December 2011-December 2012

OBJECTIVES SI ACTIVITIES for 2012

OBJECTIVE 1: **Synthesis and characterization of copolymers polysaccharide-b-bile acid polyester**

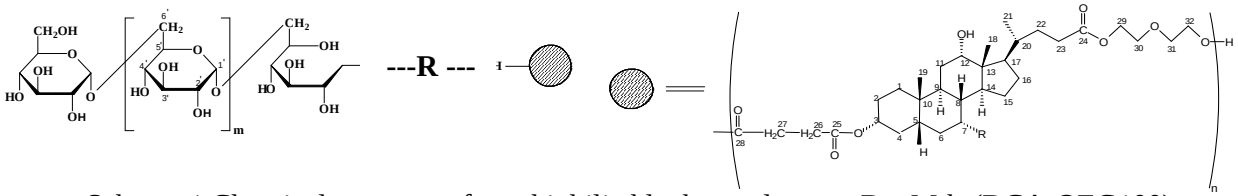
Activity 1.1. Synthesis (setting-up optima conditions)

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

Activity 1.3. Biodegradation studies

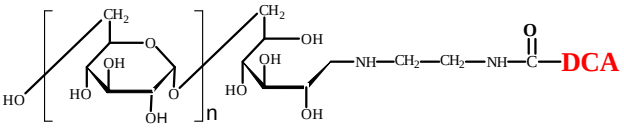
1.1. Synthesis

Synthesis of block copolymers Pz-b-BAPE (Scheme1) was performed by coupling the two preformed blocks (polysaccharide and bile acid polyester) through reaction between block end functional groups. For this purpose, dextran with M_n 6, 11 or 25 kDa, as well as deoxycholic acid-oligo(ethylene glycol) (DCA-OEGX) polyesters were used. Synthesized block copolymers were soluble in DMSO, N-methylformamide and their mixtures with THF or water. The code for the copolymers was DexM-b-(DCA-OEGX), where M is dextran molar mass, in kDa, and X is the mass of glycol used for polyester synthesis.



Scheme 1.Chemical structure of amphiphilic block copolymers DexM-b-(DCA-OEG100).

Synthesis of block-like polymers DexM-R (Scheme 2) was carried out by reductive amination of dextran end aldehyde groups with hydrophobic amines (dodecylamine, didodecylamine, hexadecylamine, 2'-aminoethylene-deoxycholyl amide) in DMSO or N-methylformamide.



Scheme 2. Chemical structure of DexM-DCA

1.2. Structural and physico-chemical properties of block copolymers

Polymer chemical structure was confirmed by elemental analysis, IR, ^{1}H- and ^{13}C-RMN spectrometry and their chemical composition was assessed by HPLC determination of the bile acid content, after polymer hydrolysis in 1N NaOH. The content was close to the theoretical value calculated for the chemical structure presented in Scheme 1 and the number average molar masses of the two blocks.

Weight average (M_w) and number average (M_n) *molar masses* were measured by GPC, using DMSO as eluent and dextran standards. All block copolymers chromatograms show single peaks with the mass close to the sum of the two component masses.

Study of thermal properties of copolymers DexM-b-(DCA-OEGX) focused on polymer thermal stability and glass transition temperatures. Thermal stability was evaluated by thermogravimetric analysis (TG/TDG), which highlighted the presence of two decomposition steps, corresponding to each block (Fig. 1a). DSC measurements allowed the detection of two values for T_g, close to the values found for each separate block (Fig. 1 b).

Low copolymers crystallinity was observed by *Wide angle X-Ray diffraction (WAXS)* measurements (Fig. 1 c) and confirmed by electronic microscopy (SEM) images (Fig 1d).

