Carbohydrate Polymers 113 (2014) 217–224

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Formation of polyelectrolyte complexes with diethylaminoethyl dextran: Charge ratio and molar mass effect

Didier Le Cerf[a,b,c], Anne Sophie Pepin[a,b,c], Pape Momar Niang[a,b,c], Mariana Cristea[d], Carole Karakasyan-Dia[a,b,c], Luc Picton[a,b,c,*]

[a] Normandie Université, France
[b] Université de Rouen, Laboratoire 'Polymères, Biopolymères, Surfaces', F-76821 Mont Saint Aignan, France
[c] CNRS UMR 6270 & FR3038, F-76821 Mont Saint Aignan, France
[d] "Petru Poni" Institute of Macromolecular Chemistry, Al. Gr., Ghica Voda 41A, 700487 Iasi, Romania

ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form 2 July 2014
Accepted 3 July 2014
Available online 17 July 2014

Keywords:
Polyelectrolyte complexes
DEAE Dextran
ITC
Polysaccharide

SUMMARY

The formation of polyelectrolyte complexes (PECs) between carboxymethyl pullulan and DEAE Dextran, was investigated, in dilute solution, with emphasis on the effect of charge density (molar ratio or pH) and molar masses. Electrophoretic mobility measurements have evidenced that insoluble PECs (neutral electrophoretic mobility) occurs for charge ratio between 0.6 (excess of polycation) and 1 (stoichiometry usual value) according to the pH. This atypical result is explained by the inaccessibility of some permanent cationic charge when screened by pH dependant cationic ones (due to the Hoffman alkylation). Isothermal titration calorimetry (ITC) indicates an endothermic formation of PEC with a binding constant around 10^5 L mol^{-1}. Finally asymmetrical flow field flow fractionation coupled on line with static multi angle light scattering (AF4/MALS) evidences soluble PECs with very large average molar masses and size around 100 nm, in agreement with scrambled eggs multi-association between various polyelectrolyte chains.

1. Introduction

Polyelectrolytes of opposite charges i.e. a polyanion (PA) and a polycation (PC) interact by means of Coulomb forces and form polyelectrolyte complexes (PECs). The formation can take place at interfaces or in bulk (Kabanov, 2005). They can be used as membranes, microparticles and/or coating in relation with biological systems (hydrophilicity, biocompatibility and permeability). Complexes between DNA and natural or synthetic PC are actually used for the cell transfection in gene therapy (Lankalapalli & Kolapalli, 2009; Ma, Lavertu, Winnik, & Buschmann, 2009; Dakhara & Anajwala, 2010).

The structure and stability of PECs strongly depend on parameters such as polyelectrolyte concentration, charge molar ratio, charge density, molar masses and chain flexibility but depend also on extrinsic parameters as pH, ionic strength and/or temperature (Kabanov, 2005). Other parameter which is very rarely discussed in literature is the position of the charged groups on the polymer chain. It can be on the backbone or on pendant branches. The accessibility of the opposite charge could be different and affected by steric hindrance. Some studies report different type of PECs structure (i.e. 'ladder' or 'scrambled eggs') according to the mode of preparation (Philipp, Dautzenberg, Linow, Kötz, & Dawydoff, 1989).

According to all these parameters, mixing oppositely charged polyelectrolytes gives two types of PECs that are mainly controlled by the charge stoichiometry: (i) soluble PECs leading to stable and transparent solutions, (ii) insoluble PECs with or without precipitation. The soluble PECs are obtained with an excess of anionic or cationic charges. On the contrary, with an equal quantity of each charged group, the total charge is zero and generally leads to a macroscopic phase separation (Feng, Leduc, & Pelton, 2008).

PECs are usually studied with suitable experimental techniques. One can quote for example optical density measurements (Leclercq, Boustta, & Vert, 2003), static and dynamic light scattering (Dautzenberg & Jaeger, 2002; Mihai, Dragan, Schwarz, & Janke, 2007), electrophoretic mobility (Ma et al., 2009), viscosity (Vishalakshi, Ghosh, & Kalpagam, 1993; Tennouga, Medjahed, Mansri, & Desbrières, 2013), atomic force microscopy (Maurstad, Kitamura, & Stokke, 2012), flow field flow fractionation coupled on line with static light scattering (Le Cerf, Simon, Argillier, & Picton, 2007). The goal of such studies is generally to investigate the structure of PECs and their stability in various environmental conditions.

