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Synthesis and characterization of thermosensitive and pH-sensitive block copolymers based on polyetheramine and pullulan with different length

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ABSTRACT

Novel thermo- and pH-sensitive block copolymers were prepared via reductive amination by end-to-end coupling reaction between the aldehyde function (reducing sugar) of pullulan with varied molar masses and the amine function of polyetheramine (i.e. methoxypoly (oxyethylene/oxypropylene)-2-propylamine) or PEO-PPO amine. The conditions of synthesis, followed by FTIR and ^{1}H NMR are well-controlled and depend strongly on pullulan length. The properties of copolymers in aqueous solution were investigated by SEC/MALS/DRI and absorbance measurements as function of temperature and pH, providing evidence of thermo- and pH-sensitive self-assembly of the copolymers.

1. Introduction

Some polymers exhibit a special behavior characterized by a lower critical solubility temperature (LCST) or a cloud point according to the concentration domain, which is followed by a precipitation when the temperature reaches a critical value. This phase separation is due to the change of polymer/water, water/water and polymer/polymer interactions (“hydrophobic effects” and/or “hydrogen bonding effects”) (Schil, 1992). Polymer, which has the LCST property can be linked to water soluble polymers giving rise to block (via copolymerization with synthetic polymers) (Deng et al., 2008) or grafted thermosensitive polymers (Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005; Kitazono & Kaneko, 2004; Padmanabha Iyer et al., 2005). Self assembly properties in aqueous solutions of systems like poly(N-isopropylacrylamide) (PNIPAM), block copolymers based on poly(ethylene-oxide)-poly(propylene oxide) (PEO-PPO) or cellulose derivatives (hydroxypropyl cellulose HPC, methyl cellulose MC) have received substantial attention mainly due to their potential applications in a number of fields including the development of novel drug carriers (Henni et al., 2007).

In recent years more attention has been focused on polysaccharides due to their main characteristics of biocompatibility, renewable origin and a great structural variety. The chemical modification of polysaccharides can be conducted on alcohol, carboxylic or amine function to obtain new families of water soluble smart polymers such as associative polysaccharides (Henni et al., 2005) in order to improve the functional properties of unmodified polysaccharides (Ghosh, Sen, Jha, & Pal, 2010). The literature reports some very interesting studies where polysaccharides are grafted by polymers, which exhibit the LCST property. Most of the published works deals with randomly branched thermosensitive polysaccharide such as chitosan (Bhattarai et al., 2005), hyaluronan (Kitazono & Kaneko, 2004), carboxymethylpullulan (Mocanu, Mihai, Dulong, Picton, & Le Cerf, 2011). Such systems generally exhibit polydisperse aggregates or macrostructures when the critical temperature is reached.

Another trigger is pH when copolymers contain ionisable functional groups like –COOH or –NH_2 which acquire a charge in a certain pH depending on pK_a. It is then possible to have both dependence temperature and pH on block copolymers as (poly(N-isopropylacrylamide-co-butylmethacrylate-co-acrylic acid) (Serres, Baudys, & Kim, 1996), (poly(ethylene oxide)-b-(poly(methoxydi(ethylene glycol)methacrylate-co-methacrylic acid) (Jiang & Zhao, 2008) or poly(2-methoxyethoxy)ethyl methacrylate)-b-(poly(acrylic acid) (Messel et al., 2011). These systems have potential applications in detergency, pharmaceutics and biomedical.

The aim of this work is to develop new thermo- and pH-sensitive surfactant systems. The possibilities, the properties and the interest of polysaccharide-containing block copolymers are described in a recent review (Schatz & Lecommandoux, 2010). The pure hydrophilic part is constituted by a linear and neutral polysaccharide composed by regularly repeating α-l,6-linked maltotriose units. The thermosensitive block is a polyetheramine composed with average of 29 PPO and 6 PEO. Moreover, PEO systems have

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Table 1
Macromolecular characterization of polymers by SEC/MALS/DRI analysis in 0.1M $LiNO_3$.

		Mn ($g\,mol^{-1}$)	Mw ($g\,mol^{-1}$)	I_p[a]
polyetheramine pH 9	PEO_6-PPO_{29}-NH_2	9500	37,000	3.9
polyetheramine pH 5.5	PEO_6-PPO_{29}-NH_2	3800	4000	1.0
Undegraded pullulan	P97000	97,000	270,000	2.8
Degraded pullulan	P37000	37,000	74,000	1.9
	P18000	18,000	36,000	1.9
	P7100	7100	11,000	1.6
	P2300	2300	3200	1.3
Copolymer pH 9	P37000-b-PPO_{29}-PEO_6	250,000	530,000	2.1
	P18000-b-PPO_{29}-PEO_6	140,000	150,000	1.1
	P7100-b- PPO_{29}-PEO_6	430,000	560,000	1.3
	P2300-b-PPO_{29}-PEO_6	370,000	490,000	1.3
	P504-b-PPO_{29}-PEO_6	210,000	310,000	1.4
Copolymer pH 5.5	P2300-b-PPO_{29}-PEO_6	5300	6800	1.2
	P504-b-PPO_{29}-PEO_6	4700	5700	1.2

[a]Polydispersity index (I_p) I_p = Mw/Mn.

evidenced great potential in therapeutic systems (Lisli, Van Es, Abuchowski, Palczuk, & Davis, 1982). In order to obtain new linear triblock copolymers the reductive amination has been involved between the reducing sugar of the polysaccharide (i.e. aldehyde) and the amine function of polyetheramine (Bosker et al., 2003). pH sensitivity is due to the amine bound between pullulan and the last PPO unit of the polyetheramine.

Pullulan has been reduced by enzymatic way to obtain various controlled molar masses (from 37,000 to 2300 $g\,mol^{-1}$). Maltotriose was used to obtain the shorter pullulan length. The aim is to obtain of various pullulan conformations (Jaud, Tobias, & Brant, 2005) (from random coil to short rods). This parameter should play an important role in the Hydrophilic/Lipophilic Balance (HLB) of the final copolymer together with the conformation of the pullulan block compared to the polyetheramine.

