Long Term Stability of Poly((2-dimethylamino)ethyl Methacrylate)-Based Gene Delivery Systems

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Purpose. To study the stability of polymer-plasmid complexes (polyplexes) both as an aqueous dispersion and in their lyophilized form.
Methods. The characteristics of the polyplexes (size, charge and transfection potential) were monitored at different temperatures. Moreover, we studied possible changes in the secondary and tertiary structure of the plasmid by agarose gel electrophoresis and by CD spectroscopy to gain insight into the mechanism of polyplex degradation.
Results. The polyplexes preserved almost their full transfection potential after aging in an aqueous solution of 20 mM Hepes (pH 7.4) containing 10% sucrose at 4 and 20°C for 10 months. On the other hand, the polyplexes aged at 40°C were rather unstable and lost their transfection capability with a half-life of around 2 months. During storage, conformational changes in the secondary and tertiary structure of DNA were observed. When naked plasmid DNA was aged at 40°C as an aqueous solution and complexed with polymer just before the transfection experiment, a slower drop in its transfection capability was observed. The freeze-dried polyplexes using sucrose as lyoprotectant almost fully retained their transfection efficiency, even when aged at 40°C for 10 months.
Conclusions. This study provides information about polyplex stability in aqueous dispersions on storage and demonstrates that freeze-drying is an excellent method to ensure long term stability.

KEY WORDS: plasmid; poly(2-dimethylamino)ethyl methacrylate; freeze-drying; long-term stability; gene therapy.

INTRODUCTION

Cationic lipids and polymers are currently under investigation to be used in non-viral gene delivery systems (1–5) Although many papers have been published on strategies to improve the transfection efficiency of the non-viral systems (e.g., by introducing homing devices, endosome disruptive agents and nuclear localization signals (6–8)), no papers have been published yet on the long term stability of these systems. To fulfil the stability requirements defined for pharmaceutical products, these systems need to be physico-chemically stable with a shelf-life of at least two years. We have recently shown that the transfection potential of a simple transfection complex composed of PDMAEMA and plasmid as well as of more sophisticated systems composed of plasmid and transferrin-conjugated polyethyleneimine or transferrin-polylysine could be fully preserved by lyophilizing these complexes in the presence of a suitable lyoprotectant (9–10). In this paper we studied the stability of polymer-plasmid complexes (polyplexes) both as an aqueous dispersion and in their lyophilized form. The characteristics of the complexes (size, charge and transfection potential) were followed in time at different temperatures. Moreover, we monitored possible changes in the secondary and tertiary structure of the plasmid by gel electrophoresis and CD spectroscopy to gain insight into the mechanism of polyplex degradation.

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MATERIALS AND METHODS

Materials

pCMV-lacZ plasmid contains a bacterial lacZ gene preceded by a Nuclear Localization Signal under control of the CMV promoter (11). The plasmid was isolated from *E. Coli.* and purified as described before (12). 2-(Dimethylamino)ethyl methacrylate (DMAEMA) was obtained from Fluka (Buchs, Switzerland). RPMI-1640 medium and DMEM (Dulbecco's modified Eagles medium) were obtained from Gibco, Breda, The Netherlands. Fetal Calf Serum (FCS) was purchased from Integron, Zaandam, The Netherlands. Cells were cultured in complete DMEM medium, which was prepared by supplementing plain DMEM with FCS (final concentration 5%), Hepes (final concentration 25 mM, pH 7.4), penicillin (final concentration 100 IU/mL), streptomycin (final concentration 100 μg/mL) and amphotericin B (final concentration 0.25 μg/mL). X-Gal (5-bromo-4-chloro-3-indoyl-β-galactopyranoside) was from Gibco, Breda, The Netherlands. Sucrose was obtained from Merck, Germany. Poly-L-aspartic acid (Mw. 15,000-50,000) was obtained form Sigma Chemical Co., St. Louis, MO, USA. Agarose gel was from Hispanagar, Burgos, Spain. All other chemicals and reagents were of analytical grade. PDMAEMA (number average molecular weight (Mn) and weight average molecular weight (M_w) were 47 * 10^3 and 280 * 10^3 g/mol, respectively) was prepared by a radical polymerization of 2-(dimethylamino)ethyl methacrylate essentially as described previously (12).

