

Novel biodegradable flocculating agents based on pullulan

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ARTICLE INFO

Article history:

Received 8 January 2010
Received in revised form 26 April 2010
Accepted 5 May 2010
Available online 11 May 2010

Keywords:

Graft copolymer
Pullulan
Flocculation
Turbidimetry
Zeta potential

ABSTRACT

New copolymers with different grafted cationic chain content and length were synthesized by graft-polymerization of (3-acrylamidopropyl)-trimethylammonium chloride onto pullulan in aqueous solution, using potassium persulfate as an initiator. Their flocculation efficiency was studied in a clay suspension as a function of the grafted chains content and length as well as of some flocculation parameters: the polycation dose, the settling time, the parent solution concentration of polycation. All the polysaccharide samples showed flocculation ability: the higher the grafted cationic chain content and length the lower the amount of pullulan derivative was required for reaching the maximum clarity degree (of about 100%). This finding together with the negative value of the zeta potential and floc size measurements, at the optimum polymer dose, point to contributions from both neutralization and bridging mechanisms for flocculation process. The residual turbidity values also varied with the settling time and the parent solution concentration of polymer.

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1. Introduction

The polyelectrolytes (PELs) ability to adsorb and interact with oppositely charged particles gives them a crucial importance in the stabilizing/destabilizing (flocculation) processes of the aqueous colloidal dispersions. The selection of ionic polymers for one of the purposes is dictated by both the intrinsic characteristics of the polyion and environment.

The flocculation process is of considerable importance in colloidal science and in various industrial fields, such as domestic/industrial waste water purification, thickening of sludges, paper-making. For water purification can be used either inorganic additives, termed coagulants agents (aluminum sulfate and ferric chloride) or macromolecular compounds. The latter are preferred due to the following advantages: (1) their versatile tailorability by controlling molar mass, chemical structure, nature of the functional groups, charge density; (2) smaller amounts are needed, 1–6 ppm compared with mineral coagulants (25–100 ppm); (3) their relative insensitivity to *pH*; (4) lower sludge volume and better flocs properties (larger, stronger, stable, low-density – flotation facilitated); (5) shorter settling time [1]. They are available in all three forms, i.e. cationic, anionic and nonionic and may be synthetic (acrylic acid polymers and copolymers, styrene sulfonate polymers and copolymers, maleic acid copolymers with nonionic monomers, derivatives of phenol–formaldehyde resin; polyalkyleneimine and

polyhydroxyalkyleneamine, diallyldimethylammonium chloride polymers and copolymers; polymers and copolymers of quaternized tertiaryaminoalkyl esters and amides with acrylamide, poly(1-aminoethylene) such as polyvinylamine (PVAm) and related polymers) [2–6] and natural (ionic derivatives of gum guar, starch, dextran, chitosan) [7–11]. Synthetic flocculants are more performant than the natural ones, requiring lower polymer dose to obtain an efficient flocculation. In the same time, they may contain toxic monomers from the synthesis and additives. On the other hand, the natural polymers have some advantages over those of synthetic ones, namely, they are non-toxic, biodegradable and fairly shear stable. In recent years, considerable attention has been paid on the synthesis of some compounds which combine the advantages of both synthetic and natural polymers. Thus, a great number of copolymers were achieved by grafting synthetic polymers, mainly, flexible chain of poly(acrylamide) (PAM) on some polysaccharide backbone, such as gum guar, starch, konjac glucomannan, chitosan [1,12–20].

Pullulan (P) is a biocompatible and biodegradable polymer, which is degraded into non-toxic oligomers or monomers [21]. Nonionic and ionic pullulan derivatives have extensively used as a coating and packaging material, sizing agent for paper or starch replacer in low-calorie food formulations, cosmetic emulsions and other biomedical applications [22–24]. Hitherto, as far as we could ascertain, no data exist on their use in solid-liquid phase separation processes. As a consequence, the goal of this study was to prepare some grafted cationic polysaccharides based on pullulan and to assess their flocculation properties on a clay suspension. The new synthesized polyelectrolytes (P-g-pAPTAC) contained various amount and length of grafted cationic chains, poly[(3-

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acrylamidopropyl)-trimethylammonium chloride] (pAPTAC) onto the pullulan backbone. The separation efficiency, determined by turbidity and zeta potential measurements, was investigated as a function of the grafted chains content and length as well as of some flocculation parameters, such as the polycation dose, the settling time, the parent solution concentration of polycation. The surface morphology and the floc size distribution measurements, at the optimum polycation dose, were also performed. The viscometric behavior of the polyelectrolytes under study will be collaterally discussed to confirm the chain conformational changes in solution and to relate them with the separation efficiency.

2. Experimental

2.1. Materials

2.1.1. Materials for synthesis of flocculant

Pullulan ($M_w = 200,000$ g/mol) was purchased from Hayashibara Lab. Ltd., Okoyama, Japan. (3-Acrylamidopropyl)-trimethylammonium chloride (75 wt.% solution in water) (Aldrich) and potassium peroxydisulfate (Fluka) (KPS) have been used as such.

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: $<20 \mu\text{m}$ 90–95%; $<10 \mu\text{m}$ 82–89%; $<6 \mu\text{m}$ 70–80%; $<2 \mu\text{m}$ 55–65%; $<1 \mu\text{m}$ 45–57%.