In this work, the controlled synthesis of such triblock smart copolymers is reported, and also their physicochemical characterization and some preliminary results on their thermoassociative behavior.

2. Materials and methods

2.1. Materials

2.1.1. Pullulan precursor

Pullulan produced by *Aureobasidium pullulans*, is a flexible and linear polysaccharide formed from α-1,4 linked glucose units and including α-1,6 maltotriose units. As Pullulan has two structural isomers, which are in thermodynamical equilibrium: one containing a glucosyl end group and the other containing an aldehyde end group. Pullulan does not show thermosensitive properties. In this study, pullulan (Mn = 97,000 $g\,mol^{-1}$; Mw = 270,000 $g\,mol^{-1}$ determined by SEC/MALS/DRI measurements) was purchased from Hayashibara Biochemical Laboratory (Okayama, Japan). Pullulan degradation has been achieved by enzymatic route. This reaction was performed with Pullulanase *Klebsiella pneumonia* (type "3") provided by Sigma–Aldrich (Pugsley, Chapon, & Schwartz, 1986; Komacker & Pugsley, 1989) (EC 3.2.1.41) which hydrolyzes the α-1,6-D-glucosidic linkages of pullulan and amylopectin. The enzymatic degradation procedure is the following:

Pullulan solution preparation: pullulan (5 $g\,L^{-1}$) was dissolved in $LiNO_3$ (0.1 M) at pH = 5.5 under stirring during 24 h.

Enzyme preparation: 1.54 $U\,mg^{-1}$, where one unit of activity is defined as the amount of enzyme required to release one μmole of D-glucose reducing sugar equivalents per minute from reducing pullulan, under the defined assay conditions: pH 5.5.

Enzymatic degradation: pullulan solution was pre-equilibrated at 60 °C, then the enzyme solution was added and the reaction was left during different times. The degradation was stopped by addition of acid to disable the enzyme, and then the solution was neutralized. The degraded pullulan was dialyzed against Milli-Q water and then lyophilized.

Thus, molar mass of pullulan decreases as function of degradation time in a controlled manner. Therefore, four molar masses have been focused: 37,000; 18,000; 7100 and 2300 $g\,mol^{-1}$ (determined by SEC/MALS/DRI measurements, in Table 1).

2.1.2. Polyetheramine

Polyetheramine contains primary amino groups attached to the end of a polyether backbone which can be a mixed of PEO–PPO block copolymers or simply based on PEO or PPO only. This chemical family could be composed of monoamines, diamines, triamines... based on this core structure. Among the very large polyetheramine family, "Jeffamine®" (a commercial name) from Huntsman has been specially chosen. Two kinds of Jeffamine® were used. For the majority of synthesis, we have taken the Jeffamine® M2005, a block copolymer of PEO_6-PPO_{29}-NH_2 (Fig. 1). The $-NH_2$ terminal group evidences a pK_a 9.2 ± 0.1, obtained by acid–base titration. This precursor is well known for its LCST behavior and its capacity to form thermoassociative copolymers (Karakasyan, Lack, Brunel, Maingault, & Hourdet, 2008). As an example, a 10 $g\,L^{-1}$ PEO_6-PPO_{29}-NH_2 solution exhibits a cloud point near 24 °C. It can be observed that under acidic conditions ($pH \ll pK_a$ PEO_6-PPO_{29}-NH_2), the terminal ionic group $-NH_3^+$ strongly modifies the association process. Indeed, its theoretical molar mass is near 2000 $g\,mol^{-1}$ (data given by Huntsman), it has been observed that PEO_6-PPO_{29}-NH_2 is self-associated (Mn and Mw about 9500 and 37,000 $g\,mol^{-1}$ respectively determined by SEC/MALS/DRI measurement in $LiNO_3$ at pH = 9.0) even under its cloud point. Under acidic conditions, pH = 5.5 ($pH \ll pK_a$ PEO_6-PPO_{29}-NH_2), the terminal ionic group $-NH_3^+$ avoid any association and Mn and Mw are 3800 and 4000 $g\,mol^{-1}$ respectively as reported in Table 1. In methylene chloride medium, PEO_6-PPO_{29}-NH_2 molar mass is 2600 $g\,mol^{-1}$ (given in polystyrene equivalent) which is in accordance with data obtained at pH = 5.5.

Jeffamine® M600, which is another polyetheramine, is composed of a block copolymer of PEO_1-PPO_9-NH_2. We used this sample in the aim to generalize this synthesis whatever the content of PEO and PPO.

2.1.3. Other reagents

Dimethyl sulfoxide (DMSO), lithium nitrate ($LiNO_3$), methylene chloride, 3,5-dinitrosalicylic acid (DNS), sodium hydroxide

