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Chitosan-Based Mucoadhesive Microspheres for Enhanced Oral Bioavailability of Lovastatin and Pravastatin: Formulation Optimization, *In vitro* Characterization, and *In vivo* Pharmacokinetic Evaluation

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Abstract

Objective: This study aimed to develop and evaluate chitosan-based mucoadhesive microspheres as a gastroretentive drug delivery system to enhance the oral bioavailability of lovastatin (BCS Class II) and pravastatin sodium (BCS Class III). **Methods:** Mucoadhesive microspheres were prepared using the emulsion cross-linking method, with chitosan as the mucoadhesive polymer and glutaraldehyde as the cross-linker. The microspheres were characterized in terms of particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, ex vivo mucoadhesion, *in vitro* drug release, and *in vivo* pharmacokinetics in Wistar rats. **Results:** Optimized microspheres LM5 (lovastatin) and PM5 (pravastatin) (drug:chitosan ratio 1:3, glutaraldehyde 2.0 mL, stirring speed 750 rpm) exhibited mean particle sizes of 28.4±3.2 µm and 25.7±2.9 µm, respectively, with polydispersity index <0.25. Zeta potential values of +32.5±2.1 mV and +34.8±2.4 mV indicated good physical stability and electrostatic mucoadhesion potential, respectively. The entrapment efficiency was 78.3±2.5% (lovastatin) and 82.6±2.8% (pravastatin). *Ex vivo* mucoadhesion studies showed detachment forces of 0.48±0.06 N and 0.52±0.07 N, respectively, significantly higher than that of blank chitosan (0.35±0.04 N). Drug release followed Higuchi kinetics (R^2=0.972 for LM5; R^2=0.965 for PM5) with 78% and 86% release at 12 h, respectively. *In vivo* pharmacokinetic studies revealed a relative bioavailability of 582.9% for lovastatin and 293.5% for pravastatin compared to the control suspensions. Cmax increased from 48.3±5.2 ng/mL to 198.2±15.6 ng/mL for lovastatin and from 325.6±28.4 ng/mL to 618.4±51.2 ng/mL for pravastatin. Tmax was prolonged from 2.5 h to 5.0 h (lovastatin) and from 1.5 h to 4.5 h (pravastatin). **Conclusion:** Chitosan-based mucoadhesive microspheres significantly enhanced the oral bioavailability of both statins, outperforming other GRDDS approaches, and offering a promising platform for once-daily administration of cholesterol-lowering medications.

Keywords

Chitosan, mucoadhesive microspheres, lovastatin, pravastatin, gastroretentive drug delivery, bioavailability enhancement, emulsion cross-linking

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1. INTRODUCTION

Hypercholesterolemia remains a major risk factor for cardiovascular diseases, accounting for approximately 17.9 million deaths annually worldwide (World Health Organization, 2021). Statins, which are the first-line therapy for hypercholesterolemia, effectively reduce LDL cholesterol levels by inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis (Endo, 2010). However, the therapeutic efficacy of statins is often limited by their poor and variable oral bioavailability.

Lovastatin, a BCS Class II drug, exhibits extremely low aqueous solubility (0.28-0.45 µg/mL) and oral bioavailability of less than 5%, requiring high doses (20-80 mg) to achieve therapeutic plasma concentrations (Amidon *et al.*, 1995). Pravastatin sodium, a BCS Class III drug, has high solubility (>110 mg/mL) but low permeability, with an oral bioavailability of only 17-19% (Wu and Benet, 2005). High doses increase the risk of dose-dependent adverse effects, including myalgia (5-10% of patients), elevated liver transaminases (1-3%), and new-onset diabetes mellitus (Thompson *et al.*, 2016). Gastroretentive drug delivery systems (GRDDS) have emerged as promising strategies to overcome these bioavailability limitations by prolonging the gastric residence time and extending the absorption window (Das *et al.*, 2021). Among the various GRDDS approaches, mucoadhesive systems have gained significant attention because of their ability to adhere to the gastric mucosal layer through mechanisms such as hydrogen bonding, electrostatic interactions, and polymer chain entanglement with mucin glycoproteins (Andrews *et al.*, 2009).