* Corresponding author at: Université de Rouen, Laboratoire 'Polymères, Biopolymères, Surfaces', F-76821 Mont Saint Aignan, France. Tel.: +33 235146543; fax: +33 235146704.
E-mail addresses: didier.le_cerf@univ-rouen.fr (D. Le Cerf), annesophiepepin@hotmail.fr (A.S. Pepin), papemomar.niang@etu.univ-rouen.fr (P.M. Niang), mcristea@icmpp.ro (M. Cristea), carole.karakasyan-dia@univ-rouen.fr (C. Karakasyan-Dia), luc.picton@univ-rouen.fr (L. Picton).

http://dx.doi.org/10.1016/j.carbpol.2014.07.015
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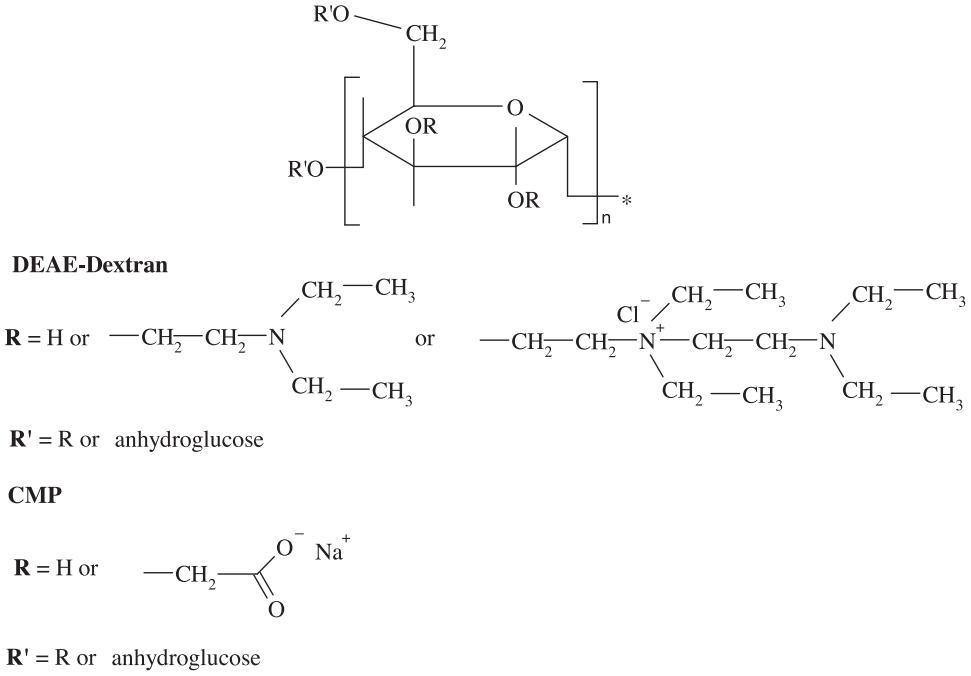

Fig. 1. Anhydroglucose substitution of DEAE-dextran and CMP.

Diethylaminoethyl dextran (DEAE Dex) is a biocompatible polymer with pharmacological and therapeutics properties. In particular, it has the capacity to form PECs by ionic interaction with proteins or DNA in order to enhance their uptake by cells. Consequently, it is an effective agent of transfection (Puchalski & Fah, 1992; Schenborn & Goiffon, 2000; Smale, 2010). Other applications are also reported for DEAE Dex based PECs, such as drug release (Delair, 2011; Abbah, Liu, Lam, Goh, & Wong, 2012) and enzyme stability (Gavalas & Chaniotakis, 2000).

In the literature the formation of PEC between DEAE Dex and DNA is not studied in terms of structure and stoichiometry. The researchers focused only on biological activities. We have found very few works based on the PEC physicochemistry between DEAE Dex and anionic polymer. Miyazaki et al. have mixed DEAE Dex and carboxymethyl dextran and formed microspheres by emulsion-solvent evaporation method (Miyazaki, Yakou, Nagai, & Takayama, 2003). Theophylline as model drug was entrapped and its released was studied as a function of pH. Elaboration of insoluble PECs based on DEAE Dex and CarboxymethylCellulose (Kikuchi & Koda, 1979) or a mixed of dextran sulfate and poly(styrenesulfonate) (Kikuchi & Sasayama, 1982) are also reported.

In the present article, we wish to bring structural information on the formation of PECs with DEAE Dex for medical scientific community. PECs were prepared from DEAE Dex and an anionic polysaccharide, carboxymethyl pullulan (CMP) with different charge ratios and molecular weights in medium with controlled ionic strength and pH. The PECs were studied by optical density measurements, dynamic light scattering, electrophoretic mobility, isothermal titration calorimetry and asymmetrical flow field flow fractionation coupled on line with multi angle static light scattering and refractometry (AsF4/MALS/DRI).

2. Materials and methods

2.1. Materials

The Scheme of CMP and DEAE Dex is given on Fig. 1.

CMP was synthesized in isopropanol/water medium (66/33, V/V) at 70 °C by the addition of sodium chloroacetate in the presence of sodium hydroxide on pullulan (Dulong, Le Cerf, Picton, & Muller, 2006). The obtained CMP was purified by dialysis (cut off 40,000 g mol^{-1}) and lyophilized. The degree of substitution (DS) was obtained by conductimetry measurements (Eyler, Klug, & Diephius, 1947). It represents the carboxyl group number per anhydroglucoside unit (AGU) and in our case it was equal to 0.94. The p*K*a is around 4.

