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RESEARCH UNIVERSITY EXCELLENCE INITIATIVE

NOVEL BIODEGRADABLE POLYANHYDRIDES BASED ON BETULIN DISUCCINATE AND SEBACIC ACID FOR MEDICAL PURPOSE

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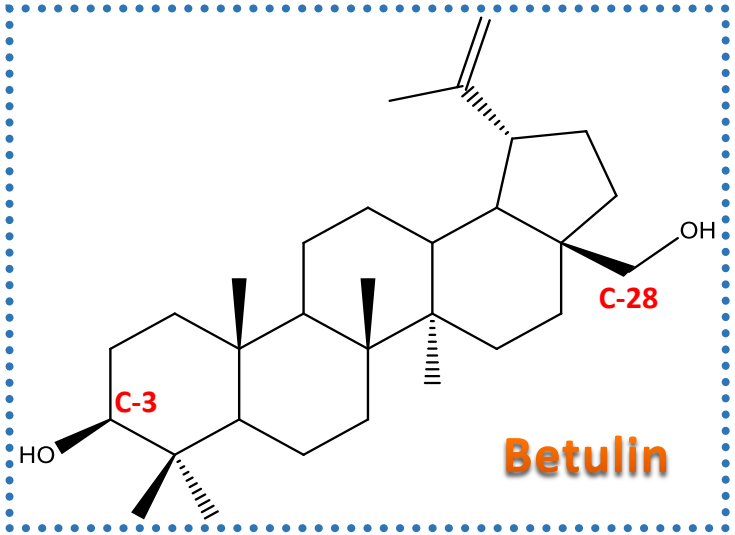
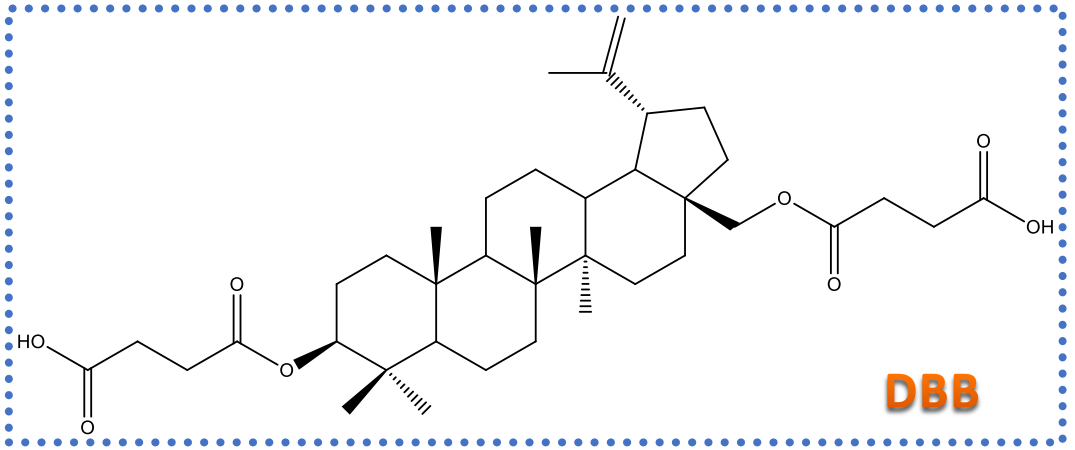
Introduction

Betulin, a lupane derivative, belongs to the pentacyclic triterpenes and occurs naturally in nature. It is obtained on a large scale from the outer layer of the birch bark. Betulin and its derivatives have a broad spectrum of biological activity.

Betulin disuccinate (DBB) is known to have antitumor activity against leishmania, as well as hypolipidemic, fungicidal, bactericidal and antiviral activity, including the Epstein-Barr virus and HIV.

Both betulin and betulin disuccinate exhibit no in vitro and in vivo toxicity. The polymers obtained from these substances can be used in the pharmaceutical industry as matrices in controlled drug delivery systems.

