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ORIGINAL CONTRIBUTION

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New polycationic polynorbornene latexes bearing an amphiphilic sugar corona of counterions: synthesis and complexation with DNA

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Abstract A new family of polycationic polynorbornene with ω-gluconamidoalkanoates and ω-lactobionamidoalkanoates as amphiphilic counterions has been prepared. These polymers spontaneously form stable latexes in water with an amphiphilic sugar corona around the particles. With these counterions, the sugar moieties are separated from the surface of latex particles by the aminoalkanoic acid residue and consequently are remote from the surface of such latexes. Such location of sugar may promote their stronger interaction with cell receptors. These original amphiphilic compartmentalized surface polycationic latexes are able to bind and efficiently complex DNA in very small complexes. Furthermore, because of the effect of the sugar corona on the formation of the complexes, there is only a slight influence of the hydrophobicity of the counterions on DNA packing.

Keywords Polynorbornene · Latex · Amphiphilic counterion · DNA · Sugar

Introduction

Stable dispersions of polymeric microspheres in water are described as polymer colloids or latexes. Synthetic latexes or polymer latexes are generally produced by emulsion polymerization or copolymerization of alkenes in water, with or without a surfactant [1–4].

The main features of latexes are their small size and volume offering a large specific surface area available for chemical reactions or adsorption. Moreover, latexes solutions have high mobility and diffusibility resulting from low viscosity and high fluidity compared to solutions containing the same amount of solid. Latex particles in dispersion are stabilized in water by electrostatic or steric repulsive interactions. Nowadays, a large variety of functionalized and calibrated latexes are produced and were found to have applications in medicine and biotechnology [5, 6].

In a previous paper, we described an original way to obtain very small nanoparticles of polynorbornene latexes in water via an organometallic vinyl polymerization [7]. This method was then extended to various functionalized norbornenes (sugar, alcohol, and ammonium) [8] to prepare biosensors [9]. Not long ago, we pointed out the influence of the nature of the counterion on the aggregation properties of a new family of polycationic polynorbornenes [10–13]. These polymers are formed by reacting acetic acid or lactobionic acid with a neutralized ammonium chloride polynorbornene. They self-organize to give stable latexes in water to be able to complex DNA into very small aggregates because of the counterion effect on the hydrophobic packing of the norbornane units. In a number of cosmetic and drug delivery systems, surfactants and polymers were formulated together which lead to new functions that polymers alone could not achieve [14, 15]. Such aggregates are formed in water as a result of electrostatic, hydrogen bounding, or hydrophobic interactions between the polymer and the surfactant [16–18]. Moreover, many polymers [19, 20] or amphiphilic [21] compounds bearing sugar residues were used to interact with sugar-specific receptors on cell membranes, leading to an attractive method for application to gene transfer targeting strategies [19, 20]. The goal of this work was to design and synthesize a new family of polycationic polynorbornenes with different carboxylic acid sugar bolaamphiphilic counterions forming an amphiphilic corona around the latex particles (Fig. 1). In such organization, the sugar moieties are separated from the surface of latex particles by the aminoalkanoic acid residue and are consequently remote from the surface. In these conditions, interactions between the latexes and the cell receptors could

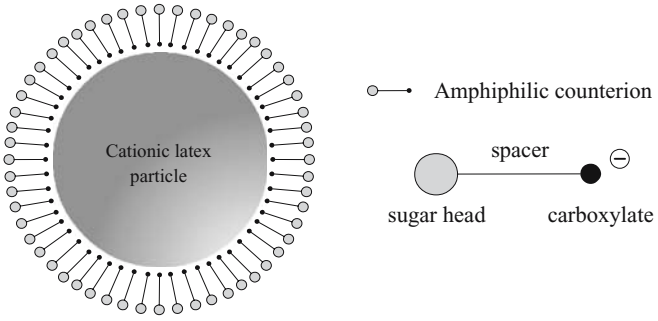

Fig. 1 Structure of a polynorbornene latex with an amphiphilic corona

be enhanced. Subsequently, these original amphiphilic compartmentalized surface polycationic latexes were complexed with DNA to study the influence of the hydrophobicity of the counterion on the DNA packing.

Experimental

Materials

All the solvents and chemicals were reagent grade and were used without further purification. Agarose and ethidium bromide (EtBr) were purchased from Sigma-Aldrich.

Synthesis of the amphiphilic counterions: sugar-amidoalkanoic acid (C_5 and C_{10}) derived from glucose and lactose

Glucose derivated: ω-gluconamidoalkanoic acid

A 50-ml methanol solution of the amino acid (6-amino-hexanoic acid or 11-aminoundecanoic acid; 11 mmol) in the presence of sodium hydroxide (11 mmol, 0.44 g) was stirred with δ-gluconolactone (11 mmol) and heated at 50°C for 24 h. After evaporation under reduced pressure, the ω-gluconamidoalkanoate sodium salts were purified by a column chromatography (acetone to water, 9:1). The ω-gluconamidoalkanoic acid derived was obtained after a 1-h treatment of the ω-gluconamidoalkanoate sodium salts (10 mmol) by an ion exchange resin (Amberlite IR-120, H-form; 1 ml) in 30 ml of methanol solution. Then the ω-gluconamidohexanoic acid was purified by a column chromatography (acetone to water, 9:1) leading to 1.23 g of product. For the ω-gluconamidoundecanoic acid, 3 g was obtained after recrystallization in water.

