

PEG-Based Positively Charged Nanoparticles for Pulmonary Delivery of Nucleic Acids

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Summary

The aim of this study was to formulate and characterize pDNA adsorbed chitosan-TPP-PEG nanoparticles for dispersion in aerosol propellants. Chitosan-TPP-PEG nanoparticles were formed using an ionic gelation process. The particles were spherically shaped with a mean diameter of 150-210 nm and a surface charge of +22 to +36 mV. These nanoparticles were freeze-dried and dispersed in a model propellant (DFP). The particle diameter was analyzed to be <500 nm and the stability of PEG based nanoparticles was determined in water and DFP. The use of trehalose as a lyoprotectant in formulations containing PEG 2000 produced aggregates, whereas it does not affect the formulation with PEG 5000. Gel electrophoresis indicated that the negatively charged nucleic acid (pDNA) adsorbed to cationic nanoparticles. The use of PEG within Chitosan-TPP nanoparticles reduced the adsorption of pDNA.

Introduction

The biodegradable and biocompatible properties of chitosan offer opportunities for biomedical applications, and hence it has been extensively used as a positively charged polymer for the formation of nanoparticles [1]. The polycationic property of chitosan provides nanoparticles with the ability to bind strongly to several mammalian cells [2]. In this study sodium tripolyphosphate (TPP) was used in an ionic gelation method as a polyanion to cross-link chitosan through electrostatic interactions. PEG moieties incorporated into nanoparticle form a protective outer layer that shields the core, giving rise to steric stabilization of the particles [3]. The current work explores the potential use of PEG 2000 and PEG 5000 for steric stabilization of the chitosan-TPP nanoparticles. In addition, the dispersion properties of the formulation in DFP and the interaction of nanoparticles with pDNA have been studied.

Material

Chitosan (Protasan UP G 113, m.w 150-200 kDa, degree of deacetylation 75-90%) was purchased from Novamatrix (Norway). Polyethylene glycol 5000 monomethyl ether and polyethylene glycol 2000 monomethyl ether were obtained from Fluka (US). D-(+)-Trehalose dihydrate, ethidium bromide, agarose and sodium tripolyphosphate 85% were purchased from Sigma-Aldrich (Germany). Gel Loading Dye Blue (6X) was bought from New England Biolabs, Plasmid DNA (MB113, Pharm. Dev), BAC tracker (Supercoiled DNA ladder) was obtained from Cambio Ltd. 2H, 3H Decafluoropentane (model propellant – DFP) was purchased from Apollo scientific.

Method

This study was performed to compare the formation of cationic nanospheres in the presence and in the absence of PEG. Nanoparticles were produced based on the concept of ionic gelation, whereby sodium tripolyphosphate solution (0.5 mg/ml) was added drop-wise to chitosan solution (1 mg/ml) in the ratio of 1:5 using a peristaltic pump (Gilson, France). A total of three different concentrations of PEG 2000 and 5000 (10 mg/ml, 15 mg/ml and 20 mg/ml) were prepared and the same protocol was used to prepare Chitosan-TPP-PEG nanoparticles by dissolving PEG along with TPP prior to its addition to chitosan. The nanoparticles were filtered through 0.45 µm pore filters (Millipore). The particle size was determined using photon correlation spectroscopy (Malvern Zetasizer), zeta potential was measured by Malvern Zetamaster. The sample was visualized for size and morphology using transmission and scanning electron microscopy.

Freeze-drying was employed to dry nanoparticles prior to dispersion in DFP. Samples were lyophilized with and without trehalose, a thermo protective excipient (in equal weight ratio as the formulation to which it was added), using a Virtis freeze-dryer (Virtis advantage high vacuum, England). The freeze-dried samples were analysed using scanning electron microscopy and dispersed in DFP. A stepwise approach was adopted to optimise dispersion properties (figure 1).

