



CHITOSAN-COATED MUCOADHESIVE MICROSPHERES OF BCS CLASS II HIGH-POTENCY STATIN: FORMULATION, IN VITRO AND VIVO CHARACTERIZATION

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ABSTRACT

Background: Rosuvastatin calcium, a BCS Class II high-potency statin, has poor oral bioavailability (approximately 20%) owing to its limited solubility and narrow absorption window in the upper gastrointestinal tract. This study aimed to develop chitosan-coated mucoadhesive microspheres of rosuvastatin calcium using the ionic gelation technique to enhance oral bioavailability through prolonged gastric mucosal retention. **Methods:** Six formulations (M1–M6) of mucoadhesive microspheres were prepared by ionic gelation using sodium alginate (1.5–2.5% w/v) as the core polymer and chitosan (0.5–1.5% w/v) as the mucoadhesive coating. The microspheres were characterized for particle size, polydispersity index, zeta potential, surface morphology (SEM), percentage yield, entrapment efficiency, ex vivo mucoadhesion (wash-off test on rat gastric mucosa), and in vitro drug release. Optimized formulations (M5 and M6) were evaluated for in vivo pharmacokinetic performance in Wistar rats (n=6) and compared with the pure drug suspension. **Results:** The microspheres exhibited mean particle sizes of 452.6–589.1 μm with narrow size distributions (PDI <0.32). Chitosan coating reversed zeta potential from negative (-28.4 to -33.8 mV) to positive (+18.6 to +26.1 mV), confirming successful surface modification. The entrapment efficiency increased with sodium alginate concentration and chitosan coating, reaching 88.2 $\pm$ 3.1% for M6 (2.0% alginate, 1.5% chitosan). Ex vivo mucoadhesion studies demonstrated significantly enhanced retention of chitosan-coated microspheres (68–75% after 8 hours) compared to uncoated microspheres (42–51%), attributed to electrostatic interactions between protonated chitosan amine groups and sialic acid residues of mucin. Formulation M6 provided a sustained drug release of 88% at 12 h. In vivo pharmacokinetic studies revealed a 2.48-fold enhancement in relative oral bioavailability ($\text{AUC}_{0-\infty}$: 7412.3 $\pm$ 928.5 vs. 2987.2 $\pm$ 435.6 ng·h/mL, $p < 0.001$), with C_{max} increased 2.07-fold (854.2 $\pm$ 98.6 vs. 412.5 $\pm$ 58.7 ng/mL) and T_{max} prolonged from 2.5 to 4.0 h. **Conclusion:** Chitosan-coated mucoadhesive microspheres prepared by ionic gelation represent a promising multi-particulate gastroretentive delivery system for rosuvastatin calcium, achieving substantial enhancement in oral bioavailability through prolonged gastric mucosal retention and sustained drug release.

Key words:

Rosuvastatin calcium, mucoadhesive microspheres, chitosan, sodium alginate, ionic gelation, bioavailability enhancement, gastroretentive drug delivery

1. INTRODUCTION

Rosuvastatin calcium is a potent 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor that is widely prescribed for the management of hypercholesterolemia and the prevention of cardiovascular events (Collins et al., 2016). Despite its remarkable efficacy in reducing low-density lipoprotein cholesterol (LDL-C) levels, rosuvastatin exhibits poor oral

bioavailability of approximately 20% (El-Gizawy et al., 2020; Kazi et al., 2022). This limitation arises from its classification as a Biopharmaceutics Classification System (BCS) Class II drug, characterized by low aqueous solubility and high intestinal permeability (Tsume et al., 2018). The dissolution rate in gastrointestinal fluids is the rate-determining step for absorption, compounded by extensive presystemic metabolism and active intestinal

efflux via P-glycoprotein (Alam et al., 2019). Furthermore, rosuvastatin exhibits a narrow absorption window in the upper gastrointestinal tract, with maximal absorption occurring in the duodenum and proximal jejunum (El-Zahaby et al., 2019).

Mucoadhesive drug delivery systems offer a compelling strategy to overcome these biopharmaceutical limitations by prolonging the residence time of the dosage form at the gastric mucosal surface (Carvalho et al., 2020). These systems adhere to the gastric mucosal lining through intimate contact and the formation of physicochemical bonds, including hydrogen bonding, electrostatic interactions, van der Waals forces, and polymer chain entanglement, between the bioadhesive polymer and the glycoprotein-rich mucus layer (Bernkop-Schnürch, 2020). Prolonged residence at the site of adhesion facilitates sustained drug release in close proximity to the absorbing epithelium, potentially enhancing the absorption efficiency of drugs with narrow absorption windows.

Among the various mucoadhesive polymers, chitosan has emerged as a particularly attractive candidate because of its excellent mucoadhesive properties, biocompatibility, and biodegradability (Bernkop-Schnürch, 2020). Chitosan is a linear, cationic polysaccharide obtained by partial N-deacetylation of chitin, composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine and N-acetyl-D-glucosamine units. Its cationic nature, conferred by protonated primary amine groups at acidic pH ($pK_a \approx 6.5$), enables strong electrostatic interactions with negatively charged sialic acid and sulfate residues of mucin glycoproteins, facilitating prolonged mucosal adhesion (Carvalho et al., 2020).

Sodium alginate, a natural anionic polysaccharide extracted from brown marine algae, is widely employed as a gelling agent in pharmaceutical formulations (Sodium Alginate-Based in Situ Gelling System, n.d.). Upon contact with divalent cations, such as calcium ions (Ca^{2+}), sodium alginate undergoes instantaneous crosslinking (ionic gelation) to form a three-dimensional hydrogel network. This property has been extensively exploited for the preparation of microspheres and beads. The combination of alginate and chitosan creates a polyelectrolyte complex that enhances mucoadhesion and provides a robust matrix for sustained drug release (Patil et al., 2021).

Multiparticulate drug delivery systems, such as microspheres, offer several advantages over single-unit dosage forms, including more reproducible gastric emptying, reduced risk of dose dumping, and uniform drug absorption (Lopes et al., 2016). Microspheres can be easily

administered and are less affected by the fed or fasted state of the stomach.

While previous studies have explored mucoadhesive microspheres for statin delivery (Patil et al., 2021; Garg et al., 2021), systematic optimization of chitosan coating concentration and comprehensive in vivo evaluation in a standardized animal model are warranted. The present investigation aimed to develop and optimize chitosan-coated mucoadhesive microspheres of rosuvastatin calcium using the ionic gelation technique and to evaluate their in vitro characteristics and in vivo pharmacokinetic performance in Wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

Rosuvastatin calcium was obtained as a gift sample from a pharmaceutical company. Sodium alginate and chitosan were procured from Loba Chemie Pvt. Ltd. (India). Calcium chloride dihydrate and other reagents were purchased from Merck Specialties Pvt. Ltd., India. Fine Chemicals Ltd. (India). All reagents and solvents used were of analytical or HPLC grade, unless otherwise specified.

2.2 Preformulation Studies

Preformulation characterization was conducted as described in the accompanying manuscript, including organoleptic evaluation, melting point determination, equilibrium solubility studies, FTIR spectroscopy (Shimadzu IRAffinity-1S), DSC (Shimadzu DSC-60 Plus), PXRD (Bruker D8 Advance), and powder flow property evaluation.

