Environmental Engineering and Management Journal

February 2019, Vol.18, No. 2, 301-310
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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BEHAVIOUR OF NEW PULLULAN DERIVATIVE SOLUTIONS WITH BIOTECHNOLOGICAL POTENTIAL

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Abstract

The synthesis of dimethylamino-propylamine pullulan, DMAPA-P, with a substitution degree of 0.4 was performed in dimethylsulfoxide using N,N'-carbonyldiimidazole as activator and dimethylaminopropylamine as amination agent. Then, two quaternary derivatives (trimethylammonium-propylamine pullulan, TMAPA-P, and benzyldimethylammonium-propylamine pullulan, BzDMAPA-P) were prepared from DMAPA-P by quaternization with methyl iodide and benzyl chloride. The behavior of new pullulan derivatives solution containing tertiary amine or quaternary ammonium groups in different polar solvents (protic or aprotic) with added low molecular salt was studied by laser light scattering. Thus, all polymers recorded positive second virial coefficients A_2 in solvents with 0.5 M salt added, and molecular masses for TMAPA-P and BzDMAPA-P increased compared with them precursor DMAPA-P. Finally, these polysaccharide derivatives (alone or in tandem with NaCl) were tested for their capacity to remove inorganic materials from synthetic turbid water. Turbidity of aqueous bentonite solutions in the presence of BzDMAPA-P or DMAPA-P increased compared with turbidity of bentonite solutions. Remarkably, the addition of NaCl improved the inorganic particles settling capacity of all three polysaccharides.

Keywords: bentonite, laser light scattering, pullulan derivatives, turbidity

Received: February, 2015; Revised final: April, 2015; Accepted: May, 2015; Published in final edited form: February, 2019

1. Introduction

As eco-friendly materials, the polysaccharides were intensively studied over time, both natural and synthetic ones. Pullulan is a special representative of them; synthesized by Aureobasidium pullulans fungus, it was isolated to use for innovative applications in food, pharmaceutical and miscellaneous industry (Singh et al., 2008; Prajapati et al., 2013). To diversify its applicability, other research groups modified this polysaccharide by different methods like carboxymethylation, sulphonation, oxidation, copolymerization or grafting (Bataille et al., 2011; Constantin et al., 2013; Prajapati et al., 2013).

Cationic polysaccharides with amino or ammonium functional groups are very helpful in a large range of applications (wastewater treatment, petroleum, textile, paper, food, cosmetic or pharmaceutical industry, as well as in molecular biology and analytical chemistry) (Prado and Matulewicz, 2014).

The scientific literature mentioned various methods and solvents, useful to determine the real properties of polysaccharides in solutions (Buchard, 2008; Corona et al., 1988; Harding et al., 1991; Prado and Matulewicz, 2014). For this purpose, specific solution properties (refractive index increment, molecular weight, radius of gyration and second virial coefficient) of polysaccharides were determined by differential refractometry and laser light scattering methods.

The access of living organisms (people, animals and plants) to cleaned waters has become a goal for life. Turbidity is related to the concentration

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of suspended solid particle in water such as inorganic materials (silt or minerals), lived/dead organic matter and biotic material (algae, viruses, and bacteria) (EPA Guidance Manual, 1999). Generally, the water treatment technology is based on coagulation, flocculation, sedimentation, and filtration processes, respectively. The kinetic of the flocculation process intensively was studied, and in this respects the Argaman and Kaufman model most widely was employed. According to this model, the aggregation of destabilized particles (as a result of various associated phenomena) and break-up are the main phenomena considered (Argaman and Kaufman, 1970; Moruzzi et al., 2013). Each step of water treatment technology is influenced by diverse factors: technical design of treatment equipments, temperature, pH, reaction time, type of impurities, and nature of coagulants/flocculants. In order to achieve best results, biotechnological methods for water treatment involved synthetic coagulants or flocculants ($FeCl_3$, $Fe_2(SO_4)_3$, polyaluminum chloride (Cheng et al., 2008; Mirzayi et al., 2012; Shen, 2005), polyamines, polydiallyldimethylammonium chloride (Polasek and Mutl, 2002), dicyandiamide and melamine formaldehyde resins, polyacrylamides) or environmentally friendly natural chemicals (date palm seeds, pollen sheath, different leguminous species and mucilages (Mishra and Bajpai, 2005), carboxymethyl inulin (Rahul et al., 2014), polyacrylamide grafted carboxymethylstarch (Sen et al., 2009), ionic dextran derivatives (Ghimici et al., 2009), poly(3-acrylamidopropyl)-trimethylammonium chloride grafted pullulan (Ghimici et al., 2010), tamarind kernel polysaccharide (Pal et al., 2009), polyacrylamide grafted oatmeal (Bharti et al., 2013), natural Strychnos Potatorum (Deshmukh et al., 2013)).

Therefore, all experiments were designed to study the relationship of different substances and treated water quality. Whereas it has been found that some substances (ex. aluminum) harm to health and it was decided to low the dosage or eliminate them from water treatment (Deshmukh et al., 2013; Wang and Cui, 2004). The natural coagulants/flocculants seem to be more sustainable. Following this trend we aimed to test some pullulan derivatives in order to eliminate inorganic particle from synthetic turbid water.