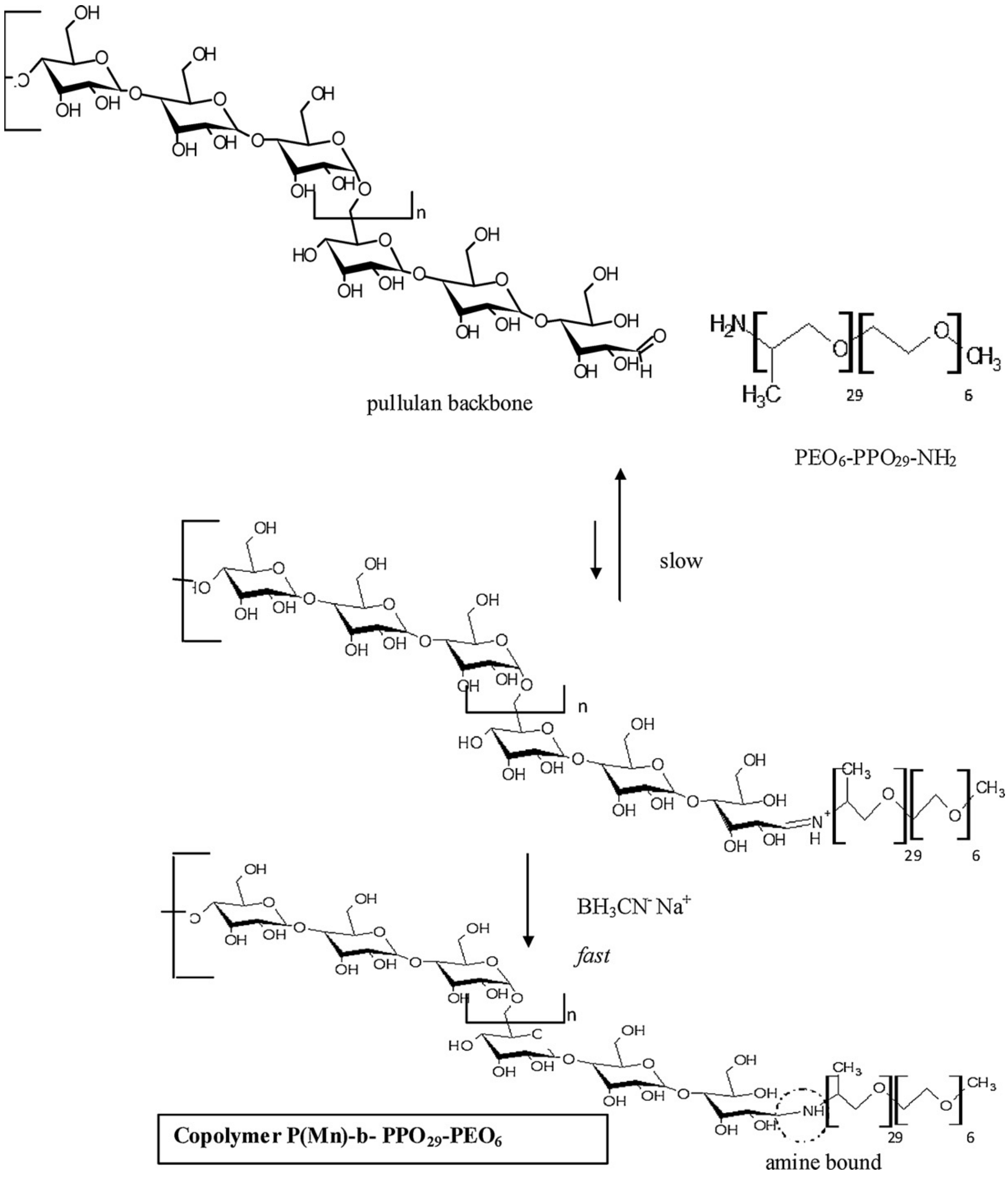

Fig. 1. Synthesis of pullulan-b-polyetheramine block copolymer.

(NaOH), benzenesulfonyl chloride, hydrochloric acid (HCl) were purchased from Acros. Glucose and maltotriose was purchased from Sigma–Aldrich. Sodium cyanoborohydride ($NaCNBH_3$) and dialysis tubing (SpectraPore) was purchased from Fisher Scientific. All organic solvents were dried in molecular sieve before use. All reagents were used without further purification. Water was purified with the Milli-Q reagent system (Millipore).

2.2. Synthesis

The synthesis strategy was based on coupling reaction between the reducing sugar of the polysaccharide and the amino-terminated polyetheramine. In Fig. 1, the last glucose unit of pullulan chain is in thermodynamical equilibrium between closed and opened form. Consequently, each polysaccharide chain reacts with only one amino terminated polyetheramine chain, resulting in only linear triblock copolymer. Conventional reductive amination of carbonyl compounds needs the presence of reducing agent such as hydride (Brown, 1961). Sodium cyanoborohydride ($NaCNBH_3$, a highly selective reducing agent, for reductive amination of aldehydes and ketones) was chosen to couple the two blocks (Borch, Bernstein, & Dupont Durst, 1971). Aldehydes or ketones are reduced with ammonia primary amines or secondary amines to give respectively secondary or tertiary amines (Abdel-Magid, Carson, Harris, Maryanoff, & Shah, 1996).

$NaCNBH_3$ was added to a mixture of pullulan and PEO_6-PPO_{29}-NH_2 dissolved, at 60 °C, in the appropriate oven-dried solvent DMSO (a polar and aprotic solvent where any association of PEO_6-

Table 2
Experimental conditions and reaction yield of the copolymers.

P(Mn)-b-PPO$_y$-PEO$_x$	Molar equivalent (eq)						
	Reaction nos.	*P*	PEO$_x$-PPO$_y$-NH$_2$	NaCNBH$_3$	Yield[a] (mol%)	Free pullulan[b] (%)	Purification method
P37000-b-PPO$_{29}$-PEO$_6$	1	1	5	10	41	50	i
P37000-b-PPO$_{29}$-PEO$_6$	2	1	5	27	45	31	i
P37000-b-PPO$_{29}$-PEO$_6$	3	1	10	27	78	30	i
P37000-b-PPO$_9$-PEO$_1$	4	1	10	27	77	29	i
P18000-b-PPO$_{29}$-PEO$_6$	5	1	10	27	75	30	i
P18000-b-PPO$_{29}$-PEO$_6$	6	1	10	27	76	30	i
P18000-b-PPO$_{29}$-PEO$_6$	7	1	10	27	74	28	ii
P7100-b-PPO$_{29}$-PEO$_6$	8	1	10	10	37	40	i
P7100-b-PPO$_{29}$-PEO$_6$	9	1	10	27	68	30	i
P2300-b-PPO$_{29}$-PEO$_6$	10	1	10	27	–	–	i
P2300-b-PPO$_{29}$-PEO$_6$	11	1	2	27	–	–	i
P2300-b-PPO$_{29}$-PEO$_6$	12	1	2	27	72	30	ii
P504-b-PPO$_{29}$-PEO$_6$	13	1	2	27	79	20	ii

[a] Yield (in mol%) is the ratio between the mole number of copolymer after reaction and the mole number of pullulan at the beginning of the reaction.