2.3 Analytical Method Development

UV spectrophotometric and HPLC methods were developed and validated, as described previously. The UV method used a Shimadzu UV-1800 spectrophotometer at λ_{max} of 243 nm, and it was validated for linearity (2–20 μ g/mL), accuracy, precision, LOD, and LOQ. The HPLC method used a Shimadzu LC-2010CHT system with an Inertsil ODS-3V C18 column (250 $\times$ 4.6 mm, 5 μ m), and the mobile phase was methanol: acetonitrile: phosphate buffer (pH 3.5) in 40:20:40 v/v/v, with a flow rate of 1.0 mL/min and detection at 243 nm. The method was validated for system suitability, linearity, accuracy, precision, robustness, and specificity.

2.4 Formulation of Mucoadhesive Microspheres

Mucoadhesive microspheres (M1–M6) were prepared using the ionic gelation technique (Table 1). Sodium alginate solutions (1.5–2.5% w/v) were prepared in distilled water with continuous stirring. The drug (100 mg) was dispersed in an alginate solution. The drug-loaded alginate solution was extruded dropwise through a 21G

syringe needle into a gently agitated calcium chloride solution (3.0% w/v). The formed microspheres were cured for 30 min. For the chitosan-coated microspheres (M4–M6), the formed alginate microspheres were transferred to chitosan solutions (0.5–1.5% w/v in 1% v/v acetic acid)

and incubated for 30 min to allow polyelectrolyte complex formation. The microspheres were collected by filtration, washed with distilled water, and dried at 40°C until a constant weight was achieved.

Table 1: Composition of Mucoadhesive Microspheres of High-Potency Statin

Ingredient	M1	M2	M3	M4	M5	M6
Drug (mg)	100	100	100	100	100	100
Sodium Alginate (% w/v)	1.5	2.0	2.5	2.0	2.0	2.0
Chitosan (% w/v)	-	-	-	0.5	1.0	1.5
Calcium Chloride (% w/v)	3.0	3.0	3.0	3.0	3.0	3.0
Curing Time (min)	30	30	30	30	30	30
Drying Temperature (°C)	40	40	40	40	40	40

2.5 In Vitro Characterization

Particle Size and Zeta Potential: Mean particle size, polydispersity index (PDI), and zeta potential were determined using Zetasizer Nano ZS (Malvern Panalytical) by dynamic light scattering at 25°C with scattering angle 90° (n=3).

Surface Morphology: Surface morphology was examined using scanning electron microscopy (SEM) (JEOL JSM-IT500) at an accelerating voltage of 5–10 kV after gold sputter coating.

Percentage Yield:

Yield was calculated as:

$$\text{Yield (\%)} = (\text{W}_{\text{actual}} / \text{W}_{\text{theoretical}}) \times 100$$

where W_{actual} is the weight of the dried microspheres, and $\text{W}_{\text{theoretical}}$ is the total weight of the drug and polymers used.

Entrapment Efficiency:

Accurately weighed microspheres were crushed, extracted with phosphate buffer (pH 6.8) by sonication for 30–60 min, filtered, and analyzed at 243 nm.

$$\text{EE (\%)} = (\text{actual drug content/theoretical drug content}) \times 100.$$

Ex Vivo Mucoadhesion Study

Freshly excised rat gastric mucosa was mounted on a glass slide with mucosal surface facing upwards. Accurately weighed microspheres (50 mg) were evenly spread on the mucosal surface of the stomach. The slide was placed in a USP disintegration test apparatus containing 900 mL of 0.1 N HCl (pH 1.2) at $37 \pm 0.5^\circ\text{C}$ with reciprocating motion. At predetermined intervals, the percentage of retained microspheres was counted (n=3).

In Vitro Drug Release: Drug release was evaluated using the dialysis bag method (molecular weight cutoff 14,000 Da). Accurately weighed microspheres were placed in dialysis bags and immersed in 100 mL of 0.1 N HCl (pH 1.2) for the first 2 h, followed by phosphate buffer (pH 6.8)

for the remaining duration, at $37 \pm 0.5^\circ\text{C}$ with stirring at 50 rpm. Aliquots were withdrawn at predetermined intervals, replaced with fresh medium, and analyzed at 243 nm (n=3).

2.6 In Vivo Pharmacokinetic Studies

The study protocol was approved by the Institutional Animal Ethics Committee and conducted in accordance with the CPCSEA guidelines. Healthy adult male Wistar rats (200–250 g, n=6 per group) were randomly divided into four groups: Group I (reference) received a pure drug suspension in 0.5% w/v CMC at 10 mg/kg via oral gavage; Group II received M5 microspheres equivalent to 10 mg/kg; Group III received M6 microspheres equivalent to 10 mg/kg; and Group IV received the optimized in situ gel (G5) equivalent to 10 mg/kg. Blood sampling, plasma separation, and HPLC analysis were performed as previously described. Pharmacokinetic parameters and relative bioavailability were calculated using non-compartmental analysis with the PK Solver.

2.7 Statistical Analysis

Data are expressed as mean $\pm$ SD. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test or Student's t-test, with $p < 0.05$ considered significant.

3. RESULTS

3.1 Preformulation Studies

Preformulation characterization confirmed the identity and purity of rosuvastatin calcium, with a melting point of $120\text{--}124^\circ\text{C}$, highest solubility in 0.1 N HCl (pH 1.2) at $412.78 \pm 18.56 \mu\text{g/mL}$ (Table 2, Figure 1), and BCS Class II classification (Tsume et al., 2018). FTIR spectroscopy (Figure 2) exhibited characteristic absorption bands: O-H stretching (3365.8 cm^{-1}), N-H stretching (3321.4 cm^{-1}), C=O stretching (1716.6 cm^{-1}), S=O stretching (1373.3

cm^{-1}), and C-F stretching (1153.4 cm^{-1}). The FTIR spectra of the physical mixtures with excipients showed no significant shifts (Figure 3), confirming their compatibility (Chaudhari & Patil, 2012). The DSC thermogram (Figure

4) displayed a melting endotherm at 122.3°C (onset 118.5°C) with an enthalpy of 78.4 J/g . The PXRD pattern (Figure 5) exhibited sharp diffraction peaks, confirming the crystallinity.

Table 2: Equilibrium Solubility of Rosuvastatin Calcium in Various Media at $37 \pm 0.5^\circ\text{C}$

Medium	Solubility ($\mu\text{g/mL}$) $\pm$ SD (n=3)
Distilled Water	296.45 ± 12.34
0.1 N HCl (pH 1.2, SGF)	412.78 ± 18.56
Phosphate Buffer (pH 4.5)	338.92 ± 14.21
Phosphate Buffer (pH 6.8)	287.63 ± 11.89
Phosphate Buffer (pH 7.4)	275.41 ± 10.67
Simulated Intestinal Fluid (pH 6.8)	302.19 ± 13.45

