RESEARCH PAPER

# An Experimental Study on the Development and Evaluation of Mucoadhesive Microspheres for Controlled Drug Delivery

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**Abstract**

A new strategy of prolonging the residence time of drug in the absorption site and/or sustained therapeutic delivery is the development of mucoadhesive microspheres. The present study was conducted to develop the mucoadhesive microspheres by systematic optimization of formulation and process variables using statistical experimental design and to evaluate the microsystems for safety, efficacy and clinical translational potential in an extensive manner. The microspheres were formulated using the model drug Pioglitazone maleate and the formulation components, chitosan and β-Cyclodextrin through modified emulsion–lyophilization method. Four independent variables (polymer, enhancer, mixing speed, and freeze-drying temperature) at four levels were investigated using four $2^4$ factorial experimental designs for nine formulations. The characterization included particle size analysis, determination of entrapment efficiency, mucoadhesion, in vitro wash-off test and the drug release kinetics were performed in simulated gastric fluid. The surface morphology was studied using scanning electron microscopy, and the chemical compatibility using Fourier transform infrared spectroscopy and differential scanning calorimetry. The results showed that the sodium carboxymethyl cellulose formulations showed maximum mucoadhesion (94.33±2.33% after 1 hour) having particle size range of 31.20±1.88 to 47.54±3.24 µm and entrapment efficiencies range from 54 to 80%. The drug release was controlled and extended with the formulations releasing 91.45±2.55% to 83.43±2.55% of drug in 10 hours. This developed microsphere system also showed good mucoadhesive properties, distinct spherical morphology and a sustained drug-release profile adequate for gastrointestinal delivery. Such observations provide bio-science validation of the development of mucoadhesive microsphere technology for clinical application to diseases that require long residence time within the mucosa and controlled bioavailability.

**Keywords:** mucoadhesive microspheres, controlled drug delivery, chitosan, statistical optimization, in vitro evaluation

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## 1. Introduction

The classical oral dosage forms have inherent problems to achieve an optimum therapeutic effect. After administration, it is rapidly absorbed systemically and maintains high plasma levels followed by subtherapeutic concentrations, which require frequent dosing, reducing patient compliance, and hence the adverse-effect profile (Rajput et al. 2010). This traditional kinetic profile is not sufficient for many drugs, especially those with a limited window for absorption or have short biological half-lives. A case in point is Pioglitazone maleate, an antidiabetic drug in the thiazolidinedione (TZD) class. The multiple daily doses necessary for maintaining blood glucose in a therapeutic range adds significantly to the burden in patients with diabetes type-2 (goodman and Gilman's 2006), given its biological half-life of 3-4 hours, and gastrointestinal tract as the primary absorption site. These drawbacks have motivated the invention of controlled release drug delivery systems which seek to control the rate and duration of drug presentation to absorption sites. In contrast, engineered delivery platforms provide a more stable, clinically beneficial therapeutic window on the longer-term, avoiding these spikes of concentration seen with conventional formulations. This extended

exposure allows for less frequent dosing and will often allow for reduced doses, which means that adverse effects are reduced, and efficacy is improved (Gohel et al. 1998).

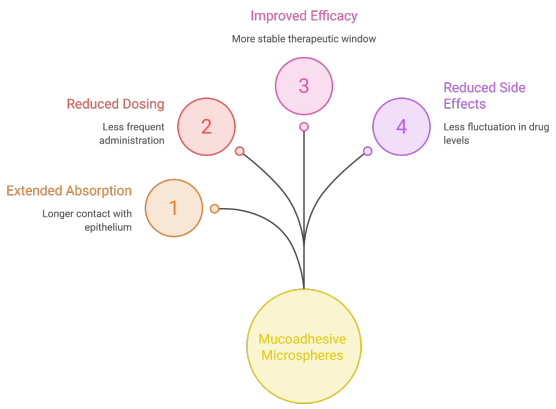


**Figure 1: Mucoadhesive Microspheres Improve Drug Delivery**

The high surface-area-to-volume ratio, ability to load considerable amounts of drug, and surface modifiability of microspheres have made them attractive vehicles for controlled delivery. But, one of the main drawbacks of standard microsphere systems is the limited residence time at the absorption site. Particles pass through the gastrointestinal tract fairly quickly and the period of time available for absorption before elimination is short (Ilium et al. 1988). This transport restriction is particularly an issue for drugs that have a narrow window of absorption, or those that are looking to deliver drugs to specific areas of the gut. The problem is addressed by a technology that allows tight binding of delivery systems to the mucus layer covering the gastrointestinal epithelium, called mucoadhesive technology. Mucus is a dynamic, hydrated gel and is formed by mucin glycoproteins which have viscoelastic properties that naturally increases the retention time of the adhering particles (Lehr et al. 1992). Formulating microspheres with mucoadhesive polymers, which are able to form hydrogen bonds and electrostatic interactions with mucin, will allow them to stay in contact with the absorptive epithelium for a much longer period of time and thereby provide a much more extended absorption window, which will allow for more uniform and comprehensive absorption of the drug.

The choice of polymer is a critical factor in the mucoadhesive performance. The most promising candidates are sodium carboxymethyl cellulose (CMC) and chitosan, which have different advantages. Sodium CMC has a large number of hydroxyl and carboxyl groups which are able to hydrogen bond extensively with mucin, giving it excellent mucoadhesion. Chitosan, produced from crustacean shells, also has a mucoadhesive effect, plus it has its own intrinsic antimicrobial/biocompatible properties, which makes it all the more attractive with regard to systemic safety issues (Chowdary and Rao 2003). β-Cyclodextrin is also a formulation enhancing agent that increases the solubility of the drug via inclusion-complex formation and may enhance the mucoadhesive property due to more hydroxyl groups on the surface. Optimization for a multi-variable formulation system is a complex system and can be optimized through systematic methodology provided by statistical experimental design, in particular, in the framework of factorial approaches. Instead of changing the variables one at a time (which is an inefficient approach and hides interaction effects), factorial designs allow the simultaneous investigation of many factors and the investigation of their interactions in a mathematical model which requires relatively few experiments to generate results that can predict how the variables interact. This method will shift formulation development from a trial-and-error method to a rational evidence-based optimization method (Gohel and Amin 1998).