[b] Determined by the D.N.S method).

PPO$_{29}$-NH$_2$ occurs). The reaction mixture was stirred for 8 days at 60 °C (Bosker et al., 2003). Different conditions of synthesis were tested (Table 2). Hereafter, the abbreviations of the different copolymers will be "P (Mn)-PPO$_y$-PEO$_x$", where P is used for pullulan, Mn is the pullulan number molar mass, *x* and *y* corresponding to the pattern number of PEO and PPO respectively.

Two purifications conditions have been tested:

Purification 1: the mixture was cooled at room temperature and poured into methanol to form a precipitate. The precipitate was filtered and washed three times with methanol to remove unreacted PEO$_6$-PPO$_{29}$-NH$_2$ and salts. Afterward, the obtained compound was precipitated in acetone to eliminate the residual PEO$_6$-PPO$_{29}$-NH$_2$. Then, the copolymer was dissolved in water, and finally dialyzed against water.

Purification 2: the mixture was dialyzed against water to eliminate salts and an extraction by methylene chloride is started to eliminate residual PEO$_6$-PPO$_{29}$-NH$_2$.

2.3. Methods measurements

2.3.1. Dosage of reducing sugar: DNS (3,5-dinitrosalicylic acid) method

The reducing sugars, assimilated to unreacted pullulan in our case, in the copolymer solution were quantified by the DNS method (Miller, 1959; Sumner & Sisler, 1944) after calibration with maltotriose (Perkin-Elmer Lambda 7UV/vis spectrophotometer, USA, at 540 nm).

2.3.2. FTIR analysis

The FTIR spectra were recorded on a spectrophotometer (ATR MKII Golden gate system Specac, UK): incidence angle of 45°, monoreflexion system, 10 co-added scan, and diamond crystal, at room temperature in the wave number range of 600–4000 cm^{-1}.

2.3.3. ^{1}H NMR analysis

^{1}H NMR analyses were performed at 30 °C with a Bruker Avance 400 spectrometer (400 MHz), UK equipped with ^{1}H probe. Samples were dissolved in deuterium oxide, D$_2$O.

2.3.4. Acid-base titration of PEO$_6$-PPO$_{29}$-NH$_2$ and copolymers

The pK_a of PEO$_6$-PPO$_{29}$-NH$_2$ and copolymers have been determined by classical acid base titration with a Metrohm 632 pH meter (Swiss). To avoid the possible insolubility of PEO$_6$-PPO$_{29}$-NH$_2$, titration was made at low temperature (under the cloud point, at 4 °C) and at 20 °C but at a low concentration (1 g L^{-1}).

2.3.5. SEC/MALS/DRI analysis of polymers

Average molar masses and molar masses distributions were determined by size exclusion chromatography (SEC) coupled online with multi-angle light scattering (MALS) and differential refractive index (DRI) detectors (Simon, Le Cerf, Picton, & Muller, 2002). The MALS apparatus is the EOS from Wyatt Technology (Ca, USA) filled with a K5 cell and a Ga–As laser ($\lambda = 690$ nm). The DRI detector is a Shimadzu RID-10A (Japan). Columns [OHPAK SB-G guard column, OHPAK SB803 HQ columns (Shodex, Japan)] were eluted with LiNO$_3$ 0.1 M at 0.5 mL min^{-1}. Solvent was filtered through 0.1 μm filter unit (Millipore, USA), degassed (Shimadzu LC-10AI, Japan). The sample (10 g L^{-1} for P2300, P7100, P18000 and their copolymers and PEO$_6$-PPO$_{29}$-NH$_2$; 5 g L^{-1} for P37000 and its copolymer and P97000) filtered on 0.45 μm unit filter (Millipore) was injected through a 100 μL full loop. The used dn/dC value is 0.148 mL g^{-1} obtained for pullulan (Duval, Le Cerf, Picton, & Muller, 2001). The collected data were analyzed using the Astra V-3-4-14 software package from Wyatt Technology (CA, USA).

2.3.6. Cloud point measurement by absorbance

The cloud point temperature (T°cp) of aqueous copolymer solution (prepared in Milli-Q at a controlled pH) was detected by absorbance measurement at 400 nm, using a Perkin-Elmer Lambda 7UV/vis spectrophotometer, USA at the inflection point of the curve. The temperature of the solution was raised at a constant rate of 0.5 °C min^{-1}.

3. Results and discussion

3.1. Reaction efficiency

3.1.1. FTIR spectra

On Fig. 2 are reported the FTIR spectra of PEO$_6$-PPO$_{29}$-NH$_2$ (A) pullulan (B), and a copolymer P2300-b-PPO$_{29}$-PEO$_6$ (C). For PEO$_6$-PPO$_{29}$-NH$_2$ (Fig. 2A), the stretching vibrations of CH, CH$_2$, and CH$_3$ are around 2900 cm^{-1} and those relating to the ether groups at 1100 cm^{-1}. Concerning pullulan (Fig. 2B) the peak at 1000 cm^{-1} can be related to ether group and the band around 3300 cm^{-1} is due to the hydroxyl groups. The copolymer (Fig. 2C) evidences the pullulan characteristics (i.e. OH and C–O (ether) stretching vibrations at 3300 cm^{-1} and 1000 cm^{-1} respectively) together PEO$_6$-PPO$_{29}$-NH$_2$ ones (CH, CH$_2$, CH$_3$ and ether stretching vibrations at 2900 cm^{-1} and 1100 cm^{-1} respectively). This is evidence of the presence of both pullulan and PEO$_6$-PPO$_{29}$-NH$_2$ in the copolymer sample. Regarding to the purification procedures, it should be a first indication of reaction efficiency.

