Evaluation of Cefixime-Loaded Chitosan Microspheres: Analysis of Dissolution Data Using DDSolver

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ABSTRACT

The objectives of this study were to fabricate cefixime-loaded chitosan microspheres with the ultimate goal of prolonging drug release and to analyze the influence of various process variables on the properties of microspheres such as mode of drug release. The cefixime-loaded chitosan microspheres were fabricated using a coacervation technique. Various process variables like volume of solvent for chitosan (glacial acetic acid), chitosan concentration, volume of phase separation agent (NaOH solution), and glutaraldehyde concentration were varied to fabricate nine different formulations. Dissolution data were evaluated using DDSolver, new software developed for the kinetic analysis of dissolution data. The microspheres were spherical, porous, and dark brown. They ranged in size from 253.13 ± 8.4 to 369.1 ± 13.7 µm, and the incorporation efficiency varied from 29.9 ± 32% to 49.3 ± 4.5%. The F5 formulation with a drug/polymer ratio of 1:3 (w/w) was the most suitable in terms of incorporation efficiency (49.3 ± 4.5%), flow characteristics (Hausner ratio = 1.4), and drug release properties. The drug release was sustained up to many hours. Fickian diffusion was the primary mode of drug release from all cefixime-loaded chitosan microsphere formulations. These results show that cefixime can be successfully microencapsulated into chitosan shells by coacervation, which is influenced significantly by formulation variables such as chitosan and glutaraldehyde concentration.

INTRODUCTION

Cefixime, a third-generation cephalosporin, is indicated for the management of uncomplicated urinary tract infection, otitis media, pharyngitis, tonsillitis, acute bronchitis, acute exacerbations of chronic bronchitis, and uncomplicated gonorrhea caused by susceptible strains of specific microorganisms. It inhibits the synthesis of mucopeptide in bacterial cell walls (1). The bioavailability, protein binding, and half-life of cefixime are approximately 50%, 60%, and 3 h, respectively. These properties require the administration of large doses (400 mg in two divided doses for up to 5–7 days) of cefixime to achieve and then maintain therapeutic levels (2).

The fabrication of a cefixime-loaded, sustained-release formulation would be useful as compared to the current dose regimens. Consequently, the aim of this study was to develop a slow-release formulation of cefixime. For this purpose, microencapsulation and chitosan were selected as the technique and polymer (release-retardant material), respectively.

Microencapsulation, which is the application of a thin polymeric coating to individual core substances (particle size range 5–5000 µm), is extensively employed in various pharmaceutical applications, like prolonging drug release to improve bioavailability (3). The coacervation technique, one of the most commonly adopted and uncomplicated methodologies of microencapsulation, has been employed to develop microspheres of a range of compounds using various polymers (4).

Currently, various studies (5–9) have been proposed using chitosan as a rate-controlling and sustaining polymer as it is substantially beneficial in fabricating biodegradable microspheres. Chitosan, a natural biopolyaminosaccharine (10), is a weak base (11) and is soluble in dilute aqueous acidic solutions like 2% acetic acid solution (12). The presence of a free amino group is helpful for bonding with drugs substances and controlling their release (13). A convenient way to synthesize chitosan microspheres is the coacervation method with the use of glutaraldehyde as a cross-linking agent (14).

A literature survey showed no studies involving the formulation and characterization of microspheres using cefixime. Thus, the primary objective of this study was to fabricate cefixime-loaded chitosan microspheres (CLCM) and secondarily to investigate various combinations of excipients such as acetic acid and NaOH solutions, drug–polymer relationships, and glutaraldehyde concentrations. These formulation variables may influence the characteristics of microspheres. Moreover, considerable attention was focused on the dissolution analysis of CLCM fabricated in these studies because in vitro dissolution analysis of solid oral formulations is an essential tool of dosage form development and quality control testing of drug release.

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properties. Dissolution data were evaluated using DDSolver, new software developed for the kinetic analysis of dissolution data. DDSolver is a menu-driven, add-on program for Microsoft Excel written in Visual Basic for Applications.

**EXPERIMENTAL**

**Materials**
Cefixime trihydrate (USP) was gifted by Mega Pharmaceuticals, Sheikhupura, Pakistan. Chitosan (deacetylation degree 85%) and glutaraldehyde were purchased from Sigma, USA. Glacial acetic acid and sodium hydroxide (NaOH) were procured from Merck, Germany.

