

## Scientific Report to the Project

# Nanosized micelles and vesicles from amphiphilic block copolymers with polysaccharides as hydrophilic blocks. A versatile route to new biomaterials

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The main objective of the project is the synthesis and evaluation of new amphiphilic block copolymers with a polysaccharide as a hydrophilic block, while the hydrophobic block is a bile acid polyester or a polymer with a temperature induced hydrophobicity.

### OBJECTIVES and ACTIVITIES for 2011

**Objective 1: Synthesis and characterization of bile acid polyesters (BAPE)**

**Activity 1.1. Synthesis (setting-up optima conditions)**

**Activity 1.2. Characterization: NMR, FT-IR, GPC, TGA, DSC, X-Ray**

**Activity 1.3. Biodegradation studies**

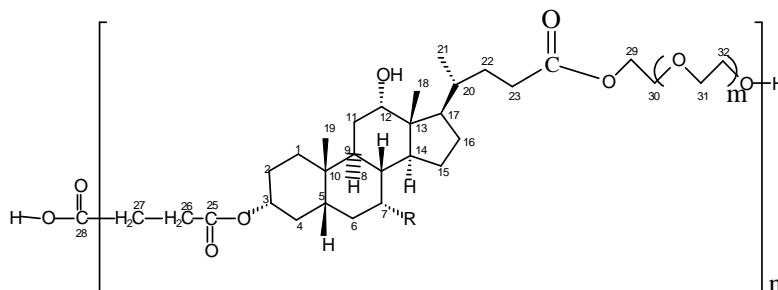
The objective was the synthesis and characterization of polyesters obtained from a bile acid (cholic or deoxycholic acid) and oligo(ethylene glycol)s (OEG) of different molar masses. The following activities were performed:

#### Synthesis of bile acid polyesters (BAPE)

Polyesters were obtained by polycondensation of a 3-succinoyloxy derivative of a bile acid and an oligo(ethylene glycol) with molar mass 100, 200, 400, 600, 1000 or 1500. Synthesis was performed in two steps.

**Synthesis of succinoyloxy** derivatives was carried out by reaction of succinic anhydride and a bile acid in anhydrous  $\text{CHCl}_3$ , in the presence of triethylamine as a catalyst, for 24 h at reflux. Acylation reaction took place selectively at OH group located at C3 of steroidal cycle, due to the much higher reactivity of this group in comparison to the OH groups in position 7 and 12 (Scheme 1).

**Polyester synthesis** was realized by polycondensation of an equimolar mixture of diacid/glycol in solution (toluene), in the presence of p-toluene sulfonic acid as catalyst, with continuous removal of condensation water in a Dean-Stark device. Different reagent concentrations and reaction times (24-72 h) were used. Chemical structure of the synthesized polyesters is presented in Scheme 1. All these polymers are soluble in THF, acetone, DMSO,  $\text{CHCl}_3$ , DMF. The polymers obtained from OEG with  $M_n \geq 1000$  are soluble in water too.



Scheme 1. Chemical structure of bile acid polyesters. R = H (deoxycholic acid) or OH (cholic acid). m = 1 (diethylene glycol-OEG100), 3 (OEG 200), 7 (OEG 200), 12 (OEG 600), 21 (OEG 1000) or 33 (OEG 1500).

Polymers obtained from cholic acid had very low molar masses (degree of polycondensation  $n \approx 2$ ), therefore, only detailed characterization of deoxycholic acid (DCA) polyester is presented in the following.

### **Polyester physico-chemical characterization**

**Chemical structure** of succinoyloxy derivatives was confirmed by elemental analysis, IR,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR, as well by mass spectrometry. Chemical composition of polyesters was calculated from the bile acid content determined by HPLC analysis after polyester complete hydrolysis in 1N NaOH. According to the data presented in Table 1, the content was very close to the theoretical one (calculated for the chemical structure presented in Scheme 1).