1. ω-gluconamidohexanoic acid
   1.1. Yield=40%
   1.2. ^{1}H NMR (D_2O): δ 1.28–3.18 (m, alkyl chain) and 3.54–4.41 (glucose part) ppm
   1.3. ^{13}C NMR (D_2O): δ 23.95–38.86 (alkyl chain), 62.60–73.40 (glucose part), 174.04 (CONH), and 179.27 (COOH) ppm
   1.4. Anal. calcd. for $C_{12}H_{22}NO_8$: C, 46.53; H, 7.43; and N, 4.52. Found: C, 46.01; H, 7.24; and N, 4.43

2. ω-gluconamidoundecanoic acid
   2.1. Yield=80%
   2.2. ^{1}H NMR (D_2O): δ 1.18–3.02 (m, alkyl chain) and 3.35–3.92 (glucose part) ppm
   2.3. ^{13}C NMR (D_2O): δ 24.50–38.22 (alkyl chain), 3.35–73.60 (glucose part), 172.19 (CONH), and 174.97 (COOH) ppm
   2.4. Anal. calcd. for $C_{17}H_{32}NO_8$: C, 53.75; H, 8.69; and N, 3.68. Found: C, 53.21; H, 8.51; and N, 3.61

Lactose derivated: ω-lactobionamidoalkanoic acid

A 50-ml methanol solution of the amino acid (6-amino-hexanoic acid or 11-aminoundecanoic acid, 11 mmol) was made to react with sodium hydroxide (11 mmol, 0.44 g), then stirred with lactobionic acid (11 mmol), and heated for 24 h at 50°C. After evaporation under reduced pressure, purification by column chromatography was necessary (ω-lactobionamidohexanoate sodium salt: $CHCl_3/CH_3OH/H_2O$ 2.5/7/0.5, ω-lactobionamidoundecanoate sodium salt: $CHCl_3/CH_3OH/H_2O$ 5/4.8/0.2); the ω-lactobionamidoalkanoate derivatives obtained were stirred for 1 h with an ion exchange resin (Amberlite IR-120, H-form 1 ml) in 30 ml of methanol. Then the ω-lactobionamidohexanoic acid was purified by column chromatography (acetone to water, 9:1) yielding 0.70 g. For the ω-lactobionamidoundecanoic acid, 1.53 g was obtained after recrystallization in water.

1. ω-lactobionamidohexanoic acid
   1.1. Yield=15%
   1.2. ^{1}H NMR (D_2O): δ 1.20–3.10 (m, alkyl chain), 3.38–4.40 (lactose part) ppm
   1.3. ^{13}C NMR (D_2O): δ 26.92–41.88 (alkyl chain), 4.10–106.46 (lactose part), 176.93 (CONH), and 182.11 (COOH) ppm
   1.4. Anal. calcd. for $C_{18}H_{30}NO_{13}$: C, 45.85; H, 7.00; and N, 2.97. Found: C, 45.79; H, 6.75; and N, 2.86

2. ω-lactobionamidoundecanoic acid
   2.1. Yield=25%
   2.2. ^{1}H NMR (D_2O): δ 1.44–3.10 (m, alkyl chain), 3.32–4.26 (lactose part) ppm
   2.3. ^{13}C NMR (D_2O): δ 24.96–40.81 (alkyl chain), 1.14–105.06 (lactose part), 172.49 (CONH), and 174.97 (COOH) ppm
   2.4. Anal. calcd. for $C_{23}H_{40}NO_{13}$: C, 51.01; H, 7.94; and N, 2.58. Found: C, 50.91; H, 7.75; and N, 2.50

Synthesis of polynorbornene methyleneammoniums and preparation of latexes

The amine precursor polymer was obtained by neutralization with NaOH in water of a polynorbornene methyleneammonium chloride (Mn=15,000 g mol^{-1}) obtained according a procedure previously described [10–13], producing a precipitate as a white powder of 0.81 mmoles. One hundred milligrams of this amine polymer in a methanol solution was then treated with one equivalent of sugar-amidohexanoic acid in methanol solution and heated for 3 days at 40°C (Fig. 2). For the sugar-amidoundecanoic acid polymer, the amine polymer was also added to sugar-amidoundecanoic acids in a methanol to water (50:50) solution and heated for 3 days at 70°C. Then these polymers precipitated slowly. After successive washings with methanol, polynorbornene with lactobionamidohexanoate or gluconamidohexanoate counterions were isolated. The degree of protonation of each polymer was determined by titration with $NaHCO_3$ (Table 1).

Polynorbornene methyleneammonium ω-gluconamidohexanoate

1. Yield=67%
2. ^{1}H NMR (D_2O): δ 0.90–3.40 (m, alkyl chain and norbornane part), 3.33–4.38 (glucose part) ppm
3. ^{13}C NMR (D_2O): δ 25.56–39.01 (alkyl chain and norbornane part), 2.46–74.05 (glucose part), 62.61 ($CH_2NH_3^+$), 173.97 (CONH), and 183.55 (COO^-) ppm

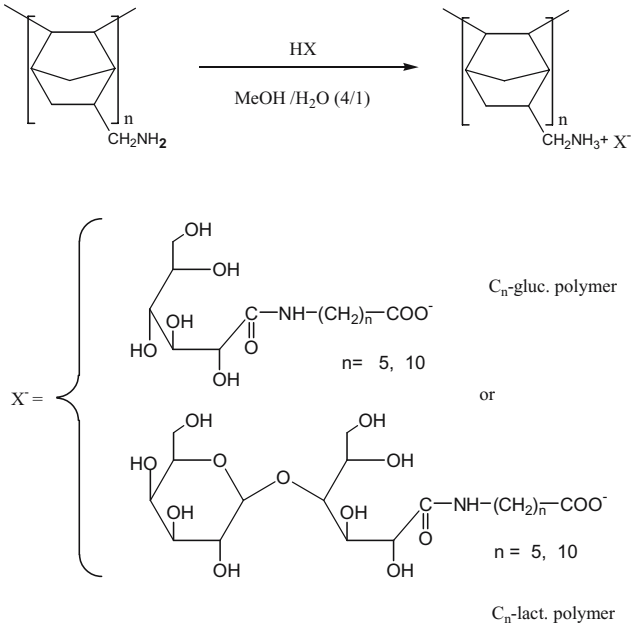

Fig. 2 Synthesis of polycationic polynorbornene with sugar-amidoalkanoic acid counterions

