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International Journal of Pharmaceutics 288 (2005) 157–168
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

pDNA loaded calcium phosphate nanoparticles: highly efficient non-viral vector for gene delivery

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Received 13 May 2004; accepted 30 July 2004
Available online 21 November 2004

Abstract

Nanoparticles of calcium phosphate encapsulating plasmid DNA (pDNA) of size 100–120 nm in diameter were prepared. XRD studies of these nanoparticles showed them to be crystalline in nature having hydroxyapatite structure. The maximum loading of pDNA and its release from nanoparticles were studied using gel electrophoresis. The time dependent size measurement of these particles demonstrated that these particles show strong aggregational behaviour in aqueous dispersion. Calcium phosphate nanoparticles were found to be dissolved even in low acidic buffer (pH 5.0) releasing the pDNA, which suggested that DNA release from these particles in the endosomal compartment was possible. In vitro transfection efficiency of these calcium phosphate nanoparticles was found to be higher than that of the commercial transfecting reagent Polyfect.

Keywords: Calcium phosphate nanoparticles; Non-viral vectors; Gene delivery; Transfection efficiency

1. Introduction

The emerging area of inorganic nanoparticles entrapping biomolecules has exhibited its diversity and potential applications in many frontiers of modern material science (Avnir et al., 1994; Dong and Chen, 2002). These inorganic particles have a number of advantages over organic ones, in the sense that they are not usually subjected to microbial attack and exhibit excellent storage stability. They can be prepared at low temperature and are relatively inexpensive. These inorganic compounds have been explored in various biomedical applications such as adjuvant for vaccination process, component in dental hygiene agents, carriers for various proteins and other growth factors, drug delivery and gene therapy vectors (Frey et al., 1997; Tischer et al., 2002; Kneuer et al., 2000; Luo and Saltzman, 2000b; Cui and Mumper, 2003). Of these inorganic compounds, the most commonly used salt is calcium phosphate whose microparticles and nanoparticles have been developed as delivery system as well as adjuvant for DNA vaccines (He et al., 2000, 2002). Cal-

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doi:10.1016/j.ijpharm.2004.07.035

cium ions are known to form ionic complexes with the helical phosphates of DNA and these complexes have easy transportability across the cell membrane via ion-channel mediated endocytosis (Truong-Le et al., 1999). Although the use of precipitated calcium phosphate for in vitro transfection (James and Grosveld, 1987) is a routine laboratory procedure, the method is hampered by the difficulty of applying it to in vivo studies, especially delivery of DNA to any particular cell type. Due to bulk precipitation of calcium phosphate, the method also suffers from variation in calcium phosphate-DNA particle size that causes variation among experiments (Luo and Saltzmann, 2000).

In the use of different types of cationic liposome (Hirko et al., 2003; Kawakami et al., 2002; Audouy et al., 2002), cationic polymers and dendrimers (Chonn and Cullis, 1998; Maurer et al., 1999; Han et al., 2000; Hashida et al., 2001) as non-viral vectors for delivery of genes, it has been observed that, in addition to cytotoxicity, these carriers do not lead to satisfactory amount of gene expression in the cells. The reasons are many, particularly, low endosomal escape, no protection of DNA from nuclease degradation and inefficient nuclear uptake (Orrantia and Chang, 1990). It was envisaged that the use of inorganic nanoparticles, a largely unexplored synthetic system that could be used as vectors for gene delivery, might eliminate many of these limitations. The phosphate salts of Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, etc. have never been explored as non-viral vectors for gene delivery although some works have been reported on the in vitro use of silica nanoparticles (Luo et al., 2004; Chen et al., 2003) inorganic nanorods (Salem et al., 2003), nanotubes (Sameti et al., 2003) and other inorganic compounds (Xiang et al., 2003).

Based on this idea we have recently reported the use of plasmid DNA (pDNA) encapsulated in calcium phosphate nanoparticles as DNA delivery carriers and have specifically targeted these particles to liver cells after appropriate surface modification (Roy et al., 2003, Maitra et al., 2003; Bhakta et al., 2003). Although the pDNA entrapped in these nanoparticles was highly protected from enzymatic degradation, the transfection efficiency of these synthetic systems was found to be about 80% of that exhibited by superfect. As prolonged ultra-sonication was a prerequisite for re-dispersion of these particles in aqueous buffer, it was envisaged that perhaps partial disintegration of DNA molecules might be a plausible reason for lower transfection efficiency. The probable loss of pDNA, therefore, led us to modify the preparative method in which the ultrasonication was completely avoided to enhance the efficiency of the transfection process.

In this paper we report an optimized method of preparation and a more detailed physicochemical characterization including the in vitro transfection studies of calcium phosphate nanoparticles. These pDNA-loaded nanoparticles have been characterized by their size, surface morphology, crystal structure, surface charge, aggregational behaviour and pH dependent pDNA release. We have also reported that by following the soft preparative method (by avoiding ultrasonication), much-improved in vitro transfection efficiency of these particles in HeLa cell lines using *pSV*β*gal* as a marker plasmid, has been observed.

