This chapter deals with the development of gellan gum beads for the controlled release (CR) of a semi-synthetic cephalosporin antibiotic, cephalexin. Results of this study are discussed in terms of effect of various formulation parameters such as pH of the counterion solution and amount of cephalexin loading on entrapment efficiency, bead size, drug release and swelling and de-swelling kinetics. Diffusional exponent values and kinetic parameters have been evaluated, which suggested that anomalous diffusion is taking place in these systems. A numerical analysis based on the Fickian diffusion model has been attempted to compute the concentration profiles and drug diffusion by assuming the spherical geometry of the formulated products.
Abstract

Gellan gum (GG) beads containing cephalexin were prepared by extruding the dispersion of cephalexin and gellan gum into a solution containing the mixture of calcium and zinc ions (counterions). Beads were prepared by changing the experimental variables such as pH of the counterion solution and amount of cephalexin loading in order to optimize the process variables on the final % drug entrapment efficiency, release rates, size and morphology of the beads. Absence of chemical interactions between drug, anionic polymer and counterions after production of the beads was confirmed by Fourier transform infrared (FTIR) spectroscopy. Differential scanning calorimetry (DSC) was used to understand the crystalline nature of the drug after its successful entrapment. These data indicated the amorphous dispersion of cephalexin in the polymer matrix. Beads were spherical in shape, as indicated by scanning electron microscopic (SEM) studies, while laser light scattering technique indicated the average bead size ranging from 925 to 1183 \( \mu \text{m} \). Cephalexin entrapment of up to 69.24 % was achieved. *In-vitro* release studies were performed in 0.1 N HCl or pH 7.4 phosphate buffer solution and cephalexin was released up to 6 h. Dynamic swelling studies were performed in 0.1 N HCl or pH 7.4 phosphate buffer. Diffusion coefficients were calculated assuming the spherical geometry of the formulated products. The release data have been fitted to an empirical relationship to estimate the transport parameters. Mathematical modeling studies were performed for the spherical geometry by solving Fick’s equation to compute the concentration profiles. These results were correlated with the release profiles of the drug through the polymeric matrices.

Over the past decades, hydrogel polymers have attracted a great deal of attention for potential carriers in controlled and site-specific delivery of drugs [1-4]. Hydrogels are hydrophilic, three-dimensional network structures having the natural propensity to absorb large quantity of water or biological fluids and they resemble those of the biological tissues. The ability of hydrogels to swell in the presence of water or biological fluids regulates the release of the encapsulated drugs. By controlling the degree of swelling due to crosslinking makes them potential carriers of drugs for the controlled release (CR) applications [5-7].

Recently, Aminabhavi and coworkers have been actively involved in the development of hydrogel-based CR formulations for the delivery of a variety of bioactive agents [8-11]. In view of their greater advantages than synthetic polymers, biocompatible polymers such as chitosan, sodium alginate, guar gum, etc., have been widely used in the literature of pharmaceutics. However, to control the release patterns, many attempts have been made to use synthetically modified natural polymers such as polyacrylamide-g-guar gum, etc [11]. In continuation of these efforts, hydrogels have been thought to be as potential CR systems. Therefore, in this chapter, experimental data on gellan gum (GG) polymer has been used for the CR of cephalexin, which is a broad spectrum bactericidal antibiotic. The drug is almost completely absorbed from the gastrointestinal tract (GTT) and its plasma half-life is about 1 h, but > 80 % of the dose is excreted unchanged in the urine within the first 6 h.

In the earlier report [12], a granule form of the sustained release cephalexin (L-Keflex) was evaluated for safety and efficacy by comparing it with Keflex (capsule of regular cephalexin). It was judged that L-Keflex (granule) had a better patient convenience than Keflex (capsule) in that it can be administered with twice daily regimen. However, there are no other reports are available on the formulations of cephalexin as the CR particulate drug...
delivery systems. The specific advantages of such systems have been discussed earlier [13]. However, of CR formulations offers many potential advantages such as sustained blood levels, attenuation of adverse effects and improved patient compliance, because it is realized that in case of antibiotics having short half-life, it is necessary to maintain constant blood levels, as otherwise microorganisms will become resistant to the antibiotic. Therefore, formulating cephalexin in CR dosage forms will increase the therapeutic efficacy and patient compliance.