Table 1 Percentage of protonated units, zeta potential of the polymers in water at 25°C and calculated log *P* of the counterions

Polymer	% of protonated units[a]±5%	Zeta potential (mV)±5 mV	Calculated log *P* of the counterion
C_5-gluc.	53	+55	−2.72
C_{10}-gluc.	36	+43	−0.63
C_5-lact.	45	+45	−4.46
C_{10}-lact.	36	+44	−2.38

[a]Determined by titration

Polynorbornene methyleneammonium ω-gluconamidoundecanoate

1. Yield=54%
2. ^{1}H NMR (D_2O): δ 0.90–3.30 (m, alkyl chain and norbornane part), 3.44–4.21 (glucose part) ppm
3. ^{13}C NMR (D_2O): δ 25.38–39.44 (alkyl chain and norbornane part), 2.53–73.37 (glucose part), 62.40 ($CH_2NH_3^+$), 173.90 (CONH), and 182.86 (COO^-) ppm

Polynorbornene methyleneammonium ω-lactobionamidohexanoate

1. Yield=61%
2. ^{1}H NMR (D_2O): δ 0.90–3.26 (m, alkyl chain and norbornane unit), 3.46–4.50 (lactose part) ppm
3. ^{13}C NMR (D_2O): δ 25.14–39.33 (alkyl chain and norbornane part), 1.10–103.46 (lactose part), 62.47 ($CH_2NH_3^+$), 173.88 (CONH), and 183.73 (COO^-) ppm

Polynorbornene methyleneammonium ω-lactobionamidoundecanoate

1. Yield=40%
2. ^{1}H NMR (D_2O): δ 0.90–3.30 (m, alkyl chain and norbornane unit), 3.46–4.50 (lactose part) ppm
3. ^{13}C NMR (D_2O): δ 25.53–39.51 (alkyl chain and norbornane part), 1.06–103.48 (lactose part), 62.47 ($CH_2NH_3^+$), 173.76 (CONH), and 180.00 (COO^-) ppm

Amplification and purification of DNA

A 4.7-kbp plasmid (pEGFP-C1, Clontech), carrying the green fluorescent protein gene under the control of the cytomegalovirus promoter was used to study the ability of the different polymers to complex the DNA. The plasmid was grown in *Escherichia coli*, extracted by alkaline lysis and purified using a Qiagen Maxiprep kit. The purity of the

plasmid, previously diluted in sterilized water, was checked by electrophoresis on a 0.8% agarose gel and the DNA concentration was determined by absorbance at 260 nm.

Zeta potentials

Measurements were made on a Malvern Instrument Zetasizer 3000. The zeta potentials of polymers and DNA-polymer complexes formed with various amounts of polynorbornene methyleneammonium polymers were determined using an electrophoretic light scattering technique. Measurements were made on complexes in water. Increasing amounts of polymer were mixed with a supercoiled plasmid DNA solution (15 μg DNA, 2.5 ml H_2O). For each measurement, 10 μl of polymer solution was added successively to the DNA solution. The concentration of polymer solution was chosen according to the NH_3^+/PO_4^- molar ratio; the volume of polymer solution added (50 μl) was kept small compared to the DNA solution. For each sample, the mean particle electrophoretic mobility was measured in a thermostatically controlled microelectrophoresis cell equilibrated at 25°C at a frequency of 1,000 Hz.

Transmission electron microscopy measurements

The polymer (C=2 μM in norbornane units) and DNA-polymer complex solutions were studied by electron microscopy. For the DNA-polymer complexes, the final DNA solution was 1 μM in nucleotide units. Complexations were induced by the addition of polynorbornene with the different counterions to the DNA solution. The solutions were studied at an NH_3^+/PO_4^- molar ratio of 1.5.

The transmission electron microscopy (TEM) grids were glow-discharged (110 mV, 60 s) before depositing the samples. A drop of sample solution was left on the carbon-coated copper grids for 1 min. Samples were negatively stained with a phosphotungstate solution (1% w/w, pH 7). Observations were performed at 60 kV with a Philips EM 301 transmission electron microscope.

Agarose gel electrophoresis

A reaction mixture of 20 μl containing 5 mM phosphate buffer (pH 7.4, 5 mM NaCl), 1 μg of DNA pEGFP-C1 (1 μg of DNA corresponds to 3 nmoles of phosphate) and various amounts of polymers were used to obtain NH_3^+/PO_4^- ratios varying from 0 to 2.3. DNA-polymer complexes were made by adding different solutions of polymer to DNA dissolved in 10 mM phosphate buffer (pH 7.4). After a 30-min incubation at room temperature, 5 μl of loading buffer (250 mM Hepes, pH 7.45, 75% glycerol, and 0.005% bromophenol blue) was added. The samples were run through a 0.8% agarose horizontal electrophoresis slab gel overnight at 20 mA using a 90 mM Tris buffer system (pH 8.0). DNA was visualized using EtBr staining.