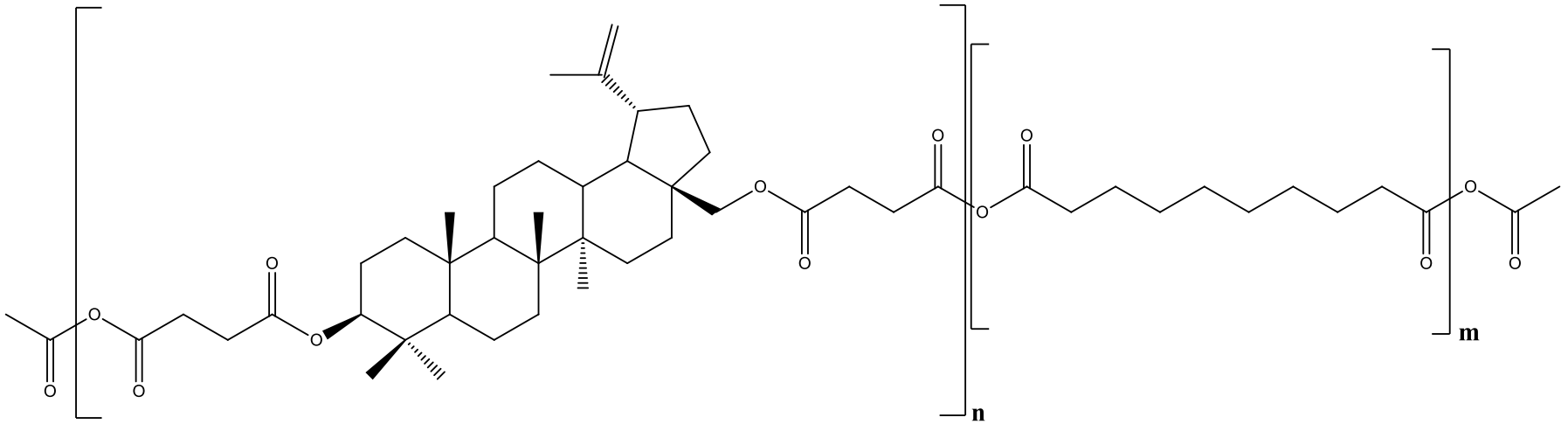




Alakurtti S. et al., *Eur. J. Pharm. Sci.* 29.1, 2006, 1-13

Aim of our study

The aim of our study was to obtain a new, biodegradable betulin-based polyanhydrides exhibiting anti-cancer activity.