2. Material and methods

2.1. Materials

Pullulan (P) (M_w = 200000 g mol^{-1}) was purchased from Hayashibara Lab. Ltd. (Okoyama, Japan). 3-dimetylamino-1-propylamine (DMAPA), methyl iodide (MeI), N, N'-carbonyldiimidazole (CDI), and N,N'-dimethylaminopropylamine (DMAP) were purchased from Fluka (Buchs, Switzerland) and have been used as received. Dimetylsulfoxide (DMSO) and dimethylformamide (DMF) were obtained from Fluka and distilled under vacuum prior the use. Na^+-saturated bentonite (BENT) from Valea Chioarului (Maramures, Romania) was supplied by S.C. Mateo S.R.L.

2.2. Methods

2.2.1. Synthesis of pullulan derivatives

Dimethylamino-propylamine pullulan (DMAPA-P) synthesis

1 g (6.17 mmol UG) of pullulan powder was dissolved in 30 mL dried DMSO, followed by the addition of N,N'-carbonyldiimidazole (0.56 g; 3 mmol), under constant stirring. After activation for 2 hours, at 25 ± 2 ºC, DMAPA (40 mg) and 3.2 mL (25.4 mmol) of 3-dimethylamino-1-propylamine (4.12 mol/mol sugar residues of pullulan) were added into the dried DMSO solution containing activated pullulan. The mixture was reacted by stirring for 48 h at room temperature. Then, the reaction mixture was poured into acetone, and the precipitate was collected by filtration. The polymer was dissolved in distilled water, dialyzed against acidic then alkaline water to remove DMAPA, and then against distilled water until total deionization (checked by conductivity measurement in the dialysis bath). Finally, the polymer was recovered by freeze-drying (0.92 g in 92% yield).

Trimethylammonium-propylamine pullulan (TMAPA-P) synthesis

DMAPA-P polymer (1 g, 2.36 mmol tertiary groups content) was dissolved in 20 mL of DMF and an appropriate volume of MeI (MeI/DMAPA molar ratio=3) solved in 5 mL of DMF was dropwise added to this solution at 20ºC for 15 min. Then the reaction was continued at 50ºC for 24h. The obtained compound was precipitated in acetone. The precipitate was dissolved in 15% (*w*/*v*) NaCl solution in order to replace the iodide ions with chloride ions followed by dialysis with deionized water for 3 days to remove inorganic materials and then freeze-dried (0.88 g in 88% yield).

Benzyldimethylammonium-propylamine pullulan (BzDMAPA-P) synthesis

Typically, at 1 g of DMAPA-P (1 g, 2.36 mmol tertiary groups content) dissolved in 20 mL DMF, a solution of BzCl (2.87 mmol; BzCl/DMAPA molar ratio=1.2) in 10 mL DMSO was dripped at 50 ºC, under stirring. The reaction mixture has being homogenous on the entire duration of reaction. The polymer was purified by precipitation in acetone, extensively washed with acetone and dried under vacuum. Then, the precipitate was solved in distilled water and washed by dialysis against distilled water until free of chloride ions (checked by $AgNO_3$ solution in the dialysis bath). The white powders obtained (0.9 g in 90% yield) were stored in vacuum desiccators over P_2O_5 for further analysis.

2.2.2. Characterization of polymers

The degree of substitution (expressed as *DS* and defined as the number of hydroxyl (OH) groups substituted per repeating structural unit of the pullulan backbone) has been determined by elemental analysis of nitrogen, conductometric titration with aqueous 0.1N HCl solution (conductivity meter CMD 210 (Radiometer, Copenhagen, Denmark)), and from ^{1}H NMR spectrum (Bruker Avance DRX 400 NMR, Rheinstetten, Germany) at 25°C, using D_2O as solvent, as previously described by Constantin et al., 2015.

Degree of substitution determined from nitrogen content (DS_N) and conductometric titration (DS_{CT}) were calculated with Eqs. (1-2):

$$DS_{\mathrm{N}} = \frac{162 \times N\%}{28 \times 100 - \mathrm{Mr} \times N\%} \tag{1}$$

$$DS_{\mathrm{CT}} = \frac{V_{\mathrm{HCl}} \times f \times c}{m} \tag{2}$$

where: N is the total nitrogen content (wt. %) of pullulan derivative and M_r=129.18; 179.67 and 255.76 is the molecular mass of the newly introduced aminopropyl dimethylamine, trimethylammonium propylamine, and benzyldimethylammonium propylamine groups. V_{HCl} is the used volume of aqueous HCl solutions in L, f is the factor of HCl solution, c is the concentration (mol/L) and m is the weight of dried sample, in g.

The degree of quaternization was also determined from ^{1}H-NMR spectra by comparing the peaks integrals assigned to the different amino substituents, before and after quaternization as detailed described in the Results and discussion section. There was a good agreement between DS determined by the three methods, therefore we used in the following only DS_{NMR}.

