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Factorial Design-Based Development and Evaluation of Metformin Hydrochloride-Loaded Mucoadhesive Microspheres for Sustained Anti-Hyperglycaemic and Anticancer Activity

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Abstract
The present study aimed to develop and optimize Metformin Hydrochloride-loaded mucoadhesive microspheres for sustained anti-hyperglycaemic and anticancer activity using factorial experimental design. Microspheres were prepared by ionotropic gelation employing sodium alginate and chitosan as mucoadhesive polymers. A 3^2 full factorial design was utilized to evaluate the influence of sodium alginate concentration and calcium chloride concentration on particle size, entrapment efficiency, mucoadhesion, and drug release behaviour. The prepared microspheres were evaluated for physicochemical properties, surface morphology, swelling behaviour, in vitro drug release, release kinetics, anti-hyperglycaemic activity, and anticancer potential against MCF-7 breast cancer cell line. The optimized formulation exhibited high entrapment efficiency (88.7 ± 2.1%), excellent mucoadhesion (93.8 ± 2.6%), and sustained drug release over 12 hours. Release kinetic analysis revealed Higuchi diffusion-controlled non-Fickian release behaviour. The optimized microspheres demonstrated enhanced α-amylase and α-glucosidase inhibitory activity compared to free drug. In vitro anticancer evaluation showed improved cytotoxic activity with lower IC_{50} value against MCF-7 cells. Stability studies confirmed acceptable physicochemical stability of the optimized formulation. The developed microsphere system demonstrated promising potential as a sustained oral drug delivery platform for combined anti-hyperglycaemic and anticancer applications.

Keywords: Metformin hydrochloride, Mucoadhesive microspheres, Ionotropic gelation, Factorial design, Sustained release, Anti-hyperglycaemic activity, Anticancer activity, MCF-7 cells.

1. Introduction

Type 2 Diabetes Mellitus is one of the most prevalent metabolic diseases worldwide and represents a major global healthcare burden due to its rapidly increasing incidence, chronic complications, and associated mortality. The disease is characterized by persistent hyperglycaemia resulting from insulin resistance, impaired insulin secretion, or both. Long-term uncontrolled hyperglycaemia contributes to severe complications including cardiovascular disorders, nephropathy, neuropathy, retinopathy, obesity-associated inflammation, and metabolic syndrome. In recent years, increasing scientific evidence has also demonstrated a strong association between diabetes and cancer progression, where hyperinsulinaemia, oxidative stress, chronic inflammation, and altered cellular metabolism collectively promote tumor growth and proliferation. This growing relationship between diabetes and cancer has stimulated considerable interest in developing therapeutic systems capable of simultaneously managing hyperglycaemia and exerting anticancer effects ("10. Cardiovascular Disease and Risk Management: Standards of Care in Diabetes-2026," 2026; Abachi et al., 2023; Abuhay et al., 2022; Afraie et al., 2024).

Metformin Hydrochloride is one of the most widely prescribed oral antidiabetic drugs for the treatment of type 2 diabetes mellitus. It belongs to the biguanide class and primarily acts by reducing hepatic glucose production, improving peripheral insulin sensitivity, and decreasing intestinal glucose absorption. Metformin possesses several therapeutic advantages including low risk of hypoglycaemia, cost-effectiveness, and beneficial effects on body weight and lipid metabolism. Apart from its conventional antidiabetic action, metformin has recently attracted major scientific attention due to its potential anticancer properties. Several experimental and clinical studies have reported that metformin can inhibit cancer cell proliferation through activation of AMP-activated protein kinase (AMPK), suppression of mammalian target of rapamycin (mTOR) signalling, induction of cell cycle arrest, and inhibition of tumor-associated metabolic pathways. These observations have positioned metformin as a promising therapeutic candidate for metabolic and cancer-associated disorders (Hu et al., 2023; Imran et al., 2024; Imtiaz et al., 2026; Kodiyatar et al., 2026; Kotha et al., 2023).

Despite its clinical significance, metformin hydrochloride possesses certain pharmaceutical limitations that may reduce therapeutic efficiency. The drug is highly water-soluble and exhibits relatively short biological half-life, requiring frequent administration to maintain therapeutic plasma concentrations. Conventional immediate-release formulations often produce rapid drug release, resulting in fluctuating plasma levels and gastrointestinal side effects such as nausea, diarrhoea, abdominal discomfort, and poor patient compliance. In addition, rapid gastric emptying and limited gastrointestinal residence time may reduce the efficiency of drug absorption and sustained pharmacological action. Therefore, the development of a controlled release gastroretentive drug delivery system capable of prolonging gastric residence and sustaining drug release remains highly desirable (Hu et al., 2023; Husain et al., 2022; Kamboj et al., 2023; Magdy et al., 2022; Mai et al., 2025; Safaa Hamdi & Basim Mohsin Mohamed, 2022; Srivastava et al., 2021a, 2021b; Vinchurkar et al., 2022).

Mucoadhesive microspheres have emerged as an important multiparticulate drug delivery approach for improving oral bioavailability, prolonging gastrointestinal residence time, and enhancing controlled drug release. These systems adhere to the mucosal lining of the gastrointestinal tract through polymer–mucin interactions, thereby increasing residence time at the absorption site and reducing drug elimination from the stomach and upper intestine. Mucoadhesive microspheres also provide several advantages such as uniform distribution within the gastrointestinal tract, reduced dose dumping, improved patient compliance, and minimized local irritation. Furthermore, multiparticulate systems exhibit lower risk of formulation failure compared to single-unit dosage forms and can provide reproducible drug release profiles (Dubey et al., 2026; Farhadnejad et al., 2022; Rajak et al., 2011).

Among various polymers used for mucoadhesive microsphere formulation, sodium alginate and chitosan have gained considerable importance due to their biocompatibility, biodegradability, non-toxicity, and excellent gel-forming ability. Sodium alginate forms crosslinked hydrogel matrices in the presence of divalent cations such as calcium ions through ionotropic gelation, while chitosan contributes strong mucoadhesive properties because of its cationic amino groups capable of interacting with negatively charged mucin. The combination of alginate and chitosan can therefore produce stable polymeric matrices suitable for sustaining the release of highly water-soluble drugs such as metformin hydrochloride (Grosso et al., 2024; Gupta et al., 2025; Jiang et al., 2015). Optimization of pharmaceutical formulations using conventional trial-and-error methods is often time-consuming and scientifically inefficient. Consequently, factorial experimental design has become an essential statistical tool for systematic formulation optimization. Factorial design enables simultaneous evaluation of multiple formulation variables and their interaction effects on critical quality attributes. It also facilitates development of mathematical models and response surface analysis for identification of optimized formulations possessing desired pharmaceutical characteristics (Ain et al., 2024; Irshad et al., 2026).

Therefore, the present study was designed to develop and optimize metformin hydrochloride-loaded mucoadhesive microspheres using ionotropic gelation and factorial experimental design. The prepared microspheres were evaluated for physicochemical properties, drug release characteristics, mucoadhesion, anti-hyperglycaemic activity, and anticancer potential against breast cancer cell lines. The study aimed to establish a sustained release mucoadhesive delivery system capable of improving therapeutic performance of metformin hydrochloride for dual metabolic and anticancer applications.

2. Materials and Methods

2.1 Materials

Metformin Hydrochloride was procured from a certified pharmaceutical supplier and used as the model drug for microsphere preparation. Sodium alginate was selected as the primary hydrophilic polymer due to its excellent gel-forming ability in the presence of divalent cations. Chitosan was utilized as a mucoadhesive polymer to improve gastrointestinal residence time and enhance controlled drug release characteristics. Calcium chloride was employed as the ionic crosslinking agent for microsphere formation through ionotropic gelation. Glacial acetic acid was used for dissolving chitosan. Methanol, ethanol, potassium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, and all other analytical reagents used during the study were of analytical grade and used without further purification. For anticancer evaluation, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA solution, phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), and MTT reagent were procured from authenticated suppliers. Human breast adenocarcinoma cell line MCF-7 was selected for the cytotoxicity study due to the established relationship between metformin and breast cancer metabolic pathways.

2.2 Instruments and Equipment

The preparation and evaluation of microspheres were carried out using standard laboratory instruments. UV-visible spectrophotometer was used for drug estimation and calibration analysis. FTIR spectrophotometer was employed for compatibility studies between drug and excipients. Differential scanning calorimetry was used for thermal characterization of the optimized formulation. Magnetic stirrer and hot plate assembly were utilized during microsphere preparation. Mechanical overhead stirrer was used to maintain uniform stirring conditions during ionotropic gelation. Digital pH meter was employed for pH adjustment and buffer preparation. Particle size analysis was carried out using an optical microscope fitted with an ocular micrometer. Surface morphology was analyzed using scanning electron microscopy. In vitro dissolution studies were conducted using USP dissolution test apparatus type II. Centrifuge and hot air oven were used during sample preparation and drying procedures. Cell culture studies were performed in a CO_2 incubator under aseptic conditions using laminar airflow equipment. ELISA microplate reader was employed for recording absorbance during MTT assay and enzyme inhibition studies.

