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Studies on the Preparation of Nanoparticles from Betulin-Based Polyanhydrides †

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Abstract: Nanoparticles were obtained by nanoprecipitation and by emulsion solvent evaporation (ESE) method. In ESE method, the size of the particles depended on the type and concentration of surfactant (in the water phase) and the polymer concentration (in the organic phase). The best results were obtained with ionic surfactants, however, the use of such compounds may accelerate the degradation process of polymers. In nanoprecipitation method, the ratio of solvent (methylene chloride) to non-solvent (hexane) has a significant influence on the particle size. The smallest particles were obtained with a solvent to non-solvent ratio of 1:150.

Keywords: betulin; polyanhydrides; biodegradable polymers; nanoparticles

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1. Introduction

Polymeric nanoparticles are considered as useful carriers in controlled drug delivery systems. Due to their small sizes and high surface area, they have many advantages. Polymer-based nanocarriers may improve solubility and bioavailability of highly insoluble hydrophobic drugs, increase the stability of volatile pharmaceutical agents or deliver a higher concentration of drugs to a desired location [1,2]. They can be used for treatment of various disease, including bacterial, fungal, hypertension, asthma or cancer [3]. Polymeric nanoparticles can be prepared from both synthetic polymers, e.g., polycaprolactone, poly(methyl methacrylate) and poly(lactide-co-glycolide), or natural polymers, such as gelatin, chitosan, sodium alginate or albumin [2,3]. Polymers used in nanoparticle formulation should be biocompatible, non-toxic and non-antigenic [4].

Polyanhydrides are FDA-approved, biodegradable polymers that possess favorable properties as the material for drug carriers, such as biocompatibility and lack of toxicity. They easily undergo hydrolytic degradation to their respective diacids, which are completely eliminated from the body within a short period of time. Polyanhydride nanoparticles can be successfully used as drug carriers [5–7].

The aim of this work was to obtain nanoparticles from polyanhydride based on betulin disuccinate (polyDBB) and to evaluate the influence of the preparation conditions, including: homogenization time, type of surfactant and concentration of the polymer solution, on the shape, morphology and size of particles. Betulin and its derivatives (e.g., betulin disuccinate) have a broad spectrum of biological relevance, including anticancer activity, thus this compounds are promising as new, potential therapeutic agents [9]. The major problem, which limits their potential pharmaceutical uses, is the poor aqueous solubility of lupane triterpenes faced when trying to formulate pharmaceutical compounds from betulin [1,9]. However, this problem can be solved by obtaining polymeric form of betulin and forming it into nanospheres, thus nanoparticles prepared from betulin-based polyanhydrides may have significant application in drug delivery systems.

2. Materials and Methods

2.1. Materials

Betulin disuccinate (obtained in the laboratory according to the procedure described earlier [9]), acetic anhydride (POCh S.A.), poly(vinyl alcohol) (M_w = 88,000 g/mol, 88% hydrolyzed) (ACROS Organics), sodium dodecyl sulfate (SDS, Fisher Scientific), cetyltrimethylammonium bromide (CTAB, ACROS Organics), methylene chloride (Chempur), hexane (Chempur), diethyl ether (Chempur) were used as supplied.

2.2. Polyanhydride Synthesis

Polyanhydride was obtained by two-step melt polycondensation of betulin disuccinate (DBB) according to the procedure described earlier [9]. DBB (5 g) was refluxed in acetic anhydride (1:10, *w*/*v*) under nitrogen flow for 40 min to get prepolymer. Excess of acetic anhydride and acetic acid formed in reaction were removed under vacuum. In the next step, prepolymer was heated at 150 °C for 2 h with constant stirring under vacuum (0.1 mm Hg) and nitrogen. The obtained polyanhydride (polyDBB) was stored in a freezer prior to be used for nanoparticles formulation.

