Stabilisation by freeze-drying of cationically modified silica nanoparticles for gene delivery

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We dedicate this paper to Professor Hans E. Junginger on his 60th birthday for his scientific approach, honesty and human warmth

Abstract

Core shell silica particles with a hydrodynamic diameter of 28 nm, an IEP of 7.1 and a zeta potential of $+35$ mV at pH 4.0 were synthesised. The role of freeze-drying for the conservation of zwitterionic nanoparticles and the usefulness of different lyoprotective agents (LPA) for the minimisation of particle aggregation were studied. The activity of the nanoparticles was measured as DNA-binding capacity and transfection efficiency in Cos-1 cells before and after lyophilisation. It was found that massive aggregation occurred in the absence of LPA. Of the various LPAs screened in the present investigations, trehalose and glycerol were found to be well suited for conservation of cationically modified silica nanoparticles with simultaneous preservation of their DNA-binding and transfection activity in Cos-1 cells.

Keywords: Freeze-drying; Gene delivery; Lyoprotective agents; Nanoparticles; Silica

1. Introduction

Silica nanoparticles have been described as drug delivery systems applied both as excipients in drug delivery systems (Ahola et al., 1999) and as a gel matrix (Bottcher et al., 1999; Kortesuo et al., 1999) with controlled release effects (Kortesuo et al., 2000). Silica nanoparticles are also used as the major component of drug carriers (Shimada et al., 1995) and for in-vitro gene transfer (Kneuer et al., 2000). With their well-defined silanol groups at the surface, the silica

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nanoparticles offer many possibilities for modification when a specific delivery and transfer system for drugs is to be designed. Generally, the disadvantage of these nanoparticles and other colloidal carriers is their tendency to agglomerate during storage, especially in liquid formulations. This is due to the immense surface area of the system and the resulting thermodynamic instability that favours aggregation of the colloidal particles. The inherent instability is a severe limitation for the practical application of nanoparticular drug carriers in medicine.

Freeze-drying is a well-established method for the preservation of unstable molecules over long periods of time, as well as a storage method under sterile conditions. Examples include biotech drugs such as DNA (Li et al., 2000), proteins (Hsu et al., 1995;

Corveleyn and Remon, 1996) and peptides (Diminsky et al., 1999). Lyophilisation as a method to stabilise inherently unstable colloids has so far gained considerably less attention. The addition of surface active molecules to reduce surface energies and valencies has remained more popular. However, as nanoscaled drug delivery systems become more widespread, the challenge of their long-term stabilisation will become of increasing importance.

Only a few examples of successful lyophilisation of nanoparticles are available in the literature. However, the focus remains on negatively charged or neutral nanoparticles. A successful freeze-drying of polymer–DNA complexes with a slightly positive zeta potential was reported by Cherng et al. (1997). Furthermore, most of the materials under investigation were stabilised by surface coatings. Recently, the role of sucrose to inhibit the aggregation of methoxy-poly(ethylene oxide)–poly(lactic acid) (MPEO–PLA) nanoparticles during freeze-drying has been investigated (Zambaux et al., 1999), and PVA nanoparticles have also successfully been lyophilised (Murakami et al., 1999). The behaviour of these PVA-coated PLGA nanoparticles was further compared with that of poloxamer-188-coated PLGA nanoparticles during the freeze-drying process (Quintanar Guerrero et al., 1998). A more systematic study was performed by Molpeceres et al. (1997), who investigated the effect of the addition of 5, 10 and 20% of the LPAs mannitol, sorbitol, trehalose and glucose on the aggregation of 100 nm poly-caprolactone nanoparticles. In these studies, both glucose and trehalose (>10%) permitted adequate reconstitution of the freeze-dried product with conservation of the encapsulated biomolecules. Similar investigations were also carried out on solid lipid nanoparticles and on lipid–DNA complexes (Allison et al., 2000; Zimmermann et al., 2000). Most studies showed a good preservation of the physico-chemical particle properties when the lyoprotectant was employed at a sufficient concentration. However, in some cases and especially when cationic and anionic polyelectrolytes were present together, the results were less satisfactory. Molina et al. (2001), for instance, observed a significant loss of biological (transfection) efficiency of polyethyleneimine–DNA nanoparticles after an initial freezing step.

