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Polycationic graft copolymers of poly(N-vinylpyrrolidone) as non-viral vectors for gene transfection

Research Article

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Abstract: Novel graft copolymers of 2-(dimethylamino)ethyl methacrylate (DMAEMA) with N-vinylpyrrolidone (NVP) were designed and synthesized by the free radical copolymerization of DMAEMA with precursor polymers of vinyl-functionalized poly(N-vinylpyrrolidone) (PVP). The ability of the PVP- grafted copolymers to bind and condense DNA was confirmed by ethidium bromide displacement assay, agarose gel electrophoresis and transmission electron microscopy. The presence of PVP in the copolymers had a favorable effect on the biophysical properties of polymer/DNA complexes. Colloidal stable complexes obtained from the copolymer systems, were shown to be separate, uniformly spherical nanoparticles by transmission electron microscopy. The approximate diameter of the complexes was 150 - 200 nm, as determined by dynamic light scattering studies. These results confirm an important role played by the PVP grafts in producing compact stable DNA complexes. The ζ-potential measurements revealed that the incorporation of the PVP grafts reduced the positive surface charge of polymer/DNA complexes. The cytotoxicity of the copolymers decreased with an increasing fraction of PVP. Furthermore, *in vitro* transfection experiments with these copolymers showed improved ability of transfection in cell culture, demonstrating an important role for PVP grafts in enhancement of the transfection efficiency.

Keywords: *Poly(2-(dimethylamino)ethy lmethacrylate) • Poly(N-vinylpyrrolidone) • Cytotoxicity • Gene transfection*

1. Introduction

Recently, there has been widespread concern over gene therapy because plasmid DNA could be introduced into cells of patients in gene therapy, to express the pharmaceutical proteins [1,2]. Gene therapy provides remarkable potential for the treatment of many genetic and non-genetic disorders, but the clinical application of this technology is severely hindered by the lack of an efficient and safe gene delivery system [3,4]. At present, viral vectors are the most efficient gene delivery system available. However their use is limited by safety problems, including a strong immune response after administration, possible insertional mutations, and toxicity [5]. The challenge of overcoming these disadvantages has led to the development of non-viral vectors such as cationic lipids and cationic polymers as promising alternatives to viral vectors [6-9].

The development of DNA delivery systems based on synthetic cationic polymers has gained increasing attention over the last few decades, because the great variety and versatility of possible chemical structures provides the potential to address issues of safety and cost [10-11]. Well known examples of such polymers are polyethyleneimine (PEI) [12-13], poly(L-lysine) (PLL) [14], polyamidoamine dendrimer [15], DEAE dextran [16], derivatized chitosan [17], and poly((2-methylamino) ethyl methacrylate) (PDMAEMA) [18]. Although cationic polymers with high charge density exhibit enhanced DNA condensation, this property also contributes to their higher cytotoxicity [19]. Among amine methacrylate-based polymers, PDMAEMA has previously been reported to be one of the efficient cationic condensing agents for DNA delivery [20], but the high positive charge and the production of large insoluble aggregates of polymer-DNA complexes remain problematic [21,22].

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To overcome these drawbacks, non-charged hydrophilic segments have been introduced as an effective modification of synthetic cationic polymeric DNA delivery systems [23-25]. Systems such as PEO-*b*-PDMAEMA [26], Pluronic-*b*-PDMAEMA [27], PEG-modified PDMAEMA [28] and PDMAEMA-*b*-PMPC (poly (methacryloyloxyethyl phosphorylcholine)) [29] have been widely studied as potential gene delivery systems.

Notably, poly(N-vinylpyrrolidone) (PVP) as a water-soluble, biocompatible and non-ionic polymer has been incorporated into PDMAEMA [30]. PVP as a hydrophilic segment offers significant advantages: with its pentatomic ring lactam structure, PVP is nontoxic [31] and biocompatible [32]. Random copolymers of DMAEMA and *N*-vinylpyrrolidone (NVP) have been synthesized and evaluated as polymeric transfectants [18]. They showed lower toxicity and improved transfection properties for copolymers with up to 50 mol% of NVP. However, little attention has been given yet to graft copolymers or block copolymers based on NVP and DMAEMA as gene transfectants.

The aim of this work is the synthesis of graft copolymers modified by non-charged hydrophililc graft segments (PVP) and to explore the potential of these systems for improved capabilities as polymeric transfectants. Here, we have investigated the potential of the novel PDMAEMA-*g*-PVP graft copolymers for gene delivery, and evaluated the improvement in the biophysical properties of complexes achieved by introducing PVP grafts. The buffering ability of the PDMAEMA-*g*-PVP graft copolymers was characterized by potentiometric titrations. Physical properties of polymer-DNA complexes have been assessed by gel electrophoresis, ethidium bromide displacement assay, ζ-potential, particle size measurements and transmission electron microscopy. Furthermore, the transfection and cytotoxicity properties of the graft copolymers were investigated and compared with PDMAEMA *in vitro*.