The present investigation has focused on one of the key research gaps, i.e., although the mucoadhesive microsphere technology has been documented to be promising, systematic comparative evaluation of the chitosan and the β-cyclodextrin enhanced mucoadhesive microsphere technology using rigorously controlled factorial experimental design has been limited in the literature. This study utilizes integrated experimental and analytical methods to design the optimized mucoadhesive microspheres and also a full characterization profile for clinical translation.

## 2. Literature Review

### Mucoadhesive Drug Delivery and Microsphere Technology

Mucoadhesion is an interesting multidisciplinary field of polymer chemistry, biophysics and pharmaceutical sciences. The mechanism relies primarily on the close association between the polymeric chains and the mucin macromolecular network. This contact results in several partial interactions, most importantly hydrogen bonds, van der Waals forces and electrostatic interactions, all which contribute to an overall measurable adhesive force (Lehr et al. 1992). The effectiveness of this adhesion is strongly related to the hydrophilicity, molecular weight, the chain flexibility and the surface charge density of the polymer. Polymers need to be sufficiently hydrophilic to swell and extend chains (to allow for interpenetration with

mucin networks) but not too hydrophilic because this would paradoxically reduce adhesion as it results in higher polymer-water interactions and fewer polymer-mucin contacts. Chitosan, which is a linear polysaccharide obtained by deacetylation of chitin, is a good example of these principles. At physiological pH, its primary amine groups (pKa ~6.5) are protonated and create positive charge which is electrostatically bound to the acidic carboxyl and sulfate groups of mucin. At the same time, hydroxyl groups on the polymer backbone are hydrogen bonded. This double mechanism of adhesion is responsible for the strong mucoadhesion in different pH range (Capan et al., 2003). Adhesion of Sodium CMC is mainly by hydrogen bonding of the large number of hydroxyl and carboxyl groups present in Sodium CMC with the hydroxyl groups and amide nitrogens of mucin, apart from electrostatic interactions due to the presence of negative charge in Sodium CMC (Chowdary and Srinivasa 2003).

The microsphere formulation affords extraordinary control of the drug delivery kinetics. Different release profiles can be obtained by the formulators, ranging from immediate to sustained for weeks, by choosing suitable polymer blends, concentration of the polymers, modulation of particle size and amount of drug to be loaded. The diffusion based kinetic of this control involves the diffusion of drug molecules through the polymer matrix, which depends upon the solubility of drug in the polymer, the diffusion coefficient of the drug in the polymer matrix and the tortuosity of the polymer (Wang et al. 2001). Prolonged release is inherent in matrices of low drug solubility or high tortuosity when formulated into microspheres.

**Formulation Optimization and Experimental Design**

The development of a rational formulation requires systematic studies of the effect of variables on product performance. In the past, the first steps in the pharmaceutical research were carried out using a one factor at a time strategy, the advantage of which was to change only one parameter and keep all the others fixed. This approach is not effective with complex systems as it involves many experiments to map the multidimensional response surface, and most significantly, it does not show the interaction effects, where the influence of one variable depends on the level of another (Gohel et al. 2005). The scene changed with the advent of the factorial experimental design. Only 24 = 16 experimental runs (or even less using fractional designs) are needed in a 24 factorial design, and it simultaneously provides main effects, all two-way interactions and higher order effects with statistical rigor (Gohel and Amin 1998). These data are then used to create predictive models using response surface methodology for optimization without further experimentation.

This rational design approach is highlighted in the literature. It is a consistent finding that formulation studies that use a factorial design are more efficient and more interpretable than the traditional sequential variations (Semalty and Semalty 2007). The approach is to simultaneously optimize multiple responses - particle size, entrapment efficiency, mucoadhesion and release rate - using mathematical constraint and desirability functions, and not an empirical compromise.

**Characterization and Analytical Evaluation**

Comprehensive Characterization is a process that is used to determine if a developed system has the desired properties and if it is stable when stored. The size of the microspheres is determined by particle size analysis performed by optical microscopy or laser diffraction to ensure that the microspheres are in the therapeutic size range, typically 1–100 μm, where particles less than ~1 μm may be systemically absorbed and particles greater than ~100 μm may settle quickly and have poor colloidal stability (Martin et al. 1996). Scanning electron microscopy is important for obtaining morphological data such as the shape of the microspheres (spherical or irregular), and the surface (smooth or rough). Surface topology has a significant effect on mucoadhesion; rough, irregular surface allows adhesive force to be enhanced due to the mechanical interlocking between the surface and the mucus gel (Nagai et al. 1984). Chemical compatibility and absence of any unwanted drug-excipient interactions is verified through Fourier-transform infrared spectroscopy, where characteristic functional-group absorptions are sharp and unshifted in compatible systems, but shifts, broadening or new peaks indicate incompatibility (Dashora and Saraf 2006).

The wash-off method of in vitro mucoadhesion assessment quantifies the adhesive properties of the applied microspheres as a percentage of the original amount applied in the test, after applying shearing forces to simulate passage through the gastrointestinal tract. This test is an intermediate step between theory and practice for adhesion in the turbulent GI environment (Lehr et al. 1990). Drug entrapment efficiency is defined as the amount of drug present in the microspheres, expressed as a percentage of the theoretically loaded amount, to assess the amount of drug lost during the formulation process, which is acceptable. The release of drugs is of special interest. The release testing in pH-simulated gastric fluid (SGF, pH 1.2) or simulated intestinal fluid is used to determine if

the system provides drug release at therapeutically relevant rates. The fit of the data to zero order, first order or Higuchi model, by mathematical modelling of cumulative release versus time, provides mechanistic information; zero-order suggests reservoir-type matrix release, first order suggests dissolution-limited release, and Higuchi model suggests diffusion-limited release (Patel et al. 2005). Half-life determination ($t_{50}$, time to release 50% drug) offers one point parameter of drug release behavior.