Ethidium bromide spectrofluorimetric assays

EtBr intercalation between DNA base pairs was determined through fluorescence spectroscopy (at excitation and emission wavelengths of 530 and 590 nm, respectively) using a PTI QM1 Photon Technology Instrument Quanta Master 1 Spectrometer.

Firstly, the ability of EtBr to intercalate into DNA complexed by polymers was investigated. For the dye exclusion assay, the plasmid pEGFP-C1 (10 μg) in sterilized water was mixed with cationic polymers in 500 μl of phosphate buffer (5 mM; pH 7.4; 15 mM NaCl) at different NH_3^+/PO_4^- molar ratios varying from 0 to 5. After standing for 20 min at room temperature, 20 μl of EtBr solution (20 μg/ml) and 1,500 μl of phosphate buffer (5 mM; pH 7.4; 15 mM NaCl) were added to the polymer/DNA mixtures and measurements were carried out.

Secondly, the EtBr displacement from DNA by cationic polymers was studied. This investigation provides an assess-

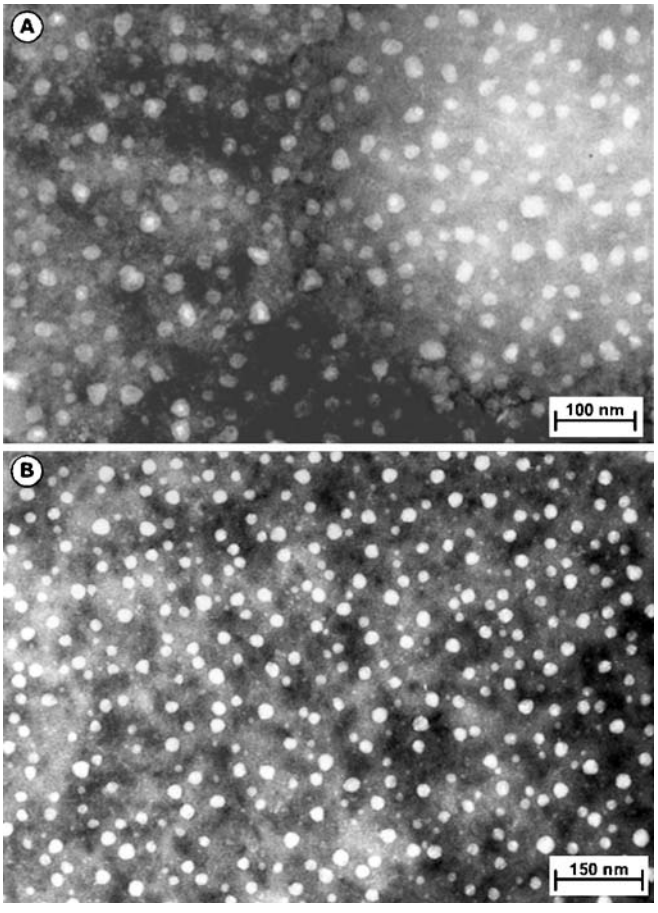

Fig. 3 Transmission electron micrographs of gluc. polymers; **a** C_5-gluc. polymer and **b** C_{10}-gluc. polymer

ment of the polycations binding onto DNA by measuring changes in the fluorescence of the EtBr probe. The plasmid pEGFP-C1 (10 μg) in sterilized water was mixed with EtBr solution (5 μl, 500 μg/ml). Then, cationic polymers in 500 μl of phosphate buffer (5 mM, pH 7.4, and 15 mM NaCl) were added to the plasmid solution to obtain polymer/DNA complexes at NH_3^+/PO_4^- molar ratios varying from 0 to 5. The samples were mixed gently and the fluorescence was measured after 20 min of incubation at room temperature.

The relative fluorescence was calculated as below:

$$\%\,\text{Relative fluorescence} = \frac{\text{Fluorescence}_{(\text{obs})} - \text{Fluorescence}_{(\text{EtBr})}}{\text{Fluorescence}_{(\text{DNA+EtBr})} - \text{Fluorescence}_{(\text{EtBr})}}$$

where

$\text{Fluorescence}_{(\text{obs})}$ = fluorescence of [DNA + EtBr + polymer]
$\text{Fluorescence}_{(\text{EtBr})}$ = fluorescence of EtBr alone
$\text{Fluorescence}_{(\text{DNA+EtBr})}$ = fluorescence of [DNA + EtBr]

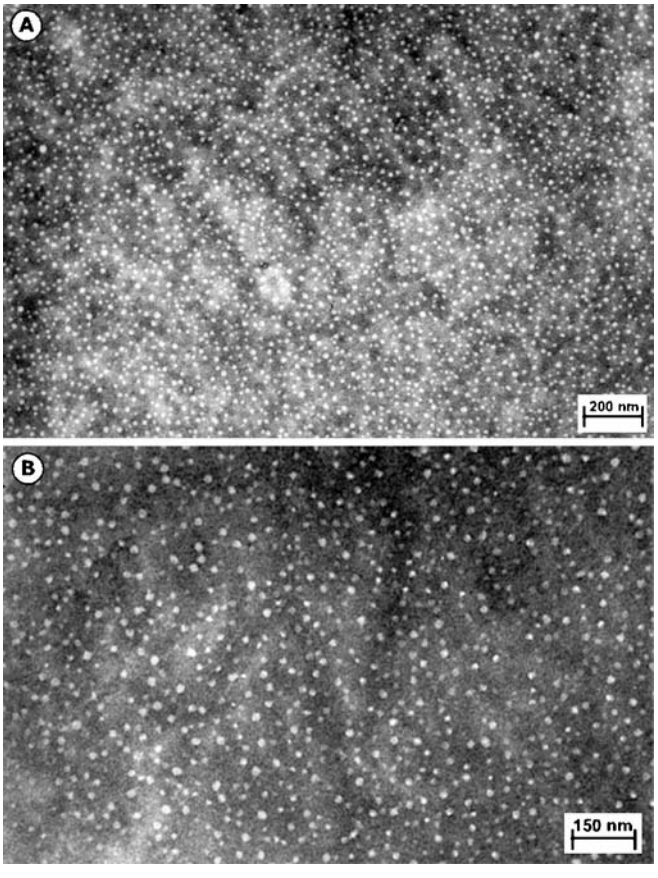

Fig. 4 Transmission electron micrographs of lact. polymers; **a** C_5-lact. polymer and **b** C_{10}-lact. polymer

