Cationic Nanoparticles based on pH Responsive Polyion Complexes containing Plasmid GFP DNA

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ABSTRACT

Cationic nanoparticles were formulated from a Polyhydroxylamine (PT) with low pKa and PolyAcrylic Acid (PA) that form binary polyion complexes (PICs). Particles spontaneously form on aqueous mixing with sizes between 80-700nm and particle charge 3-30mV. Plasmid containing GFP (pGFP) was incorporated by prebinding pGFP to PT and assessed by Agarose gel electrophoresis.

Keywords: nanoparticle, DNA, polyion, pGFP, pH.

1 INTRODUCTION

Nanoparticle polymer systems below 1 micron in size have potential roles as carrier systems in fields of biotechnology and emerging nanotechnology.

Recent advances in understanding the physico-chemical structure of PICs has rekindled interest in their application to various fields [1,2]. We describe here the formulation of nanoparticles based on a polycation, Polyhydroxylamine (PT), with PA as a model polyanion to form binary polyelectrolyte complexes (PICs). Plasmid containing Green Fluorescence Protein (pGFP) DNA was also incorporated as a carried gene (polyanion) within the PEC nanoparticle. An advantage of using polyhydroxlamine based PICs is that they should allow release of bound DNA as a polyanion from a PEC at relatively mild pH conditions, due to the low pKa (below 9) of the PT. Such nanoparticles have potential use as cationic transfection agents that can release under benign physiological conditions compared to similar particles formulated with 'hard' polyamines with higher pKa (>pH9).

2 EXPERIMENTAL METHODS

2.1 Cationic Nanoparticle Preparation

Aqueous dispersions of a cationic polymer, Polyhydroxylamine (PT), were complexed by simple equal volume mixing of aqueous solutions (w/w) of polycation with polacrylic acid. In addition two molecular weights of PT (PT15:15k MWt 0.1% w/v +5.4mV, PT240: 240k 0.1% w/v +3.6mV) were each complexed with two molecular weights of Polyacrylic acid (PA) (PA15:15k, –7.1mV and PA240: 240k M.Wt, -1.7mV.) all at pH4.5 in 20mM K Acetate, 14mM KCl. Clean-up was performed as serum replacement by centrifugation (13k rpm) and resuspension. Factorial (2x2) addition of the polycation with polyanion was performed and the order of addition was also used as a formulation factor. Post-treatment with PT was also applied to some dual component particles. pGFP (5.1k, pCS2*mt-SGP [a gift of Matt Guille], 1µg endotoxin free in 30µl DW) was added to 1ml 0.2% PT solution at pH4 and used in PEC formulation. All particles were twice washed between treatments. Particle types prepared were PA to PT:

1.PT15-PA15, 2. PT240-PA15, 3. PT15-PA240, 4. PT240-PA240, and PT to PA:

5. PA15-PT15, 6. PA240-PT15, 7. PA15-PT240, 8. PA240-PT240.

Post-treatment of PT240 was applied to types 1, 2 and 3. giving bead types 9. PT15-PA15-PT240, 10. PT240-PA15-PT240 and 11. PT15-PA240-PT240.

GFP treatments [PL} were applied to some of the PEC bead types by prebinding pGFP to the PT.

2.2 Nanoparticle Characterization

Particle size and electrophoretic mobility (reported here as zeta potential) were determined on resultant particle dispersions using a Malvern Zetasizer 3000HSa at pH4.00 and 2.0mS conductivity, 22'C. Size reported in nanometres (nm) standard error (se) and Polydispersity (PD).

2.3 GFP plasmid incorporation

Presence of plasmid GFP (pGFP) was determined by agarose gel electrophoresis on 6µl subsamples of final nanoparticle stock. Two further particle types: 12. a PS bead bearing an outer layer of PT and surface bound pGFP and 13. PT240 polymer with pGFP (PT240-PL) are included on the gel.