2.2. Methods

2.2.1. Graft copolymerization

The grafting reaction was carried out under nitrogen atmosphere in a three-necked flask equipped with a nitrogen inlet and a reflux condenser immersed in a constant temperature water bath. The typical synthesis procedure is as follows: 1.0 g pullulan was dissolved in 30 mL of distilled water under constant stirring and nitrogen was purged for 30 min. Then 0.1 g of potassium peroxydisulfate has been added. After 15 min supposed to be enough to induce free radicals onto pullulan chains, the desired quantity of APTAC was added to the flask and the reaction was continued for 5 h, after which it was stopped by letting air into the reactor. The reaction temperature was maintained at 80 ± 1 °C. The homogeneous solution was precipitated into a large excess of acetone. The crude material has been further extracted with methanol for 72 h using a soxhlet apparatus to remove the homopolymer (pAPTAC) and the un-reacted monomer. Finally, the grafted pullulan was solubilized in distilled water, dialysed for 5 days at 20 °C for removal of water soluble species, and recovered by freeze-drying. Three samples of P-g-pAPTAC were synthesized by altering initiator and monomer concentration. The details are given in Table 1.

Table 1
Synthesis parameters of graft copolymerization.

Polymer	P^a (mmol)	APTAC (mmol)	KPS (mmol)	Product		$[\eta]$ (mL g^{-1})	R^c	c^* (g mL^{-1})
				pAPTAC (wt.%)	Graft ratio ^b (%)			
P-g-pAPTAC1	6.17	4.84	0.369	22.53	29.09	67	0.999	0.015
P-g-pAPTAC2	6.17	9.67	0.369	29.05	40.94	500	0.995	0.002
P-g-pAPTAC3	6.17	4.84	0.924	34.51	52.69	77	0.998	0.013

^a 1 mol $P = 1$ mol of anhydroglucose unit (UGU) = 162 g.

^b Graft ratio is calculated with the equation: (weight of grafted polymer – weight of substrate)/weight of substrate.

^c R = regression squared coefficient of linear fits to experimental data.

2.2.2. Infrared (IR) spectral analysis

The IR spectra of representative grafted and ungrafted pullulan were run as KBr pellets on VERTEX 7 FTIR spectrophotometer in the frequency ranges of 400–4000 cm^{-1} .

2.2.3. Grafted copolymer composition

The copolymer composition was determined by ^1H NMR spectroscopy. ^1H NMR spectra were recorded on a Bruker Avance DRX 400 NMR, at 50 °C, using deuterated water as the solvent.

2.2.4. Gravimetric analysis

Percent yield of grafting was calculated by the Eq. (1):

$$\text{yield (\%)} = \frac{W_{\text{CP}} - W_{\text{P}}}{W_{\text{P}}} \times 100\% \quad (1)$$

where W_{CP} is the weight of the grafted copolymer and W_{P} is the weight of pullulan

2.2.5. Viscosity measurement

Viscometric measurements of the polyelectrolyte solutions were carried out with an Ubbelohde viscometer (capillary 10/l, viscometer constant $K = 0.01025 \text{ mm}^2/\text{s}^2$) at 25 ± 0.1 °C; the viscometer allows a reliability of the flow times with an accuracy of 0.03 s. Polymer solutions were made up in double-distilled water. All viscosity measurements were repeated at least twice in order to check the reliability of the data which was within $\pm 3\%$.

2.2.6. Flocculation characteristics

Both the aqueous grafted polymer solutions and clay dispersions were prepared by using distilled water. The concentration of the model suspensions was 1 g L^{-1} . The initial pH of the suspension was 4.5. After preparation each sample was sonicated for 30 min using an ultrasonicator (Bandelin Sonorex RK510H, Berlin, Germany) followed by vigorous stirring for 15 min. This procedure was found to be effective in fully dispersing the clay powder. All the flocculation experiments were conducted at room temperature. 500 mL of the clay suspension were placed into 600 mL glass beakers. A Teflon bar was inserted and then different volumes of polycation solution were added at 1000 rpm. Stirring was continued with the same speed for about 3 min, and then it was decreased to about 200 rpm for 15 min. After settling times of 15, 30, 60 and 120 min the reading of the supernatant turbidity was performed. Turbidity measurements were carried out using a HACH 2100 An turbidimeter (HACH Company, Dusseldorf, Germany). A blank experiment was performed in the absence of grafted polysaccharide to evaluate the “natural” decantation of the suspension under the selected experimental conditions (pH , concentration of suspended mater). The residual turbidity ($T\%$) was expressed as percent of the initial turbidity of the clay suspension, at time zero, in the absence of polymer.