1.3.Polymer (bio) degradation

Hydrolytic degradation of bile acid polyester is very slow under conditions simulating physiological media (pH 7.4, 37 ^{o}C), what was found also for aliphatic polyesters such as poly(ε-caprolactone) or poly(glycolides). Coupling of these polyesters to a hydrophilic polymer such as dextran determined an

increase of hydrolytic degradation rate (Fig. 2), the extent of the increase being enhanced by longer dextran chains. Nevertheless, degradation rate was still low, being as high as 5g% after 5 weeks for block copolymers obtained from polyester DCA-OEG100.

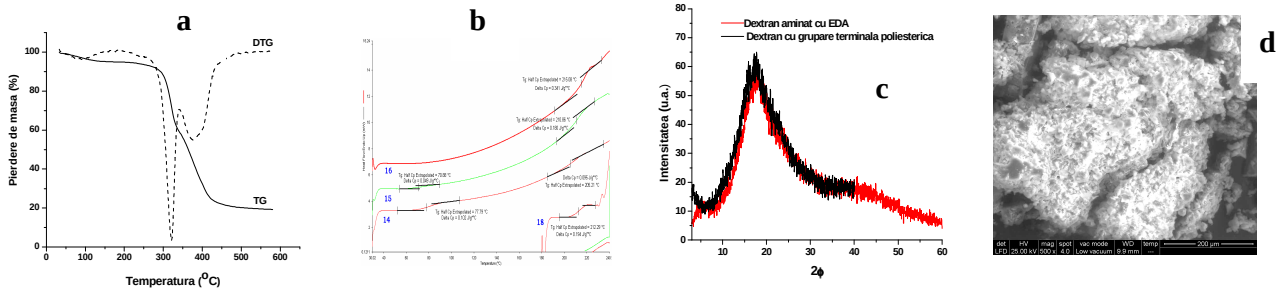


Fig. 1. Properties of copolymers Dex6-b-(DCA-OEG100) measured by (a) TG/DTG; (b) DSC; (c) WAXD; (d) SEM

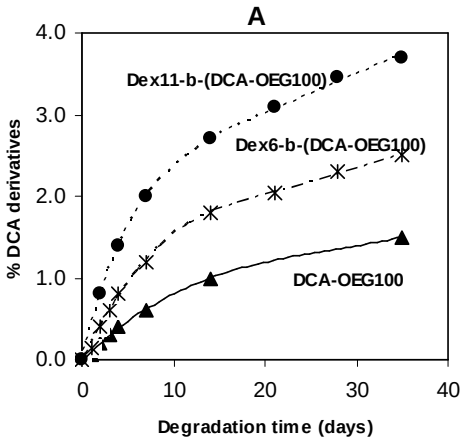


Fig. 2. Hydrolysis of polyester DCA-OEG100 and its block copolymers with dextran, in phosphate buffer at pH 7.4 and 37°C. Hydrolysis process was followed by HPLC analysis of DCA derivative amount released in degradation medium.

OBJECTIVE 2*:* **<u>Study of block copolymers self-assembly</u>**

Activity 2.1. Micelles (vesicles)preparation

Activity 2.2. Micelles/vesicles characterization

2.1. Micelles (vesicles) preparation. Copolymers DexM-b-(DCA-OEGX) were dissolved in DMSO and dialyzed against water. The resulting solution/suspension with concentration in the range 0.1-0.5 g/dL were stable for several months. Block-like polymers DexM-R form micelles by direct dissolution in water at a concentration higher than their critical aggregation concentration (*CMC*).

2.2. <u>Aggregate characterization</u> was performed by fluorescence, light scattering (DLS), AFM and TEM.

Fluorescence studies in the presence of pyrene as fluorophore give information about aggregate properties, like critical concentration at which aggregate formation starts (*CMC*), polarity of the aggregate hydrophobic core (evaluated from the maximum values of pyrene parameter I_3/I_1), and aggregation number (N_{agg}, the number of polymeric chain forming one aggregate), the latter being determined by pyrene fluorescence quenching studies in the presence of cetylpyridinium bromide. *CMC* values for copolymers DexM-b-(DCA-OEGX) are very low (0.5-3 mg/L) (Fig. 3a), and slightly increase with increasing dextran molar mass. By comparison, *CMC* values for DexM-R are 3-4 order of magnitude higher (Fig. 3d), micelle core polarity is lower and their aggregation number (8-20) decreases with increasing dextran molar mass.