GG used in this study is a linear anionic polysaccharide obtained from exocellular secretion of the microorganism, *Pseudomonas elodea* [14]. The natural form of GG is composed of the linear structure of a repeating tetrasaccharide unit of glucose, glucuronic acid and rhamnose in a molar ratio of 2:1:1 [15]. It is partially acetylated with acetyl and L-glyceryl groups located on glucose residues [16]. However, the presence of acetyl groups interferes in ion bonding ability. On the other hand, commercially available GG is a deacetylated product obtained by treatment with alkali [14]. Because of the presence of free carboxylate groups in GG, it is anionic in nature and thus, would possess the characteristic property of undergoing ionic gelation in the presence of mono and divalent cations. However, the affinity for divalent cations is much stronger than the monovalent cations [17].

The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water [18]. GG has a wide variety of applications mainly in food and pharmaceutical industries. In view of the characteristic property of cation-induced gelation, it has been widely used in the formulation of *in-situ* gelling ophthalmic preparations [19,20] as well as *in-situ* gelling oral sustained release formulations [21-23]. Quigley and Deasy [24] developed the sustained release beads containing GG for the CR of sulfamethizole prepared by hot extrusion method into the chilled ethyl acetate. The GG beads containing salbutamol
sulfate have also been studied for sustained release applications [25]. Recently, Kedzierewicz et al., [26] have developed the GG beads for the CR of propranolol hydrochloride and found that the drug release was rapid. However, the ionotropic gelation with GG offers new opportunities in the field of bioencapsulation.

Chan and Heng [27] recently reported that a combination of calcium and zinc ions could produce more sustained release of drugs from the alginate microspheres compared to calcium ions alone. In the earlier literature, no CR formulations of GG beads containing cephalexin have been studied. This prompted us to develop GG beads containing cephalexin by a rather simple procedure using the cation-induced gelation of GG. In this work, we have used the combination of calcium and zinc ions to induce cationic gelation of GG. Various formulation and process variables affecting the preparation of beads and in-vitro release characteristics of cephalexin were investigated.

Drug-loaded beads have been characterized by DSC and FTIR to understand crystallinity of the drug and drug-polymer interactions, respectively. Morphology of the formulated beads was investigated by SEM. The swelling and deswelling kinetics of GG beads were studied to understand their suitability in oral dosage formulations. Drug release results were analyzed using an empirical equation proposed by Ritger and Peppas [28]. Furthermore, diffusion coefficients were computed from Fick's equation. Diffusion data were discussed in terms of concentration profiles calculated using a numerical scheme [29] to understand the transport of drug/liquid through the spherical beads.

IV.2. Results and Discussion

IV.2.1. Preparation and Characterization of Beads

GG is an anionic deacetylated exocellular polysaccharide. Aqueous solution of GG is known to form hydrogels upon warming to body temperature (37°C) as well as in the presence of cations [30].
formation involves the formation of double helical junction zones followed by the aggregation of double helical segments to form a three-dimensional network by complexation with cations and hydrogen-bond formation with water [18]. The strategy for ensuring hydrogelation in the present work is similar to that used earlier for the preparation of beads as well as microspheres from the ionic polymers such as alginate, chitosan, etc. [22]. The GG contains carboxylate side groups in glucuronosyl residues and hence, it can be crosslinked by inducing the ionic gelation with cations.

