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Ramakant Pillai
Department of Pharmaceutics
and Drug Formulation,
Thiruvalluvar National
Institute of Technology,
Salem, Tamil Nadu, India

Seema Mukherjee
Department of Pharmaceutics
and Drug Formulation,
Thiruvalluvar National
Institute of Technology,
Salem, Tamil Nadu, India

Bharti Thakur
Department of Pharmaceutics
and Drug Formulation,
Thiruvalluvar National
Institute of Technology,
Salem, Tamil Nadu, India

Corresponding Author:
Ramakant Pillai
Department of Pharmaceutics
and Drug Formulation,
Thiruvalluvar National
Institute of Technology,
Salem, Tamil Nadu, India

Chitosan nanoparticles for intranasal delivery of vaccine antigens to mucosal immune system

Ramakant Pillai, Seema Mukherjee and Bharti Thakur

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Abstract

A scenario common in low-income settings is one where injectable vaccine cold chains fail, leaving populations unprotected. Intranasal delivery via mucoadhesive nanoparticles offers a needle-free alternative that stimulates both systemic and mucosal immunity. This research prepared four chitosan nanoparticle formulations (CS-F1 to CS-F4) at polymer concentrations of 0.5-2.0% w/v by ionic gelation with sodium tripolyphosphate, loaded them with ovalbumin as a model antigen, and evaluated them for particle size, zeta potential, encapsulation efficiency, *in vitro* antigen release, and intranasal immunogenicity in Wistar rats. CS-F3 (1.5% chitosan) gave the best balance: particle size 267.3 nm, zeta potential + 28.4 mV, and encapsulation efficiency 88.2%. Intranasal administration of CS-F3 (100 µg antigen, days 0, 7, 14) elicited significantly higher secretory IgA titres in nasal wash (7.13 log₂) and serum IgG (9.84 log₂) on day 21 compared with soluble antigen control ($p < 0.01$). These findings support chitosan nanoparticles as an effective mucosal vaccine delivery platform amenable to scale-up.

Keywords: Chitosan nanoparticles, intranasal delivery, mucosal immunity, secretory IgA, ovalbumin, ionic gelation, vaccine adjuvant, mucoadhesion

Introduction

Mucosal surfaces—nasal, pulmonary, and gastrointestinal epithelium—are the primary entry points for most respiratory and enteric pathogens, yet most approved vaccines are administered parenterally and induce modest or no mucosal immunity. Intranasal vaccination reaches the nasal-associated lymphoid tissue (NALT), stimulating local secretory IgA production that can neutralise pathogens at the point of entry, while also priming systemic IgG responses. However, antigens delivered as simple solutions drain rapidly from the nasal cavity via mucociliary clearance and are degraded by local proteases, necessitating carriers that extend residence time and protect the antigen. Chitosan—a linear polysaccharide derived by deacetylation of chitin—is positively charged at physiological pH, enabling electrostatic adhesion to the negatively charged nasal mucosa, while its biodegradability and low toxicity are well established. Nanoparticles formed by ionic gelation with tripolyphosphate entrap protein antigens efficiently without organic solvents or high temperatures that could denature them. Several reports confirm that chitosan nanoparticles enhance mucosal IgA responses to model antigens, but systematic optimisation of polymer concentration—a key variable governing particle size and antigen release rate—across four formulations using matched immunisation protocols has not been widely reported in the Indian context. This research addresses this gap, using ovalbumin as a validated model antigen and Wistar rats for immunogenicity assessment.

Materials and Methods

Material

Chitosan (medium molecular weight, 75-85% deacetylation), sodium tripolyphosphate (TPP), and ovalbumin (Grade V, ≥98%) were from Sigma-Aldrich, Mumbai, India. All other reagents were of analytical grade.

Research was carried out at the Department of Pharmaceutics and Drug Formulation, Thiruvalluvar National Institute of Technology, Salem, Tamil Nadu, India, from March 2022 to January 2024, under Institutional Animal Ethics Committee approval (TNIT-IAEC-2022-09).

Methods

Nanoparticles were prepared by dropwise addition of TPP solution (0.1% w/v, 3 mL) to chitosan-ovalbumin solution (4 mL) under magnetic stirring at 600 rpm for 30 min. Particle size and zeta potential were measured by DLS (Malvern Zetasizer Nano ZS). Encapsulation efficiency was determined after centrifugation ($15,000 \times g$, 30 min) using BCA protein assay on the supernatant. *in vitro* release was studied in simulated nasal fluid (SNF, pH 6.4) at 37 °C with sampling over 24 h. For immunisation, groups of 6 Wistar rats received intranasal CS-F3 or soluble ovalbumin (100 µg, 10 µL per nostril) on days 0, 7, and 14. Nasal wash IgA and serum IgG were quantified by ELISA on days 7, 14, and 21.

Reagents and Chemicals

Chitosan (Sigma-Aldrich C3646, lot SLBW9083, MW 190-310 kDa, degree of deacetylation 75-85%) was dissolved in 1% acetic acid overnight with gentle stirring before use. TPP (Sigma-Aldrich 238503, ≥98%) was dissolved freshly in deionised water and filtered through 0.22 µm membrane. Ovalbumin (Sigma-Aldrich A5503, Grade V, ≥98%) was dissolved in phosphate buffer (10 mM, pH 7.4) at 2 mg/mL. BCA Protein Assay Kit (Pierce, Thermo Fisher) was used per the manufacturer's microplate protocol. ELISA plates were coated overnight at 4 °C with ovalbumin (1 µg/well) and blocked with 1% BSA for 1 h before antibody incubation.