Light scattering (DLS) provided aggregate size (hydrodynamic diameter) which was 20-30 nm for DexM- R and 60-100 nm for DexM-b-(DCA-OEGX) (Fig. 3 b,e).

AFM images (Fig. 3 c,f) allowed to estimate aggregate size, shape and organization type of aggregates. According to these images, block copolymers and block-like polymers form spherical micelles with sizes similar to those observed by DLS.

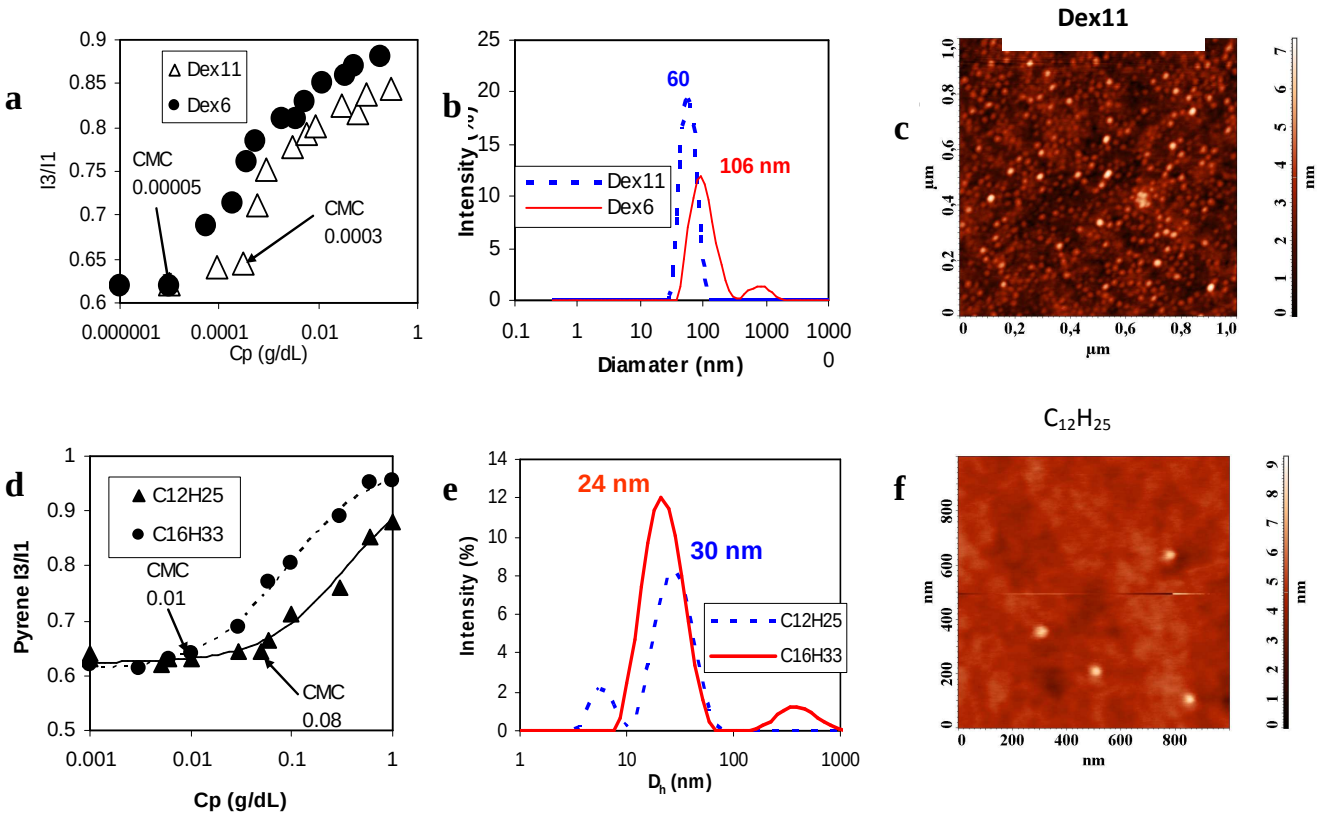


Fig. 3. Characteristics of aggregates formed by DexM-b-(DCA-OEG100) (a, b, c) and Dex11-R (d,e,f) provided by fluorescence (a,d), DLS (b,e) and AFM (c,f) measurements

OBJECTIVE 3*:* **<u>Block copolymer functionalization</u>**

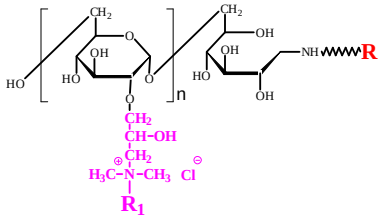
Activity 3.1. Introduction of ionic groups

Activity 3.2. Crosslinking

Activity 3.3. Folic acid attachment

3.1. <u>Introduction of ionic groups</u> can lead to preparation of multifunctional polymers. Besides their capacity to self-assemble with formation of a hydrophobic core acting as a reservoir for hydrophobic molecules, these functionalized polymers can act as polyelectrolytes with various applications in retention (binding) of charged hydrophilic or hydrophobic molecules, as flocculants or antibacterial