75 years
of the Silesian University
of Technology

Silesian
University
of Technology

RESEARCH
UNIVERSITY
EXCELLENCE INITIATIVE
Ministry of Science
and Higher Education

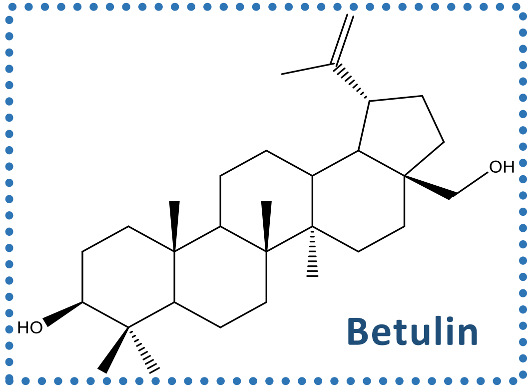
STUDIES ON THE PREPARATION OF NANOPARTICLES FROM BETULIN-BASED POLYANHYDRIDES

Daria Niewolik*, Grzegorz Dzido, Katarzyna Jaszcz***

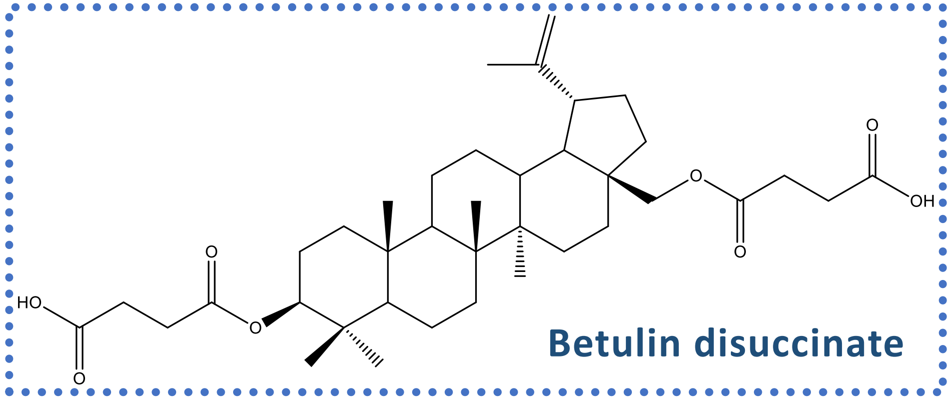
**Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, Gliwice, Poland*
*** Department of Chemical Engineering and Process Design, Silesian University of Technology, Gliwice, Poland*
daria.niewolik@polsl.pl

Introduction

- Betulin, a lupane derivative, belongs to the pentacyclic triterpenes and occurs naturally in nature, i.a. in the outer layer of the birch bark. 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.
- The poor aqueous solubility of lupane triterpenes faced when trying to formulate pharmaceutical compounds from betulin, limits their potential pharmaceutical uses.
- This problem can be solved by obtaining polymeric form of betulin and forming it into nanoparticles, thus nanoparticles prepared from betulin-based polyanhydrides may have significant application in drug delivery systems.