**Fabrication of Microspheres**
Cefixime-loaded microspheres were fabricated using a coacervation technique. A chitosan solution was prepared by dissolving a weighed quantity of chitosan in 2% acetic acid with continuous magnetic stirring at 500 rpm. To this solution, a weighed quantity of cefixime was added and mixed. Coacervation was induced by changing the pH of the system through the addition of a 2 M NaOH solution. Consequently, the swollen gummy material was stirred manually for 5 min and then by magnetic stirrer at 600 rpm for 2 h. Subsequently, microspheres were separated by filtration and dried in an oven at 40 °C for 24 h. The variables studied are listed in Table 1.

**Size and Morphological Characterization**
The external morphology of dried CLCM was studied using a scanning electron microscope (Hitachi S 3000H, Japan). After the dried CLCM were fixed to the plate surface with double-sided adhesive tape, a thin coating of gold was applied and surface features were observed (15). The microsphere size was evaluated using an optical microscope (Nikon, Japan) interfaced to a computer. Briefly, the glass slide of CLCM suspended in distilled water was observed under the microscope, and micrographs were obtained for the analysis of microsphere size (16).

**Flow Characteristics of Microspheres**
The following relationship was used to evaluate the Hausner ratio (flow property) of the microspheres fabricated in this study (3):

\[
\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}}
\]

**Preparation of Calibration Curve**
Calibration curves \((n = 3)\) for the selected drug were prepared in 0.05 M potassium phosphate buffer pH 7.2 \((17)\) \((6.8 \text{ g of monobasic potassium phosphate in 1000 mL of water, adjusted to pH 7.2 with 1 N NaOH, 900 mL})\). The concentration range was 2–30 µg/mL, and the regression coefficient \((R^2 = 0.9965, \text{good linearity})\) of the plots was evaluated. No interference of polymers with the subsequent analyses was observed.

**Drug Incorporation Efficiency**
To determine drug incorporation efficiency, weighed microspheres were soaked in a small volume of 0.05 M potassium phosphate buffer pH 7.2 for 24 h to extract the drug, and then filtered through a 0.45-µm filter. The filtrate was analyzed using a UV–vis spectrophotometer (Shimadzu 1601, Japan) at 230 nm \((18)\) in triplicate. The drug concentration was determined using a calibration curve, and drug incorporation efficiency was calculated using the following equation \((12, 19)\):

\[
\text{Drug incorporation efficiency} = \left(\frac{\text{Actual drug content}}{\text{Theoretical drug content}}\right) \times 100
\]

The actual drug content was determined using:

\[
\text{Theoretical drug content} = \left(\frac{\text{Weight of drug}}{\text{Weight of microparticles}}\right) \times 100
\]

**Swelling and Erosion Studies**
The weighed CLCM \((W)\) were gently shaken in 0.05 M potassium phosphate buffer pH 7.2 at room temperature \((32 \, ^{\circ}C)\) for 6 h to study erosion using a sonicator. Subsequently, the microspheres were separated by

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Cefixime conc. (g)</th>
<th>Acetic Acid vol. (mL)</th>
<th>Chitosan (g)</th>
<th>Vol. NaOH (mL)</th>
<th>Glutaraldehyde conc. (mL)</th>
<th>Size (µm)</th>
<th>Incorporation efficiency (%)</th>
<th>Swelling (%)</th>
<th>Erosion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>50</td>
<td>3</td>
<td>290.13 ± 9.5</td>
<td>42.4 ± 3.1</td>
<td>192 ± 8.9</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>F2</td>
<td>20</td>
<td>150</td>
<td>20</td>
<td>50</td>
<td>3</td>
<td>287.54 ± 11.17</td>
<td>39.1 ± 3.8</td>
<td>176.9 ± 9.2</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>F3</td>
<td>20</td>
<td>200</td>
<td>20</td>
<td>50</td>
<td>3</td>
<td>283.13 ± 8.4</td>
<td>34.5 ± 4.2</td>
<td>151.3 ± 1.2</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>F4</td>
<td>20</td>
<td>100</td>
<td>40</td>
<td>50</td>
<td>3</td>
<td>317.35 ± 10.5</td>
<td>43.1 ± 5.6</td>
<td>201.6 ± 6.7</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>F5</td>
<td>20</td>
<td>100</td>
<td>60</td>
<td>50</td>
<td>3</td>
<td>369.1 ± 13.7</td>
<td>49.3 ± 4.5</td>
<td>218.5 ± 9.3</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>F6</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>100</td>
<td>3</td>
<td>305.23 ± 9.6</td>
<td>33.1 ± 2.9</td>
<td>190.2 ± 7.8</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>F7</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>150</td>
<td>3</td>
<td>321.82 ± 7.8</td>
<td>29.9 ± 3.2</td>
<td>198.6 ± 6.7</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>F8</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>50</td>
<td>6</td>
<td>280.3 ± 8.9</td>
<td>44.9 ± 3.9</td>
<td>176.4 ± 7.9</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>F9</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>50</td>
<td>9</td>
<td>267.9 ± 12.4</td>
<td>46.2 ± 4.3</td>
<td>153.9 ± 8.3</td>
<td>3.0 ± 0.7</td>
</tr>
</tbody>
</table>
centrifugation at 1000g for 10 min, dried in an oven at 40 °C for 24 h, and weighed (Wf) to determine erosion (%)

