Novel thermosensitive flocculanting agent based on pullulan

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ABSTRACT

New thermosensitive polysaccharide (P-g-pNIPAAm) was synthesized by graft-polymerization of p(N-isopropylacrylamide) (pNIPAAm) onto pullulan (P) using Ce(IV) ion as initiator. The grafted polysaccharide was characterized by FT-IR and ^{1}H NMR spectroscopy and elemental analysis. Its flocculation efficiency was studied in a clay suspension, in comparison with p(NIPAAm) homopolymer, as a function of the polymer dose, temperature and settling time. The thermosensitive polysaccharide could induce clay particle flocculation both below and above the lower critical solution temperature (LCST), but the process was more effective above the LCST. A lower value of the residual turbidity at the optimum polymer dose and a wider flocculation window were recorded at temperature above the LCST. The floc size distribution and surface morphology revealed bigger size flocs when the flocculation was performed above the LCST. The re-dispersion effect was negligible for the flocs obtained in the presence of P-g-pNIPAAm.

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1. Introduction

Flocculation of different types of contaminants in wastewaters using polymers is an important operation in the separation processes. Several characteristics related to particles (charge, concentration and size), polymers (the chemical structure, the molar mass, the charge density, the polymer chain hydrophobicity) and medium (the ionic strength, pH and temperature) greatly influence the polymer chain conformation in solution and at solid/liquid interface and hence, the flocculation efficiency [1–7]. Experimental studies revealed that quite often the impact of these parameters on the polymer-induced flocculation process is not clear. For example, the increasing temperature led to improving separation process in some cases, like the flocculation of barium chromate with poly(diallyldimethylammonium chloride) [8] and of kaolin suspension with cationic starches of different substitution degree [9] or cationic konjac glucomannan [10]; the opposite effect was noticed when some dyes were removed from the waste waters using two commercial polycations [11]. The temperature effect is very important in the case of the thermosensitive polymers, which become soluble (hydrophilic) at low temperatures and insoluble (hydrophobic) at high temperatures. The temperature at which the phase separation occurs is known as the lower critical solution temperature (LCST) [12]. The most known thermosensitive polymer is poly(N-isopropylacrylamide) (pNIPAAm) with the transition temperature in water of 32 °C. pNIPAAm and its copolymers have been investigated as temperature responsive flocculant at

temperature above the LCST for separation of TiO$_2$ [13,14], kaolin [15,16], silica and alumina [17] suspensions. Moreover, it was used as reversibly responsive flocculant and dispersant [15,18,19]. The reversibility of the state of aggregation and dispersion can be used to induce both rapid sedimentation (at temperature above LCST) and sediment consolidation and dewatering (at temperature below LCST) [19]. Nichifor and Zhu [20] investigated N-alkylacrylamide-styrene copolymers as thermally reversible dispersants/flocculants for emulsions (dodecane/water) and kaolin suspension. The thermosensitive polymers usually used in the solid/liquid separation processes are synthetic flocculants. It is well known that the commercial forms of synthetic flocculants have some drawbacks including high costs of petroleum feedstocks, very slow biodegradation in soil and water and toxicity of residual monomer which is usually present in small amounts. Lately, natural polymers and their derivatives obtained by grafting synthetic polymers on some polysaccharides backbone (gum guar, starch, konjac glucomannan, chitosan) are gaining importance as they combine the advantages of both synthetic (low polymer dose) and natural (biodegradability and low toxicity) polymers [21]. So far, the thermoresponsive grafted polysaccharides were extensively used in bio-related applications such as drug delivery, tissue engineering, wound healing, cell culture, bioseparation, biomedical implants, bionanotechnology [22,23]. However, to our knowledge there have no reported studies with regard to their use in the flocculation process. As a consequence, the goal of this study was to prepare a new thermoresponsive grafted polysaccharide, namely pullulan grafted pNIPAAm copolymer (P-g-pNIPAAm) and to assess its flocculation properties in comparison to p(N-isopropylacrylamide) (pNIPAAm) in a clay suspension. The separation efficiency, determined by optical density measurements, was investigated as a function of the

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temperature, polymer dose and the settling time. The surface morphology and the floc size distribution measurements, at the optimum polymer dose, were also performed.

2. Experimental part

2.1. Materials

2.1.1. Materials for synthesis of graft copolymer (P-g-pNIPAAm) and homopolymer (pNIPAAm)

Pullulan (P) (M_w = 200 000 g/mol), was purchased from Hayashibara Laboratories Ltd. (Okayama, Japan). N-isopropylacrylamide (NIPAAm), supplied from Aldrich Chemical Corp. (Milwaukee, WI, USA), was recrystallized from hexane. N,N′-azobisisobutyronitrile (AIBN) were supplied from Fluka AG (Buchs, Switzerland). AIBN was purified by recrystallization from methanol before use. Ceric ammonium nitrate (CAN) and other chemicals were purchased from Fluka AG (Buchs, Switzerland) and used without further purification. 1,4-Dioxane, from Fluka AG, was purified by refluxing.