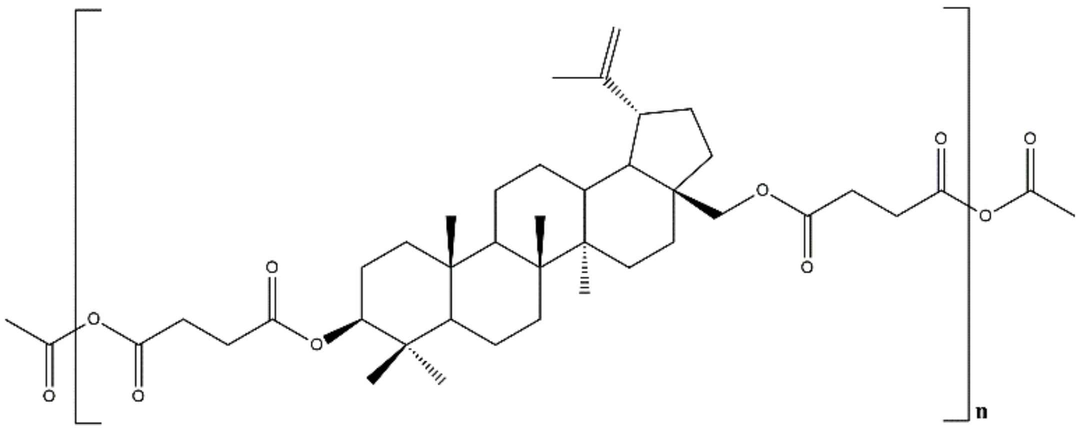
Betulin



Betulin disuccinate

Aim of our study

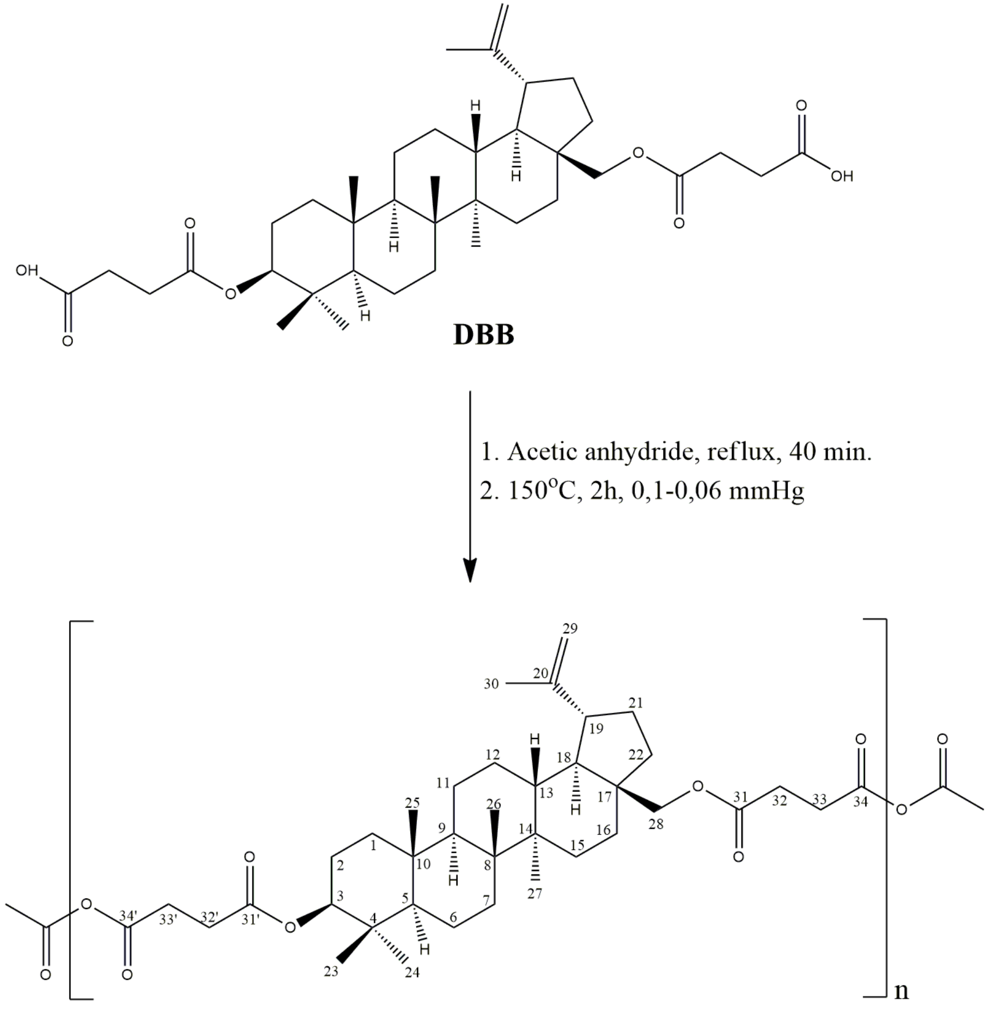
The aim of our study was to obtain polymeric nanoparticles from polyanhydride based on betulin disuccinate (DBB) and to evaluate the influence of the preparation conditions (homogenization time, type of surfactant, concentration of the polymer solution) on the shape, morphology and size of the particles.



Chemical structure of polyanhydride based on betulin disuccinate (polyDBB)

Polyanhydride based on DBB

- PolyDBB was obtained by two-step melt polycondensation of betulin disuccinate with the use of acetic anhydride.
- The use of DBB was intended to obtain polyanhydrides with potential antitumor activity.
- PolyDBB serves as a promising degradation rate controlled drug delivery system for either DBB or other drugs, including cytostatic agents.