The macromolecular characterization of pullulan derivatives was carried out in a comparative study that involved different solvents. Usually, aqueous solutions of polysaccharides presented aggregates. Therefore the choice of solvent was taken in account when the molar mass of individual chains was required. Aqueous solutions were prepared with bidistilled water and NaCl or KCl from Sigma Aldrich. Organic solvents, such as dimethylsulfoxide DMSO (Fluka), are commonly used in the analysis of polysaccharides due to their non-aggregative nature and better solubilization properties. DMSO solutions still require some salt (e.g. LiCl, Sigma Aldrich) to aid dissolution and prevent aggregation by hydrogen bonding (Schmitz et al., 2009).

Na^+-saturated bentonite (BENT) from Valea Chioarului (Maramures, Romania) is a mixture of silicates, mainly montmorillonite (over 70%), with different size particles, and minor components such as quartz, mica, chlorite and feldspar (Copcia et al., 2010). BENT is presented as a light cream colored powder with specific surface area of 25-30 m^2 g^{-1} and density of 260-300 g L^{-1}. Bulk chemical composition of BENT (Table 1) indicates a very high content of silica and sodium, and a low content of iron oxide, as compared with other Romanian bentonites (Cotea et al., 2008).

2.3. Preparation of synthetic turbid water

Bentonite slurry was used as model system. Synthetic turbid water was prepared by dispersion of bentonite in bidistilled water. The solid fraction of inorganic material was 1 mg mL^{-1}. For a uniform dispersion, the magnetic stirring (1000 rpm, 1 h) and ultrasonication (100 watt cm^{-2}, 2 h) were used.

2.4. Laser light scattering and differential refractometry

The refractometric and static light scattering measurements were made on unfractionated samples. The light scattering methods allow the exploration of different associations of (bio)macromolecules and require suitable solvents for the molecular dissolution of the components (Grigoras et al., 2013). Also, to determine the characteristic solution properties (weight-average molecular weight $\overline{M}_w$, radius of gyration R_g and second virial coefficient A_2) it was necessary to know the specific refractive index increments dn/dc of sample-solvent pair. The values of dn/dc are dependent on the measurement temperature, solvent type and wavelength of incident light, solution concentration and molecular weight of the polymer (Podzimek, 2011).

Laser light scattering of macromolecular compounds was described by Zimm equation (Eqs. 3-4):

$$Kc/R_\theta = 1/\overline{M}_w + 2A_2c + (16\pi^2 R_g^2/3\lambda_0\overline{M_w})\sin^2(\theta/2) + \ldots \tag{3}$$

with

$$K = 4\pi^2(dn/dc)^2 n^2/N\lambda_0^4 \tag{4}$$

where K is the optical constant for vertically polarized incident light, c concentration (g mL^{-1}) of the scattering species in solution, R_θ Rayleigh ratio (directly proportional to the ratio of the scattered light intensity at angle θ to the incident light intensity), N is Avogadro number, n solution refractive index, and λ_0 is the wavelength of the incident light. In the following calculations, we assumed that the concentration is sufficiently low to neglect the terms containing the higher virial coefficients than A_2. The R_g value can be found from the initial slope of Kc/R_θ versus $\sin^2(\theta/2)$ plot, $2A_2$ from slope of Kc/R_θ versus c graph, and 1/$\overline{M}_w$ from intercepts of mentioned plots with the ordinate (Podzimek S., 2011).

Table 1. Chemical composition of the native bentonite from Chioar Valley (Maramures, Romania)

Component	***SiO_2***	***Al_2O_3***	***Fe_2O_3***	***CaO***	***MgO***	***Na_2O+K_2O***	***H_2O***
%	75.04	14.32	1.64	1.16	2.41	1.86	3.57

The second virial coefficient A_2 is an important parameter that quantitatively characterizes the thermodynamic interaction between solute molecules at a specific temperature. The polymer-solvent interactions dominate the polymer-polymer interactions when $A_2>0$. In the case of $A_2=0$, both interactions are energetically equivalent and the solvent is called theta solvent. For $A_2<0$, polymer-polymer contact is preferred to polymer-solvent and the solvent is a poor one for the given polymer (Czechowska-Biskup et al., 2007). The Zimm plots were computed using Berry formalism (Eq. 5) and the scattering angle dependence was modeled by first-order and second-order polynomial functions in case of aqueous salted solution and solution in DMSO, respectively. For the sake of clarity, the noisy signals of some detectors were rejected.

$$\sqrt{Kc/R_\theta} = \mathrm{f}\,(\sin^2(\theta/2) + \mathrm{a}\,c) \qquad (5)$$

2.5. Turbidity

Turbidity as a qualitative parameter of water contamination is based on interaction of incident light with organic and inorganic matter from water sample. Using a primary standard (e.g. formazin, kaolin) and a calibration curve, turbidity measurement became a quantitative parameter expressed in different units, usually Formazin Nephelometric Unit (FNU) or Nephelometric Turbidity Unit (NTU) according to international standards (Sadar, 2004). In our study, qualitative estimation of turbidity for synthetic water was made by spectrophotometric method and clarity was related with transmittance of solution.