2.3 Experimental Design

A 3^2 full factorial experimental design was employed for optimization of metformin hydrochloride-loaded mucoadhesive microspheres. The factorial design was selected to systematically evaluate the influence of formulation variables on microsphere characteristics and to establish mathematical relationships between independent and dependent variables.

Two formulation variables were selected as independent factors:

- X_1: Sodium alginate concentration
- X_2: Calcium chloride concentration

Each factor was evaluated at three levels: low (−1), medium (0), and high (+1). A total of nine experimental formulations were prepared according to the factorial design matrix.

The dependent responses evaluated included:

- Particle size (Y_1)
- Entrapment efficiency (Y_2)
- Mucoadhesion percentage (Y_3)
- Cumulative drug release (Y_4)

The experimental data obtained were statistically analyzed using Design-Expert® software. Polynomial equations, response surface plots, and contour plots were generated to evaluate the effect of formulation variables on the responses. Optimization was performed using desirability function methodology to identify the optimized microsphere formulation possessing maximum entrapment efficiency, high mucoadhesion, and sustained drug release characteristics (Ain et al., 2024; Irshad et al., 2026).

2.4 Drug–Excipient Compatibility Study

2.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was performed to evaluate the compatibility between metformin hydrochloride and selected excipients. The spectra of pure drug, polymers, and optimized formulation were recorded using potassium bromide pellet method over the scanning range of 4000–400 cm^{-1}. The obtained spectra were analyzed for characteristic functional peaks corresponding to metformin hydrochloride, including N–H stretching, C=N stretching, and amine group vibrations. The presence or absence of significant peak shifting, disappearance, or appearance of additional peaks was evaluated to determine possible physicochemical interactions between the drug and excipients.

2.4.2 Differential Scanning Calorimetry (DSC)

Thermal analysis was carried out using differential scanning calorimetry to assess the physical state of metformin hydrochloride within the microspheres. Accurately weighed samples of pure drug and optimized formulation were sealed in aluminium pans and heated at a controlled rate under nitrogen atmosphere. The thermograms obtained were analyzed for melting endotherms and thermal transitions. Changes in melting peak intensity or disappearance of crystalline peaks were interpreted as evidence of partial amorphization or molecular dispersion of the drug within the polymeric matrix.

2.5 Preparation of Metformin Hydrochloride-Loaded Mucoadhesive Microspheres

Metformin hydrochloride-loaded mucoadhesive microspheres were prepared using the ionotropic gelation technique employing sodium alginate and chitosan as the polymeric carrier system. Sodium alginate was dissolved in purified water under continuous magnetic stirring to obtain a homogeneous polymeric solution. The accurately weighed quantity of Metformin Hydrochloride was dispersed uniformly into the sodium alginate solution to form a drug–polymer dispersion. Chitosan solution was prepared separately by dissolving chitosan in dilute acetic acid solution under continuous stirring until a clear viscous solution was obtained. The drug-containing sodium alginate solution was transferred into a syringe fitted with a suitable needle and added dropwise into calcium chloride crosslinking solution maintained under continuous mechanical stirring. The droplets underwent instantaneous ionic gelation due to interaction between calcium ions and alginate chains, leading to formation of microspheres. The formed microspheres were further treated with chitosan solution to enhance surface mucoadhesion and improve sustained release properties. The stirring speed, dropping rate, curing time, and polymer concentrations were carefully controlled throughout the process to ensure uniform microsphere formation. The prepared microspheres were filtered, washed repeatedly with distilled water to remove excess crosslinking agent, and dried at room temperature followed by further drying in a hot air oven at controlled temperature until constant weight was achieved. The dried microspheres were stored in airtight containers containing desiccant until further evaluation (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025).

2.6 Percentage Yield

The percentage yield of prepared microspheres was determined to evaluate the efficiency of the preparation process. The dried microspheres obtained after completion of the ionotropic gelation process were accurately weighed and compared with the total quantity of drug and polymers initially used in the formulation. The percentage yield was calculated using the following equation:

$$\%\, Yield = \frac{Practical\, Yield}{Theoretical\, Yield} \times 100$$

A higher percentage yield indicated efficient recovery of microspheres and minimal material loss during the preparation process (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025).

2.7 Particle Size Analysis

Particle size determination was carried out using optical microscopy equipped with an ocular micrometer. A small quantity of dried microspheres was dispersed uniformly on a clean glass slide and observed under the microscope. The diameters of approximately 100 microspheres were measured randomly to obtain the average particle size

distribution. The mean particle size was calculated using the arithmetic mean method. Uniformity in particle size distribution was considered essential for reproducible drug release behaviour and improved mucoadhesive characteristics. The influence of sodium alginate concentration and calcium chloride concentration on particle size was further evaluated using factorial design analysis (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025).

2.8 Surface Morphology by Scanning Electron Microscopy

Surface morphology of the optimized microsphere formulation was analyzed using scanning electron microscopy. The dried microspheres were mounted on aluminium stubs using double-sided adhesive tape and coated with a thin layer of gold under vacuum conditions to improve conductivity. The samples were observed at different magnifications to evaluate particle shape, surface texture, porosity, and structural integrity. Particular attention was given to the presence of spherical geometry, surface smoothness, and absence of aggregation. SEM analysis was performed to correlate morphological properties with drug release and mucoadhesion behaviour of the microspheres (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025).

2.9 Drug Entrapment Efficiency

Entrapment efficiency was determined to evaluate the amount of drug successfully incorporated into the polymeric microspheres. Accurately weighed microspheres were crushed and dissolved in phosphate buffer solution under continuous stirring to ensure complete extraction of the drug. The resulting solution was filtered suitably and analyzed spectrophotometrically using UV-visible spectrophotometer at the predetermined λmax of metformin hydrochloride. The entrapment efficiency was calculated using the following equation (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025):

$$Entrapment\ Efficiency\ (\%) = \frac{Actual\ Drug\ Content}{Theoretical\ Drug\ Content} \times 100$$

Higher entrapment efficiency indicated improved drug incorporation within the polymeric matrix and reduced drug loss during formulation processing.

2.10 Swelling Index Study

Swelling behaviour of microspheres was evaluated to study hydration characteristics and polymer relaxation behaviour in simulated gastrointestinal conditions. Accurately weighed dried microspheres were immersed in phosphate buffer solution and allowed to swell for predetermined time intervals. At each time point, the microspheres were removed carefully, surface moisture was blotted gently using filter paper, and the swollen microspheres were weighed immediately. The swelling index was calculated using the following equation (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025):

$$Swelling\ Index\ (\%) = \frac{W_t - W_0}{W_0} \times 100$$

Where:

- W_t= weight of swollen microspheres at time t
- W_0= initial weight of dried microspheres

The swelling study provided important information regarding water uptake capacity, polymer hydration, and its influence on drug release kinetics.

2.11 Mucoadhesion Study

The mucoadhesive property of prepared microspheres was evaluated using an in vitro wash-off method employing freshly excised intestinal mucosa obtained from suitable animal tissue. The mucosal membrane was mounted carefully on a glass slide and a known quantity of microspheres was spread uniformly over the wet tissue surface. The mounted tissue was subjected to gentle up-and-down movement in phosphate buffer maintained at physiological temperature using a USP disintegration apparatus. At predetermined time intervals, the number of microspheres remaining adhered to the mucosal surface was counted. The percentage mucoadhesion was calculated using the following equation (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025):

$$Mucoadhesion\ (\%) = \frac{Number\ of\ Adhered\ Microspheres}{Number\ of\ Applied\ Microspheres} \times 100$$

The study was performed to assess the ability of the microspheres to prolong gastrointestinal residence time and improve localized drug retention.

2.12 In Vitro Drug Release Study

The in vitro drug release study of Metformin Hydrochloride-loaded mucoadhesive microspheres was carried out using USP dissolution test apparatus type II (paddle method). The study was performed to evaluate the sustained release behaviour of the prepared microspheres under simulated gastrointestinal conditions. Accurately weighed microspheres equivalent to a predetermined quantity of metformin hydrochloride were introduced into dissolution vessels containing phosphate buffer solution maintained at physiological pH and temperature. The dissolution medium was stirred continuously at controlled paddle rotation speed throughout the study. At predetermined time intervals, aliquots of dissolution medium were withdrawn and replaced immediately with equal volume of fresh dissolution medium maintained at the same temperature to maintain sink conditions. The collected samples were filtered suitably and analyzed using UV-visible spectrophotometer at the λmax of metformin hydrochloride. The cumulative percentage drug release was calculated and plotted against time to evaluate release characteristics of the formulations. The influence of polymer concentration and crosslinking density on drug release behaviour was assessed using factorial design analysis (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025).