Preparation of PDMAEMA-Plasmid Particles

PDMAEMA was dissolved in Hepes buffer (20 mM, pH 7.4) to a concentration of 300 μg/mL. The plasmid stock solution was diluted to a concentration of 400 μg/mL in the same buffer. Next, 100 μL plasmid solution was mixed with 500 μL aqueous solution of Hepes buffer (20 mM, pH 7.4) containing sucrose (20% w/v). Subsequently, 400 μL polymer solution was added and gently mixed for 5 s (Vortex Genie 2, Scientific Industries, INC., Bohemia, NY. USA). Thus, the final dispersions contained 20 mM Hepes, 0.12 mg/mL PDMAEMA, 0.04 mg/mL plasmid, and 10% sucrose. In case of freeze drying, a 30 min incubation time at ambient temperature was used and, subsequently, the formed complexes were frozen and freeze-dried (see next section).

Freeze-Drying

Aliquots of PDMAEMA-plasmid dispersions of 1 mL were filled into 10 mL glass vials and frozen for 60 min by placing them on the shelf (pre-cooled by circulating silicone oil of

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−40°C) of a Leybold GT4 pilot-production freeze-dryer. The set pressure was 16 Pa which corresponds to the vapor pressure of ice at about −38°C. During the primary drying process, the temperature was kept below the collapse temperature of sucrose (−32°C) (13). After 40 h, the shelf temperature was raised to +20°C with a rate of 10°C/h and kept at this temperature (under a pressure of 1 Pa) for 2 h. Finally, the vials were closed with a closing device under vacuum. For freeze-drying of plasmid DNA, 100 μL plasmid solution (400 μg/mL in Hepes buffer) was mixed with 500 μL aqueous Hepes buffer plus sucrose (20% w/v) and freeze-dried according to the same procedure.

Aging of Polyplexes and Plasmid DNA

The freeze-dried and aqueous dispersions of polyplexes as well as the solutions of naked DNA were aged at 4°C, 20°C, and 40°C for 10 months. Samples were taken at regular time points and evaluated for their physico-chemical properties (size, zeta-potential, DNA secondary and tertiary structure) and their transfection potential.

Moisture Analysis

Residual moisture contents of the freeze-dried cakes were determined by the Karl-Fisher method using a Mitsubishi moisturemeter (model CA-05, Tokyo, Japan).

Rehydration of the Freeze-Dried Cakes

After freeze-drying, the cakes of the polyplexes were reconstituted in 1 mL water and their size and zeta-potential were determined. For transfection studies, 7 mL RPMI-1640 medium was further added to this dispersion, resulting in a final concentration of 15 and 5 μg/mL for the polymer and plasmid, respectively. Freeze-dried naked plasmid was reconstituted in 600 μl water and 400 μl polymer solution (300 μg/ml) was added. This sample was used for size and zeta-potential measurements. For transfection, the same procedure as used for freeze-dried polyplexes was followed.

Particle Size and Zeta-Potential Measurements

The z-average particle size and polydispersity index (p.d.) of the PDMAEMA-plasmid particles were determined by dynamic light scattering (DLS) at 25°C with a Malvern 4700 system using 25 mW He-Ne laser (NEC Corp., Tokyo, Japan) and the automeasure version 3.2 software (Malvern Ltd, Malvern, UK). As a measure of the homogeneity of the colloid dispersion, the system reports a polydispersity index (p.d.). This index ranges from 0.0 for an entire homogeneous up to 1.0 for a completely inhomogeneous dispersion. For data analysis, the viscosity (0.8905 mPa.s) and refractive index (1.333) of water were used for the aqueous Hepes solution at 25°C. The refractive index and viscosity of a 10% sucrose solution were determined using a refractometer and by measuring the diffusion coefficient of a latex with known diameter (100 nm) essentially according to the method described by De Smidt and Crommelin (14), respectively.

Zeta-potential measurements were conducted by determining the electrophoretic mobility at a temperature of 25°C with a Zetasizer 2000 (Malvern Ltd., Malvern, UK). For, viscosity and refractive index values: see above.

Agarose Gel Electrophoresis

Freeze-dried naked plasmid and aqueous plasmid solutions aged at 40°C up to 10 months were analyzed by electrophoresis at 5.6 V/cm using a 0.7% agarose gel in Tris-acetate-EDTA buffer (pH 7.6). For each sample, 0.5 μg plasmid DNA was applied per slot. DNA was visualized by ethidium bromide (0.6 μg/mL in gel). To analyze plasmid complexed with polymer, 20 μl aliquot of the samples (0.8 μg plasmid and 2.4 μg polymer) was incubated with 5 μl of a polyaspartic acid solution (20 mg/mL PBS) to dissociate the polyplexes (15). After 18 h incubation at ambient temperature, the samples were applied on a 0.7% agarose gel.