Therefore, our aim was to study the effects of different classes of lyoprotective agents (LPA) on the lyophilisation behaviour of nanoparticles that possess negative as well as positive surface charges and which are not pre-coated with a stabilising surface layer. The influence on physicochemical characteristics as well as that on suitable markers of biological activity should be studied. Cationically modified silica particles provide a very suitable model for this purpose. After reaction of the silanol groups on the particle surface with aminoalkylsilanes, both anionic silanol $(Si-O^-)$ as well as cationic amino $(-NH_3^+)$ groups will be present. In addition, this model system is very sensitive to particle collisions as there is a high probability of the condensation of silanol groups into stable $Si-O-Si$ bridges and hence permanent particle aggregation (Harding et al., 1997). In addition, such cationically modified silica particles possess an interesting biological activity that can be readily measured. They have recently been shown to bind plasmid DNA and to transfect cultured cells in vitro (Kneuer et al., 2000). Both activities were sensitive to particle properties including size and charge. Therefore, we also compared the DNA-binding capacity and the transfection efficiency, before and after lyophilisation, as a measure of biological activity.

2. Materials and methods

2.1. Synthesis and surface modification of silica nanoparticles

Nanoparticles were synthesised by modification of commercially available silica particles (IPAST, NISSAN Chemical Industries, Tokyo, Japan) with N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS) as follows: 60.0 g particle suspension (corresponding to 20.0 g particle dry weight), 200 g demineralised water and 40 ml concentrated acetic acid were stirred for 20 h at 80 °C in a round bottom flask under reflux with 20 g AEAPS. The product was chilled, mixed with 300 ml ethylene glycol, rotated for 4 h under vacuum (20 mbar) at 50 °C and dialysed five times for 24 h against 20 l of deionised water. The particle suspension was filtered through a 0.2 μm filter resulting in 9 mg/ml concentrated suspension. Elemental analysis (CHN) of dehumidified particles was performed using a CHN analyser RC-900 (Leco, St. Joseph, MI, USA).

2.2. Determination of particle size

Particle size was determined by photon correlation spectroscopy (PCS) on an ALV 5000 (Laser Vertriebsgesellschaft GmbH, Langen, Germany) at a scattering angle of 90° (sampling time: 200 s). Autocorrelation was performed using the "contin" method. For PCS measurements, all samples were diluted at least 50-fold in demineralised water, resulting in comparable viscosities. Hence, no corrections for the effect of the additives were necessary.

2.3. Zeta potential measurements

Surface charge of nanoparticles was judged by zeta potential measurement on a Malvern Zetasizer 2000 HS (Malvern, UK) with a flow measurement cell connected to a Mettler DL 25 (Mettler-Toledo, Giessen, Germany) autotitrator via a circulating system. Within the 250 ml sample container at the titrator, 5–10 ml of nanoparticle suspension or resuspended samples were diluted with demineralised water to a final volume of 200 ml. Different amounts of sample were required to maintain the detected count-rates in the measurement chamber (High Sensitive M3 cell) at a constant level. The pH was adjusted to 3.0 by using HCl (1 N) before titration to pH 10 with NaOH (0.1 N) Measurement of the zeta-potential was carried out at 0.5 pH increments. The temperature of the measurement cell was adjusted to 25 °C during the measurement. Autocorrelation was performed using the software "PCS" version 1.40. Kinematic viscosity measurements of diluted samples at 25 °C on an Ubbelohde viscometer (Schott & Gen, Mainz, Germany) showed a viscosity $\nu = 1.272$ cSt for the untreated particle suspension and a ν between 1.269 and 1.275 cSt for the batches containing additives. Because of these insignificant differences in viscosity, no corrections in this field were performed. The instrument was calibrated routinely with a −50 mV latex standard.

2.4. Freeze-drying

Ten millilitres of the nanoparticle suspension (9 mg/ml beads) or a mixture thereof with the desired amount of LPA were filled in 25 ml glass vials. The lyoprotective agents glucose, trehalose, mannitol, sorbitol, acetic acid or glycerol were added at concentrations of 5, 10 or 20% (w/v). The sample pH was 6.8–7.0 (neutral beads) in case of sugars and 4.0 in case of acetic acid as LPA. After rapid freezing of the nanoparticle suspension in liquid nitrogen, the samples were placed into the drying chamber of an alpha IIa freeze-dryer (Martin Christ GmbH, Osterode, Germany), pre-cooled to −40 °C. Drying was performed at a pressure of 0.05 mbar for 48 h. After this period, a secondary drying step for 96 h at −20 °C and 0.01 mbar was applied before the vials were removed and sealed. The freeze-dried samples were resuspended in demineralised water and evaluated for size, surface charge, DNA-binding and transfection efficiency.

2.5. DNA

The plasmid pCMVβ (7.6 kbp) was purchased from ATCC (Manassas, VA, USA) and transformed into *Escherichia coli DH5α*. A Gigaprep from 2500 ml of overnight culture was performed according to the manufacturer's instructions (QIAGEN, Hilden, Germany). The DNA was precipitated in 70% ethanol, washed and reconstituted in water to approximately 1 mg/ml.