\[
\text{Erosion} \% = \frac{(W_i - W_f)}{W_i} \times 100
\]

To study swelling (%), the weighed microspheres (Wf) were gently shaken in 0.05 M potassium phosphate buffer pH 7.2 at room temperature (32 °C) for 24 h; subsequently the swollen microspheres were weighed (WF) to determine swelling or swelling ratio (RW) using (11):

\[
R_W = \frac{(W_f - W_i)}{W_f} \times 100
\]

The experimentation was repeated in triplicate.

**In Vitro Drug Release Study**

In vitro drug release from CLCM was performed using USP Apparatus 1 in 900 mL of 0.05 M potassium phosphate buffer pH 7.2 stirred at 37 °C and 100 rpm maintaining sink conditions (17). The accurately weighed CLCM were enclosed in a sieve, placed in the basket, and processed for dissolution testing. All the CLCM stayed in the basket during 24-h dissolution testing (i.e., no particles diffused out of the sieve). Dissolution samples (5 mL) were withdrawn at regular intervals (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, and 24 h) using an autosampler with replacement of equal volumes of fresh medium. The samples were filtered through a 0.45-µm filter and analyzed spectrophotometrically at 230 nm (18) in triplicate. Drug concentration was calculated using a calibration curve.

**Kinetic Analysis**

The dissolution data were analyzed using various model-dependent approaches like zero order, Higuchi, and Korsmeyer–Peppas and a model-independent approach like the similarity factor (f2) to determine the kinetics of drug release. Similarity factor can be defined as the logarithmic reciprocal square root transformation of one plus the mean squared (the average sum of squares) differences of drug percent dissolved between the test and the reference products (4). The times for 25% and 50% release of drug (T25% and T50%, respectively) were also calculated from the regression equation of the zero-order model. The zero-order model illustrates the systems where the rate of drug release is independent of the initial concentration of drug (20):

\[
M_t = k_0t
\]

where \( k_0 \) is the zero-order rate constant and \( M_t \) is the amount of drug released at time \( t \).

The release of drug from an insoluble matrix is a function of the square root of time as shown in the Higuchi equation (21):

\[
M_t = K_t \sqrt{t}
\]

where \( K_t \) is the Higuchi rate constant, which represents the design variables of the system.

The following graphs were constructed: cumulative percent drug released (CPDR) versus time (zero-order kinetic model); ln (natural log) of CPDR versus time (first-order kinetic model); CPDR versus square root of time (Higuchi model), and ln of CPDR versus ln of time (Korsmeyer–Peppas model) (22).

To determine the mode of drug release, the initial 60% drug release values were fitted to the Korsmeyer–Peppas model:

\[
\frac{M_t}{M_\infty} = Ke^n
\]

where \( M_t/M_\infty \) is the fraction of drug released at time \( t \), \( K \) is the drug release rate constant, and \( n \) is the release exponent. The \( n \) value is employed for the characterization of different release modes for cylindrical-shaped solid formulations. The drug release exponent \( n \) and drug release mode are related as: \( n = 0.45 \), Fickian diffusion; \( 0.45 < n < 0.89 \), Anomalous (non-Fickian) diffusion; \( n = 0.89 \), Case-II transport; and \( n > 0.89 \), Super case-II transport (22). Dissolution data were evaluated using DDSolver.

**Statistical Analysis**

Experimental results are expressed as mean ± SD. The statistical significance of all the results was elaborated using one way ANOVA. The probability was considered significant at a \( p \)-value of less than 0.05.