The zeta potential of clay suspensions in the presence and absence of grafted polymer was measured with a Zetasizer Nano-ZS, ZEN-3500 model (Malvern Instruments, Malvern, England). The equipment measures the electrophoretic mobility of the particles

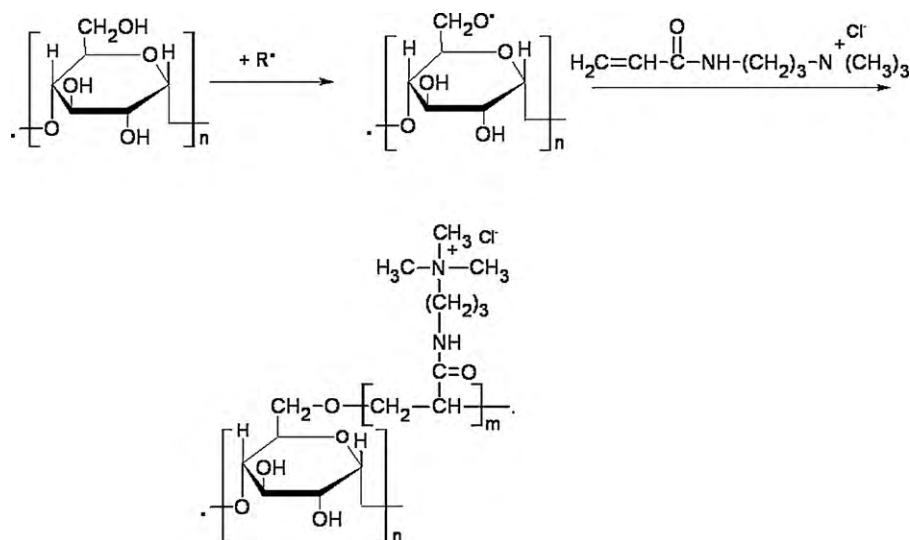


Fig. 1. The mechanism route for grafting APTAC onto pullulan.

and converts it to the zeta potential using the von Smoluchowski equation. The reading of the supernatant zeta potential was performed after a settling time of 15 min.

Measurement of particle dimensions was done using 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 unit of the sample (Hydro 2000A) type 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 polycation dose was allowed to settle for 15 min and the settled flocs were collected and rapidly transferred to the dispersion unit of the equipment.

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, operating at 30 kV (referinta) and 10 kV (flocs) with secondary electrons in low vacuum.

In the following, the parent solution concentration of polymer, c_p , means the concentration of polyelectrolyte solution added to the clay suspension, and polymer dose refers to the polyelectrolyte concentration in its mixture with clay suspension.

3. Results and discussion

3.1. Synthesis

Table 1 summarizes the synthesis parameters for the three samples of pullulan-based grafted copolymers prepared by grafting APTAC onto pullulan by KPS initiation. During the preparation of the first two samples, P-g-pAPTAC1 and P-g-pAPTAC2, the APTAC concentration varied at fixed P and KPS concentrations; whereas for the samples P-g-pAPTAC1 and P-g-pAPTAC3 the initiator concentration varied but the concentrations of P and APTAC were kept constant.

It is believed that the mechanism of KPS initiated graft copolymerization is the abstracting hydrogen atom from pullulan and producing PO^\bullet macroradical at the OH sites of the polysaccharide molecule [25–27]. The suggested reaction path for the graft reaction of pullulan is shown in Fig. 1. The average number of grafting sites per backbone of the pullulan molecule depends on the ratio of the concentration of $\text{SO}_4^{\bullet-}$ and OH^\bullet radicals to that of pullulan.

A relatively low concentration of initiator will initiate few grafting sites, resulting in longer pAPTAC chains against shorter pAPTAC chains at higher concentration. This is reflected in their corresponding intrinsic viscosity (Table 1). The viscometric behavior of these compounds in saltless aqueous solution will be discussed in Section 3.3.

3.2. Characterization of graft copolymers

The graft copolymer samples were followed gravimetrically. The increase in weight of the residue for each sample, over that of the corresponding control, indicates possible grafting.

The grafting of the monomer on the pullulan backbone is confirmed by the presence in the IR spectrum (Fig. 2 curve b) of the peak at 1480 cm^{-1} which corresponds to the methyl groups of ammonium [28,29], the peak at 1417 cm^{-1} referenced as the C–N stretching vibration, 1729 cm^{-1} due to C=O stretching vibration, 1560 cm^{-1} (secondary amide N–H stretching, amide II band) and 1655 cm^{-1} (amide I band), 2850 cm^{-1} ($-\text{CH}_3$ symmetric stretch-

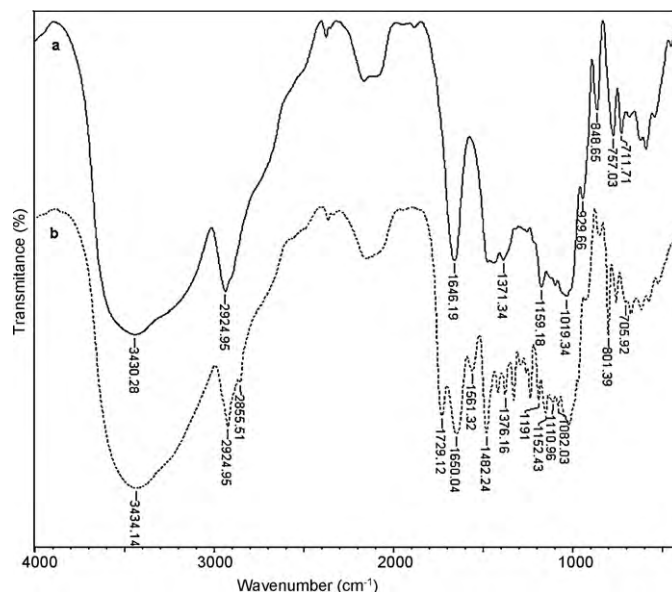


Fig. 2. FTIR spectrum of pullulan (a) and P-g-pAPTAC (b).