agents. At this end, cationic polymers with chemical structure depicted in Scheme 3 were obtained and their aggregation capacity was studied by fluorescence, DLS and AFM, in comparison with their neutral (unfunctionalized) predecessors.

According to the fluorescence measurements, cationic groups attachment slightly increases *CMC* values, but significantly increases polarity (and reduces compactness) of hydrophobic core. The polarity parameter (the ratio I_3/I_1), depends on the R_1 substituent chemical structure and decreases in the order: R_1 = Octyl > Benzyl > Ethyl (Fig. 4a). Polymer self-assembly capacity is impaired by increase of cationic group content and disappears at a degree of substitution > 30 mol%. Presence of cationic groups decreases N_{agg} (from 22 for Dex11-$C_{12}H_{25}$, to 8 for Dex11- $C_{12}H_{25}$-QOct25), and increases the aggregates size (until 200-400 nm, Fig. 4b and c).



Scheme 3. Chemical structure of cationic polymers prepared by reaction of DexM-R with an equimolar mixture of epichlorohydrine and a tertiary amine, in an aqueous medium. Cationic group content (Y) was 16-30 mol% (0.8-1.4 meq/g) and R_1 was ethyl (Et), benzyl (Bz), or octyl (Oct). Polymer code is DexM-R-QR_1Y