2.1.2. Materials for flocculation

Clay powder-SSM Blend 14888 (gift sample from Romance-ram Co., Romania) was used to prepare model suspensions in all experiments. Mineralogical composition: 47% kaolin, 22% montmorillonite, 31% quartz. Chemical composition (wt%): SiO_2, 66–72; Al_2O_3, 23–26; TiO_2, max 1.8; Fe_2O_3, max 1.5; Na_2O, 0.1; K_2O. 1.9–2.4; particle size distribution: <20 μm 90–95%; <10 μm 82–89%; <6 μm 70–80%; <2 μm 55–65%; <1 μm 45–57%.

2.2. Methods

2.2.1. Synthesis of graft copolymer (P-g-pNIPAAm) and homopolymer (pNIPAAm)

1.0 g (6.17 mmol) of pullulan was dissolved in 12 mL of 0.01 M nitric acid solution in a three-necked flask equipped with a nitrogen inlet and a reflux condenser immersed in a thermostated water bath. The solution was purged with nitrogen for 30 min and the nitrogen atmosphere was maintained throughout the polymerization period. Then, 0.88 g (1.6 mmol) CAN solubilized in 4 mL 0.01 M nitrate acid solution were added. After 10 min supposed to be enough to induce free radicals onto pullulan chains, 1 g of NIPAAm (10 mmol) were added to flask, and the reaction continued for 20 h at 25 °C. The reaction was stopped by addition of 1 N of sodium hydroxide aqueous solution. The reaction mixture was finally poured into methanol, filtrated, washed and extracted with methanol for 24 h in order to remove pNIPAAm homopolymers. The product was dried under vacuum at 60 °C.

Synthesis of linear homopolymer (pNIPAAm) was carried out by free radical copolymerization in 1,4-dioxane with AIBN as initiator, according to Fundueanu et al. [24]. Typically, 1.13 g of NIPAAm and 0.010 g of AIBN were solubilized in 6 mL of 1,4-dioxane. Dried nitrogen was bubbled through the solution for 30 min prior to polymerization. The reaction mixture was allowed to react at 70 °C until the gel point was reached (about 4 h). Then 6 mL of dioxane was added and the reaction was allowed to continue for 16 h. The polymer was precipitated into diethyl ether and dried under vacuum. Finally, the polymer was solubilized in distilled water, dialyzed for 5 days at 20 °C and recovered by freeze-drying.

2.2.2. Extraction of grafted pNIPAAm

In order to estimate the amount of grafted pNIPAAm onto pullulan and found out the grafted chains' molar mass, the grafted pNIPAAm chains were separated from pullulan by hydrolyzing the graft copolymer in a 72% (v/v) solution of sulfuric acid at room temperature for 7 h. The separated pNIPAAm chains were recovered

from solution by precipitation with n-hexane and the obtained precipitates were solubilized and dialyzed against water at 4 °C for 3 days (M_w cut-off of dialysis tube 2000), and then freeze-dried to constant weight.

The viscosity-average molar mass of pNIPAAm grafts and homopolymer was determined by viscometric measurements and calculated according to the following equation [25]:

$$[\eta] = 5.8 \times 10^{-5} M_\eta^{0.78} \tag{1}$$

Intrinsic viscosity [η], was measured in THF at 27 °C in an Ubbelohde-type viscometer.

2.2.3. Polymer characterization

The copolymer composition was determined by nitrogen analysis and ^1H NMR spectroscopy. ^1H NMR spectra were recorded on a Bruker Avance DRX 400 NMR, using deuterated water as the solvent.

The grafted and un-grafted pullulan samples were dried under vacuum at 50 °C for 24 h, then were prepared accordingly to the KBr technique and analyzed with a Fourier transform infrared spectrophotometer (VERTEX 70, Bruker, Austria) in the frequency ranges of 400–4000 cm^{-1}.

The molar mass of the samples was determined by using a static light scattering (SLS) technique with a Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, England). Static light scattering (SLS) is a non-invasive technique used for characterizing macromolecules in solution. A beam of monochromatic light is directed through a sample and the intensity of the light scattered at an angle of 173° by the molecules is measured. Aqueous polymer solutions (1–10 mg mL^{-1}) were filtered using 0.4 μm Millipore filters before any measurement. Each sample was analyzed in triplicate, and the reported data represent the mean values.

The lower critical solution temperature was determined from the dependence of the absorbance change at 450 nm on temperature. The UV-Vis Specord 200 spectrophotometer (Analytic Jena, Jena, Germany), coupled with a temperature controller, was used. The polymer solution (1%, w/v) was prepared in distilled water. The heating rate was 1 °C every 10 min. Cloud point (CP) was defined as the temperature at 10% absorbance in the curve of the absorbance versus temperature.