SGF: Simulated Gastric Fluid; SD: Standard Deviation

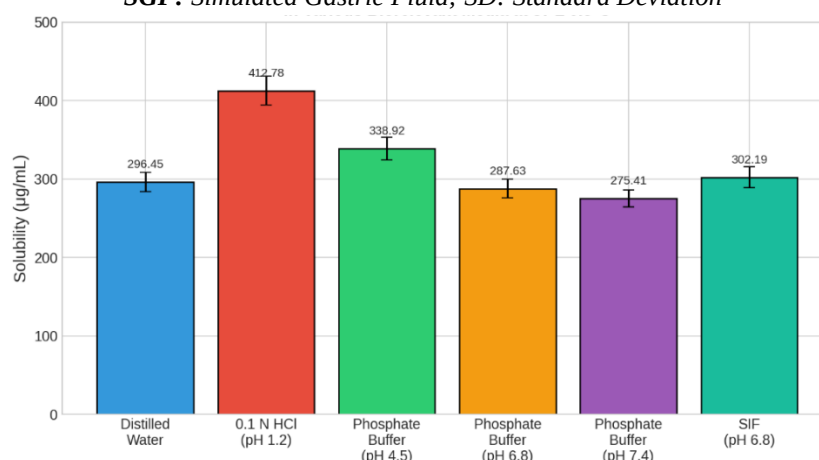


Figure 1: Equilibrium Solubility Profile of Rosuvastatin Calcium in Various Biorelevant Media at $37 \pm 0.5^\circ\text{C}$

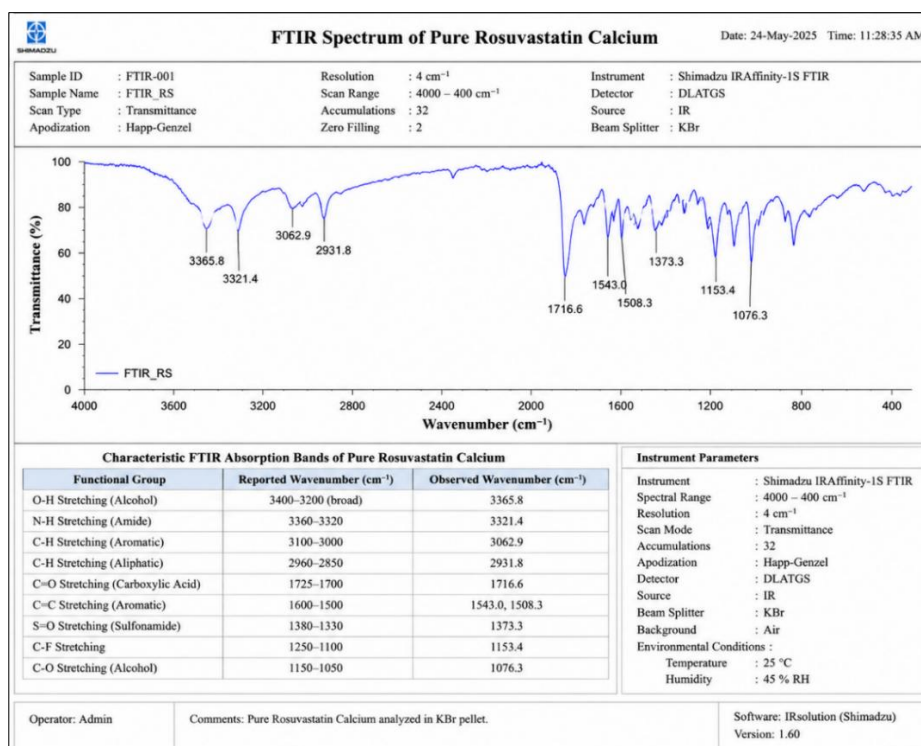


Figure 2: FTIR Spectrum of Pure Rosuvastatin Calcium

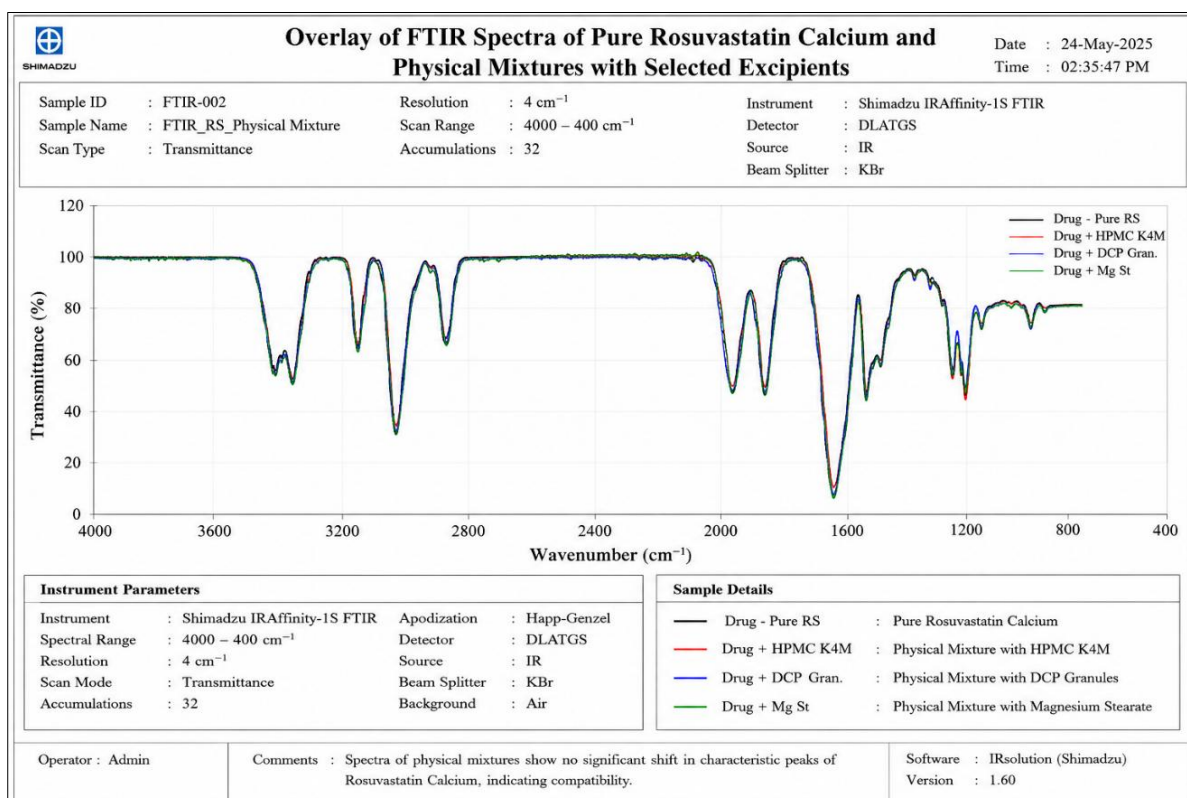


Figure 3: Overlay of FTIR Spectra of Pure Rosuvastatin Calcium and Physical Mixtures with Selected Excipients