2. Experimental

2.1. Materials

Surfactant, Aerosol OT (or AOT), i.e. bis (2-ethylhexyl) sulphosuccinate, of analytical grade, was obtained as a 99% pure product from Sigma, USA. *n*-Hexane (AR grade), was purchased from SRL (India). Analytical grade agarose, calcium chloride, disodium hydrogen phosphate, gel-loading dye (bromophenol blue), ethidium bromide were procured from ACROS (USA). Fetal calf serum (FCS) was purchased from Sigma-Aldrich (USA). Cell culture media (DMEM), antibiotics penicillin, streptomycin and amphotericin B were obtained from Genetix, India. X-Gal was purchased from Genei, Bangalore, (India). All other chemicals used were of the high purity grade commercially available from the local market. pUC19, *pSV*β*gal* and pEGFP-N1 (expressing GFP protein) were extracted and purified in the laboratory of Dr Daman Saluja of ACBR, Delhi University. Transfection studies were undertaken in the tissue culture unit of Prof D. Sarkar of Department of Biochemistry, University of Delhi South Campus using HeLa cell lines, which were gifted by Prof Sarkar.

2.2. Plasmid isolation by alkaline lysis method

Plasmid isolation was done using alkaline lysis method (Sambrook and Russell, 2001). The detailed

method is as follows: 1–5 ml culture was centrifuged at 10,000 rpm for 5 min at 4 °C. Alkaline lysis of pelleted cells was carried out, followed by treatment of the cell lysate with potassium acetate solution. After incubation in ice for 5 min, the lysed cells were centrifuged at 10,000 rpm for 5 min at 4 °C. Five hundred microlitres of 1:1 (v/v) of phenol and chloroform mixture was added to the supernatant, mixed thoroughly and centrifuged at 10,000 rpm for 5 min at 4 °C. To 500 μl of this mixture, 500 μl of isopropanol was added and centrifuged at 10,000 rpm for 5 min at 4 °C. Pellet containing DNA was washed twice with 70% ethanol and dried in air. The pellet was dissolved in Tris–EDTA buffer followed by RNase treatment.

2.3. Preparation of pUC 19, pSVβgal and pEGFP-N1 DNA loaded calcium phosphate nanoparticles

The basic method of preparation of pDNA loaded nanoparticles of calcium phosphate is similar to that reported in our earlier communication (Roy et al., 2003), but with some modifications. More specifically the details of the method is as follows: 0.1 M AOT solution in hexane was prepared. In 25 ml of AOT in hexane, 70 μl aqueous solution of 1.36 M calcium chloride and aqueous solution of 2.94 μg of pDNA, were added by continuous stirring for 12 h to form microemulsion A. In another 25 ml of AOT solution, 50 μl of 0.2 M Tris–HCl buffer (pH 7.4), 70 μl aqueous solution of 0.35 M Na_2HPO_4 and aqueous solution of 2.94 μg of pDNA, were dissolved by continuous stirring for 12 h to form microemulsion B. Before stirring both the microemulsions, excess water was added to make total volume of water to 450 μl, to adjust w_o, i.e. the molar ratio of water to AOT at 10. Both the microemulsions were optically clear solutions. Microemulsion B was, then, added to microemulsion A at an extremely slow rate (20 drops per min) with continuous stirring at 4 °C. The resulting solution was, then, further stirred for another 12 h in the above cold condition. Development of translucency indicated the nanoparticle formation in the aqueous core of the microemulsion droplets. Hexane was completely removed from the resulting solution, and the nanoparticles containing the solid mass of AOT was dissolved in 10 ml of absolute ethanol (99.9%) by vortexing. The solutions were centrifuged for half an hour at 8000 rpm at 4 °C in a cold centrifuge (Sigma 3K18). The pelleted nanoparticles were washed with absolute ethanol three times. Finally, the pelleted nanoparticles were dispersed in double distilled water at 4 °C by vortexing to give clear dispersion. The dispersed nanoparticles were dialysed overnight in a cold room using 12 kD dialysis membrane bag, and were lyophilized to dry powder (yield ∼1 mg) for further characterization.

2.4. Size and morphology of the nanoparticles

2.4.1. Dynamic light scattering (DLS)

In aqueous dispersion, these particles aggregate very rapidly to form the bigger particles. Therefore, the size of the primary particles formed within the aqueous core of microemulsion droplets were determined by DLS experiment.

The measurements were done with a Brookhaven BI8000 instrument fitted with a BI200SM goniometer. An argon-ion air-cooled laser was operated at 488 nm as a light source and the measurements were done on a scattering angle of 90°. The time dependent autocorrelation function was derived using a 128-channel digital photon correlator. The particle size was calculated from the autocorrelation function using Stokes–Einstein equation: $d = kt/3\pi\eta D$, where D = translational diffusion coefficient, d = particle diameter, η = viscosity of the liquid in which particles were suspended, k = Boltzmann's constant and T = absolute temperature. The particle size of calcium phosphate nanoparticles dispersed in aqueous buffer as well as the size of the aggregated nanoparticles were also measured by DLS.

2.4.2. Transmission electron microscopy (TEM)

One drop of the aqueous dispersion of nanoparticles followed by one drop of 1% phosphotungstic acid were put on a forvmar coated copper grid (1% solution of forvmar was prepared in spectroscopic grade chloroform) and air dried in a vacuum desiccator. The dried grid was then examined under an electron microscope (JEOL JEM 2000 EX 200 model).

2.5. X-ray diffractogram (XRD) of nanoparticles

The X-ray source used was Cu K_α radiation at 40 kV and 20 mA, and diffraction was analyzed with a PHILIPS PW 3710 diffractometer. 0.2 g of lyophilized

nanoparticles was put inside the diffractometer for analysis.

2.6. Determination of plasmid loading capacity

In order to determine the maximum loading in calcium phosphate nanoparticles, different amounts of pUC19 DNA, (from 3 to 9.0 μg) were added in the microemulsions at the time of preparation of nanoparticles, keeping the total amount of metal ion and phosphate ion concentrations same for all the cases. These nanoparticles dispersed in water were then subjected to gel electrophoresis. After saturated loading, the excess pDNA remained as unentrapped free pDNA in aqueous buffer, which was visible as distinct bands in the gel.