To study the influence of molecular weight, we have degraded the CMP sample by ultrasonic way (Vibra Cell, Sonics and Materials). The condition was 2 g of CMP in 200 mL of water, 20 W for 6 h.

The Average molecular weights and the gyration radii are obtained by size exclusion chromatography (SEC) (columns OHPAC 806 –804 Shodex) with on line multiangle light scattering (MALS) and differential refractometry index (DRI). The results are summarized in Table 1.

DEAE dextran was purchased from Sigma-Aldrich. It is obtained by the reaction of 2-chloro-*N*,*N*-diethylaminoethane on dextran. As previously reported, the structure is more complex due to the possible reaction of an other 2-chloro-*N*,*N*-diethylaminoethane on DEAE dextran (Souguir, Roudesli, Picton, Le Cerf, & About-Jaudet, 2007a; Souguir, Roudesli, Le Cerf, About-Jaudet, Picton, 2007b; Demirbilek & Dinç, 2012). Consequently three basic groups are present with different p*K*a values (Heinze, Liebert, Heublein, & Hornig, 2006). One group is 2-*N*,*N*-diethylaminoethyl. Two other groups are on 2-*N*,*N*-diethylaminoethyl diethylaminonium ethyl due to the Hoffman alkylation. Only the last basic group is not pH dependant. The quantification of each amino group was realized by conductimetric measurements and nitrogen elementary analysis (Souguir et al., 2007a,b). The first method permits to obtain only the quantity of tertiary amino group without distinction between the two p*K*as. Determination of nitrogen percentage on the chemical structure gives the number of both tertiary and quaternary amino groups per anhydroglucose unit. The studied DEAE Dex has presented a degree of substitution of tertiary amino group (DS_N) equal to 0.29 and a degree of substitution of quaternary amino group (DS_{N+}) equal to 0.26.

Table 1
Macromolecular characteristics of used polysaccharides.

	M_n (g mol^{-1})	M_w (g mol^{-1})	PDI	Rg_w (nm)
CMP	140,000	230,000	1.7	35
CMP LW	27,000	43,000	1.6	<20*
DEAE Dex	350,000	970,000	2.8	30
DEAE Dex LW	70,000	100,000	1.4	25

Where M_n and M_w are the average number and weight molar masses, respectively, PDI is the polydispersity index and Rg_w is the average weight gyration radius.
* Indicating the limit of anisotropic scattering.

As for CMP, a DEAE Dex solution (2 g in 200 mL of water, 20 W for 4 h) has been sonicated with the aim to obtain a low molar mass sample.

Acetic acid and sodium acetate, ammonia and ammonium chloride, hydrochloric acid, sodium hydroxide and sodium chloride were purchased from Acros Organics. All compounds were used without any further purification and for all the experiments Milli-Q water (Millipore) was used as solvent at fixed pH and ionic strength.

2.2. Methods

2.2.1. Electrophoretic mobility measurements

Electrophoretic mobility measurements were carried out with a Zetasizer ZS (Malvern Instruments) at 25 °C in special cell (DTS 1060 disposable zeta cell, Malvern instruments). The zeta potential values were calculated using the Smoluchowski approximation.

2.2.2. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) was made using a Nano ITC 2G microcalorimeter from TA Instruments (Dv, USA) as previously reported for PEC titrations (Vieira, Cestari, Airoldi, & Loh, 2008; Ma et al., 2009; Maurstad et al., 2012). The sample cell (1040 μL) was filled with an acetic acid buffered solution of PA or PC. A solution of the opposite polyelectrolyte (PC or PA) in the same buffer was placed in a 200 μL syringe fitted with a helix that ensure a continuous stirring (300 rpm) in the cell. 20 consecutive aliquots of 10 μL were injected into the cell with intervals of 300 s. All measurements were performed at 25 °C. Heats of dilution/mixing were determined by injecting aliquots (10 μL) of polyelectrolyte buffer solution into buffer solution without polyelectrolyte. Data analysis was carried out using the ITC Run 1.4.0 Software™ (TA Instruments Dv, USA). For each system, 3 measurements were performed with a good repeatability.

2.2.3. AsF4/MALS/DRI

The average molar masses (M_n and M_w) and sizes (gyration radii (R_g) and hydrodynamic radii (R_h)) were determined by Asymmetrical Flow Field Flow Fractionation (AsF4) coupled on line with Multi Angle Light Scattering (MALS) and Differential Refractometer Index (DRI) (Le Cerf et al., 2007). The system is composed of a degasser (DGU 20A3, Shimadzu, Japan), a pump (LC10Ai, Shimadzu, Japan), an AsF4 (Eclipse 3, Wyatt Technology, CA, USA), a MALS (EOS, Wyatt Technology (CA, USA) and DRI (RID10A Shimadzu, Japan). The eluent (0.05 mol L^{-1} sodium acetate pH 4.5) has been filtered through 0.1 μm filter (Millipore, USA). 100 μL of a polymer solution at 0.5 g L^{-1} filtrated on 8 μm is injected. The linear flow was maintained at 1 mL min^{-1} during all the analysis. The cross-flow was equal to 1.6 mL min^{-1} during 6 min then suddenly decreased at 1.2 mL min^{-1} for 3 min (relaxation) following by linear decrease from 1.2 to 0 mL min^{-1} during 30 min. To ensure the end of the analysis, no cross-flow was applied during 3 min. Data acquisition and macromolecular characterization determinations were performed using the software Astra V5.4.1. Refractive index increment (dn/dC) of both polysaccharides was 0.150 mL g^{-1}.