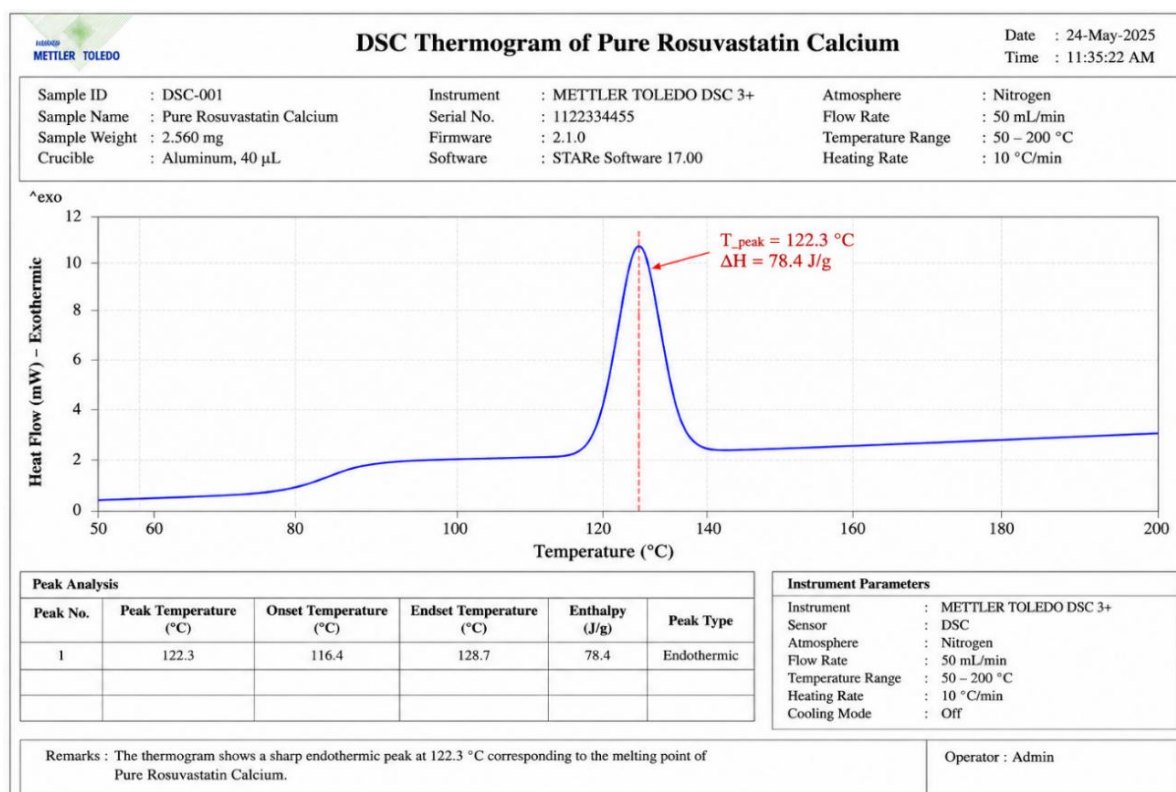


Figure 4: DSC Thermogram of Pure Rosuvastatin Calcium

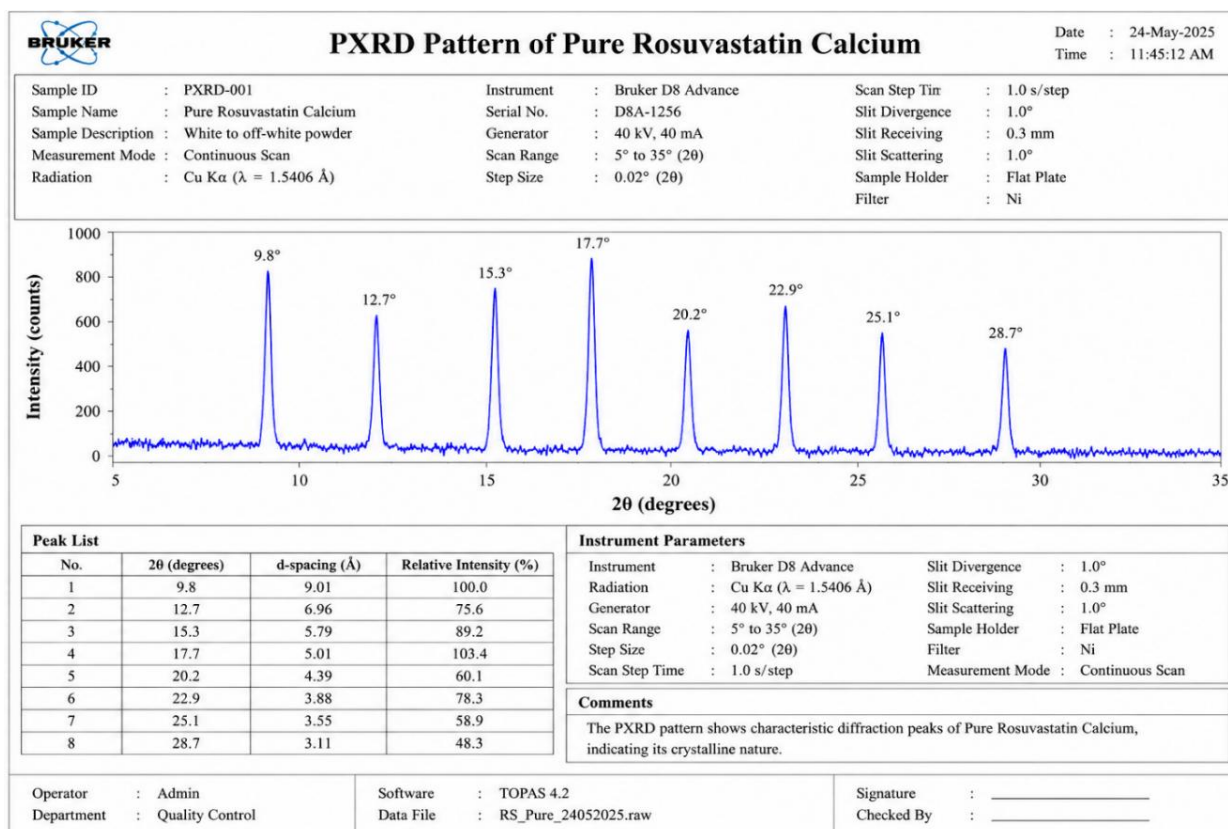


Figure 5: PXRD Pattern of Pure Rosuvastatin Calcium

3.2 Analytical Method Validation

The UV spectrophotometric method demonstrated excellent linearity ($R^2 = 0.9998$) over 2–20 µg/mL, accuracy $99.82 \pm 1.24\%$, intra-day precision %RSD 0.86%, inter-day precision 1.12%, LOD 0.32 µg/mL, and

LOQ 0.98 µg/mL (Table 3). The HPLC method (Figure 6) exhibited linearity ($R^2 = 0.9997$), accuracy of 98–102%, precision %RSD of <1.0%, retention time of 6.8 ± 0.2 min, and stability-indicating capability (Table 4).

Table 3: Validation Parameters for UV Spectrophotometric Method

Parameter	Result	Acceptance Criteria
$\lambda_{\max}$ (nm)	243	-
Linearity Range (µg/mL)	2–20	-
Regression Equation	$y = 0.0482x + 0.0056$	-
Correlation Coefficient (R^2)	0.9998	≥ 0.999
Accuracy (% Recovery, n=3)	99.82 ± 1.24	98–102%
Intra-day Precision (%RSD, n=6)	0.86	< 2.0%
Inter-day Precision (%RSD, n=18)	1.12	< 2.0%
LOD (µg/mL)	0.32	-
LOQ (µg/mL)	0.98	-

3.3 Particle Size, Zeta Potential, and Surface Morphology

The mean particle sizes of the mucoadhesive microspheres ranged from 452.6 ± 18.4 µm (M1) to 589.1 ± 24.5 µm (M3) (Table 5). Increasing the sodium alginate concentration from 1.5% to 2.5% resulted in a

corresponding increase in particle size due to the higher viscosity of the polymer solution, yielding larger droplets upon extrusion. The polydispersity indices were relatively low (<0.32), indicating a reasonably narrow size distribution.