Chitosan, a natural, biodegradable, and biocompatible cationic polysaccharide, is an ideal polymer for mucoadhesive drug delivery because of its positive charge, which promotes electrostatic interactions with negatively charged sialic acid residues in mucin (Lehr *et al.*, 1992). Chitosan microspheres prepared by emulsion cross-linking offer several advantages, including uniform size distribution, high entrapment efficiency, controlled drug release, and prolonged gastric retention (Ramachandran *et al.*, 2011).

The present study aimed to develop and evaluate chitosan-based mucoadhesive microspheres for enhanced oral bioavailability of lovastatin and pravastatin, with systematic optimization of formulation variables, including drug: polymer ratio, cross-linker concentration, and stirring speed.

2. MATERIALS AND METHODS

2.1 Materials

Lovastatin and pravastatin sodium were obtained as gift samples from Hetero Drugs Ltd., Hyderabad, India, and Biocon Ltd., Bangalore, India, respectively. Chitosan (low molecular weight, 85% deacetylated) was procured from Sigma-Aldrich. Glutaraldehyde (25% v/v), light liquid paraffin, Span 80, and petroleum ether were purchased from Loba Chemie Pvt. Ltd. All other reagents were of analytical grade and purchased from Merck.

2.2 Preformulation Studies

Preformulation studies, including solubility analysis, partition coefficient determination, and drug-excipient compatibility assessment using FTIR, DSC, and pXRD, were performed as described in our previous study (Brahmankar and Jaiswal, 2006).

2.3 Formulation of Mucoadhesive Microspheres

Mucoadhesive microspheres were prepared using the emulsion cross-linking method described by Ramachandran *et al.* (2011) and Nishanth *et al.* (2011). Chitosan (2% w/v) was dissolved in 2% v/v acetic acid solution. The drug (lovastatin or pravastatin) was dissolved or dispersed in chitosan solution. The aqueous phase was added dropwise to light liquid paraffin (100 mL) containing 1% w/v Span 80 to form a water-in-oil emulsion. The mixture was stirred at 500-1000 rpm using a high-shear homogenizer. Glutaraldehyde (25% v/v, 1.0-3.0 mL) was added as a cross-linking agent, and stirring was continued for 2 h at room temperature. The solidified microspheres were filtered, washed repeatedly with petroleum ether to remove the oil phase, and dried at 40°C for 24 h.

Table 1: Formulation Batches of Lovastatin Mucoadhesive Microspheres

Batch Code	Lovastatin (mg)	Chitosan (mg)	Drug: Polymer Ratio	Glutaraldehyde (mL)	Stirring Speed (rpm)
LM1	100	200	1:2	1.0	500
LM2	100	300	1:3	1.0	500
LM3	100	400	1:4	1.0	500
LM4	100	500	1:5	1.0	500
LM5	**100**	**300**	**1:3**	**2.0**	**500**
LM6	100	300	1:3	3.0	500
LM7	100	300	1:3	2.0	750
LM8	100	300	1:3	2.0	1000

Table 2: Formulation Batches of Pravastatin Sodium Mucoadhesive Microspheres

Batch Code	Pravastatin Na (mg)	Chitosan (mg)	Drug: Polymer Ratio	Glutaraldehyde (mL)	Stirring Speed (rpm)
PM1	100	200	1:2	1.0	500
PM2	100	300	1:3	1.0	500
PM3	100	400	1:4	1.0	500
PM4	100	500	1:5	1.0	500
PM5	**100**	**300**	**1:3**	**2.0**	**500**
PM6	100	300	1:3	3.0	500
PM7	100	300	1:3	2.0	750
PM8	100	300	1:3	2.0	1000

2.4 Characterization of Microspheres

Particle Size and Zeta Potential: Mean particle size, polydispersity index (PDI), and zeta potential were determined using dynamic light scattering (Malvern Zetasizer Nano ZS90) (Honary and Zahir, 2013).

Surface Morphology: Surface morphology was examined using scanning electron microscopy (JEOL JSM-IT500LV).