**Weight average molar mass** ( $M_w$ ) was determined by GPC, using THF as eluent and polystyrene standards.

**Number average molar mass** ( $M_n$ ) was determined by several techniques:

- (a) GPC. The ratio  $M_w/M_n$  allowed calculation of polydispersity index PI.
- (b) NMR spectra. The polycondensation degree could be calculated as the ratio of integrals corresponding to the internal protons of glycol (positions 31 and 32 in Scheme 1) (3.7 ppm) and to the final protons of glycol bound to carboxylic groups (position 29 in Scheme 1) (4.25 ppm).
- (c) End group determination. End carboxylic groups were quantified by colorimetric titration (in the presence of thymol blue) or conductometric titration with 0.02 N KOH. End OH groups were reacted with acetic anhydride in the presence of triethylamine, then the total carboxylic groups were titrated with 0.2 N KOH. All titration experiments were performed in THF solutions with methanolic KOH solutions.

Molar mass values obtained by these procedures are presented in Table 1.  $M_n$  values obtained by different methods agree well, except for the values calculated from end group titration, which are lower than those given by GPC. The difference can be assigned to the difference in flexibility of polystyrene used as standard and polyesters, latter being polymers with a rigid structure. Molar masses are low (polycondensation degree  $\sim 5$ -10), they are influenced by monomer concentration and reaction time, and decrease with increasing glycol molar mass.

**Polyester thermal properties** studied were thermal stability, transition temperatures and potential thermosensitive properties.

- (a) Thermal stability was evaluated by thermogravimetric analysis (TG/TDG), which allowed calculation of mass loss ( $\Delta W$  %) and characteristic temperatures: temperature for the degradation start ( $T_i$ ), temperature corresponding to the maximum mass loss ( $T_{max}$ ) and temperature at the end of decomposition ( $T_f$ ).  $T_i$  was mainly used for the comparison of the polyester thermal stability (Table 1) and it was  $\geq 250$  °C, suggesting a good stability.  $T_i$  decreases gradually with glycol molar mass increase due to thermal instability of ether bonds.
- (b) Differential scanning calorimetry (DSC) measurements allowed determination of glass transition temperature ( $T_g$ ) and, in particular cases, melting temperature ( $T_m$ ) (Table 1).  $T_g$  decreases with increasing glycol molar mass and increases with increasing polyester molar mass.  $T_m$  was observed in case of polyesters obtained from OEG with molar mass  $\geq 1000$ .
- (c) UV-Vis analysis of aqueous solutions of polyesters VIII and IX obtained from OEG 1000 and 1500 proved their thermosensitivity (Fig. 1). Transition temperature (cloud point,  $T_c$ ) was 45 °C si 63 °C, respectively, at 1% (g/g) concentration.  $T_c$  values depend on polymer concentration and ionic strength of aqueous solutions. The thermosensitive behavior, similar to that of poly (N-isopropylacrylamide), will be the subject of a more detailed and separate study.

Table 1. Characteristics of polyesters obtained from deoxycholic acid derivative and oligo(ethylene glycol)s with different molar masses.

| Polyester code | Glycol   | $M_w^{(a)}$ | $M_n$ |      |           | $PI^{(a)}$ | $n^{(b)}$ | $T_i^{(c)}$<br>°C | $T_g^{(d)}$<br>°C | $T_m^{(e)}$<br>°C | $T_c^{(f)}$<br>°C | DCA content (%) <sup>(g)</sup><br>Found (calculated) |
|----------------|----------|-------------|-------|------|-----------|------------|-----------|-------------------|-------------------|-------------------|-------------------|--|
|                |          |             | GPC   | NMR  | End group |            |           |                   |                   |                   |                   |  |
| I              | DEG      | 19850       | 10800 | -    |           | 4          | 18        | -                 | 68                | -                 | -                 | 65.  |
| II             | DEG      | 11600       | 5922  | 4280 | 3720      | 2          | 9         | 320               | 61                | NO                | NO                | 63.5 (65.1)  |
| III            | DEG      | 7161        | 5922  | -    | -         | 2          | 9         | -                 | -                 | NO                | NO                | -  |
| IV             | OEG 200  | -           | -     |      | 13400     | -          | 20        | 310               | 26                | NO                | NO                | 53.2 (55.5)  |
| V              | OEG 200  | 8620        | 6633  | 5800 | -         | 1.3        | 9         | 310               | -1                | NO                | NO                | 56.0 (55.5)  |
| VI             | OEG 400  | -           | -     | -    | 2650      | -          | 3         | 300               | -14               | NO                | NO                | 42.3 (42.8)  |
| VII            | OEG 600  | -           | -     | -    | 6900      | -          | 6         | 285               | -29               | NO                | NO                | 34.8 (34.2)  |
| VIII           | OEG 1000 | -           | -     | 2000 | 2500      | -          | 2         | 250               | -47               | 13                | 45                | 24.2 (25.3)  |
| IX             | OEG 1500 | -           | -     | -    | -         |            |           |                   | -                 | -                 | 63                | -  |

