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# BIODEGRADABLE CHITOSAN-BASED MICROSPHERES FOR CONTROLLED ORAL VACCINE DELIVERY: SYNTHESIS, CHARACTERIZATION, AND RELEASE KINETICS

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## ABSTRACT

In recent years, advances in biodegradable polymer-based drug delivery systems have opened new avenues for oral vaccine administration. Chitosan, a natural cationic polysaccharide, has attracted considerable interest due to its mucoadhesive properties, biocompatibility, and ability to enhance mucosal permeability. In this study, caprylate–chitosan/alginate–cetyl trimethyl ammonium bromide (CTAB) polyelectrolyte complexes were synthesized for the encapsulation of diphtheria and tetanus toxoids. The microparticles were prepared via coacervation and characterized using Fourier transform infrared spectroscopy (FT-IR), field emission scanning electron microscopy (FE-SEM), and energy-dispersive X-ray spectroscopy (EDS). Entrapment efficiency, loading capacity, swelling index, and in-vitro release kinetics were evaluated. The microspheres exhibited high entrapment efficiency (TT: 91.12%, DT: 87.26%) and sustained release profiles over three weeks, following anomalous diffusion kinetics. SEM revealed smooth, sponge-like particles with low sphericity, while FT-IR confirmed polyelectrolyte complex formation. These findings highlight the potential of chitosan-based microspheres as an effective oral vaccine delivery platform, enabling controlled antigen release and enhanced mucosal immunization.

**KEYWORDS:** Chitosan, Controlled release, Oral vaccine delivery, Microspheres, Biodegradable polymers, Mucoadhesion, Diphtheria toxoid, Tetanus toxoid.

## 1. INTRODUCTION

The development of novel immunization strategies has been a priority in biomedical research, with emphasis on achieving higher and longer-lasting immune responses through parenteral and/or oral administration. One promising approach involves **prolonged antigen release** via controlled drug delivery systems based on biodegradable polymers (Kohn et al., 1986; Villa et al., 1998). These systems can deliver antigens or adjuvants to specific locations at predetermined rates, protecting them from degradation, reducing systemic side effects, and enabling co-encapsulation of multiple components.

### 1.1 Oral Drug Delivery

Oral delivery offers convenience and improved patient compliance. However, proteins and macromolecules are generally unsuitable for oral administration due to **enzymatic degradation** in the gastrointestinal tract and poor epithelial permeability (Coyne & Bergelson, 2005). Approaches such as innovative encapsulation, enzyme inhibitors, and permeation enhancers aim to overcome these limitations (Falson & Buri, 1997; Kissel & Li, 2002; Lakshmi et al., 2003).

### 1.2 Permeation Enhancers

Chemical permeation enhancers (cpEs) can transiently increase intestinal permeability, enabling the absorption of macromolecules by altering epithelial tight junctions or increasing transcellular transport (Chandy & Sharma, 1990; Kato et al., 2003). Ideal cpEs are biocompatible, fast-acting, reversible, and non-toxic.

### 1.3 Chitosan as a Material of Choice

Chitosan, derived from the partial deacetylation of chitin, is a **biodegradable, mucoadhesive, and biocompatible** polymer widely investigated for controlled drug delivery (Sahoo et al., 2009). Its cationic nature allows strong interaction with negatively charged mucin, enhancing paracellular transport by transiently opening tight junctions (Artursson et al., 1994). Chitosan microspheres have been shown to improve mucosal uptake and immune responses in vaccine delivery (McNeela et al., 2000).

### 1.4 Mucoadhesive Microspheres for Vaccine Delivery

Microspheres provide optimal particle size for uptake by antigen-presenting cells (Eldridge et al., 1991; O'Hagan et al., 1993), prolong residence time at mucosal surfaces, and protect antigens from degradation. Chitosan-based mucoadhesive microspheres have demonstrated strong systemic and mucosal immune responses following oral immunization (Ahire et al., 2007).