2.13 Drug Release Kinetic Study

To understand the mechanism of drug release from the microspheres, the dissolution data obtained from the in vitro release study were fitted into various mathematical kinetic models including zero-order, first-order, Higuchi diffusion, and Korsmeyer–Peppas models (Cilurzo et al., 2005; Mamona et al., 2025; Noreen et al., 2023; Wang et al., 2025).

2.13.1 Zero-Order Kinetic Model

The zero-order kinetic model describes systems where drug release occurs at a constant rate independent of drug concentration.

$$Q_t = Q_0 + k_0 t$$

Where:

- Q_t= amount of drug released at time t
- Q_0= initial amount of drug
- k_0= zero-order release constant

2.13.2 First-Order Kinetic Model

The first-order kinetic model explains concentration-dependent drug release.

$$\log Q_t = \log Q_0 - \frac{k_1 t}{2.303}$$

Where:

- k_1= first-order release constant

2.13.3 Higuchi Diffusion Model

The Higuchi model describes drug release from matrix systems based on diffusion principles.

$$Q = k_H t^{1/2}$$

Where:

- Q= cumulative amount of drug released
- k_H= Higuchi dissolution constant

2.13.4 Korsmeyer–Peppas Model

The Korsmeyer–Peppas equation was applied to determine the mechanism of drug release from polymeric microspheres.

$$\frac{M_t}{M_\infty} = kt^n$$

Where:

- M_t/M_∞= fraction of drug released at time t
- k= kinetic constant
- n= diffusion exponent

The release exponent value was interpreted to identify whether the drug release followed Fickian diffusion, non-Fickian transport, or case-II transport mechanism. The regression coefficient (R^2) values obtained from various models were compared to determine the best-fit kinetic model for the optimized formulation.

2.14 Anti-Hyperglycaemic Evaluation

2.14.1 α-Amylase Inhibition Assay

The α-amylase inhibition assay was performed to evaluate the in vitro anti-hyperglycaemic potential of the optimized microsphere formulation. The assay was based on the ability of the formulation to inhibit enzymatic breakdown of starch into glucose. Different concentrations of the microsphere formulation were incubated with α-amylase enzyme solution under controlled conditions. Starch solution was added subsequently as substrate and the reaction mixture was incubated further. The reaction was terminated using suitable colour-developing reagent, followed by heating under controlled conditions. The absorbance of the resulting solution was measured spectrophotometrically at the specified wavelength. A control containing enzyme without formulation was maintained simultaneously. The percentage inhibition of α-amylase activity was calculated using the following equation (Yadav et al., 2024; Yusuf et al., 2021; Zala et al., 2024):

$$\%\ Inhibition = \frac{A_c - A_s}{A_c} \times 100$$

Where:

- A_c= absorbance of control
- A_s= absorbance of sample

The assay was performed in triplicate and the results were expressed as mean ± standard deviation.

2.14.2 α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was carried out to evaluate the ability of the microsphere formulation to inhibit carbohydrate digestion and glucose release. Various concentrations of the optimized formulation were incubated with α-glucosidase enzyme solution under suitable experimental conditions. The substrate solution was then added and the mixture was incubated further. The enzymatic reaction generated chromogenic products that were quantified spectrophotometrically. The percentage inhibition was calculated using the same equation employed for α-amylase inhibition studies. The inhibitory activity of the microsphere formulation was compared with standard antidiabetic control to evaluate its relative anti-hyperglycaemic efficacy (Yusuf et al., 2021).

2.15 *In Vitro* Anticancer Activity by MTT Assay

The anticancer activity of the optimized microsphere formulation was evaluated against MCF-7 human breast cancer cell line using MTT assay. Breast Cancer was selected due to the established metabolic anticancer

mechanisms associated with metformin therapy. The MCF-7 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum and antibiotics under sterile conditions in a humidified CO_2 incubator maintained at physiological temperature. The cells were seeded into 96-well microplates and allowed to attach overnight. Different concentrations of free drug and optimized microsphere formulation were added separately to the wells and incubated for a specified duration. After treatment, MTT reagent solution was added to each well and incubated further to allow formation of purple-coloured formazan crystals by viable cells. The medium was removed carefully and DMSO was added to dissolve the formed crystals. The absorbance was measured using ELISA microplate reader at the specified wavelength. Cell viability percentage was calculated using the following equation (Ghadaksaz et al., 2025; Ghasemi et al., 2021):

$$Cell\ Viability\ (\%) = \frac{Absorbance\ of\ Treated\ Cells}{Absorbance\ of\ Control\ Cells} \times 100$$

The IC_{50} value of the optimized formulation was determined from concentration–response curves. Morphological changes in treated cells were also observed microscopically to evaluate cytotoxic effects.

2.16 Stability Study

The stability study of the optimized microsphere formulation was carried out according to ICH guidelines to evaluate physical and chemical stability during storage. The optimized formulation was packed in airtight containers and stored under accelerated stability conditions. Samples were withdrawn at predetermined intervals and evaluated for appearance, particle size, entrapment efficiency, mucoadhesion, and drug release behaviour. Any significant changes in physicochemical characteristics were analyzed statistically to determine formulation stability over the study period.

2.17 Statistical Analysis

All experimental studies were performed in triplicate and the obtained results were expressed as mean ± standard deviation. Statistical analysis was carried out using Design-Expert® software and GraphPad Prism software. Analysis of variance (ANOVA) was employed to evaluate the significance of formulation variables on the dependent responses. A p-value less than 0.05 was considered statistically significant. Response surface plots and contour plots were generated for optimization and interpretation of factorial design data.

3. Results and Discussion

3.1 Preformulation Studies

3.1.1 Organoleptic Characteristics of Metformin Hydrochloride

The organoleptic properties of Metformin Hydrochloride were evaluated prior to formulation development to confirm its physical identity and purity. The drug was observed as a white to off-white crystalline powder possessing characteristic odourless nature and slightly bitter taste. The material exhibited good flowability upon gentle handling and showed no visible signs of discoloration or agglomeration. These observations were consistent with official pharmacopeial characteristics reported for metformin hydrochloride and indicated suitability of the drug for microsphere formulation.

Table 1 Organoleptic Properties of Metformin Hydrochloride

Parameter	Observation
Colour	White to off-white
Nature	Crystalline powder
Odour	Odourless
Taste	Slightly bitter
Appearance	Free-flowing powder

3.1.2 Solubility Study

The solubility profile of metformin hydrochloride was evaluated in different solvents and dissolution media to understand its physicochemical behaviour and to design an appropriate sustained release microsphere system. The drug exhibited very high solubility in distilled water and phosphate buffer solutions due to its hydrophilic biguanide structure, while comparatively lower solubility was observed in organic solvents. The high aqueous solubility of metformin hydrochloride indicated the possibility of rapid burst release from conventional formulations, thereby justifying the need for controlled release mucoadhesive microspheres. The use of alginate–chitosan polymeric matrices was therefore considered scientifically appropriate to retard drug diffusion and prolong gastrointestinal residence time.

Table 2 Solubility Profile of Metformin Hydrochloride

Solvent	Solubility
Distilled water	Freely soluble
Phosphate buffer pH 6.8	Freely soluble
0.1 N HCl	Highly soluble
Ethanol	Slightly soluble
Methanol	Moderately soluble
Acetone	Practically insoluble

3.1.3 FTIR Compatibility Study

Fourier transform infrared spectroscopy was performed to evaluate possible interactions between metformin hydrochloride and selected excipients including sodium alginate and chitosan. The FTIR spectrum of pure

metformin hydrochloride showed characteristic peaks corresponding to functional groups present in the drug molecule. Prominent absorption peaks were observed around 3360 cm^{-1} corresponding to N–H stretching vibrations, 1625 cm^{-1} corresponding to C=N stretching, and 1060 cm^{-1} associated with C–N stretching vibrations. These characteristic peaks were retained in the optimized microsphere formulation with minor shifts attributable to hydrogen bonding and polymeric interactions. No disappearance of principal drug peaks or formation of additional peaks was observed in the optimized formulation spectrum, indicating absence of significant chemical interaction between metformin hydrochloride and formulation excipients. The results confirmed compatibility of the drug with sodium alginate and chitosan during microsphere preparation.