3. Results and discussion

3.1. PEC formation

The choice of pH is important as it determines the charge density of each polyelectrolyte. The neutralization of the two polyelectrolytes was followed by pH measurements. These assays give the charge ratio per anhydroglucose unit (α) as a function of pH (Fig. 2) and shows that at pH 4.5, both PA and PC evidence the same ionization rate (IR = 0.55). Consequently, we decided to study the PEC in acetate buffer (0.05 mol L^{-1}) at pH 4.5.

Three total polymer concentrations (C_p) have been studied: 0.5; 2 and 5 g L^{-1}. The quantity of PA and PC is given by the weight ratio or by the molar ratio, but also by the charge ratio CR.

$$\mathrm{CR} = \frac{n-}{n+} = \frac{[(m_{\mathrm{CMP}}/Mo_{\mathrm{CMP}})\alpha_{\mathrm{CMP}}]}{[(m_{\mathrm{DEAEDex}}/Mo_{\mathrm{DEAEDex}})\alpha_{\mathrm{DEAEDex}}]}$$

where m is the polysaccharide weight and Mo the molecular weight of the structural unit.

Several options are possible to form PECs: (i) polyanion in polycation or polycation in polyanion, (ii) successive additions of low quantity or single addition (one shot).

Successive additions method is known to form a ladder structure with fixed ionic cross-links (Philipp et al., 1989). In our case, we have fixed the equilibrium time between additions at 1 hour with a soft screening (150 rpm) for all measurements excepted for ITC (300 rpm).

One shot method gives a more chaotic scrambled-egg structure with statistical charge compensation (Michaels & Miekka, 1961). In this study, analyses were realized 24 h after mixing.

In a first approach, a set of PECs was elaborated by one shot method. The obtained solutions were photographed in Fig. 3.

With a high excess of charges (CR = 0.2 or 5), the PECs appear soluble. Curiously insoluble PECs (evidenced on the photo by a clear solution in which invisible solid particles are fallen down at the bottom of the vial meaning a precipitation) are obtained with CR of about 0.6 and not for the charge stoichiometry as usually observed. With lower or higher CR than 0.6, PECs are insoluble without precipitation and form colloidal system. This value of 0.6 is surprising and is not in accordance with many studies (Philipp et al., 1989; Wang, Kimura, Huang, Dubin, & Jaeger 1999; Dautzenberg & Jaeger, 2002; Drogoz, David, Rochas, Domard, & Delair, 2007 Chiappisi, Hoffmann, & Gradzielski, 2013) where precipitation is obtained with a CR = 1. This atypical behaviour must be confirmed by other technical measurements, whatever the method of preparation.

We have performed electrophoretic mobility measurements of PECs with different charge ratio and according to the two methods of preparation i.e. successive addition and one-shot (Fig. 4).

It appears that electrophoretic mobilities motilities data do not depend on the elaboration protocol. For CR lower than 0.5, the surface charge is globally positive and electrostatic repulsions between such positive charges stabilize the colloidal system. The same is obtained when CR is higher than 0.7 with a negative global charge. Between these two behaviours, we observed a neutral charge for CR about 0.6 according to the visual determination of the PEC

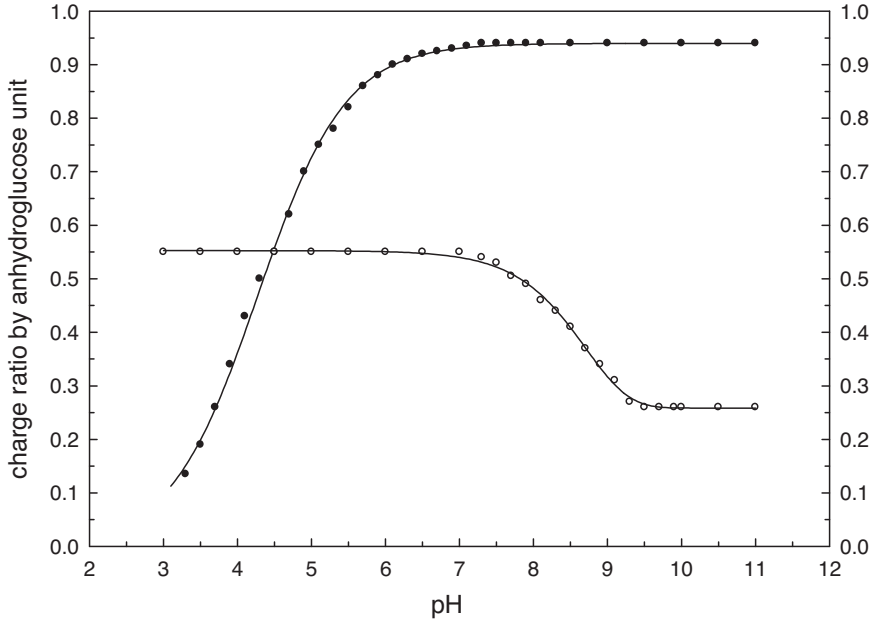

Fig. 2. Evolution of charge ratio by anhydroglucose unit for CMP (black point) and dextran (white point).