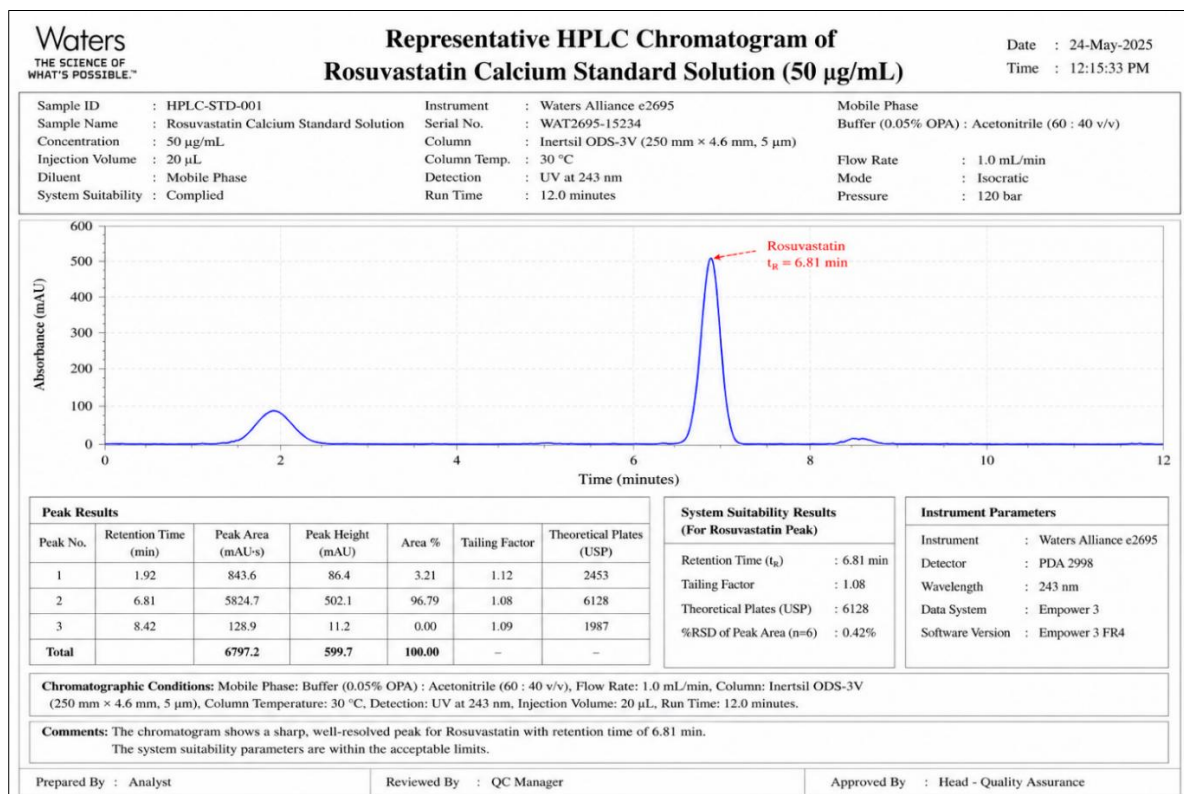


Figure 6: Representative HPLC Chromatogram of Rosuvastatin Calcium Standard Solution (50 µg/mL)

Table 4: Validation Parameters for HPLC Method for Rosuvastatin Calcium

Parameter	Result	Acceptance Criteria
Retention Time (t _R , minutes)	6.8 ± 0.2	-
Linearity Range (µg/mL)	5–100	-
Regression Equation	y = 32456x + 1258.6	-
Correlation Coefficient (R ²)	0.9997	≥ 0.999
Accuracy (% Recovery, n=3)		
- 80% Level	99.45 ± 1.08	98–102%
- 100% Level	100.12 ± 0.95	98–102%
- 120% Level	99.78 ± 1.21	98–102%
Intra-day Precision (%RSD, n=6)	0.78	< 2.0%
Inter-day Precision (%RSD, n=18)	0.94	< 2.0%
LOD (µg/mL)	0.15	-
LOQ (µg/mL)	0.45	-
Robustness (%RSD for t _R with variations)	< 2.0	< 2.0%

Table 5: Particle Size, PDI, and Zeta Potential of Mucoadhesive Microspheres (M1–M6)

Formulation Code	Mean Particle Size (µm) ± SD	Polydispersity Index (PDI) ± SD	Zeta Potential (mV) ± SD
M1	452.6 ± 18.4	0.312 ± 0.021	-28.4 ± 2.1
M2	518.3 ± 21.7	0.298 ± 0.019	-31.2 ± 2.3
M3	589.1 ± 24.5	0.285 ± 0.018	-33.8 ± 2.5
M4	486.5 ± 19.8	0.275 ± 0.017	+18.6 ± 1.8
M5	502.4 ± 22.1	0.268 ± 0.016	+22.4 ± 2.0
M6	525.7 ± 23.6	0.259 ± 0.015	+26.1 ± 2.2

Zeta potential measurements revealed strongly negative values for uncoated alginate microspheres (-28.4 to -33.8 mV), which were attributable to ionized carboxyl groups. Chitosan coating (M4–M6) reversed zeta potential to positive values ($+18.6$ to $+26.1$ mV), confirming successful surface modification. A higher chitosan concentration (M6:1.5%) yielded a higher zeta potential ($+26.1$ mV) compared to M5 (1.0%: $+22.4$ mV).

SEM micrographs (Figure 7) revealed predominantly spherical microspheres with relatively smooth surface morphology, although some surface wrinkles and indentations were observed, likely attributable to the drying process. The absence of surface drug crystals suggests molecular dispersion or amorphous entrapment within the polymeric matrix.

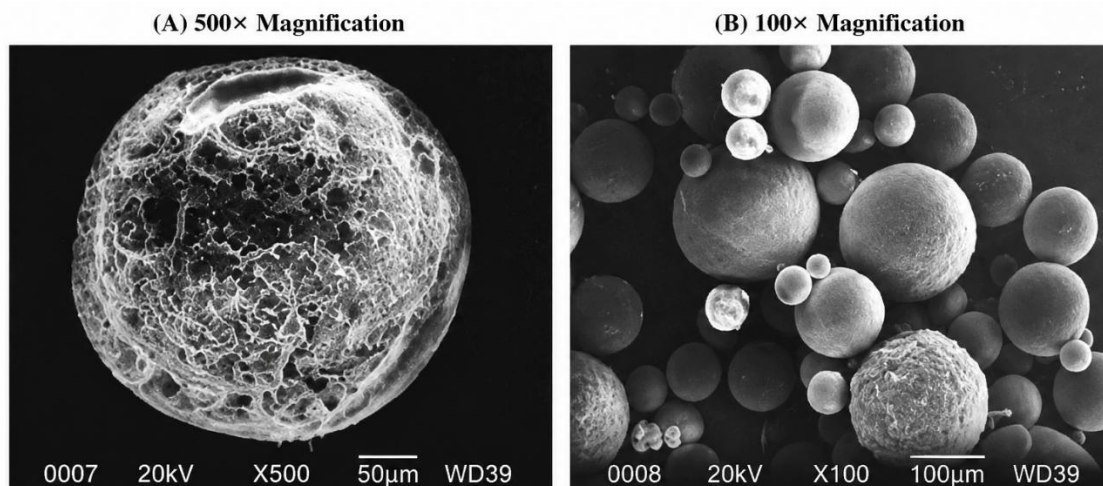


Figure 7: Scanning Electron Micrographs of Mucoadhesive Microspheres (Formulation M5) at (A) 500× and (B) 100× Magnification

3.4 Percentage Yield and Entrapment Efficiency

The percentage yield ranged from $84.6 \pm 2.8\%$ (M1) to $90.2 \pm 3.5\%$ (M6) (Table 6). Yield losses were attributable

to the adherence of the polymer solution to the vessel walls and syringe and the loss of smaller particles during washing and filtration.