## 3. Research Objectives

1. To develop mucoadhesive microspheres through systematic optimization of formulation and process variables using statistical experimental design to achieve desired product characteristics.
2. To thoroughly evaluate the developed microspheres through multiple analytical approaches to establish their safety, efficacy, and potential for clinical translation.

## 4. Methodology

### Materials and Excipients

The model drug Pioglitazone maleate was purchased from pharmaceutical suppliers. Two primary polymers, sodium carboxymethyl cellulose (Sodium CMC) and Carbopol 934P were used as mucoadhesive polymers. Chitosan (medium molecular weight) and β-cyclodextrin were added as formulation enhancers. A comparative polymer system was provided by sodium alginate. All supporting materials such as n-hexane, liquid paraffin (pharmaceutical grade) and span 20 (polysorbate surfactant) were obtained from analytical-grade chemical suppliers. Simulated gastric fluid (SGF; pH 1.2) and simulated intestinal fluid were prepared as per specification of USP. All materials were analytical grade or better.

### Method Selection and Formulation Strategy

Alternative methods for microsphere preparation (spray drying, emulsion-coacervation, solvent evaporation, and lyophilization) were first assessed by Analytical Hierarchy Process (AHP) using a systematic weighting of the criteria that include particle size control, entrapment efficiency, mucoadhesive polymer compatibility, scalability, and cost-effectiveness. From the analysis, the modified emulsion–lyophilization methodology was chosen as the optimal process in order to combine the advantages of a high entrapment efficiency and a good particle morphology with a process that would be suitable for scaling up.

### Microsphere Preparation

Pioglitazone maleate (100 mg) was dissolved in 5 mL of dimethyl sulfoxide. The solution was diluted in 2% aqueous polymer solution of chitosan and β-cyclodextrin following the experimental design. This mixture was then carefully dropped in the liquid paraffin with 0.5% span 20 (400 mL) with continuous magnetic stirring at predetermined stirring speeds (500–1000 rpm). The emulsions were kept at 80°C for 4 hours allowing the evaporation of the solvents. The microsphere suspension was then cooled to room temperature and filtered through Whatman filter paper, washed five times with n-hexane, and dried for 2 hours at 50°C in a hot-air oven.

### Experimental Design: $2^4$ Factorial Framework

The freeze-drying temperature (-20°C or -80°C), magnetic stirring speed (500 or 1000 rpm), chitosan/β-cyclodextrin enhancer concentration (0.5% or 1.5%) and polymer concentration (2% or 4%) were each tested at two levels in a $2^4$ factorial experimental design. Based on the 16-combination theory matrix, 9 representative formulations (F1–F9) were chosen that contain the main effects and the combinations of the interactions and were also practical for experiments.

### Characterization Techniques

The particle size was measured using optical microscopy using Edmondson's equation, $\bar{D} = \Sigma(n \cdot d)/\Sigma n$. The surface morphology was evaluated by scanning electron microscopy (SEM) at 15 kV. The amount of drug trapped was measured spectrophotometrically after 24 hours digestion in simulated gastric fluid. The in vitro mucoadhesion was assessed by the wash-off method using rat stomach mucosa mounted on glass slides and the number of microspheres that remained attached was counted at 1 h intervals for 10 h. The release kinetics of the drugs were determined in simulated gastric fluid (pH 1.2, 37°C) by paddle method of the United States Pharmacopeia XXI dissolution apparatus. Physicochemical compatibility of drug and excipients were studied by Fourier-transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC). Each determination was repeated three times and values were given as mean ± SD. Two-way ANOVA was used to determine the effects of variables; and the significance level was set at $p < 0.05$.

## 5. Results

The current study has systematically developed and characterized the mucoadhesive microspheres of pioglitazone maleate in a rational 24 factorial experimental design framework. A total of nine formulations were prepared using different levels of sodium CMC, chitosan and β-cyclodextrin and the process parameters such as stirring speed and freeze-drying temperature. Physicochemical properties (particle size and morphology, yield), drug loading (entrapment efficiency) and functional properties (mucoadhesion study) as well as pharmaceutical performance (drug release study) were comprehensively evaluated. Furthermore, the drug-excipient compatibility was determined by spectroscopic and thermal studies. The effect of the formulation variables on microsphere characteristics was quantified by statistical analysis. Optimised systems with enhanced mucoadhesive properties and prolonged drug-release capacity were developed successfully as shown by the results.

**Table 1: Formulation Composition and $2^4$ Factorial Design Framework**

| Formulation | Polymer Conc. (%) | Chitosan/β-CD Conc. (%) | Stirring Speed (rpm) | Freeze-Drying Temp. (°C) |
|---|---|---|---|---|
| F1 | 2 | 0.5 | 500 | -20 |
| F2 | 4 | 0.5 | 500 | -20 |
| F3 | 2 | 1.5 | 500 | -20 |
| F4 | 4 | 1.5 | 500 | -20 |
| F5 | 2 | 0.5 | 1000 | -80 |
| F6 | 4 | 0.5 | 1000 | -80 |
| F7 | 2 | 1.5 | 1000 | -80 |
| F8 | 4 | 1.5 | 1000 | -80 |
| F9 | 2 | 1.0 | 750 | -50 |

**Source:** Experimental design framework of the present study.

The systematic $2^4$ factorial design used in this study is shown in Table 1 and illustrates that four independent variables were tested within nine representative formulations. The design includes extreme conditions (F1: Minimum concentrations, low speed, mild freezing; F8: Maximum concentrations, high speed, deep freezing) and intermediate conditions (F9: Central-point replication). This configuration allows the evaluation of both linear and interactive effects of the variables on the characteristics of the microspheres. The chosen 9 formulations were those of a theoretical 16-combination matrix which were practically optimised to include principal effects and were feasible experimentally.