2.3. Formulation of Nanoparticles Using ESE Method

Nanoparticles were prepared by solvent evaporation from O/W polymer emulsion. The polymer solution in methylene chloride (20 mL, concentration 10, 30 or 50 mg/mL) was emulsified in 400 mL of aqueous solution (1% *w*/*w*) of surfactant (PVAl, SDS or CTAB), using ULTRA-TURRAX T18 homogenizer, for 60 s. The speed of homogenizer was 18,000 rpm. Afterwards, the emulsion was subjected to ultrasonication under ice cooling for 180 s in a pulse regime (50% pulses, 50% power) using a Omni Sonic Ruptor 250 W and 3/4″tip. The emulsion was then stirred with a magnetic stirrer at 1100 rpm at room temperature for 3 h to evaporate the organic solvent. After that nanoparticles were collected by centrifugation at 5000 rpm for 5 min, washed 3 times with distilled water, lyophilized and stored in freezer.

2.4. Formulation of Nanoparticles by Nanoprecipitation

The polymer solution in methylene chloride (concentration 50 mg/mL) was added dropwise via a syringe into non-solvent bath of hexane at a solvent to non-solvent ratio 1:50, 1:100 or 1:150, which was continually stirred with a magnetic stirrer at 1100 rpm at room temperature. Upon contact with the non-solvent, the polymer spontaneously precipitated from solution, forming polymeric nanospheres. The suspension was stirred for 2 h. After that hexane was decanted and nanoparticles were filtered, dried under vacuum and stored in freezer.

2.5. Characterization of the Particles

The average size and size distribution of polymer particles were determined by dynamic light scattering (DLS) using a Zetasizer Nano S90. Before measurements, particles were dispersed in distilled water.

The morphological characterization of particles was carried out using a Phenom ProX scanning electron microscope (SEM) using accelerating voltage of 10 kV. Samples were coated with a 10 nm gold layer under vacuum using sputter coater Quorum Q150R ES. The particle size was measured by imaging software (ImageJ).

The content of anhydride groups in polyanhydride after the nanoparticles fabrication process was monitored by ^{1}H NMR. ^{1}H NMR spectra of polyDBB nanoparticles in $CDCl_3$ were recorded on a Varian 600 MHz spectrometer with TMS as an internal standard.

3. Results and Discussion

Polyanhydride (polyDBB) was obtained by melt polycondensation of betulin disuccinate. The use of DBB was intended to obtain polyanhydride with potential antitumor activity. Chemical structure of polyDBB (Figure 1) was confirmed by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Molecular weight of polyDBB was calculated from ^{1}H NMR and determined by GPC. The molecular weight was approx. 8500 g/mol. The detailed characteristic of polyDBB were described previously [9].

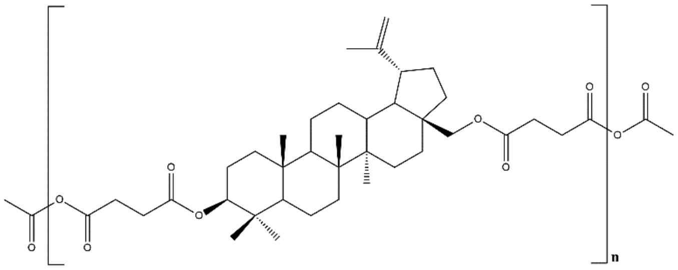

Figure 1. Chemical structure of polyDBB.

Nanoparticles were prepared using two methods: solvent evaporation (ESE) method and nanoprecipitation.

3.1. Nanoparticles Obtained by ESE

Particles obtained by solvent evaporation method were in the range 250–2400 nm for different formulation conditions. The nanoparticles were spherical in shape and had smooth surface. The morphology of the polyDBB particles are shown in Figure 2.