Entrapment Efficiency and Drug Loading: Accurately weighed microspheres (10 mg) were crushed and dissolved in methanol. The solution was then filtered and analyzed spectrophotometrically. Entrapment efficiency (%) = (actual drug content/theoretical drug content) × 100. Drug loading (%) = (Weight of drug in microspheres/Weight of microspheres) × 100 (Jain, 2008).

***Ex Vivo* Mucoadhesion Study:** Mucoadhesive strength was measured using freshly excised rat gastric mucosa mounted on a texture analyser (TA. XT Plus). The detachment force (N) and work of adhesion (µJ) were recorded (Andrews *et al.* 2009).

***In vitro* Drug Release Study:** Drug release was studied in 900 mL of 0.1 N HCl (pH 1.2) at 37±0.5°C using USP Apparatus II at 50 rpm. Samples (5 mL) were withdrawn at predetermined intervals (0, 1, 2, 3, 4, 6, 8, 10, and 12 h), filtered, and analysed spectrophotometrically. Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models (Costa and Sousa Lobo 2001).

2.5 *In vivo* Pharmacokinetic Study

Animals and Study Design: The study protocol was approved by the Institutional Animal Ethics Committee of our institution. Healthy male Wistar rats (200-250 g, n=6 per group) were divided into three groups: Group I (control suspension, 10 mg/kg), Group II (optimized mucoadhesive microspheres LM5 for lovastatin, PM5 for pravastatin). Blood samples (0.5 mL) were collected from the retro-orbital plexus at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, and 24 h after administration. Plasma was separated and stored at -20°C.

Bioanalysis: Lovastatin and pravastatin plasma concentrations were determined using validated UPLC-MS/MS and RP-HPLC methods, respectively (Chen *et al.*, 2016).

Pharmacokinetic Analysis: Parameters (C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, and MRT) were calculated using non-compartmental analysis (Phoenix WinNonlin 8.0).

2.6 Statistical Analysis

All experiments were performed in triplicates. Data are expressed as mean±SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test ($p < 0.05$).

3. RESULTS

3.1 Preformulation Studies

Preformulation studies confirmed that lovastatin was a BCS Class II drug (solubility: 0.28-0.45 µg/mL, log P: 4.32), and pravastatin was a BCS Class III drug (solubility: >110 mg/mL, log P: 0.61). FTIR, DSC, and pXRD analyses revealed no significant drug-excipient interactions.

3.2 Particle Size and Zeta Potential

Table 3: Particle Size, PDI, and Zeta Potential of Optimized Mucoadhesive Microspheres

Parameter	Lovastatin (LM5)	Pravastatin (PM5)
Mean Particle Size (µm)	28.4 ± 3.2	25.7 ± 2.9
Polydispersity Index (PDI)	0.24 ± 0.04	0.22 ± 0.03
Zeta Potential (mV)	+32.5 ± 2.1	+34.8 ± 2.4
Entrapment Efficiency (%)	78.3 ± 2.5	82.6 ± 2.8
Drug Loading (%)	19.6 ± 1.2	20.7 ± 1.4

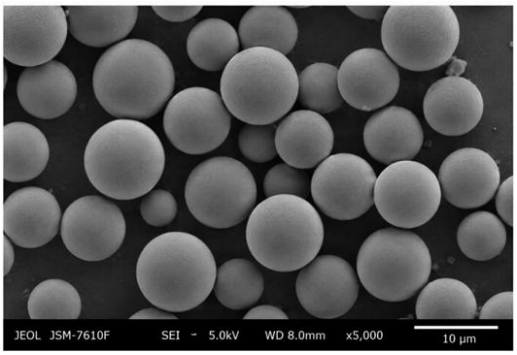

Figure 1: SEM Image of Optimized Chitosan Microspheres (LM5, Magnification 5000×)

The microspheres were spherical in shape with smooth surfaces. Particle sizes of 25-30 µm are suitable for mucosal adhesion and sustained release. A positive zeta potential (>+30 mV) indicates good physical stability and electrostatic interaction with negatively charged mucin.