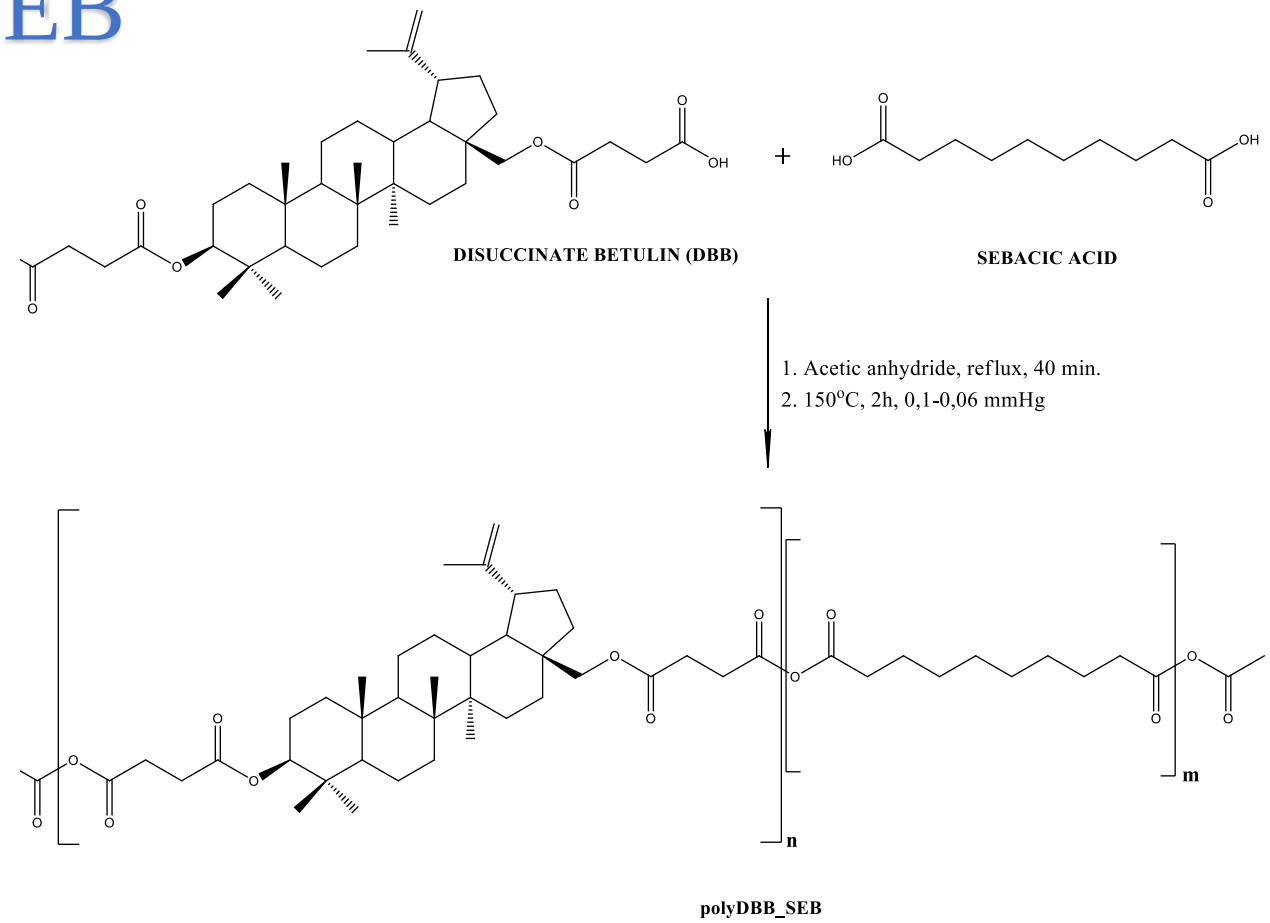
Chemical structure of polyanhydride based on betulin disuccinate and sebacic acid

Synthesis of polyDBB_SEB

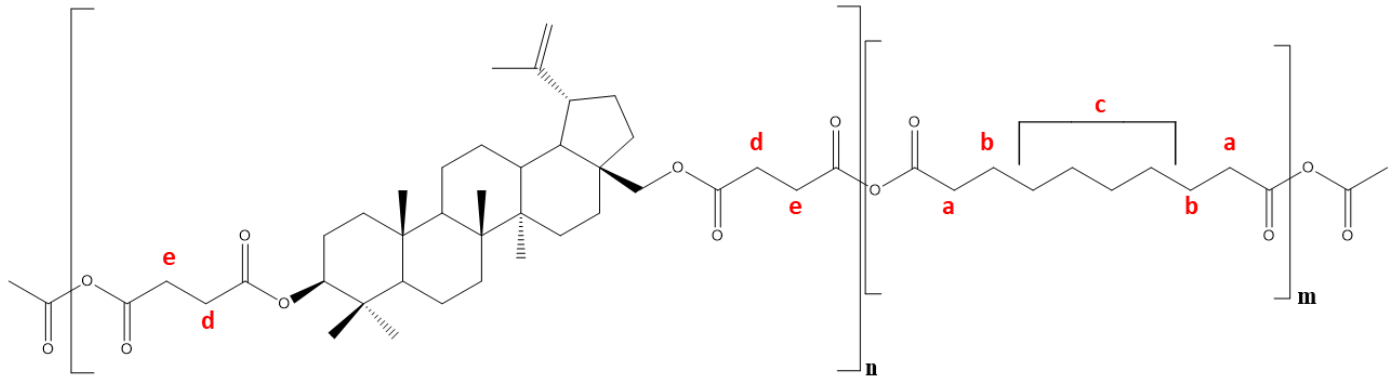
Polyanhydrides were obtained by two-step melt polycondensation of betulin disuccinate and sebacic acid with the use of acetic anhydride. The content of sebacic acid in obtained copolymers was from 20 to 80 wt %.