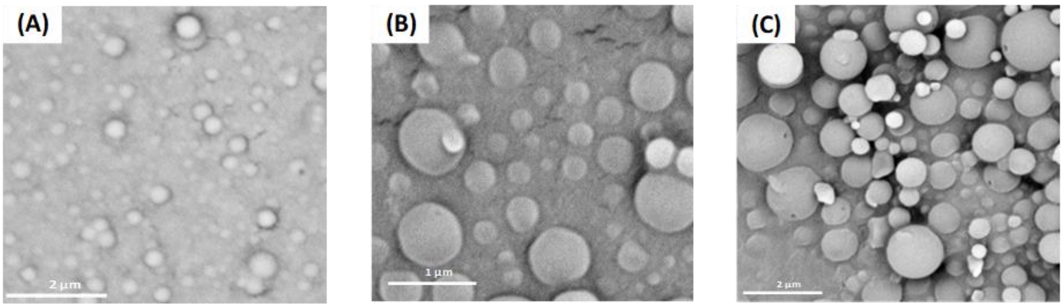

Figure 2. SEM images of polyDBB nanoparticles obtained by ESE method with the use of: (**A**) SDS, (**B**) CTAB and (**C**) PVAl as surfactant.

Different process parameters, including homogenization time, type of surfactant and concentration of the polymer solution have significant influence of the particle size. Changing the homogenization time from 30 s to 180 s reduced the diameter of particles. The obtained results showed that extending the homogenization time above 60 s slightly reduces the particle size, therefore in further experiments the homogenization time was 60 s.

3.1.1. Effect of the Polymer Concentration and Surfactant Type

During the preparation of nanoparticles such parameters as: concentration of polymer solution and type of surfactant were changed. These parameters plays an important role in the final size of particles, but they didn’t affect the surface morphology of nanoparticles. The particle sizes were determined by DLS and also calculated from the SEM images. Due to the tendency of nanoparticles to agglomeration, the sizes determined by DLS were larger than sizes calculated from SEM (except for particles obtained using PVAl as surfactant). The diameters of particles obtained by ESE in different conditions are listed in Table 1.

Table 1. The sizes of polyDBB nanoparticles with different polymer concentration and type of surfactant.

Surfactant	**Polymer Concentration in Organic Phase**								
	50 mg/mL			**30 mg/mL**			**10 mg/mL**		
	Particle Size [nm]		**PDI** [1]	**Particle Size [nm]**		**PDI** [1]	**Particle Size [nm]**		**PDI** [1]
	SEM	**DLS**		**SEM**	**DLS**		**SEM**	**DLS**	
1% SDS	820.0	802.9	0.212	770.0	1008.0	0.296	560.0	732.8	0.294
1% CTAB	504.2	859.8	0.434	330.0	1000.0	0.445	250.0	1268.0	0.386
1% PVAl	2420.0	2436.0	0.261	1870.0	1548.0	0.427	1420.0	896.8	0.356

[1] PDI = polydispersity index determined by DLS. Homogenization = 18,000 rpm.

Influence of concentration of polymer solution and surfactant type on characteristic of nanoparticles was determined using homogenization speed at 18,000 rpm. Nanoparticles with the smallest diameters were obtained with use of polymer solution of concentration 10 mg/mL. Increasing the polymer concentration increases the diameters of particles. Additionally, when the smaller particles were formed, the tendency to agglomeration was higher. A comparison of the data in Table 1 shows that the use of ionic surfactants (anionic SDS and cationic CTAB) leads to smaller particles compared to the non-ionic surfactant (PVAl). However the use of such compounds may accelerate the degradation process of polymers.

3.1.2. Effect of the Use of Ultrasound

Another parameter influencing the size of the obtained nanoparticles is the use of ultrasound. The diameters of particles obtained with the use of ultrasonication are listed in Table 2.

Table 2. The sizes of polyDBB nanoparticles after ultrasonication.