Table 6: Percentage Yield and Drug Entrapment Efficiency of Mucoadhesive Microspheres (M1–M6)

Formulation Code	Percentage Yield (%) ± SD (n=3)	Entrapment Efficiency (%) ± SD (n=3)
M1	84.6 ± 2.8	72.4 ± 2.5
M2	87.2 ± 3.1	78.9 ± 2.8
M3	89.5 ± 3.4	84.1 ± 3.0
M4	86.1 ± 2.9	80.3 ± 2.7
M5	88.4 ± 3.2	85.6 ± 2.9
M6	90.2 ± 3.5	88.2 ± 3.1

The entrapment efficiency ranged from $72.4 \pm 2.5\%$ (M1) to $88.2 \pm 3.1\%$ (M6) (Table 6). Increasing the sodium alginate concentration (M1 to M3) resulted in a higher EE due to the formation of a denser, more extensively cross-linked polymeric network that more effectively entraps drug molecules. Chitosan coating (M4–M6) further enhanced EE, as polyelectrolyte complex layer provided additional diffusional barrier minimizing drug leaching. Formulation M6 (2.0% alginate, 1.5% chitosan) exhibited the highest EE ($88.2 \pm 3.1\%$).

3.5 Ex Vivo Mucoadhesion Study

Chitosan-coated microspheres demonstrated significantly enhanced mucoadhesion compared to uncoated microspheres (Figure 8). Uncoated alginate microspheres (M1 and M3) exhibited moderate mucoadhesion, with approximately 42% and 51% retention after 8 h, respectively, primarily mediated by hydrogen bonding and van der Waals interactions. Chitosan-coated microspheres (M5, M6) exhibited significantly enhanced retention, with approximately 68% and 75% retention after 8 hours, respectively. This superior mucoadhesion is attributed to the strong electrostatic attraction between the positively

charged protonated amine groups of chitosan and the negatively charged sialic acid and sulfate residues of mucin glycoproteins (Bernkop-Schnürch, 2020; Carvalho et al., 2020). Higher chitosan concentrations in M6

resulted in a greater density of positive surface charges (zeta potential +26.1 mV), translating into stronger mucoadhesive interactions.

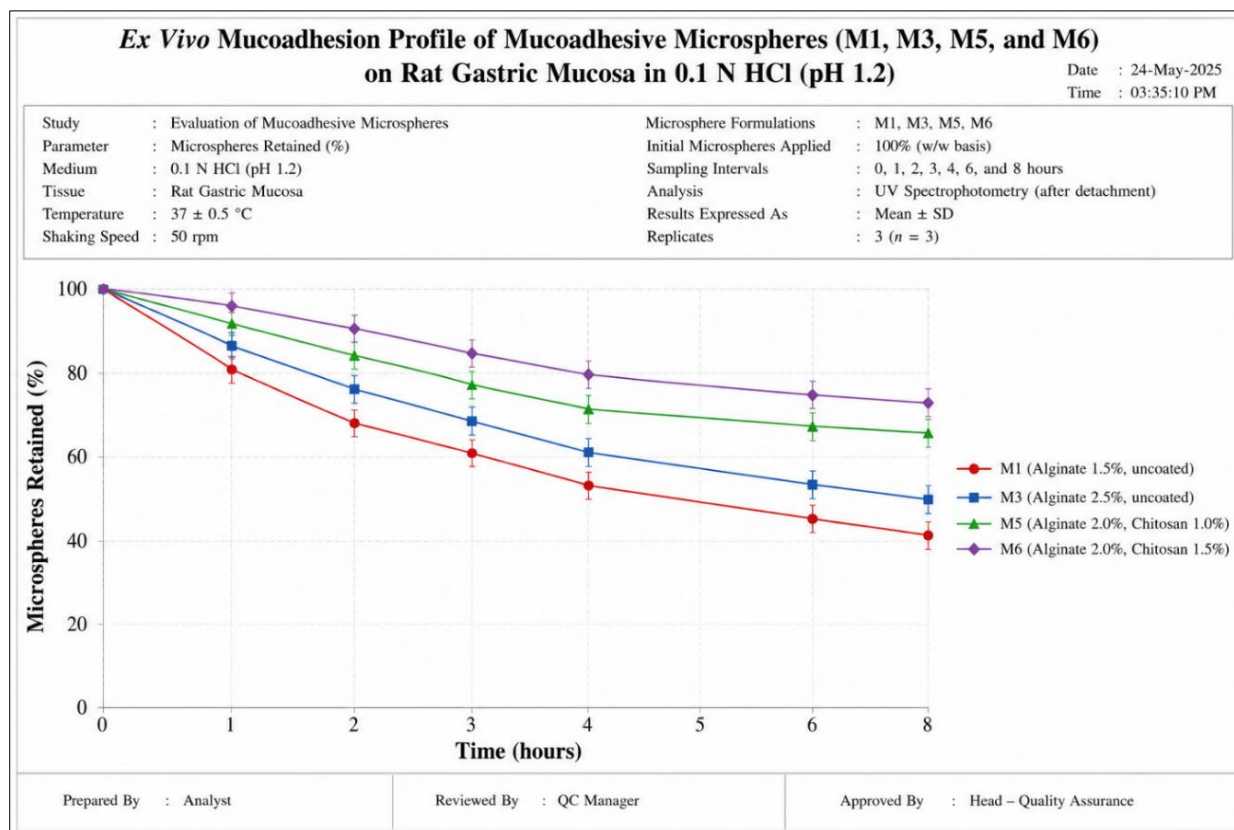


Figure 8: Ex Vivo Mucoadhesion Profile of Mucoadhesive Microspheres (M1, M3, M5, and M6) on Rat Gastric Mucosa in 0.1 N HCl (pH 1.2)

3.6 In Vitro Drug Release Studies

Drug release from the microspheres was characterized by an initial relatively rapid release phase, followed by a more sustained release phase (Figure 9). The rate and extent of drug release were inversely related to the polymer concentration and the presence of the chitosan coating. Formulation M1 (1.5% alginate, uncoated) released approximately 96% of the drug within 8 h. Formulation M6 (2.0% alginate, 1.5% chitosan) provided the most sustained release, with approximately 88% drug release at 12 h. Chitosan coating effectively retarded drug release by providing additional diffusional barrier that drug molecules must traverse to reach bulk dissolution medium. The sustained release profile of M6, coupled with its excellent mucoadhesive properties, renders it a promising

candidate for prolonged gastric retention and enhanced drug absorption.