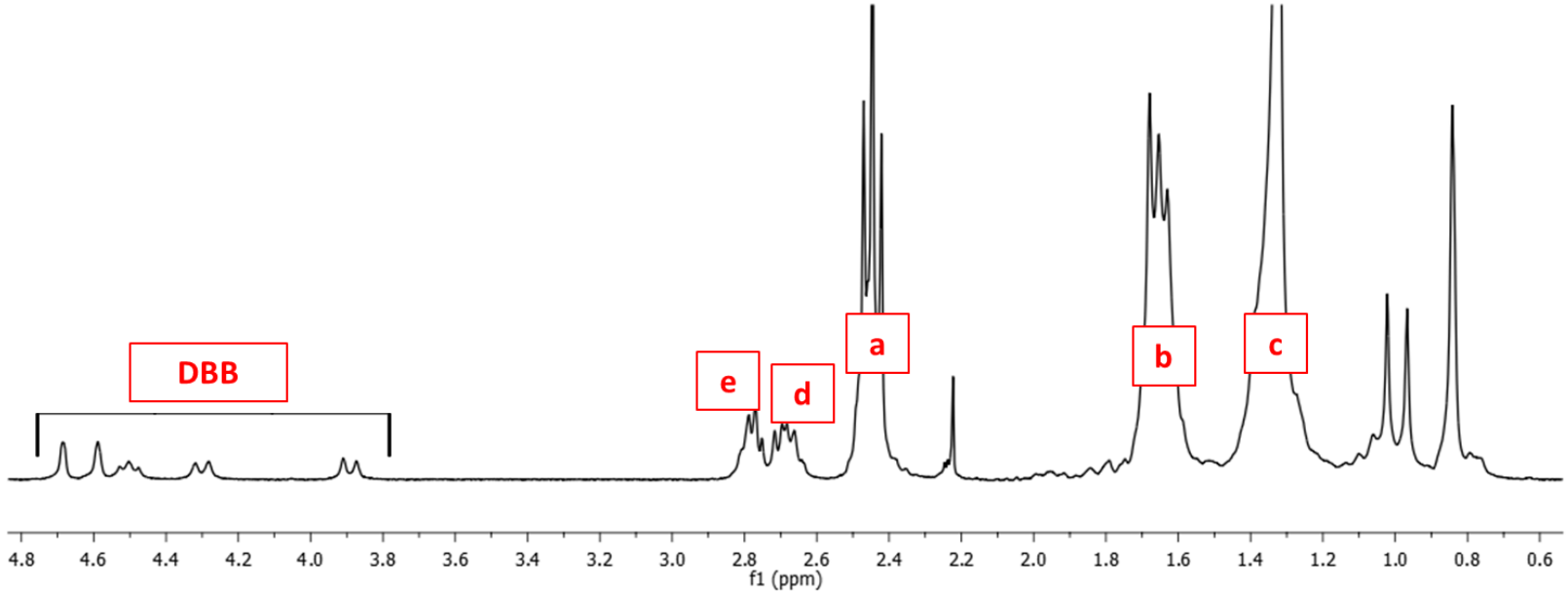
The use of DBB was intended to obtain polyanhydrides with potential antitumor activity and the use of sebacic acid as a comonomer was intended to increase the crystallinity.

The obtained polymers were solid materials, amorphous when containing more DBB, and crystalline when containing more sebacic acid.