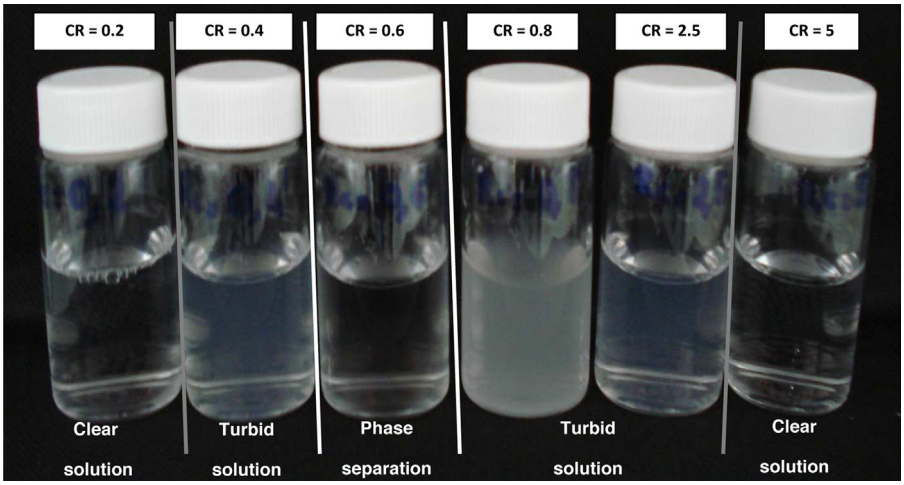

Fig. 3. Visual turbidity for PECs with different charge ratio (CR). C_p = 0.5 g L^{-1} in acetate buffer 0.05 M, pH 4.5. M_n CMP = 140,000 g mol^{-1}, M_n DEAE Dex = 350,000 g mol^{-1}.

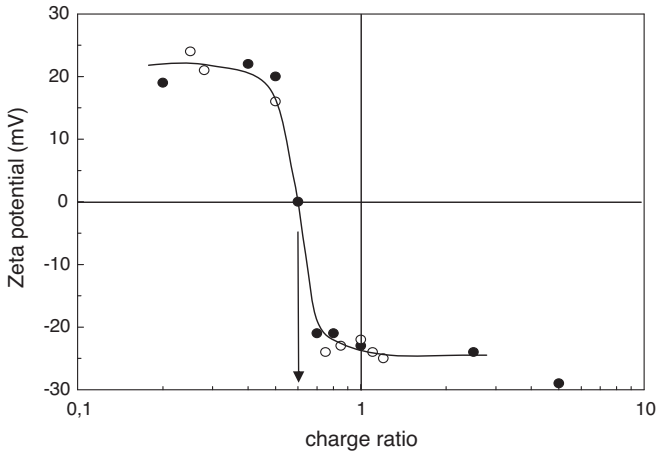

Fig. 4. Zeta potential of PECs obtained with different charge ratio for the two procedures: successive addition (white point) and one shot (black point).

precipitation. These data confirm that the phase precipitation does not occur at a CR = 1.

Isothermal titration calorimetry (ITC) is usually used to study PECs formation between polyelectrolytes via a thermodynamic approach (Vieira et al., 2008; Maurstad et al., 2012). In order to avoid potential enthalpic phenomena from the dilution, a blank has been monitored (injections of buffered solutions of PA (or PC) aliquots in buffer without the opposite polyelectrolyte) and subtracted to the complex formation measurement.

In Fig. 5A, we can see the heat signal for each injection according to the energy produced by ionic interaction (i.e. complex formation). When the quantities of PA and PC are suitably chosen, one can obtain a strong decrease of heat (that becomes equal to the heat of dilution/mixing) indicating that no more ionic interaction occurs. After integrating the heat as a function of the molar ratio between the two reactants for each injection, the integration curve give the stoichiometry of binding (Fig. 5B). Then, we can estimate the binding constant and the enthalpy of binding (expressed in kJ per mole of injectant), which were used in order to estimate the Gibbs free energy and the entropy of binding.

Table 2
ITC thermodynamical parameters at 25 °C. Ionization rate is 0.55 for both polymers pH = 4.5 acetate buffer 0.05 mol L^{-1}.