2.7. Entrapment efficiency (E%)

The pDNA-loaded nanoparticles in reverse micelles were separated after ultra centrifugation (40×10^3 rpm for 4 h at 4° C) and the pellets after washing with hexane was dissolved in acidic buffer (pH = 3.0). The amount of pDNA released, $[pDNA]_r$ from the nanoparticles was estimated spectrophotometrically by measuring the OD at $\lambda = 260$ nm. The entrapment efficiency was then calculated from the amount of pDNA originally added in the microemulsion, ($[pDNA]_0$), using the equation $E\% = [pDNA]_r/[pDNA]_0 \times 100$.

2.8. Gel electrophoresis experiments

One percent Agarose gel was used for electrophoresis experiments. The entrapment of DNA in the nanoparticles, its protection from external Dnase degradation and the pH dependent release of pDNA from these nanoparticles were investigated by gel electrophoresis. In each set of 100 μl of aqueous buffers (pH = 5 and 8), 5 mg of lyophilized *pSVβgal* loaded calcium phosphate nanoparticles was dispersed and the mixtures were incubated at 37° C for specific time intervals (0, 1, 2, 4 and 24 h). Twenty microlitre of the sample was removed from each mixture, loaded in the well and was subjected to electrophoresis.

2.9. Confocal microscopy

HeLa cells preparations were observed with a laser scanning confocal microscope (Biorad 206 Confocal Microscope). The microscope settings were as follows: excitation at 488 nm, emission at 507 nm/30 BP into channel 1 to record GFP fluorescence, 63×1.4 NA oil-immersion lens at an Airy disc setting of 0.9. When necessary, serial images were taken, with 1.2 μm steps to get an overall image of fluorescent objects within the cell. The experiments were done at the Department of Pathology, All India Institute of Medical Sciences, New Delhi.

2.10. In vitro transfection studies in HeLa cell line

For in vitro studies, *HeLa* cell line were transfected with free form as well as encapsulated plasmid, *pSVβgal*. For these studies, *HeLa* cell lines were grown in DMEM medium containing 10% fetal calf serum, antifungal drug, Amphotericin B and 1.6% (w/v) penicillin and streptomycin solutions. Twelve well plates were seeded with 1×10^5 cells per well, after checking for cell viability using trypan blue exclusion, and appropriate dilution. Cells were grown under standard conditions for 24 h till 80% confluency. 1ml of aqueous dispersion of pDNA loaded calcium phosphate nanoparticles (total amount of pDNA = 588 ng) was added in each well. After an appropriate time interval of 4 h, the added material was replaced with fresh medium containing 10% (v/v) FCS and antibiotics, and incubated for another 36 h. After 36 h, cells were lysed with 200 μl of lysis buffer (pH 7.4) containing 250 mM Tris–HCl and 0.5% Triton X-100. Ten microlitres of X-Gal solution was added to each well and the reaction was continued for another 36 h. The reaction was, then, stopped by the addition of 50 μl of 1M sodium carbonate solution. The blue colour developed was measured spectrophotometrically at $\lambda_{max} = 616$ nm and the quantity of β-galactosidase produced were calculated from a calibration curve (amount of β-galactosidase versus OD at 616 nm).

3. Results and discussion

Nanoparticles encapsulating pDNA were formed in the aqueous core of the AOT microemulsion in hexane. The strategy involved the precipitation of the calcium phosphate in the presence of pDNA in the aqueous core of the microemulsion droplets. A flow chart of the preparative method adopted is represented by Fig. 1.