Surfactant	**Ultrasonication**	**Particle Size [nm]**		**PDI** [1]
		SEM	**DLS**	
1% SDS	—	820.0	802.9	0.212
	before stirring	330.0	288.3	0.225
	after 1.5 h of stirring	930.0	1218.0	0.325
1% CTAB	—	504.2	859.8	0.434
	before stirring	440.0	480.9	0.278
	after 1.5 h of stirring	549.5	1064.0	0.237
1% PVAl	—	2420.0	2436.0	0.261
	before stirring	918.4	1552.0	0.305

[1] PDI = polydispersity index determined by DLS. Polymer concentration in organic phase = 50 mg/mL, ultrasonication time = 3 min.

Ultrasonication reduces the size of the obtained nanoparticles. However, it is important at which point of the formulation of nanoparticles the ultrasound will be used. Nanoparticles with the smallest diameters were obtained when ultrasonication was used directly after homogenization process. Ultrasonication used at a later stage, results in the formation of particles with a larger size compared to the particles obtained without ultrasound.

3.2. Nanoparticles Obtained by Nanoprecipitation

Nanoprecipitation was used for the preparation of polyDBB nanoparticles, varying the solvent to non-solvent ratio. Obtained particles were in the range 370–950 nm. Nanoparticles with the smallest diameters were obtained with the solvent to non-solvent ratio of 1:150, thus it can be concluded that increasing the hexane to methylene chloride ratio reduces the particle diameter. The diameters of particles obtained by nanoprecipitation are listed in Table 3.

Table 3. The sizes of polyDBB nanoparticles obtained by nanoprecipitation.

Sample	Solvent to Non-Solvent Ratio	Homogenization	Ultrasonication	Particle Size [nm]		PDI [1]
				SEM	DLS	
1	1:50	—	—	—	953.2	0.354
2	1:100	—	—	—	867.4	0.416
3	1:150	—	—	440.0	682.0	0.240
4	1:100	—	3 min.	370.0	703.9	0.262
5	1:100	18,000 rpm/1 min.	—	—	689.5	0.191

[1] PDI = polydispersity index determined by DLS.

Taking into account the hydrolytic instability of the polyanhydrides, nanoprecipitation is the more suitable method, because it does not require the use of water. However, obtained nanoparticles indicated very high tendency to agglomeration, which can be observed at the SEM image.

In this method it is also difficult to control the shape of the particles. The morphology of the nanoparticles are shown in Figure 3. Among the spherical particles, many irregular ones were also observed (Figure 3).

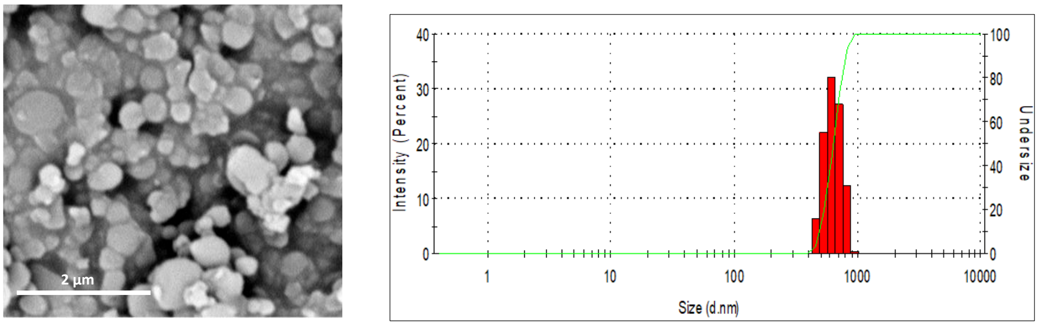

Figure 3. SEM image (**left**) and DLS size distribution profile (**right**) of polyDBB nanoparticles obtained by nanoprecipitation with the solvent to non-solvent ratio of 1:150.

As in the case of the ESE method, the use of additional homogenization and/or ultrasonication during the particle formation process, reduces the particle size.