3. Experimental

3.1. Laser light scattering

Static light scattering measurements (SLS) were achieved in "batch mode" using a DAWN-DSP photometer (Wyatt Tech.), equipped with a HeNe laser (633 nm; 5 mW). This detector was calibrated with toluene (Sigma Aldrich) and normalized with dextran (M_p=6430 g mol^{-1}; Sigma-Aldrich). Light scattering intensities, recorded at 18 angles between 14°-152° were processed by ASTRA software 4.90.07 according to Zimm procedure.

For differential refractometry and light scattering experiments, the stock and diluted solutions (in range of 1×10^{-3} – 3×10^{-4} g mL^{-1}) were prepared. All solvents were filtered through 0.02 μm Anotop filters (Whatman). Before dilution stage, every stock sample was filtered through Whatman membrane filter with pore size of 0.2 μm. Then, all solutions were transferred into cylindrical quartz glass scattering cells (Quartz SUPRASIL, Hellma GmbH & Co. KG, Germany), immediately sealed with plastic caps, and kept 2 days for degassing. Thus, polymeric solutions were prepared for "batch mode" laser light scattering measurements.

3.2. Differential refractometry

The values of refractive index increments *dn/dc* were determined by differential refractometry using an Optilab-rEX refractometer from Wyatt Technology (Santa Barbara, CA, USA). Measurements were realized in off-line mode at 25°C and 633 nm.

3.3. Turbidity

In case of turbidity tests, the operation procedure was as follows: to 10 mL of fresh synthetic turbid water, 1 mL of aqueous or salted (0.5 M NaCl) solution of polymer was added so that polymer dose included 3 mg mL^{-1} of DMAPA-P, TMAPA-P or BzDMAPA-P. In this way, dilute concentration regime of polymer was the same with that in laser light scattering measurements, when macromolecular chains were separated from each other and behaved more or less independently.

Turbidity measurements were carried out at room temperature (25 °C) with a Spekol 1300 spectrophotometer (Analytik Jena AG), using a 1 cm^2 quartz cuvette and ± 2 nm wavelength accuracy. Initially, the samples were 3D stirred for 1 minute. After definite settling time of flocs, all measurements were carried out at 15, 30, 60, 90, 120, 150, and 180 min. Transmittance (T) values were recorded against distilled water as blank at 560 nm, wavelength characteristic to bentonite (Balch, 1931). Supernatant turbidity was defined as (100 – T) %. The bentonite removal was expressed as percent of the initial turbidity recorded for the bentonite suspension, at time zero, in the absence of polymer (residual turbidity %).

4. Results and discussion

4.1. Synthesis of pullulan derivative containing tertiary amine groups and its quaternization reactions

Polymer-analogues reactions are very well studied, especially for the quaternization of dimethylamino- (Bogoeva-Gaceva and Andonova, 1993; Deboudt et al., 1995) and vinylpyridine- (Arcus et al., 1964; Boucher, 1978) homopolymers.

In the present study, pullulan has been firstly modified by amidation reaction with 3-dimethylaminopropylamine in DMSO and in the presence of NMAP as catalyst as previously described (Constantin et al., 2015). Then, this pullulan derivative containing tertiary amine groups (DMAPA-P) was used as precursor in the synthesis of quaternary ammonium derivative possessing methyl or benzyl groups (TMAPA-P and BzDMAP-P). The chemical structure of the three pullulan derivatives is presented in Fig. 1.

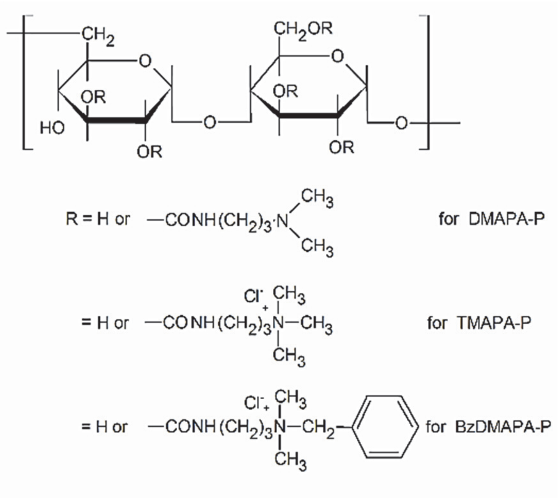

Fig.1. The chemical structure of synthesized pullulan derivatives

The quaternization of DMAPA-P was carried out in DMF with MeI and DMF/DMSO mixture (1/1, v/v) with BzCl, respectively. Structure characterization, degree of substitution (*DS*) and extent of quaternization of all derivatives was assessed from ^{1}H-NMR spectra. For example, Fig. 2A shows the ^{1}H NMR spectrum of the DMAPA-P in D_2O with the signals belonging to the aminopropyl substituents assigned as follows: 1.76 ppm to the -CH_2 protons of the propyl group, 2.26 ppm to the protons of the methyl on the tertiary amine group, 2.44 ppm to the -CH_2-N^+ and 3.17 ppm to the NH-CH_2- protons.