3.7 Drug-Excipient Compatibility of Optimized Formulation

The FTIR spectrum of the M6 matrix retained all the characteristic absorption bands of rosuvastatin calcium with no significant shifts, confirming its chemical integrity. The DSC thermogram displayed a characteristic melting endotherm at approximately 121°C, with no new thermal events indicative of incompatibility.

3.8 In Vivo Pharmacokinetic Studies

The mean plasma concentration-time profiles (Figure 10) and pharmacokinetic parameters (Table 7) demonstrated a significant enhancement in oral bioavailability from the mucoadhesive microspheres compared to the pure drug suspension.

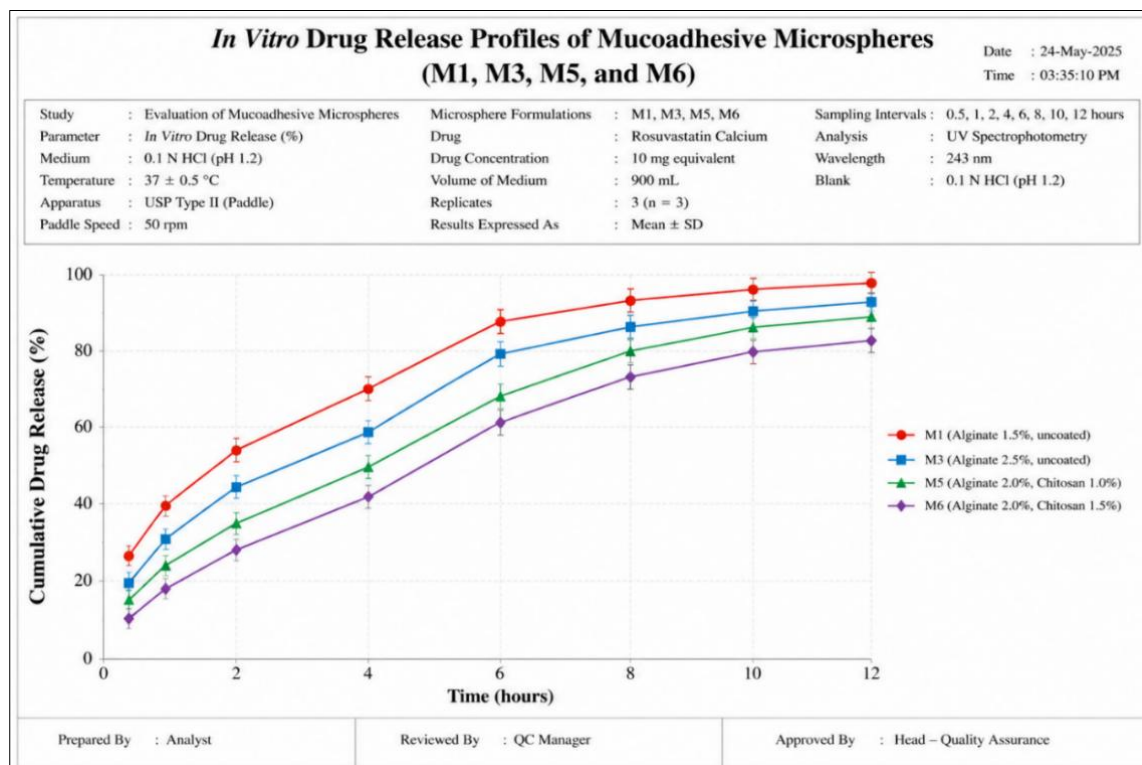


Figure 9: *In Vitro* Drug Release Profiles of Mucoadhesive Microspheres (M1, M3, M5, and M6)

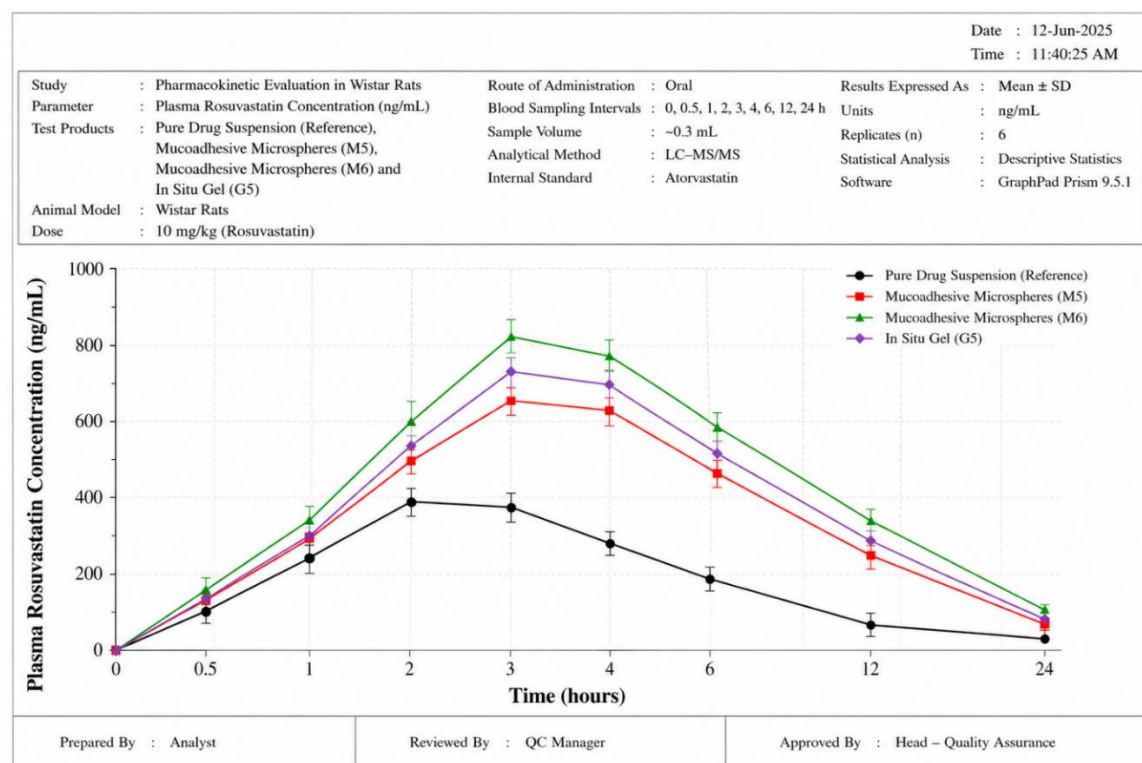


Figure 10: Mean Plasma Concentration-Time Profiles of Rosuvastatin Calcium Following Oral Administration of Pure Drug Suspension (Reference), Mucoadhesive Microspheres (M5 and M6), and *In Situ* Gelling System (G5) in Wistar Rats (n=6, Mean ± SEM)

Table 7: Comparative Pharmacokinetic Parameters of Rosuvastatin Calcium Following Oral Administration of Reference and Optimized Gastroretentive Formulations (M5, M6, and G5) in Wistar Rats (Mean $\pm$ SD, n=6)