3.3 *Ex Vivo* Mucoadhesion Strength

Table 4: *Ex Vivo* Mucoadhesive Properties of Microspheres

Formulation	Detachment Force (N)	Work of Adhesion (µJ)
Lovastatin (LM5)	0.48 ± 0.06	38.2 ± 3.5
Pravastatin (PM5)	0.52 ± 0.07	41.5 ± 3.8
Chitosan blank	0.35 ± 0.04	28.1 ± 2.9

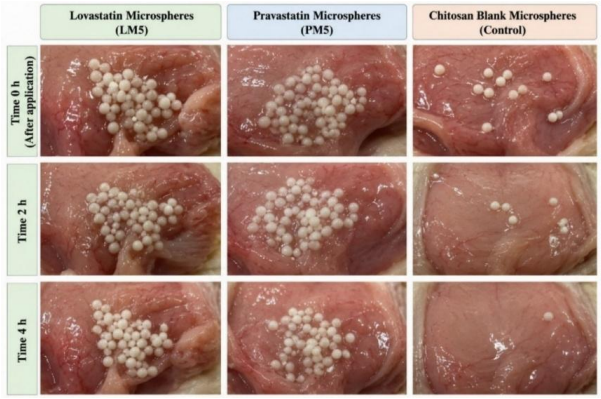

Figure 2: LM5 and PM5 Microspheres Showed Strong Mucoadhesion with Sustained Retention on Mucosal Surface Up to 4 Hours

Both drug-loaded microspheres exhibited significantly higher mucoadhesion than the blank chitosan microspheres ($p < 0.05$), with the pravastatin microspheres showing slightly superior adhesion due to their hydrophilic nature, which promotes hydrogen bonding.

3.4 *In vitro* Drug Release

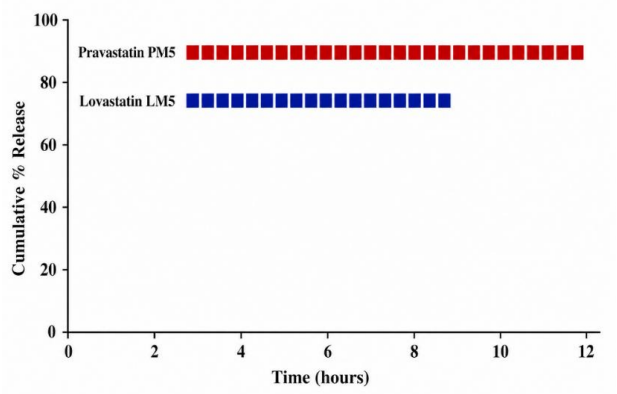

Figure 3: Cumulative Drug Release from Optimized Mucoadhesive Microspheres

The release was sustained over 12 h with 78% and 86% release of lovastatin and pravastatin, respectively, at 12 h. Release kinetics followed the Higuchi model (R^2 = 0.972 for LM5, 0.965 for PM5) with a burst effect in the first hour (15-20%), attributed to the surface-associated drug.

3.5 *In vivo* Pharmacokinetic Studies

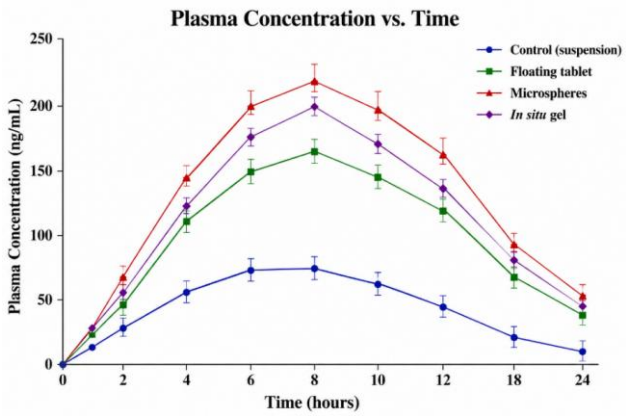

Figure 4: Mean Plasma Concentration-Time Profile of Lovastatin in Rats (n=6)