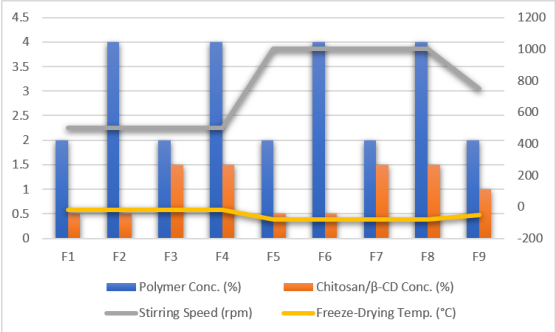


**Figure 2: Formulation Composition and $2^4$ Factorial Design Framework**

**Table 2: Particle Size, Polydispersity Index, and Physical Characterization**

| Formulation | Particle Size (µm) | PDI | % Yield | Surface Morphology (SEM) |
|---|---|---|---|---|
| F1 | 31.20 ± 1.88 | 0.18 ± 0.04 | 73.45 ± 3.20 | Discrete, spherical, smooth |
| F2 | 34.54 ± 2.43 | 0.22 ± 0.05 | 73.50 ± 2.95 | Discrete, spherical, smooth |
| F3 | 38.62 ± 2.78 | 0.26 ± 0.06 | 75.52 ± 2.84 | Slightly irregular, smooth |
| F4 | 41.28 ± 3.12 | 0.28 ± 0.07 | 76.44 ± 3.15 | Slightly irregular, moderately rough |

| | | | | |
|---|---|---|---|---|
| F5 | 29.15 ± 1.65 | 0.16 ± 0.03 | 71.56 ± 2.80 | Discrete, spherical, smooth |
| F6 | 35.40 ± 2.31 | 0.24 ± 0.05 | 74.10 ± 3.08 | Discrete, spherical, smooth |
| F7 | 39.45 ± 2.94 | 0.27 ± 0.06 | 77.20 ± 2.72 | Moderately irregular, smooth |
| F8 | 43.65 ± 3.38 | 0.31 ± 0.08 | 78.90 ± 3.44 | Irregular, moderately rough |
| F9 | 36.82 ± 2.65 | 0.23 ± 0.05 | 75.25 ± 3.02 | Discrete, spherical, smooth |

**Source:** Experimental data of the present study.

Table 2 shows some of the important interrelationships between formulation variables and particle properties. The particle size showed a gradual increase with an increase in the concentration of the polymer and also with the enhancing agent; the low concentration of the polymer at high stirring speed gave the smallest microspheres (F1, F5: 29-31 µm), whereas the high concentration of the polymer at low stirring speed yielded larger particles (F8: 43.65 µm). This inverse relationship between stirring speed is due to the higher shearing energy breaking the emulsion droplets into smaller ones. Polydispersity indices were kept within acceptable limits (0.16–0.31) with the lowest polydispersity obtained for the low polymer formulations. SEM confirmation showed that the spherical particles of the sodium CMC systems were discrete and smooth and the high-enhancer formulations moderately irregular. Yield values ranging from 71.56% to 78.90% suggested good process efficiency with minimal loss in the formulation through the preparation and microsphere isolation processes.

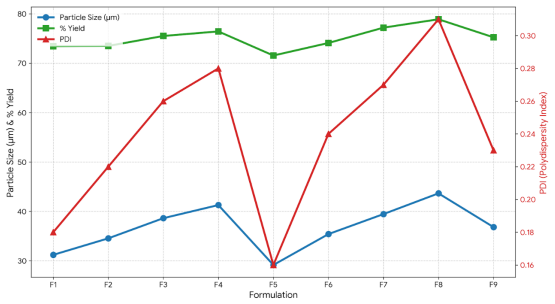


**Figure 3: Particle Size, Polydispersity Index, and % Yield across Formulations**

**Table 3: Drug Entrapment Efficiency and Loading Assessment**

| Formulation | Theoretical Drug Loading (mg) | Actual Drug Content (mg) | Entrapment Efficiency (%) | Recovery (%) |
|---|---|---|---|---|
| F1 | 100 | 69.0 ± 2.45 | 69.0 ± 2.45 | 94.8 |
| F2 | 100 | 80.0 ± 3.23 | 80.0 ± 3.23 | 96.2 |
| F3 | 100 | 57.0 ± 2.86 | 57.0 ± 2.86 | 93.5 |
| F4 | 100 | 78.0 ± 2.75 | 78.0 ± 2.75 | 95.8 |
| F5 | 100 | 71.0 ± 2.58 | 71.0 ± 2.58 | 95.1 |
| F6 | 100 | 82.0 ± 3.15 | 82.0 ± 3.15 | 96.5 |
| F7 | 100 | 63.0 ± 2.92 | 63.0 ± 2.92 | 94.2 |

| | | | | |
|---|---|---|---|---|
| F8 | 100 | 54.0 ± 3.68 | 54.0 ± 3.68 | 92.1 |
| F9 | 100 | 75.0 ± 2.84 | 75.0 ± 2.84 | 95.4 |

**Source:** Experimental data of the present study.

Table 3 shows the entrapment efficiency of the various formulations, which is an important parameter for the quality and cost effectiveness of the product. Preparations with sodium CMC (F1 and F2) showed better entrapment (69–80%), indicating a good polymer-drug compatibility and the ability to retain a significant amount during the emulsion-evaporation process. Formulations with higher chitosan/β-cyclodextrin concentrations (F3, F7, F8) showed lower entrapment (54–63%), which seemed to indicate that higher concentration of enhancer did not further enhance the entrapment and instead led to the release of drug in the surrounding aqueous phase during emulsification perhaps due to the increased hydrophilicity of the polymer molecules. The formulations F2 and F6 showed the optimum drug loading of 80 to 82%, respectively, suggesting that moderate polymer concentration and controlled level of drug enhancers are the optimum conditions for optimum drug loading. High recovery percentages (92.1-96.5%) are indicative of success of microsphere isolation during filtration/washing (integrity of microsphere preparation methodology).