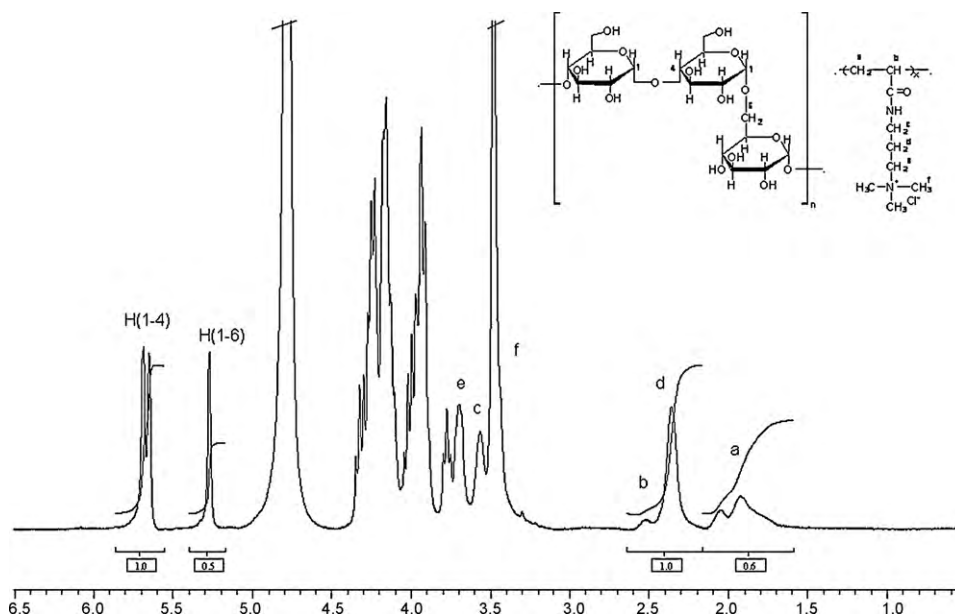


Fig. 3. ^1H NMR spectrum of P-g-pAPTAC copolymer.

ing), 2925 cm^{-1} (asymmetric $-\text{CH}_2-$ stretching). C–O–C stretching vibration at 1110 cm^{-1} confirms the attachment of monomer molecules to substrate through ether linkage at OH sites of substrate [30].

The chemical structure of the graft copolymer was also examined by ^1H NMR spectroscopy. As shown in the spectrum of the copolymer (Fig. 3), the characteristic resonance peaks corresponding to the protons in saccharide ring of pullulan at 3.4–5.8 ppm are present [31] and the peaks a, b, c, d, e and f which result from grafted APTAC units [32,33]. The molar ratio of APTAC units grafted on structural unit of pullulan was calculated from the report of the integral value of methylene protons at 1.9–2 ppm which correspond to the APTAC and that of H-1 and H-6 protons of pullulan moieties observed at 5.6 and 5.2 ppm.

This content was confirmed from the nitrogen analysis by the Kjeldahl method. The weight content of pAPTAC in the grafted copolymers was calculated from N content using following Eq. (2):

$$\% \text{ grafted APTAC} = \frac{\% \text{N}}{14.01} \times 206.72 \quad (2)$$

where 206.72 represent molar mass of the monomer.

3.3. Viscometric measurements

The viscometric behavior of a polymer is one of the most frequently used method to receive information about the chains conformation and their hydrodynamic dimensions in solution, polymer–polymer interactions, excluded volume effects governed by polymer–solvent interactions and chain stiffness. In order to discuss the influence of the chain conformational changes in solution on the flocculation behavior of the cationic polysaccharides viscosity measurements were performed.

The viscosity results obtained in water for the grafted copolymers P-g-pAPTAC are illustrated in Fig. 4.

All copolymers exhibited polyelectrolyte behavior in water, that is, a typical upturn of the $\eta_{\text{red}}=f(c)$ plots at low concentration arising from intra- and intermolecular repulsive electrostatic interactions. Comparing the data, at a constant concentration, reduced viscosities are seen to increase in the order P-g-pAPTAC1 < P-g-pAPTAC3 < P-g-pAPTAC2, that means with increasing the content as well as the grafted chain length. A greater content of pAPTAC chains,

even shorter, means a higher content of the quaternized units in the copolymer P-g-pAPTAC3 than that in the copolymer P-g-pAPTAC1, which implies stronger electrostatic repulsive forces between the charged groups of the macroion; the higher the quaternized units content, the more extended polymer chains, and hence higher viscosity. On the other hand, a fewer number but longer of pAPTAC chains in P-g-pAPTAC2 than P-g-pAPTAC1 brought about much stronger increase of the coil dimension and hence, the reduced viscosity of the former sample.