3 RESULTS AND DISCUSSION

3.1 Polyion Complex Nanoparticles

Polyion complexes (PIC) formed from PA addition to PT [PT-PA] resulted in nanoparticles between 95 and 182 nm, with a charge range of +3.5 to +17.7 mV (Table1). Conversely formulations performed by PT addition to PA

gave particles in the range 80 to 700 nm, with a charge range of +7.6 to +30.9 mV (Table2).

Type PA to PT	Zeta [mV] (se)	Size [nm] (se) PD
1. PT15-PA15	+4.1 (1.7)	95 (75) 0.443
2. PT240-PA15	+17.7 (1.8)	182 (23) 0.785
3. PT15-PA240	+3.5 (0.4)	142 (98) 0.797
4.PT240-PA240	+3.6 (1.6)	136 (62) 0.578

Table 1: PIC nanoparticles formed from Polyanion PA addition to Polycation PT. Type 1-4

Post-treatment of type 1,2 & 3 with further addition of polycation resulted in types 9-11 (Table 3), with type 10 PT240-PA15-PT240 demonstrating a significantly reduced particle zeta potential +17.7 (1.8) to +2.7 (1.0) as type 3; PT240-PA15. The most noticeable difference in nanoparticles produced is in the importance of addition order; PA to PT or PT to PA. These results are summarized in Figures 1 and 2.

Type PT to PA	Zeta Potential [mV] (se)	Size [nm] (se) PD
5. PA15-PT15	+25.6 (1.2)	112 (173) 0.688
6. PA240-PT15	+7.6 (3.4)	82 (67) 0.874
7. PA15-PT240	+30.9 (0.5)	346 (11) 0.151
8. PA240-PT240	+21.0 (1.3)	724 (527) 0.850

Table 2: PIC nanoparticles formed from Polyanion PA addition to Polycation PT. Type 5-8.

Type PT-PA-PT	ZetaPotential [mV] (se)	Size [nm] (se) PD
9. PT15-PA15-PT240	+2.8 (1.1)	122 (49) 0.654
10. PT240-PA15-PT240	+2.7 (1.0)	112 (86) 0.721
11.PT15-PA240-PT240	+3.4 (0.9)	69 (80)

Table 3: PIC nanoparticles formed after further PT240 addition to PT-PA type particles 1, 2 and 3.

All formulations were found to be positively charged under mildly acidic conditions (+3+30 mV) pH 4.00 (Fig1). Addition of PA to PT produces a smaller range of particle types in both size and charge, when compared to similar solutions mixed as PT to PA to form the PIC (Figure 2).

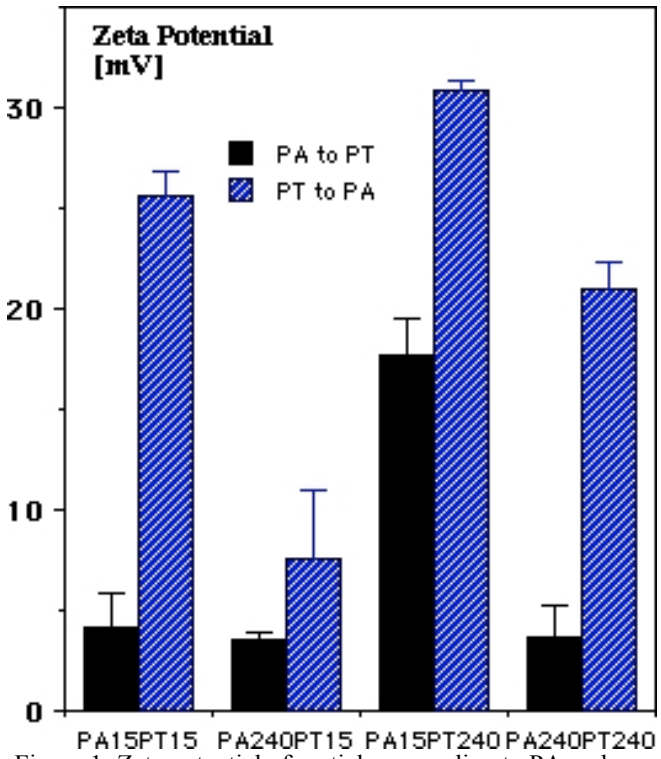

Figure 1: Zeta potential of particles according to PA and PT combination and direction of addition.