2.2.4. Flocculation characteristics

The aqueous polymer solution and clay dispersion were prepared with distilled water. The concentration of the model suspension was 1 g L^{-1}. The initial pH of the suspension was 4.5. Fifty milliliters of the clay suspension was placed into 100-mL glass beakers, sonicated for 30 min using an ultrasonicator (Bandelin Sonorex RK51OH, Berlin, Germany) followed by vigorous stirring for 15 min at 1000 rpm to fully disperse the clay powder. The flocculation experiments were conducted at two temperatures, below (20 °C) and above (60 °C) the copolymer sample critical transition temperature (LCST = 34 °C, see Table 1).

In the former case, different volumes of polymer solutions with a concentration of 1 g dL^{-1} were added to clay suspension at 1000 rpm and stirring was continued with the same speed for about 25 min and decreased to about 200 rpm for 15 min. In the latter case, the polymer solution was added at room temperature and stirring proceeded at 1000 rpm for 10 min. Afterwards, the beakers were placed in a water bath thermostated at 60 °C and the rapid stirring was continued for 15 min, followed by a slower one (200 rpm) for 15 min. After settling times of 15, 30, 60 and 120 min, the reading of the supernatant optical density (D^{500}) was performed at 500 nm using a Spekol 1300 Spectrophotometer (Analytik Jena AG, Germany). A blank experiment was performed in the absence of grafted polysaccharide to evaluate the "natural" decantation of the
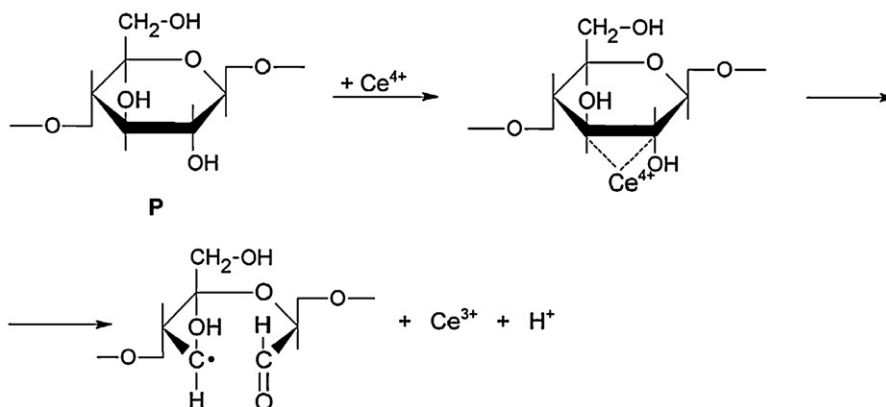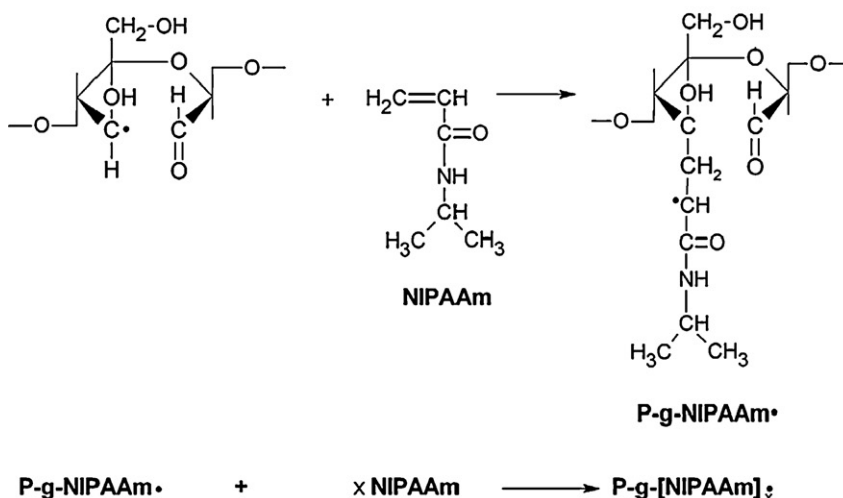
Initiation

Propagation

Termination

$$P\text{-}g\text{-}[NIPAAm]_x^{\bullet} \quad + \quad P\text{-}g\text{-}[NIPAAm]_y^{\bullet} \quad \longrightarrow \quad P\text{-}g\text{-}[NIPAAm]_{x+y}^{\bullet}$$

Fig. 1. Mechanism of graft copolymerization of NIPAAm on pullulan.

suspension under the selected experimental conditions (pH, concentration of suspended mater). Each experiment was performed in triplicate and the average values were taken. The residual turbidity was expressed as percent of the initial optical density of the clay suspension, at time zero, in the absence of polymer.

Measurement of particle dimensions was done using the laser diffraction technology with a Mastersizer 2000 system (version 5.31) (Malvern Instruments, Malvern, England). The system is constituted of an optical bank which uses laser light He–Ne 632 nm/2 mW, a dispersion unity of the sample (Hydro 2000G)

Table 1
Characteristic data of P and P-g-pNIPAAm copolymer.