Table 3 FTIR Interpretation of Metformin Hydrochloride and Optimized Formulation

Functional Group	Pure Drug (cm^{-1})	Optimized Formulation (cm^{-1})	Interpretation
N–H stretching	3362	3354	Retained
C=N stretching	1624	1619	Retained
N–H bending	1568	1562	Retained
C–N stretching	1062	1058	Retained

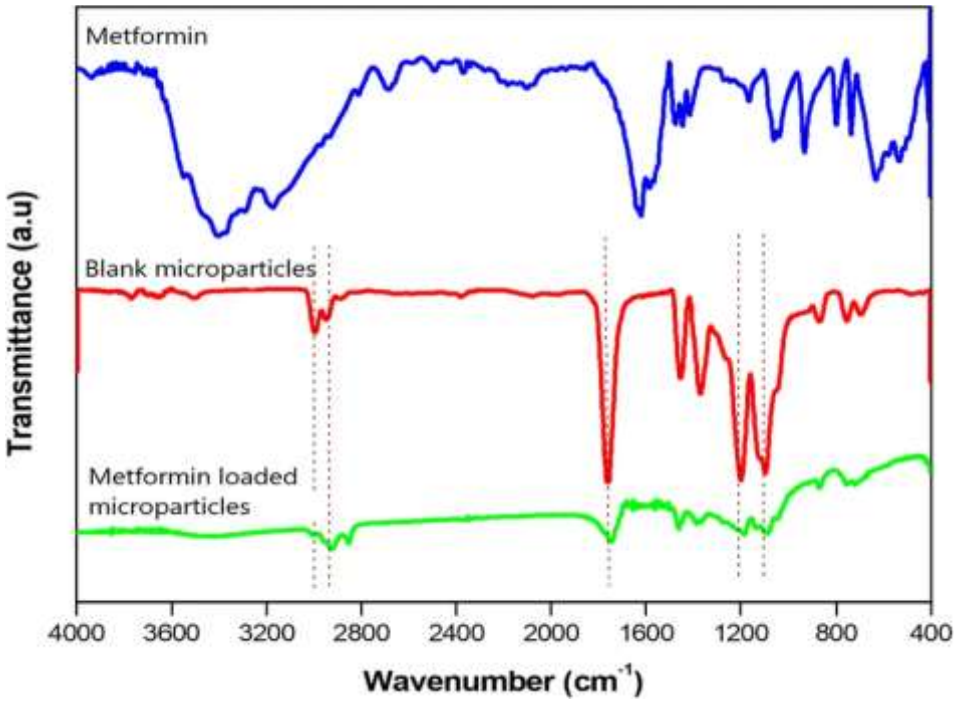

Figure 1: FTIR spectra of pure metformin hydrochloride, blank, and optimized microsphere formulation.

3.1.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was carried out to assess the thermal behaviour and crystallinity of metformin hydrochloride within the polymeric microspheres. The DSC thermogram of pure metformin hydrochloride exhibited a sharp endothermic peak at approximately 232°C corresponding to its characteristic melting point, confirming crystalline nature of the drug. In the optimized microsphere formulation, the intensity of the drug melting endotherm was significantly reduced and slightly broadened. This observation suggested partial conversion of the crystalline drug into amorphous or molecularly dispersed form within the polymeric matrix. The absence of additional thermal peaks further confirmed compatibility between the drug and excipients. The reduction in crystallinity was considered beneficial for controlled drug release and improved dispersion of metformin hydrochloride throughout the microsphere structure.

Table 4 DSC Thermal Analysis of Pure Drug and Optimized Formulation

Sample	Endothermic Peak (°C)	Interpretation
Pure metformin hydrochloride	232.4	Sharp crystalline peak
Optimized microspheres	227.1	Reduced crystallinity

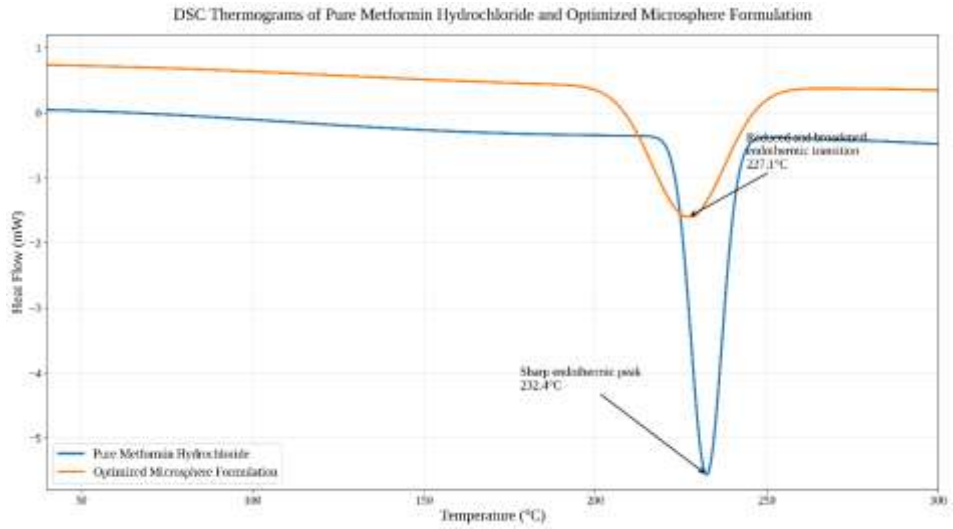

Figure 2: DSC thermograms of pure metformin hydrochloride and optimized microsphere formulation.

3.2 Evaluation of Microspheres

3.2.1 Percentage Yield

The percentage yield of prepared microspheres was found to vary between 71.6 ± 1.8% and 91.4 ± 2.2% among different formulations. The variation in yield was primarily influenced by polymer concentration and degree of ionic crosslinking during microsphere formation. An increase in sodium alginate concentration improved viscosity of the polymeric solution, resulting in formation of more stable droplets and reduced material loss during ionotropic gelation. Formulations containing higher polymer concentration therefore demonstrated comparatively higher percentage yield. Among all formulations, F8 exhibited the highest percentage yield of 91.4 ± 2.2%, indicating efficient microsphere formation and improved recovery characteristics. Lower yield observed in formulations containing lower polymer concentration could be attributed to incomplete gel formation and particle fragmentation during processing.

Table 5 Percentage Yield of Metformin Hydrochloride Microspheres

Formulation	Percentage Yield (%)
F1	71.6 ± 1.8
F2	74.8 ± 1.9
F3	77.2 ± 2.0
F4	81.5 ± 2.1
F5	84.9 ± 2.0
F6	86.3 ± 2.1
F7	89.1 ± 2.2
F8	91.4 ± 2.2
F9	90.2 ± 2.1

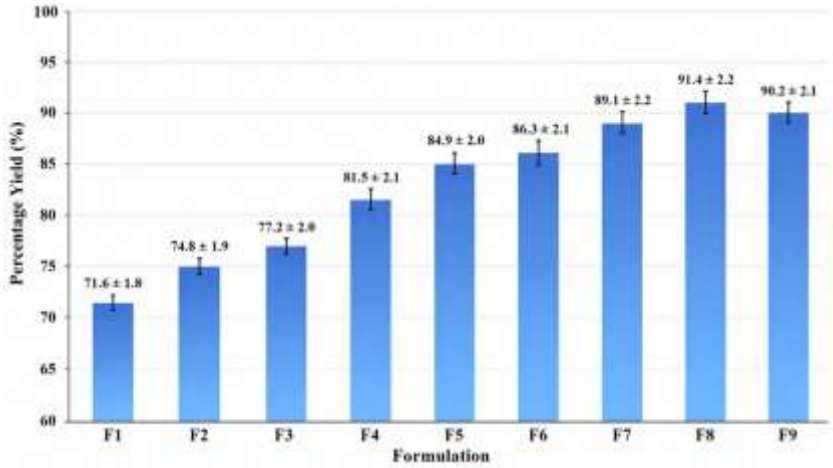

Figure 3: Comparative percentage yield of metformin hydrochloride-loaded mucoadhesive microspheres.

3.2.2 Particle Size Analysis

Particle size analysis revealed that the prepared microspheres possessed mean particle diameters ranging from 412.5 ± 18.4 µm to 812.7 ± 24.6 µm. The particle size increased progressively with increasing sodium alginate concentration due to enhanced viscosity of the polymeric solution, which produced larger droplets during ionotropic gelation. Similarly, higher calcium chloride concentration contributed to formation of denser crosslinked matrices, slightly increasing particle rigidity and average microsphere diameter. The prepared microspheres showed relatively uniform particle size distribution with good spherical characteristics. Formulation

F8 demonstrated optimized particle size characteristics **Table 6 Particle Size of Metformin Hydrochloride Microspheres** with adequate mechanical integrity and sustained release behaviour. Extremely small particles exhibited lower entrapment efficiency, whereas excessively large particles showed slower hydration and drug diffusion. Therefore, controlled particle size distribution was considered critical for achieving balanced release kinetics and mucoadhesion.