Additionally, the relevant signals of pullulan at 4.98 ppm, 5.38 and 5.42 ppm were assigned to the (1→6) and (1→4) anomeric protons. The hydrogen atoms in the pullulan rings give signals around 3.44 - 4.04 ppm (Bruneel et al., 1993). *DS* was calculated from the rapport of the integral value of CH_2 protons at 1.76 ppm divided by a factor of 2, and that of *H-1* anomeric protons of pullulan moieties at 5.6 and 4.98 ppm. It was found = 0.4.

After quaternization of this pullulan derivative with MeI and its change in chloride form (Fig. 2B), nine quaternary ammonium protons appear at 3.14 ppm. Comparing of this peak integral with that of the methylene proton signal at 2.05 ppm indicates 100% quaternization. Fig. 2C shows the ^{1}H-NMR spectrum of the ~ 97% benzylated DMAPA-P. In this case the degree of quaternization was calculated by comparing the peak integral for the five aromatic protons at 7.5 ppm with that of the six dimethylamino protons at 3.1 ppm.

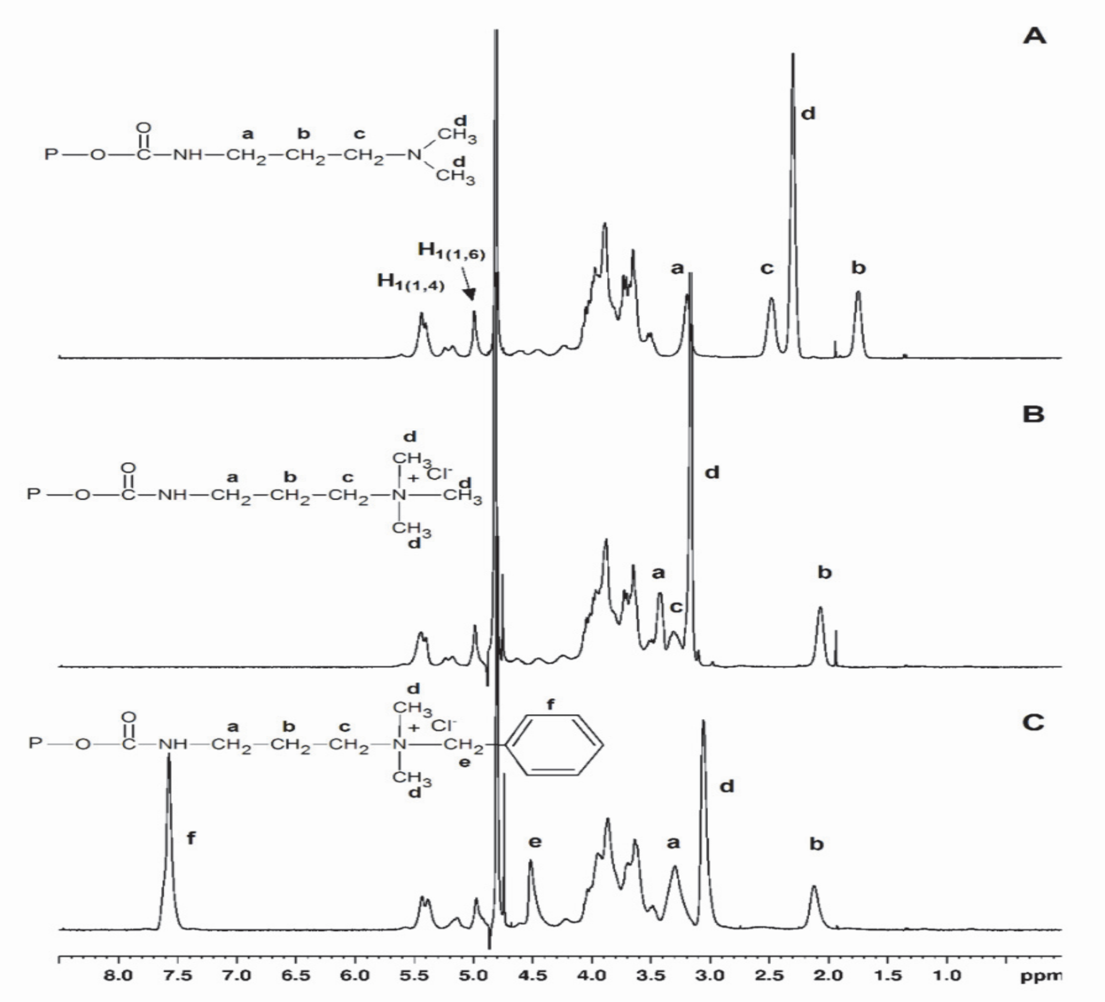

Fig. 2. ^{1}H-NMR spectra of DMAPA-P (A), TMAPA-P (B) and BzDMAPA-P (C) in D_2O

4.2. Laser light scattering and refractometric data

Usually, the characterization of polysaccharide solution was not realized in pure solvent, but with salt adding, thus allowing a more expanded conformation for macromolecular chains. The presence of salt in solution weakens the interaction between charged regions of same macromolecular chain or hinders the autoassociation phenomena.