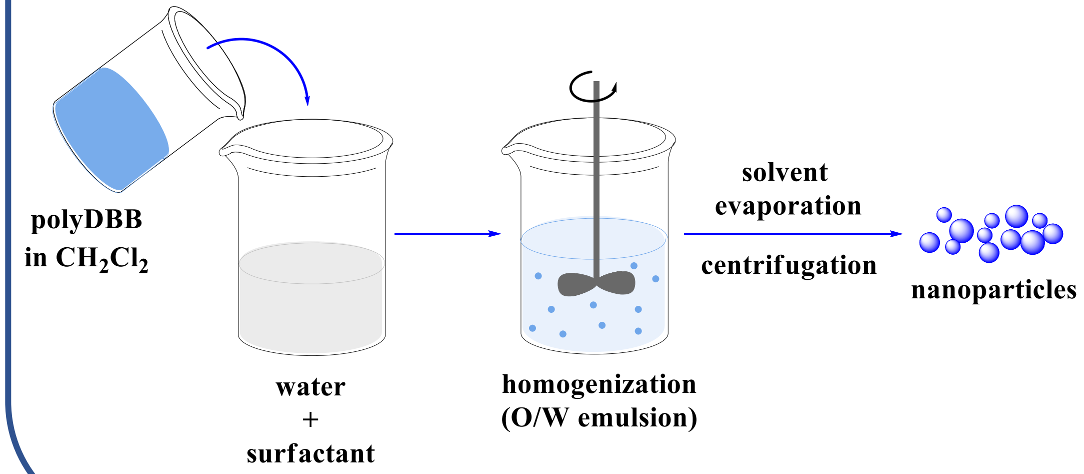


1. Acetic anhydride, reflux, 40 min.
2. 150°C, 2h, 0,1-0,06 mmHg

Reaction scheme of the synthesis of polyanhydrides based on DBB

Preparation of polyDBB-based nanoparticles

ESE method



polyDBB
in CH_2Cl_2

water
+
surfactant

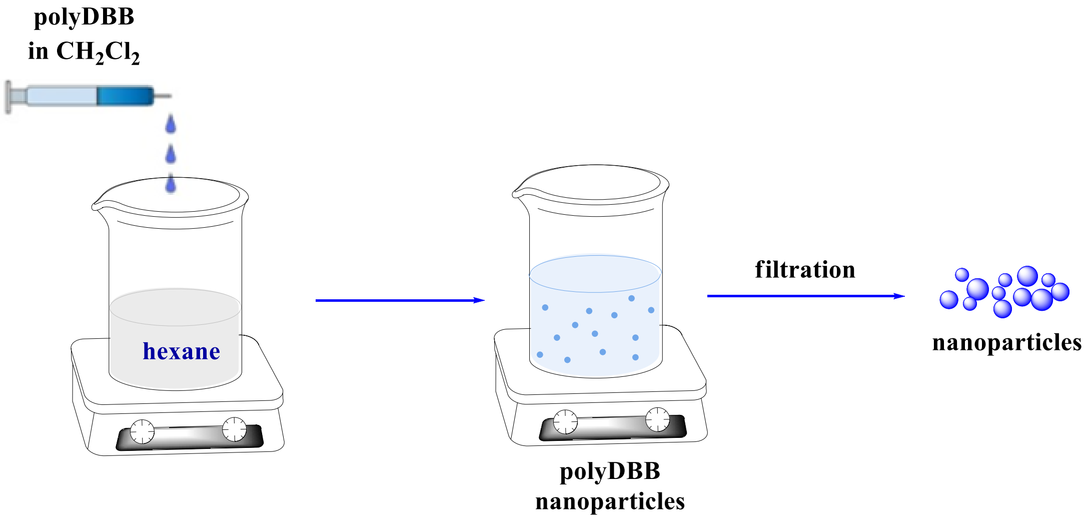
homogenization
(O/W emulsion)