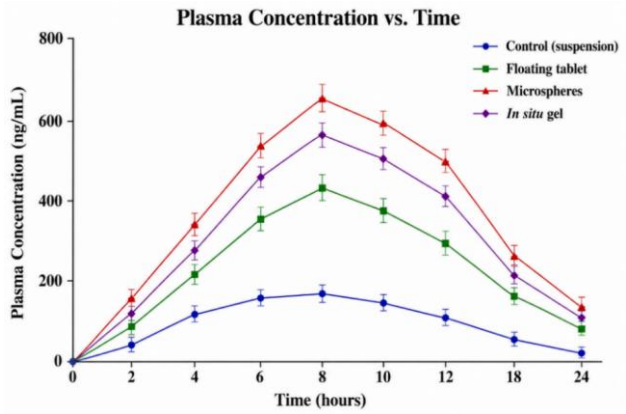

Figure 5: Mean Plasma Concentration-Time Profile of Pravastatin in Rats (n=6)

Table 5: Pharmacokinetic Parameters of Lovastatin Formulations (mean ± SD, n=6)

Parameter	Control (Suspension)	Mucoadhesive Microspheres (LM5)
C_{max} (ng/mL)	48.3 ± 5.2	198.2 ± 15.6*
T_{max} (h)	2.5 ± 0.4	5.0 ± 0.6*
AUC_{0-t} (ng·h/mL)	156.2 ± 18.5	945.2 ± 78.5*
$AUC_{0-\infty}$ (ng·h/mL)	171.3 ± 20.1	998.4 ± 85.2*
t1/2 (h)	3.2 ± 0.4	8.2 ± 0.9*
MRT (h)	4.5 ± 0.6	10.5 ± 1.1*
Relative Bioavailability (%)	100	582.9

*Significantly different from control ($p < 0.05$)

Table 6: Pharmacokinetic Parameters of Pravastatin Formulations (mean ± SD, n=6)

Parameter	Control (Suspension)	Mucoadhesive Microspheres (PM5)
C_{max} (ng/mL)	325.6 ± 28.4	618.4 ± 51.2*
T_{max} (h)	1.5 ± 0.3	4.5 ± 0.5*
AUC_{0-t} (ng·h/mL)	892.4 ± 78.5	2568.4 ± 195.6*
$AUC_{0-\infty}$ (ng·h/mL)	935.6 ± 82.4	2745.8 ± 212.4*
t1/2 (h)	2.8 ± 0.3	7.2 ± 0.8*
MRT (h)	3.8 ± 0.4	9.1 ± 1.0*
Relative Bioavailability (%)	100	293.5

*Significantly different from control ($p < 0.05$)

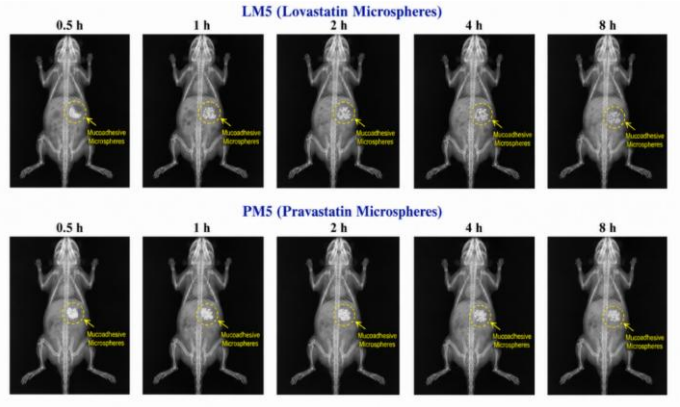

Figure 6: X-ray Images of Mucoadhesive Optimized Formulations (LM5 and PM5) in Rats at Different Time Intervals

The X-ray imaging study confirmed that the microspheres remained visible within the stomach for up to 8 h, indicating effective adhesion to the gastric mucosa and prolonged gastric residence.

4. DISCUSSION

The emulsion crosslinking method successfully produced spherical chitosan microspheres with a uniform size distribution (PDI < 0.25) and a positive zeta potential (>+30 mV). The positive surface charge is critical for electrostatic interactions with negatively charged sialic acid residues in gastric mucin, which is the primary mechanism of mucoadhesion for chitosan (Lehr *et al.*, 1992). A mean particle size of 25-30 µm is optimal for mucosal adhesion, as particles smaller than 10 µm may be taken up by Peyer's patches, whereas particles larger than 100 µm may cause irritation (Vyas and Khar, 2012).