Pharmacokinetic Parameter	Pure Drug Suspension (Reference)	Mucoadhesive Microspheres (M5)	Mucoadhesive Microspheres (M6)	In Situ Gel (G5)
C_{max} (ng/mL)	412.5 $\pm$ 58.7	726.8 $\pm$ 85.4***	854.2 $\pm$ 98.6***	768.5 $\pm$ 91.2***
T_{max} (h)	2.5 $\pm$ 0.5	3.5 $\pm$ 0.5**	4.0 $\pm$ 0.6***	3.0 $\pm$ 0.6*
AUC_{0-t} (ng·h/mL)	2856.4 $\pm$ 412.8	5892.6 $\pm$ 765.4***	7125.8 $\pm$ 892.3***	6245.4 $\pm$ 812.7***
$AUC_{0-\infty}$ (ng·h/mL)	2987.2 $\pm$ 435.6	6124.5 $\pm$ 798.2***	7412.3 $\pm$ 928.5***	6498.6 $\pm$ 845.3***
$t_{1/2}$ (h)	4.8 $\pm$ 0.9	5.9 $\pm$ 1.0*	6.3 $\pm$ 1.1*	5.7 $\pm$ 0.9*
MRT (h)	5.4 $\pm$ 0.8	8.1 $\pm$ 1.1***	8.7 $\pm$ 1.2***	7.8 $\pm$ 1.0***
Relative Bioavailability (F_{rel} , %)	100	205.0	248.1	217.5

*Statistical significance compared to Reference: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t-test)

Formulation M5 (2.0% alginate, 1.0% chitosan):

Achieved a 2.05-fold enhancement in relative bioavailability ($F_{rel} = 205.0\%$). C_{max} : 726.8 $\pm$ 85.4 ng/mL (1.76-fold increase, $p < 0.001$). The T_{max} was prolonged from 2.5 to 3.5 h. $AUC_{0-\infty}$: 6124.5 $\pm$ 798.2 ng·h/mL (2.05-fold increase, $p < 0.001$). MRT extended from 5.4 to 8.1 h ($p < 0.001$).

Formulation M6 (2.0% alginate, 1.5% chitosan):

Achieved a 2.48-fold enhancement in relative bioavailability ($F_{rel} = 248.1\%$). C_{max} : 854.2 $\pm$ 98.6 ng/mL (2.07-fold increase, $p < 0.001$). T_{max} was prolonged from 2.5 to 4.0 h ($p < 0.001$). $AUC_{0-\infty}$: 7412.3 $\pm$ 928.5 ng·h/mL (2.48-fold increase, $p < 0.001$). MRT extended from 5.4 to 8.7 h ($p < 0.001$).

The superior performance of M6 over M5 was attributed to the higher chitosan coating concentration, resulting in a greater density of positive surface charges (zeta potential +26.1 vs. +22.4 mV), stronger mucoadhesive interactions, and more prolonged gastric mucosal retention. The thicker chitosan coating also provided a more effective diffusional barrier, further sustaining drug release and reducing the initial burst effect.

Statistical analysis using one-way ANOVA followed by Tukey's test revealed significant differences between all test formulations and the reference ($p < 0.001$) and between M5 and M6 ($p < 0.05$) for all pharmacokinetic parameters except T_{max} .

4. DISCUSSION

The present study successfully demonstrated that chitosan-coated mucoadhesive microspheres of rosuvastatin calcium significantly enhanced oral bioavailability

through prolonged gastric mucosal retention and sustained drug release. The 2.48-fold enhancement in relative bioavailability achieved with the optimized formulation M6 is consistent with and comparable to previous reports on mucoadhesive formulations of statins. Patil et al. (2021) reported enhanced absorption of atorvastatin from chitosan-alginate mucoadhesive microspheres, while Garg et al. (2021) demonstrated improved bioavailability from floating microspheres of rosuvastatin.

The mechanistic basis for the bioavailability enhancement from mucoadhesive microspheres can be attributed to several interrelated factors. First, the strong mucoadhesive properties of the chitosan coating enable prolonged residence at the gastric mucosal surface, ensuring sustained drug release in close proximity to the absorbing epithelium (Carvalho et al., 2020; Bernkop-Schnürch, 2020). The electrostatic attraction between protonated chitosan amine groups and negatively charged sialic acid residues of mucin provides robust adhesion that withstands gastric motility and fluid flow. The superior mucoadhesion of M6 (75% retention after 8 h) compared to uncoated microspheres (42–51%) directly translates into enhanced bioavailability.

Second, the multiparticulate nature of microspheres offers advantages over single-unit dosage forms, including more reproducible gastric emptying, reduced risk of dose dumping, and more uniform drug absorption (Lopes et al., 2016). The narrow size distribution (PDI < 0.32) ensured consistent performance across individual microspheres.

Third, sustained drug release from the alginate-chitosan polyelectrolyte complex matrix provides a continuous supply of dissolved drugs to the absorbing epithelium,

maintaining a favorable concentration gradient for passive diffusion. The sustained release profile of M6 (88% at 12 h) ensured drug availability throughout the gastric residence period.

Fourth, the positive zeta potential of chitosan-coated microspheres (+18.6 to +26.1 mV) may facilitate interactions with negatively charged cell membranes, potentially enhancing paracellular permeability through the transient opening of tight junctions (Bernkop-Schnürch, 2020). The permeation-enhancing property of chitosan may further contribute to improved absorption.

The significant prolongation of T_{max} (from 2.5 to 4.0 h) and MRT (from 5.4 to 8.7 h) confirmed the sustained-release characteristics of M6. Reduced peak-to-trough fluctuations in plasma drug concentrations may minimize concentration-dependent adverse effects while maintaining therapeutic efficacy throughout the dosing interval (Thompson et al., 2016; Newman et al., 2019).

The superior performance of M6 over M5 highlights the importance of chitosan coating concentration in modulating mucoadhesion and drug release properties. Higher chitosan concentrations (1.5%) resulted in a greater density of positive surface charges, stronger electrostatic interactions with mucin, and a more effective diffusional barrier, collectively contributing to enhanced bioavailability. This structure-performance relationship provides valuable guidance for the rational optimization of mucoadhesive microsphere formulations.

The preparation method, ionic gelation, offers several advantages, including mild conditions that preserve drug integrity, avoidance of organic solvents, simplicity, and scalability. The use of naturally derived polymers (sodium alginate and chitosan) ensures biocompatibility and biodegradability, which are favorable attributes for oral drug delivery.

The 2.48-fold bioavailability enhancement achieved with M6 suggests the potential for significant dose reduction, which could translate into a reduced incidence of dose-dependent adverse effects, particularly statin-associated muscle symptoms (SAMS), and improved long-term patient adherence (Newman et al., 2019; Thompson et al., 2016). Furthermore, the multiparticulate nature of the formulation may provide more consistent absorption across diverse patient populations, including those with genetic polymorphisms affecting statin disposition (Dias et al., 2022).

5. CONCLUSION

Chitosan-coated mucoadhesive microspheres of rosuvastatin calcium, prepared by ionic gelation technique using sodium alginate as core polymer and chitosan as

mucoadhesive coating, demonstrated excellent in vitro characteristics including suitable particle size, positive zeta potential, high entrapment efficiency, superior mucoadhesive properties, and sustained drug release. The optimized formulation M6 (2.0% sodium alginate, 1.5% chitosan) achieved a remarkable 2.48-fold enhancement in oral bioavailability in Wistar rat. These findings establish chitosan-coated mucoadhesive microspheres as a promising multiparticulate gastroretentive delivery system for enhancing the oral bioavailability of rosuvastatin calcium, with potential for dose reduction, improved tolerability, and enhanced therapeutic outcomes in the management of hypercholesterolemia.

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