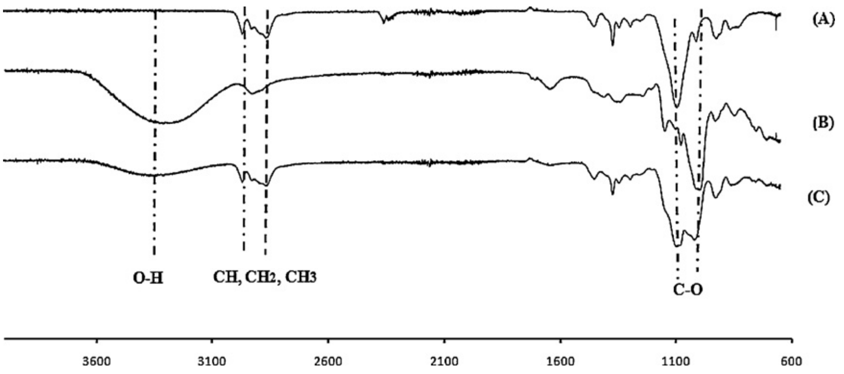

Fig. 2. FTIR spectra of (A): PEO$_6$-PPO$_{29}$-NH$_2$ (B): pullulan; (C) copolymer (P2300-b-PPO$_{29}$-PEO$_6$).

3.1.2. ^{1}H NMR

Fig. 3 shows the ^{1}H NMR spectra of the PEO$_6$-PPO$_{29}$-NH$_2$ (A), pullulan (B), and the copolymer P7100-b-PPO$_{29}$-PEO$_6$ (C) in deuterium oxide (D$_2$O). A typical spectrum of pullulan (Fig. 3B) is obtained (Lack, Dulong, Picton, Le Cerf, & Condamine, 2007). Proton signals, observed at 3.40–3.80 ppm (e) correspond to non anomeric protons, and 4.70–4.90 ppm (d) as well as 5.31–5.35 ppm (d) can be attributed to anomeric protons. PEO$_6$-PPO$_{29}$-NH$_2$ (Fig. 3A) is characterized by 0.92–1.00 ppm (a′) doublet of CH$_3$–CH– from PPO near the amine extremity; 1.10–1.12 ppm (a″) to the **CH$_3$**–CH– from PPO; 3.27 ppm (b) to the –OC**H$_3$**; 3.50–3.65 ppm (c) to C**H**–C**H$_2$** from PPO and C**H$_2$**–C**H$_2$** from PEO. The copolymer spectrum (Fig. 3C) evidences the chemical shifts of methyl protons of PPO (–CHC**H$_3$**) at 1.10–1.12 ppm (a″) (doublet corresponding to all the –C**H$_3$** groups of PPO except the one near the amine group). All chemical shifts of pullulan and PEO$_6$-PPO$_{29}$-NH$_2$ protons were identified in the copolymer spectrum.

In order to check if any unreacted PEO$_6$-PPO$_{29}$-NH$_2$ remains with the copolymer, a mix of a copolymer (P7100-b-PPO$_{29}$-PEO$_6$) and 5 wt.% of PEO$_6$-PPO$_{29}$-NH$_2$ was analyzed by ^{1}H NMR (Fig. 3D). This spectrum reveals the presence of a doublet at ppm 0.92–1.00 ppm (a′) due to the –C**H$_3$** group which is near the primary amine group. Since this doublet is not present in the copolymer spectrum (Fig. 3C), it can be assume that if it remains free unreacted PEO$_6$-PPO$_{29}$-NH$_2$ in the copolymer, this is necessary less that 5%.

3.2. Condition of synthesis

For each synthesis, the yield of the reaction was determined (i.e. ratio between the mole number of copolymer after reaction and the mole number of pullulan at the beginning of the reaction). The possible amount of unreacted pullulan at the end of the reaction has also been determined thanks to the dosage of reducing sugar by the DNS method. A large number of syntheses with the experimental conditions and their characteristics have been reported in Table 2.

As a first result, it has to be noticed that unreacted pullulan is always present. Purification is not effective to remove the unreacted pullulan. The quantity of unreacted pullulan is almost around 30% if the proportion of reducing agent is high (27 eq) but reaches 40 or 50% if the reducing agent is added in lower proportion (10 eq, reactions 1 and 8). The reproducibility of the reaction was well checked (reactions 5 and 6). In order to control this reaction (yield and free pullulan), different parameters have been studied.

3.2.1. Effect of PEO$_6$-PPO$_{29}$-NH$_2$ amount

2; 5 and 10 molar equivalent of PEO$_6$-PPO$_{29}$-NH$_2$ have been tested for the same molar mass of pullulan and pullulan/NaCNBH$_3$ ratio (reactions 2 and 3). It appears that 10 molar equivalents are well adapted to obtain a good yield (78%) and few unreacted pullulan (30%). A large excess of amine (five times higher) compared to aldehyde is often required for reductive amination by a hydride (Bosker et al., 2003). However, this proportion could not be used for pullulan of smaller molar masses (reaction 10) because the unreacted PEO$_6$-PPO$_{29}$-NH$_2$ was very difficult to eliminate from the copolymer. In this case, only 2 equivalents PEO$_6$-PPO$_{29}$-NH$_2$ is well adapted (reaction 12).

3.2.2. Effect of amount of NaCNBH$_3$

10 or 27 molar equivalents of NaCNBH$_3$ have been used for the same pullulan molar mass and pullulan/PEO$_6$-PPO$_{29}$-NH$_2$ ratio (reaction 8 and 9 respectively). 27 molar equivalent of NaCNBH$_3$ led to higher yields (68% instead of 37%) and lower unreacted pullulan (30% instead of 40%).