Calculated log *P*

The partition coefficient for *n*-octanol/water or log *P* was calculated using "ChemDraw" (CambridgeSoft) by the atomic increment method considering the functional group contribution based upon the chemical structure and environment of the fragment. Due to the limitation of the table of increments, the log *P* for all the counterions was calculated in their acid form.

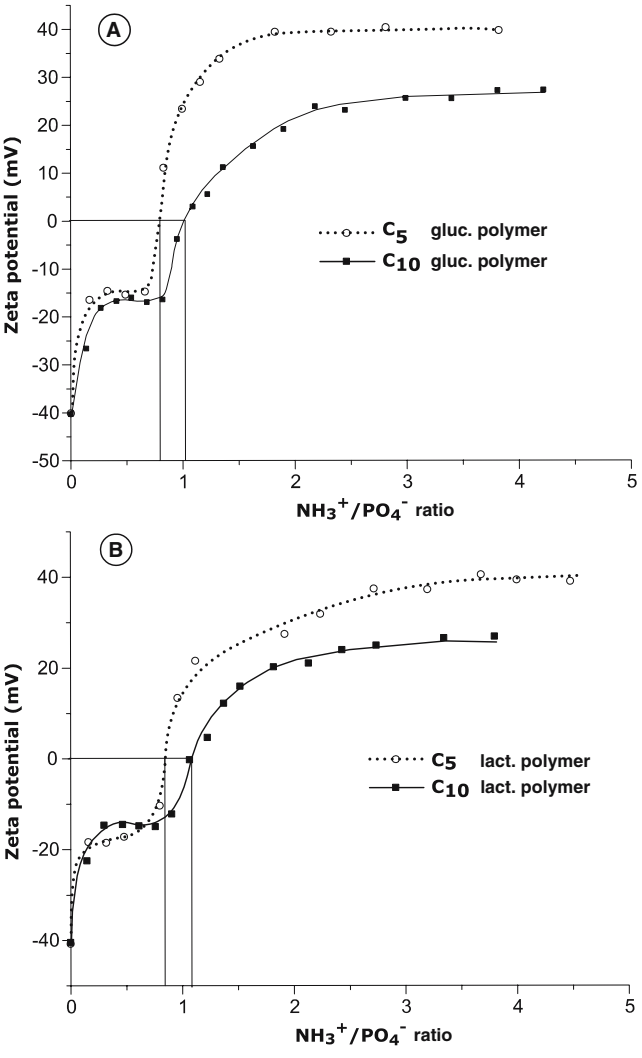

Fig. 5 Zeta potential of DNA-lact. polymer complexes as a function of their charge ratio; **a** DNA-gluc. polymer complex and **b** DNA-lact. polymer complex

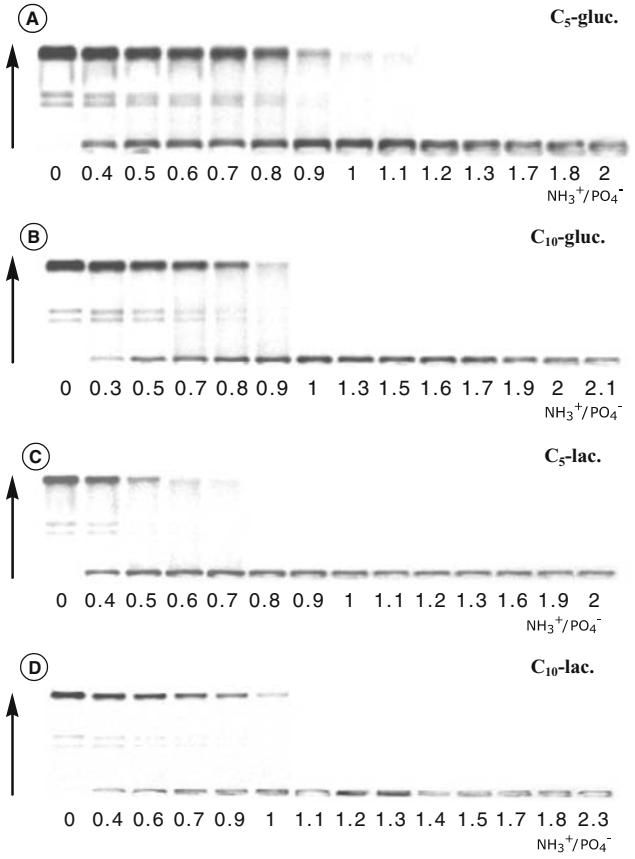

Fig. 6 Effect of the polymers on the electrophoretic mobility of the DNA at different NH_3^+/PO_4^- molar ratios; **a** C_5-gluc. polymer, **b** C_{10}-gluc. polymer, **c** C_5-lact. polymer, and **d** C_{10}-lact. polymer

Results

Physicochemical study of the latexes

Zeta potential

The zeta potential of these polymers in water solution was determined by laser Doppler microelectrophoresis (Table 1). For all the polymers, zeta potential values are similar. The value for C_{10}-glucose (gluc.) polymer is +43 mV, +44 mV for C_{10}-lactose (lact.) polymer, +55 mV for C_5-gluc., and +43 mV for the C_5-lact. polymer.