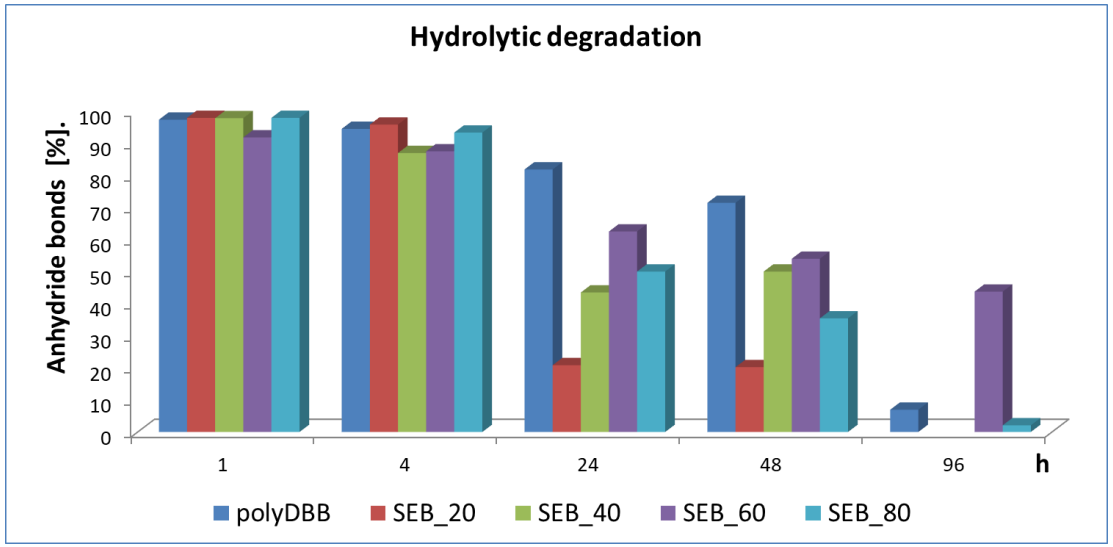
Reaction scheme of the synthesis of polyanhydrides based on DBB and sebacic acid

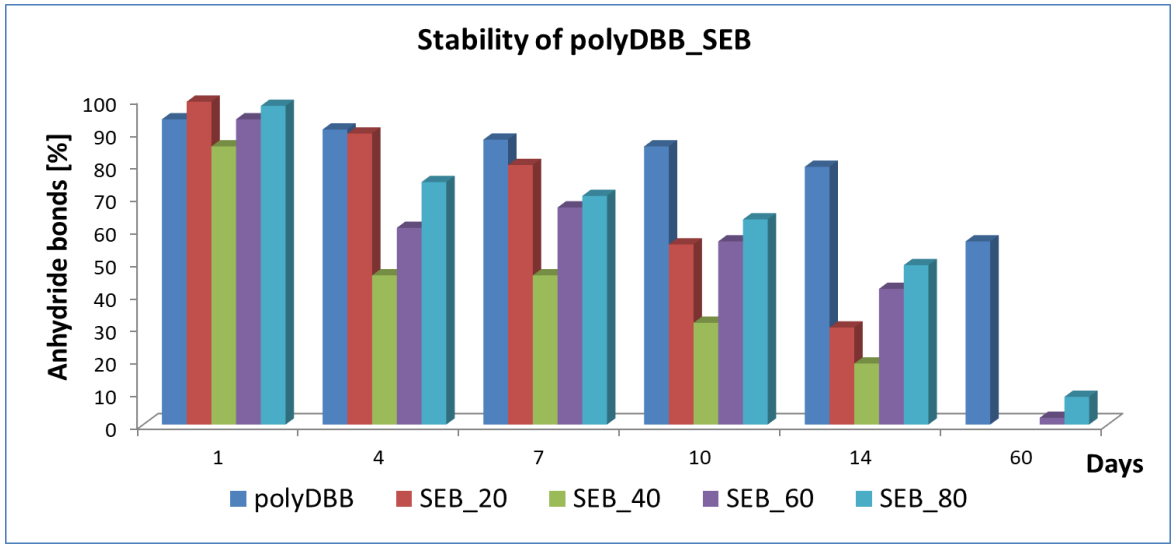




^{1}H NMR spectrum of polyanhydrides based on betulin disuccinate and sebacic acid

Hydrolytic degradation





Anhydride bonds loss of copolymers based DBB and sebacic acid during hydrolytic degradation in phosphate buffer conducted at 37°C (left) and in the air at 25 °C (right).