2.6. Agarose gel electrophoresis and determination of unbound DNA

Nanoparticle–DNA complexes were prepared by mixing the nanoparticles with plasmid at a concentration of 10 μg/ml in 25 mM HEPES (pH 7.4) and electrophoresed on an agarose gel (1% ethidium bromide included for visualisation) for 90 min at 5 V/cm. Images were acquired using a Geldoc 2000 gel documentation system (Bio-Rad, Munich, Germany) equipped with a UV transluminator. Molecular Analyst, version 1.1 software (Bio-Rad) was used for band integration and background correction.

2.7. Cell culture

Cos-1 cells were obtained from DSMZ (Braunschweig, Germany) and maintained in Dulbecco's modified eagle's medium (DMEM) (Sigma, Deisenhofen, Germany) supplemented with 10% fetal calf serum (Gibco-BRL). The cultures were incubated at 37 °C with 5% CO_2 in air and sub-cultured two times a week.

2.8. Transfection assay

Cos-1 cells were seeded in 96-well plates at a density of 10,000 cells/well and incubated overnight. Nanoparticle–DNA complexes were prepared as follows: 300 μl of an appropriate particle suspension were added to an equal volume of plasmid DNA (20 μg/ml) in water and mixed by gentle shaking. After 15 min equilibration at room temperature, the complexes were diluted with one volume of two times concentrated DMEM containing 200 μM chloroquine. The ratio bead–DNA varied from 10 for untreated sample to 20 for freeze-dried samples with a constant amount of plasmid–DNA. The semiconfluent Cos-1 cells were washed two times with HEPES (pH 7.4) and incubated with 200 μl of the samples for 4 h at 37 °C with 5% CO_2 in air. The cells were washed and cultured for a further 48 h in DMEM supplemented with 10% FCS to allow for transgene expression.

2.9. β-Galactosidase detection

The level of β-galactosidase expression was determined using the luminescence coupled Galacto-Star assay system (PE Biosystems). Wells were washed twice with PBS and lysed with 100 μl of mammalian cell lysis buffer for 20 min at 4 °C. Fifty microlitre lysate were transferred to black isoplates, mixed with 100 μl of freshly prepared reaction solution and incubated for 90 min at RT to reach the plateau of light emission. Luminescence was measured in a Wallac 1450 Microbeta Trilux (PE Biosystems) over an integration time of 1 s. Purified recombinant β-galactosidase (Sigma) was used as standard. With the protocol described above, one unit of galactosidase per well resulted in an increase in the luminescence signal of 8.9×10^7 LCPS.

2.10. Analysis of behaviour on reconstitution

All lyophilisates were resuspended in 20 ml water and the resuspension properties were observed immediately after (i) addition of the fluid, (ii) simple manual shaking by inverting the tubes, (iii) an additional 1 min vortexing and (iv) 15 min of ultra sonication. The samples were classified by the type of treatment (i–iv) that resulted in complete resuspension visually defined as a transparent suspension without any sediment.

2.11. Atomic force microscopy

Untreated particles and reconstituted particles after freeze-drying were adsorbed to the surface of silicon wafers and the wet surface was examined by atomic force microscopy on a Nanoscope IIIa Dimension 5000 (Digital Instruments, Veeco) in tapping mode using a Si_3N_4 cantilever with a spring constant of about 34 N/m and a resonance frequency of about 200 kHz. Scanning was performed at a scan speed of 0.5 Hz with a resolution of 512 × 512 pixels. The tip loading force was minimised to avoid structural changes of the sample.

2.12. Other materials

If not stated otherwise, all chemicals were at least of analytical grade and obtained from Sigma.

3. Results

3.1. Particle properties after synthesis

Size and size distribution was analysed by dynamic light scattering (PCS). The mean hydrodynamic particle diameter was found to be 28 nm (relative width: ±29%). PCS revealed a unimodal size distribution. Zeta potential titration provided proof of successful cationic surface modification (Fig. 1). At acidic pH
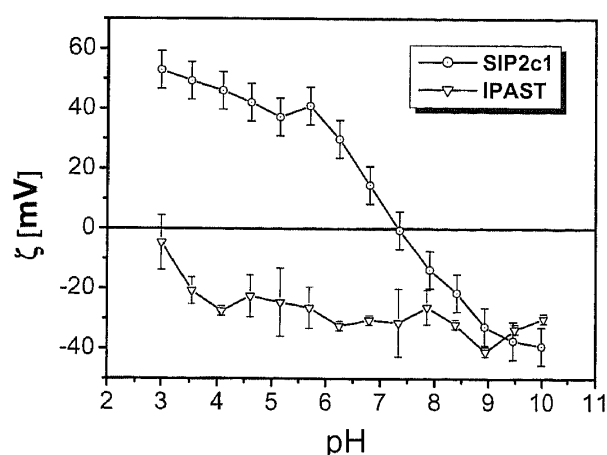