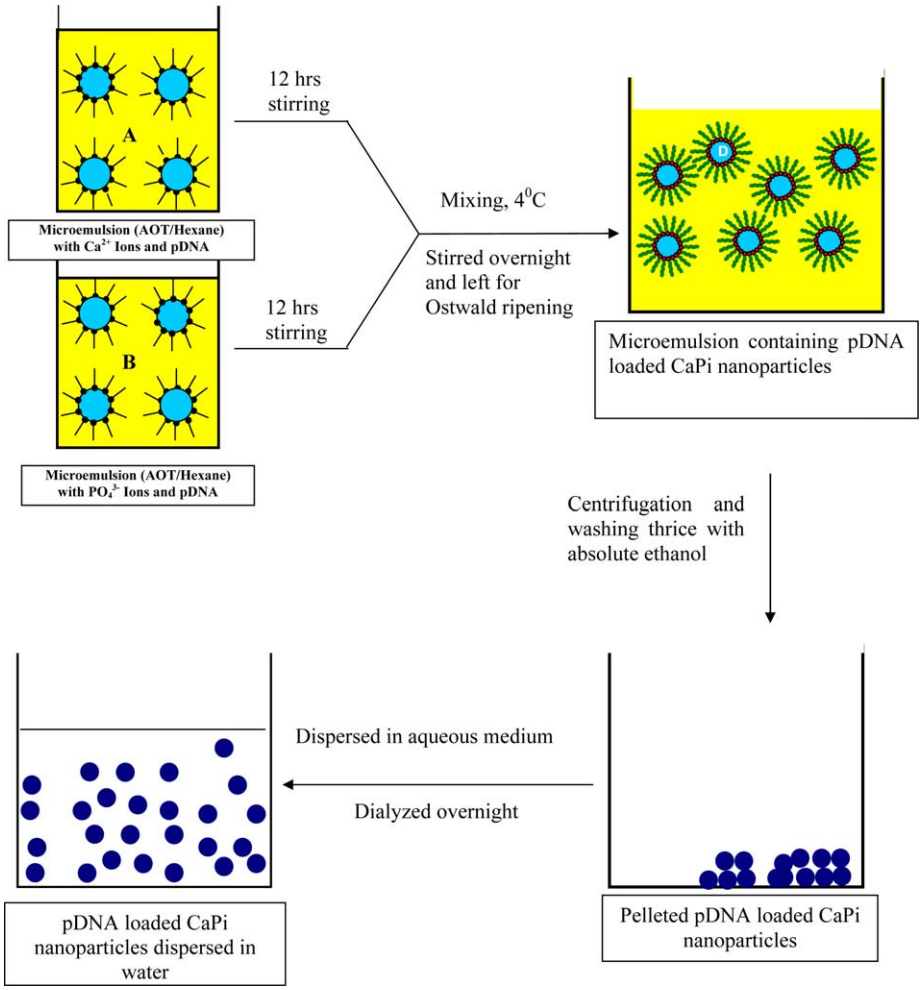

Fig. 1. Flowchart for the preparation of pDNA loaded calcium phosphate nanoparticles.

The centrifuged pellets could be easily redispersed in aqueous buffer. The maximum amount of pDNA, which could be loaded into the nanoparticles, was checked by preparing samples with different amount of pDNA and finally subjecting the aqueous dispersions of these particles to electrophoresis on 1% agarose gel. It was clearly observed that 5.88 μg of pDNA per mg of calcium phosphate could be easily loaded in the nanoparticles. To measure the entrapment efficiency of calcium phosphate nanoparticles, 1mg of pUC19 loaded nanoparticles was dissolved in pH = 3.0 buffer by overnight incubation and the amount of pDNA released was estimated spectrophotometrically at 260 nm and the entrapment efficiency (*E*%) was found to be more than 99%.

The physiochemical characteristics and the aggregation behaviour of the calcium phosphate particles were studied using DLS, TEM and X-Ray diffractometry. The size of the nanoparticles formed was found to be dependent on w_o values (i.e. the molar ratio of water to AOT). The mean size distribution of calcium phosphate nanoparticles while dispersed in microemulsuion at $w_o = 10$ was in the range of 30–40 nm. Fig. 2 is a representative size distribution profile of calcium phosphate nanoparticles encapsulating *pSVβgal* in AOT microemulsion. The particle size, while dispersed in mi-

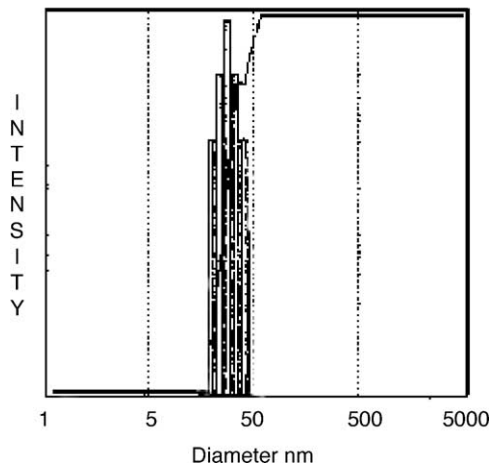

Fig. 2. Representative DLS of pDNA loaded calcium phosphate nanoparticles in microemulsion.

croemulsion, depends, however, on the time of stirring. Instead of 12 h of stirring as reported in the present protocol, if the microemulsion was stirred for 24–36 h, the particle size was found to be increased from 30–40nm to about 80–90 nm diameter. The size of the particles in aqueous dispersion was also measured and it was found to be in the range of about 100–130 nm diameter. The time dependent size measurement studies at 20° C (Fig. 3) clearly indicated that the particles aggregated rapidly with time (more than 100 nm jump after 24 h) thus leading to the bigger size of the particles.

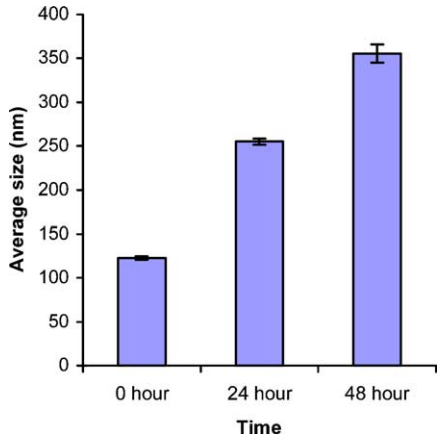

Fig. 3. Time dependent aggregation studies of pDNA loaded calcium phosphate nanoparticles in aqueous medium at 20° C.

The transmission electron micrographs of pUC 19 loaded calcium phosphate nanoparticles (Fig. 4a) and void calcium phosphate nanoparticles (Fig. 4b) revealed the formation of dense particles with spherical morphology.

The surface charge of calcium phosphate nanoparticles was determined by measuring the zeta potential in the pH range between 6.0 and 10.0. The pH dependent zeta potential as shown in Fig. 5 indicated that the particles were positively charged in neutral aqueous buffer and were turned negatively charged in strongly alkaline solution above pH ∼9.5.

The crystallinity of the calcium phosphate nanoparticles was studied by means of an X-ray powder diffractometry. Fig. 6a and b show the diffraction patterns of pUC19 encapsulated as well as void calcium phosphate nanoparticles respectively. The diffractograms obtained for void and DNA loaded particles indicated the formation of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals as evidenced from the characteristic peak at $2\theta = 31.8$ degree (Shimabayashi et al., 1995).