Hydrolytic degradation of copolymers was carried out in phosphate buffer solution (pH 7.4) at 37°C in order to determine the loss of anhydride bonds. The resulting polymers under physiological conditions (37°C, pH = 7.4) are hydrolytically degradable to the starting reagents: betulin disuccinate, whose biological activity is known and confirmed and to sebacic acid that is physiologically acceptable. The stability of copolymers on air at room temperature was also investigated. It can be concluded that the obtained polymer is quite stable on the air.

Cytostatic activity

Compound	Cytostatic activity IC_{50} [µg/ml]					
	HeLa	A-549	U-87 MG	KB	HepG2	HDF
DBB	8.25±0.81	7.09±0.01	7.37±0.26	7.17±0.93	8.02±0.04	14.80±0.06
polyDBB	16.23±0.72	16.19±0.31	16.07±0.02	17.81±0.03	15.93±0.12	27.13±0.01
SEB_20	4.61±0.03	4.15±0.24	4.99±0.05	4.28±0.01	4.15±0.01	7.31±1.92
SEB_40	8.22±0.09	8.15±0.01	8.12±0.91	8.04±0.03	8.77±0.23	13.55±0.22
SEB_60	8.10±0.74	8.29±0.55	8.41±0.01	8.28±0.01	8.39±0.16	8,22±1.17
SEB_80	11.48±0.22	11.29±0.18	11.06±0.37	11.39±0.51	11.12±0.46	18.01±0.02
polySEB	47.94±1.62	48.88±1.37	48.41±0.94	48.66±0.73	48.17±1.29	75.01±2.81

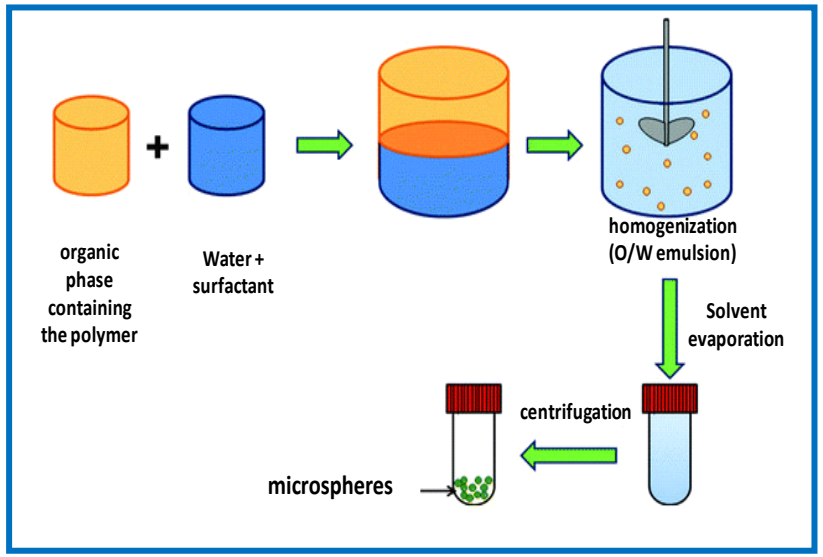
Cytostatic activity of polyanhydrides against various cancer cell lines as well as a normal control (HDF), expressed as IC_{50}

Polyanhydrides containing DBB and sebacic acid were studied to determine their cytostatic activity against selected cancer cell lines. Cell lines representing cervix, lung, liver, central nervous system and nasopharynx tumors were used in these studies to find concentrations causing inhibition of cell growth in culture by 50% (IC_{50}).