Sample	NIPAAm/AGU (mol%) determined from		Av. graft points of pNIPAAm per P backbone	Length of pNIPAAm grafts, $M_v \times 10^{-4}$ [a]	$M_w \times 10^{-4}$ [b]	LCST in water (°C)
	%N	¹H NMR				
P	0/100	0/100	0	–	23.1	–
P-g-pNIPAAm	54.34/45.66	56.61/43.38	0.47	6.13	26.0	34
pNIPAAm homopolymer	100/0	100/0	–	7.92	n.d.	33

[a] Calculated from viscosity measurements.
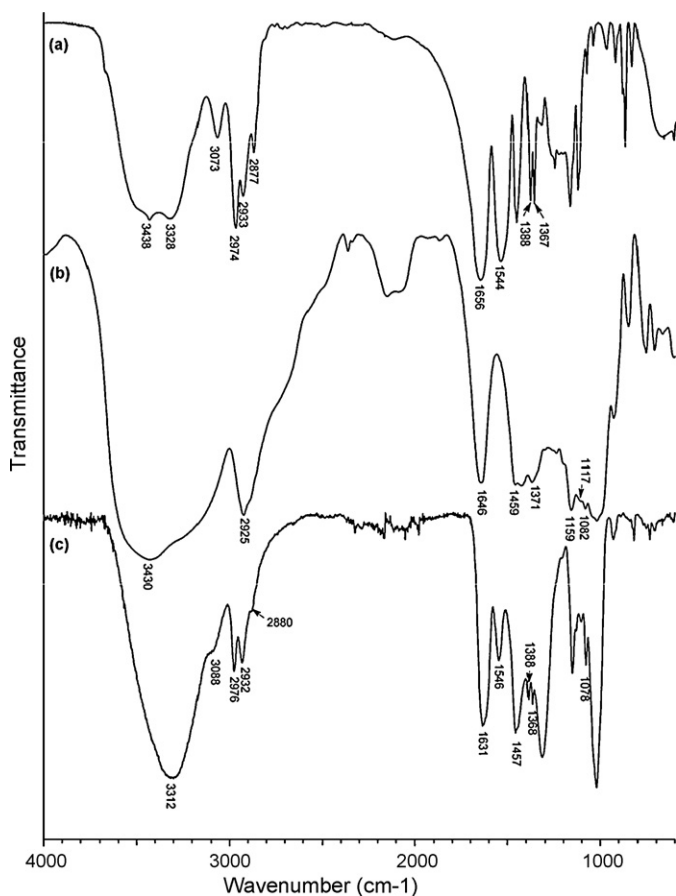[b] Obtained by SLS in neutral water.

Fig. 2. FT-IR spectra for pNIPAAm (a), pullulan (b), and P-g-pNIPAAm (c).

equipped with stirrer, recirculating pump, ultrasonics and software to record and process results on a computer. Dispersion obtained with pullulan derivative corresponding to the optimum polymer dose was allowed to settle for 30 min and the settled flocs were collected and rapidly transferred to the dispersion unit of the equipment. In order to avoid the agglomeration of the flocs, the sample under investigation is stirred at 1750 rpm by means of the stirrer integrated in the dispersion unity.

The surface morphology of the untreated as well as of the treated clay particles was examined by using an Environmental Scanning Electron Microscope (ESEM) type Quanta 200, in powdered form.

3. Results and discussion

3.1. Synthesis

Several research articles have been published regarding the graft copolymerization of various acrylic monomers onto polymer backbone containing hydroxyl groups, using Ce(IV) ion as initiator [26–29]. This method was used in the present paper for preparing grafted pullulan (P-g-pNIPAAm) by a mechanism described in Fig. 1.

3.2. Characterization of graft copolymer

The resultant copolymer P-g-pNIPAAm was fully characterized by FT-IR (Fig. 2), ^{1}H NMR (Fig. 3), and elemental analysis.

Thus, Fig. 2, curve *a*, shows absorbance bands of the pure NIPAAm homopolymer at 1656 cm^{-1} and 1544 cm^{-1}, assigned to the carbonylamide and N–H stretching, respectively. The sharp peaks at 1388 and 1367 cm^{-1} correspond to the characteristics

absorbance of isopropyl in pNIPAAm [26,30]. From Fig. 2, curve *b*, one observes two bands located around 1117 and 1080 cm^{-1}, attributed to the stretching of ether bands in pullulan. An intense band at 3430 cm^{-1} represents the stretching vibration of –OH in pullulan [31]. The P-g-pNIPAAm copolymer exhibits both the absorption peaks at 1631 cm^{-1} and 1546 cm^{-1}, characteristics of the amide groups of pNIPAAm (Fig. 2, curve *c*), and the characteristic stretching vibrations of the hydroxyl groups (at 3400 cm^{-1} and 1078 cm^{-1}) of pullulan.