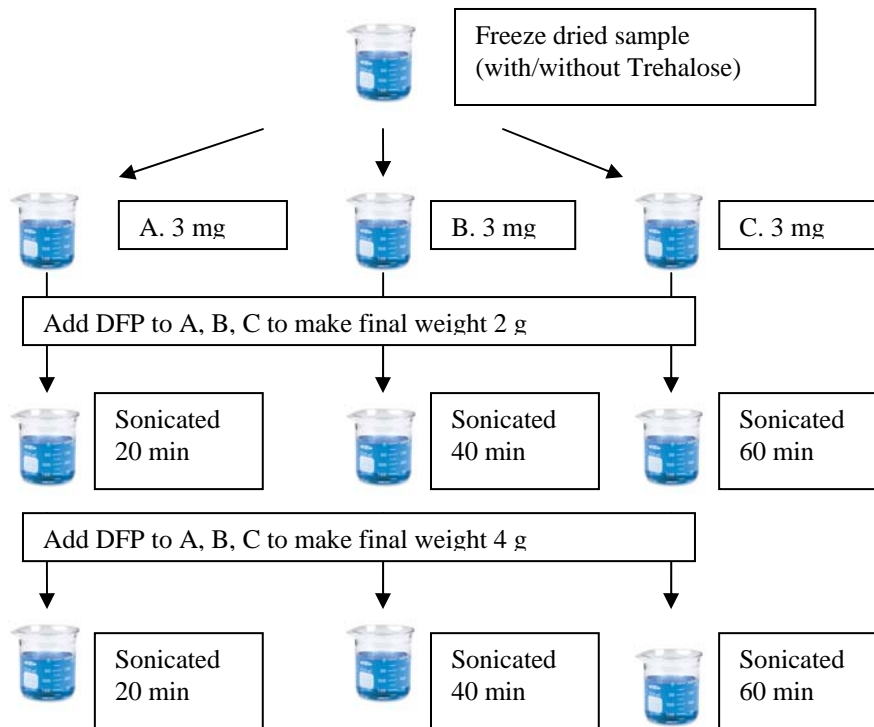


Figure 1. Method for dispersion of nanoparticles in DFP

Freeze-dried samples, with or without trehalose, were divided into three sections of equal weight (3 mg). To each, DFP was added to make the final weight 2 g. These samples were sonicated, in a bath sonicator, for 20, 40 and 60 min respectively. Finally, the total weight of these three samples was made up to 4 g with DFP and was again sonicated for 20, 40 and 60 min respectively.

Gel electrophoresis was performed to examine the degree of binding of different formulations of charged nanoparticles (Chitosan-TPP (5:1) and Chitosan-TPP-PEG5000 (5:1:30)) with plasmid DNA ranging from 50:1 to 2.5:1. Following mixing, samples were kept for an hour. The studies were conducted using 0.7% agarose gel formed with 0.5x Tris Borate EDTA (TBE) buffer at pH 8.2. Ethidium bromide (4 μ l) was added to the agarose gel (100ml) in order to stain the plasmid DNA. The sample and loading dye were mixed in the ratio of 6:1 and were injected onto the well. Gel electrophoresis was carried out at a voltage of 65 V for 2 hours. The movement of stained plasmid DNA was detected when exposed to ultraviolet light using a UV transilluminator based image analyzer (Syngene G-Box).

Result and Discussion

Characterization of nanoparticles

The particle diameter was found to be 150 to 210 η m (table 1). The zeta potential for all formulations was in the range of + 22 to +36 mV. However, in the case of ionic gelation, factors such as weight ratio of chitosan:TPP and pH of the chitosan solution also affects the particle size. Any alteration in the weight ratio of chitosan:TPP (5:1) leads to aggregation. It was further observed that forming the nanoparticles at lower pH (<6) leads to more positively charged chitosan nanoparticles with a smaller particle size.

Table1. Characteristics of chitosan-TPP nanoparticles (n=3 \pm s.d.)