One of the primary reasons for the low transfection efficiency obtained with non-viral vectors, including the precipitated bulk calcium phosphate is the incomplete protection of the plasmid DNA by the encapsulating material. Such partial protection makes the DNA highly susceptible to aggressive DNase attack in the body as well as inside the cell. In order to check the level of protection being offered to the encapsulated DNA, we subjected the calcium phosphate nanoparticles to extensive DNase treatment followed by electrophoresis on 1% agarose gel (Fig. 7). We found that while free plasmid DNA (pUC 19) moved at its usual position in the gel, pUC19 encapsulated in the matrix of the nanoparticle was right at the top of the gel and hardly moved. Moreover, while free pDNA was completely digested by DNase treatment, encapsulated plasmid was totally protected. As expected, this was quite contrary to the plasmid DNA adsorbed on the surface of the nanoparticles. In this case, we found that the level of protection offered to the DNA was extremely low and the DNA was highly prone to nearly total degradation by DNase. These results clearly demonstrated that DNA was completely encapsulated in the rigid matrix of the calcium phosphate nanoparticles, which enabled the particles to protect the nucleic acid from external DNase environment.

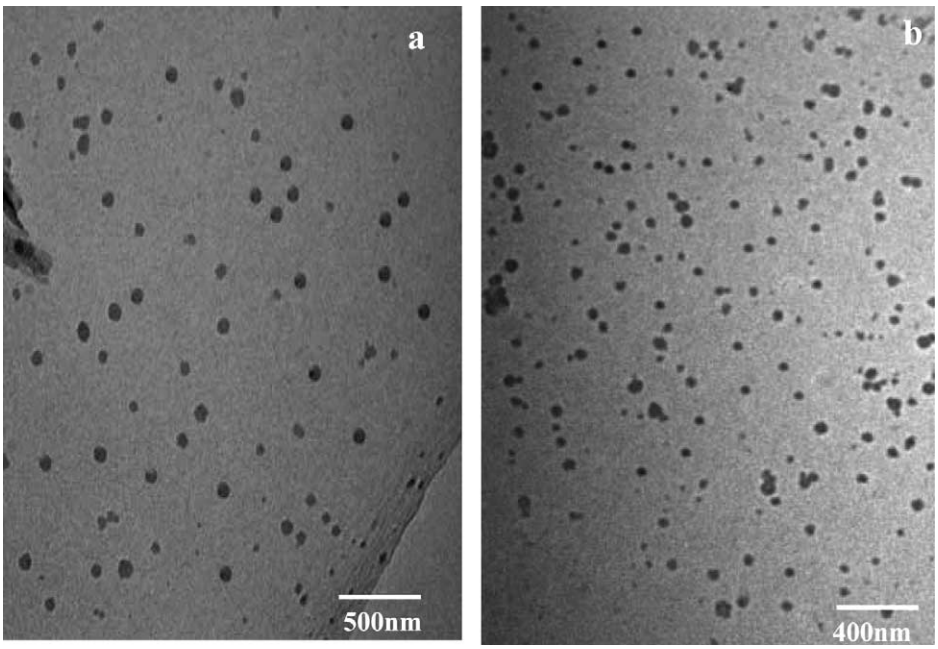

Fig. 4. Transmission electron micrographs of (a) pDNA loaded and (b) void calcium phosphate nanoparticles.

The primary purpose of using carriers for gene delivery is to protect and transport the genetic material into the cell, and ultimately into the nuclear compartment avoiding intracellular degradation. But, most non-viral vectors are known to internalize into the cells by endocytosis, and this endocytic uptake of particulate carriers, leads to ultimate transport of the vesicles to the lysosomal compartment, where subsequent degradation of the particles as well as the encapsulated DNA takes place. Thus, as has been previously indicated by several workers (Cifti and Levy, 2001; Wagner, 1998; Zauner et al., 1998), low transfection efficiency of non-viral vectors may be due to the intracellular degradation of input DNA in the endosomes and/or lysosomes. Also, according to them, DNA degradation can be inhibited either by inactivating the lysosomal enzymes

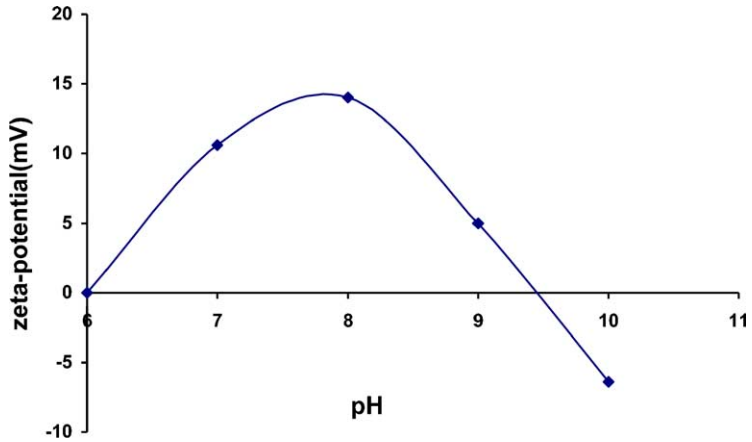

Fig. 5. pH Dependent zeta potential of calcium phosphate nanoparticles dispersed in aqueous buffers.