2. Experimental Procedures

2.1. Materials

2.1.1. Reagents and Buffers

N-Vinylpyrrolidone (NVP, 99%) and N,N-dimethylaminoethyl methacrylate (DMAEMA, 98%) were obtained from Arcos Co. and purified by distillation under reduced pressure before use. *N*-Succinimidyl acrylate (NSA, 98%) and 2-aminoethanethiol hydrochloride (AET×HCl, 98%) were purchased from Arcos Co. and used as received. *N*,*N*'-Azobisisobutyronitrile (AIBN) was purchased from Shanghai Chemical Reagent Co. and used after recrystallization from methanol. Triethylamine (TEAM), 1,4-dioxane and N,N-dimethylformamide (DMF) were used after purification by standard methods. Acryloyl chloride was synthesized in our laboratory. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT, 99%) was purchased from Sigma Chemical Co. Modified Eagle Medium (MEM) and phosphate-buffered saline (PBS) were obtained from Gibco Co. and used without further purification.

2.1.2. DNA

Two forms of DNA were used for this study. A 12.6-kb Hepatitis C virus plasmid DNA donated by China Center of Type Culture Collection was used for ethidium bromide displacement, ζ-potential, particle size measurements and agarose gel electrophoresis. PDsRed1-N1 DNA containing the red fluorescent reporter gene, obtained from Sigma, was used for transfection studies. All DNA was used as obtained without further treatment.

2.2. General polymerization protocol

2.2.1. Synthesis of vinyl-functionalized PVP and the homopolymer of DMAEMA

NVP (4.5 g, 40 mmol), 2-Aminoethanethiol hydrochloride (0.226 g, 2 mmol) and AIBN (45 mg) were dissolved in 20 mL dioxane and purged with high purity nitrogen for 15 min. The solution was then stirred and heated to 60°C for 10 h under nitrogen atmosphere. After cooling to room temperature, the amino-terminated polymers ($PVP-NH_2$) were recovered by precipitation in 10-fold diethyl ether. The product was again purified by repeated reprecipitation from dioxane into diethyl ether three times, and dried in vacuum to constant weight. This polymerization afforded approximately 4 g of polymer of M_n = 4,700 and a M_w/M_n of 1.72.

A polymerizable vinyl group was introduced into the amino semitelechelic $PVP-NH_2$ using an amide condensation reaction between amino groups in PVP and NAS (molar ratio 1:10) in DMF at 4°C for 2 days. This synthetic procedure resulted in a macromonomer (vinyl-PVP) of M_n = 5,100 and a M_w/M_n of 1.78.

The homopolymer of DMAEMA was synthesized by radical polymerization as previously described in detail [20]. This synthesis afforded PDMAEMA of M_n = 138,000 and a M_w/M_n of 1.62.

2.2.2. Synthesis of graft copolymer PDMAEMA-*g*-PVP

The predetermined amount of macromonomer vinyl-PVP, DMAEMA (0.5 g, 3.5 mmol) and AIBN (10 mg) were dissolved in 10 mL DMF. The solution was degassed by bubbling with nitrogen for 30 min. The reaction mixture was stirred and heated to 60°C for 10 h and then cooled to room temperature. The solution was put into a dialysis sack (molecular weight cut-off: 8,000~10,000 g mol^{-1}, Shanghai Chemical Reagent Co.) and subjected to dialysis against distilled water for 4 days to remove the unreacted macromonomer, and then lyophilized before further use.

2.2.3. Polymer to DNA ratios

Polymer to DNA ratios are expressed as monomer: nucleotide molar ratio (N/P mole/mole) in terms of DMAEMA monomer: DNA nucleotides. Monomer of DMAEMA was calculated according to molecular weight. Nucleotide was calculated to have an average molecular weight of 308.

2.3. GPC measurements

Number- and weight-average molecular weights (M_n and M_w, respectively) of the polymers obtained were determined by a gel-permeation chromatographic (GPC) system equipped with a Waters 2690D separations module and Waters 2410 refractive index detector. DMF was used as the eluent at a flow rate of 0.3 mL min^{-1}. Waters millennium module software was used to calculate molecular weight on the basis of a universal calibration curve generated by a polystyrene standard of narrow molecular weight distribution.

2.4. ^{1}H NMR measurements

^{1}H NMR spectra were recorded on a Mercury VX-300 spectrometer (USA) at 300 MHz using $CDCl_3$ as the solvent.

2.5. Potentiometric titrations

Copolymer (5.0 mg) was diluted in distilled water 10 mL in a glass beaker and stirred with a magnetic stirrer. The pH was measured using PHS-3C pH meter (CHINA). Titrations were carried out using 4 μL aliquots of 0.25 mol L^{-1} HCl solution. The titrant was added manually by a micropipette and pH readings were allowed to reach a constant value. Titration was carried out at ambient temperature.