Preliminary studies on these pullulan derivatives revealed negative A_2 values in water and lower positive values of A_2 in 0.1 M salted solutions. To improve interactions between polysaccharide chains and solvent molecules, all polymers were dissolved in solutions with 0.5 M salt, consequently.

As solvents for light scattering and refractometric measurements, the following solvent systems were selected: 0.5 M aqueous solution of sodium chloride (NaCl), 0.5 M aqueous solution of potassium chloride (KCl), and dimethylsulfoxide (DMSO) containing 0.5 M lithium chloride (LiCl). The influence of these three univalent salts (NaCl, KCl and LiCl) on macromolecular solution properties ($\overline{M}_w$, R_g and A_2) were analyzed. The resulted parameters for polysaccharide samples were summarized in Table 2, together with refractive index increment values.

The lower values of *dn/dc* for pullulan derivatives studied in DMSO compared with those values for pullulan derivatives dissolved in aqueous solutions, generally were characteristic to polysaccharides.

The positive values of second virial coefficient A_2 for all polymers dissolved in protic/aprotic polar solvent with 0.5 M salt indicated the presence of favorable polymer-solvent interactions. This salt concentration was sufficient to reduce the macromolecular interchain associations and to ensure an efficient screening of positive ammonium groups. Regarding to molecular masses of studied compounds it was observed that in any solvent, the $\overline{M}_w$ values for quaternary ammonium salts (TMAPA-P and BzDMAPA-P) increased compared with them precursor (DMAPA-P). This result sustained the ^{1}H-NMR spectroscopy data, meaning that the quaternization of tertiary amine has been successful, and increasing of molecular weight of quaternized compounds was in agreement with the increasing of molecular size of substituent (methyl or benzyl).

Some authors agree with the idea that the accuracy of R_g and final data of laser light scattering measurements were highly dependent on extrapolation of Zimm plots to zero θ and on fitting degree (first order or second order) (Harding et al., (1991); Burchard, (2008); Podzimek, (2011)). Generally, in case of our compounds dissolved in aqueous solutions, the values for R_g extracted from MALLS measurements were bigger in solutions with NaCl addition due to the fact the sodium chloride molecules more easily penetrated between polymer chains than KCl (a salt with a bigger crystal radius, but a smaller hydration capacity), and thus ensured the development of biggest associations for these polymers. Such low values of R_g for high molar mass polysaccharides in KCl aqueous solutions indicated a highly compact conformation in solution. Moreover, even if the $\overline{M}_w$ values recorded in 0.5 M NaCl aqueous solutions for BzDMAPA-P were bigger than for TMAPA-P, the radius of gyration R_g had almost the same value for both polymers. It was expected that BzDMAPA-P developed a bigger macromolecular coil compared with the other quaternary ammonium salts, but the bulky benzyl groups induced in some extent a hydrophobic character to this pullulan derivative and diminished the grade of extension for this polysaccharide. Thus, BzDMAPA-P presented an amphiphilic behavior compared with hydrophilic TMAPA-P.

Table 2. Refractometric and laser light scattering data for pullulan derivatives

Sample	***Solvent***	***dn/dc* (*mL g^{-1}*)**	**R_g (*nm*)**	**$\overline{M}_w$ (*g mol^{-1}*)**	**A_2 (*mol mL g^{-2}*)**
DMAPA-P	0.5 M NaCl aq.sol.	0.177	63.9	3.795 E+5	19.23E-4
	0.5 M KCl aq.sol.	0.133	44.1	3.808 E+5	3.838 E-4
	DMSO/0.5M LiCl	0.031	ND	5.384 E+5	4.580 E-4
TMAPA-P	0.5 M NaCl aq.sol.	0.112	78.1	7.467 E+5	1.089 E-4
	0.5 M KCl aq.sol.	0.105	71.6	5.304 E+5	1.451 E-4
	DMSO/0.5M LiCl	0.035	ND	5.942 E+5	2.517 E-4
BzDMAPA-P	0.5 M NaCl aq.sol.	0.170	77.2	1.823 E+6	7.949 E-4
	0.5 M KCl aq.sol.	0.139	ND	2.003 E+6	6.275 E-4
	DMSO/0.5M LiCl	0.032	ND	8.036 E+5	9.343 E-5

ND = not detected

The shape of Zimm plots depended on the adopted conformation of polymer in each solvent (Grigoras et al., 2013). For polymers in salted aqueous solutions, the Zimm plots (Berry formalism) with first order polynomial fit were linear and characteristic to flexible chains and quasimonodisperse samples. In case of DMSO with added LiCl, the polysaccharides adopted a spherical coil conformation in solution due to lack of involvement of the polar aprotic solvent in hydrogen bonding, compared with water (a protic polar solvent). For instance, the Zimm plots (Berry formalism) for BzDMAPA-P were presented in Fig. 3.