The entrapment efficiency was higher for pravastatin (82.6%) than for lovastatin (78.3%), which can be attributed to pravastatin's higher water solubility, which facilitates its incorporation within the hydrophilic chitosan matrix (Jain, 2008). A drug loading of approximately 20% indicates efficient incorporation of the drug within the polymer matrix.

The ex vivo mucoadhesion studies demonstrated detachment forces of 0.48-0.52 N for drug-loaded microspheres, significantly higher than blank chitosan (0.35 N). This enhanced mucoadhesion can be attributed to the combined effect of chitosan's inherent mucoadhesive properties and the presence of the drug, which may increase the polymer chain mobility and hydrogen bonding capacity (Andrews *et al.*, 2009; Smart, 2005).

The *in vitro* drug release followed Higuchi kinetics (R^2 > 0.96), indicating that the release was primarily diffusion-controlled through the swollen polymer matrix (Higuchi, 1963). The initial burst release (15-20% in the first hour) is attributed to surface-associated drugs, while the subsequent sustained release up to 12 h confirms the ability of the cross-linked chitosan matrix to control drug release (Ramachandran *et al.*, 2011).

An *in vivo* pharmacokinetic study revealed a remarkable enhancement in the oral bioavailability of both statins. For lovastatin (BCS Class II), the mucoadhesive microspheres achieved 582.9% relative bioavailability with a 4.1-fold increase in C_{max} (from 48.3±5.2 ng/mL to 198.2±15.6 ng/mL) and a prolongation of T_{max} from 2.5 h to 5.0 h. For pravastatin (BCS Class III), the relative bioavailability was 293.5%, with a 1.9-fold increase in C_{max} (from 325.6±28.4 ng/mL to 618.4±51.2 ng/mL) and prolongation of T_{max} from 1.5 h to 4.5 h.

The greater bioavailability enhancement observed for lovastatin compared to that for pravastatin is consistent with their different BCS classifications. Lovastatin, which is poorly soluble, benefits from prolonged gastric retention, which extends the dissolution window and potentially saturates intestinal CYP3A4, reducing first-pass metabolism (Neuvonen *et al.*, 2008). Pravastatin, being highly soluble but poorly permeable, benefits from a maintained concentration gradient for passive paracellular absorption, but to a lesser extent (Lennernäs, 2003).

The mucoadhesive microspheres outperformed our previously reported floating tablets (486.2% and 244.7% relative bioavailability for lovastatin and pravastatin, respectively), which can be attributed to the combined advantages of the microsphere system, including a higher surface area-to-volume ratio, stronger mucoadhesive properties, more uniform distribution along the gastric mucosa, and reduced irritancy compared to single-unit systems (Vyas and Khar, 2012).

The X-ray imaging study confirmed that the microspheres remained localized in the stomach for up to 8 h, validating the mucoadhesive gastroretentive potential of the formulation.

5. CONCLUSION

Chitosan-based mucoadhesive microspheres were successfully developed using the emulsion cross-linking method for enhanced oral delivery of lovastatin and pravastatin. The optimized formulations (LM5 and PM5) exhibited favorable physicochemical properties, including uniform particle size (25-30 µm), positive zeta potential (>+30 mV), high entrapment efficiency (78-83%), and strong mucoadhesive properties (detachment force 0.48-0.52 N). *In vitro* drug release followed Higuchi kinetics with sustained release over 12 h. *In vivo* pharmacokinetic studies demonstrated significantly enhanced oral bioavailability, with relative bioavailability of 582.9% for lovastatin and 293.5% for pravastatin compared to conventional suspensions. The greater enhancement observed for the BCS Class II drug confirms that mucoadhesive microspheres are particularly beneficial for poorly soluble drugs. The X-ray imaging study validated prolonged gastric retention for up to 8 h. These findings suggest that chitosan-based mucoadhesive microspheres represent a promising platform for once-daily administration of cholesterol-lowering medications, with the potential for improved therapeutic efficacy and patient compliance.

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