<sup>(a)</sup> Determined by GPC

<sup>(b)</sup> Polycondensation degree, calculated from  $M_n$  values

<sup>(c)</sup> Temperature for decomposition start, determined by TG

<sup>(d)</sup> Glass transition temperature, determined by DSC

<sup>(e)</sup> Melting temperature, determined by DSC

<sup>(f)</sup> Cloud point, determined from UV-Vis absorbance measurements (420 nm)

<sup>(g)</sup> Determined by HPLC, after complete hydrolysis in 1N NaOH

NO – not observed

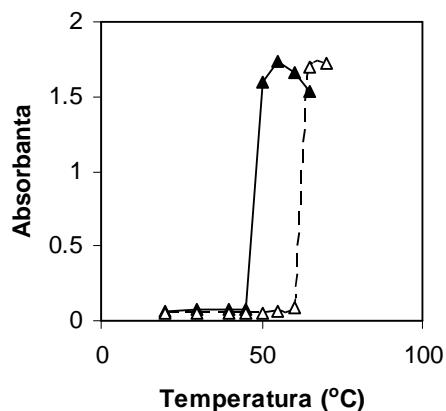


Fig.1. Variation of absorbance with temperature for polyester VIII (solid triangle) and IX (open triangle)

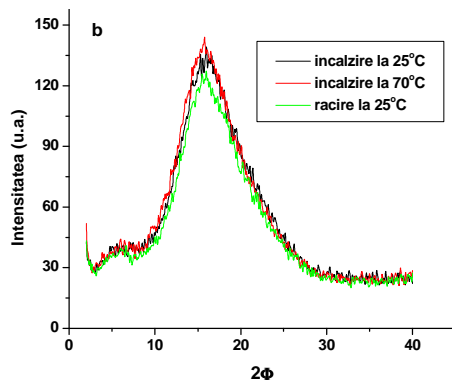


Fig. 2. X-Ray diffractograms of polyester II at ambient temperature and 70 °C.

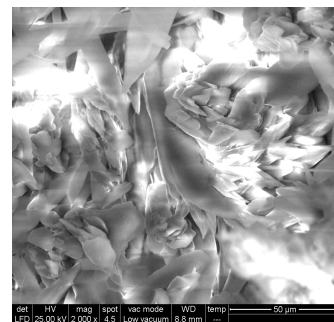


Fig. 3. SEM image for polyester II

**Wide angle X-Ray diffraction (WAXS)** witnessed for a partially crystalline structure (about 30 % crystallinity for polyester II) (Fig. 2), which does not modify after heating at a temperature higher than  $T_g$ . Crystallinity increases with increasing OEG molar mass and was about 51 % for polyester IX.

**Morphology of polyesters** was observed by electronic microscopy (SEM) (Fig. 3), which confirmed the partial crystallinity of these polymers. Crystalline component has a lamellar structure, with lamellae size in the range 10 -100  $\mu\text{m}$ .

### Study of polyester (bio)degradation

One of the main objectives of the project was the design of block copolymers with at least one (bio) degradable block. The presence of ester bonds in bile acid polyesters suggests their potential for degradation by chemical and/or enzymatic hydrolysis. Preliminary studies showed that polyester degradation under conditions simulating physiological environment (pH 7.4, 37 °C) is very slow (Fig. 4A), a behavior which is similar to aliphatic polyesters such as poly( $\epsilon$ -caprolactone) or poly(glycolide)s. In order to study the influence of chemical structure on polyester propensity to chemical hydrolysis, several experiments were performed in 1 N NaOH and the amount of free DCA released in time was monitored by HPLC analysis. A clear enhancement of hydrolysis rate with increase of glycol molar mass was observed. Hence, the time required for a complete hydrolysis increases from several hours in case of the polyester VII (DCA-OEG600) to 48 h in case of polyester II (DCA-OEG100)(with DEG) (Fig. 4B). DCA amount released by hydrolysis increases monotonously, which suggests a hydrolysis at the end of polyester chain. The hydrolysis experiments in the presence of enzymes (lipases) will be performed in the near future.