Electron microscopy

By electron microscopy we observed very small particles of latexes for all the polymers. For the glucose-derived polymers, particles displayed similar sizes, 15–30 nm diameters for the C_5-gluc. and the C_{10}-gluc. (Fig. 3). For the lactose-derived polymers (Fig. 4) there was little difference between the diameters of the C_5-lact. polymer particles (15–25 nm) and the C_{10}-lact. polymer particles (10–20 nm).

Table 2 NH_3^+/PO_4^- molar ratios inhibiting the DNA migration on an agarose gel for the different polymers

Polymer	C_5-gluc.	C_{10}-gluc.	C_5-lact.	C_{10}-lact.
NH_3^+/PO_4^- molar ratio at the neutralization	1.2	1	0.8	1.1

Interaction between the latexes and DNA

Zeta potential

The zeta potentials of polymer-DNA complexes at different NH_3^+/PO_4^- molar ratios are shown in Fig. 5. The zeta potential of the resulting complexes changes from a negative value to a positive value when the quantity of polymer increases. For the two C_{10} polymers the isoelectric point (zeta of 0) was around an NH_3^+/PO_4^- molar ratio of 1.1. For the two C_5 polymers, neutralization (zeta of 0) appeared around an NH_3^+/PO_4^- molar ratio of 0.8.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed to evaluate the efficiency of the different polymers to bind to DNA. The plasmid DNA preparation used in these experiments contained mainly supercoiled DNA but also a low amount (1%) of relaxed circular and linear DNA. The order of migration from the well was relaxed circular, linear, and supercoiled DNA. As shown in Fig. 6, all polymers were able to retard and inhibit DNA migration on an agarose gel. Total retention of DNA for all the polymers was observed at NH_3^+/PO_4^+ molar ratio around 1 (Table 2). Moreover, the values of NH_3^+/PO_4^- molar ratios at neutralization are in good accordance with the zeta potential experiments.

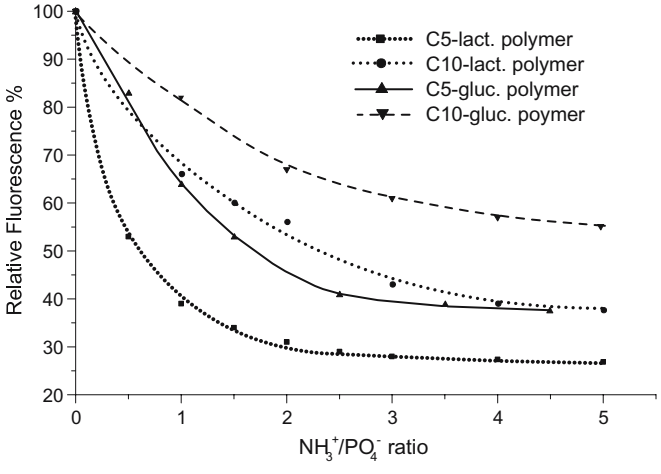

Fig. 7 Assay of EtBr displacement by polynorbornene methyle neammonium polymers. Each point is expressed as percentage of the maximum fluorescence obtained with naked DNA

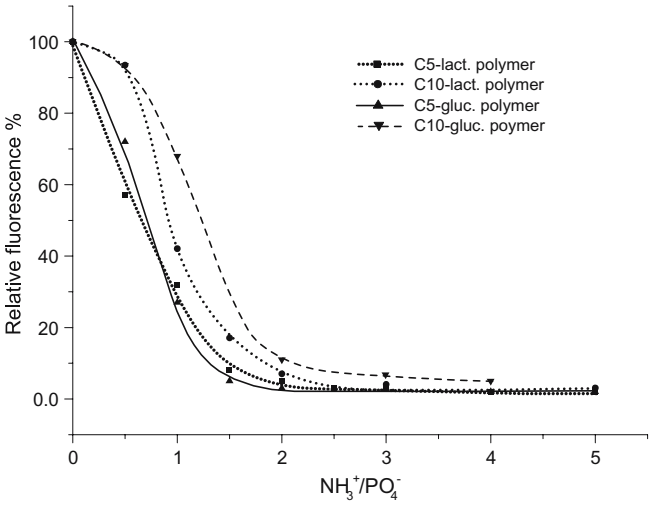

Fig. 8 Assay of EtBr exclusion by polynorbornene metyleneammonium polymers. Each point is expressed as percentage of the maximum fluorescence obtained with naked DNA

Ethidium bromide displacement assay

EtBr displacement is a simple and reproducible method to monitor the interaction of DNA with the polymer [22]. This method was used to assess the ability of the different polymers to bind to DNA. Sequential addition of these polymers to DNA/EtBr complex in solution caused a decrease in relative fluorescence because of the displacement of the dye by the polymer. For all the polymers we observed a gradual decrease in fluorescence as a plateau was reached (Fig. 7). The higher residual fluorescence is observed for the C_{10}-gluc. polymer (57%) and the lower for the C_5-lact. polymer (27%). We have an intermediate value (37%) for the C_5-gluc. and the C_{10}-lact. polymer.