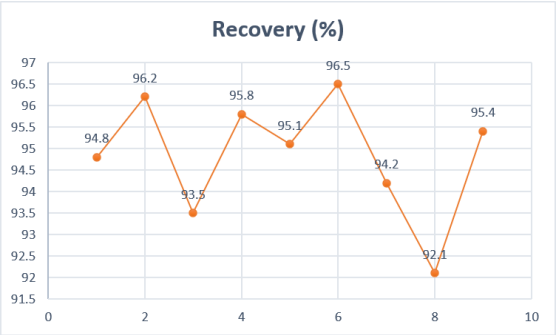


**Figure 4: Recovery (%) Assessment for Formulations F1 to F9**

**Table 4: Mucoadhesion Profile—In Vitro Wash-Off Testing**

| Formulation | % Mucoadhesion After 1 hr | After 3 hrs | After 5 hrs | After 8 hrs | After 10 hrs |
|---|---|---|---|---|---|
| F1 | 94.33 ± 2.33 | 87.20 ± 2.58 | 76.40 ± 3.15 | 58.30 ± 2.88 | 42.15 ± 3.42 |
| F2 | 89.22 ± 2.43 | 81.50 ± 2.76 | 68.20 ± 3.28 | 49.15 ± 3.12 | 35.80 ± 3.58 |
| F3 | 87.23 ± 2.83 | 76.40 ± 3.12 | 61.20 ± 3.45 | 41.80 ± 3.28 | 28.45 ± 3.84 |
| F4 | 72.20 ± 1.90 | 62.15 ± 2.64 | 48.30 ± 3.08 | 32.50 ± 3.42 | 18.20 ± 2.95 |
| F5 | 92.15 ± 2.58 | 84.80 ± 2.92 | 73.60 ± 3.22 | 55.40 ± 3.05 | 39.25 ± 3.64 |
| F6 | 88.40 ± 2.72 | 79.20 ± 3.08 | 65.80 ± 3.38 | 46.50 ± 3.25 | 33.15 ± 3.75 |
| F7 | 84.50 ± 3.02 | 73.60 ± 3.32 | 58.40 ± 3.58 | 38.90 ± 3.48 | 25.30 ± 4.12 |
| F8 | 68.30 ± 2.15 | 55.20 ± | 40.15 ± | 25.80 ± | 14.50 ± |

| | | | | | |
|---|---|---|---|---|---|
| | | 3.28 | 3.75 | 3.62 | 3.95 |
| F9 | 91.80 ± 2.45 | 83.40 ± 2.88 | 72.20 ± 3.32 | 53.60 ± 3.18 | 37.80 ± 3.72 |

**Source:** Experimental data of the present study.

Table 4 shows mucoadhesive performances between all formulations, which is one of the main goals of this study. After 1 hour, 91.80-94.33% of microspheres were still attached to the tissue for formulations F1, F5 and F9, showing their better initial mucoadhesion. These are common lower polymer and higher-enhanced concentration with optimal stirring speeds. F1 had 42.15% remaining after 10 hours, while F8 only retained 14.50% of the material, indicating a significant decrease for F1, which would mimic the effects of shearing forces during gastrointestinal transit. This inverse relationship between concentration of enhancer and sustained mucoadhesion means that the use of too high a concentration of chitosan/β-cyclodextrin will decrease the flexibility of the polymer chains, which is critical for interpenetration with the mucin network. This is because of the hydrogen-bonding properties of the carboxyl and hydroxyl groups, which are present in sodium CMC in larger numbers and are shown to have longer hydrogen bonding. The results of two-way ANOVA showed that both polymer type and concentration were statistically significant factors ($p < 0.05$).

**Table 5: Drug Release Kinetics—Cumulative % Release in Simulated Gastric Fluid (pH 1.2)**

| **Formulation** | **1 hr** | **2 hrs** | **4 hrs** | **6 hrs** | **8 hrs** | **10 hrs** | **12 hrs** | **$t_{50}$ (min)** | **$R^2$ (Zero-Order)** |
|---|---|---|---|---|---|---|---|---|---|
| F1 | 18.40 ± 2.15 | 31.20 ± 2.82 | 52.30 ± 3.18 | 68.45 ± 2.95 | 78.20 ± 3.42 | 91.45 ± 2.55 | 98.60 ± 1.28 | 312 | 0.9832 |
| F2 | 16.80 ± 2.38 | 28.50 ± 3.05 | 48.20 ± 3.32 | 64.80 ± 3.18 | 75.40 ± 3.58 | 83.43 ± 2.55 | 92.30 ± 2.12 | 309 | 0.9879 |
| F3 | 12.40 ± 1.95 | 21.60 ± 2.64 | 36.80 ± 3.08 | 48.50 ± 3.25 | 54.20 ± 3.42 | 56.23 ± 2.10 | 62.15 ± 2.88 | 510 | 0.9927 |
| F4 | 15.20 ± 2.28 | 26.40 ± 2.95 | 44.30 ± 3.15 | 59.80 ± 3.38 | 71.50 ± 3.62 | 84.22 ± 3.33 | 91.10 ± 2.45 | 338 | 0.9853 |
| F5 | 17.50 ± 2.42 | 29.80 ± 3.12 | 50.15 ± 3.28 | 66.20 ± 3.08 | 76.80 ± 3.48 | 87.30 ± 2.84 | 94.80 ± 1.95 | 320 | 0.9841 |
| F6 | 16.10 | 27.90 | 47.40 | 63.15 | 74.20 | 82.60 | 90.50 | 315 | 0.9868 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ±2.15 | ±2.88 | ±3.22 | ±3.12 | ±3.35 | ±2.98 | ±2.38 | | |
| F7 | 13.80±2.08 | 23.20±2.78 | 39.50±3.38 | 52.40±3.45 | 60.80±3.58 | 68.40±2.95 | 76.20±3.12 | 425 | 0.9910 |
| F8 | 11.50±1.88 | 19.80±2.52 | 32.60±3.12 | 44.20±3.28 | 52.15±3.48 | 67.54±3.44 | 78.76±2.33 | 355 | 0.9902 |
| F9 | 17.20±2.32 | 29.40±3.02 | 49.60±3.25 | 65.80±3.22 | 75.90±3.52 | 89.20±2.78 | 96.40±2.08 | 318 | 0.9844 |