Formulation	Mean Size (η m)	Mean Charge (mV)
Chitosan:TPP (5:1)	165 \pm 15	+29 \pm 7
Chitosan:TPP:PEG 2000 (5:1:20)	174 \pm 25	+28 \pm 2
Chitosan:TPP:PEG 2000 (5:1:30)	179 \pm 25	+30 \pm 4
Chitosan:TPP:PEG 2000 (5:1:40)	193 \pm 6	+31 \pm 1
Chitosan:TPP:PEG 5000 (5:1:20)	181 \pm 16	+29 \pm 2
Chitosan:TPP:PEG 5000 (5:1:30)	196 \pm 5	+31 \pm 2
Chitosan:TPP:PEG 5000 (5:1:40)	199 \pm 10	+30 \pm 2

Table 1 shows that PEG 2000 and 5000 do not have a great effect on the particle diameter and zeta potential of the particles. Nanoparticles were visualized under transmission electron microscopy and scanning electron microscopy, prior to and after freeze-drying. Both methodologies indicated that discrete particles in the nanometre size range were produced (figure 2).

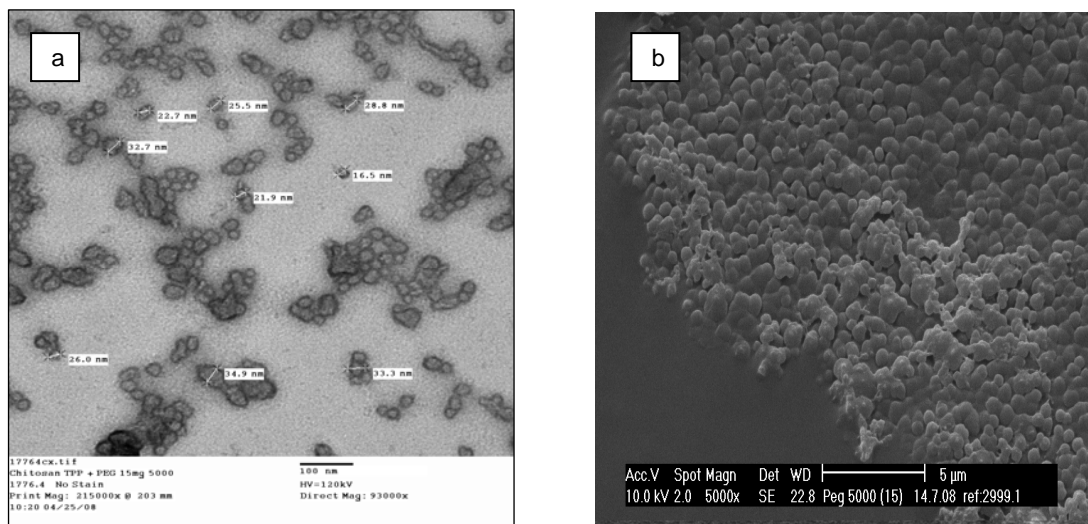


Figure 2. Chitosan-TPP-PEG5000 (5:1:30), a) Transmission electron micrograph of nanoparticles dispersed in water, b) Scanning electron micrograph of freeze-dried nanoparticles.

Nanoparticle stability in water

Nanoparticles formed by Chitosan-TPP were stable (no aggregates formed) for 1 week, whereas, the Chitosan-TPP-PEG nanoparticles showed stability for 3 weeks in water.

The nanoparticles were freeze-dried and it was observed that when trehalose was used along with the formulation containing PEG 2000 it showed less bulk as compared to the same formulation without trehalose, while formulations with PEG 5000 (with and without trehalose) showed no difference in bulk properties (figure 3).

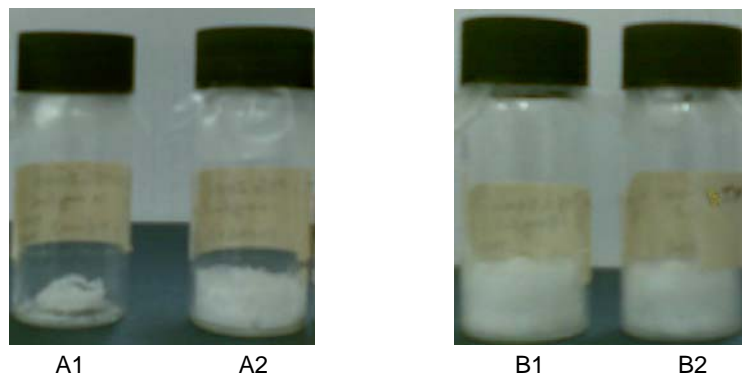


Figure 3. Freeze-dried nanoparticles: A1) Chitosan TPP PEG2000 with trehalose, A2) Chitosan TPP PEG2000 without trehalose, B1) Chitosan TPP PEG5000 with trehalose, B2) Chitosan TPP PEG5000 without trehalose.