**RESULTS AND DISCUSSIONS**

The CLCM were fabricated successfully using the coacervation technique. During incorporation of NaOH solution into the drug–polymer solution, it was observed that magnetic stirring did not disperse the matrix into distinct droplets and thus did not adequately produce fine microspheres; rather, aggregated granules were achieved. Very high-speed magnetic stirring also could not disperse the matrix system uniformly. However, fine, spherical microspheres were obtained when manual stirring was done initially, and after 5 min, the system was stirred using a magnetic stirrer at 600 rpm. Therefore, this strategy of stirring was used during the fabrication of all microspheres.

In all cases, formulations exhibited good flow characteristics (Hausner ratio = 1.4). This indicates that CLCM can be easily handled during processing (23). The size and incorporation efficiency ranges were 253.13 ± 8.4 to 369.1 ± 13.7 µm and 29.9 ± 32 to 49.3 ± 4.5%, respectively.

**Effect of Acetic Acid Volume**

The size, incorporation efficiency, swelling, and erosion of cefixime-loaded chitosan microspheres decreased significantly \( (p < 0.05) \) with an increase in the acetic acid volume used to dissolve chitosan. The reason for the decrease in the value of all dependant variables of the microspheres may be the decrease in solution viscosity, which increases the ease of solution stirring, thus fewer drug molecules contact polymer. Ultimately, microsphere size and incorporation efficiency are reduced (24). This logic may count for the decrease in microsphere swelling and erosion. The microspheres obtained (F1, F2, and F3) were spherical with a porous surface (Figure 1) and were dark brown.
Effect of Chitosan Concentration

Microsphere size, incorporation efficiency, and swelling increased significantly (p < 0.05) with increasing chitosan concentration, which may contribute to the increase in solution viscosity and thus low leakage of drug molecules from the network of polymer chains. The increase in solution viscosity decreased stirring efficiency and thus increased microsphere size and incorporation efficiency (12). However, microsphere erosion decreased with the increase in chitosan concentration, which could be due to the increase in viscosity resulting in a strong polymeric network and thus a decrease in erosion (%). It was noted that increased chitosan concentration produced nonspherical microspheres (F4 and F5) with rougher porous surfaces. The microspheres were dark brown (Figure 2).

Effect of Sodium Hydroxide Volume

Microsphere size increased with an increase in NaOH volume. This could be due to the increased bulk volume, which decreases solution stirring efficiency, thereby increasing microsphere size due to greater accumulation of chitosan molecules (12). In contrast, incorporation efficiency decreased significantly (p < 0.05) due to the increase in NaOH volume. Thus a decrease in incorporation efficiency could be due to the increase in fluid surrounding the microspheres and the subsequent leakage of drug substance, ultimately decreasing the amount of drug (19). No significant effect (p > 0.05) of NaOH volume on the swelling and erosion of microspheres was observed. Increased NaOH volume used in microencapsulation produced no change in microsphere (F6 and F7) morphology and color, and the particles were similar to those of F1 (Figure 1).

Effect of Glutaraldehyde Concentration

Increased glutaraldehyde concentration produced microspheres (F8 and F9) with rougher porous surface and nonspherical shape. The microspheres were dark brown (Figure 3). The increase in glutaraldehyde (cross-linking agent) concentration caused stiffening of the polymeric matrix and thus a decrease in accommodation of solvent molecules in the polymer network such that microsphere size, swelling, and erosion decreased with an increase in concentration of cross-linking agent (11). In addition, water uptake by hydrogels is influenced by the extent of hydrodynamic free volume and the provision of hydrophilic functional groups for the water to make hydrogen bonds. Because of the presence of hydroxyl and amino groups, chitosan is highly hydrated in an aqueous environment. When glutaraldehyde is exposed to chitosan, the amino groups of chitosan develop imine bonds with the aldehyde groups of glutaraldehyde (25). Chitosan microspheres formulated with a higher glutaraldehyde concentration develop a greater number of covalent bonds, thus the polymeric matrix becomes stiffer, which is...
responsible for low microsphere size, swelling, and erosion. However, incorporation efficiency increases significantly ($p < 0.05$) with an increase in glutaraldehyde concentration, which may be due to stronger retention of a large number of drug molecules with a minimum possibility of leakage and ultimately an increase in incorporation efficiency (19).