Due to the concave upward of the η_{sp}/c – c curves it is not possible to extrapolate the reduced viscosity to zero concentration in order to determine the intrinsic viscosity $[\eta]$. This problem can be solved either through screening of charges by addition of low-molar-mass electrolyte or by means of empirical equations. In order to obtain $[\eta]$, the viscometric data were linearized by means of Rao equation [34]. This empirical equation is applicable for describing the viscosity of diluted to moderately concentrated neutral polymer solution and relative viscosity (η_r) values from 1 to about 100. As η_r values for all samples under study lie in this range, the curves shown in

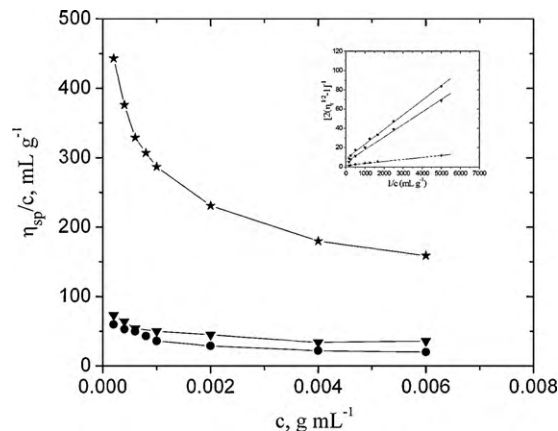


Fig. 4. Reduced viscosity (η_{sp}/c) dependence on the grafted cationic polysaccharide concentration (c) for: (circle) P-g-pAPTAC1; (star) P-g-pAPTAC2; (triangle) P-g-pAPTAC3; inset, fitting of the Rao equation for: (circle) P-g-pAPTAC1; (star) P-g-pAPTAC 2; (triangle) P-g-pAPTAC3.

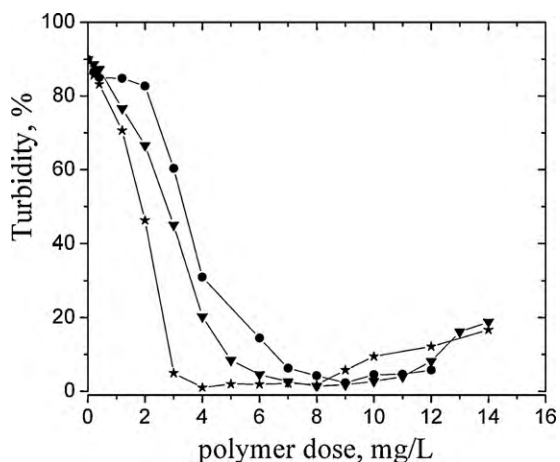


Fig. 5. Residual turbidity ($T\%$) dependence on the grafted cationic polysaccharide dose for: (circle) P-g-pAPTAC1; (star) P-g-pAPTAC2; (triangle) P-g-pAPTAC3; settling time 15 min.

Fig. 4 were linearized applying the above mentioned Eq. (3):

$$\frac{1}{2\left(\eta_r^{1/2} - 1\right)} = \frac{1}{[\eta]c} - \frac{a-1}{2.5} \quad (3)$$

where a is a constant for a given polymer–solvent system.

As can be observed straight lines were obtained for all polycations (Fig. 4, inset), showing that the Rao equation is suitable for describing the viscometric behavior of these samples and estimating the $[\eta]$ values in salt-free solutions. The calculated values for $[\eta]$ are summarized in Table 1. The applicability of the Rao equation to describe the viscosity of the polymer solution was also found in the case of other polyelectrolytes [35–37].

The overlap concentration, c^* , which is the limit between the dilute and semidilute regime, can be estimated from the intrinsic viscosity values by means of the Eq. (4) [38]:

$$c^* = \frac{1}{[\eta]} \quad (4)$$

where $[\eta]$ = intrinsic viscosity. The results obtained for c^* are also listed in Table 1. As it was expected, the higher content as well as the longer charged grafted chains resulted in higher hydrodynamic dimension of the macromolecular coils and therefore in higher $[\eta]$ and, as a consequence, in lower c^* values.

3.4. Turbidity measurements

3.4.1. Effects of polycation dose on flocculation efficiency

The effect of any polymer on the flocculation process is influenced by the polymer dose, the flocculation efficiency decreasing at lower or higher doses than the optimum dose. The flocculation performance can be explained in terms of the residual turbidity of the supernatant liquid. The lower the residual turbidity, the better the flocculating polymer is. For all grafted copolymers investigated, a downtrend of residual turbidity with increasing polycation dose was evidenced, reaching a minimum, and then increased again (Fig. 5).

These findings can be explained in the followings. The main mechanisms by which polymers can bring about flocculation are bridging (where tails and loops of a few polymers with high affinity to the particle surface make bridges between two or more particles simultaneously), charge neutralization (where the particle surface charges are neutralized by the oppositely charged groups of the chain so that the particles attract each other by van der Waals forces), or a charge patch mechanism (when aggregation

occurs as a result of the electrostatic attraction between oppositely charged regions on partially covered particles) [5]. These flocculation mechanisms can act alone or in combination, depending on the properties of particles and polymers in solution.

The P-g-pAPTAC samples have a comb like structure. The data reported in the literature regarding the flocculation properties of the grafted copolymers reveal that the bridging mechanism plays the most important role in the separation process [12,14,20]. Furthermore, 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 [39]. From the above information one may infer that the bridging mechanism, where grafted polymer chains adsorb on different particles linking them together, operated in the flocculation of clay particles. In addition, the grafted polymer segments on the pullulan backbone bear positive charges. Hence, the electrostatic attraction between the negative charges of the suspended particle and the positive ones of the polyelectrolyte, also, played an important role in the separation process. The presented herein results suggest that the bridging mechanism as well as the charge neutralization one operated in the flocculation process of the clay dispersion with pullulan derivatives under study. At lower dosage, on the one hand, there was insufficient polymer to form adequate bridging links between particles and, on the other hand, the surface charge of the particles remained negative and the particle aggregation was slow due to the repulsive Coulombic forces. As the polymer dose increased more particles were linked together causing destabilization of suspension. Moreover, the charge of the particles was neutralized by the adsorbed polymer chains and consequently, the particles aggregated more rapidly due to the attractive van der Waals forces. At a polymer dose higher than that corresponding to the minimum value of the residual turbidity, called here the optimum polymer dose, there was no longer enough bare particle surface available for attachment of polymer segments and no floc will form; hence, the suspended particles are not easily removed. The suspended particles may also be surrounded by the grafted charged chains, and the solution was re-stabilized. Furthermore, steric hindrance between the extended polymer segments could hinder the bridge formation. Of course, the higher viscosity at higher doses might also impair the flocculation of the clay particles.