Nanoparticle stability in DFP

Freeze-dried nanoparticles (with or without trehalose) were redispersed in the model pMDI propellant (DFP). Chitosan-TPP and Chitosan-TPP-PEG 2000 nanoparticles showed evidence of aggregation, whereas nanoparticles containing PEG 5000 (with and without trehalose) formed a milky dispersion, without aggregates. The mean size of re-dispersed PEG 5000 nanoparticles in DFP was less than 500 nm, with additional processing yielding size less than 300 nm for some formulations. The measured size did not change over a period of 72 hours. It was visually observed that this formulation creamed within 1 min, as the density of formulation was less than DFP.

Gel electrophoresis

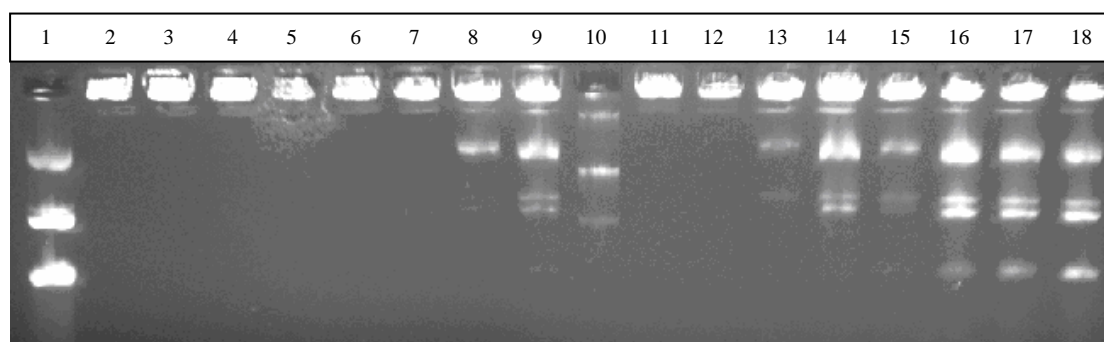


Figure 4. Analysis of the binding of pDNA to nanoparticles in different weight ratios of nanoparticles; Lane 1- pDNA standard. Lanes 2 to 9 pDNA to nanoparticle (Chitosan-TPP) weight ratio 1:50, 1:40, 1:30, 1:20, 1:15, 1:10, 1:5, 1:2.5. Lane 10 – pDNA ladder. Lanes 11 to 18- pDNA to nanoparticle (Chitosan-TPP-PEG5000) weight ratio 1:50, 1:40, 1:30, 1:20, 1:15, 1:10, 1:5, 1:2.5.

It was observed that the negatively charged pDNA was able to adsorb on to cationic nanoparticles. The pDNA:nanoparticle (Chitosan-TPP) complex showed strong binding in ratios from 1:50 to 1:10 whereas 1:5 and 1:2.5 showed unbound pDNA. Nanoparticles containing PEG showed unbound pDNA in ratios from 1:30 to 1:2.5. This indicates that PEG is present at the surface of nanoparticles and hinders the adsorption of pDNA at higher ratios.

Conclusion

In conclusion, we are able to generate a nanoparticles size of less than 200 nm. The use of PEG 5000 in this formulation prevents aggregation against undesirable interactions with impertinent surroundings and improves the stability of the dispersion. Moreover, the use of trehalose as a thermo protective agent in freeze drying, did not aid in re-dispersion. When the lyophilized formulation was re-dispersed in DFP a particle size of less than 500 nm was achieved. It is known that nanoparticles have higher intracellular uptake than micro particles and are taken up less by macrophages and thus this formulation offers considerable potential for pulmonary delivery. In terms of nucleic acid interaction, plasmid DNA/nanoparticles complex at ratios above 1:30 showed strong binding and could potentially be used for pMDI preparations.

Acknowledgements

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References

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