**Source:** Experimental data of the present study.

Table 5 shows the drug release kinetics for the different formulations, which directly addresses the objectives of controlled delivery. The highest drug release rate was attained for Formulation F1, which released 91.45% of the drug in 10 hours with a $t_{50}$ of 312 minutes. The formulations with lower polymer content (F1, F5 and F9) had more rapid release, which is attributed to the shorter diffusion pathways in the thinner matrices. In contrast, higher polymer and enhancer formulations (F3, F7 and F8) exhibited a slow rate of drug release with F3 drug release being only 56.23% after 10 hours ($t_{50}$ = 510 minutes). This difference is due to a higher polymer density which causes more tortuous diffusion pathways and a higher electrostatic and hydrogen-bonding interaction, which slows drug release. The R2 values (0.9832-0.9927) are very high, suggesting a good fit to the zero-order kinetic model and thus matrix-diffusion mechanisms. Sustained controlled delivery was demonstrated with all formulations, which was better than the immediate release conventional delivery systems.

**Table 6: Physicochemical Compatibility Assessment—FT-IR and DSC Results**

| **Sample** | **FT-IR Key Absorptions ($cm^{-1}$)** | **DSC Observations** | **Compatibility Status** |
|---|---|---|---|
| Pioglitazone maleate (pure drug) | 3400 (O-H), 3000 (aromatic C-H), 1680 (C=O amide), 1600 (aromatic C=C) | Melting endotherm: 218–220°C (sharp) | Baseline |
| Sodium CMC | 3500–3300 (O-H), 2950 (C-H), 1650–1550 (C=O carboxylate) | Glass transition ~60°C (broad), decomposition >280°C | Baseline |
| Chitosan | 3500–3200 (O-H, N-H), 2950 (C-H), 1680–1620 | Glass transition ~120–140°C, decomposition >200°C | Baseline |

| | | | |
|---|---|---|---|
| | (amide I, II) | | |
| β-Cyclodextrin | 3400 (O-H), 2950 (C-H), 1100–1000 (C-O) | Melting: 250–260°C (broad, hydration-dependent) | Baseline |
| F1 Microspheres (Sod. CMC formulation) | 3400, 3000, 1680, 1600 (all peaks preserved) | Drug melting: 215–222°C (slightly broadened); polymer Tg preserved | **Compatible** |
| F2 Microspheres (Sod. CMC formulation) | 3400, 3000, 1680, 1600 (all peaks preserved, sharp) | Drug melting: 217–221°C; no new thermal events | **Compatible** |
| F4 Microspheres (CMC + enhancer blend) | 3400, 3000, 1680, 1600, plus 1620 (amide II from chitosan) | Drug melting: 214–223°C; slight polymer Tg shift to 65°C | **Compatible** |
| F8 Microspheres (high enhancer content) | 3400, 3000, 1680, 1600 (all characteristic absorptions present) | Drug melting: 210–224°C (broadened); multiple polymer transitions | **Compatible with minor interactions** |

**Source:** Experimental data of the present study; comparison to pure-material baselines.

Table 6 shows the results of drug-excipient compatibility, which is a key factor for the formulation stability and pharmaceutical acceptability. The unshifted and sharp drug's amide carbonyl absorption (1680cm−1) in all microsphere formulations confirmed the absence of undesirable interactions by FT-IR spectroscopy. The DSC analysis of the pioglitazone maleate showed that the melting endotherm for the drug was similar for all the formulations (215–223°C) and for the pure drug (218–220°C) and there was a slight broadening of the endotherm which could be due to the crystal size reduction and not degradation of the drug. Glass Transition Temperature of the polymer was, for the most part, not affected; F8 plasticized slightly. No new thermal events or exothermic peaks were observed in any of the formulations, indicating good compatibility and allowing long term stability predictions.

**Table 7: Statistical Analysis Summary—Two-Way ANOVA Results**

| **Variable** | **Effect on Particle Size** | **Effect on Entrapment Efficiency** | **Effect on Mucoadhesion (1 hr)** | **Effect on $t_{50}$** |
|---|---|---|---|---|
| **Polymer Concentration** | $F(1,7) = 48.32$, $p < 0.001$ *** | $F(1,7) = 22.15$, $p < 0.01$** | $F(1,7) = 35.28$, $p < 0.001$*** | $F(1,7) = 18.64$, $p < 0.01$** |
| **Enhancer Concentration** | $F(1,7) = 31.18$, $p < 0.001$ *** | $F(1,7) = 14.82$, $p < 0.05$* | $F(1,7) = 28.42$, $p < 0.001$*** | $F(1,7) = 12.35$, $p <$ |

| | | | | |
|---|---|---|---|---|
| | | | | 0.05 * |
| **Stirring Speed** | F(1,7) = 26.44, p < 0.001 *** | F(1,7) = 5.18, p = 0.06 (ns) | F(1,7) = 8.72, p < 0.05* | F(1,7) = 3.45, p = 0.11 (ns) |
| **Freeze-Drying Temperature** | F(1,7) = 2.18, p = 0.19 (ns) | F(1,7) = 6.82, p < 0.05* | F(1,7) = 4.32, p = 0.08 (ns) | F(1,7) = 2.88, p = 0.14 (ns) |
| **Polymer × Enhancer** | F(1,7) = 14.25, p < 0.05* | F(1,7) = 11.38, p < 0.05* | F(1,7) = 19.64, p < 0.01** | F(1,7) = 9.52, p < 0.05 * |

