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Sub-100 nm chitosan-triphosphate-DNA nanoparticles for delivery of DNA vaccines

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Abstract: Intramuscular delivery is one of the main route for DNA vaccines administration. However, it requires large amounts of the DNA administered and external stimulation to encourage the internalization of the DNA. In this work we consider routes for less invasive administration route, and develop drug delivery systems (DDS) for intranasal administration. Chitosan polyplexes using sodium tripolyphosphate (TPP) as a crosslinker were prepared using the ionic gelation method. Our method allowed preparation of nanoparticles with the size bellow 50nm that is at least two times lower than previously reported. Moreover, despite small size, we obtained DNA encapsulation efficiencies about 70%. Parameters that may affect the encapsulation efficiency were investigated, including different chitosan-TPP ratios and concentrations of DNA. We found that encapsulation efficiency of DNA inside the particles increases with the increasing TPP-chitosan ratio. Moreover, increasing the DNA concentration also leads to a higher encapsulation efficiency. Small (<50nm) chitosan nanoparticles hold enormous potential as DNA carriers through physiological barriers and subsequent internalization

Keywords: chitosan; DNA vaccines; tripolyphosphate; nanoparticles; ionic gelation

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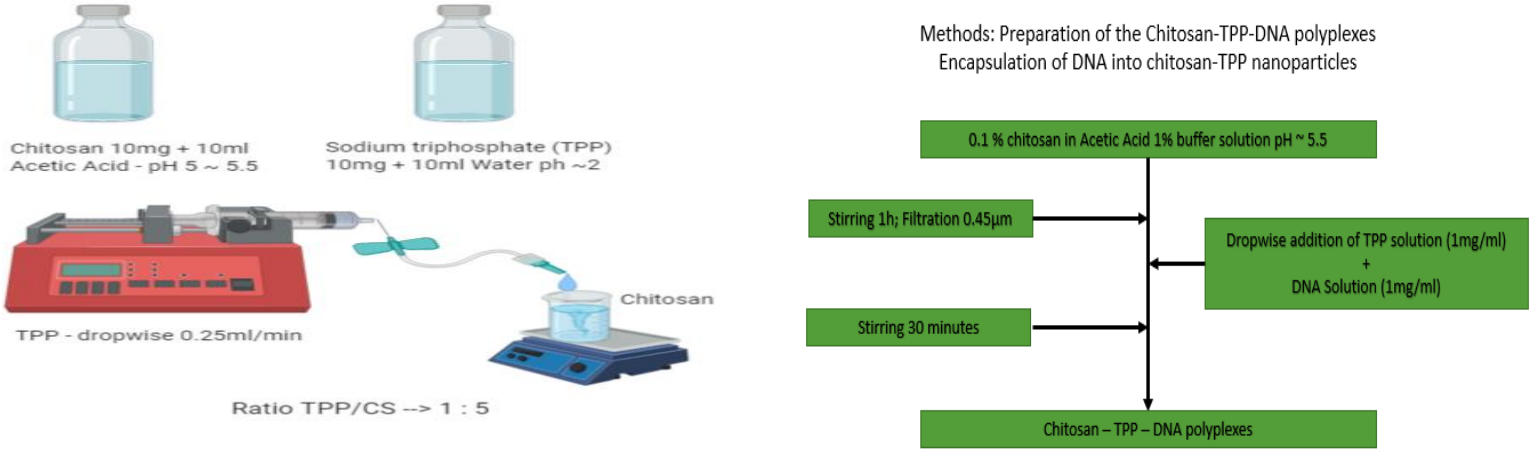
Methods

Ionotropic gelation:

Chitosan → polymer

Sodium tripolyphosphate (TPP) → crosslinker



(a) (b)

Figure 1. (a) Schematic illustration of chitosan-TPP nanoparticles (b) Flow chart of the nanoparticle with DNA preparation First solutions with optimized concentrations were prepared. Then TPP with DNA was added to chitosan solution dropwise while stirring.

- Dropwise addition of TPP into CS solution
- Particles with and without DNA
- Negative and Positive control

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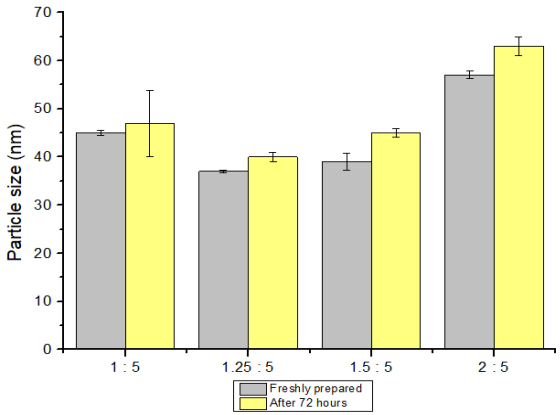
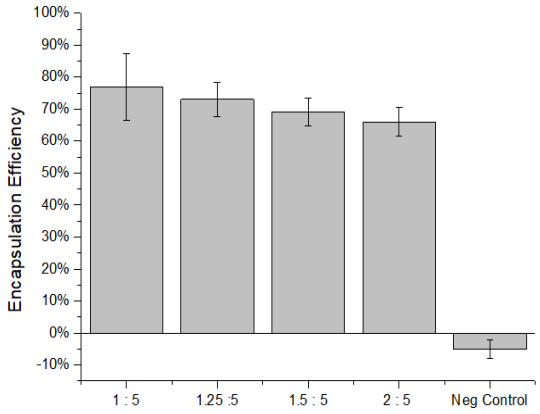
Results and Discussion

