration (w/v)	(HLB = 29)	(HLB = 16.7)	(HLB = 11)	(HLB = 6.7)	
0	722.1				
0.1	548.9	362.6	474.4	706.2	
0.2		353.8	455.3	>5000	
0.3	705.3	318.8	390.5	>5000	
0.5		376.2	453.9		
0.6	490.0	319.7	>5000		
0.9	605.8		>5000		
1		310.7			
2	850.7				

 TABLE 2.

 PARTICLES PREPARED BY THE COMBINATION OF GELATIN A AND ANOTHER NONIONIC SURFACTANT

>5000 means that part particles in sample was larger than 5 μm

higher surfactant concentrations coarse particles were obtained. The observations could be explained by the fact that the higher concentration of e.g. Tween 20 increases HLB of the system too far from the optimal range which obviously is the HLB interval where the most stable emulsions and finest particles are obtained.

As it is evident, the addition of Pluronic in some concentration interval reduced particles z-averages. However, for concentrations 0.6, 0.9 and 2 PSD got an upward trend and for the latter particles larger than those without the addition of Pluronic were



Fig. 2. Dependence of z-average on concentration of surfactants in mixture with 0.5% Gelatin. \Box , Pluronic F68 (HLB=29); \blacksquare , Tween 20(HLB=16.7); \bullet , Tween 85(HLB=11). Error bars correspond to twice standard deviation (n=3).

formed. Increasing PSD and its inhomogeneity with the rise of Pluronic can be observed in the Fig. 3.

In the case of addition of Tween85, first particle z-averages decrease slightly to achieve the minimal value of z-average, with increasing concentration the value raised and got over 5 μ m limit. The observations could be again explained by the effect of optimal HLB value for the system. Gelatin HLB was lowered by addition of low HLB value surfactant to achieve some optimal value when the smallest particles are formed. Further addition of low HLB value surfactant led to an excessive decrease of HLB, destabilizing the system and coarse particles formation.

4. Conclusions

It could be concluded that gelatin A proved to be a suitable biodegradable surface active agent and emulsion stabilizer in the process of PLA submicroparticles preparation. Gelatin A probably combines effects of steric protection and its surface properties. On order to active get PLA submicroparticles with lowest diameter and narrow distribution HLB of the system has to set to the optimum value which appears to be under about 16. In case of the combination of gelatin A, with apparently high HLB, and another nonionic surfactant the reduction of the aggregate HLB value of the system could better be achieved with an addition of a surfactant having HLB closer to the optimum like Tween 20 or Tween 85.



Fig. 3. Scanning electron microscopy of polymer submicroparticles $(5000\times)$; A, prepared with using 0.5% solution of Gelatin A; B, prepared with using mixture 0.5% Gelatin A and 1% Tween20 solution; C, 0.5% Gelatin A and 0.6% Pluronic F68 solution; D, 0.5% Gelatin A and 0.9% Pluronic F68; E, 0.5% Gelatin A and 2% Pluronic solution.

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