M_n PA (g mol^{-1})	M_n PC (g mol^{-1})	PA (mmol L^{-1})*	PC (mmol L^{-1})*	Log K_a**	$\Delta H°$ (kJ mol^{-1})	$\Delta G°$ (kJ mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})	CR Stoich
PA dropped in PC								
140,000	350,000	15.0	2.3	5.6	1.50	−31	110	0.54
140,000	70,000	15.0	2.4	5.5	1.37	−31	110	0.52
27,000	350,000	17.6	3.2	4.9	1.14	−29	100	0.52
27,000	70,000	17.6	3.3	4.9	1.09	−29	100	0.53
PC dropped in PA								
140,000	350,000	1.4	28.4	4.3	0.95	−26	90	0.66
140,000	70,000	1.4	28.2	4.3	0.74	−26	90	0.52
27,000	350,000	1.7	28.4	4.0	0.90	−23	80	0.68
27,000	70,000	1.7	28.2	4.0	0.69	−23	80	0.62

* Concentration in mmol of charge per L.
** K_a in L mol^{-1}.

Measurements were performed with PA or PC in syringe (Table 2, highlighting in gray). In both cases, the stoichiometry of binding is still around 0.6 as it was obtained from optical and electrophoretic mobility measurements. According to the nature of the injectant, we observe a slight difference between the thermodynamical data without explanation at this step of the paper. However, we can conclude that the enthalpy of binding is endothermic and the affinity is high regarding the values of K_a and Gibbs free energies. Finally the binding between the two polyelectrolytes can be considered as entropically driven by the release of counterions and the restructuring of water molecules around the charged sites during complexation (Ou & Muthukumar, 2006).

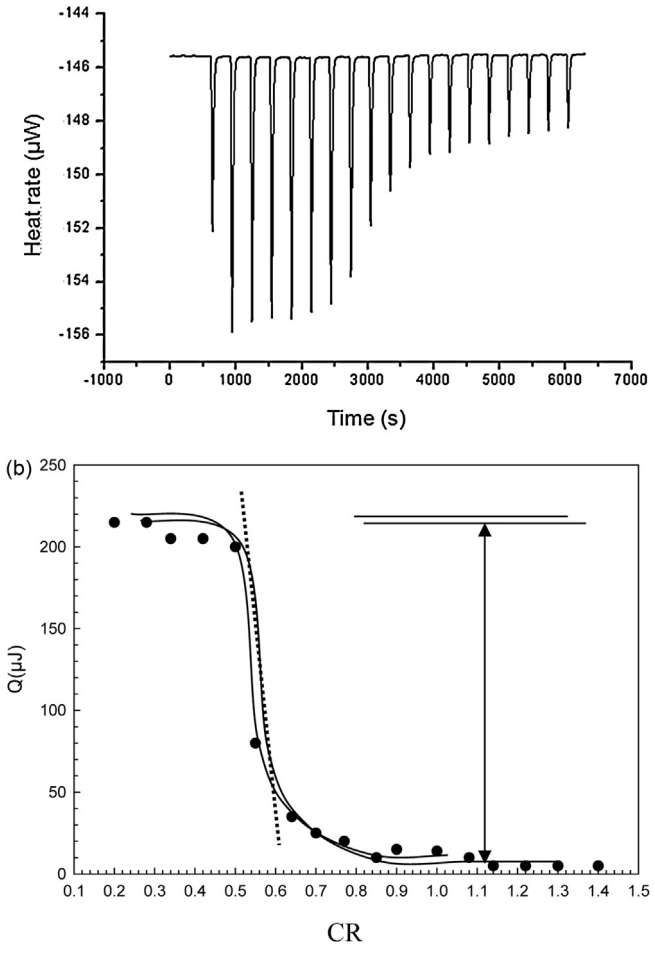

Fig. 5. Typical isothermal titration calorimetry data obtained for the binding interaction of PC (2.3 mM) in acetate buffer (0.05 mol L^{-1}) at pH 4.5 to a solution of PA at 15 mM in acetate buffer (0.05 mol L^{-1}) at pH 4.5 and 25 °C. (A) Shows endothermic heat releases upon injection of 10 mL aliquots of PC into PA solution. (B) Shows integrated heat data, giving a differential binding curve in function of CR.

3.2. Deviation of the 1:1 stoichiometric charge ratio

Such deviations from a 1:1 stoichiometric charge ratio have been previously observed (Dautzenberg & Jaeger, 2002; Philipp et al., 1989; Wang et al., 1999; Chiappisi et al., 2013). Many explanations could be exposed always based on accessibility considerations.

Concentrations in semi-dilute regime could have a negative effect on Coulombic interactions due to entanglement of chains (Vishalakshi et al., 1993). In our case, polymer concentrations are situated in dilute regime at 0.5 g L^{-1}. Other experiments were made at 2 and 5 g L^{-1} and we have obtained similar results with electrophoretic mobility measurements.

A great difference in the molecular dimensions between the two polyelectrolytes has also been reported to explain a deviation from 1:1 stoichiometry (Vishalakshi et al., 1993; Schwarz et al., 2006). To test this hypothesis, the study by ITC was continued with samples obtained by ultrasonic degradation (Table 2). No strong differences have been reported when changing the molar masses of the polyelectrolytes. Only slight differences according to the nature of the injectant have been confirmed.