Table 1. Average particle size, PDI and encapsulation efficiency of chitosan-TPP-DNA polyplexes with different TPP/CS ratios

TPP/CS Ratio	Z-Average Size (nm)	Polydispersibily index (PDI)	Z - Average Size after 72h (nm)	(PDI) after 72h	Encapsulation efficiency (%)
1 : 5	45 ± 0.5	0.55 ± 0.01	47 ± 6.9	0.51 ± 0.006	77 ± 10
1.25 : 5	37 ± 0.3	0.50 ± 0.03	40 ± 0.9	0.48 ± 0.004	73 ± 5
1.5 : 5	39 ± 1.7	0.46 ± 0.04	45 ± 0.9	0.40 ± 0.009	69 ± 4
2 : 5	57 ± 0.8	0.50 ± 0.02	63 ± 1.9	0.46 ± 0.010	66 ± 4





- Small particles → below 70 nm
- Hight TPP/CS ratio → small increase in size
- No significant increase after 72h
- No interference of blank particles in dEE
- Good efficiency of encapsulation
- Increasing TPP/CS ratio → small decrease in E.E. (10%)

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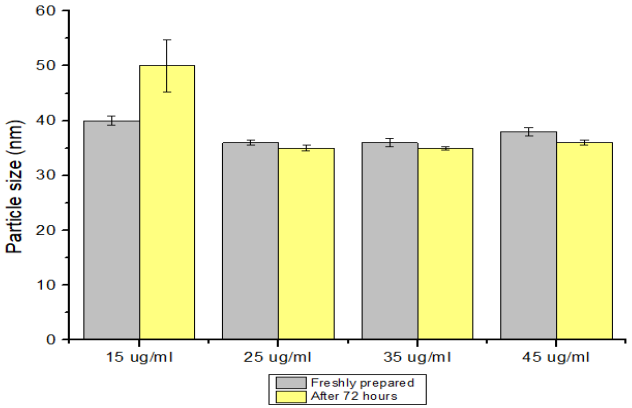
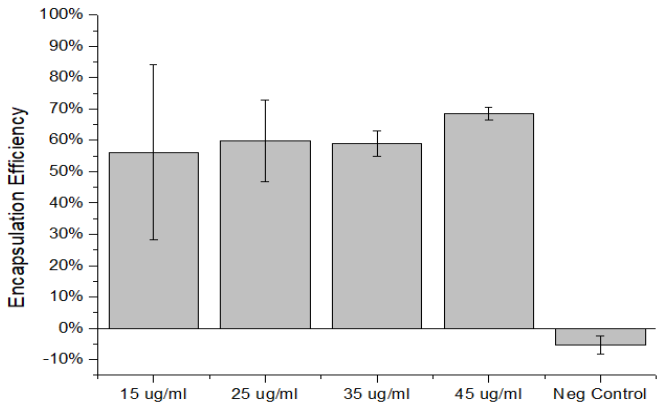
Results and Discussion

Table 2. Average particle size, PDI and encapsulation efficiency of chitosan-TPP-DNA polyplexes with different DNA concentrations

DNA Concentration (µg/ml)	Z-Average Size (nm)	Polydispersibily index (PDI)	Z - Average Size after 72h (nm)	(PDI) after 72h	Encapsulation efficiency (%)
15 ± 5	40 ± 0.8	0.59 ± 0.01	50 ± 4.8	0.39 ± 0.26	56 ± 28
25 ± 5	35 ± 0.5	0.48 ± 0.05	35 ± 0.5	0.53 ± 0.006	60 ± 13
35 ± 5	36 ± 0.8	0.47 ± 0.05	35 ± 0.3	0.56 ± 0.006	59 ± 4
45 ± 5	38 ± 0.7	0.47 ± 0.04	36 ± 0.4	0.55 ± 0.01	69 ± 2





- Small particles → below 50 nm
- Changing DNA concentration → no difference in size
- Small increase about 10nm in the sample with 15 µg/mL after 72h
- Good efficiency of encapsulation
- Increasing DNA concentration → Increase in E.E.

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Conclusions

- Ionic gelation of CS/TPP nanoparticles → promising delivery system for nucleic acids
- Nanocarriers below 100nm in size → Efficient internalization of NPs by relevant cells
- High quantities of DNA can be encapsulated using TPP/CS nanoparticles
- Delivery of DNA vaccines demands → high quantity of DNA to achieve the effective immunization and therapeutic effects

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