solvent
evaporation

centrifugation

nanoparticles

Nanoprecipitation



polyDBB
in CH_2Cl_2

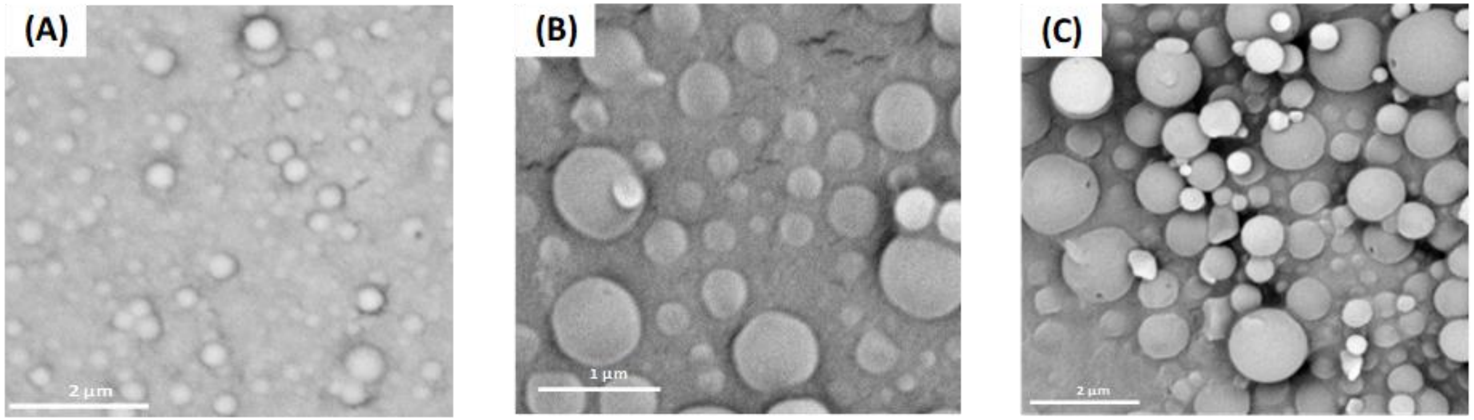
hexane

polyDBB
nanoparticles

filtration

nanoparticles

Results – ESE method



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

- Particles obtained by solvent evaporation method were in the range 250-2400 nm for different formulation conditions. The nanoparticles were spherical in shape and were had smooth surface.

- Changing the homogenization time from 30 s to 180 s reduced the diameter of particles. The 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.

Results – ESE method

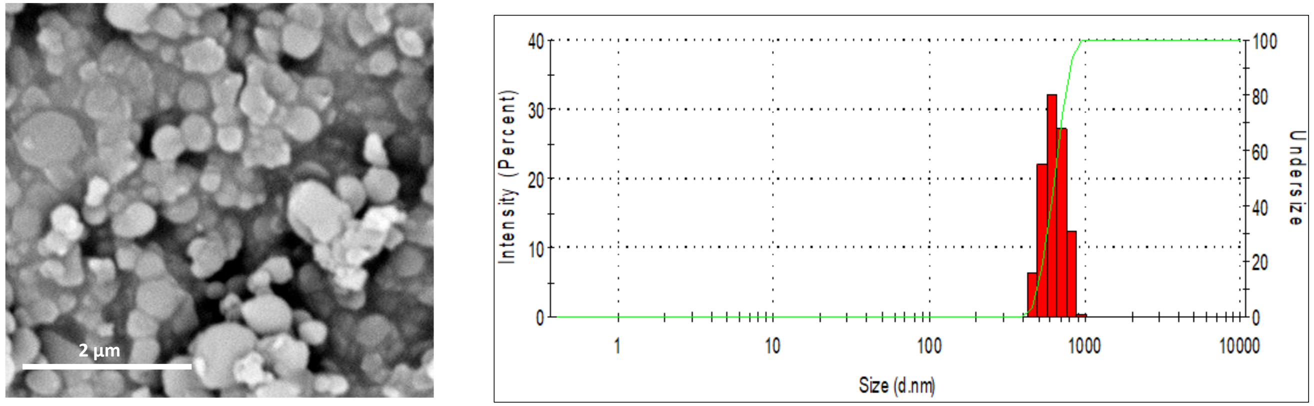
Table 1. The sizes of polyDBB nanoparticles with different polymer concentration and type of surfactant.

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.

- 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).
- 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.
- The smallest particles were obtained with ionic surfactants, however, the use of such compounds accelerate the degradation process of polymers.

Results – Nanoprecipitation



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.

- Particles obtained by nanoprecipitation were in the range 370-950 nm.
- Nanoparticles with the smallest diameters were obtained with the solvent to non-solvent ratio of 1 : 150.
- Increasing the hexane to methylene chloride ratio reduces the particle diameter.

Results – influence of ultrasound on particle size

Table 2. The sizes of polyDBB nanoparticles obtained by ESE method 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 1.5h 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 1.5h of stirring	549.5	1064.0	0.237
1% PVAl	—	2420.0	2436.0	0.261
	before stirring	918.4	1552.0	0.305

Table 3. The sizes of polyDBB nanoparticles obtained by nanoprecipitation before and after ultrasonication.

Solvent to non-solvent ratio	Ultrasonication	Particle size [nm]		PDI [1]
		SEM	DLS	
1 : 100	—	—	867.4	0.416
1 : 100	3 min.	370.0	703.9	0.262

[1] PDI = polydispersity index determined by DLS.

- In both method, ultrasonication reduces the size of the obtained nanoparticles.
- In ESE method, 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.

Conclusions

- ESE method provided regularly spherical particles with diameters of 250-2400 nm for different formulation conditions, while among the particles obtained from the nanoprecipitation method (with diameter of 370-950 nm), 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). Increasing the polymer concentration increases the diameters of particles.
- The smallest particles were obtained with ionic surfactants (SDS, CTAB), 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.
- An important factor influencing the particle size in both methods was the use of ultrasonication. Ultrasonication used during the particle formation process reduces the particle size.