2.6. Ethidium bromide displacement assay

Ethidium bromide (2 μg) was added to 10-fold diluted PBS (2 mL) in a fluorimetry cuvette and mixed by gentle agitation. The fluorescence of the solution was measured at excitation wavelengths of 560 nm and emission wavelengths of 605 nm using fluorescence spectrometer (RF-5301PC, Shimadzu, Japan). DNA (10 μg) was added, the solution was mixed by gentle agitation and the fluorescence was measured again. Aliquots of the copolymer solution were then added in a stepwise manner, mixed gently and the fluorescence measured after each addition. The fluorescence readings for every sample were conducted in triplicate. The relative fluorescence was calculated as below:

$$\%\text{Relative Fluorescence} = \frac{I_{(obs)} - I_{(EtBr)}}{I_{(DNA+EtBr)} - I_{(EtBr)}} \times 100$$

$I_{(obs)}$ = Fluorescence of DNA + ethidium bromide + polymer;
$I_{(EtBr)}$ = Fluorescence of ethidium bromide alone;
$I_{(DNA+EtBr)}$ = Fluorescence of DNA + ethidium bromide.

2.7. Agarose gel electrophoresis

The electrophoretic mobility of the polymer/DNA complexes at different N/P molar ratios in PBS buffer at pH 7.4 was determined by gel electrophoresis using 2.0% agarose gel mixed with 20 μg ethidium bromide. Experiments were run at 100 V for 60 min. DNA was visualized under UV (254 nm) illumination.

2.8. ζ-potential and particles size measurements

The measurements were carried out using the Malvern Zetasizer Zs90 (United Kingdom) at different N/P molar ratios in distilled water at 25°C.

2.9. Transmission electron microscopy (TEM)

The morphology and size of polymer/DNA complexes were observed using JEM-100CXII transmission electron microscope (Japan) at an acceleration voltage of 80 kV. First, one drop of the complexes was deposited on carbon-coated grids. After 5 min on the grids, 1.5% phosphotungstic acid (PTA) was added to negatively stain the complexes for additional 5 min, and then the complexes were recorded on films with TEM.

2.10. Cytotoxicity assays

Evaluation of cytotoxicity was performed using the MTT assay. MTT solution (5 mg mL^{-1}) was prepared by dissolving 50 mg MTT into 10 mL PBS (pH=7.4). SGC-7901 cells were seeded at a density of 1.5×10^4 cells/well in a 96-well plate and were incubated for 48 h in a 5% CO_2 incubator at 37°C. The cells were then incubated for another 24 h after 200 μL of a copolymer solution in MEM media at different concentrations were added to each well. To every copolymer concentration, eight wells were simultaneously used. Then 20 μL MTT solution were added to each well and the cells incubated for an additional 4 h at 37°C.The MTT solution was then carefully removed from each well, and 120 μL DMSO was added to dissolve the MTT formazan crystals. Absorbance was measured spectrophotometrically at

490 nm using a MuLTiskAn MK3 unit. The cell viability (%) was calculated according to the following equation: Cell viability(%) = $OD_{490(sample)}$ / $OD_{490(control)}$ × 100. Here $OD_{490(sample)}$ is the absorbance of the cells in the presence of copolymer and $OD_{490(control)}$ represents the absorbance of the cells in the absence of copolymer.

