

OBJECTIVES and ACTIVITIES for 2013

Objectives of this stage were synthesis, characterization and functionalization of block copolymers polysaccharide-b-poly(N-izopropylacrylamide) (PNIPAM).

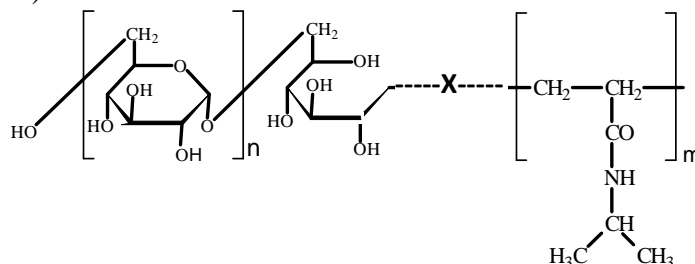
OBJECTIVE 1: Block copolymers polysaccharide-b-PNIPAM. Synthesis and characterization

Activity 1.1. Synthesis (setting-up optima conditions)

Activity 1.2. Structural and physico-chemical characterization

1.1. Synthesis

Several synthetic procedures have been employed for preparation of copolymers polysaccharide-*b*-PNIPAM (Scheme1). These procedures were evaluated in relation to the yield in the final product and the presence of thermosensitivity (occurrence of a sol-gel phase transition at a certain temperature called low critical solution temperature, LCST).



Scheme 1. General chemical structure of block copolymers DexM-PNIPAM. The linkage X between the two blocks is determined by the method used for synthesis.

Some results concerning the synthesis and properties of the obtained polymers are included in Table 1. According to these data, the best yields were obtained by method 1.1.b, the lowest by method 1.1.a. However, the method 1.1.b provided block copolymers with low molar masses for PNIPAM, and this fact led in several cases to the absence of thermosensitivity. Consequently, the method 1.1.c was considered as the most advantageous for synthesis of block copolymer in moderate yield and displaying the desired thermosensitive properties.

Table 1. a, b, c define the synthetic procedures employed.

Code Block copolymer	M _n		M _n copolymer		Yield,* %	LCST, °C
	Dextran	PNIPAM	Calculated	GPC		
Dex-PNIPAM(a-1)	4500	2000	7500	6920	25	34
Dex-PNIPAM(a-2)	4500	4000	8500	7860	20	33
Dex-PNIPAM(b-1)	4500	1000	5500	4873	65	38
Dex-PNIPAM(b-2)	4500	1500	6000	5500	68	37
Dex-PNIPAM(b-3)	8000	1000	9000	8755	58	NO**
Dex-PNIPAM(c-1)	4500	2000	7500	7120	60	34
Dex-PNIPAM(c-2)	4500	4000	8500	8100	53	33
Dex-PNIPAM(c-3)	8000	4000	12000	11000	50	33

* Related to the dextran amount used in synthesis

** Not observed – polymer is not thermoresponsive

1.2. Structural and physico-chemical characterization of the block copolymers

Chemical structure of precursors and block copolymers was certified by elemental analysis, IR, ¹H- and ¹³C-NMR spectroscopy. Chemical composition of block copolymers (molar and gravimetric ratio of the

blocks) was calculated from $^1\text{H-NMR}$ spectra, from the ratio of integrals corresponding to the characteristic peaks of the blocks: 4.9 ppm (anomeric CH of dextran) and 1.15 ppm (CH_3 of PNIPAM).

Average weight (M_w) and numeric (M_n) molar mass were measured by GPC, with a mixture 0.1N NaOH /DMSO 80/20 v/v as eluent and dextran standards. Block copolymer chromatograms displayed one single peak, with molecular mass close to that corresponding to the sum of the two blocks. Molar mass of PNIPAM blocks from copolymers was measured on copolymer samples after hydrolysis in HCl under condition allowing a complete depolymerisation of dextran to glucose.

Study of copolymers Dex-PNIPAM thermal properties was performed in order to highlight their thermosensitive behavior and determine the phase transition temperatures (LCST). At this end, UV absorbance (at 450 nm) of copolymer aqueous solutions (1% g/v) was measured at temperature in the range 20-60 °C. Sol-gel phase transition is accompanied by an increase in solution turbidity, due to the temperature induced insolubilization of PNIPAM blocks. All studied polymers showed temperature induced phase transition, except for polymers with a low content in PNIPAM block (under 15 % g/g, ex. Dex-PNIPAM (b-3). LCST values (33-38 °C), taken as temperatures at which phase separation process starts, were relatively close to PNIPAM' LCST (32.4°C). According to Fig. 1, when copolymers have the same dextran block molar mass (4500), a slight increase of LCST values with decreasing PNIPAM' block molar mass can be observed (Fig. 1, curves 1-3). An increase of dextran molar mass determined a slower, less cooperative phase transition (Fig. 1, curves 4, 5).