Ethidium bromide exclusion assay

The ability of EtBr to access to DNA inside complexes was monitored by a titration assay at different NH_3^+/PO_4^- molar ratios (Fig. 8). A solution of EtBr was added to the complexes and the fluorescence of the solutions was

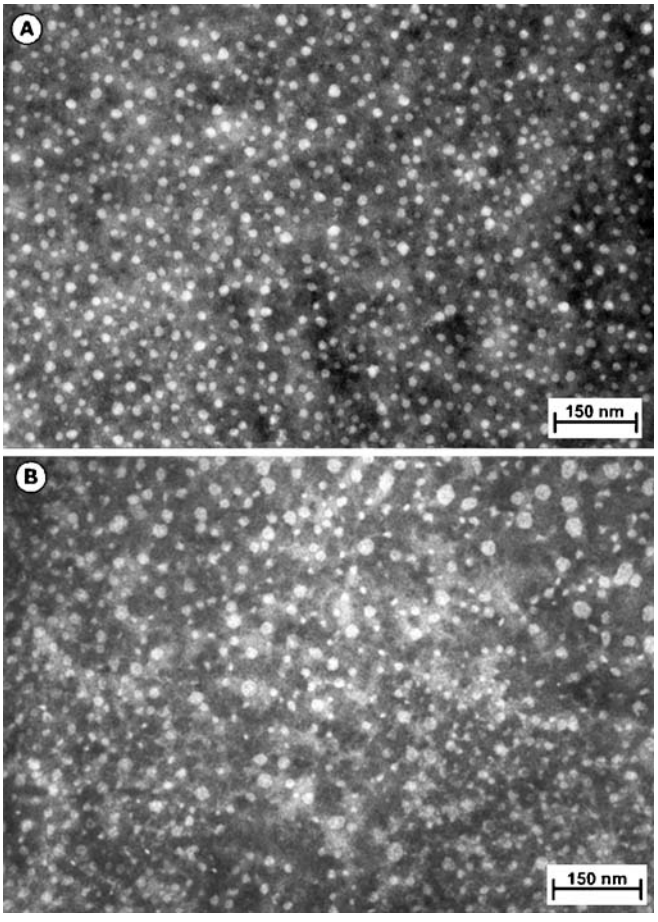

Fig. 9 Transmission electron micrographs of polymer-DNA complexes; **a** C_5-gluc. polymer-DNA complex and **b** C_{10}-gluc. polymer-DNA complex

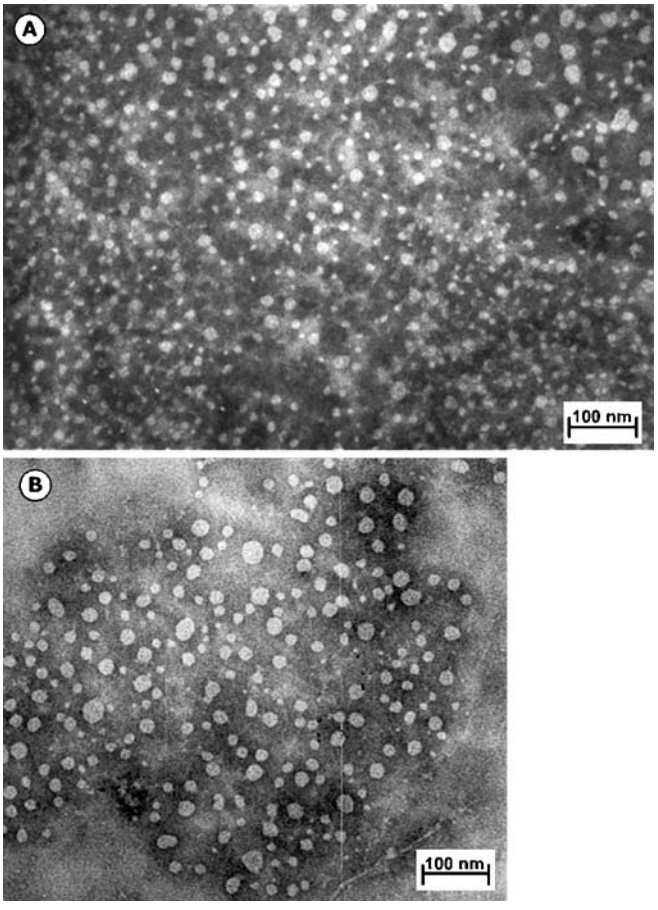

Fig. 10 Transmission electron micrographs of lact. polymer-DNA complexes; **a** C_5-lact. polymer-DNA complex and **b** C_{10}-lact. polymer-DNA complex

measured. For all the polymers, the exclusion profiles was quite similar and can be divided in two regions: (1) between 0 and 1.5 NH_3^+/PO_4^- molar ratio there is an important fall of the fluorescence and (2) the relative fluorescence percentage tailed off at around 5 for ratios higher than 2.

Electron microscopy

Characterization of polymer-DNA complexes was performed in water at an NH_3^+/PO_4^- molar ratio of 1.5 using TEM. For the gluc. polymer-DNA complexes we observed uniformly dispersed particles. The diameter of these very small particles was 10–25 nm for C_5-gluc. polymer-DNA and 15–30 nm for C_{10}-gluc. polymer-DNA (Fig. 9). With lact. polymer-DNA complexes (Fig. 10), we also observed uniformly dispersed particles. The diameters of the particles were measured as 10–25 nm for C_5-lact. polymer-DNA and 10–30 nm for C_{10}-lact. polymer-DNA.

Discussion

Synthesis and physicochemical study of the latexes

Sugar-specific receptor mediated gene transfer is an attractive method to target DNA to specific cell receptors like lectins [23, 24]. For this reason we selected two families of sugar bolaamphiphile counterions, one derived from glucose and the other derived from lactose. The counterions described are ω-gluconamidoalkanoic acids and ω-lactobionamidoalkanoic acids synthesized according to a patented method [25].