2.11. Transfection studies

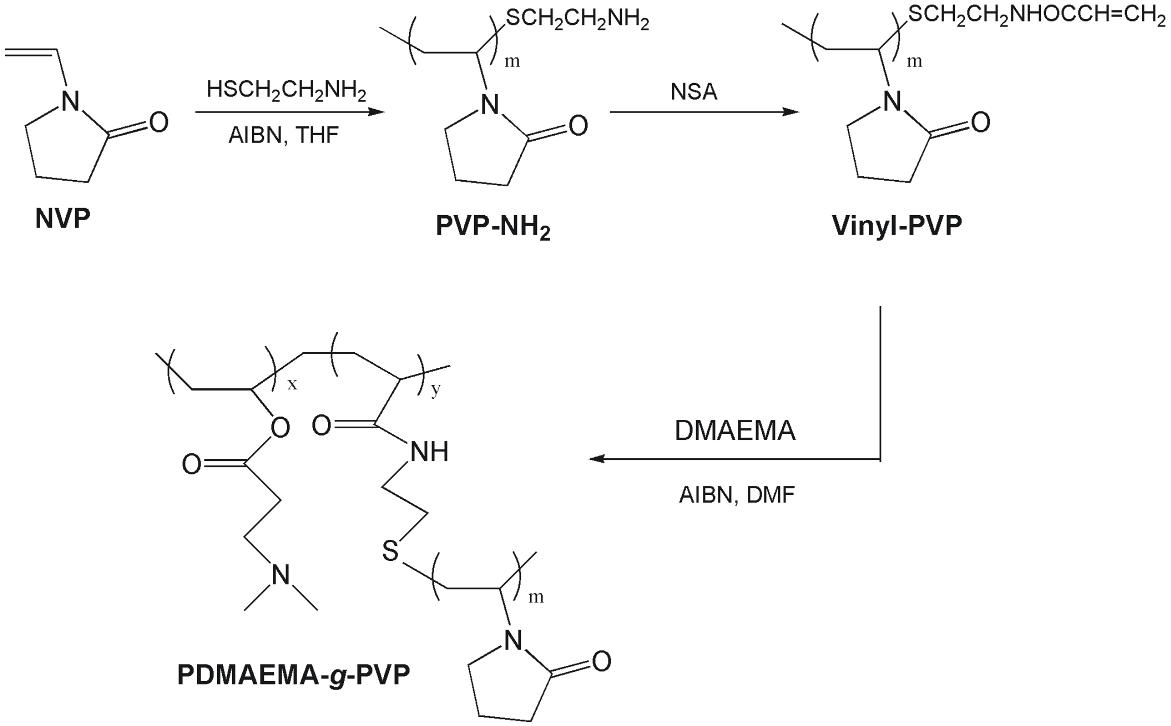
Transfection experiments were performed with SGC-7901 cells using the PDsRed1-N1 plasmid DNA. Cells were seeded at a concentration of 5×10^4 cells/well in 12-well plates and the cells were incubated at 37°C and 5% CO_2 for 24 h. On the day of the transfection, the medium was aspirated off from the wells and gently replaced with DNA-copolymer complexes. The cells were incubated at 37°C and 5% CO_2 for 4 h. After removal of the transfection complexes, RMPI-1640 medium was added and the cells were further cultured for 48 h before analysis. The transfection efficiency was evaluated using a microscope equipped with fluorescence to determine the total number of living cells and the number of fluorescent cells. Living cells were counted under visible light, whereas fluorescent cells were counted using a hemocytometer at an excitation wavelength of 488 nm. The transfection efficiency was calculated as the number of fluorescent cells in a well divided by the total number of cells in the same well.

3. Results and Discussion

3.1. Synthesis and Characterization of the PDMAEMA-g-PVP copolymers.

The two graft copolymers P30 and P60 were synthesized by a two-step synthetic pathway: the synthesis of vinyl-functioned macromonomer (vinyl-PVP) and the copolymerization of vinyl-PVP and DMAEMA. The synthesis is summarized in Scheme 1. In the first step, amino-terminated polymer ($PVP-NH_2$) was prepared by the radical polymerization of NVP with AET•HCl as a chain transfer agent, and then an amide condensation reaction between amino groups in PVP and NAS was carried out. In the second step, the copolymerization of vinyl-PVP and DMAEMA was carried out by radical polymerization and PVP was transferred to graft arms of PDMAEMA main chain. The molecular weights of two graft copolymers with different composition were given in Table 1.

The ^{1}H-NMR spectra of P60 are shown in Fig. 1 with signal assignments. To evaluate the composition of PVP grafts in each main chain, the relative number of graft PVP arms in the copolymers was determined from the relative intensities at 4.18 ppm ($-OC\mathbf{H}_2C\mathbf{H}_2-$ of the PDMAEMA segments) and 3.79 ppm ($-CH_2CHN-$ of the PVP segments). This observation confirmed that there had been successfully quantitative incorporation of the macromonomer vinyl-PVP into the graft copolymer.



Scheme 1. The synthetic pathway of the graft copolymers

Table 1. Characterization and Polymerization Details for Copolymers.

polymer	m/n[a)]	m/n[b)]	M_n[g mol^{-1}]	M_w/M_n[c)]
P30	100 : 30	100 : 14	152,200	1.83
P60	100 : 60	100 : 33	140,700	1.75

[a)] *Feed molar ratio of DMAEMA to vinyl-PVP.*
[b)] *Experimental molar ratio, estimated by ^{1}H-NMR.*
[c)] *Molecular-weight distribution, determined by GPC analysis.*

3.2. Potentiometric titrations the copolymers

The “proton sponge” effect was expected and confirmed by potentiometirc titration. PDMAEMA with a low pK_a value around 7.5 are partially deprotonated at the physiological pH [33,34], which should endow this polymer with protection of complexed DNA from lysosomal nucleases and the endosome disruptive function. The buffering capacity of the obtained copolymer was evaluated by potentiometirc titration. On the titration of copolymer solution with hydrochloric acid, the pH titration curves are shown in Fig. 2. The considerable buffer capacity of both copolymers over the pH range was observed from their respective buffer capacity profiles, which indicated that the introduction of PVP grafts does not significantly reduce the buffer capacity of the copolymers. However, a slightly lower buffer capacity was observed for P60 in comparison with P30, which is consistent with the lower content of DMAEMA in P60. Increasing the content of DMAEMA in copolymers improved the “proton sponge” effect of copolymers, indicating the dependency of buffer capacity on the content of PDMAEMA in graft copolymer. The result of potentiometric titration indicates that these copolymers might show a slight “proton sponge” effect when located within acidic endosomal membrane after endocytosis as expected.