Chan and Heng [27] reported that calcium and zinc cations exert varying effects on the morphology and drug release profiles of the alginate microspheres and they suggested that a combination of calcium and zinc cations could be employed to produce microspheres with more sustained release characteristics. Kedzierewicz et al [26] studied the CR of a highly water-soluble propranolol hydrochloride from GG beads prepared by using calcium as a counterion. However, drug release was too rapid for practical applications. These observations prompted us to use the combination of calcium and zinc as counterions for hydrogelation of GG and thereby, for easy encapsulation of cephalexin.

The % entrapment efficiency (see Table IV.1) of the GG beads for cephalexin varied between 47 and 69, depending upon the pH of the counterion media used for encapsulation. The % entrapment efficiency was the lowest for beads prepared in pH 5 media, whereas the highest % entrapment efficiency was observed for beads produced in pH 9 media. This difference in % entrapment efficiency may be attributed to the degree of ionization of carboxyl groups of GG in different pH media [30; 31]. For instance, if lesser number of carboxylate groups present on GG at pH 5, then calcium and zinc ions are less likely to crosslink and form a dense matrix that can entrap cephalexin successfully.
Table IV.1
Results of % Entrapment Efficiency, Bead Size, Parameters $k$ and $n$, Correlation Coefficient ($r$) Calculated from Eq. (IV.14) and Diffusion Coefficients ($D$) Calculated from Eq. (IV.7) for Sorption and Desorption Processes in pH 7.4 Phosphate Buffer

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>% Entrapment efficiency</th>
<th>Volume mean particle size ($\mu$m)</th>
<th>$k$</th>
<th>$n$</th>
<th>$r$</th>
<th>$D_{(sorption)} \times 10^5$ (cm$^2$/s)</th>
<th>$D_{(desorption)} \times 10^8$ (cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>47.58</td>
<td>925</td>
<td>0.105</td>
<td>0.69</td>
<td>0.964</td>
<td>5.80</td>
<td>7.64</td>
</tr>
<tr>
<td>F2</td>
<td>54.50</td>
<td>958</td>
<td>0.109</td>
<td>0.66</td>
<td>0.958</td>
<td>5.82</td>
<td>7.71</td>
</tr>
<tr>
<td>F3</td>
<td>55.92</td>
<td>943</td>
<td>0.115</td>
<td>0.65</td>
<td>0.974</td>
<td>5.93</td>
<td>7.62</td>
</tr>
<tr>
<td>F4</td>
<td>59.22</td>
<td>934</td>
<td>0.118</td>
<td>0.62</td>
<td>0.982</td>
<td>6.01</td>
<td>7.70</td>
</tr>
<tr>
<td>F5</td>
<td>50.31</td>
<td>1029</td>
<td>0.086</td>
<td>0.72</td>
<td>0.966</td>
<td>2.18</td>
<td>7.15</td>
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<tr>
<td>F6</td>
<td>58.50</td>
<td>1060</td>
<td>0.090</td>
<td>0.71</td>
<td>0.988</td>
<td>2.38</td>
<td>7.24</td>
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<tr>
<td>F7</td>
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<td>1048</td>
<td>0.093</td>
<td>0.70</td>
<td>0.972</td>
<td>2.49</td>
<td>7.18</td>
</tr>
<tr>
<td>F8</td>
<td>64.21</td>
<td>1039</td>
<td>0.099</td>
<td>0.70</td>
<td>0.985</td>
<td>2.72</td>
<td>7.23</td>
</tr>
<tr>
<td>F9</td>
<td>55.38</td>
<td>1183</td>
<td>0.075</td>
<td>0.76</td>
<td>0.969</td>
<td>0.629</td>
<td>6.83</td>
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<tr>
<td>F10</td>
<td>61.65</td>
<td>1122</td>
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<td>0.74</td>
<td>0.971</td>
<td>0.631</td>
<td>6.77</td>
</tr>
<tr>
<td>F11</td>
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<td>1136</td>
<td>0.081</td>
<td>0.73</td>
<td>0.986</td>
<td>0.653</td>
<td>6.72</td>
</tr>
<tr>
<td>F12</td>
<td>69.24</td>
<td>1151</td>
<td>0.086</td>
<td>0.72</td>
<td>0.978</td>
<td>0.688</td>
<td>6.91</td>
</tr>
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<td>44.28</td>
<td>914</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>C2</td>
<td>49.13</td>
<td>1050</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>C3</td>
<td>53.36</td>
<td>1171</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