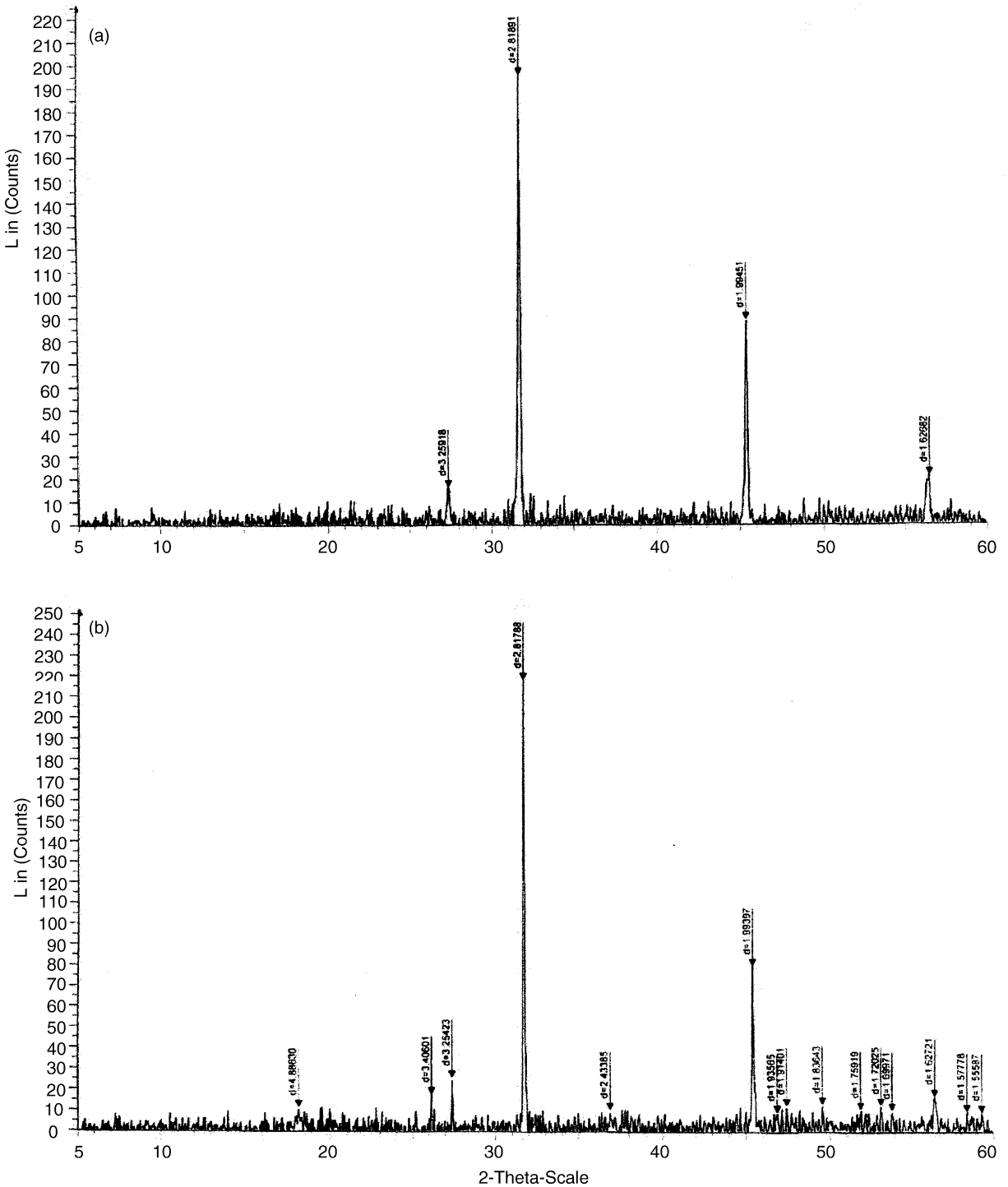

Fig. 6. Powder XRD patterns for (a) pDNA loaded and (b) void calcium phosphate nanoparticles.

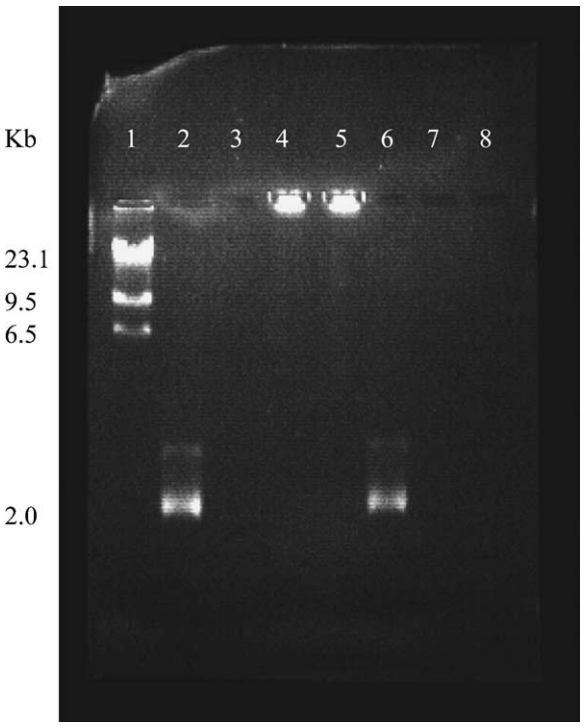

Fig. 7. Agarose (1%) gel electrophoresis of free, entrapped and adsorbed pUC19 DNA. Lane 1: λ DNA digested with *Hin*dIII enzyme. Lane 2: Free pUC19 DNA. Lane 3 pUC19 DNA treated with DnaseI. Lane 4: calcium phosphate nanoparticles containing entrapped pUC19 DNA. Lane 5: calcium phosphate nanoparticles containing entrapped pUC19 treated with DnaseI. Lane 6: pUC19 DNA adsorbed on void calcium phosphate nanoparticles. Lane 7: pUC19 DNA adsorbed on void calcium phosphate nanoparticles and then treated with DnaseI.