It has been shown that if one of the polyelectrolyte shows an aggregation tendency, a deviation from 1:1 stoichiometry towards a lower charge ratio is observed. This result was obtained between chitosan and carrageenan that are effectively well known for their aggregation capacity (Hugerth, Caram-Lelham, & Sundelöf, 1997). Therefore this explanation cannot be proposed in our case because CMP and DEAE Dex evidence flexible backbone without aggregation (Souguir et al., 2007a,b; Dulong, Mocanu, Picton, & Le Cerf, 2012). A difference of the repartition of charges on the polymer backbone between the oppositely charges polyelectrolytes could be another explanation but the chain flexibility must minimize this effect.

The last possibility is an overestimation of charge number able to interact with an opposite charge. This hypothesis may be envisaged when considering the peculiar chemical structure of DEAE Dex due to Hoffman reaction (Souguir et al., 2007a,b; Demirbilek & Dinç, 2012). As presented above (Fig. 1), two charges can be found on the same sugar. In this case, the first one is a tertiary amino group, which is pH dependent, and the second is a quaternary amino group, covered by the first one. Our hypothesis considers the difficult accessibility of the quaternary cationic amino group when covered by the cationic tertiary amino group. To check this idea, we have conducted electrophoretic mobility measurements at different pH (mainly towards alkaline conditions) in order to neutralize the tertiary amino group to free the quaternary amino group and perhaps obtain the stoichiometry of binding. This has been done and the Fig. 6 shows the evolution of CR at the precipitation as a function of the pH.

Table 3
Macromolecular characteristics of PECs via asymetrical F4/MALS/DRI.

M_n of CMP (g mol^{-1})	M_n of DEAE Dex (g mol^{-1})	Polyelectrolyte in excess		PECs	
		M_n (g mol^{-1})	M_w (g mol^{-1})	M_n (g mol^{-1})	M_w (g mol^{-1})
140,000	350,000	480,000	700,000	6 700,000	8 300,000
140,000	70,000	/	/	330,000	780,000
27,000	350,000	230,000	390,000	3 700,000	6 400,000
27,000	70,000	/	/	200,000	430,000

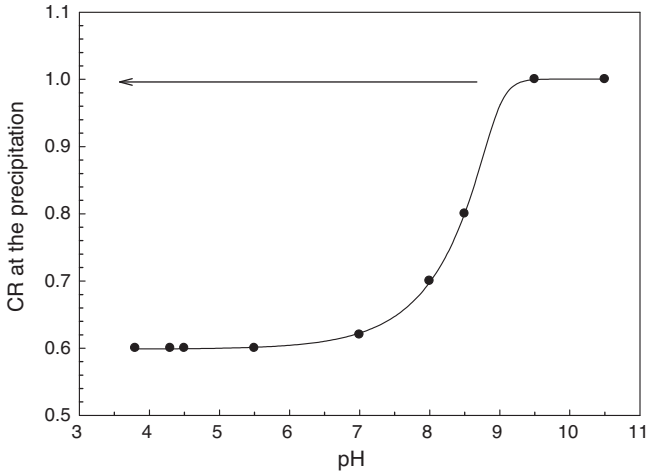

Fig. 6. Evolution of the CR at the precipitation obtained by electrophoretic mobility measurements in function of pH. C_p = 0.5 g L^{-1} in acetate buffer 0.05 M, pH 4.5. M_n CMP = 140,000 g mol^{-1}, M_n DEAE Dex = 350,000 g mol^{-1}.

If it remains constant up to pH 7, we observe an increase until pH 9.5 reaching the value of 1. This result clearly indicates that when the accessibility of quaternary amino group is liberated, the behaviour becomes consistent with the majority of the systems studied in the literature. New calculus without consideration of the Hoffman alkylation give CR = 1 for insoluble electroneutral PECs whatever the pH. Consequently, we can conclude that the quaternary amino groups are not accessible to form Coulomb interactions with anionic charges when tertiary amino group are protonated because of steric hindrance and/or great proximity of the two cationic charges. This result has been fully confirm by ITC measurement with the titration of the PC (M_n = 70,000 g/mol^{-1}) by the PA (M_n = 27,000 g mol^{-1}) in pH9.5 buffer which has given a stoichiometric ratio of 1.1 and a K_a of 6.104 (largely higher than that obtained at pH 4.5).

Now, one can return to the slight difference observed in the thermodynamical data (Table 2) according to the nature of the injectant, i.e. 'PA dropped in PC' or 'PC dropped in PA'. Enthalpic data are related to the molar concentration of charges. When PA (injectant) is dropped in PC all anionic charges effectively react with cationic charges and the enthalpy can be considered as correct. Now, when PC (injectant) is dropped into PA, and regarding our previous conclusion, only the part of accessible charges (i.e. tertiary ammonium) can react with the anionic charges of PA. So if we correct the enthalpy for systems 'PC dropped in PA' by the ratio tertiary ammonium/global ammonium (about 0.5), the recalculated data becomes very near from that we have obtained for the system 'PA dropped in PC'. This seems to be new evidence that the accessibility of cationic charge can be involved in the observed behaviour.