The chemical structure of the graft copolymer was also examined by ^{1}H NMR spectroscopy in D$_2$O at room temperature (Fig. 3). In the spectrum of pullulan, the resonance peaks at 4.96 ppm, 5.36 and 5.40 ppm were assigned to the $(1 \rightarrow 6)$ and $(1 \rightarrow 4)$ anomeric protons, while the signals of the ring protons of pullulan skeleton range between 3.4 and 5.8 ppm [32]. For P-g-pNIPAAm, besides those of pullulan, new characteristic signals at 1.15 ppm (the two methyl groups), 1.46–2.14 ppm (the chain CH$_2$ and CH groups) and 3.9 ppm (the isopropyl methyl groups) appear [27,28]. The molar ratio of pNIPAAm units grafted on structural unit of pullulan was calculated from the rapport of the integral value of methyl protons at 1.15 ppm which correspond to the NIPAAm divided by a factor of 6, and that of H-1 protons of pullulan moieties observed at 5.4 and 4.96 ppm. This content was confirmed by the nitrogen analysis (Keldhal method). The content of pNIPAAm in grafted copolymer (Table 1) was calculated from N content using the following equation:

$$\% \text{ grafted NIPAAm} = \frac{\%N}{14.01} \times 113$$

where 113 represents molar mass of the monomer.

The average molar mass of P and P-g-pNIPAAm was measured by static light scattering (Table 1). In order to find out the average graft length and graft density (the number of grafts per P backbone), the backbone of P-g-pNIPAAm copolymer was fully degraded with sulfuric acid, and then the pNIPAAm grafts were recovered for viscosity measurements. The graft densities were calculated from the composition, total molar mass of the copolymer, and the molar mass of the grafts (see Table 1).

3.3. Effects of polymer dose and temperature on flocculation efficiency

The residual turbidity of the supernatant is commonly used to estimate the performance of the flocculation process.

Fig. 4 presents the variation of the residual turbidity as a function of polymer dose at 20 °C and 60 °C for P-g-pNIPAAm copolymer and pNIPAAm homopolymer with a molar mass close to that of the pNIPAAm grafted chains (M_v = 79 200) (see Table 1). The pNIPAAm homopolymer was not able to flocculate the clay suspension neither at temperature below nor above the LCST = 33 °C (see Table 1), while the opposite effect was noticed for the pullulan grafted pNIPAAm copolymer; the addition of different doses of copolymer determined the rapid decrease of the residual turbidity values until a plateau was reached (much larger at 60 °C than that at room temperature), followed by an increase with a further increase in polymer dose.

The behavior of these samples in the flocculation process can be explained in the followings. For effective flocculation, polymers need to be adsorbed on particles. The polymer adsorption takes place by means of several interactions: electrostatic and/or hydrophobic attractions, hydrogen bonding, ion binding [33]. Thus, it is generally believed that the hydrogen bonding and/or hydrophobic attractions operate for the adsorption of nonionic polymers while electrostatic attraction becomes the key factor for the attachment of ionic polymers [1].
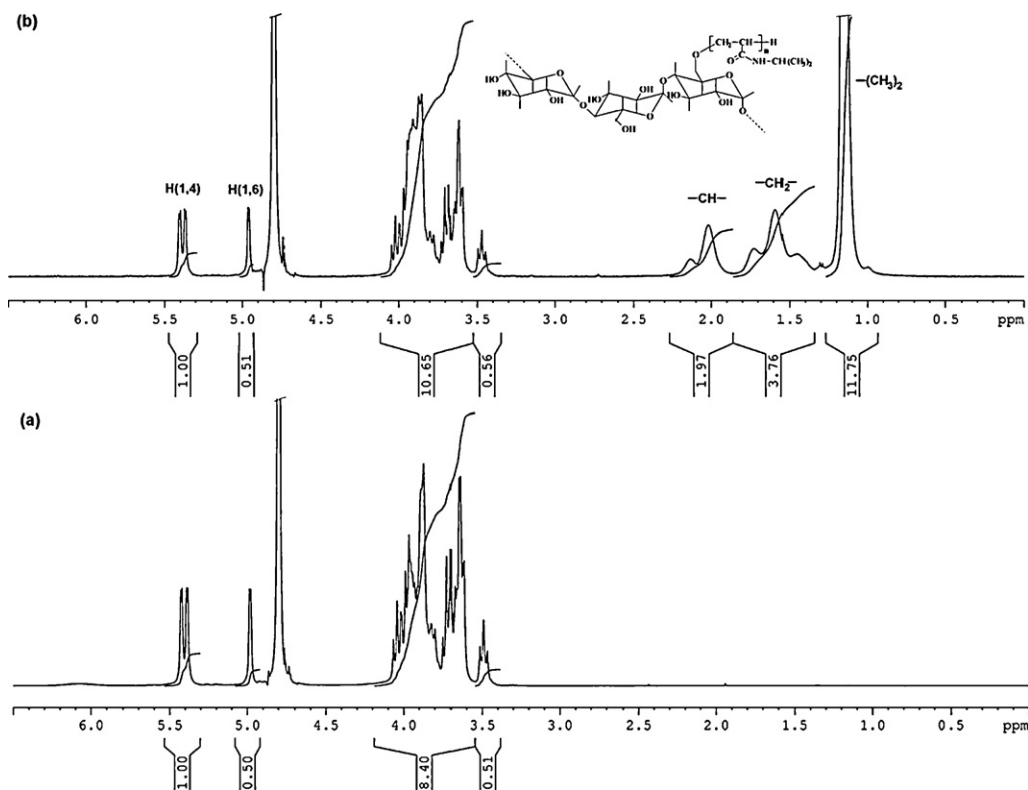
Fig. 3. ^{1}H NMR spectra in D$_2$O for pullulan (a) and P-g-pNIPAAm (b).