3.2.3. Effect of the pullulan molar masses

Four different molar masses of pullulan (i.e. P37000, P18000, P7100 and P2300) have been tested in the same conditions (reactions 3; 5; 9 and 10). Higher yields were obtained with the higher molar masses (37,000 g mol^{-1} and 18,000 g mol^{-1}). The used purification method (i) should lead to a better copolymer recovery for higher Mn. This method consists in a precipitation in methanol (to remove salts and unreacted PEO$_6$-PPO$_{29}$-NH$_2$ then a precipitation in acetone (leaving unreacted PEO$_6$-PPO$_{29}$-NH$_2$) and a dialysis against water (using adequate cut off Mw). To check the influence of purification method with low pullulan molar masses, another method of purification was performed (method ii) that consists in dialyzing against water and extraction by methylene chloride. For sufficient high molar masses (i.e. 18,000 g mol^{-1}), results are similar whatever the purification method (reactions 6 and 7). For lower pullulan molar masses (i.e. 2300 g mol^{-1}), the method of purification (ii), reaction 12, when compared to the method (i), reaction 11, appears well adapted since the yield and free pullulan become similar to the best obtained results. To sum up, these two purification types could be used for high pullulan molar mass. For pullulan of smaller molar masses, the precipitation in methanol does not occur, or is less efficient (7100 g mol^{-1}) leading to lower yields (37%). The second purification method (ii) should be chosen for these small copolymers. The smallest pullulan unit (maltotriose named P504) was tested (reaction 13) in the same experimental conditions that reaction with P2300 (reaction 12) leading to similar results.

3.2.4. Effect of the polyetheramine structure

In order to check if there is an influence of the polyetheramine structure, the syntheses done with PEO$_6$-PPO$_{29}$-NH$_2$ (reaction 3) and another polyetheramine PEO$_1$-PPO$_9$-NH$_2$ (reaction 4) have been compared (in same experimental conditions). Both syntheses are similar meaning that any polyetheramine should be used in these conditions.

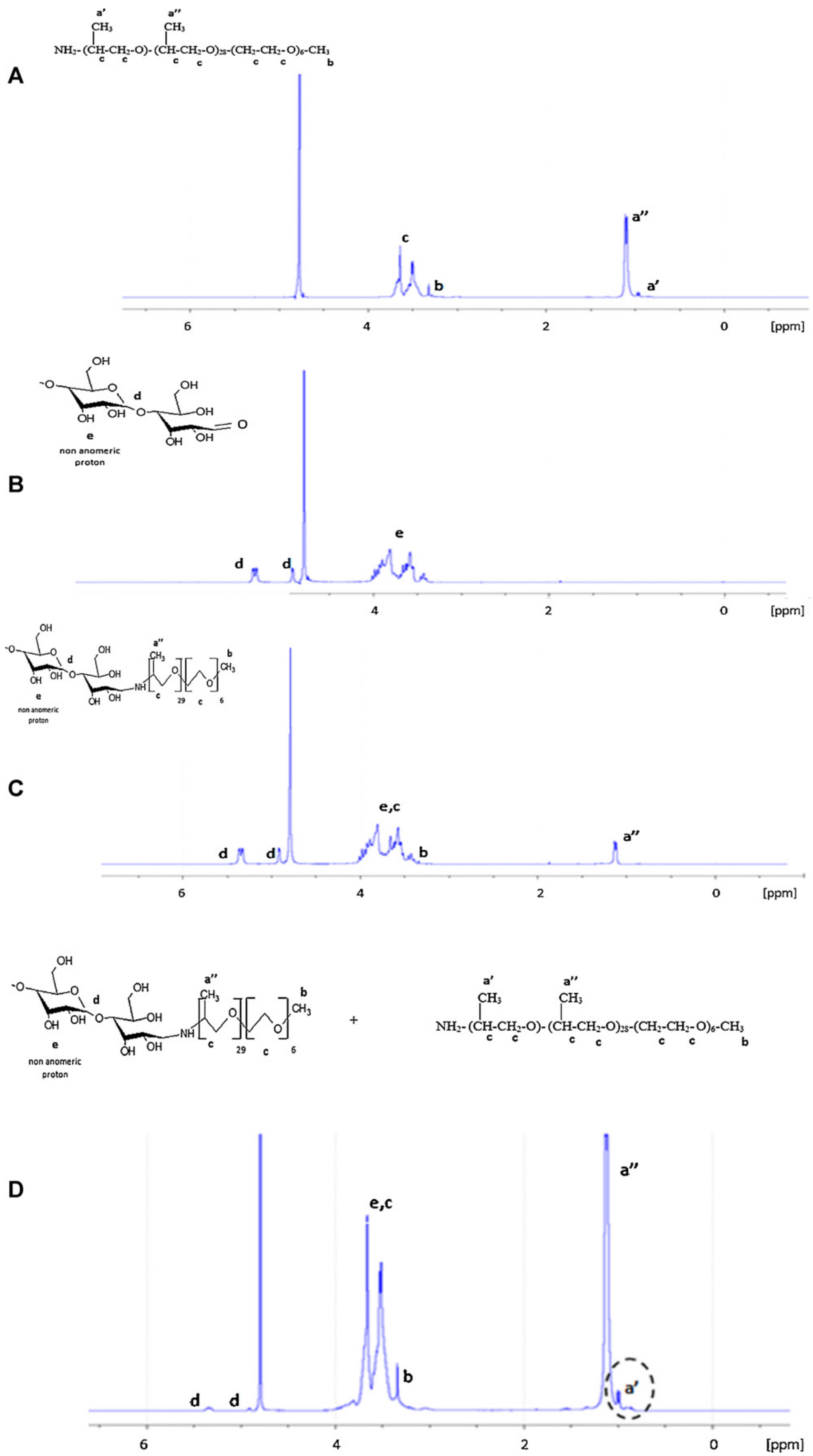

Fig. 3. ^{1}H NMR spectrum of: (A) PEO_6-PPO_{29}-NH_2/(B) degraded pullulan/(C) copolymer pullulan-b-PEO_6-PPO_{29}-NH_2 (P7100-b-PPO_{29}-PEO_6)/(D) copolymer pullulan-b-PPO_{29}-PEO_6 (P7100-b-PPO_{29}-PEO_6) + 5% PEO_6-PPO_{29}-NH_2 in D_2O.