3.3. Ethidium bromide displacement assays

Ethidium bromide (EtBr) binds DNA by intercalating into the DNA base pairs and stretches the double helix of DNA, leading to fluorescence under illumination [35]. The EtBr displacement assay provides an assessment of the polycations’ ability to form complexes with DNA by measuring changes in the fluorescence of EtBr-DNA complexes. Loss of EtBr fluorescence is thought to the result of polyelectrolyte binding to DNA-polymer complexes, resulting in expulsion of intercalated EtBr molecules [36]. In this study, the assay is used to assess the ability of the graft copolymers to bind to DNA (Fig. 3). Increasing the molar ratios of copolymer to DNA resulted in a rapid decrease in the relative fluorescence at N/P ratios between 0.2 and 2 for the copolymers, indicating the ability of the copolymers to bind to DNA. The decrease in fluorescence intensity reached a plateau at about 30% in the high N/P ratios (> 2), implying that about 70% of intercalated EtBr molecules were displaced by the copolymer. Interestingly, the displacement profiles of the copolymers are comparatively similar, but P60 exhibited slightly higher relative fluorescence at various N/P ratios. This result can be attributed to the higher PVP content of the copolymer. It confirmed that the presence of a highly hydrophilic and non-condensing segment in the copolymers may be expected to decrease affinity for binding to DNA due to an unfavorable entropy change [37].

3.4. Agarose gel electrophoresis

To investigate the effect of DNA condensation and the form of polymer/DNA complexes, a gel retardation assay

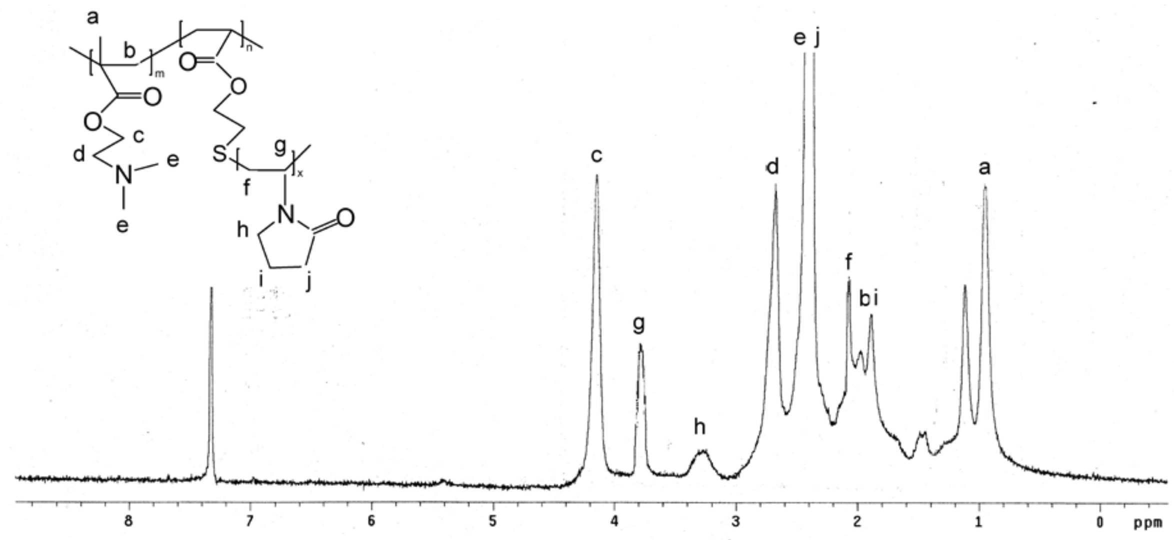


Figure 1. ^{1}H-NMR Spectra of P60 with signal assignments. Recorded at 300 MHz in $CDCl_3$ at ambient temperature.

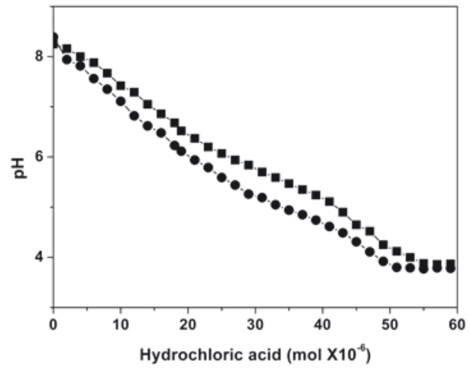


Figure 2. Potentiometric titration curve for copolymers in distilled water.The copolymer concentration was 0.5 mg mL^{-1}. P 30 (■), P 60 (●).