P (N-isopropylacrylamide) is a nonionic polymer and, as it is well known, at temperature below LCST is hydrophilic and interacts with the suspension particles by the H bonds established between the amide groups and the OH groups on the particle surface. The low flocculation performance of the sample investigated may be caused by the low molar mass. Consequently, the polymer chains could not produce interparticles attractive forces that were strong enough to induce efficient flocculation. This finding is in agreement with that reported by Li et al. [34] who investigated the flocculation of silica suspension with pNIPAAm of different molar masses and found out that the sample with the lowest molar mass ($M_w = 0.23$ MDa) (higher than that of the sample investigated in this study) was not an effective flocculant neither at room temperature, nor at 50 °C.

Grafting polymerization of pNIPAAm on hydrophilic backbone (pullulan) yielded a new thermoresponsive compound which, as shown in Fig. 4, had a great impact on the separation of clay suspension. The P-g-pNIPAAm sample has a comb like structure. The Singh's Easy Accessibility Model (EAM) postulates the graft copolymers are more effective in the flocculation process, compared to the linear ones, because of their greater approachability to the contaminants, which could be due the spreading effect of the dangling grafted chains on the polysaccharide chains [35].

Based on this model, we assume that at temperature below the LCST, the grafted pNIPAAm chains adsorb on the surface of suspended particles and at a certain polymer dose, called here the optimum polymer dose ($dose_{op}$), the particles aggregated and separated rapidly from suspension leading the lowest residual turbidity value. At overdose, there was no longer enough bare particle surface available for attachment of polymer segments and no flocculation took place. Moreover, the suspended particles may also be surrounded by the grafted chains and the steric repulsion between highly hydrated chains stabilizes the suspension.

Regarding the flocculation tests at temperature higher than LCST, we recall the reader that the copolymer adsorption was performed at room temperature and then the suspension was heated at 60 °C. Above the LCST the pNIPAM chains, and hence the particle surface, become amphiphilic as a result of dehydration of the polymer chains (the hydrogen bonds between amide groups of pNIPAAm and water molecules are destroyed) resulting in the flocculation of the suspended particles due to the hydrophobic associations. One observes that even the optimum polymer dose value was the same, $dose_{op} = 60$ mg L^{-1}, the corresponding residual turbidity value was lower at temperature above LCST (1.57%) than at room temperature (6%), suggesting a more efficient flocculation
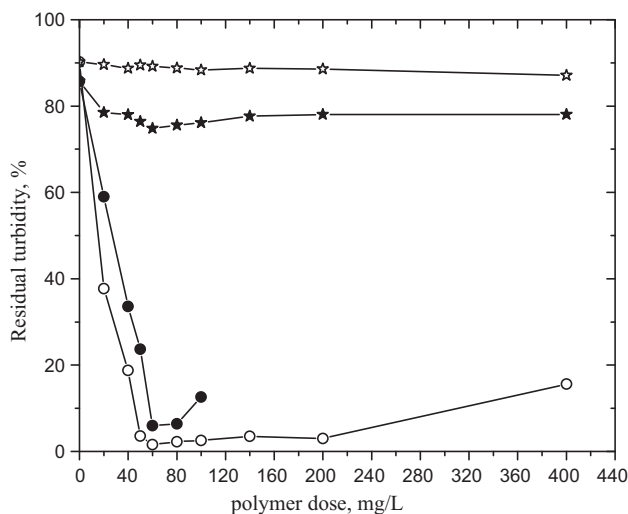

Fig. 4. Residual turbidity (%) dependence on the polymer dose at 20 °C (solid symbol) and 60 °C (open symbol): (star) pNIPAAm; (circle) P-g-pNIPAAm; settling time 30 min.
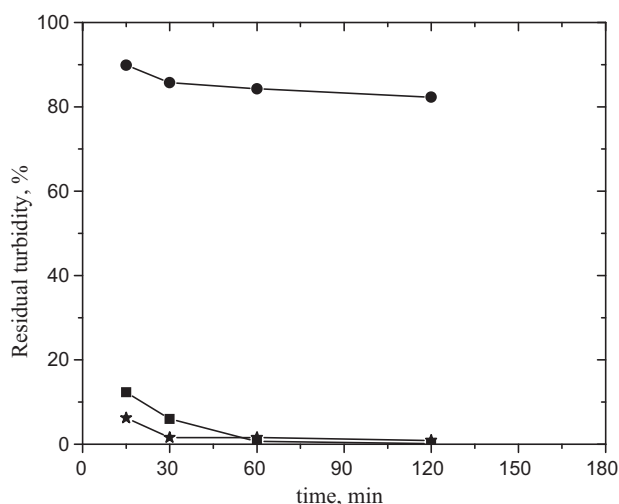
Fig. 5. Residual turbidity (%) dependence on the settling time for clay suspension: in the absence (circle) and in the presence of grafted polysaccharide at 20 °C (square) and at 60 °C (star); polymer dose: 60 mg L^{-1}.