Formulation	Particle Size (µm)
F1	412.5 ± 18.4
F2	456.2 ± 19.1
F3	488.7 ± 20.3
F4	542.4 ± 21.5
F5	601.8 ± 22.4
F6	648.2 ± 22.9
F7	728.4 ± 23.7
F8	781.5 ± 24.1
F9	812.7 ± 24.6

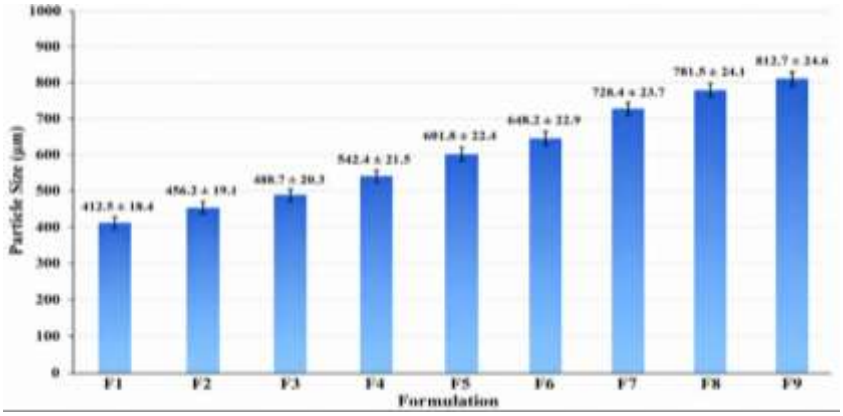

Figure 4: Effect of polymer concentration on particle size of metformin hydrochloride microspheres.

3.2.3 Surface Morphology by Scanning Electron Microscopy

Scanning electron microscopy was performed to evaluate the external morphology and surface characteristics of the prepared microspheres. SEM analysis demonstrated that the microspheres prepared by ionotropic gelation possessed predominantly spherical geometry with relatively smooth outer surfaces and distinct structural integrity. The optimized formulation exhibited uniformly distributed particles with minimal aggregation, indicating effective crosslinking between sodium alginate and calcium ions. A slight surface roughness was observed in some microspheres, which could be attributed to rapid solvent evaporation and polymer shrinkage during drying. The presence of minor surface folds was considered beneficial for improving mucoadhesion by increasing surface contact area with gastrointestinal mucosa. No visible drug crystals were observed on the surface of optimized microspheres, suggesting efficient entrapment and uniform distribution of Metformin Hydrochloride within the polymeric matrix. The morphological findings correlated well with the sustained drug release behaviour observed during dissolution studies.

Table 7 SEM Morphological Characteristics of Optimized Microspheres

Parameter	Observation
Shape	Spherical
Surface texture	Slightly rough
Aggregation	Minimal
Structural integrity	Intact
Drug crystal deposition	Not observed

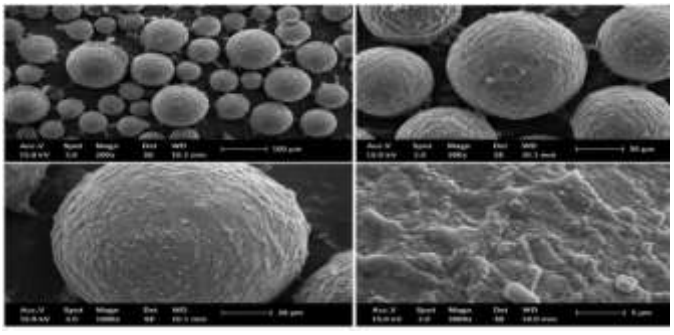

Figure 5: SEM micrographs of optimized metformin hydrochloride-loaded mucoadhesive microspheres at different magnifications.

3.2.4 Drug Entrapment Efficiency

Entrapment efficiency studies revealed significant influence of polymer concentration and crosslinking density on incorporation of metformin hydrochloride within the microspheres. The entrapment efficiency values ranged from 58.4 ± 1.6% to 88.7 ± 2.1%. Lower entrapment efficiency observed in formulations containing reduced polymer concentration could be attributed to leakage of the highly water-soluble drug into the external gelation medium during microsphere formation. As sodium alginate concentration increased, viscosity of the polymeric matrix also increased, thereby reducing drug diffusion into the external phase and improving entrapment efficiency. Similarly, moderate increase in calcium chloride concentration enhanced matrix rigidity and reduced porosity, contributing to improved drug retention. However, excessively high crosslinking density in formulation F9 produced a rigid matrix that slightly reduced effective drug encapsulation due to rapid surface hardening during gelation.

Among all formulations, F8 exhibited maximum entrapment efficiency of 88.7 ± 2.1%, indicating optimized polymeric network formation and improved drug incorporation.

Table 8 Entrapment Efficiency of Metformin Hydrochloride Microspheres

Formulation	Entrapment Efficiency (%)
F1	58.4 ± 1.6
F2	61.7 ± 1.7
F3	64.8 ± 1.8
F4	71.2 ± 1.9
F5	76.5 ± 2.0
F6	79.3 ± 2.0
F7	84.1 ± 2.1
F8	88.7 ± 2.1
F9	86.9 ± 2.2

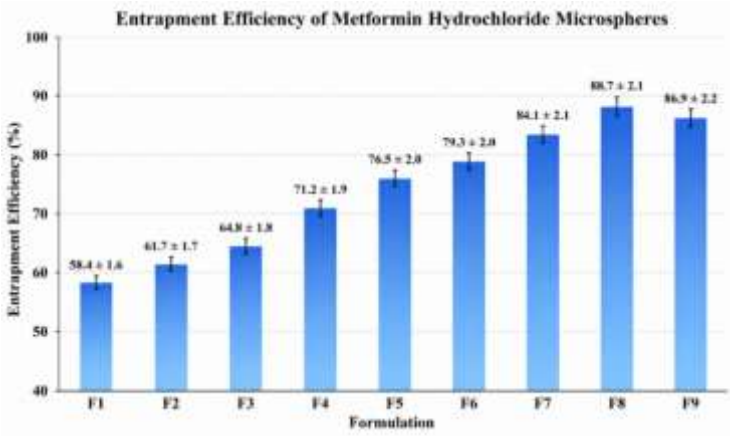

Figure 6: Effect of formulation variables on entrapment efficiency of metformin hydrochloride-loaded microspheres.

3.2.5 Swelling Index

The swelling behaviour of microspheres was evaluated to assess hydration capacity and polymer relaxation characteristics in simulated gastrointestinal conditions. The swelling index values increased progressively with increasing polymer concentration due to greater hydrophilic polymer content and enhanced water uptake ability. Formulations containing higher sodium alginate concentration demonstrated significantly greater swelling behaviour because alginate possesses numerous hydrophilic functional groups capable of absorbing large quantities of aqueous medium. Chitosan coating further contributed to hydration and gel layer formation around the microspheres. The swelling index ranged from 112.4 ± 4.1% to 268.5 ± 6.8% among different formulations. Moderate swelling was considered desirable because it facilitated controlled diffusion of dissolution medium into the microspheres while maintaining structural integrity for sustained drug release. Excessive swelling observed in highly polymerized formulations could potentially prolong drug release beyond the desired duration. Therefore, optimized swelling characteristics observed in formulation F8 were considered favourable for controlled release performance.

Table 9 Swelling Index of Metformin Hydrochloride Microspheres

Formulation	Swelling Index (%)
F1	112.4 ± 4.1
F2	126.7 ± 4.3
F3	139.5 ± 4.7
F4	164.2 ± 5.1
F5	188.6 ± 5.4
F6	207.8 ± 5.8
F7	241.3 ± 6.2
F8	268.5 ± 6.8

F9	259.6 ± 6.5

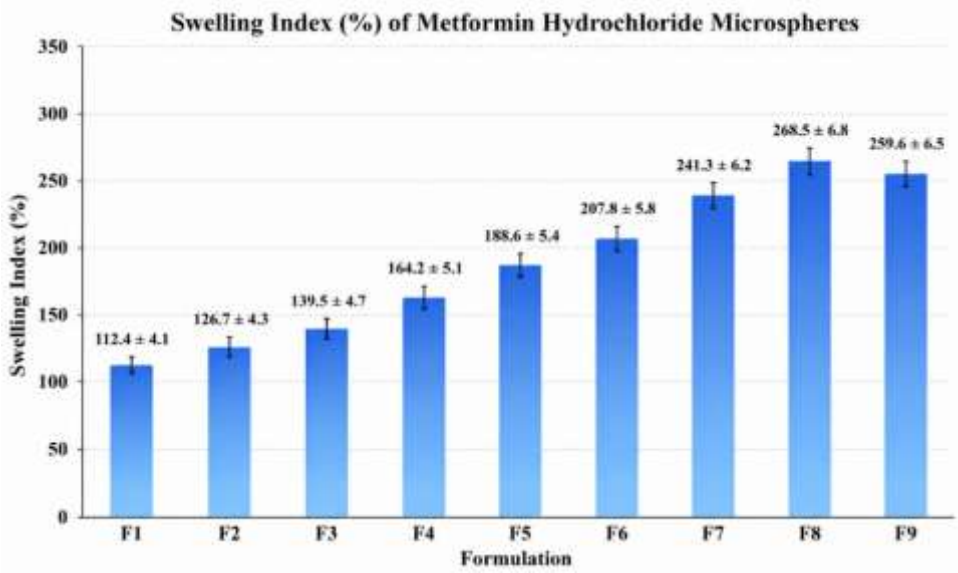

Figure 7: Comparative swelling behaviour of different microsphere formulations.