In the first step the ω-gluconamidoalkanoate sodium salts were obtained by condensation of the ω-amino carboxylate sodium salt (C_5 or C_{10}) with the δ-gluconolactone. Finally, the ω-gluconamidoalkanoate salts were converted to their acid forms by an acidic cation-exchange resin. For ω-lactobionamidoalkanoic acid synthesis the method was similar but the sodium salts were obtained by making ω-amino carboxylate sodium salt (C_5 or C_{10}) react with the lactobionic acid in equilibrium in solution with its lactone form. The sugar-amidoalkanoic acid yields were low to moderate depending on the sugar and the chain length.

The polymers with ω-gluconamidoalkanoate and ω-lactobionamidoalkanoate counterions were obtained by reaction of the sugar-amidoalkanoic acids with the polynorbornene methylenamine in methanol or methanol/water solution. In these conditions, the reaction lead to a rapid precipitation of the polymer exchanged, and consequently, the degree of protonation depended on the solubility of the final polymer in solution. Furthermore, the reactivity on polymers are often difficult due to their limited solubility, resulting in partially folded chains not easily accessible to the reagents [26, 27]. If we compare the protonation degrees (Table 1), we have the smaller values for the two long chains counterions (C_{10}-gluc. and C_{10}-lact.) and the higher values for the C_5-gluc. and C_5-lact. counterions. These differences could be explained by the increase of the counterion chain length that increased the precipitation of the polymer during the protonation.

Concerning the zeta potential, if we compare the effect of the four counterions and considering the accuracy of the measurements, we have the same values for all the polymers.

The mean diameters of the particles formed in solution was determined by electron microscopy using a staining technique. These latex particles were very small (Figs. 3 and 4) with a diameter ranging from 10 to 30 nm for all polymers. The latex particles consist of a packed core formed by the norbornane units surrounded by a corona of the amphiphilic counterions (Fig. 1). It is interesting to note that the diameter of the latexes did not seem to be greatly affected by the length of the counterion spacer (C_5 or C_{10}).

Interaction between the latexes and DNA

Concerning the DNA complexation, there were no major differences between the four polymers. The variation of the zeta potential during the titration of the DNA by the polymers shows classical sigmoid curves with neutralization around $NH_3^+/PO_4^-=1$. From a general point of view we observed a very sharp change of slope around neutralization for the C_5-polymer-DNA complexes indicating a more abrupt complexation of the DNA than for the C_{10}-polymer-DNA complexes. Furthermore, the zeta potentials obtained at high NH_3^+/PO_4^- molar ratios were higher for the C_5-polymer-DNA complexes than for the C_{10}-polymer-DNA complexes in accordance with the difference of hydrophobicity between the counterions.

The neutralization ratios were also confirmed by agarose gel electrophoresis of polymer-DNA complexes at various NH_3^+/PO_4^- molar ratios. Moreover, the electrophoresis experiment showed the presence of small amounts of linear and circular relaxed DNA, and we observed that these two forms of DNA were also complexed by the polymers.

For the C_{10}-gluc. polymer (Fig. 7), the EtBr displacement assay showed that a gradual decrease in fluorescence as a plateau was reached at a high ratio with a relative fluorescence of 57%. This high value could be explained by a partial displacement of the EtBr from the DNA. Comparatively with the others polymers we observed here a lower affinity for the DNA. For this counterion the highest hydrophobicity (log P=−0.63) is obtained, so in this case, the corona of counterions is very stable and not easily displaced by the DNA. For the C_5-lact. polymer, the displacement profile can be divided in two regions. In the first region, between NH_3^+/PO_4^- molar ratio of 0 and 1, a rapid fall in fluorescence was observed. At higher ratios in

the second region, values tailed off at relative fluorescence percentage around 27. In this case we have the less hydrophobic counterion (log P=−4.46) weakly binding to the particle and more easily displaced by the DNA. For the C_5-gluc. and C_{10}-lact. polymers we have an intermediate behavior with quite the same displacement profile. We observed a gradual decrease in fluorescence until 37% at a NH_3^+/PO_4^- molar ratio of 5. This behavior is also correlated with the fact that we have quite the same calculated log P for these two counterions (Table 1).

It appeared that by EtBr exclusion assay (Fig. 8) we have a similar binding accessibility for all the polymers independent to the hydrophobicity of the counterions.

Analysis by TEM of the complexes with DNA showed the presence of very small particles of complexes as we have previously observed with lactobionate counterion [12]. It is interesting to note that there is a weak influence of the size and the hydrophobicity of the counterions on the size and the morphologies of the particles of polymer-DNA complexes in water. This counterion effect is very different to that previously observed with chloride, acetate, and lactobionate as counterions of polynorbornene polycationic polymers [10–13]. Because of the amphiphilic nature of the counterions and the possibility of intermolecular hydrogen binding between the sugars (sugar corona effect), they led to small differences in the size of the latexes and indeed the interactions with DNA led to similar sized complexes.

Conclusion

We have synthesized an original family of polycationic latexes with sugar counterions. These new polymers spontaneously form latexes in water and complex DNA in very small particles. Overall, the results show that these new polymers could be potential vectors for targeted DNA transfection. We have observed for all these polymers, which are independent of the nature of the amphiphilic sugar counterion, the same behavior concerning their complexation with DNA. This observation could be interesting for DNA targeting, introducing the possibility of changing the nature of the sugar part to enhance the interaction of the complex with the cell receptors. Furthermore, for these complexes, because of the amphiphilic nature of the counterion, the sugar moieties are separated from the surface of latex particles by the aminoalkanoic acid residue and consequently are farther from the latex surface. In these conditions, interactions between the latexes and the cell receptors could be enhanced.

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