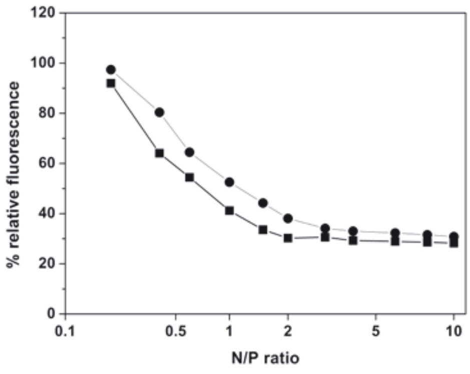


Figure 3. Ethidium bromide displacement by copolymers interacting with Herrin Sperm DNA at pH 7.4. P 30 (■), P 60 (●).

was used. Images for a series of monomer: nucleotide molar ratios are shown in Fig. 4. At low molar ratios of copolymer to DNA (N/P ratios of 0.1, 0.2, 0.5 and 1.0), free DNA bands were observed, displaced toward the anode, due to the presence of excess uncomplexed DNA. These free DNA bands gradually became dimmer with increasing N/P molar ratio, due to retarded migration of DNA in the gel, demonstrating that the graft copolymers obtained were capable of binding with DNA. At N/P ratios of 1.5 : 1 (Fig. 4a, lane 7) and 2.0 : 1 (Fig. 4b, lane 8), free DNA bands were no longer observed, but luminance could still be observed within the loading well, indicating the presence of non-compact, near-neutral polymer/DNA complexes, with some sites remaining available for ethidium bromide displacement. At high molar ratios (lane 10), the luminance band eventually disappeared and luminance could not be observed within the loading well. This suggests that DNA was complexed with copolymers so completely as to prevent the intercalation of ethidium bromide.

The effect of copolymer composition on condensation ability was also evaluated. For P30 (Fig. 4a), DNA was fully retained and no luminance was observed within the well at a ratio of 1.5 : 1 (lane 7). A similar phenomenon was not observed until the ratio reached 2 : 1 for P60 (lane 8). This suggests a slightly reduced ability to complex with DNA for graft copolymer with a higher PVP content.

3.5. ζ-potential and particles size measurements

There is evidence that, in order to transfect cells, polymer/DNA complexes with a size of about 0.15 - 0.2 μm and with a slightly positive ζ-potential are favorable [20,38]. Previous studies involving homopolymer PDMAEMA/DNA complexes showed that the ζ-potentials of the complexes increased to about +24 mV at high polymer/DNA ratios, when the N/P ratio is more than 12 [18]. To investigate the surface charge of the synthesized copolymer complexes with DNA, ζ- potential measurements were carried out in distilled water solution, to avoid the substantial masking of ζ- potentials by the high salt content in PBS solution. Fig. 5 shows the surface ζ-potentials of synthesized copolymer/DNA complexes at different N/P ratios. As show in Fig. 5, the naked plasmid DNA possesses a negative ζ- potential of about -29 mV. With the increasing

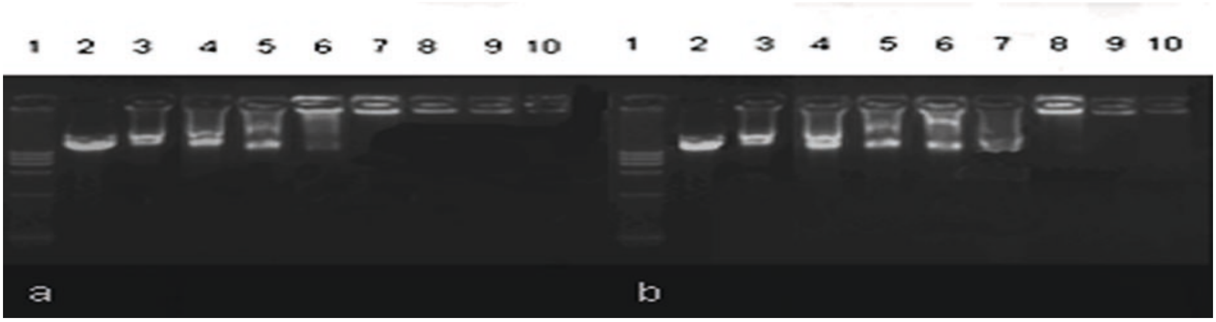


Figure 4. Agarose gel retardation assay. Lane 1 is molecular weight Marker. Lane 2 is DNA only. Lines 3 to10 correspond to polymer/DNA complexes in PBS at different N/P ratios of 0.1, 0.2, 0.5, 1.0, 1.5, 2, 3, and 5. P30 (a), P60 (b).

of N/P ratios, the ζ- potential of the complexes becomes less negative due to the neutralization of the negative charges on the DNA by PDMAEMA segments with the positive charges. When the N/P ratio was increased to about 3, the surface charge of polymer/DNA complexes was completely neutralized. This is consistent with a degree of protonation (α), of the amine groups in the graft copolymer of about 30% in distilled water [39]. Further increase of N/P ratios led to positive saturation values of the surface ζ-potential of the polymer/DNA complexes at 17.5 mV (P30) and 12.7 mV (P60) respectively, This can be attributed to the nonionic PVP grafts covering the surface of the complexes. The plateau value of the surface ζ- potential is slightly lower for the copolymer with higher PVP graft content, which can be ascribed to the reduction in charge density of the copolymer with increasing PVP content. Thus, incorporation of nonionic PVP grafts into cationic PDMAEMA can effectively decrease the surface charge of polymer/DNA complexes, which is deemed to be in favor both of transfecting cells and of alleviating the cytotoxicity of the complexes.