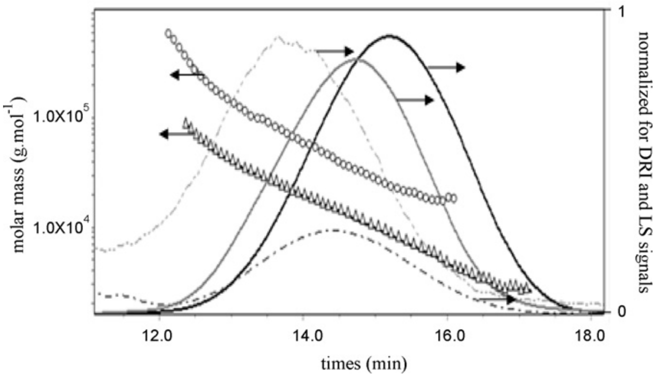

Fig. 4. Elution profiles from refractive index (full lines) and light scattering (dashed lines) of P7100 (black symbol) and P7100-b-PPO_{29}-PEO_6 (grey symbol) together with molar mass distributions of P7100 (△) and P7100-b-PPO_{29}-PEO_6 (◯) determined by SEC/MALS/DRI in $LiNO_3$ 0.1 M, pH 9.0.

For the following, samples from syntheses leading to the highest yield and the lowest free pullulan have been investigated.

3.3. Chemical and physical characterization

The copolymers have been characterized by SEC/MALS/DRI and their thermal behavior in aqueous medium has been studied by absorbance measurements.

3.3.1. SEC/MALS/DRI analysis of polymers

SEC/MALS/DRI analyses were performed to evaluate the average molar masses and possible aggregation states of copolymers at about 10 g L^{-1} or 5 g L^{-1} (depending on molar mass of pullulan) in $LiNO_3$ 0.1 M, pH = 9.0 (near PEO_6-PPO_{29}-NH_2 pK_a and copolymer pK_a, 9.2 ± 0.1 and 9.5 ± 0.1 respectively) and at 15 °C (controlled room temperature). Analyses were done, always in the same condition i.e. just after filtration at cold temperature (4 °C) to avoid copolymer precipitation. As an example, Fig. 4 illustrates the chromatogram profiles (both DRI and LS at 90° responses) together with the molar masses distribution for P7100 and P7100-b-PPO_{29}-PEO_6. In these conditions the polymer recovery (ratio between recovered polymer from integrated DRI peak and injected polymer) of all samples is always about 70–80%. A shift of the peaks towards higher size and an important increase of the molar mass distribution for the copolymers have been seen. The whole results are reported on Table 1. If any association occurs, the copolymers molar mass (Mn) must be approximately the addition of pullulan and PEO_6-PPO_{29}-NH_2 molar masses (Table 3). The found copolymer molar masses are always largely higher than the expected results of isolated macromolecule. These results clearly evidence an aggregation (self association) of the copolymers in these conditions of solvent and concentration. The aggregate number (Z) in the used experimental conditions could be estimated by using Eq. (1) which is the ratio between the obtained molar mass of the aggregated copolymer and the expected isolated one.

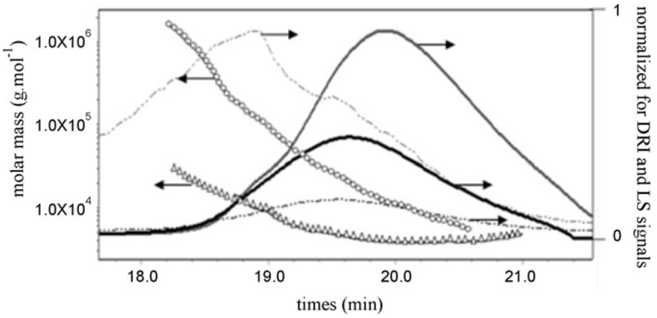

Fig. 5. Elution profiles from refractive index (full lines) and light scattering (dashed lines) of P504-b-PPO_{29}-PEO_6 at pH = 5.5 (black symbol) and pH = 9.0 (grey symbol) together with molar mass distributions of P504-b- PPO_{29}-PEO_6 at pH = 5.5 (△) and pH = 9.0 (◯) determined by SEC/MALS/DRI in $LiNO_3$ 0.1 M.

$$Z = \frac{M_n\text{aggregats}}{M_n\text{copolymer}} \qquad (1)$$

As a result, Z increases when the pullulan length in the copolymer is diminishing. This trend can be correlated to both HLB and stiffness.

Pullulan has flexible chain. When the pullulan chains are reduced to few maltotriose units, it is well known that a stiff appears. For block copolymers, a stiffness asymmetry in the two blocks should facilitate the self assembly (Borsali, Lecommandoux, Pecora, & Benoit, 2001).

The HLB data are compiled in Table 3 according to the following Eq. (2), from Griffin's method (Pasquali, Taurozzi, & Bregni, 2008).

$$\text{HLB} = 20 \cdot \left(\frac{M_{\text{PEO}} + M_{\text{pullulan}}}{M_{th\,\text{copolymer}}}\right) \qquad (2)$$

where MPEO, Mpullulan and Mth copolymer are respectively the molar mass of Poly(ethylene oxide), pullulan and copolymer (theoretical in the last case).

It can be noticed that this equation can only be employed for non-ionic surfactant, thus it is considered that at pH 9, the copolymer and the polymer amine are by a majority under unprotonated form.