*Experiments not performed

Correlation coefficient ($r$) calculated at 95% confidence limit

The GG beads were spherical as revealed by SEM images shown in Figure IV.1. The pH of the counterion media appears to have a significant effect on the morphology and size of the beads. For instance, beads prepared in pH 9 have smooth surfaces, while those prepared in pH 5 have a porous structure. This could be due to a more rapid approach of calcium and zinc ions to the surface of the GG droplet that has a higher anionic character at higher pH and thus, it forms a dense matrix. Size and size distribution of the beads were assessed by the laser light diffraction technique (Mastersizer-2000, Malvern, UK). On a population basis, particle size distribution was found to be unimodal with narrow size distributions that are displayed for formulations F1, F5 and F9 in Figure IV.2.
Calculated values of volume-mean particle size of the beads are included in Table IV.1. These data showed that with increasing pH of the counterion media, bead size also increased. The higher bead size at higher pH may be the result of rapid migration of calcium and zinc ions to the surface of the GG droplet that has the higher anionic character at higher pH. This has resulted in the immediate crosslinking of the droplets, which might have prevented the erosion of GG from the droplets.

It would be instructive to suggest a correlation between % entrapment efficiency with the size of the beads (see Table IV.1). For instance, by increasing the pH of the counterion media from 5 to 9 (formulations F1, F5 and F9), the % entrapment efficiency also increased considerably from 47.6 to 55.4, while the bead size increased systematically from 925 to 1183 μm. On the other hand, for formulations F2, F6 and F10, the % entrapment efficiency increased systematically from 54.5 to 61.7, while the bead size increased from 958 to 1122 μm. For formulations F3, F7 and F11, the % entrapment efficiency increased from 55.9 to 65.2, while the bead size showed a systematic variation from 943 to 1136 μm. Similarly, for formulations F4, F8 and F12, both % entrapment efficiency and bead size increased systematically from 59.2 to 69.2 and 934 to 1151, respectively.
Figure IV.1. SEM images of beads produced in (a) pH 9 media and (b) pH 5 media.
Figure IV.2. Particle size distribution of beads prepared in three different pH media.
The size and % entrapment efficiency of the beads prepared in counterion media with pH 5, 7 or 9 are further statistically evaluated by analysis of variance (ANOVA). The $F$ value for bead size was found to be 111.7 (df = 35, $P < 0.01$), indicating a highly significant difference in the sizes of the beads prepared in three different pH media. The ANOVA was further extended by the least significant difference (LSD) procedure. The results indicate that beads prepared in pH 5 media are significantly different in size from those prepared in pH 7 media and also, beads prepared in pH 7 media are significantly different in size from those prepared in pH 9 media. Similarly, $F$ value for % entrapment efficiency was found to be 7.298 (df = 35, $P < 0.05$), which indicates that there is a significant difference in the % entrapment efficiency of beads prepared in three different pH media. Here also, the ANOVA was extended by the least significant difference (LSD) procedure.

The results indicate that % entrapment efficiency of the beads prepared in pH 5 media is significantly different than those prepared in pH 9 media. Similarly, the % entrapment efficiency of the beads prepared in pH 7 media is significantly different from those prepared in pH 9 media; however, there is no significant difference in the % entrapment efficiency between the beads prepared in pH 5 and pH 7 media. A similar trend is followed for the control formulations C1, C2 and C3, in which % entrapment efficiencies and bead sizes increased systematically from 44.3 to 53.4 and 914 to 1171 μm, respectively with increasing pH of the counterion media from 5 to 9.