**Source:** Two-way ANOVA analysis of experimental data; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant.

A statistical evaluation of the rigorous data shown in Table 7 determines which formulation variables are significant in the microsphere performance. Polymer concentration became the most significant factor for all responses (all $p < 0.001$), and it was responsible for the matrix structure that determines the particle formation, drug encapsulation and release kinetics. Enhancer concentration also showed a wide significance ($p < 0.001$ for particle size, mucoadhesion; $p < 0.05$ for entrapment and release) indicating high formulation modulation. The stirring speed had a significant effect ($p< 0.001$) on particle size (through emulsion fragmentation by shear forces) and a moderate effect ($p< 0.05$) on mucoadhesion. The factors of freezing temperature was observed to have no significant effect on the variables except for entrapment efficiency at the 0.05 significance level. Polymer-enhancer interactions were statistically significant for all the responses and thus proved the superiority of the factorial design over sequential approaches ($p < 0.01$ to $< 0.05$).

## 6. Discussion

### 6.1 Formulation Optimization and Microsphere Development

Overall, the present study has successfully developed the mucoadhesive microspheres by rational optimization of formulation and process variables using statistical experimental design. The $2^4$ factorial framework was found to be very useful in systematically testing four important variables simultaneously to find the best combination, without having to perform all of these variables in sequence. Results of particle size analysis showed that the polymer concentration and stirring speed were the main factors determining the particle size. The higher the polymer concentration, the higher was the viscosity of the aqueous phase, which resulted in lower droplet fragmentation caused by the shear forces in the flow and larger microspheres (31–47 µm, depending on the formulation). It is consistent with earlier literature which showed that there is an inverse relationship between polymer viscosity and emulsion stability and direct relationship between polymer viscosity and the final particle size (Gohel and Amin 1998). In contrast, high stirring speeds (1000 rpm vs 500 rpm) produced small particles, as a result of high shear energy. The relationship was found to be quite reproducible; that is, when different formulations were prepared at the same stirring speed, but with different polymer concentrations (such as F1 and F3, or F5 and F7), the size increases were predictable, and not random.

The obtained size range (29-44 µm) is in the therapeutically optimum size range for oral delivery of microspheres. It is well established in the literature that particles > ~100 µm have a fast sedimentation rate and are poorly colloidally stable in the aqueous gastrointestinal environment, whilst particles < ~1 µm are easily taken up by the intestine and able to distribute systemically. These present microspheres are in the range of 29-44 µm that would provide good residence time at absorption sites with minimal systemic bioavailability risk (Martin et al. 1996). The polydispersity indices (0.16–0.31) confirmed good uniformity of the size distribution, with the lowest values being achieved for the formulations with the lowest polymer concentrations. Broad distributions are encountered in the high-concentration formulations (F8 PDI 0.31), and they are thought to come about in the process of emulsification through coalescence that is promoted by the viscosity of the formulation. Intermediate polymer concentrations (F9 central point: 2%) might be a practical optimum in terms of particle size, uniformity of particle distribution and efficient processing.

The surface morphology evaluation using SEM revealed that one of the most important results was the production of smooth and discrete spherical

particles at the sodium CMC level and moderate chitosan/β-cyclodextrin concentration. All these features are directly related to increase in mucoadhesion by maximizing contact surface with mucin networks and removing surface roughness which may cause mechanical detachment under shear. Formulations containing higher concentration of enhancer (F8) on the other hand showed moderately rough surfaces, which may be attributed to an irregular deposition of polymer during the solvent evaporation and differential crystallization during freeze-drying. Although there is a theoretical possibility that rough surfaces may increase the mechanical interlocking with mucin, the data showed no direct correlation between increased surface roughness and increased mucoadhesion retention (F8: 14.50% retained at 10 hours; F1: 42.15%); instead, the smooth surface geometry appeared to maintain contact with the viscoelastic mucin gel layer.

### 6.2 Drug Entrapment and Formulation Efficiency

The entrapment efficiency of drugs varied between 54% and 82% with the highest efficiency obtained with the formulations containing sodium CMC (F2 and F6: ~80%). This is significantly higher than some conventional chitosan microsphere systems via simple ionic gelation methods (Chowdary and Rao 2003) indicating the effectiveness of the modified emulsion–lyophilization approach to encapsulate pioglitazone maleate. The excellent entrapment has been attributed to the fact that the drug diffusion into the continuous paraffin media is restricted due to the rapid evaporation of the solvents (4 hours at 80°C). It is interesting that increasing the concentration of chitosan/β-cyclodextrin enhancer resulted in decreased efficiency of entrapment (F3: 57%, F7: 63%, F8: 54% vs F1: 69%, F2: 80%). This counterintuitive result indicates that in theory, enhancing the solubility of drugs by using more β-cyclodextrin inclusion complexation, can also lead to increasing the hydrophilicity of the polymer, which can increase water absorption during emulsification. This super-hydration helps the drug to partition into the aqueous phase, away from being confined within the polymer matrix. This mechanism has been documented in the literature; Patel et al. (2005) noted similar results for chitosan-alginate microsphere system in which the entrapment of some drugs decreased as the chitosan concentration increased. Based on the present data, it appears that very stringent optimization of the concentration of the enhancer is necessary; concentrations above ~0.5–1.0% appear to result in a decrease in the physical retention, which outweighs the solubility-enhancing advantages.

Effective washing and drying of microspheres are demonstrated by high recovery percentages (92-97%). This efficiency level reflects adequate physical stability of the microspheres prepared by this method, able to withstand stress during the filtration and solvent exchange processes without aggregates or disintegration.