3.3. Molecular weights of soluble PECs

Soluble PECs with a CR = 0.2 obtained with the 'one shot' method, were studied by AsF4/MALS/QELS/DRI. Elution profiles (both differential refractive index (DRI) and light scattering at 90° (LS) responses) together with the molecular weights distribution are shown in Fig. 7.

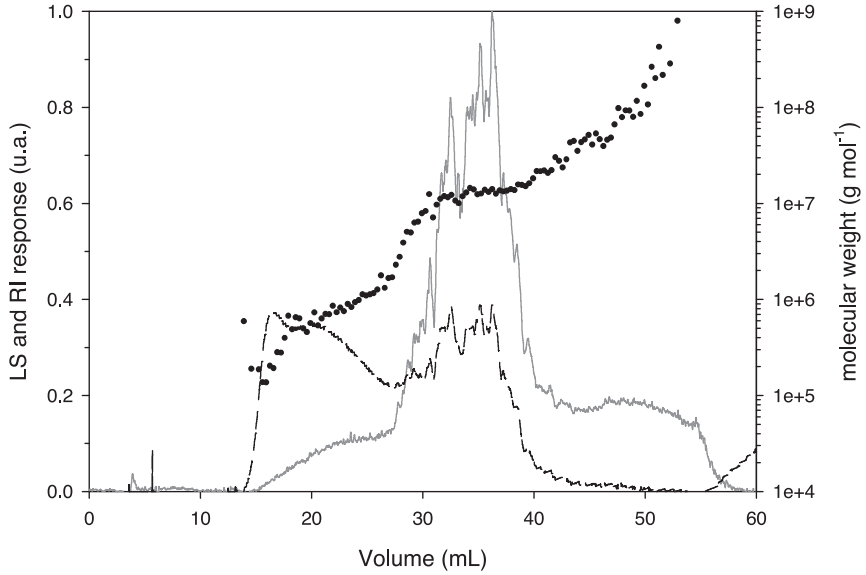

Fig. 7. Elution profiles from refractive index (black line) and light scattering at 90° (grey line) of PEC obtained with CR = 0.2 and C_p = 0.5 g L^{-1} in acetate buffer 0.05 M, pH 4.5. M_n CMP = 140,000 g mol^{-1}, M_n DEAE Dex = 350,000 g mol^{-1} together with molar mass distributions (●) determined by AsF4/MALS/DRI in $LiNO_3$ 0.1 mol L^{-1}.

It has been verified by comparison between the integration of the DRI response and the initial quantity of injected PECs (according to the loop volume and the concentration) that no loss of product occurs during the filtration step and/or in the AsF4 channel. The elution profiles can be divided into two parts. The first one (between 15 and 27 mL) evidences an important DRI response and a weak LS response. With regard to corresponding molecular weight, this peak should represent the uncomplexed PC (eventually some PECs with small size). The second one (between 27 and 56 mL) is characterized by a very important LS response which is the sign of very large molar masses, very probably due to PECs for which many macromolecular chains are associated in agreement with the "scrambled egg" model. The structure is relatively compact with an average gyration radius around 100 nm even when PECs are (Table 3).

To confirm these results, we have studied (via AsF4/MALS/QELS/DRI keeping the same elution method) the PECs obtained with polyelectrolytes elaborated from PA and PC which evidence the lower M_n (Table 3). Free polycations are visible only when they have an important molecular weight. We find again large molecular weights for PECs. They are the assembly of many chains with an excess of polycation.

4. Conclusions

We have used electrophoretic mobility and isothermal titration microcalorimetry to characterize the formation of DEAE Dex and CMP complexes in dilute aqueous solution. We found that the PECs are insoluble when the charge ratio is around 0.6 and not 1 as it is classically obtained. This mismatch is explained by the peculiar structure of DEAE Dex with two possible cationic charges (a quaternary ammonium and a tertiary ammonium) on the same anhydroglucose due to Hoffman addition. The quaternary ammonium becomes inaccessible when covered by cationic tertiary amino groups. By using the pH dependence of such tertiary ammino group, we have clearly evidenced that the 1:1 binding stoichiometry is reached when tertiary amino group are deprotonated in alkaline condition (i.e. above the p*K*a). ITC measurements have shown that the complexation is an endothermic process governed by entropic effect. Soluble cationic PECs evidence very large molar masses in agreement with the scrambled egg model.

These findings reveal important relationships of DEAE Dex structure for the formation of PEC between DEAE Dex and DNA and important guidelines in the design of gene transfection systems.

Acknowledgments

This research was supported by a Grant between CNRS (France) (Grant No. 23720) and Romanian Academy of Science. We thank the "French Ministère de l'Education Nationale, de la Recherche et de la Technologie" and the "Région Haute-Normandie" for financial support.

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