Circular Dichroism (CD) Measurements

CD spectra were recorded from 220 to 350 nm at ambient temperature in quartz cells with a path length of 1 cm using a spectropolarimeter J-600 (Jasco, Tokyo, Japan). The scan rate was 20 nm/min and each measurement was the average of three repeated scans in steps of 2 nm. The values of λ and Δε are presented after subtraction of the CD scan of the appropriate buffer. The DNA concentration was 13.3 μg/ml ($4 * 10^{-5}$ M, based on number of DNA bases).

Cell Culture and Transfection

For the gene transfer studies, COS-7 cells (cells of SV-40-transformed African green monkey kidney) were used essentially as described previously (12). The cells were seeded in a flat-bottom 96-well plate (1×10^4 cells per well (0.38 cm^2)) 24 h before transfection in complete DMEM. Thereafter, the PDMAEMA-plasmid complexes (volume 200 μl) were added to the cells and incubated for 1 h at 37°C and 5% CO_2. After removal of the transfection complexes, the cells were cultured for an additional 2 days in complete DMEM and then evaluated for transfection efficiency.

Expression of the pCMV-lacZ gene was established by incubation of fixed cells (0.25% glutaraldehyde; 5 min at 4°C) with a X-gal solution (0.8 mg/mL in phosphate buffer pH 7.4) together with 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$ and 2 mM $MgCl_2$ for 24 h at 37°C. By using a light microscope, transfected cells were made visible as blue spots and were quantified by counting the number of blue spots in each well. Transfection values (relative transfection efficiency) were normalized to the number of transfected cells found after incubation of the cells with freshly prepared polymer/plasmid complexes (polymer/plasmid (w/w) ratio of 3, in Hepes buffer (20 mM, pH 7.4)).

RESULTS

Experimental Set-Up

Polyplexes were aged for a period of up to 10 months at three different temperatures (4°C, 20°C, and 40°C) as aqueous dispersions as well as in the freeze-dried state. As a control naked plasmid was aged at 40°C. Sucrose was selected as a lyoprotectant since we demonstrated that this is an excellent compound to preserve the short term transfection potential of polyplexes (9) Moreover, 40°C was selected as highest aging temperature since at this temperature the matrix is still in its glassy state (9) (Tg = 50°C). We evaluated the aged polyplexes

for both their physico-chemical characteristics and their transfection potential. In a recent study we demonstrated that no significant hydrolysis of the ester side chains of PDMAEMA occurred even at high temperature and extreme pH values, indicating that the polymer is stable (16). Therefore, we focused our attention on changes which might occur in the secondary and tertiary structure of the plasmid both in its free form and complexed with polymer.

Physicochemical Characteristics of the Aged Polyplexes

Dynamic light scattering measurements revealed that after rehydration of the cake, the size of the lyophilized polyplexes remained constant in time (diameter around 150 nm, Fig. 1). This figure also shows that for the polyplexes aged as aqueous dispersions at 4°C and 20°C no change in particle size was observed either. On the other hand, the size of the polyplexes aged at 40°C gradually increased with time (from 150 to 350 nm after 10 months) suggesting that limited aggregation occurred (t-test, $p < 0.05$). The size of the polyplexes obtained by complexing polymer with aged plasmid (lyophilized or aqueous) was not affected by the aging time and amounted to 150 nm (results not shown). All polyplexes studied have a p.d. of around 0.2 indicating narrow size distribution. No changes in the charge of the polyplexes (zeta potential 25–30 mV) were observed for the polyplexes aged under different conditions (results not shown).

Transfection Efficiency of Aged Polyplexes

Figure 2a and Fig. 2b show the transfection potential of polyplexes aged in aqueous dispersions containing 10% sucrose and in the freeze-dried state, respectively. The polyplexes almost fully preserved their transfection potential after aging at 4 and 20°C (Fig 2a). But, polyplexes aged at 40°C were rather unstable and lost their transfection potential (half-life around 2 months). When naked plasmid DNA was aged in solution at 40°C and complexed with polymer just before the transfection experiment, a reduction in its transfection capability was observed as well. However, for naked plasmids loss of transfection efficiency was slower than for the polyplexes (t-test, $p < 0.05$). Polyplexes and naked plasmid were also aged in aqueous media without sucrose at 40°C. Again, the plasmid in the polyplexes lost its transfection efficiency faster than naked plasmid: half-life 2 and 4 months, respectively (data not shown).