Fig. 1. Zeta potential titration of SIP2c1. The pH-dependent surface charge of the SiO_2 nanoparticle (IPA-ST) and aminoalkylsilane modified SiO_2 nanoparticle.
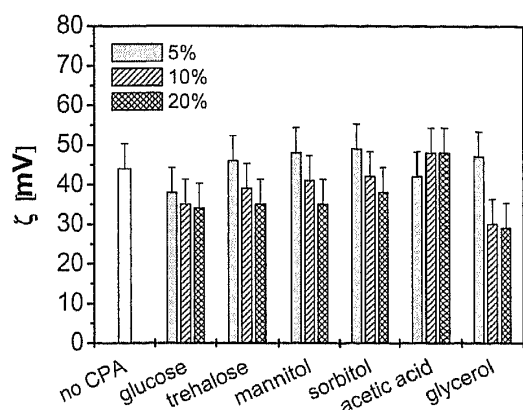
Fig. 2. Change of zeta potential of SIP2c1 after addition of ly-oprotectants. The surface charge of SIP2c1 was measured at pH 4.0 before (no LPA) and after addition of the LPAs.

(3.0) the particles displayed a surface potential of $+46\,mV$ which decreased to $-37\,mV$ at pH 10. Electrophoretic neutrality was observed at a pH of 7.1. This titration was also shown to be reversible. The extent of surface modification was determined by CHN analysis. The carbon content increased from 1.05 to 2.28% after modification and the average number of attached amino functions was calculated as 65 nitrogen atoms per nanoparticle.

3.2. Immediate effects after addition of LPA

The change of zeta potential at pH 4.0 after addition of lyoprotectants is shown in Fig. 2. No significant changes were observed on addition of 5% any of the LPAs under investigation. However, a decrease of the positive zeta potential was recorded on addition of 10 or 20% LPAs, with the exception of acetic acid. It could be that LPA molecules change the solvating shell at the particle surface.

Further, we assessed the influence of addition of the lyoprotectants on particle–solute and particle–particle interactions by dynamic light scattering. No significant changes in hydrodynamic properties were observed for glycerol, trehalose, sorbitol and acetic acid. However, we observed a remarkable increase in the size of the particles on addition of 10 and 20% of glucose or mannitol, an increase up to 142% of the reference value being noted (data not shown). This cannot be attributed to increased viscosity, as all samples were diluted extensively.

3.3. Particle properties after freeze-drying

After lyophilisation the residues appear voluminous and snow-like for trehalose, sorbitol, mannitol and acetic acid, condensed and salt-like for glucose and fluid when glycerol was used. Representative examples for each case are shown in Fig. 3.

All of the lyophilisates were re-suspendable in water, even those produced without addition of any lyoprotectant. However, it was necessary to take
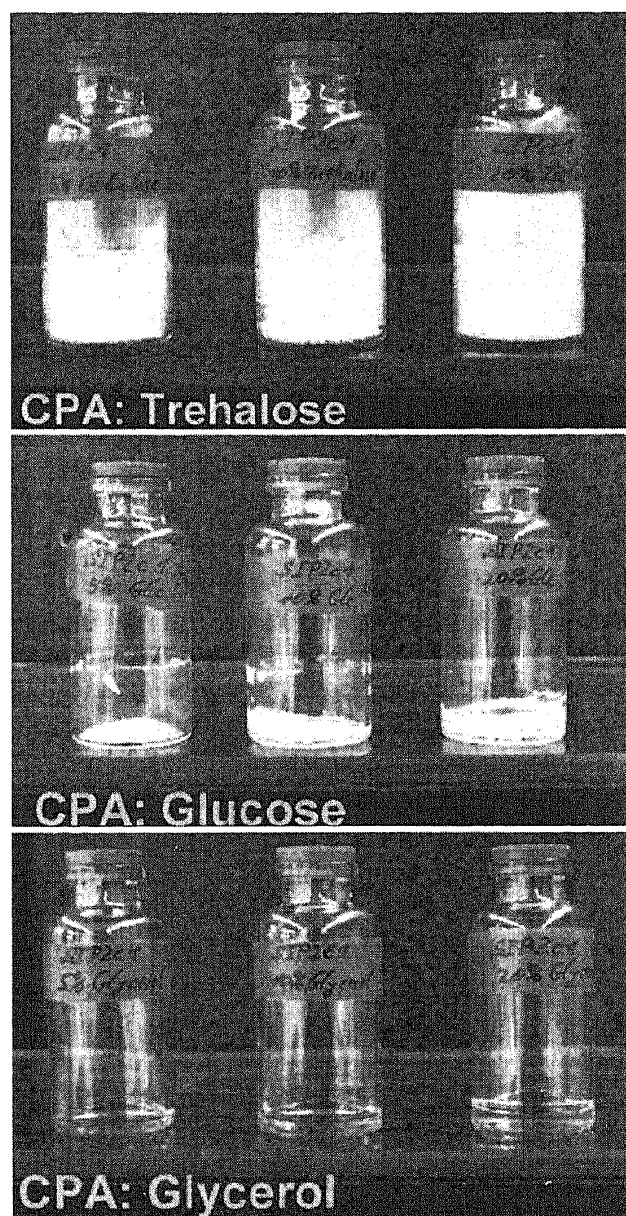