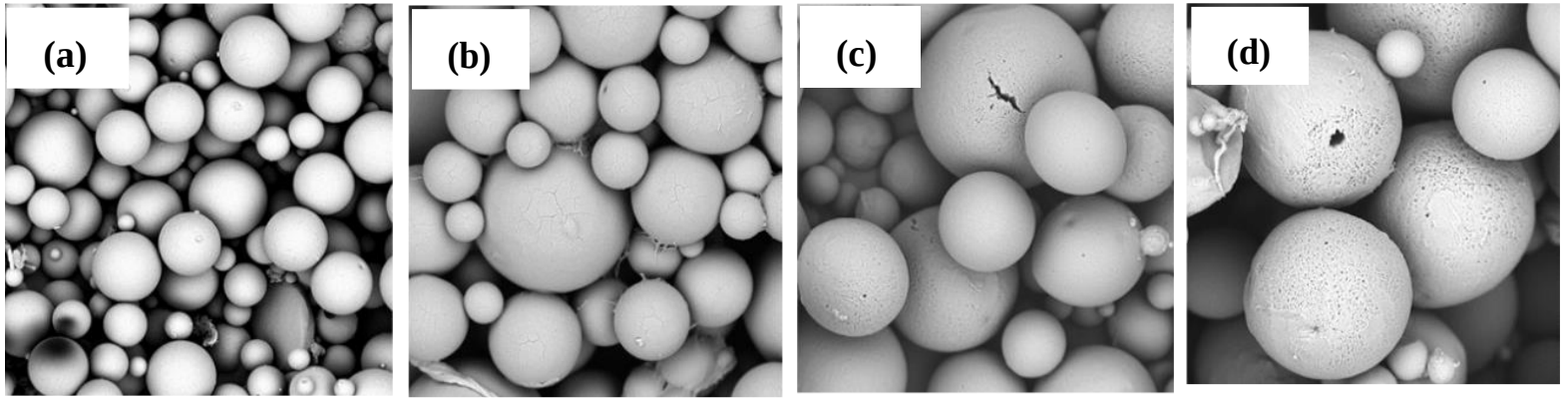
Cytostatic tests indicated the effectiveness of obtained copolymers in inhibition of growth of cancer cells, with limited cytotoxicity towards normal cells.

The results shows that cytostatic activity of polyanhydrides based on betulin disuccinate can be modified by changing the kind of comonomer and its content, allowing to optimize the degradation rate and tailor the period of cytotoxicity.

Microspheres



Preparation of microspheres by emulsion (O/W) solvent evaporation technique



SEM images of microspheres obtained from: (a) polyDBB_SEB_20; (b) polyDBB_SEB_40; (c) polyDBB_SEB_60 and (d) polyDBB_SEB_80, by using homogenization speed of 3000 rpm

Particle size (D_n)			
SEB_20	SEB_40	SEB_60	SEB_80
18.6 µm	16.4 µm	19.1 µm	20.8 µm

Conditions				
Concentration of the polymer solution	Concentration of PVAl	Oil to water ratio [v:v]	Homogenization time	Speed of homogenization [rpm]
50 mg/ml	1%	1:20	30s	3000

Conclusions

- A new polyanhydrides, based on betulin disuccinate and sebacic acid were obtained and characterized.
- Under physiological conditions copolymers undergoes hydrolytic degradation to betulin disuccinate, whose biological activity is known and confirmed and to sebacic acid approved by FDA for use in drug delivery systems.
- The use of sebacic acid as a comonomer increases the crystallinity of polymers, which affects the characteristics of microspheres.
- Cytotoxicity tests indicated the effectiveness of obtained polyanhydrides in inhibition of growth of cancer cells, with limited cytotoxicity towards normal cells.
- The use of these polymers in biological systems will lead to the release of DBB, controlled by the rate of polymer degradation.
- The obtained polymers can be used in the pharmaceutical industry as matrices in controlled drug delivery systems.