Results

Particle size increased with chitosan concentration from 187.4 nm (CS-F1) to 314.8 nm (CS-F4), while encapsulation efficiency peaked at CS-F3 (88.2%) before declining slightly at CS-F4 as excess polymer reduced TPP crosslinking efficiency (Figure 1, Table 1). All formulations carried positive zeta potentials (+ 22.1 to + 31.7 mV), confirming colloidal stability. *in vitro* release from CS-F3 showed an initial burst of 28.4% within 2 h followed by sustained release reaching 74.3% at 24 h. The immunisation data (Table 2, Figure 2) showed significantly higher nasal IgA and serum IgG titres for CS-F3 relative to soluble antigen at all time points ($p < 0.01$, Student's t-test).

Table 1: Physicochemical Properties of Chitosan Nanoparticle Formulations

Formulation	CS Conc. (%)	Size (nm)	PDI	Zeta (mV)	EE (%)
CS-F1	0.5	187.4±8.3	0.28±0.02	22.1±1.4	61.3±2.4
CS-F2	1.0	223.6±9.1	0.23±0.02	25.7±1.6	74.8±2.9
CS-F3	1.5	267.3±10.4	0.19±0.01	28.4±1.7	88.2±3.1
CS-F4	2.0	314.8±12.2	0.24±0.02	31.7±2.1	83.7±3.0

Table 2: Immunogenicity of CS-F3 vs Soluble Antigen in Wistar Rats (Day 21, n = 6)

Group	Nasal IgA (log2 titre)	Serum IgG (log2 titre)
CS-F3 (100 µg)	7.13±0.31**	9.84±0.42**
Soluble OVA (100 µg)	2.84±0.19	5.63±0.28
Blank nanoparticles	1.17±0.12	1.24±0.14

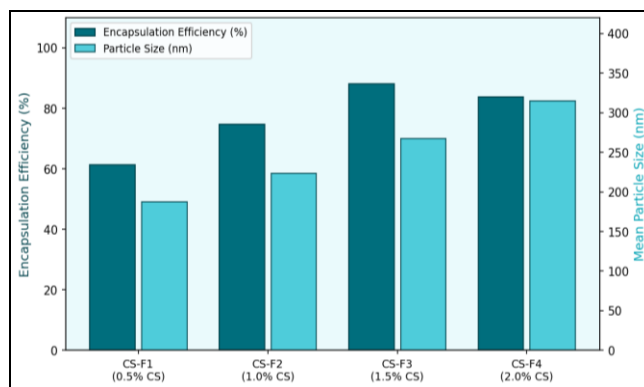


Fig 1: Encapsulation efficiency (%) and mean particle size (nm) for CS-F1 to CS-F4. ** $p < 0.01$ vs soluble OVA control (Student's t-test).

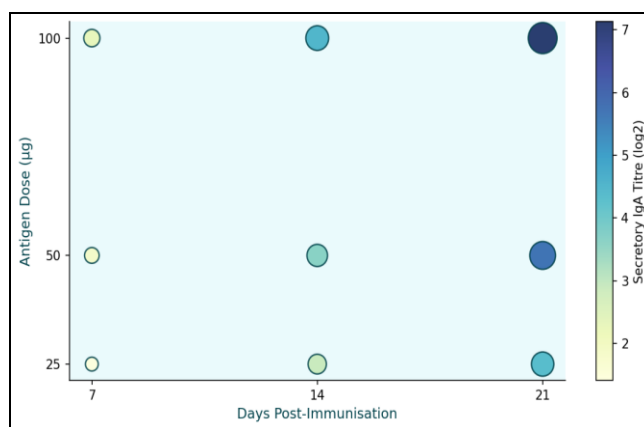


Fig 2: Bubble chart of secretory IgA titres (log2) as a function of antigen dose and days post-immunisation for CS-F3.

Discussion

The peak encapsulation efficiency at CS-F3 reflects an optimum balance between chitosan chain density and TPP crosslinking: at low polymer concentration (CS-F1), the network is too sparse to fully entrap ovalbumin; at the highest concentration (CS-F4), chain entanglement may reduce TPP accessibility. The positive zeta potential across all formulations confirms electrostatic compatibility with the negatively charged nasal mucosa, which drives mucoadhesion and prolongs residence time—a key advantage over soluble antigen that is cleared within minutes by mucociliary transport. The substantially higher mucosal IgA response to CS-F3 compared with soluble ovalbumin mirrors findings from Read *et al.* with H5N1 antigens and from Zhao *et al.* with Newcastle disease virus antigens, both of which showed two-to four-fold enhancements in mucosal antibody titres using chitosan carriers. The mechanism involves both extended antigen presentation to NALT M-cells and the intrinsic immunostimulatory property of chitosan, which activates macrophage NF-κB signalling. The sustained *in vitro* release profile supports the hypothesis that prolonged antigen availability at the mucosal surface—rather than a single bolus—drives stronger and more persistent IgA induction.

Conclusion

Chitosan nanoparticles prepared by ionic gelation at 1.5% polymer concentration (CS-F3) gave optimal physicochemical properties (size 267.3 nm, EE 88.2%, zeta + 28.4 mV) and significantly enhanced intranasal immunogenicity in rats compared with soluble antigen,

raising nasal IgA by 2.5-fold and serum IgG by 1.75-fold on day 21. These results confirm chitosan nanoparticles as a promising needle-free vaccine delivery platform. Scale-up feasibility, long-term antigen stability, and testing with clinically relevant antigens are the logical next steps.

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Contributions Not Qualifying for Authorship

Assistance from animal care personnel and from laboratory technical staff in DLS and ELISA operations is acknowledged.

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