3.3. Stability of Polyanhydride in Nanoparticle Formulation Process

Because the polyDBB is hydrolytically degradable, it is necessary to quantify the degree of hydrolytic degradation that occurs during the nanoparticles formulation. For this purpose, the anhydride bonds content were determined by ^{1}H NMR spectroscopy for neat polymer (before nanospheres formulation) and polymer after nanospheres preparation, according to the formula described earlier [9]. The anhydride bonds content was calculated using the intensity of the signals at $\delta = 2.74$–2.64 ppm and at $\delta = 2.82$–2.77 ppm, which are assigned to methylene protons close to ester and anhydride groups, respectively. When the nanospheres were produced by ESE method, the disappearance of anhydride bonds was less than 10%, when using PVAL as a surfactant, but the almost 20% when SDS or CTAB was used. The observed results confirmed that the presence of ionic groups on the particle surface accelerate the polyanhydrides degradation rate, due to the better water access. When the nanoprecipitation was used to produce the nanoparticles, the degradation of polyDBB also occurred, but was less significant than in the case of ESE method (the disappearance of anhydride bonds was less than 5%).

4. Conclusions

In this work polyanhydride based on disuccinate betulin were successfully fabricated into nanoparticles. Nanoparticles were obtained by two methods. The process parameters influence the characteristic of obtained particles. ESE method provided regularly spherical particles, while among the particles obtained from the nanoprecipitation method, many irregular ones were observed. In ESE method, the size of the particles depended on the type of surfactant (in the water phase) and the polymer concentration (in the organic phase). The smallest particles were obtained with ionic surfactants, however, the use of such compounds accelerate the degradation process of polymers. In nanoprecipitation method, the ratio of solvent (methylene chloride) to non-solvent (hexane) has a significant influence on the particle size. The smallest particles were obtained with a solvent to non-solvent ratio of 1:150.

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References

1. Rao, J.P.; Geckeler, K.E. Polymer nanoparticles: Preparation techniques and size-control parameters. *Prog. Polym. Sci.* **2011**, *36*, 887–913.
2. Nagavarma, B.V.N.; Yadav, H.K.; Ayaz, A.V.L.S.; Vasudha, L.S.; Shivakumar, H.G. Different techniques for preparation of polymeric nanoparticles—A review. *Asian J. Pharm. Clin. Res.* **2012**, *5*, 16–23.
3. Moritz, M.; Geszke-Moritz, M. Recent developments in the application of polymeric nanoparticles as drug carriers. *Adv. Clin. Exp. Med.* **2015**, *24*, 749–758.
4. Michalak, G.; Głuszek, K.; Piktel, E.; Deptuła, P.; Puszkarz, I.; Niemirowicz, K.; Bucki, R. Polymeric nanoparticles—A novel solution for delivery of antimicrobial agents. *Med. Stud./Stud. Med.* **2016**, *32*, 56–62.
5. Jain, J.P.; Modi, S.; Domb, A.J.; Kumar, N. Role of polyanhydrides as localized drug carriers. *J. Control. Release* **2005**, *103*, 541–563.
6. Wafa, E.I.; Geary, S.M.; Goodman, J.T.; Narasimhan, B.; Salem, A.K. The effect of polyanhydride chemistry in particle-based cancer vaccines on the magnitude of the anti-tumor immune response. *Acta Biomater.* **2017**, *50*, 417–427.
7. Lee, W.C.; Chu, I.M. Preparation and degradation behavior of polyanhydrides nanoparticles. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2008**, *84*, 138–146.
8. Alakurtti, S.; Mäkelä, T.; Koskimies, S.; Yli-Kauhaluoma, J. Pharmacological properties of the ubiquitous natural product betulin. *Eur. J. Pharm. Sci*. **2006**, *29*, 1–13.
9. Niewolik, D.; Krukiewicz, K.; Bednarczyk-Cwynar, B.; Ruszkowski, P.; Jaszcz, K. Novel polymeric derivatives of betulin with anticancer activity. *RSC Adv.* **2019**, *9*, 20892–20900.