Fig. 3. Photographs after freeze-drying of SIP2c1. Photographs depicting different physical appearances after freeze-drying 10 ml of the nanoparticle suspension containing 5, 10 and 20% of different lyoprotectants.

Table 1

Reconstitution properties of lyophilisates

LPA added	5%	10%	20%
Glucose	*	*	*
Trehalose	***	***	**
Mannitol	*	*	*
Sorbitol	**	**	*
Acetic acid	*	*	*
Glycerol	****	****	****
No LPA	*		

Reconstitution scores: full re-suspension after (****) addition of 20 ml water, (***) mixing 10 times, (**) vortexing for 1 min and (*) sonication for 15 min.
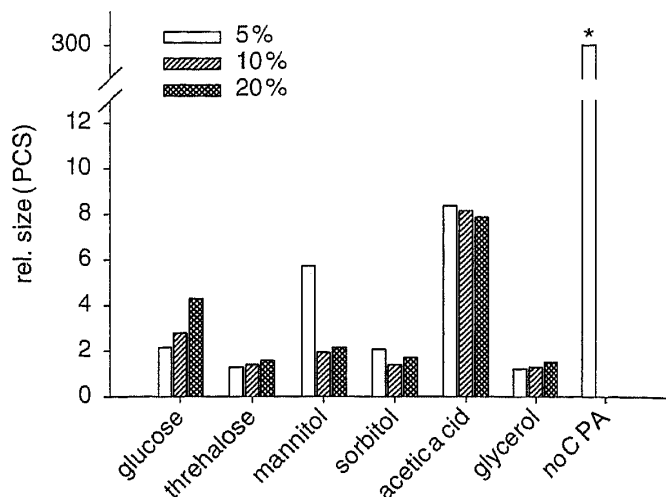

Fig. 4. Effect of addition of lyoprotective agents on the aggregation of SIP2c1 during lyophilisation. Trehalose, glucose, mannitol, sorbitol, acetic acid or glycerol was added at concentrations of 5, 10 and 20% to 10 ml of the particle suspension containing 0.9% of particle dry weight. The hydrodynamic size of the lyophilised particles was determined by re-suspending in 20 ml of water. Size increase was calculated as relative size compared to the average value for the untreated particle suspension (*: value above the measuring range).

additional actions such as shaking, vortexing or sonication to achieve full resuspension after addition of 20 ml of water in most cases (Table 1). Only the samples containing 5, 10 or 20% glycerol did not require these additional measures. Trehalose containing lyophilisates dissolved easily after inverting the tube 10 times, although vortexing was required for the highest concentration of trehalose. When glucose, mannitol or acetic acid was used as LPA or when the LPA was omitted, the reconstitution was difficult and good resuspensions could only be achieved by sonication for 15 min. The effect of sorbitol was in between these two extremes but far from optimal.

After successful resuspension of all samples, the hydrodynamic size of the dispersed particles was re-examined and compared to the initial data (Fig. 4). A particle size of above 5 μm was only observed in the samples without any LPA. Addition of trehalose (5–20%), mannitol (10, 20%) sorbitol (5–20%) and glycerol (5–20%) was found to prevent permanent particle aggregation, while only 5% mannitol or acetic acid were not effective. Paradoxically, particle aggregation increased with higher LPA concentration in lyophilisates that were produced from glucose containing particle suspensions. The results obtained by PCS could be confirmed by atomic force microscopy. An untreated, non-lyophylised SIP2c1 sample contained almost exclusively single particles of similar size (Fig. 5A). The average diameter could be estimated as approximately 30 nm, which is in agreement with the PCS data. After freeze-drying of the pure suspension, a number of large aggregates was observed (Fig. 5B). Only a fraction survived the procedure as single particles. An informative image could

be obtained for the 5% trehalose batch (Fig. 5C). It can be readily seen, that trehalose formed a matrix into which the nanoparticles were interdispersed. All particles were nicely separated by the matrix. In addition, the trehalose formed a coat that surrounded the individual particles.