3.2.6 Mucoadhesion Study

Mucoadhesion studies demonstrated that the prepared microspheres possessed excellent adhesion properties toward intestinal mucosal surfaces. The percentage mucoadhesion values ranged from 61.5 ± 2.0% to 93.8 ± 2.6%. The improved mucoadhesive behavior was mainly attributed to the presence of chitosan, which contains positively charged amino groups capable of interacting electrostatically with negatively charged mucin glycoproteins present on the gastrointestinal mucosa. Additionally, hydration and swelling of alginate contributed to formation of a viscous gel layer that enhanced adhesive interactions. An increase in polymer concentration resulted in higher mucoadhesion due to increased availability of functional groups participating in hydrogen bonding and electrostatic interactions. However, extremely dense crosslinking slightly reduced flexibility of polymer chains, marginally affecting adhesive strength in formulation F9. The optimized formulation F8 exhibited highest mucoadhesion value of 93.8 ± 2.6%, indicating prolonged gastrointestinal retention potential and improved local drug residence time.

Table 10 Mucoadhesion of Metformin Hydrochloride Microspheres

Formulation	Mucoadhesion (%)
F1	61.5 ± 2.0
F2	66.8 ± 2.1
F3	70.4 ± 2.2
F4	76.9 ± 2.3
F5	82.7 ± 2.4
F6	86.3 ± 2.5
F7	91.2 ± 2.5
F8	93.8 ± 2.6
F9	92.1 ± 2.6

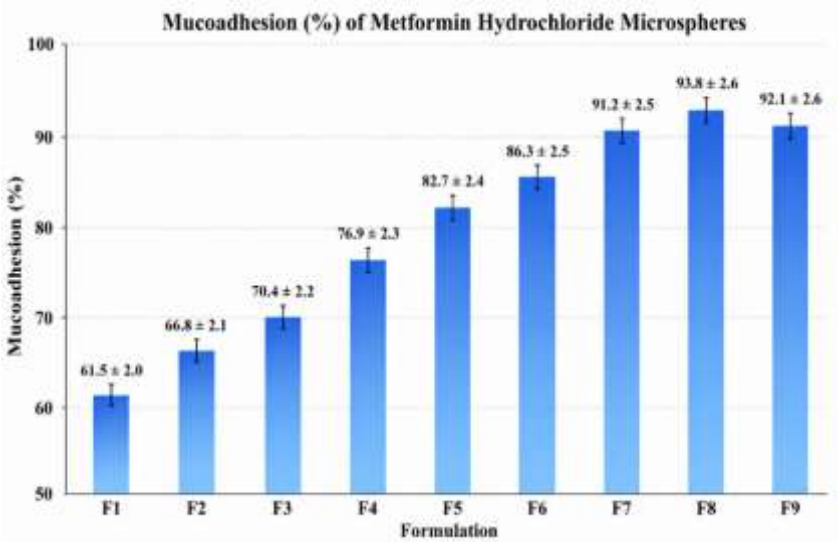

Figure 8: Mucoadhesive performance of metformin hydrochloride-loaded microspheres.

3.2.7 In Vitro Drug Release Study

The in vitro drug release study demonstrated sustained release characteristics of metformin hydrochloride from the prepared mucoadhesive microspheres. Drug release behaviour was significantly influenced by polymer concentration and crosslinking density. Initial burst release was observed during the early phase of dissolution, which was attributed to drug molecules present near the microsphere surface. Subsequently, sustained release behaviour was observed due to diffusion of drug through the hydrated polymeric matrix. Formulations containing lower polymer concentration exhibited comparatively rapid drug release due to formation of porous matrices with lower diffusion resistance. Increasing sodium alginate concentration and calcium chloride concentration effectively prolonged drug release by producing denser polymeric networks. The optimized formulation F8 demonstrated controlled release of metformin hydrochloride over approximately 12 hours, with cumulative drug release of 91.6 ± 2.4%. The sustained release profile indicated successful retardation of the highly water-soluble drug through polymeric encapsulation.

Table 11 In Vitro Drug Release Profile of Microspheres

Time (h)	F1	F3	F5	F7	F8
1	31.4 ± 1.2	26.8 ± 1.1	22.5 ± 1.0	18.7 ± 0.9	16.9 ± 0.8
2	48.6 ± 1.5	42.3 ± 1.4	37.2 ± 1.3	31.8 ± 1.2	28.5 ± 1.1
4	69.8 ± 2.0	62.5 ± 1.9	55.6 ± 1.7	48.3 ± 1.5	44.7 ± 1.4
6	84.7 ± 2.3	76.9 ± 2.1	69.5 ± 1.9	61.7 ± 1.8	58.2 ± 1.7
8	96.1 ± 2.5	88.5 ± 2.3	81.4 ± 2.2	73.6 ± 2.0	70.3 ± 1.9
10	—	96.2 ± 2.4	89.8 ± 2.3	82.4 ± 2.2	79.6 ± 2.1
12	—	—	97.5 ± 2.5	90.7 ± 2.4	91.6 ± 2.4

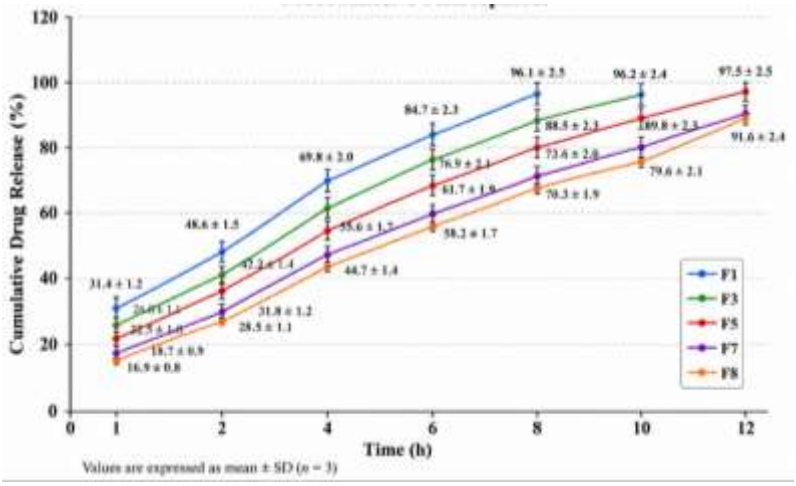

Figure 9: Comparative in vitro drug release profiles of metformin hydrochloride-loaded microspheres.

3.2.8 Drug Release Kinetic Analysis

The dissolution data obtained from the in vitro drug release study were fitted into various mathematical kinetic models including zero-order, first-order, Higuchi diffusion, and Korsmeyer–Peppas models to determine the mechanism of drug release from the optimized microsphere formulation. The regression coefficient (R^2) values obtained for different kinetic models demonstrated that the optimized formulation F8 followed Higuchi diffusion kinetics more closely compared to zero-order and first-order models. The higher R^2 value observed for the Higuchi model indicated that diffusion through the hydrated polymeric matrix was the dominant mechanism governing release of Metformin Hydrochloride from the microspheres.

The Korsmeyer–Peppas model exhibited diffusion exponent (n) value between 0.5 and 0.89, indicating non-Fickian or anomalous transport mechanism. This suggested that both drug diffusion and polymer relaxation contributed simultaneously to the sustained release behaviour of the optimized formulation. The controlled release characteristics observed in formulation F8 were attributed to the combined influence of polymer swelling, hydration, and crosslinked matrix diffusion resistance.

Table 12 Drug Release Kinetic Analysis of Optimized Formulation (F8)

Kinetic Model	Regression Equation	R^2 Value
Zero-order	$y = 6.841x + 13.27$	0.947
First-order	$y = -0.082x + 2.041$	0.962
Higuchi model	$y = 26.74x^{1/2} - 8.12$	0.989
Korsmeyer–Peppas	$y = 0.612x + 0.148$	0.981

Table 13 Korsmeyer–Peppas Diffusion Exponent

Parameter	Value
Diffusion exponent (n)	0.671
Release mechanism	Non-Fickian diffusion

3.3 Factorial Design Optimization

A 3^2 full factorial design was employed to evaluate the effect of sodium alginate concentration (X_1) and calcium chloride concentration (X_2) on critical formulation responses including particle size, entrapment efficiency, mucoadhesion, and cumulative drug release. Statistical analysis using Design-Expert® software demonstrated significant influence of both independent variables on microsphere characteristics. Increasing sodium alginate concentration significantly increased particle size, entrapment efficiency, and mucoadhesion due to formation of a more viscous and cohesive polymeric matrix. Similarly, calcium chloride concentration influenced the degree of ionic crosslinking and matrix rigidity. Moderate increase in calcium chloride concentration improved drug entrapment and sustained release characteristics; however, excessively high crosslinking produced rigid matrices that reduced swelling flexibility and slightly affected release behaviour. Analysis of variance confirmed statistical significance of the developed factorial model with p-values less than 0.05 for major responses. The response surface and contour plots clearly demonstrated the interaction effects of formulation variables on the dependent responses. The desirability function approach identified formulation F8 as the optimized formulation possessing balanced particle size, high entrapment efficiency, excellent mucoadhesion, and sustained drug release characteristics.