On the other hand, at high N/P ratios (> 4 : 1), relatively small complexes (size around 150 nm) were formed (Fig. 5). It has been confirmed that the synthesized copolymers are effectively able to condense the structure of DNA. The complexes of the P30 with DNA had larger size than those of the other copolymer throughout the N/P ratio range studied, and it was also obvious that the particle size of P30/DNA gradually decreased with increasing N/P ratio at low N/P ratios (< 4 : 1). Notably, the size of P60/DNA complexes exhibited little change throughout the N/P ratio range studied, suggesting that stable micelle-like complexes were formed even at relatively low N/P ratios. A similar phenomenon was reported in a recent study: random copolymers of DMAEMA with high NVP content yielded small particles independent of the N/P ratio when complexed with DNA. We therefore hypothesize that the introduction of nonionic PVP grafts is effective in forming stable micellar complexes and preventing aggregates of neutral polymer/DNA complexes due to steric repulsion associated with the hydrophilic interactions.

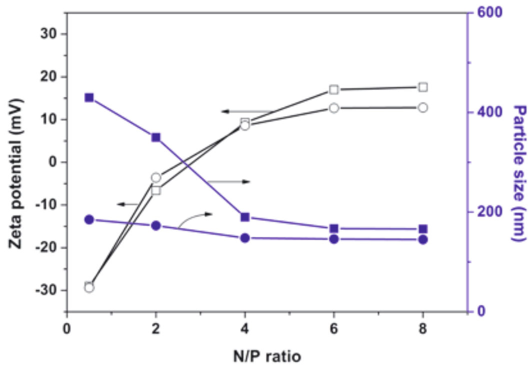


Figure 5. Surface ζ-potential and size of polymer/DNA complexes. The plasmid concentration was 2 $\mu g\ mL^{-1}$. P30 (■,□), P60 (●,○)

3.6. Morphology of polymer/DNA complexes

Examination of the morphology of the DNA complexes obtained in this study and of complexes with homopolymers of DMAEMA has led to markedly improved understanding of favorable effects on aggregation. All the electron micrographs of DNA complexes shown in Fig. 6 were obtained at a 1:1 N/P ratio. The images obtained clearly demonstrate significant morphological differences with varying content of PVP grafts in the copolymers. As previous articles have reported, DMAEMA homopolymer complexes tend to produce more aggregates (Fig. 6a). However, P30 complexes appeared to be more weakly associated and somewhat aggregated (Fig. 6b) and P60 complexes are uniformly discrete and spherical (Fig. 6c). A similar spherical morphology has recently been reported using amine methacrylate-based systems and PEG copolymers [22,36]. The reasons for the small size and uniform complexes formed using PVP grafted copolymer could be explained by a micelle-like

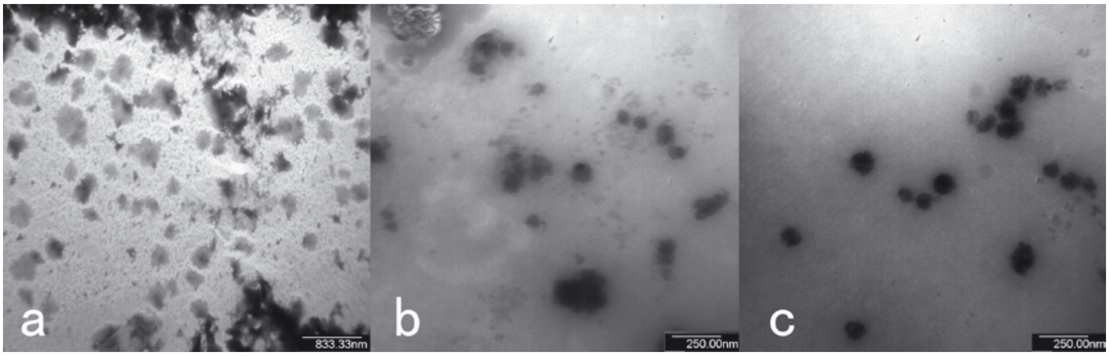


Figure 6. TEM images of polymer / DNA complexes at N/P ratio = 1:1. PDMAEMA/DNA complexes (a), P30/DNA complexes (b), P60/DNA complexes (c).