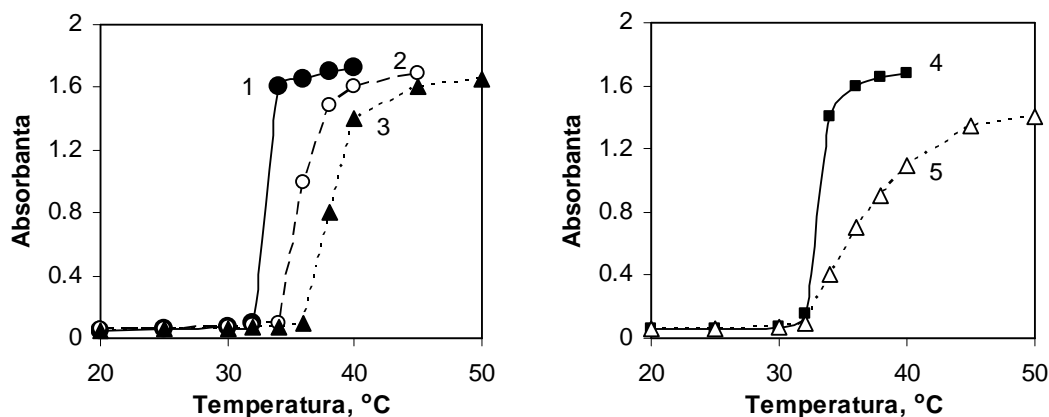


Fig.1. Absorbance variation with temperature for 1g% aqueous solutions of polymers PNIPAM (1), Dex-PNIPAM (a-1) (2), Dex-PNIPAM(b-2), Dex-PNIPAM(c-2) si Dex-PNIPAM(c-3). Sample codes correspond to the copolymers listed in table 1..

OBJECTIVE 2: Micelle/vesicles for PNIPAM copolymers

Activity 2.1. *Micelles/vesicle preparation (at $T > \text{LCST}$)*

Activity 2.2. *Properties measurement (size, polarity, morphology)*

2.1. Aggregate preparation from copolymers Dex-PNIPAM was carried out by heating polymer aqueous solutions of different concentration at a temperature higher than LCST (50 °C).

2.2. Aggregate characterization was performed in this stage by fluorescence and light scattering (DLS).

Fluorescence studies in the presence of pyrene as fluorophore provided the critical temperature at which aggregates are formed (T_{CA}) and polarity of aggregate hydrophobic core (minimum value of the polarity parameter I_1/I_3). The plots of I_1/I_3 ratio variation with temperature (Fig. 2A) present inflexion points, which correspond to T_{CAs} , and T_{CAs} values are close to LCST values determined by absorbance measurements. The lowest I_1/I_3 values indicate an increase of aggregates hydrophobicity with decreasing dextran block molar mass.

Light scattering (DLS) can be used for aggregate size measurements (hydrodynamic radius) (Fig. 2B). At $T < \text{LCST}$, very low sizes (< 10 nm) prove the presence of individual (non-aggregated) copolymer chains. When temperature increases, PNIPAM chains start to dehydrate and become hydrophobic,

determining their hydrophobic associations with formation of big aggregates ($D_h > 100$ nm). The presence of hydrophilic dextran blocks with higher molar masses (Dex-PNIPAM(c-3)) led to formation of bigger aggregates. As one can notice from Fig. 2B, temperature at which the increase in size starts is very close to the LCST and TCA values determined by absorbance and fluorescence measurements, respectively.

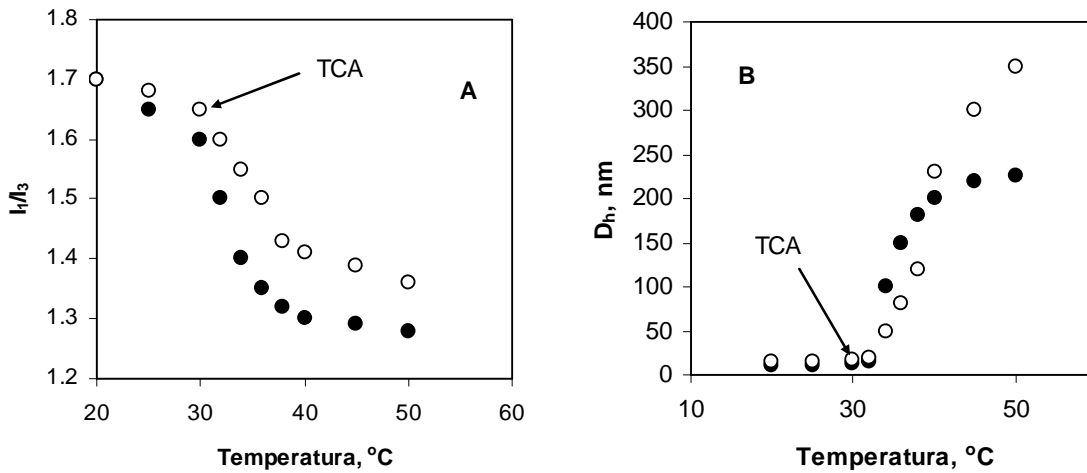


Fig. 2. Variation of polarity parameter I_1/I_3 (calculated from fluorescence emission spectra obtained in the pyrene presence) (A) and hydrodynamic diameter (D_h) (B) with temperature for block copolymers Dex-PNIPAM(c-2) (open circles) and Dex-PNIPAM(c-3) (solid circles). Measurements were performed with 1 g% aqueous solutions.