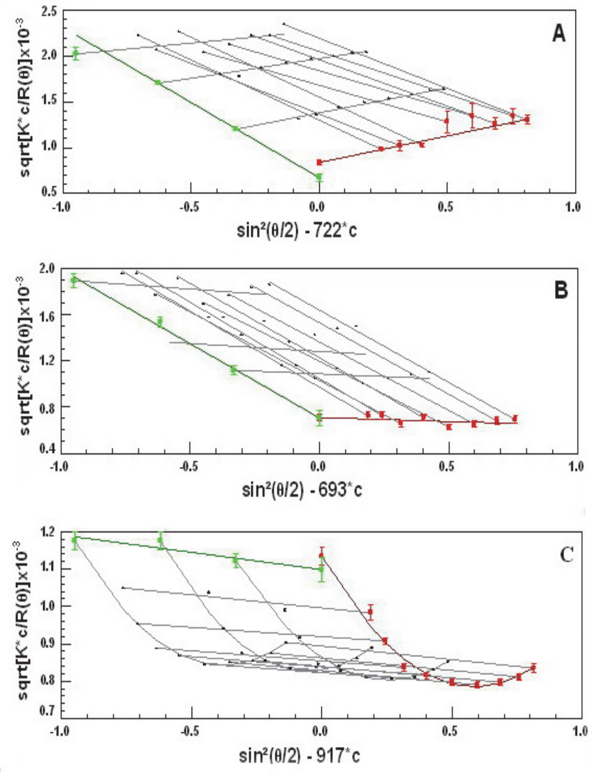

Fig. 3. Zimm plots (Berry formalism) for BzDMAPA-P in 0.5M NaCl aq.sol. (A),0.5M KCl aq.sol. (B) and DMSO/0.5M LiCl (C)

4.3. Turbidity data

Bentonite is composed of numerous tiny platelets negatively charged on their planed surfaces or positively charged on their edges. Bentonite swells in water like a porous sponge allowing electrical attraction and seizure of oppositely charged impurities in its cavities. There is a difference between *adsorption* and *absorption* characteristics of bentonite. The *adsorption* phenomenon consists in an exchange of ions, usually between positive ions of toxins, viruses, bacteria, fungus, parasites, heavy metals or pesticides, and negative ions of the outer surface of the clay molecule such that the ionic bonds which surround the clay molecule edges are satisfied (Lipson and Stotzky, 1985). By *absorption*, the clay molecule draws other foreign substances into its internal structure after it has undergone a chemical change, allowing such substances then to enter into clay's molecular inner structure therefore, acting like a sponge. As the foreign substances are absorbed, the clay expands filling the spaces between the clay molecule's stacked layers. Absorbent clay will only absorb positively charged impurities and ignore negatively charged ones (Jelinek et al., 2014; Mohammadi et al., 2015).

There is a series of models or mechanisms proposed to explain the flocculation process between charged polymers and different contaminant particles. According to *electrostatic patch mechanism*, the charges from on a polyelectrolyte type substance firstly self-organize in the form of a "patch" and then will be surrounded by the areas of opposite charges. In the second step, different particles bearing oppositely charged areas will be adsorbed and interact by strong attraction with those "patched" islands. *Singh's Easy Approachability Model* states that dangling branches of ionic moiety have easy approachability when they are grafted onto the rigid polysaccharide bone and more is the branched base polysaccharide, the ionic moiety chain grafted onto polysaccharide has more opportunity to form aggregates of the contaminants. So, hyperbranched polysaccharides provide the best flocculation characteristics. *Brostov, Paul and Singh's flocculation model* assume a connection between radius of gyration R_g of macromolecules in solution and settling velocity of particles out of suspension. A good polymeric flocculant should have higher R_g. In this way, an effective flocculant fills large volumes of liquid medium exceeding by far the dimensions of that chain (Sanghi and Singh, 2012).

The stability of bentonite suspensions with c = 1 mg mL^{-1}, both in the absence and presence of polymers, with and without NaCl, was followed during 3 hours (Fig. 4). In the absence of polymer, the turbidity values are almost constant (removal turbidity = 84 % without salt and 82 % with NaCl), signifying that the bentonite suspension is stable.

It was noted that the turbidity of the unsalted bentonite suspensions in the presence of DMAPA-P and BzDMAPA-P increased compared with that of the initial bentonite suspensions (Fig. 4A). This effect may be due to the polymer chains which are adsorbed on the outer surface of the clay molecule but not adhered to inorganic particles to initiate their settling process. For the more hydrophilic polymer (TMAPA-P) with a more expanded coil conformation, the turbidity decrease continuous until 120 min, then an almost constant turbidity value was observed. The removal capacity (%) for this polymer after 3 hours was 34% (Fig. 4C). Instead, in the presence of NaCl, the settling capacity of all polysaccharides was improved (Fig. 4B), the best flocculant being the one with the biggest radius of gyration R_g (TMAPA-P). Once again, the more extended coil conformation of polymer in a thermodynamically "good" solvent/medium represented an important factor that favors interactions between clay platelets and polymer chains.