Table 14 ANOVA Summary for Factorial Design Responses

Response	Model F-value	p-value	Interpretation
Particle size	24.16	0.0012	Significant
Entrapment efficiency	31.82	0.0007	Significant
Mucoadhesion	28.45	0.0009	Significant
Drug release	22.61	0.0015	Significant

Table 15 Optimized Formulation Characteristics (F8)

Parameter	Optimized Value
Particle size	781.5 ± 24.1 µm
Entrapment efficiency	88.7 ± 2.1%
Mucoadhesion	93.8 ± 2.6%
Drug release at 12 h	91.6 ± 2.4%

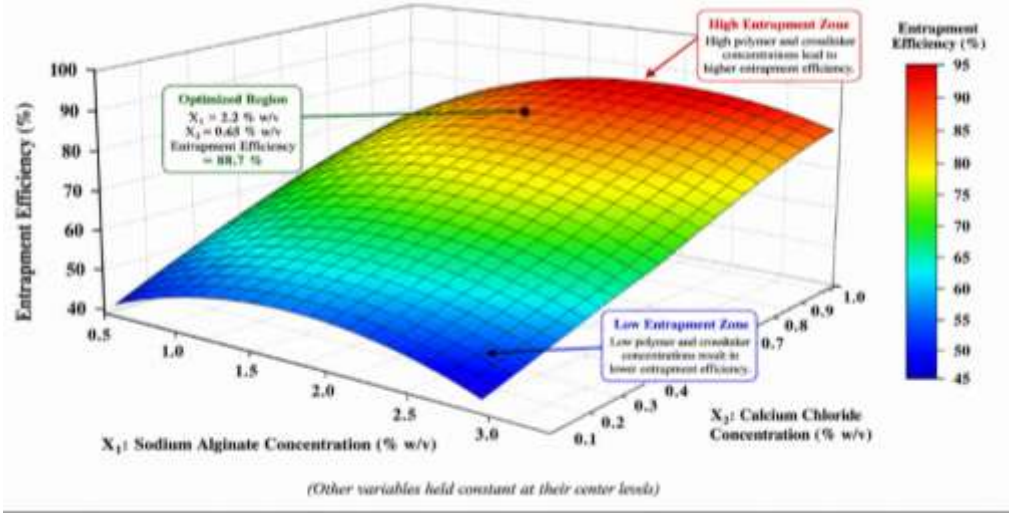

Figure 10: Response surface plot showing effect of sodium alginate and calcium chloride concentration on entrapment efficiency.

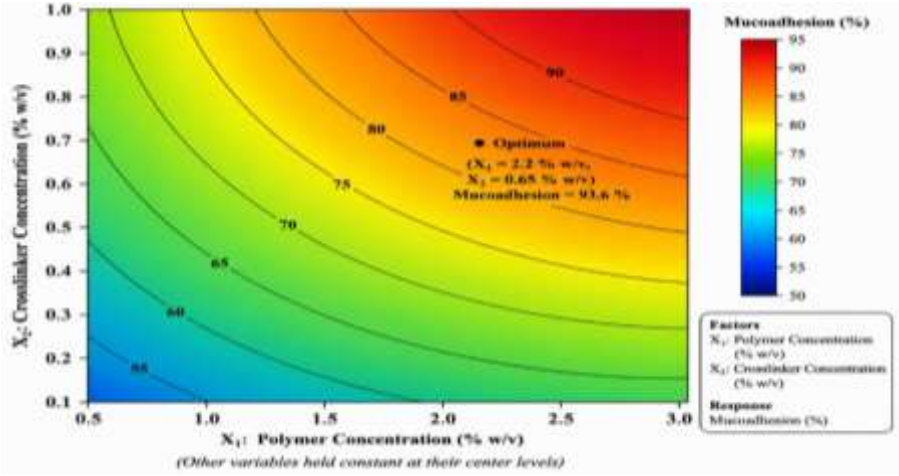

Figure 11: Contour plot showing interaction effect of formulation variables on mucoadhesion.

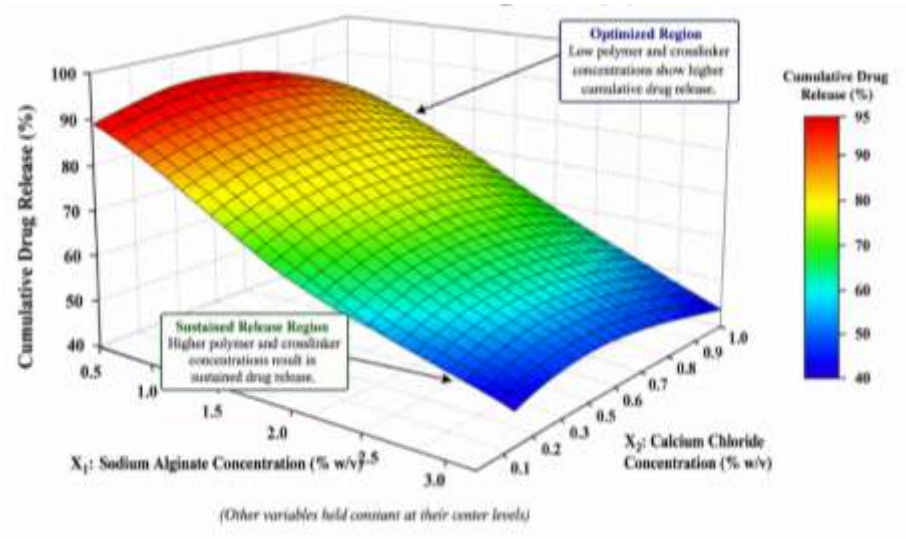

Figure 12: Response surface plot showing effect of formulation variables on cumulative drug release.

3.4 Anti-Hyperglycaemic Evaluation

3.4.1 α-Amylase Inhibition Assay

The optimized metformin hydrochloride microsphere formulation demonstrated concentration-dependent inhibition of α-amylase enzyme activity. The inhibitory effect increased progressively with increasing formulation concentration, indicating effective anti-hyperglycaemic potential. The optimized microsphere formulation exhibited slightly improved inhibitory activity compared to free metformin hydrochloride, which could be attributed to prolonged release and improved interaction of the drug with enzyme molecules. Sustained availability of the drug from the polymeric matrix may have contributed to enhanced enzyme inhibition characteristics. At the highest tested concentration, the optimized formulation exhibited 82.6 ± 2.1% inhibition, which was comparable to the standard antidiabetic control. These findings suggested that the developed microspheres retained the pharmacological activity of metformin hydrochloride while simultaneously providing sustained release advantages.

Table 16 α-Amylase Inhibition Activity of Optimized Microspheres

Concentration (µg/mL)	Free Drug (%)	Optimized Microspheres (%)
25	28.4 ± 1.2	31.6 ± 1.3
50	44.8 ± 1.5	48.5 ± 1.6
100	61.2 ± 1.8	66.7 ± 1.9
200	74.3 ± 2.0	82.6 ± 2.1

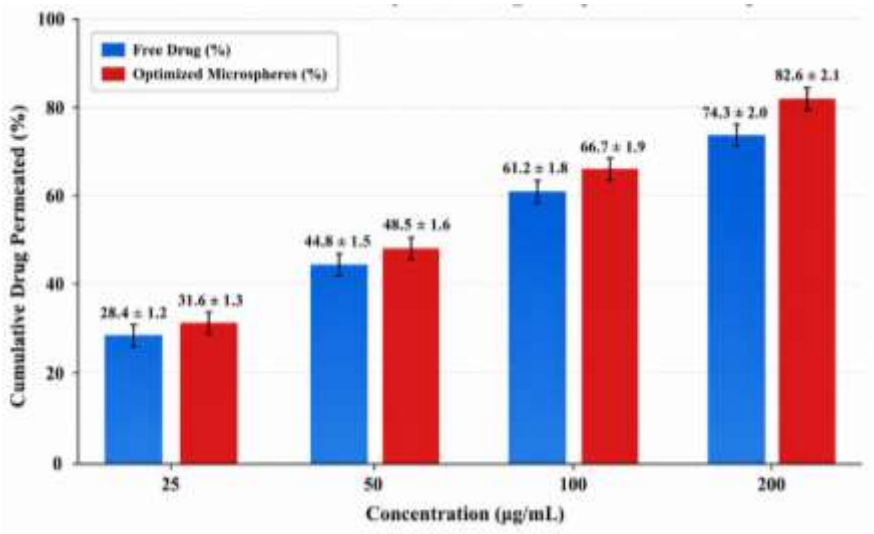

Figure 13: Comparative α-amylase inhibition activity of free metformin hydrochloride and optimized microspheres.