**In Vitro Release Study**

The in vitro drug release testing (Figures 4–7) exhibited a biphasic mode of drug release from the microspheres. There was an initial rapid release of drug, known as burst effect, due to fast dissolution of drug molecules attached to the surface of the microspheres, and a subsequent slow release phase, during which drug molecules present in the core of the microspheres diffused out (Figures 4–7). The initial burst effect decreased with an increase in polymer concentration as is evident from $T_{50\%}$ and $T_{25\%}$ values. These results show that $T_{50\%}$ was more than three times $T_{25\%}$. However, $T_{50\%}$ for formulations F1, F2, F3, F6, and F7 was about seven times longer than $T_{25\%}$, while $T_{50\%}$ was just 3–4 times $T_{25\%}$ in the case of F4, F5, F8, and F9 (Figure 5). These results show that an increase in polymer and cross-linking agent concentrations reduced the burst effect significantly ($p < 0.05$), which is in accordance with previously reported results (7, 8).

Theoretically, the rate of drug release from microcapsules decreases with an increase in polymer concentration due to the prolongation of the diffusion route of drug (13). The results of the present study are in accordance with this theory as is evident from Table 2 (i.e., $T_{50\%}$ for F1, F4, and F5 are 7.840, 8.726, and 10.889 h, respectively), indicating a decrease in drug release with an increase in chitosan concentration (Table 2, Figure 5). These results are further supported by the drug release rate constants for formulations F4 and F5. These two formulations have low values of release rate constants as compared with F1. It was also noted that the cumulative amount of drug released after 24 h was less than 100% for all formulations (Figures 4–7); this could be due to the relatively slow erosion of the polymeric substance under dissolution test conditions, with a consequential slow release of entrapped drug from the matrices (11).

However, significant ($p < 0.05$) decrease in the initial burst release occurred with an increase in glutaraldehyde concentration as evident from the values of $T_{50\%}$ (Table 2, Figure 7). The values of $T_{50\%}$ for F1, F8, and F9 are 7.840, 8.444, and 9.565 h, respectively, indicating a decrease in drug release with an increase in chitosan concentration. These results are further verified by the drug release rate constants for formulations F8 and F9. These two
formulations have low values of release rate constants as compared with F1. The value of \( T_{50%} \) was higher for those formulations that used higher concentrations of glutaraldehyde. The reason could be same as mentioned previously (i.e., stiffer polymeric matrix), and thus the increase in matrix density retards the permeation of dissolution medium into the microspheres with subsequent slow diffusion of drug medium from the microspheres (14).

Drug release profiles of formulations F2–F9 were compared with that of the reference product (F1) to evaluate the effect of process variables using the similarity factor \( f_2 \). The formulation showing an \( f_2 \) value near 100 exhibited no effect of the parameter varied for that formulation and vice versa. The results in Table 2 show that the \( f_2 \) values for formulations F2, F3, F6, and F7 are closer to 100. However, formulations F4, F5, F8, and F9 exhibited the lowest \( f_2 \) values among all the formulations; this indicates a significant effect from polymer and cross-linking agent concentrations.

There was a nonsignificant \( (p > 0.05) \) effect of acetic acid and NaOH concentrations on the release behavior of the microspheres (Table 2, Figures 4 and 7).

**Kinetic Analysis**

The dissolution data were plotted according to different models, and these curves were used to draw some conclusions regarding the mode of drug release from the microspheres. The kinetic analysis proved that the Higuchi model (linear nature of curve) best fit the dissolution data. This was confirmed by high values of regression coefficients obtained in all cases, which illustrates that the rate of drug release was directly proportional to the square root of time (26). All other models produced curvilinear plots with low values of regression coefficients. It also indicates diffusion-controlled drug release. The Korsmeyer–Peppas equation was used to calculate the value of release point \( n \). The values of \( n \) for formulations F1–F3 and F6–F9 suggest a Fickian diffusion mode of cefixime release from chitosan microspheres. The term Fickian diffusion indicates a gradient-dependent release of drug from formulations. Formulations F4 and F5 exhibited anomalous diffusion of drug, which means a dual mode of drug release (i.e., diffusion plus erosion). The anomalous mode of drug release from F5 and F6 could be due to the use of a higher polymer concentration with a constant concentration of cross-linking agent (glutaraldehyde).

**CONCLUSION**

This study presents successful microencapsulation of cefixime into chitosan coats. Formulation variables like chitosan concentration and the quantity of glutaraldehyde (cross-linking agent) influence microsphere properties such as size, drug incorporation efficiency, swelling, erosion, and drug release behavior. The release behavior was Fickian diffusion and Higuchian.

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**REFERENCES**