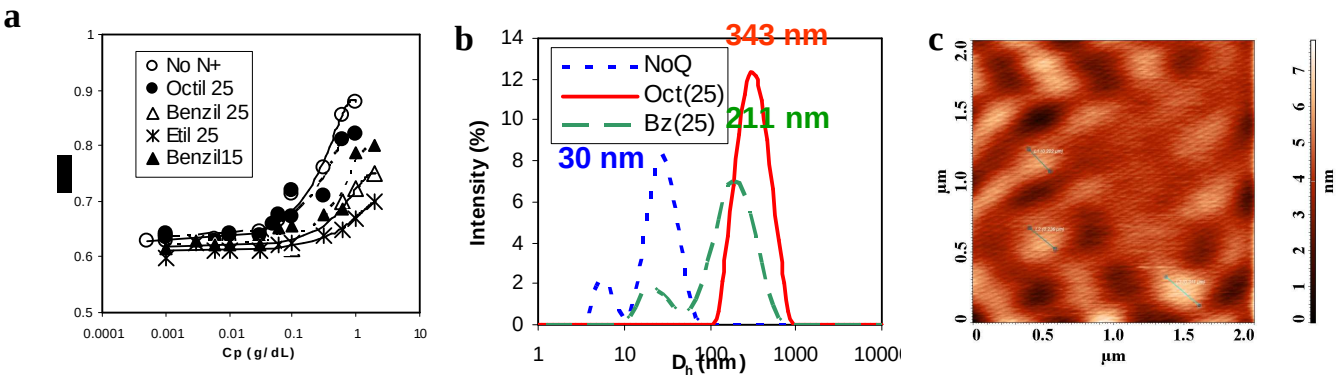


Fig. 4. Properties of cationic amphiphilic block-like polymers Dex11- $C_{12}H_{25}$-QR_1Y (R_1 = Octyl for AFM)

3.2. <u>Crosslinking</u>. The drop of concentration under *CMC* value of amphiphilic block copolymers can lead to aggregate disappearance, therefore, aggregate crosslinking may improve their stability to dilution, for example when they are used as drug carriers and are injected into blood stream. Presence of a polysaccharide as a micelle outer shell (corona) facilitates aggregate crosslinking due to numerous reactive OH groups. Crosslinking of block copolymers and block-like polymers was performed with divinyl sulfone (DVS), in aqueous medium, in the presence of a catalyst (NaOH, pH 12, or DABCO, pH 9). The reaction was carried out under conditions which can supply stable particles and preserve the size and shape of un-crosslinked aggregates: polymer concentration 0.1-0.2 g/dl (higher than *CMC*), DABCO as a catalyst and the ratio DVS/glucosidic unity = 1.5-2).

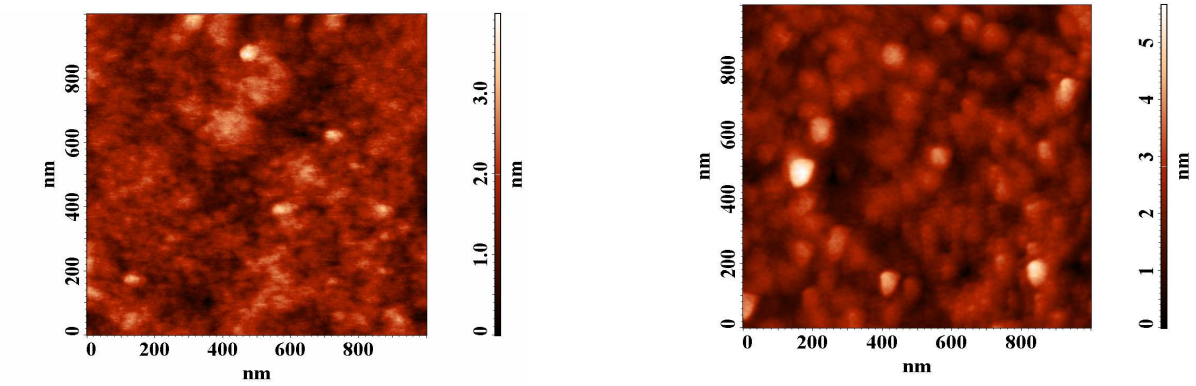
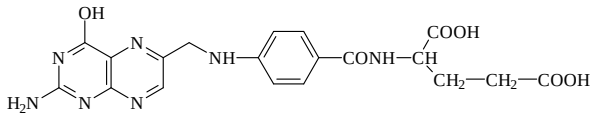


Fig. 5. AFM images of crosslinked amphiphilic polymers (a) Dex25-$C_{16}H_{33}$, crosslinked with 5 % DVS, size (DLS) 64 nm (100 %); (b) Dex11-b-(DCA-OEG100), crosslinked with 11 % DVS, size (DLS) 60 nm (98 %), 244 nm (1.2%).