As already mentioned PEO_6-PPO_{29}-NH_2 has a pK_a of 9.2 ± 0.1 and the copolymer's pK_a is 9.5 ± 0.1 (determined by acid-base titration). In order to follow the influence of cationic charge onto the copolymer via the amine function, a SEC/MALS/DRI analysis was conducted at pH 5.5 on both P2300-b-PPO_{29}-PEO_6 and P504-b-PPO_{29}-PEO_6. The results, reported on Table 3 and Fig. 5, show that the obtained molar masses are consistent with the isolated copolymer. The presence of ionic group $-NH_2^+$ on the copolymer leads to an enhanced overall solubility and a hindered of association tendency due to the modification of HLB and to repulsive forces between macromolecules.

Table 3
Experimental and theoretical molar masses of polymers by SEC/MALS/DRI analysis in 0.1 M $LiNO_3$ and HLB values of copolymers calculated from their theoretic chemical formulas.

	$Mn_{\text{theoretical}}$ (g mol^{-1})	Mn $_{\text{experimental}}$ (g mol^{-1})	Z^b	HLB
P37000-b-PPO_{29}-PEO_6 pH 9	39,600	250,000	6	19.2
P18000-b-PPO_{29}-PEO_6 pH 9	20,600	140,000	7	18.3
P7100-b-PPO_{29}-PEO_6 pH 9	9700	430,000	47	16.2
P2300-b-PPO_{29}-PEO_6 pH 9	4900	370,000	83	11.9
P2300-b-PPO_{29}-PEO_6 pH 5.5	4900	5300	1.1	–
P504-b-PPO_{29}-PEO_6 pH 9	3104	210,000	80	6.2
P504-b-PPO_{29}-PEO_6 pH 5.5	3104	4700	1.5	–

[b] Aggregate number (Z) Z = Mn aggregats/Mn copolymer.

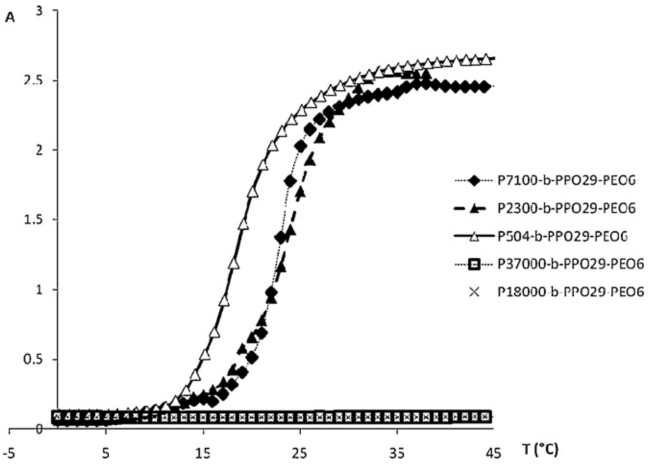

Fig. 6. Evolution of absorbance as function the temperature for P504-b-PPO$_{29}$-PEO$_6$, P2300-b-PPO$_{29}$-PEO$_6$, P7100-b-PPO$_{29}$-PEO$_6$, P18000-b-PPO$_{29}$-PEO$_6$ and P37000-b-PPO$_{29}$-PEO$_6$ copolymer solution at 10 g L^{-1}, in pure water, pH=11.0 (pH $\gg$ p$K_{a\ \mathrm{copolymer}}$).

3.3.2. Thermosensitive properties

Macroscopic behaviors of copolymers solution have been followed at 10 g L^{-1} as a function of temperature by measurement of the absorbance (Fig. 6). The critical temperature (i.e. cloud point or T°cp) was defined as the temperature corresponding to the inflection point in the normalized absorbance versus temperature curve. A clear transition is found for P7100-b-PPO$_{29}$-PEO$_6$ and P2300-b-PPO$_{29}$-PEO$_6$ between 20 and 25 °C. The very small P504-b-PPO$_{29}$-PEO$_6$ evidences a lower transition temperature of about 17 °C. Copolymers with longer pullulan blocks (i.e. P18000-b-PPO$_{29}$-PEO$_6$ and P37000-b-PPO$_{29}$-PEO$_6$) do not present a temperature transition on the base of a macroscopic cloud point.

To know if unreacted pullulan influence the macroscopic behavior, we have added artificially pullulan with the same molar mass to the copolymer. Absorbance measurements are identical with the same cloud point. It seems that free pullulan does not interact with copolymer.

These absorbance measurements allow two remarks. The first one is that the thermal transition depends on the molecular scale of observation (Fettaka et al., 2011). The same copolymer, as the P18000-b-PPO$_{29}$-PEO$_6$ evidences any transition regarding the absorbance measurements whereas a clear aggregation has been showed by light scattering (with $Z \sim 7$). The second remark concerns the clear influence of the HLB on the thermal behavior. When hydrophilic character is increased (i.e. longer pullulan length in the copolymer), the thermosensitive characteristics are diminishing as seen by the disappearance of cloud point transition and by the decrease of Z.

Other investigations should be conducted mainly thanks to μDSC, fluorescent probe techniques and size measurements. The emulsifying properties have also to be studied as a function of both pH and temperature. These future studies will be reported in forthcoming papers.

4. Conclusion

A novel linear thermo and pH-sensitive copolymer, containing pullulan of different length and polyetheramine have been successfully synthesized thanks to a reductive amination of aldehyde group of the reducing sugar. The efficiency of the reaction has been checked by FTIR and ^{1}H NMR analysis. The synthesis and purification conditions have been optimized according to the length of used pullulan notably.

SEC/MALS/DRI and absorbance measurements clearly evidence the association of copolymers which is both temperature and pH sensitive. As preliminary results it appears that evidencing the copolymers associations and the thermal transition strongly depend on the used technique and notably on the scale of observation.

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