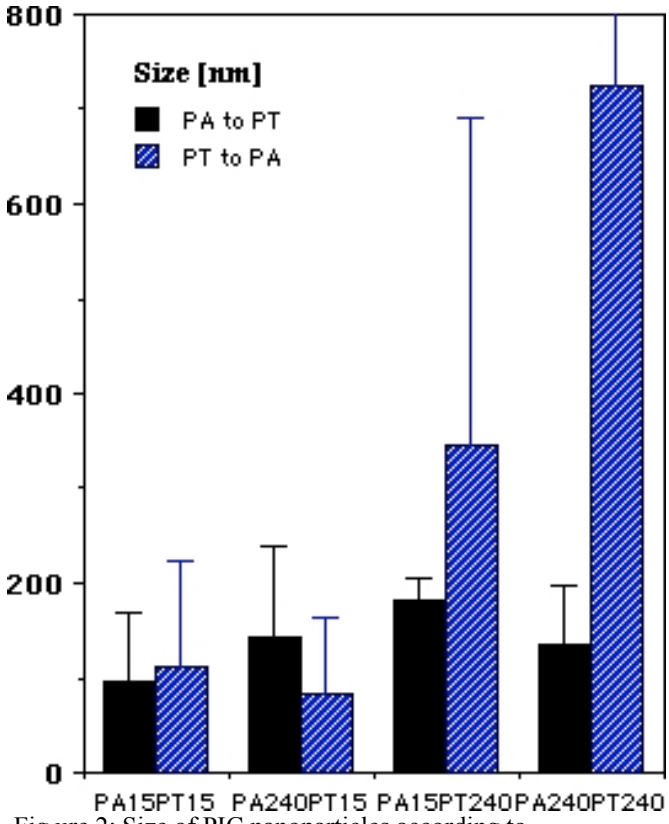

Fig ure 2: Size of PIC nanoparticles according to combination of PT and PA, and order of addition to create PIC.

3.2 pGFP incorporation

pGFP is confirmed to be incorporated into the PIC when prebound to the PT component (Figure 3).

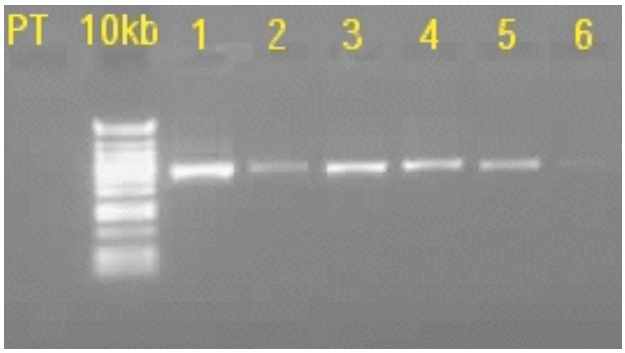

Figure 3: Agarose Gel of various pGFP (5.1kb) bearing nanoparticles. c. 200ng beads/well. Key: [PL: plasmid GFP], PT –Polyhydroxylamine 240k control, 10kb - mwt ladder, 1.PT240-PL (Type 13), 2. Core-PT[PL] (Type 12), 3.PT15[PL]-PA15,4.PT15[PL]-PA15-PT15, 5.PT240[PL]/PA15 , 6. former 5. +PT240.

4 CONCLUSION

Simple addition of polyhydroxylamine and poly-acrylic acid (w/w), under mildly acidic conditions, causes spontaneous formation of cationic nanoparticulate polyion complex (PIC) dispersions with a range of size (80 and 700 nm), and charge (+4+30mV).

Vector of addition, as PA to PT or PT to PA, influences particle size and charge. Addition of further polyhydroxylamine to particles formed as PA added to PT results in reduced zeta potential (+17.7 to +2.7 mV). Plasmid GFP can be incorporated into the particle by prebinding pGFP to the polyhydroxylamine before PIC formation.

REFERENCES

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