Plotting of the relative increase in size during lyophilisation against the relative zeta potential measured in presence of the LPA (versus in absence of LPA) revealed some correlations (Fig. 6). Those LPAs which reduced the measurable surface potential most effectively at high concentrations were also most suited to avoid permanent particle aggregation. These agents were trehalose, sorbitol and glycerol.

3.4. DNA-binding activity

Based on the results of the physicochemical tests described above, a selection of promising lyophilised batches was made for further examination of their activity. This selection included samples containing 5% trehalose, 10% sorbitol or 10% glycerol.

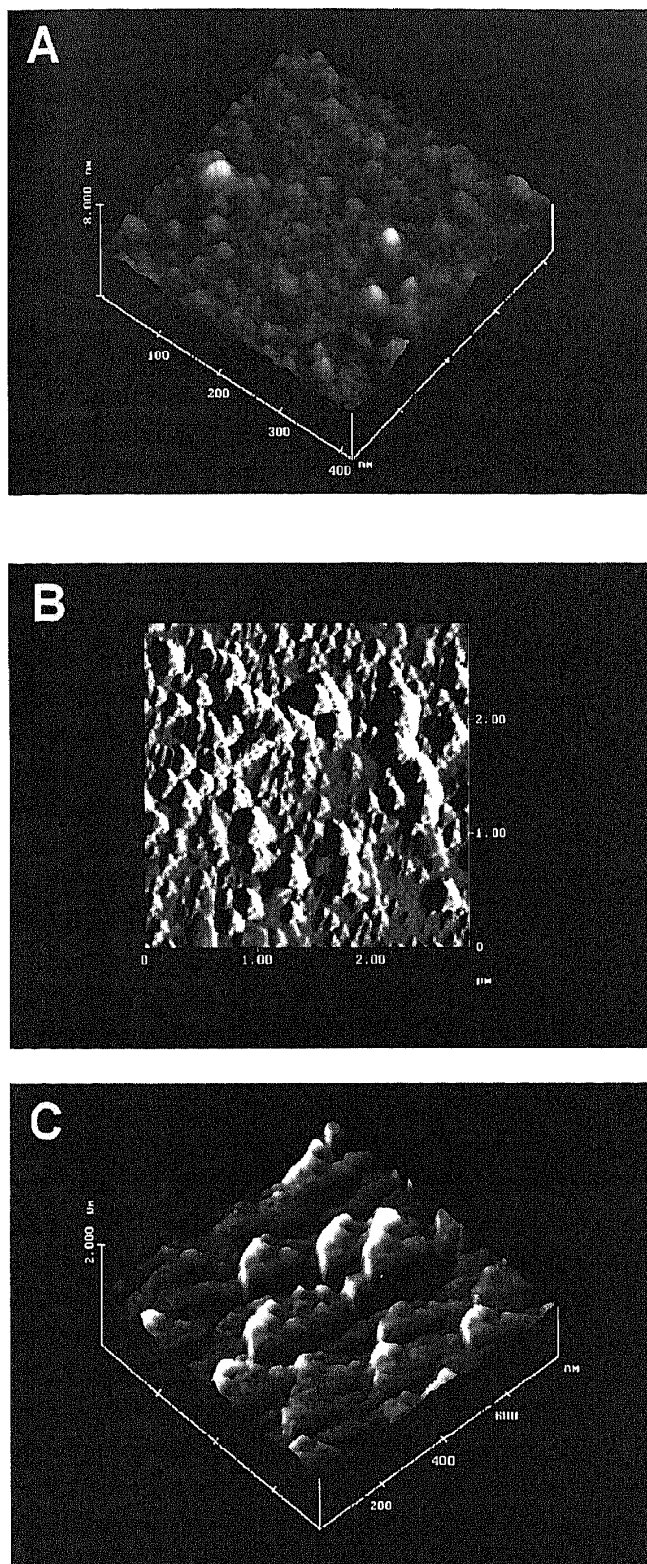Agarose gel electrophoresis was used to quantify the capacity of selected batches to bind plasmid DNA.