To investigate the possible chemical interactions of the drug with the hydrogel matrix, we have analyzed (a) pure cephalexin, (b) placebo beads and (c) cephalexin-loaded beads using FTIR. These spectra are displayed in Figure IV.3. In case of placebo beads, a broad peak appeared at 3419 cm$^{-1}$ due to the presence of hydroxyl groups of glucopyranose ring that are hydrogen-bonded to various degrees. The bands appearing at 1622 and 1412 cm$^{-1}$ are due to asymmetric and symmetric stretching vibrations of the carboxylate group. The band at 2925 cm$^{-1}$ is due to the stretching vibration of $-\text{CH}_2$ group, while those
appearing at 1159 and 1029 cm\(^{-1}\) are due to ethereal and hydroxylic C–O stretchings. Bending vibration of C–H appeared at 887 cm\(^{-1}\).

Cephalexin has shown a characteristic band at 3438 cm\(^{-1}\) due to the amide N–H stretching vibrations. The bands at 3271 and 3209 cm\(^{-1}\) are due to intermolecular hydrogen-bonded amine groups, while a band at 3042 cm\(^{-1}\) is due to the acidic hydroxyl groups. Characteristic bands appearing at 1758 and 1690 cm\(^{-1}\) are due to four-membered lactam carbonyl and secondary amide carbonyl groups, respectively. The bands at 1591 and 2919 cm\(^{-1}\) are due to the N–H bending vibrations and C–H stretching vibrations, respectively. The bands appearing at 1455, 1406 and 1350 cm\(^{-1}\) are due to C–H bending vibrations. The C–N stretching vibrations are observed at 1283 cm\(^{-1}\), whereas C–O stretching vibrations are observed at 1073 cm\(^{-1}\). Characteristic peaks due to monosubstituted phenyl groups appeared at 745 and 696 cm\(^{-1}\), whereas the peak due to C–S stretching vibrations has merged at 745 cm\(^{-1}\).

Spectra of the drug-loaded GG beads are not characteristically different from the spectra of pristine GG beads. Peaks appearing at 1752, 1412, 1283, 1079 and 702 cm\(^{-1}\) for cephalexin have also appeared in the drug-loaded beads, indicating the chemical stability of cephalexin after entrapment. However, bands appearing at 1690 cm\(^{-1}\) in cephalexin and 1622 cm\(^{-1}\) in GG have appeared in the drug-loaded beads at 1635 cm\(^{-1}\), indicating weak interactions of the quaternary ammonium salt type formed between the primary amine group of the drug with that of the carboxylate group of GG. This kind of interaction disappears to release the primary amine group of the drug effectively in basic pH conditions of the intestine.
Figure IV.3. FTIR spectra of (a) pure cephalexin, (b) cephalexin-loaded beads, and (c) placebo beads.
DSC thermograms for pure cephalexin (a), placebo beads (b) and cephalexin-loaded beads (c) are presented in Figure IV.4. Crystallinity of cephalexin and melting temperature ($T_m$) of the polymer were determined. Placebo beads have shown an endothermic peak at 252°C, indicating the melting temperature of the polymer, whereas cephalexin-loaded beads showed an endothermic peak at 248°C. This slight decrease in melting temperature may be due to minor physical and morphological changes taking place in the beads after drug loading. For pure cephalexin, an endothermic peak appeared at 194°C due to the melting of the drug, but this peak has not appeared in all the cephalexin-loaded beads, indicating an amorphous dispersion of the drug into the polymer matrix.