OBJECTIVE 3: Functionalization of PNIPAM copolymers

Activity 3.1. Crosslinking

Activity 3.2. Introduction of ionic groups, folic acid

3.2. Crosslinking. Crosslinking of aggregates formed by amphiphilic polymers is often required for the increase in aggregate stability to dilution, since a decrease of polymer concentration under a critical value lead to aggregate disintegration. In case of aggregates formed only above a certain temperature (LCST) crosslinking allows the preservation of size and number of aggregates, irrespective of temperature value. Presence of a polysaccharide as the outer shell (corona) of the aggregate is very advantageous for crosslinking due to numerous polysaccharide OH reactive groups. In the present case, crosslinking was performed with divinyl sulfone (DVS) in aqueous medium, in the presence of a catalyst (NaOH, pH 12, or DABCO, pH 9). Study was intended for finding the optima reaction conditions under which stable particle are obtained, with the shape and size similar to those of starting non-crosslinked polymer. The most important studied parameters were polymer concentration (0.1-1 g/dl), temperature (10-20 °C above LCST), catalyst (NaOH, DABCO) and crosslinking agent concentration (ratio DVS/UG = 1.5-3).

3.2. Introduction of ionic groups, folic acid

Quaternary ammonium groups were attached to the dextran block by reaction of polysaccharide OH groups with an equimolar mixture N,N-dimethyl-N-benzylamine/ epichlorohydrine in aqueous medium. Reaction was carried out on crosslinked Dex-PNIPAM copolymers, and the final content in amine groups was about 10-20 mol%. UV absorbance studies showed that aminated polymers displayed thermosensitivity, LCST was close to that of precursor neutral polymer, but the speed (cooperativity) of the phase transition process significantly decreased. According to preliminary experiments performed in solutions with high content in NaCl (0.1-1 M), transition process might be accelerated by the increase of medium ionic strength.

Covalent attachment of the folic acid was realized by its reaction with dextran OH groups, in DMSO, in the presence of N,N- dicyclohexylcarbodiimide as coupling agent and N,N-dimethylaminopyridine as

catalyst. UV analysis of the final product indicated a substitution degree of 1-2 mol % (related to dextran glucosidic units).

4. Prospective study for the application of amphiphilic polymers. Application of amphiphilic polymers based on polysaccharides as controlled drug delivery systems or flocculants was studied mainly using ionic linear or crosslinked polymers.

Conclusions

New amphiphilic block copolymers based on a polysaccharide, dextran, as hydrophilic block were synthesized and characterized. Hydrophobic block was a temperature sensitive polymer, poly (N-isopropylacrylamide) (PNIPAM), which becomes hydrophobic only at a temperature higher than 35 °C (temperature induced hydrophobicity). Block copolymers Dex-PNIPAM were synthesized by three different methods, with the aim at obtaining good yields and polymer with desired properties. Copolymers with different chemical compositions were obtained by variation of block precursor properties, with a special focus on dextran and PNIPAM molar mass (blocks chain length). Block copolymers were further chemically modified by the attachment of crosslinking agents (divinyl sulfone), ionic groups or folic acid, to the dextran block. In order to establish their chemical structure and composition, all synthesized polymers were characterized by elemental analysis, FTIR and NMR spectroscopy, GPC, potentiometric titrations. Fluorescence and DLS techniques were used to emphasize polymer capacity to form ordered aggregates in aqueous solution as a function of temperature, and to determine aggregate characteristics. The results can be summarized in the following:

- Block copolymers Dex-PNIPAM with thermosensitive properties can be obtained in good yield by specific procedure adapted to the each block properties.
- Synthesized block copolymers form nanosized aggregates in aqueous solutions at temperature higher than LCST, the value of which is close to the PNIPAM'LCST. Aggregate size and polarity of the hydrophobic domain built by association of dehydrated PNIPAM chains depend mainly on the dextran block length.
- Chemical modification of these amphiphilic polymers preserves their thermosensitivity. Crosslinking under mild condition did not affect the shape and size of aggregates. Attachment of ionic groups can provide polymers with improved applicability and possible dual sensitivity to pH and temperature.

Results obtained in this stage were dissaminated in 3 articles published in 2013 and an article in draft.

- L. Ghimici, M. Nichifor, Separation of TiO₂ particles from water and water/methanol mixtures by cationic dextran derivatives, Carbohydrate Polymers 98, 1637– 1643 (2013).
- G. Mocanu, M. Nichifor, Cationic amphiphilic dextran hydrogels with potential biomedical applications, Carbohydrate Polymers 99, 235-241 (2014).
- G. Bai, Y. Wang, M. Nichifor, M. Bastos, Critical role of the Degree of Substitution on the Interaction of Biocompatible Cholic Acid-Modified Dextrans with Phosphatidylcholine Liposomes, Langmuir 29 (43), 13258–13268, 2013.
- M. Nichifor, C.M. Stanciu, New thermosensitive block copolymers based on dextran and poly(isopropylacrylamide). Draft manuscript.

Project Director,