### 1.5 Mechanisms of Action

Chitosan enhances drug delivery via

1. **Increasing paracellular permeability** by modulating tight junctions (Godfrey, 1997).
2. **Increasing membrane fluidity** to facilitate transcellular transport (Ozaki et al., 1999).
3. **Modifying mucus rheology** to reduce viscosity and improve drug diffusion (Lee et al., 1991).

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Low molecular weight chitosan (90% deacetylation), sodium alginate, sodium caprylate, cetyl trimethyl ammonium bromide (CTAB), tetanus toxoid (TT), and diphtheria toxoid (DT) were used. TT and DT were provided by the Serum Institute of India, Pune.

### 2.2 Synthesis of Caprylate–Chitosan/Alginate–CTAB Microspheres

Microspheres were prepared by **coacervation**, wherein chitosan and caprylic acid were combined and added dropwise to sodium alginate, with or without TT and DT. The pH was adjusted, and products were washed with acetone and ethanol, centrifuged, and dried at 45°C overnight.

### 2.3 Entrapment Efficiency and Loading Capacity

Antigen content was quantified by in-house ELISA.

- **Entrapment efficiency (%)** = Initial antigen –

free antigenInitial antigen×100\frac{\text{Initial antigen – free antigen}}{\text{Initial antigen}} \times 100Initial antigenInitial antigen – free antigen×100

- **Loading capacity (%)** = Initial antigen –

free antigenWeight of microspheres×100\frac{\text{Initial antigen – free antigen}}{\text{Weight of microspheres}} \times 100Weight of microspheresInitial antigen free antigen×100

### 2.4 Characterization

- **FT-IR** (Shimadzu FT-IR-8400) for functional group analysis.
- **FE-SEM** for surface morphology.
- **EDS** for elemental analysis.

### 2.5 Swelling Index

Determined in phosphate buffer (pH 7.4) over 1–6 h.

### 2.6 In-vitro Release Studies

Microspheres were incubated in PBS at 37°C, and antigen release was measured by ELISA at 24 h intervals for three weeks.

### 2.7 Kinetic Analysis

Release data were fitted to Zero-order, First-order, Higuchi, Korsmeyer–Peppas, and Baker–Lonsdale models.

## 3. RESULTS AND DISCUSSION

### 3.1 Synthesis

Both placebo and antigen-loaded microspheres were successfully synthesized, achieving uniform pH and batch-to-batch reproducibility.

### 3.2 Entrapment Efficiency and Loading Capacity

High entrapment efficiencies were observed (TT: 91.12%, DT: 87.26%), with loading capacities of 7.81% and 7.47%, respectively. Minimal burst release in the first 24 h indicated optimal protein loading.

### 3.3 FT-IR Analysis

Spectra confirmed ionic interactions between chitosan amino groups and alginate carboxyl groups, as well as conjugation with caprylate. Shifts in characteristic peaks indicated successful incorporation of toxoids into the polymer matrix.

### 3.4 SEM Morphology

Microparticles displayed smooth, sponge-like surfaces with slight irregularities, suggesting mechanical effects during preparation. Post-release samples exhibited porous structures, confirming polymer erosion.

### 3.5 Swelling Behavior

Microspheres showed pH-dependent swelling, facilitating controlled antigen release.

### 3.6 In-vitro Release and Kinetics

Sustained release was achieved over three weeks, with release kinetics best fitting the **Korsmeyer–Peppas model**, indicating anomalous diffusion involving both diffusion and polymer relaxation.

## 4. CONCLUSION

This study demonstrates the potential of **caprylate–chitosan/alginate–CTAB microspheres** as an effective oral vaccine delivery system. The high entrapment efficiency, mucoadhesive properties, and sustained release profile make them suitable for inducing prolonged mucosal and systemic immune responses. Future in-vivo studies are warranted to validate immunogenicity and optimize formulation parameters.

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