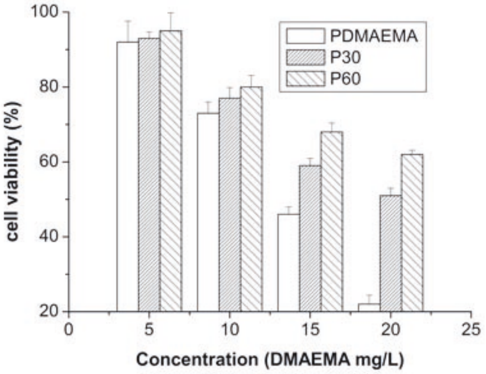


Figure 7. Effects of various polymers on relative SGC-7901 cell viability. The concentration of copolymer is expressed as the concentration of DMAEMA monomer.

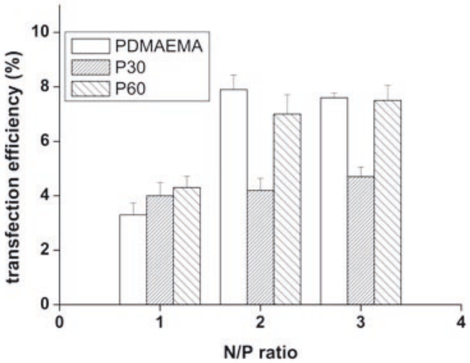


Figure 8. Transfection efficiency of various polymers with different N/P ratios.

structure. Oriented micellisation of the copolymer would be expected to lead the close association of polycationic segment with negatively charged DNA. This may allow the hydrophilic PVP grafts to be oriented outwards from the DNA containing core, producing a hydrophilic steric barrier to stabilize the complexes and prevent aggregation with other species [20,22,30]. These results confirm improved aggregation behavior of complexes with polymer modified by the inclusion of PVP grafts.

3.7. Cytotoxicity

The influence of the polymer concentration on the cell viability was evaluated in cell culture for the different polymers by MTT assays. To eliminate the influence of different DMAEMA content of the copolymers, the MTT assays for different polymers were performed with the same concentration of DMAEMA. Fig. 7 shows the effect of the polymer concentration on the cell viability. The cytotoxicity substantially increased with increasing polymer concentration. Interestingly, an increase in the incorporated PVP graft content of the copolymers used led to a lower cytotoxicity than PDMAEMA especially at high polymer concentration. The relatively high cytotoxicity of PDMAEMA might be ascribed to its ability to destabilize endosomes [10]. For P60, toxicity was almost the same at low concentration, but there was a 3-fold lower toxicity than for PDMAEMA at high concentration, such as 20 mg L^{-1}. A similar phenomenon has been reported before for other polymers [18,23,24]. Due to the biocompatibility of PVP [32], cytotoxicity can be expected based on the DMAEMA content of the polymer. The reduced cytotoxicity can therefore be attributed to the masking of effects of cationic charges in the polymer against cells by PVP grafts.

3.8. Transfection Studies

The transfection abilities of PVP-grafted PDMAEMA copolymers and of PDMAEMA homopolymer have been assessed to study the effect of PVP grafts on the transfection efficiency. The influence of the N/P ratio on the transfection efficiency was evaluated for the different polymers. Fig. 8 shows the relation between the N/P ratio and the transfection efficiency. For PDMAEMA, at low N/P ratio (N/P ratio = 1), a lower transfection efficiency was observed. This can be explained by the formation of the large aggregated particles, which obviously could not enter the cell easily. At high N/P ratio (N/P ratio = 3), the transfection efficiency is slightly lower than observed at intermediate N/P ratio (N/P ratio = 2). This is attributed to the presence of an increasing amount of free polymer, resulting in a cell viability decrease and a reduction of the transfection efficiency. For P60, at low N/P ratio, the transfection efficiency was higher than observed for PDMAEMA. The results suggested that at low N/P ratio, due to the formation of the uniformly discrete and spherical particles (size around 150 nm), the copolymer/plasmid complexes could enter the cell easily, resulting in better transfection abilities. However, there is slightly lower transfection efficiency than PDMAEMA at high N/P ratio. One possible explanation is that the presence of PVP provides a steric barrier around these complexes, inhibiting contact with the cells, with a consequent reduction in transfection efficiency. Another possible explanation is that due to the formation of the stable and lower positive charged copolymer/plasmid complexes, the ability to destabilize endosomes was relatively low as compared the homopolymer of DMAEMA.

4. Conclusions

Hydrophilic and biocompatible PVP grafts were successfully introduced to PDMAEMA and polycationic graft copolymers of poly(N-vinylpyrrolidone) were prepared. The copolymers were found to bind and condense DNA. The experiments showed that the copolymer/DNA complexes develop compact stable particles with diameters of 150 - 200 nm. Subsequently, a less positive surface charge of copolymer/DNA complexes and slightly lower cytotoxicity with the increasing fraction of PVP were confirmed. Furthermore, the transfection studies show some favorable effects for the copolymer systems on transfection efficiency *in vitro*. Thus, this study demonstrates improved biophysical properties for polycationic polymers incorporating PVP grafts as non-viral transfection agents.

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