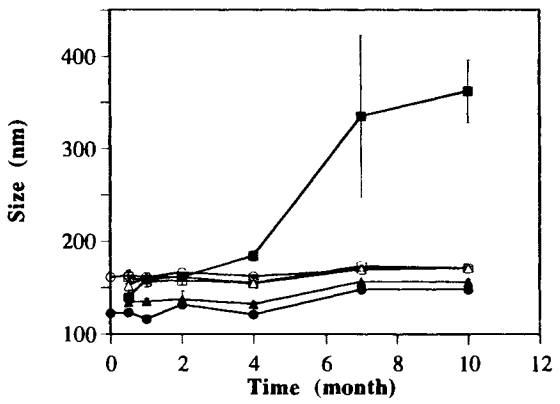

Fig. 1. Size of polyplexes aged in 10% sucrose/aqueous solutions at 4°C (●), 20°C (▲) and 40°C (■) and in the freeze-dried state at 4°C (○), 20°C (Δ) and 40°C (□). The results are expressed as mean values ± S.D. of 3 experiments.

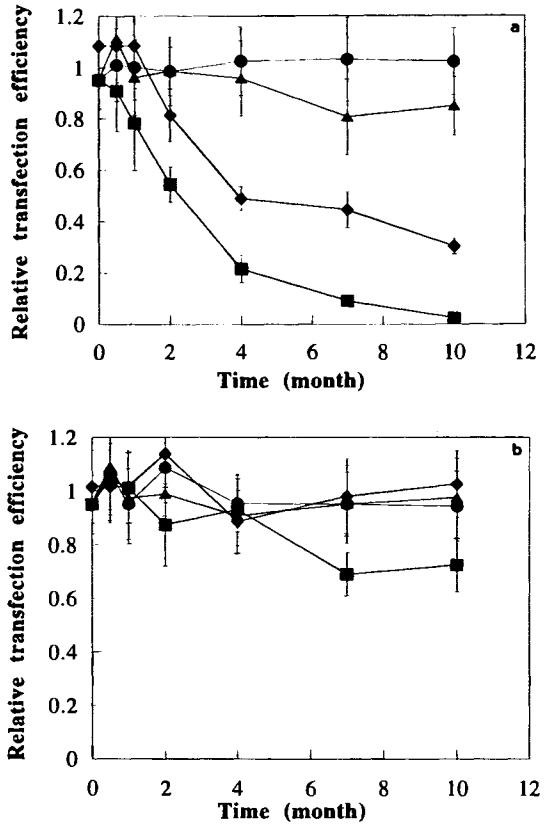

Fig. 2. Relative transfection efficiency of aged polymer/plasmid complexes at 4°C (●), 26°C (▲) and 40°C (■) and naked plasmid at 4°C (◆). Aging was done in 10% sucrose/aqueous solutions (Fig. 2a) or in freeze dried form (10% sucrose, Fig. 2b); naked plasmid was complexed with polymer just before the transfection experiment. The results are expressed as mean values ± S.D. of 3 experiments.

After 10 months, no change in the morphology of the freeze-dried samples was observed by visual inspection. The moisture contents (0.5%) remained constant in time as well. As observed for the polyplexes aged in aqueous media at 4°C and 20°C, freeze-dried polyplexes aged at the same temperatures preserved their full transfection potential (Fig. 2b). This figure also shows that, although some reduction in transfection efficiency was observed for the freeze-dried polyplexes aged at 40°C (20–30% reduction after 10 months), their stability was superior compared to polyplexes aged in aqueous media. Interestingly, the naked plasmid in freeze-dried form fully preserved its transfection potential, which suggests that plasmid complexed with polymer in freeze dried form also lost its transfection potential faster than naked plasmid.

DNA Tertiary Structure

Electrophoresis in agarose gels was used to study possible changes in the tertiary structure of plasmid DNA. Polyaspartic acid was used to dissociate the plasmid from the polymer in the polyplex. The plasmid used in this study consisted of a

mixture of supercoiled and open circular DNA, with some traces of multimers (Fig. 3, lane 1). The freshly prepared plasmid shows an identical pattern on agarose gel as free plasmid after the storage for 10 months at 4°C. When polyplexes were treated with polyaspartic acid shortly after their preparation, the same pattern was observed (lane 2), which indicates that complexation of polymer with plasmid does not lead to changes in the tertiary structure of the plasmid. Also, when the plasmid was liberated from lyophilized polyplexes with polyaspartic acid after 10 months of aging, the same electrophoretic pattern was observed as for freshly prepared naked plasmid (lane 6–8). No large changes in the tertiary structure of the plasmid were observed either when the polyplexes were aged as an aqueous dispersion at 4°C (Fig. 3, lane 3). However, supercoiled and open circular plasmid were hardly found in the polyplexes aged in aqueous media at 20°C and 40°C after 10 months and an intense band in and close to the application site was observed, particularly for the sample aged at 40°C (lane 4 and 5, Fig. 3, respectively). Apparently, polyaspartic acid could not dissociate these polyplexes.