3.4.2. Effect of the grafted charged chains content and length in the P-g-pAPTAC on the flocculating effect

Fig. 5 also reveals that the flocculation ability was also affected by the pAPTAC grafted chains content (%) in the P-g-pAPTAC. The results indicate that increasing the pAPTAC content, the flocculation efficiency of P-g-pAPTAC increased. Thus, for P-g-pAPTAC3, the maximum removal of the turbidity was achieved at the optimum dose of 8 mg L^{-1} , while for P-g-pAPTAC1, the minimum turbidity was obtained at the optimum dose of about 9 mg L^{-1} . Increasing the charged chains content of the cationic polymer increased its electrostatic attraction to the negatively charged surface of the clay particles. On the other hand, the highest rise in the flocculation efficiency for P-g-pAPTAC2 (optimum dose = 4 mg L^{-1}) may be due to the longest cationic pAPTAC chains grafted on the pullulan, which could bind the highest number of colloidal particles and also caused the hydrodynamic coil volume to be more extended (see the viscometric data in Fig. 4 and Table 1) and thus, more effective in flocculation. These data agree with some experimental results reported in the literature [1,12,13,19]. Furthermore, this sample showed a wider optimum concentration range (flocculation window) compared with the other two samples. The maximum flocculation induced by P-g-pAPTAC2 occurred at a dose interval ranging from 4 to 9 mg L^{-1} ; the possibility of the longest grafted

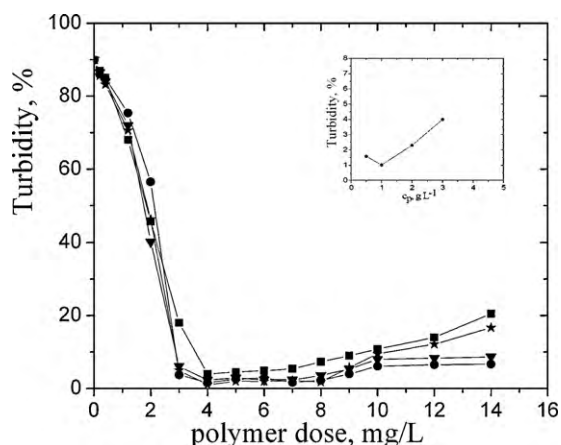


Fig. 6. Residual turbidity ($T\%$) dependence on the grafted cationic polysaccharide dose for P-g-pAPTAC2 for different parent solution concentration (c_p): (circle) 0.5 g L^{-1} ; (star) 1 g L^{-1} ; (inverted triangle) 2 g L^{-1} ; (square) 3 g L^{-1} ; settling time 15 min; inset, residual turbidity ($T\%$), at the optimum polycation dose, dependence on the parent solution concentration (c_p).

cationic chains to be adsorbed onto a higher number of particles means a less number of free (nadsorbed) cationic segments and hence, less pronounced repulsion between the polyelectrolyte segments, resulting in a delayed redispersion.

3.4.3. Effects of parent solution concentration of polycation on flocculation efficiency

In the flocculation process the adsorption of polymer chains on the particle surface takes place during the mixing of the flocculant parent solution and the clay suspension. Thus, the initial conformation of the flocculant chains at the moment of their addition to the clay suspension may influence the flocculation performance even if the final polymer concentration in suspension is the same. In order to check this assumption, similar experiments were performed with different parent solution concentrations, c_p , of the cationic polysaccharides P-g-pAPTAC2 (the sample with the best flocculating performance). The polycation concentration varied in the range $0.5\text{--}3 \text{ g L}^{-1}$. Fig. 6 shows a similar variation of the residual turbidity with the polycation dose for all flocculant parent solution concentrations, that is, the residual turbidity decreased with increasing polycation dose and then increased due to the excess of the grafted chains adsorbed on the particles, resulting in restabilization of clay suspension. An optimum polycation dose was found for each $T\%$ –polycation dose curve. The optimum polymer dose values are almost the same, irrespective of c_p : about 4 mg L^{-1} .

The residual turbidity value, at a given polycation dose, however, changed with changing c_p . Fig. 6 (inset) illustrates the residual turbidity ($T\%$) dependence on c_p at optimum polycation dose. The residual turbidity values decreased with increasing c_p from 0.5 to 1 g L^{-1} and increased for higher c_p . A close inspection of Table 1 reveals that the c_p values are located below and above the overlap concentration, $c^* = 2 \text{ g L}^{-1}$. At $c_p = c^*$ or higher ($c_p = 2$ and 3 g L^{-1} ; semidilute concentration regime) the polycation chains are overlapped or even entangled which may hinder the bridge formation. Moreover, this resulted in reduced availability of positively charged groups of the grafted polymer chains and, consequently, lower flocculation efficiency. So, an appropriate concentration of the parent solution is necessary to reach the best flocculation. Taking in consideration the highest clarity degree (of about 100%) at optimum polymer dose, one may consider $c_p = 1 \text{ g L}^{-1}$ as the optimum parent solution concentration (c_{op}) for P-g-pAPTAC2, in the concentration range investigated.