Fig. 5. Atomic force microscopy of SIP2c1 before and after lyophilisation and in the presence of 5% trehalose. (A) Representative image of untreated control particles without aggregation. (B) Massive aggregation of particles, lyophilised in absence of lyoprotectant imaged in μm range. (C) Matrix formation and particle coating by addition of 5% of trehalose (after lyophilisation).
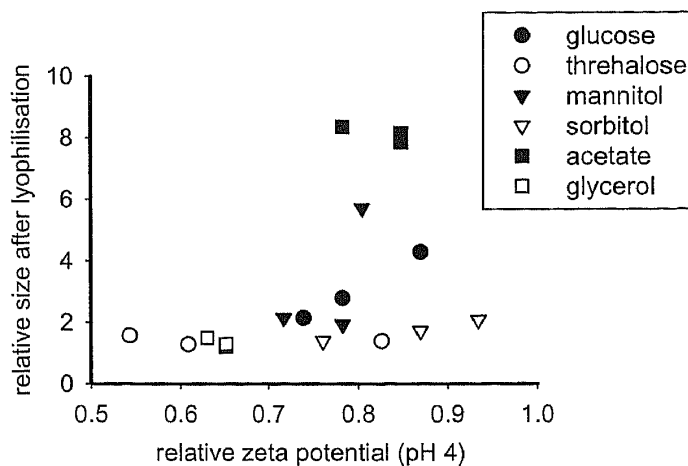

Fig. 6. Correlation of particle shielding and stabilisation during lyophilisation. The zeta potential of SIP2c1 at pH 4.0 was measured in presence of 5, 10 and 20% of the lyoprotectants. A relative zeta potential was calculated by dividing the measured value by the zeta potential of the non-lyophilised control. The relative size increase during lyophilisation as a measure for particle aggregation (see Fig. 4) was then plotted against this value. All samples were diluted at least 50-fold prior to the measurement to avoid artefacts by changes in viscosity or density.

With untreated silica particles SIP2c1, 30 weight equivalents were found sufficient to immobilise all DNA in solution. As shown in Fig. 7, all lyophilised samples retained their DNA-binding activity. However, there was a slight change in equilibrium of this interaction which can be described as a right-shift of the binding curves. We found that about 44 weight
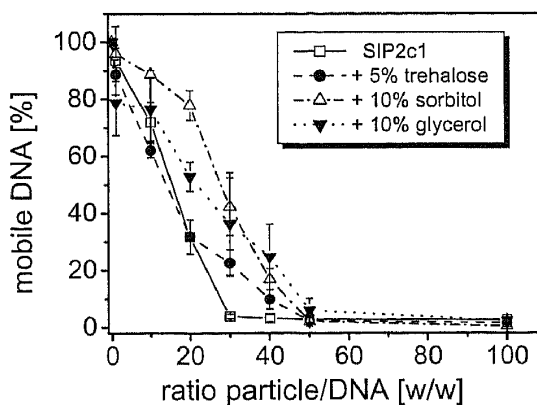

Fig. 7. DNA-binding behaviour of selected lyophilised nanoparticle batches. The amount of supercoiled plasmid DNA remaining unbound after addition of increasing weight equivalents of nanoparticle was determined by agarose gel electrophoresis. Particles lyophilised in the presence of trehalose (5%), sorbitol (10%) and glycerol (10%) were compared to an untreated control. All measurements were performed in triplicate.

equivalents of the particles lyophilised in the presence of 10% glycerol or 10% sorbitol, respectively, were required to bind the plasmid. This is significant when compared to the untreated SIP2c1 and the batch lyophilised in the presence of 5% trehalose (30 weight equivalents), i.e. trehalose caused only minor differences with regards to DNA-binding capacity of the nanoparticles.

3.5. Transfection efficiency

The ability of the selected lyophilised batches to transfect the reporter gene plasmid pCMVβ was examined in Cos-1 fibroblast like cell cultures and compared to the untreated control. All experiments were performed in the absence of serum but in presence of the endosomolytic agent chloroquine. Fig. 8 shows the average gene expression levels at the optimised ratio of SIP2c1:DNA for all four samples. It could be observed that there was only small and insignificant differences in the activity between the control and the batches containing either 5% trehalose or 10% glycerol. Both lyoprotectants were able to fully conserve the transfection activity of the nanoparticles. In con-
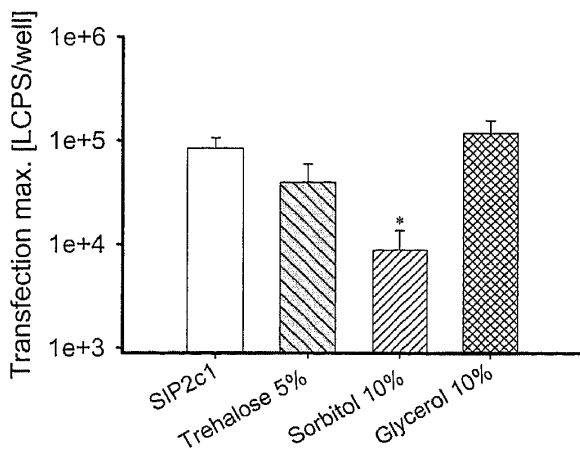