We also analyzed the tertiary structure of naked plasmid aged as an aqueous solution and in the lyophilized state. After 10 months of storage of naked plasmid in freeze-dried form at 40°C, the supercoiled and open circular form of the plasmid were still present (lane 8, Fig 3); the electrophoretic pattern was not significantly different from that of fresh plasmid. However, naked plasmid aged in an aqueous solution for 10 months at 40°C showed the presence of about 50% linear and 50% open-circular forms; supercoiled plasmid being virtually absent (results not shown).

To gain insight into the kinetics of the changes in the plasmid tertiary structure, polyplexes as well as naked plasmid were aged in aqueous solution at 4 and 40°C for 4 months. It was demonstrated that at 4°C no detectable changes in the structure occurred for both the polyplexes and naked plasmid (Fig. 4, lane 3 and 8, respectively). However, already after one month of aging at 40°C, no bands of supercoiled and open circular DNA were found in the polyplexes and most of the DNA hardly moved into the gel and stayed at the application site. Aging of naked plasmid in an aqueous solution at 40°C for one month showed that a major part of the DNA was in the open circular form, whereas only a minor part was still in the supercoiled form. Further aging resulted in the disappearance of the supercoiled form and the concomitant formation of open-circular and linear DNA, and an evidence of existence of oligomeric DNA or aggregates (Fig. 4).

DNA Secondary Structure

The CD spectrum of naked plasmid shows a positive peak with a maximum at about 274 nm and a negative peak with a minimum at 246 nm, which indicates a typical B-form DNA (17–18) (Fig. 5). Complexation with polymer resulted in a shift of both peaks to a higher wavelengths (290 and 254 nm, respectively), which suggests that a B to C transition had occurred (19). Moreover, complexation of plasmid and polymer is associated with an increase in the intensity of the negative peak. This can possibly be ascribed to formation of DNA helices which are oriented parallel to each other (so called Ψ DNA (20). The freeze-dried polyplexes aged for 10 months at different temperatures (4°C, 20°C and 40°C) and the polyplexes stored as an aqueous dispersion at 4°C showed similar CD spectra as those obtained for freshly prepared polyplexes (see Fig. 6, results of freeze-dried samples not shown). Even after 10 months, the changes in the CD spectrum of polyplexes aged as an aqueous dispersion at 20°C only concerned the $\Delta\varepsilon$, not the peak maxima/minima (Fig. 6). But, the CD spectrum of polyplexes aged as an aqueous dispersion at 40°C showed a gradual shift in the intensity and the wavelengths of both the maximum positive and the minimum negative peak (position

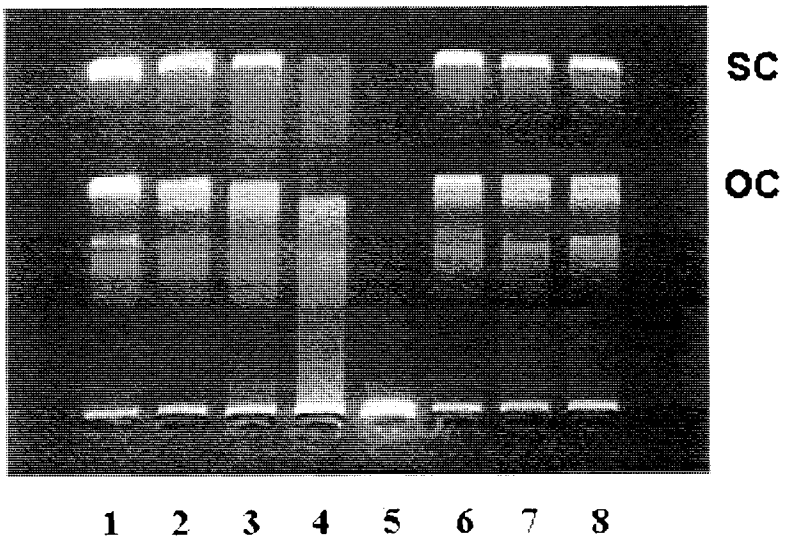

Fig. 3. Agarose gel electrophoresis of plasmid. Naked plasmid (after storage for 10 months at 4°C) (lane 1); plasmid after dissociation of freshly prepared polyplexes (lane 2); plasmid after dissociation of aged polyplexes in sucrose/aqueous solutions after 10 months at 4°C (lane 3), 20°C (lane 4) and 40°C (lane 5) plasmid after dissociation aged polyplexes in freeze-dried form after 10 months at 4°C (lane 6), 20°C (lane 7) and 40°C (lane 8). Dissociation of the polyplexes was established by polyaspartic acid.