### Conclusions

At this stage, synthesis and characterization of bile acid based polyesters were performed, the chemical composition of which was varied by used of bile acids with 3 or 2 OO groups (cholic and deoxycholic acid, respectively) and oligo(ethylene glycol)s with different chain length.

- Oligoesters with polycondensation degree  $n = 2-20$  were obtained as a function of bile acid and glycol chemical structure, their concentration in polycondensation medium and reaction time.
- Synthesized oligoesters display a good thermal stability, glass transition temperatures varying between 70 °C and -50 °C as a function of glycol chain length, and partial crystallinity.

- Polyesters based on OEG 1000 and OEG 1500 are water soluble and display sol-gel phase transitions as a function of temperature, therefore they can be considered thermosensitive.
- Polyester degradation under physiological condition is slow, degradation rate increases with increasing medium pH and increasing polymer hydrophilicity (given by the glycol chain length)..

Polyesters based on deoxycholic acid and DEG or OEG 200 have glass transition temperatures higher than 20°C, which are convenient for handling and processing, therefore they were selected for the studies regarding synthesis of amphiphilic block copolymers. Other polyester synthetic route will be taken into account in the future, in order to obtain polymers with higher molar mass.

Polyesters with thermosensitive properties will be evaluated as thermosensitive blocks, which could replace poly(izo-propylacrylamide).

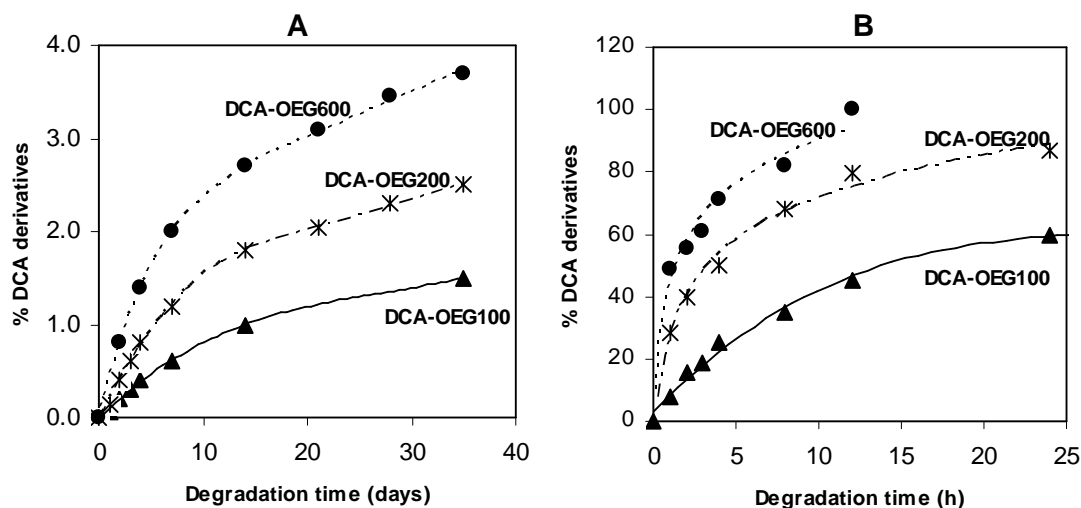


Fig. 4. Variation in time of deoxycholic acid (DCA) released during polyester hydrolysis in phosphate buffer pH 7.4 (A) or 1N NaOH (B), at 37 °C. Polyester sample code indicates OEG' molar mass.

**Results obtained during this stage were included in 2 articles published in 2013.**

1. M. Silion, M. C. Stanciu, M. Nichifor, Mass Spectral Analysis of New Biocompatible Polyesters based on Bile Acids under Electrospray Ionization Condition, *Revista de Chimie* 64, 330-333 (2013).
2. M. C. Stanciu, M. Nichifor, New degradable polyesters from deoxycholic acid and oligo(ethylene glycol)s, *Polymer International*, 62, 1236–1242 (2013)

Project Director,

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