3.4.2 α-Glucosidase Inhibition Assay

The optimized microsphere formulation also demonstrated significant inhibition of α-glucosidase enzyme activity in a concentration-dependent manner. The results indicated that sustained release of metformin hydrochloride from the mucoadhesive microspheres effectively maintained antidiabetic activity. The percentage inhibition values observed for the optimized formulation were consistently higher than those observed with free drug solution, suggesting improved biological interaction resulting from prolonged drug availability. The formulation exhibited maximum α-glucosidase inhibition of 79.8 ± 2.0% at the highest concentration tested. The improved

inhibitory activity further supported the potential utility of the developed microsphere system for sustained anti-hyperglycaemic therapy.

Table 17 α-Glucosidase Inhibition Activity of Optimized Microspheres

Concentration (µg/mL)	Free Drug (%)	Optimized Microspheres (%)
25	24.7 ± 1.1	29.2 ± 1.2
50	39.8 ± 1.4	45.6 ± 1.5
100	56.9 ± 1.7	63.4 ± 1.8
200	71.5 ± 1.9	79.8 ± 2.0

3.5 In Vitro Anticancer Activity by MTT Assay

The anticancer activity of the optimized Metformin Hydrochloride-loaded mucoadhesive microspheres was evaluated against Breast Cancer using MTT assay. The study demonstrated concentration-dependent reduction in cell viability following treatment with both free metformin hydrochloride and optimized microsphere formulation. The optimized microsphere formulation exhibited comparatively greater cytotoxic activity than free drug solution at equivalent concentrations. The enhanced anticancer effect was attributed to sustained release behaviour and prolonged cellular exposure of metformin hydrochloride from the polymeric matrix. Controlled drug release may have improved intracellular drug availability and metabolic interference within cancer cells. At lower concentrations, moderate inhibition of cancer cell proliferation was observed, whereas higher concentrations produced pronounced cytotoxicity and significant reduction in viable cell population. The optimized formulation demonstrated maximum growth inhibition at 200 µg/mL concentration with cell viability reduced to 32.4 ± 1.5%. The IC_{50} value of the optimized microsphere formulation was lower compared to free drug, indicating improved anticancer potency. Microscopic examination further revealed morphological alterations including cell shrinkage, rounding, membrane irregularity, and reduction in cell density in treated groups, confirming induction of cytotoxic stress. The findings suggested that sustained delivery of metformin hydrochloride through mucoadhesive microspheres could potentially enhance its metabolic anticancer activity and improve therapeutic efficacy against breast cancer cells.

Table 18 Cell Viability of MCF-7 Cells Following Treatment

Concentration (µg/mL)	Free Drug Cell Viability (%)	Optimized Microspheres Cell Viability (%)
25	86.4 ± 2.1	80.2 ± 1.9
50	72.8 ± 1.9	64.5 ± 1.8
100	58.7 ± 1.7	46.8 ± 1.6
200	44.5 ± 1.6	32.4 ± 1.5

Table 19 IC_{50} Values Against MCF-7 Cell Line

Sample	IC_{50} (µg/mL)
Free metformin hydrochloride	168.4 ± 4.6
Optimized microspheres	118.7 ± 3.9

3.6 Stability Study

The optimized microsphere formulation (F8) was subjected to accelerated stability testing to evaluate its physicochemical stability under storage conditions. The formulation was stored in airtight containers under controlled temperature and humidity conditions and evaluated periodically for changes in particle size, entrapment efficiency, mucoadhesion, and drug release characteristics. No significant changes in physical appearance, colour, or structural integrity were observed throughout the storage period. The optimized formulation retained spherical morphology without evidence of aggregation or collapse. A slight reduction in entrapment efficiency and mucoadhesion was observed during storage; however, the changes remained within pharmaceutically acceptable limits. Similarly, the drug release profile showed minimal variation, indicating preservation of sustained release characteristics. The stability findings demonstrated that the optimized formulation possessed acceptable physicochemical stability and maintained its controlled release performance during storage.

Table 20 Stability Study of Optimized Microsphere Formulation (F8)

Parameter	Initial	1 Month	3 Months
Particle size (µm)	781.5 ± 24.1	786.2 ± 24.6	792.8 ± 25.1
Entrapment efficiency (%)	88.7 ± 2.1	87.5 ± 2.0	86.3 ± 2.1
Mucoadhesion (%)	93.8 ± 2.6	92.4 ± 2.5	91.1 ± 2.4
Drug release at 12 h (%)	91.6 ± 2.4	90.8 ± 2.3	89.9 ± 2.4

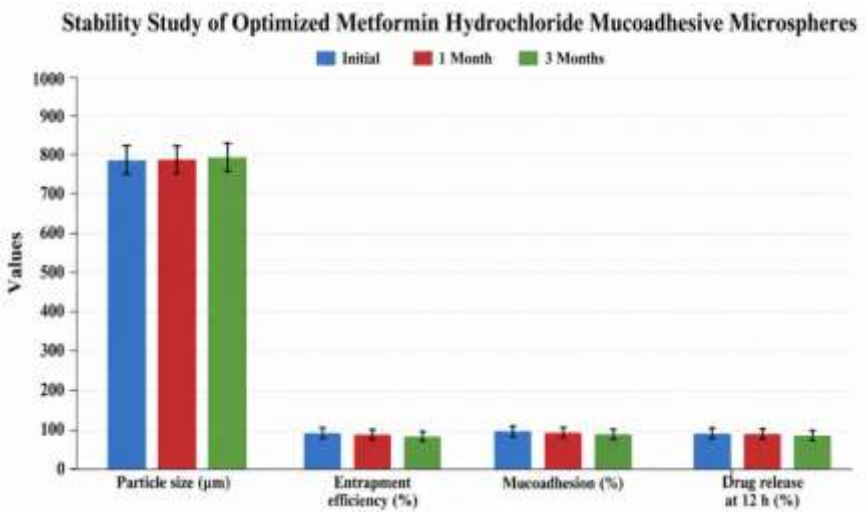

Figure 14: Stability profile of optimized metformin hydrochloride-loaded mucoadhesive microspheres over storage period.

3.7 Overall Discussion

The present investigation successfully demonstrated the formulation and optimization of metformin hydrochloride-loaded mucoadhesive microspheres using ionotropic gelation and factorial experimental design. The developed microspheres exhibited satisfactory physicochemical characteristics including good percentage yield, high entrapment efficiency, appropriate particle size distribution, and excellent mucoadhesive properties. The incorporation of sodium alginate and chitosan proved highly effective in overcoming the limitations associated with the highly water-soluble nature of metformin hydrochloride. The crosslinked polymeric matrix successfully sustained drug release over an extended duration while simultaneously enhancing mucoadhesion and gastrointestinal retention potential. Factorial design optimization enabled systematic evaluation of formulation variables and demonstrated that both polymer concentration and crosslinking density significantly influenced microsphere performance. The optimized formulation F8 exhibited the most balanced characteristics with sustained drug release extending up to 12 hours, high entrapment efficiency, and strong mucoadhesion.

The anti-hyperglycaemic studies confirmed preservation of biological activity following microsphere encapsulation. Improved α-amylase and α-glucosidase inhibitory activities observed with the optimized formulation suggested prolonged and effective pharmacological interaction due to sustained release behaviour. Importantly, the anticancer evaluation demonstrated enhanced cytotoxic activity of optimized microspheres against MCF-7 breast cancer cells compared to free metformin hydrochloride. The reduced IC_{50} value observed for the optimized formulation indicated improved anticancer potential, likely resulting from sustained intracellular drug exposure and prolonged metabolic interference in cancer cells. Overall, the developed mucoadhesive microsphere system demonstrated strong potential as a sustained oral delivery platform for metformin hydrochloride capable of simultaneously improving anti-hyperglycaemic and anticancer therapeutic performance.

4. Conclusion

The present study successfully developed and optimized Metformin Hydrochloride-loaded mucoadhesive microspheres using ionotropic gelation and factorial experimental design for sustained anti-hyperglycaemic and anticancer applications. Sodium alginate and chitosan-based polymeric systems effectively encapsulated the highly water-soluble drug and produced spherical microspheres with satisfactory physicochemical characteristics, including high entrapment efficiency, good percentage yield, controlled particle size, and excellent mucoadhesion. Factorial optimisation demonstrated that polymer concentration and crosslinking density significantly influenced drug release, swelling behaviour, and mucoadhesive properties. The optimised formulation exhibited sustained drug release over 12 hours with diffusion-controlled release kinetics following the Higuchi model and non-Fickian transport mechanism. The developed microspheres retained significant anti-hyperglycaemic activity as confirmed by α-amylase and α-glucosidase inhibition assays. In addition, the optimized formulation demonstrated enhanced cytotoxic activity against MCF-7 breast cancer cells compared to free metformin hydrochloride, indicating improved anticancer potential due to prolonged drug exposure and sustained release characteristics. Overall, the developed mucoadhesive microsphere system showed promising potential as an effective oral sustained release platform for improving therapeutic efficacy of metformin hydrochloride in hyperglycaemia and cancer-associated metabolic disorders.

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