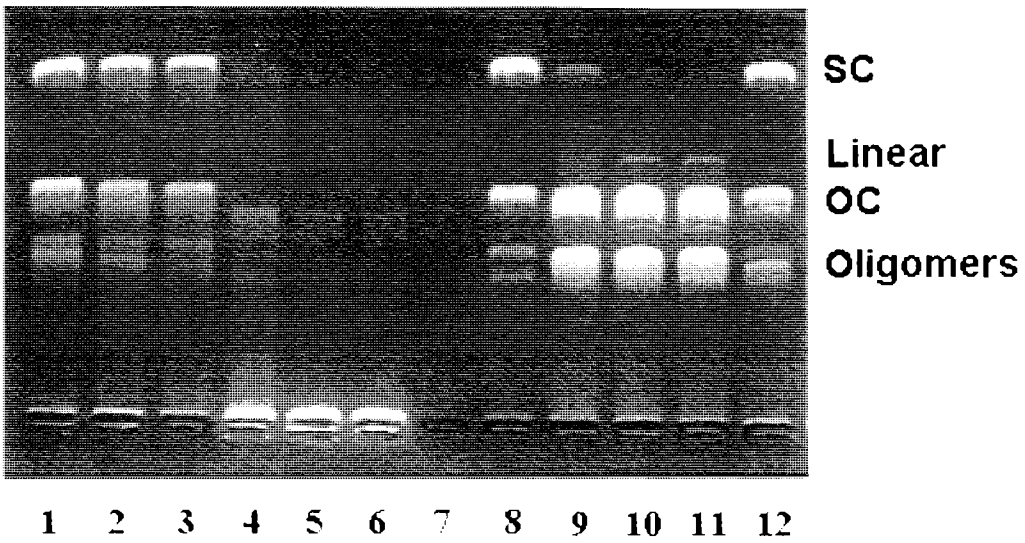

Fig. 4. Agarose gel electrophoresis of plasmid. Naked plasmid (lane 1, 8 and 12); plasmid after dissociation of freshly prepared polyplexes (lane 2 (freshly prepared) and lane 3 (stored for 10 months at 4°C); plasmid after dissociation of polyplexes aged as an aqueous dispersions (Hepes, 20 mM, pH 7.4) at 40°C after 1 month (lane 4), 2 months (lane 5) and 4 months (lane 6). Naked plasmid aged as an aqueous dispersion (Hepes, 20 mM, pH 7.4) at 40°C for 1 month (lane 9), 2 months (lane 10) and 4 months (lane 11).

after 10 months: 300 and 260 nm, respectively). Moreover, the change in $\Delta\varepsilon$ of the negative peak over time indicated that more Ψ DNA is formed. Both observations suggest that the interaction between the polymer and plasmid became stronger in time.

The CD spectrum of naked plasmid aged in aqueous solution for 10 months was not significantly different from a fresh plasmid sample. Interestingly, when aged plasmid was complexed with polymer only a slight change in the CD spectrum was observed compared to the naked plasmid (slight shift of the position of both the maximum positive and the minimum negative peak to a higher wavelength; Fig. 6).

DISCUSSION

This paper shows that aqueous dispersions of polymer-plasmid complexes preserve their transfection efficiency over a 10 months period of storage when kept at 4°C and 20°C; but,