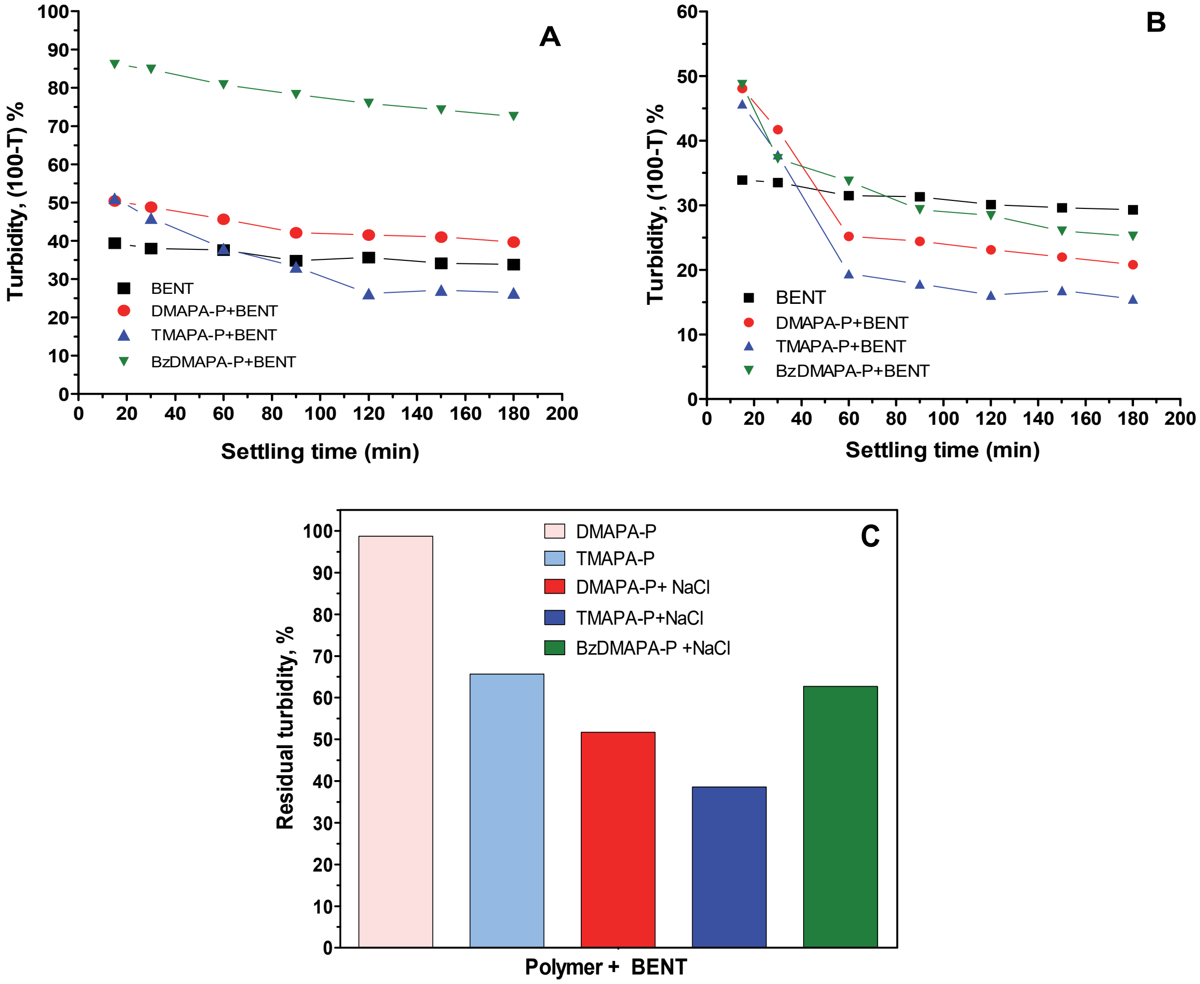

Fig. 4. Turbidity data dependence on the settling time in aqueous solutions without (A) and with 0.5M NaCl (B). Residual turbidity after 180 min as function on the polymer/BENT mixture (C)

Fig. 4C shows the residual turbidity after 3 hours for all polymer derivatives studied. It is obvious that the addition of polymers and NaCl led to a decrease of the residual turbidity in the investigated interval. The more pronounced decrease of the residual turbidity was recorded for TMAPA-P (38.55 %) in the presence of salt. None of above-mentioned flocculation models fails to explain in a unitary way the behavior of the three polymers. In all these aqueous systems containing negatively charged inorganic particle, charged polymer, with or without low molecular weight salt, a competition between adsorption, charge neutralization, and hydrophobic interactions exists. To find details about flocculation mechanism (by charge neutralization, bringing or electrostatic patch), stability and size distribution of flocs, optimum dose of flocculants and windows of flocculation, a future study will be developed.

5. Conclusions

1. The physico-chemical properties of pullulan derivatives depended on low molecular salt type used to dissolution and the solvent type.

2. All tested solvents with 0.5 M added salt were thermodynamically „goods" for pullulan derivatives as A_2 positive values revealed.

3. Using an appropriate polynomial fit for plotting the Zimm graphs, determination of real solution parameters was allowed. Also, the shape of Zimm plots were related with the state of macromolecular chains in solution.

4. According to preliminary turbidity tests, the new pullulan derivatives showed good flocculation potential in water treatment, and the presence of NaCl improved their removal capacity.

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