or obliterating endosome fusion to lysosomes using lysosomotropic agents. Further, prevention of DNA destruction is also dependent on its release from the endosomal compartment prior to fusion of the endosome and the lysosome. Upon internalization, endosomal compartments undergo continuous acidification from the initial cell-surface pH (∼7) ultimately to that found in the lysosomes through the action of ATP-dependent proton pumps acting in conjunction with other ion transporters found in the membranes. Ideally, the entire delivered vector should escape from the endosome before degradation along the trafficking route, either by vector fusion with the endosomal membrane or through endosome disruption, in either event, ultimately resulting in the release of the nucleic acid (with or without the protective vehicle) into the cytosol (Varga et al., 2000). It is envisaged that the dissolution of inorganic nanoparticles in the acidic endosomal compartment may lead to an osmotic disbalance and consequent disruption of the endosomal compartment so that DNA can come out in the cytosol. Ca^{2+} in the form of nascent calcium phosphate microprecipitates and other lysosomolytical agents facilitate endosomal/lysosomal release by their fusigenic and membranolytic activity (Haberland et al., 1999; Haberland et al., 2000). Therefore, keeping in mind the endosomal pH (5.0–5.5) and to assess the possible release profile of pDNA from the calcium phosphate nanoparticles, *pSV*β*gal* release was visualized using agarose gel electrophoresis following incubation of the loaded particles in buffers (pH = 5 and 8) for varied time intervals (Fig. 8). From the gel picture it is apparent that at pH = 5, there is no release of *pSV*β*gal* from calcium phosphate nanoparticles at zero hours but the release was observed at 2, 4 and 24 h. On the other hand there was no release of the plasmid from the nanoparticles at pH = 8 even after 24 h. The supercoiled DNA (sc DNA) and double stranded DNA (ds DNA) were the most active and stable forms of the DNA released from the nanoparticles. Electrophoresis in an agarose gel permitted the identification of the supercoiled, open circular and the linear forms of the DNA. Interestingly the supercoiled form was clearly observed in the released DNA even after incubation in acidic pH for 24 h (Fig. 8). Although, previously it has been reported (Walter et al., 1999; Tinsley-Brown et al., 2000), that plasmid DNA was greatly damaged at acidic pH, our observations indicated to the contrary. Thus, the stabilization of the plasmid in the nanoparticles has also been addressed with the observation of plasmid release in the supercoiled form.

The endosomal escape as well as nuclear uptake of the plasmid and subsequent expression had been observed in vitro through confocal microscopy. In the present study, we observed the pathway of FITC-dextran in cytosol after endosomal escape in the whole cell based on the Z-series images sequentially obtained by confocal laser scanning microscopy. FITC-dextran loaded calcium phosphate nanoparticles were added in vitro to HeLa cells and within four hours of addition, the green fluorescence due to FITC, in the entire cytosolic region of the cell was observed (Fig. 9a). The plasmid expressing GFP encapsulated in calcium phosphate nanoparticles was also added in vitro to HeLa

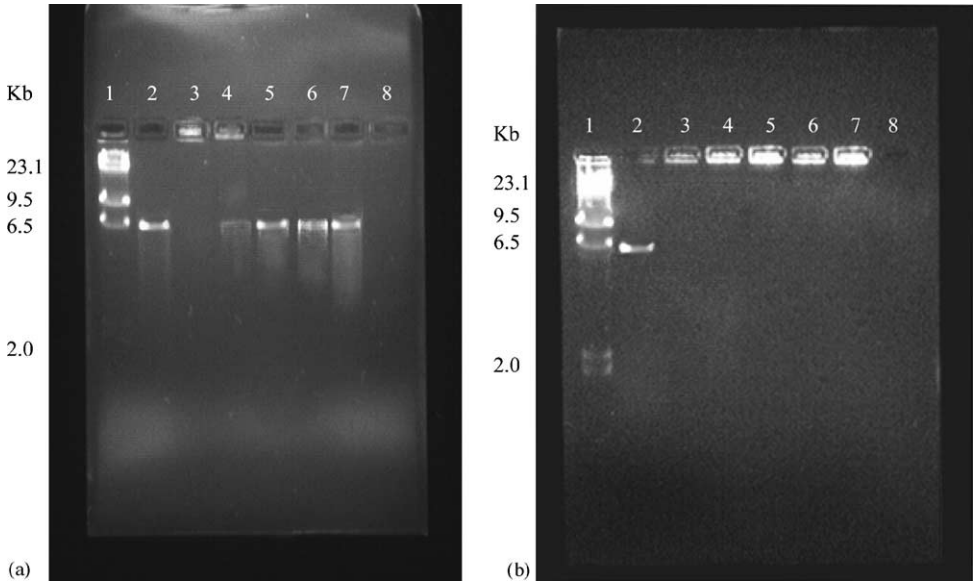

Fig. 8. Agarose (1%) gel electrophoresis to show DNA release at (a) pH 5.0 and (b) pH 8.0. Lane 1 (in a and b): λ DNA digested with *Hin*dIII enzyme. Lane 2 (in a and b): free *pSV*β*gal* DNA. Lane 3–7 (in a and b): DNA released on incubation at 37 °C from calcium phosphate nanoparticles after 0, 1, 2, 4 and 24 h.