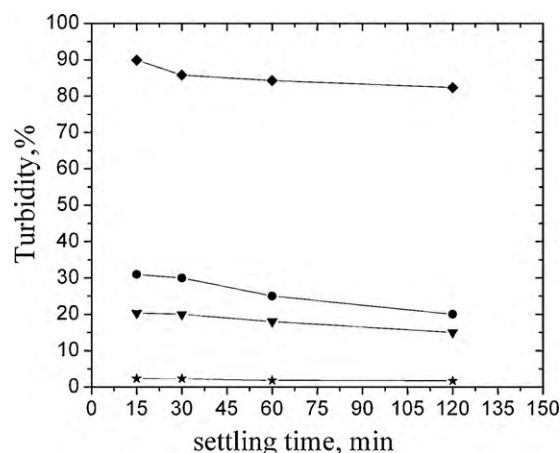


Fig. 7. Residual turbidity ($T\%$) dependence on the settling time for clay suspension: (diamond) in the absence of polymer samples and in the presence of polymer samples: (circle) P-g-pAPTAC1; (star) P-g-pAPTAC2; (triangle) P-g-pAPTAC3; polymer dose 4 mg L^{-1} .

This parameter was also determined in the case of other polymeric flocculants, namely, three kind of polyacrylamide (linear, star and hybrid) [40] and cationic dextran derivatives [11].

3.4.4. Effects of the settling time on flocculation efficiency

Fig. 7 displays the settling time impact on the flocculation of clay suspension in the presence and absence of grafted polysaccharides, at a polymer dose of 4 mg L^{-1} . Without pullulan samples the natural decantation of clay suspension was very slow, and the residual turbidity did not decrease below 80% even after 120 min of settling. The suspension was very stable. The addition of the modified pullulan samples significantly improved the settling efficiency. However, the effect was highly dependent on the polycation grafted chains content and length, the settling time decreasing drastically for the polycation with the longest grafted chain; this may be due to the greater spreading effect of the dangling grafted chains on the polysaccharide chains, and hence binding the highest number of colloidal particles and thus, more effective in flocculation. Moreover, it was found for all polycations that the residual turbidity decreasing was more pronounced in the first 15 min.

3.5. Flocculation mechanism

More supporting evidences for the flocculation mechanisms proposed above for the samples used in this work come from zeta

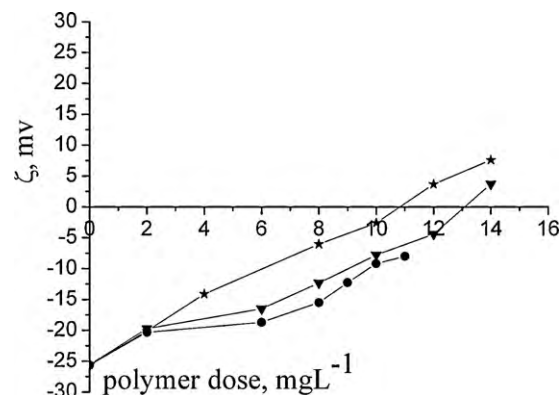


Fig. 8. Zeta potential (ζ) dependence on the polycation dose for the grafted cationic polysaccharides: (circle) P-g-pAPTAC1; (star) P-g-pAPTAC2; (triangle) P-g-pAPTAC3; settling time 15 min.

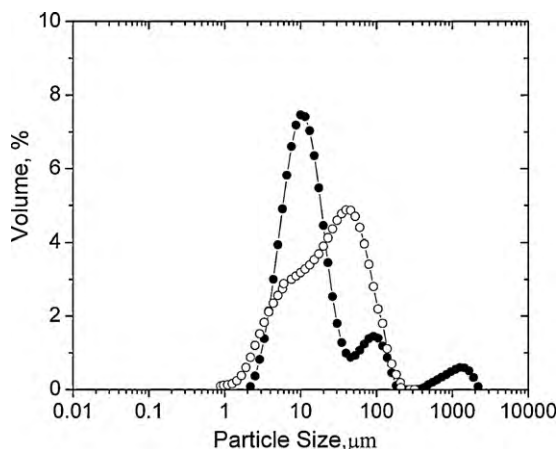


Fig. 9. Floc size distribution at the optimum polymer dose for: (solid circle) P-g-pAPTAC2; (empty circle) no polymer.