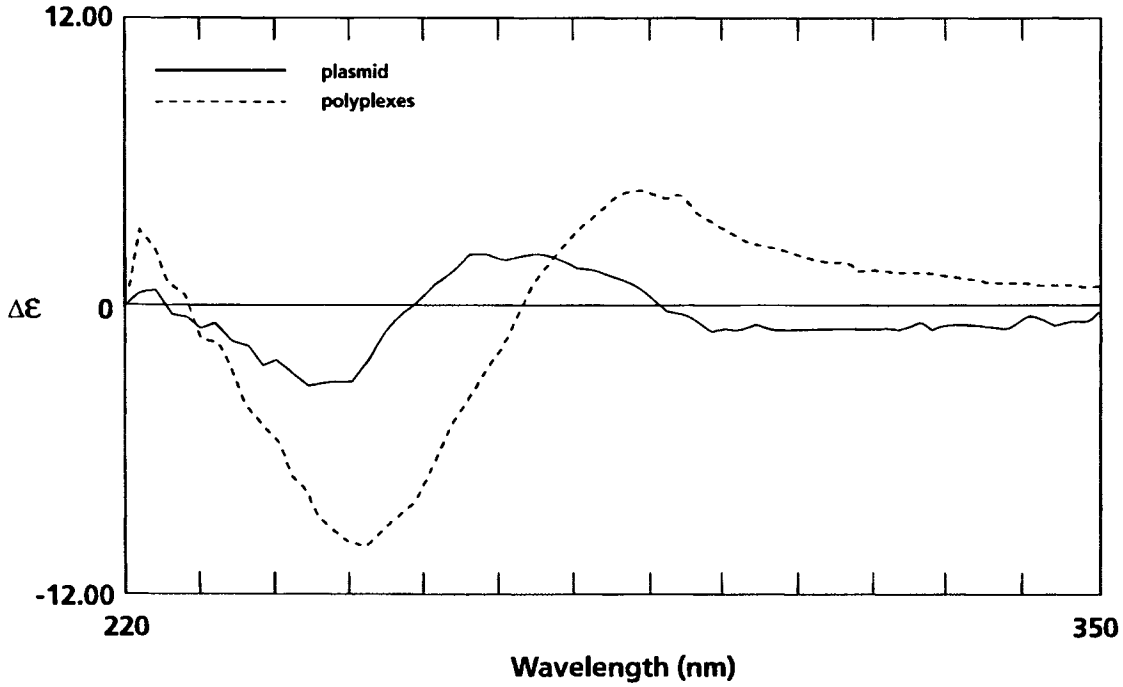

Fig. 5. Typical CD spectra (n = 3) of free plasmid and freshly prepared polyplexes. The plasmid concentration was 13.3 μg/mL, the polymer/plasmid ratio was 3/1 (w/w).

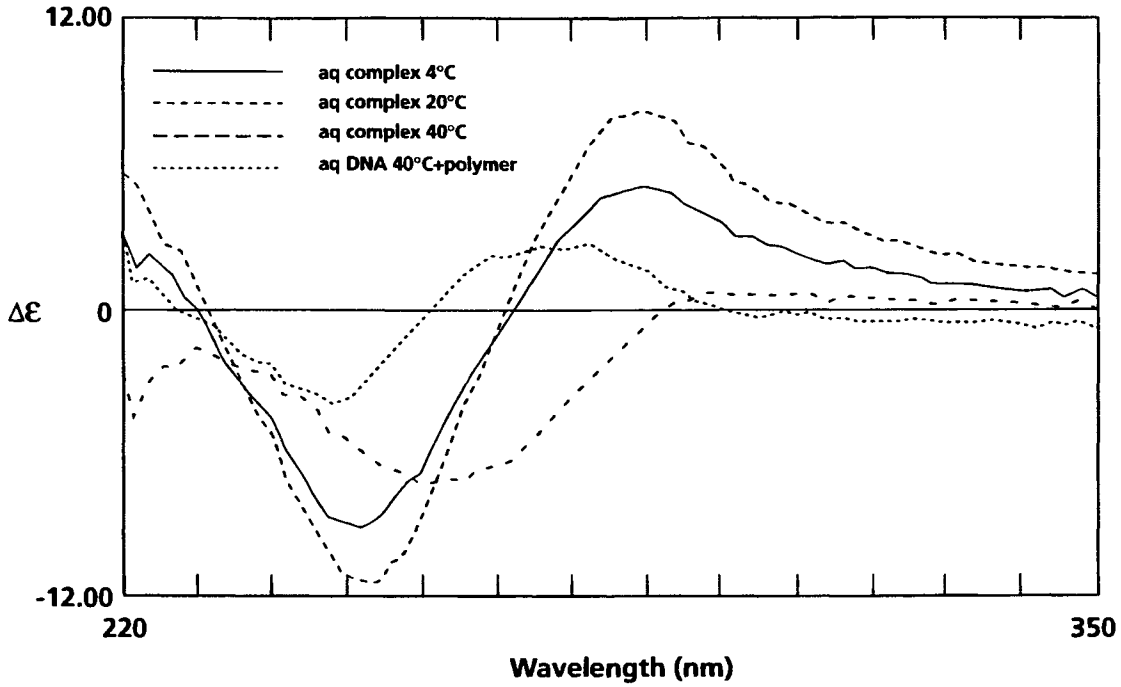

Fig. 6. Typical CD spectra (n = 3) of polyplexes aged in 10% sucrose/aqueous solutions for 10 months at 4°C, 20°C and 40°C. The spectrum of aged plasmid (40°C, 10 months) and then complexed with polymer is also shown. The plasmid concentration was 13.3 μg/mL plasmid; the polymer/plasmid ratio was 3/1 (w/w).