Fig. 6. Floc size distribution for P-g-pNIPAAm (a): in the absence (star) and in the presence of grafted polysaccharide at 20 °C (open circle) and at 60 °C (solid circle).

in the former case. Fig. 4 further shows the temperature effect on the flocculation window width (range of the optimum doses). Thus, the residual turbidity above the LCST was maintained at low level (1.6–3.6%) from 60 to 200 mg L^{-1}, while the maximum flocculation induced at room temperature occurred at a narrow dose interval, ranging from 60 to 80 mg L^{-1}. The above finding may be explained by the fact that supplementary adsorbed P-g-pNIPAAm chains, by hydrophilic/hydrophobic transition, determine further intermolecular hydrophobic associations and particles aggregation, and hence, the wider range of polymer dose where the supernatant clarity was reasonable.

3.4. Effects of the settling time on flocculation efficiency

Fig. 5 depicts the settling time influence on the flocculation of clay suspension in the presence and absence of the thermosensitive grafted polysaccharide at room temperature as well as at 60 °C. The experiments were performed at optimum polymer dose (60 mg L^{-1}). The natural decantation of clay suspension was very slow as the residual turbidity did not decrease below 80% even after 120 min of settling. The addition of P-g-pNIPAAm significantly improved the settling efficiency, the residual turbidity decrease being more pronounced in the first 15 min (about 88%). One further notices that at all settling time investigated, the flocculation efficiency of P-g-pNIPAAm copolymer was higher (lower residual turbidity values) at 60 °C than that at the room temperature.

3.5. Particle size measurements and the morphology of clay particles

In order to obtain more information about the flocculation mechanisms effect on the floc size and morphology, particle size measurements and SEM analysis at the polymer doses located in the flocculation window range were performed. Thus, the particle size distribution (presented as volume fraction versus particle diameter) for flocs obtained in the presence of P-g-pNIPAAm both below (polymer dose = 60 mg L^{-1}) and above LCST (polymer dose = 140 mg L^{-1}) as well as of the initial clay suspension is shown in Fig. 6.

Some features may be noticed for the floc size distribution depending on the temperature. A unimodal distribution in flocs size together with smaller size compared with the untreated clay was
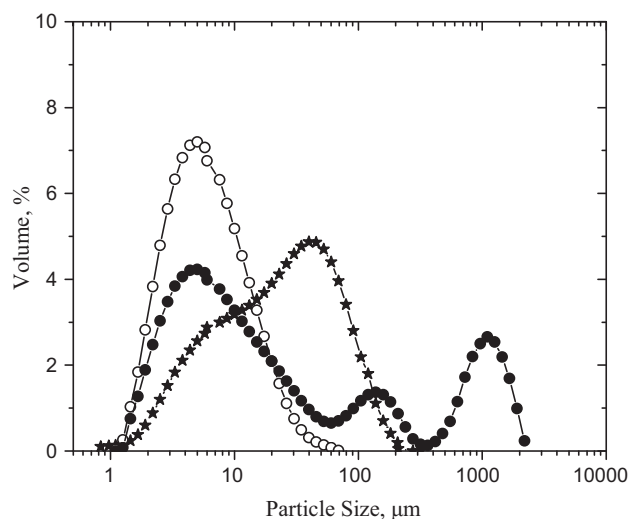
noticed for the flocs obtained below the LCST, where the aggregation took place as an outcome of the H bonds established between the amide groups and the OH groups on the particle surface. Two additional peaks, compared to the size distribution below the LCST, were recorded at temperature above the LCST. These peaks, corresponding to larger size flocs than the original clay, indicate that a part of the clay particles covered with hydrophobic chains could participate to inter-particle associations, which resulted in bigger flocs.

The morphology of the clay particles in the absence (Fig. 7a) and in the presence of P-g-pNIPAAm both below (polymer dose = 60 mg L^{-1}) (Fig. 7b) and above LCST (polymer dose = 140 mg L^{-1}) (Fig. 7c) is shown in scanning electron micrographs.

The SEM pictures revealed that the clay particles in the absence of thermosensitive polymer are almost separate. On the contrary, in the presence of P-g-pNIPAAm the particles are assembled together in high size aggregates (below LCST) and even large network (above LCST).