Fig. 8. Transfection efficiency of selected lyophilised nanoparticle batches. The gene expression levels measured in luminescence counts (LCPS) were determined 48 h after incubation of Cos-1 cells with complexes of the galactosidase reporter gene plasmid pCMVβ and nanoparticles lyophilised in presence of different lyoprotectants (5% trehalose, 10% sorbitol or 10% glycerol). The values obtained for the optimised composition of each type of complex were compared to the results of the complexes of the non-lyophilised control particle (SIP2c1). All measurements were performed in six replicates (* indicates significant difference from SIP2c1, $P \leq 0.05$).

trast, the LPA sorbitol (10%) was not comparably successful although it gave similar results as trehalose and glycerol in the physicochemical tests. The highest average gene expression level that could be obtained for the batch lyophilised in the presence of 10% sorbitol was only one-tenth of the untreated control.

4. Discussion

Although Chacon et al. (1999) concluded from their studies that the properties of 80 nm sized PLGA nanoparticles cannot be maintained during freeze-drying by addition of various LPAs, however, from the present investigations, we could say that this is possible with silica nanoparticles. This observation is in line with the work of other authors such as Molpeceres and colleagues (1997), Heiati et al. (1998) and De Jaeghere et al. (1999) who respectively, worked on polycaprolactone and solid lipid nanoparticles as well as poly-lactic acid-co-ethylene oxide nanoparticles. These authors reported the usefulness of various sugars, especially glucose and trehalose. It may therefore not appear very surprising, that we did also find that the lyoprotectant trehalose was most suitable in this comparative study. However, due to the presence of reactive silanol groups on the particle surface and the complicated electrolytic properties, the modified silica particles provided a different model to the polymer complexes studied before.

From comparison of the zeta potentials (Fig. 2) it can be concluded that some LPAs did already show detectable interaction with the particle surface before lyophilisation. These included trehalose, mannitol, sorbitol and glycerol. Such interactions were also reported by Foissy and Persello (1998). All four LPAs were also very effective with regard to the preservation of the physical particle properties during freeze-drying (Fig. 4). On the other hand, glucose was much less effective, its addition having only a minor effect on the zeta potential of the particles, compared to the LPA-free suspension. In addition, 15 min sonication could not dissolve all the aggregates that formed during freeze-drying suggesting imperfect particle isolation.

The most likely explanation for the observed effects may indeed be found in the different interactions of the lyoprotectant with the particle surface. The in-

crease of particle concentration during freeze-drying does favour the condensation of Si–OH groups (which are in equilibrium with Si–O–) into stable Si–O–Si bridges. The velocity of this reaction is lowest in a pH range between 3.0 and 5.0 (Foissy and Persello, 1998). In the case of the investigated particles, reactive groups are present on the core itself and the modified shell, including Si–OH, SiO–, NH_2 and NH. Presence of acetic acid influences the equilibrium between Si–OH and Si–O– towards Si–OH, thereby stabilising the suspension. Sugars (hydroxyl groups), on the other hand, can react with Si–OH resulting esters or form hydrogen bonds via dipole–dipole interactions (Abdelmouleh et al., 2002). These interactions protect the Si–OH groups from condensation with reactive groups of other particles and hence inhibit permanent particle agglomeration. This is also described as the particle-isolation hypothesis. Both ester and hydrogen bonds between the silica bead and the sugar will partly resolve after dissolution in an excess of water.

Such a mechanism has already been suggested for the stabilisation of nanoscaled lipid–DNA complexes by lyoprotective agents as opposed to the glass formation theory applicable to small molecules (Allison et al., 2000). The authors of that study could observe a good preservation with mono- and disaccharides which probably also allowed interdispersion of the nanoparticles within the LPA-matrix due to low surface tension, while high-molecular weight matrices such as starch caused their aggregation.

Finally, the preservation of the DNA-binding activity of these cationic silica particles by addition of either 5% trehalose or 10% glycerol before lyophlisation confirms that this method may be useful to stabilise even nanoscaled drug delivery systems. It remains unclear why sorbitol was less effective than trehalose and glycerol in preserving biological activity, especially as the physico-chemical tests did not show any differences in behaviour. A possible explanation may be that sorbitol interfered with particle–DNA-binding even after dilution. This is suggested by the results of the DNA immobilisation test shown in Fig. 6.

In conclusion, trehalose was confirmed as an effective LPA for freeze-drying of cationically modified silica nanoparticles; glycerol may be an interesting alternative. Effective particle isolation and matrix formation are equally important, but it has to be considered that the interaction between LPA and the colloidal particles may influence their biological activity as exemplified by sorbitol.

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