they lost a substantial part of their transfection potential when aged at elevated temperatures (40°C) (Fig. 2a). Since only the polyplexes stored at 40°C grew in size (Fig. 1), their reduced transfection capacity might be ascribed to aggregation. However, the size of the freshly prepared polyplexes with aged plasmid was always around 150 nm, independent of aging time. As their transfection efficiency dropped over storage time, the observed drop in transfection efficiency (Fig. 2a) can not be ascribed to aggregation, but should be caused by changes occurring in the plasmid molecule. Interestingly, the naked plasmid was less susceptible for transfection capacity loss than plasmid complexed with polymer (Fig. 2a). DNA can undergo a depurination reaction which is often followed by a β-elimination leading to a strand scission (21). This degradation process is associated with the conversion of supercoiled to open circular and linear forms of DNA (22–23). Indeed, electrophoresis demonstrated (Fig. 4) that upon aging of naked plasmid and polyplexes, the supercoiled plasmid disappeared ultimately leading to the formation of open-circular DNA, a small amount of linear DNA and oligomers. Recently, some studies demonstrated that the relative transfection potential of the supercoiled form ≥ open-circular form > linear form (24–26). The observed degradation of plasmid might therefore very well explain the reduced (or even absent) transfection capability of the aged polyplexes. The higher degradation rate of plasmid complexed with polymer as compared to naked plasmid is most likely caused by the accelerating effect of the tertiary amine groups of the polymer on the depurination reaction (27–28).

The results presented in this paper suggest that, besides chemical degradation of DNA, also physical aging processes occurred in the polyplexes. Firstly, electrophoretic analysis showed that upon incubation of the polyplexes with polyaspartic acid, the amount of DNA which remained on the application site of the gel increased in time (Fig. 4), suggesting that the strength of the polymer-plasmid interaction increased. Secondly, CD analysis revealed that the positive peak increased in intensity upon storage at 20°C for 10 months, also indicating that the DNA-polymer interaction increased in strength with time. A time-dependent maturation process in lipid/DNA complexes which resulted in both an increase in size and serum resistance has been reported before (29). This means that this maturation process might help to decrease the transfection capability of the aged polyplexes. In our study no direct relationship between maturation and transfection efficiency could be established with gel electrophoretic analysis as the application slots of lane 4, 5 and 6 in Fig. 4 (representing polyplexes after 1, 2 and 4 months of storage at 40°C) show the same unsuccessful dissociation of the polyplexes with polyaspartic acid: the plasmid remains at the application site. However, this maturation did not affect the transfection efficiency in a parallel fashion as transfection dropped between 1 and 4 months of storage of the polyplexes at 40°C (Fig. 2a). Interestingly, the plasmid aged in an aqueous dispersion at 40°C has the same CD spectrum as the freshly prepared one (data not shown). This is in an agreement with the study of Patrick *et al.* (30), who showed only modest CD changes above 210 nm on heat denaturation of T_7 phage DNA. Upon addition of the polymer to the plasmid aged at 40°C, no changes in the CD spectrum were found indicating that the DNA bases of aged plasmids could not be dehydrated anymore (17). The exact physical structure of the latter complexes is not clear yet, but the complexes are apparently less packed so water is not excluded from DNA bases.

Evaluation of the freeze dried samples revealed that polyplexes aged at 4 and 20°C preserved their physico-chemical characteristics as well as their transfection potential. In addition, neither electrophoretic nor CD analysis showed significant changes in the secondary and tertiary structure in the plasmid molecules. Although naked plasmid was stable in the freeze-dried form at 40°C, some degradation in freeze-dried polyplexes occurred under the same conditions (Fig. 2b). This again indicates that the polymer, once complexed with plasmid, accelerates the depurination of the DNA. However, the stability of the polyplexes in the freeze-dried form was considerably better than the stability when kept as an aqueous dispersion. In terms of transfection efficiency, the half-life of the polyplexes at 40°C amounted to 1–2 months and 20–30 months (estimated) in an aqueous dispersion and in freeze-dried form, respectively.

In conclusion, we have demonstrated that PDMAEMA-pCMV lac Z plamid complexes are stable when stored in aqueous solution at low temperature. The stablity of this system could even be inceased by lyophilization. A lyphilized formulation preserved almost its full transfection potential when aged just below the glass transition temperature of the sucrose matrix. Altough these findings are stricly speaking only applicable for PDMAEMA-pCMV lacZ plasmid formulations, the results might be extended to other polyplex and lipoplex formulations.

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