Moreover, the next experiment demonstrated that the re-dispersion effect of the clay particles is negligible when are flocculated with P-g-pNIPAAm in the experimental conditions used in this study. Thus, both flocculated suspensions (copolymer dose of 60 mg L^{-1}) obtained at 60 °C (residual turbidity 1.67%) as well at room temperature (residual turbidity 6%) were stirred for 60 min at 200 rpm. After 30 min of settling time, the residual turbidity values were 3% for the supernatant of the former suspension and 10% for the supernatant of the latter one. This behavior suggests that even if, the pNIPAAm chains become hydrophilic and the interparticle hydrophobic associations are destroyed at room temperature, the P-g-pNIPAAm chains do not readily desorb from the particles surface. This is likely, because the polymer adsorption onto the suspended particles took place by means of many functional groups in the polymer chain, as stated by Sakohara et al. [15]. The reasonable residual turbidity recorded for the supernatant below the LCST (10%) confirms this assumption. In addition, the particle size distribution of the flocs formed both below and above the LCST, after re-dispersion were performed (Fig. 8). One observes quite resistant flocs in the former case (the curves almost overlap) while the greater size flocs obtained above the LCST were not so shear resistant; the peaks corresponding to larger size flocs disappeared. Even so, the flocs re-dispersion in both cases was minor.
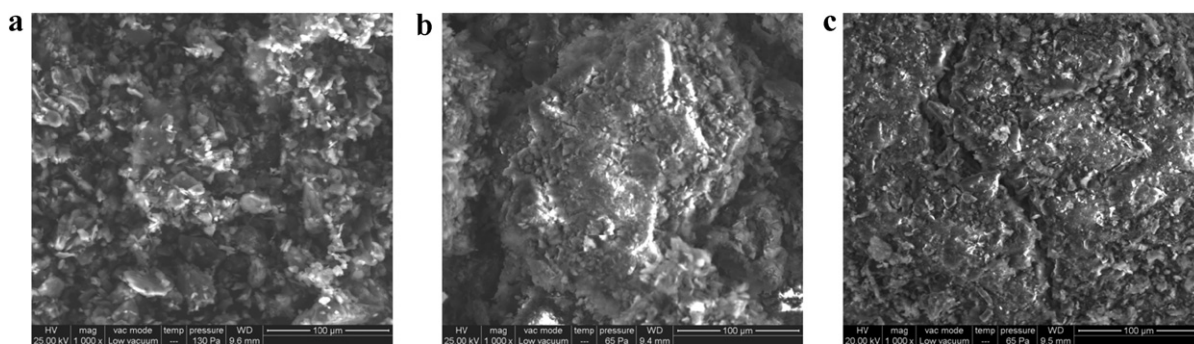
Fig. 7. SEM micrographs of clay flocs in the absence (a) and in the presence of P-g-pNIPAAm at 20 °C (b) and 60 °C (c).
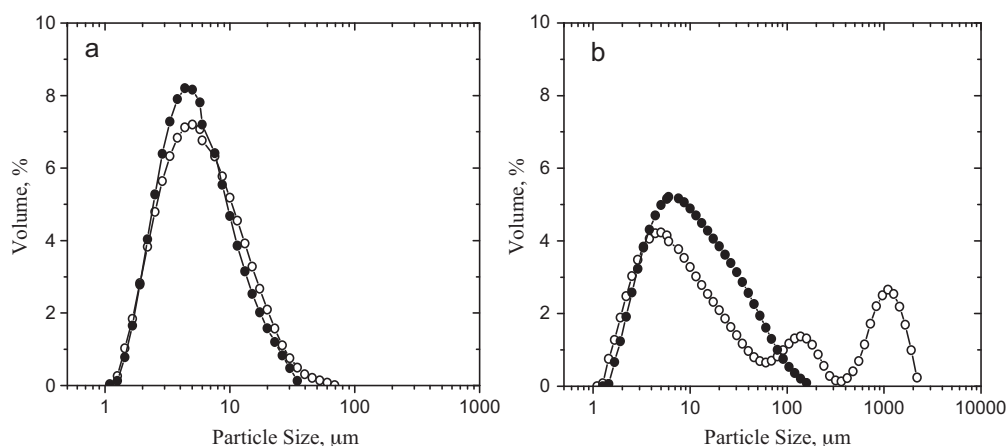

Fig. 8. Particle size distribution of the flocs formed at 20 °C (a) and at 60 °C (b): before (open symbol) and after (solid symbol) re-dispersion.

4. Conclusions

A novel thermosensitive flocculant was obtained by graft-polymerization of pNIPAAm onto pullulan. The flocculation study showed that the polymer based on pullulan exhibited good flocculation properties for clay suspension at temperature both below and above LCST. The combined information supplied by turbidity, laser diffraction and SEM measurements emphasized the temperature played an important role in the flocculation process, influencing the residual turbidity at optimum polymer dose and flocculation window width. These results recommend this polymer as new biodegradable flocculant which requires a proper choice of temperature in order to achieve an increased efficiency of the separation process. The presence of pullulan, and hence biodegradable linkages, in the P-g-pNIPAAm sample could confer it self-degradation characteristics which will diminish, unlike non-self degradable pNIPAAm homopolymer, the "secondary pollution" of the environment. This makes possible the using of the P-g-pNIPAAm in the real waste waters treatment.

Acknowledgements

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