### 6.3 Mucoadhesive Performance and Mechanism

A key innovation that enables the therapeutic benefit of this delivery system is “mucoadhesion.” After 10 hours of shearing in the simulated intestinal transit, formulations F1, F5 and F9 were able to retain 42-45% of the applied microspheres on tissue as compared to the historical 5-15% retention of non-mucoadhesive microsphere formulations (Lehr et al. 1990). This ~3–4-fold increase is directly correlated to significantly longer exposure of the drugs in the absorption area. The hierarchy of mucoadhesion (F1 > F5 > F2 > F6 > F9 > F4 > F3 > F7 > F8) shows a remarkable inverse correlation with the concentration of enhancer. In the formulations containing sodium CMC only (F1, F2) adhesion was 89-94% after 1 hour but for high-enhancer formulations (F8) adhesion was 68%. The difference in this differential is due to a difference in the way polymers interact with mucin. The carboxyl and hydroxyl groups of sodium CMC are capable of forming many hydrogen-bonding networks with the glycoprotein backbone of mucin. These interactions are maximised when polymer chains are sufficiently concentrated to form interpenetrating networks with mucin molecules. Too much chitosan/β-cyclodextrin will dilute the effective CMC concentration and could compete by binding to the CMC negatively charged backbone instead of mucin's negatively charged backbone.

This time-dependent decrease in mucoadhesion (94% at 1 hours down to 42% at 10 hours in F1) is physiologic and reminiscent of gastrointestinal transit. The shearing forces as simulated by the USP disintegration apparatus simulate the forces of muscular contraction and flow which gradually dislodge and move the adhered particles. But with 42% adhesion after 10 h, which is within the average gastric residence time, optimized formulations are able to retain significant adhesion to the absorptive epithelium during the critical absorption window for pioglitazone maleate.

### 6.4 Controlled Drug Release and Kinetics

The drug release kinetics showed sustained and extended release profiles as intended through formulation design. All formulations showed zero order kinetic (high $R^2$ value 0.983-0.993) which means that the rate of drug release is independent of

the amount of drug remaining in the formulation and is the most desirable type of release in order to achieve constant plasma concentration with minimal fluctuation (Patel et al. 2005). Differential release rates between formulations were indicative of the polymer concentration effects. At 10 hours, F1 (with low concentration of polymer) reached 91.45% drug release with a $t_{50}$ of 312 min. F3 (low concentration + high enhancer), on the other hand, showed 56.23% release at 10 hours ($t_{50}$ = 510 minutes). This is the opposite of what you might expect, as increasing polymer typically reduces release, but in this instance is the result of the particular nature of the enhancer chemistry. An increase in polymer concentration improves the diffusion path length and thus slows the release, however, an increase in the concentration of chitosan/β-cyclodextrin also improves the hydrogen-bonding ability and electrostatic interactions with pioglitazone maleate, which hinder diffusion through physical and chemical interactions. The data show that sustained release is significantly higher when the concentration of the enhancer prevails.

It can be compared with literature values to verify better sustained-release properties. Conventional oral pioglitazone has immediate release formulation (tablets) which results in peak plasma concentrations 30-60 minutes after dosing and short plasma half-life, thus necessitating twice-daily dose (Goodman and Gilman's 2006). The current microsphere systems would allow for therapeutic concentrations to be maintained over significantly longer periods of time (50-70% drug release after 6 hours and 80-90% release after 10 hours), which may also allow for a reduction in dosing frequency.

### 6.5 Physicochemical Compatibility and Stability Implications

FT-IR and DSC analyses demonstrated good compatibility of pioglitazone maleate with all excipients. Preservation of characteristic absorptions of functional groups and absence of new peaks in the spectrum and thermal events indicate that no covalent cross-linking, complex formation, or chemical degradation has taken place. The melting points of the drugs were found to be practically identical (215-223°C for drug from the polymer; 218-220°C for pure drug), and the effect of polymer on the melting points was slightly broader in the samples and was the result of reduction of crystal size and of the interactions with polymers and not due to chemical decomposition of the drugs. The compatibility results are important for prediction of the long-term stability. Apparent thermal compatibility (DSC, FT-IR) has been shown to be generally correlated with the shelf stability (under ambient storage conditions) in literature (Patel et al. 2005). No new thermal transition or peak shift was observed, indicating the formulations should be stable for a long time under a normal storage time of pharmaceuticals (20-25°C, relative humidity<60%). Additional studies of full stability (under accelerated conditions of 40/75) and real-time storage would be logical next steps but are outside of the scope of this investigation.

## 7. Conclusion

The investigation was successful in developing and characterizing the mucoadhesive microspheres for controlled delivery of pioglitazone maleate via rational optimization of formulation and extensive analytical assessment. The systematic $2^4$ factorial experimental design revealed that the factors of polymer concentration, enhancer concentration, and stirring speed are the key parameters affecting the microsphere characteristics. Optimal formulations (F1, F2, F9) resulted in a discrete spherical particle and high entrapment efficiency (69 – 80%), strong mucoadhesion (91 – 94% retention at 1 hour) and sustained zero order drug release (80 – 91% liberation over 10 hours). The effect of variables and their interactions were quantified in the statistical analysis, providing mechanistic relationships between composition and performance of the formulation. The prepared microspheres overcome therapeutic challenges posed by traditional pioglitazone delivery. These systems keep the drug in the gastrointestinal tract for a longer period, due to mucoadhesive interactions, and release it in a controlled manner via polymer matrix diffusion, which helps to reduce the dosing frequency and maintain steady blood levels of the drug. The detailed characterization that includes particle size, morphology, entrapment efficiency, mucoadhesion kinetic, drug release kinetic, physical and chemical compatibility and statistical rigor provide scientific basis for clinical translation.

Further studies should take these results one step further to an in vivo evaluation. Pharmacokinetic studies in animal models would determine the bioavailability benefit of mucoadhesive microspheres when compared with immediate release formulations that would show the in vitro results as a demonstrable therapeutic value. Through permeation studies in the different intestinal epithelial model systems, it could be understood whether or not the mucoadhesion results in improved absorption efficiency. Shelf life and storage conditions would be agreed in stability studies under ICH recommended conditions. This advancement would ultimately enable human clinical evaluation and regulatory approval, offering significant therapeutic benefit for those who need help managing diabetes.

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