3.3.<u>Folic acid attachment</u>. Attachment of some specific vector molecules are very useful in targeting a drug delivery system to its site of action. Folic acid is a very useful vector for the treatment of many cancer types. Besides, it can easily react with another molecule through its carboxylic group in *γ* position (Scheme 4), without loosing recognition capacity.



Scheme 4. Chemical structure of folic acid

Covalent binding of folic acid was realized by its reaction with dextran OH groups, in DMSO, in the presence of N,N- dicyclohexylcarbodiimide as a coupling agent and N,N-dimethylaminopyridine as a catalyst. Content in bound folic acid, as determined by UV measurements, was 1-2 mol % (related to

glucosidic units).

4. <u>Evaluation of synthesized amphiphilic polymers' application potential.</u> Amphiphilic polymers based on polysaccharides, especially those with ionic charges (carboxylic or quaternary ammonium groups) and/or crosslinking bridges were evaluated as carriers for controlled drug delivery or as flocculants.

Conclusions

Amphiphilic polymers based on a polysaccharide (dextran) as a hydrophilic block were synthesized and characterized. Hydrophobic block was either a deoxycholic acid polyester (amphiphilic block copolymers), or a hydrophobic chain (alkyl, bile acid) (block-like end-modified amphiphilic polymers). Polymers with different chemical compositions were obtained by variation of each block properties: dextran molar mass, molar mass of the oligo(ethylene glycol) used for polyester preparation, presence of ionic groups (quaternary ammonium groups), crosslinking bridges (divinyl sulfone) or targeting vectors (folic acid) along the dextran backbone. All synthesized polymers were characterized by fluorescence, DLS, AFM, in order to estimate their ability to form ordered aggregates in aqueous media and to determine aggregate properties..

- Block copolymers DexM-b-(DCA-OEGX) form aggregates at much lower concentrations (*CMC* = 0.5-3 mg/L), than classical surfactants. *CMC* values increase with increasing dextran molar mass. Aggregates have a spherical shape, nanometric size (hydrodynamic radius = 60-100 nm) and a narrow size distribution.
- Block copolymer degradation under physiological conditions is slow, but the degradation rate increases with increasing dextran molar mass.
- Block-like polymers DexM-R form aggregates at concentrations close to *CMC* of classical surfactants (0.01-0.1g/dL), they are small in size (20-30 nm) and have spherical shape. Micelle core polarity is higher than that of block copolymers (ratio I_3/I_1 = 0.95-1.2, as a function of R hydrophobicity, while maximum I_3/I_1 = 0.9 for block copolymers). Aggregation capacity is preserved after attachment of a moderated number of cationic groups along the dextran main chain (maximum 30 mol%), but core polarity increases.
- Both types of amphiphilic polymers aggregates could be crosslinked under mild conditions without significant change in aggregate size and shape.
- Attachment of folic acid to the dextran shell enhances the potential of these nanosized aggregate as controlled drug delivery systems. Evaluation of this potential, as well as prospective applications in other fields will be studied in the following stages of the project.

Studies performed during this stage were disseminated in 2 articles published in 2012, 1 article sent to publication (published in 2014) and 1 article in manuscript:

- G. Mocanu, Z. Souguir, L.Picton, D.Le Cerf, Multi-responsive carboxymethyl polysaccharide crosslinked hydrogels containing Jeffamine side-chains, Carbohydrate Polymers 89, 578– 585 (2012).
- L. Ghimici, M. Nichifor, Flocculation by cationic amphiphilic polyelectrolyte: Relating efficiency with the association of polyelectrolyte in the initial solution, Colloids and Surfaces A: Physicochem. Eng. Aspects 415, 142– 147 (2012).
- M. Nichifor, G. Mocanu, C. M. Stanciu, Micelle-like association of polysaccharides with hydrophobic end groups, sent to Carbohydrate Polymers
- G. M. Stanciu, M. Nichifor, Block copolymers with dextran as hydrophilic part. Manuscript.

Project Director,

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