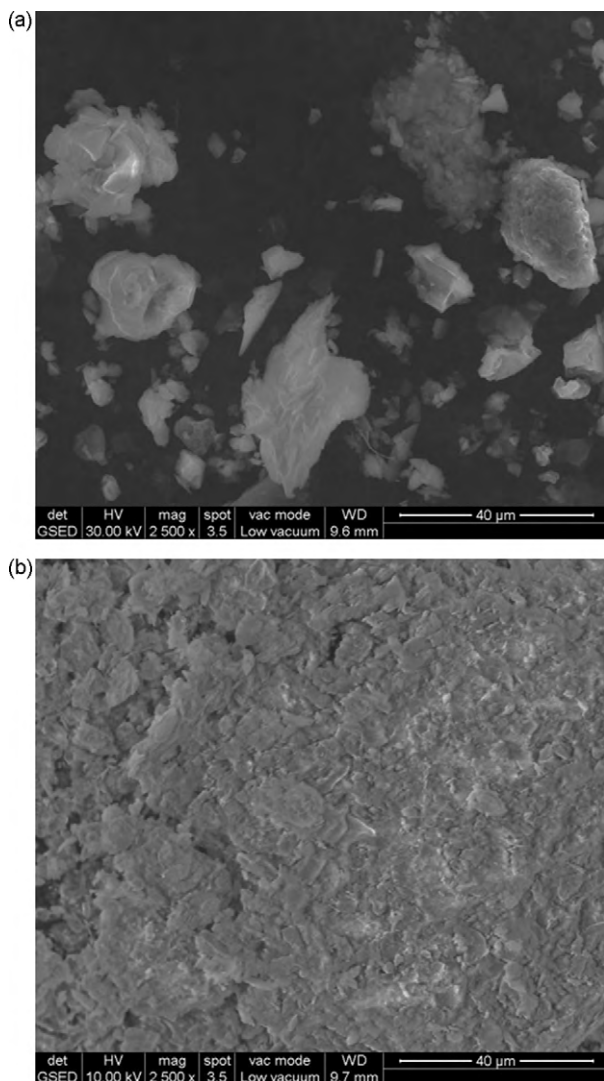


Fig. 10. SEM micrographs of clay flocs in the absence of P-g-pAPTAC2 (a) and in the presence of P-g-pAPTAC2 (b).

potential, particle size measurements and the morphology of clay particles which are shown in the following.

Fig. 8 shows the zeta potential of clay suspension as a function of polymer dose.

Prior to flocculant addition, the clay suspension has a zeta potential of -25.6 mV. An increase in polycation dose led to the ζ values increase as an outcome of the electrostatic attraction forces between the charges on the polycation chains and the particle surface. In the same time, it is worth noting the negative value of ζ at the optimum polycation dose; this finding pleads for the contribution, alongside the neutralization mechanism, of the bridging mechanism for the clay removal by the pullulan derivatives. In the case of the neutralization mechanism, the optimum flocculation occurs at polyelectrolyte dose around that needed to give a zeta potential close to zero [41].

The particle size distribution, which is presented as volume fraction versus particle diameter, for P-g-pAPTAC2 at the optimum polycation dose as well as of the initial clay suspension is shown in Fig. 9.

Three size peaks may be noticed for the clay floc size distribution in the presence of P-g-pAPTAC2: (1) An unimodal distribution in flocs size together with smaller size and narrower distribution compared with the untreated clay, that points to the neutralization mechanism as the dominant mechanism for the particle clay flocculation by this polycation sample. (2) Two larger size peaks (smaller volume percentage) which indicates that a certain portion of the particles was flocculated via bridging mechanism. This was likely possible, because the grafted chains could participate to inter-particle associations, which resulted in bigger flocs.

The morphology of the clay particles in the absence and in the presence of P-g-pAPTAC2 at the optimum polymer dose (4 mg L^{-1}) is shown in scanning electron micrographs (Fig. 10a and b). The SEM pictures reveal the P-g-pAPTAC2 flocs comprise minor substructures linked together in a large network.

SEM analysis together with the data gathered with the zeta potential and particle size distribution measurements provide experimental evidence for the simultaneous contributions from both neutralization and bridging mechanisms for the flocculation of clay dispersion by the grafted pullulan derivatives investigated.

4. Conclusions

The main conclusions drawn from the study are as follows:

- Some novel flocculants were obtained by graft-polymerization of (3-acrylamidopropyl)-trimethylammonium chloride (APTAC) onto pullulan (P). Variation in the synthesis parameters resulted in cationic polymers with different content and length of the grafted pAPTAC chains
- The viscometric measurements highlighted a strong influence of the grafted chains length on the viscosity of grafted cationic polysaccharides in saltless aqueous solutions. A longer pAPTAC chains brought about much stronger increase of the coil dimension and hence, the intrinsic viscosity.
- For all our grafted cationic polysaccharides, the residual turbidity values ($T\%$) decreased with increasing polycation dose, reaching a minimum, and then increased again.
- Flocculation experiments revealed that among the three samples, the one having longer pAPTAC chains (P-g-pAPTAC2) performed better separation efficiency than those with shorter pAPTAC chains (P-g-pAPTAC1 and -g-pAPTAC3).
- The optimum polymer dose value for P-g-pAPTAC2 was the same, irrespective of the parent solution concentration (c_p), but the lowest residual turbidity value was obtained at $c_{op} = 1 \text{ g L}^{-1}$.

- SEM analysis together with the data gathered with the zeta potential, floc morphology and particle size distribution measurements point to simultaneous contributions from both neutralization and bridging mechanisms for flocculation of clay dispersion by grafted pullulan derivatives.

Acknowledgements

The authors thank the group of Micro- and nanostructures characterization Laboratory ("P. Poni" Institute of Macromolecular Chemistry, Iasi, Romania) for performing zeta potential and particle dimension measurements. This study was supported by grant from Ministry of Education and Research of Romania (CNCSIS, PCE "Idei" nr. 644/2009).

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