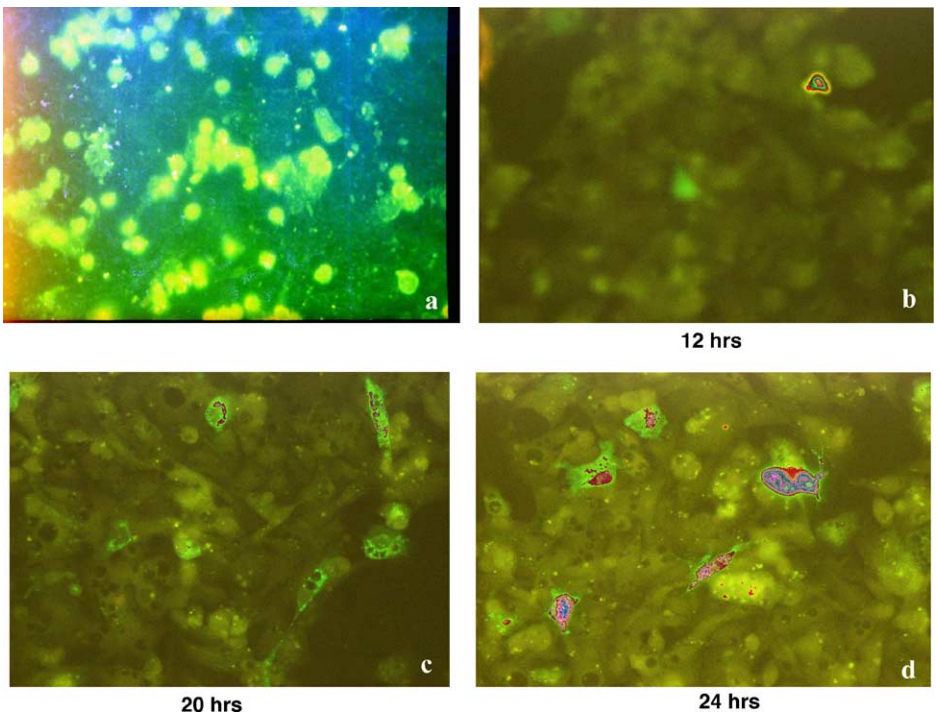

Fig. 9. (a) Fluorescence picture of FITC-Dx loaded calcium phosphate nanoparticles incubated with HeLa cells. The picture was taken 4 h after the addition of nanoparticles. (b–d) Time dependent pEGFP-N1 expression in HeLa cells (b) 12 h, (c) 20 h and (d) 24 h.

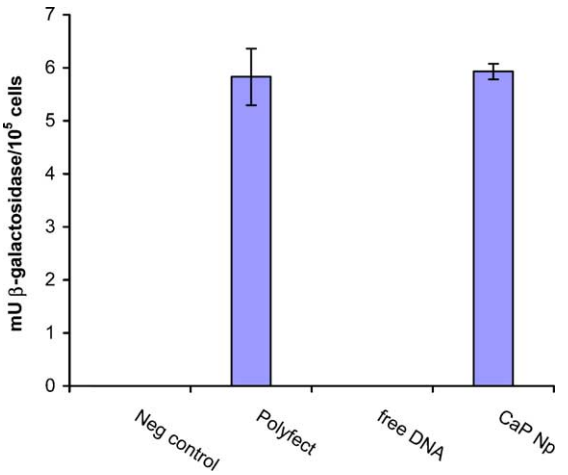

Fig. 10. In vitro transfection studies in HeLa cell-line.

cells and the expression was seen. It was observed (Fig. 9b–d) that, in the initial 12 h, there was practically no GFP expression. But after 12 h of incubation the green fluorescence appeared which increased with time indicating more and more of expression of the plasmid.

To estimate the transfection efficiency on mammalian cells using these nanoparticles, Hela was selected and *pSV*β*gal* was chosen as a marker DNA. For comparing the extent of transfection a standard laboratory transfecting reagent, Polyfect (a polyamidoamine dendrimer), was used. From the results (Fig. 10), it could be seen that the calcium phosphate nanoparticles showed higher transfection efficiency than exhibited by Polyfect. Considering that Polyfect is a highly competent vector used for in vitro transfection (Tang et al., 1996), our results indicate that under in vitro conditions calcium phosphate nanoparticles are more effective as non-viral vectors than Polyfect. In our previous communication (Roy et al., 2003) the transfection efficiency of calcium phosphate nanoparticles was noted to be less as compared to that of the Polyfect reagent. The reason could be attributed, besides others, to the partial degradation of DNA due to ultrasonication.

4. Conclusion

Calcium phosphate nanoparticles encapsulating different plasmids were prepared and characterized. The size dependent studies of these particles clearly showed the high tendency to aggregation resulting in their larger size. The plasmid DNA was well protected inside these nanoparticles and these particles were easily dissolved in mild acidic environment releasing DNA. In vitro transfection studies indicated that the transfection efficiency of these carriers was as high as Polyfect, a commercially available transfecting reagent. We presume that these calcium phosphate nanoparticles have well-defined role in DNA delivery so far as endosomal escape, protection against nuclease degradation and nuclear uptake are concerned and, therefore, can be used as an effective non-viral vector in gene therapy.

Acknowledgements

The authors thank the Department of Science and Technology, Government of India for financial assistance in the form of a research project. Thanks are also due to Prof D.Sarkar, Department of Biochemistry, University of Delhi South Campus for allowing us to use his tissue culture facility. We thank Dr Daman Saluja of Ambedkar Centre of Biomedical Research (ACBR), Delhi University, for providing us facilities for extraction and purification of plasmids and Prof. A.K.Dinda of AIIMS, New Delhi for assisting in confocal microscopic studies.

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