**IV.2.2. Dynamic Swelling Studies**

Swelling studies of the formulations are important to assess the hydrogelation of the beads formed. Dynamic swelling experiments were carried out gravimetrically in 0.1 N HCl or pH 7.4 phosphate buffer. Since no significant difference ($P < 0.01$) was observed in the sorption of beads in both the media studied and hence, only % weight gain raw data obtained in pH 7.4 phosphate buffer are displayed in Figure IV.5 for some representative formulations viz., F1, F5 and F9. It is observed that higher weight gain of the beads in pH 5 media was more prominent just before the attainment of equilibrium swelling or more appropriately, sorption. However, the beads prepared in pH 9 media showed a more rigid structure and hence, lesser swelling than those prepared in pH 5 or 7 media. The formulated beads (F9) prepared in pH 9 media attained equilibrium sorption quite fast (ca. 100 min), but experiments were continued until longer time (ca. 300 min) to ensure complete equilibration. Beads produced in pH 5 media have shown the maximum swelling up to 175% of the dry mass of the polymer.
Figure IV.4. DSC thermograms of (a) pure cephalaxin, (b) placebo beads and (c) cephalaxin-loaded beads.
IV.2.3. Drying Rate of the Beads

In order to optimize the drying conditions and to evaluate the effect of processing variables on the drying of beads, few beads representative of the batch were selected. The raw data of weight loss (mg) as a function of time are displayed in Figure IV.6 for the formulations that were chosen for swelling studies i.e., F1, F5 and F9. These results indicate that beads produced in pH 5 media dried quicker than those produced in pH 9 media. This could be attributed to the porous nature of the beads produced in pH 5, thereby allowing higher evaporation loss of the liquids from the beads. Also, it could be attributed to the rigidity of the hydrogel matrix formed at higher pH.

IV.2.4. Diffusion Coefficients for Liquid Transport Through Spherical Beads

In the present study, cephalaxin is dispersed in almost spherically shaped beads. It would be of interest to model the transport process to compute the diffusion coefficient, \( D \) and to study its effect in terms of concentration profiles generated within the beads. Diffusion occurs due to the immersion of the beads into the medium of interest. Mathematical models are available [29,32] to describe the sorption and desorption processes under the simulated test conditions. For a spherical geometry, change in concentration inside the spherical bead of radius, \( r \) can be described by Fick’s equation [32].

\[
F = -D \left[ \frac{\partial C}{\partial r} \right]
\]  

(IV.1)

Here, \( F \) is flux (matter transported per unit area and unit time) and \( \frac{\partial C}{\partial r} \) represents the concentration gradient. Following assumptions were considered to predict the concentration profiles of liquids within the spherical beads.
Figure IV.5. Effect of pH of counterion media on % water uptake by the beads.

Figure IV.6. Effect of pH of counterion media on the drying rate of the beads.
1. Dosage form is spherical and drug is uniformly dispersed in it (as studied by DSC).

2. Radial transport is considered, wherein matter transport takes place simultaneously i.e., (i) liquid enters the polymer and (ii) drug leaves the dosage form. Both these transports are controlled by the transient diffusion and are related to each other.

3. Diffusion coefficients of both the transports depend upon the concentrations of the drug and the liquid.

4. Despite matrix swelling, a frame of reference is fixed with respect to the initial dosage form for all the calculations.

Fick's equation for the radial diffusion with a concentration-dependent diffusivity is given as:

\[
\frac{\partial C}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left[ D \cdot r^2 \frac{\partial C}{\partial r} \right] \quad \text{(IV.2)}
\]

Diffusion in spherical beads can be described by using Laplace transformation of Fick's equation to calculate mass uptake (or more appropriately release in the present context), by the beads using,

\[
\frac{M}{M_\infty} = 6 \sqrt{\frac{Dt}{\pi r}} \left( \frac{1}{\sqrt{\pi}} + 2 \sum_{n=1}^{\infty} \text{erf} \left( \frac{nr}{\sqrt{Dt}} \right) \right) - 3 \frac{Dt}{r^3} \quad \text{(IV.3)}
\]

where \( M \) is the amount of liquid released at time, \( t \) and \( M_\infty \) is the total amount of liquid in the spherical bead. Equation (IV.3) is quite complicated to solve, but Baker and Lonsdale [33] have derived the equation that is appropriate for the present case using the initial and boundary conditions set as follows:

Initial: \( t = 0 \quad r < R \quad C = C_{in} \) \quad (inner part of beads)

Boundary: \( t > 0 \quad r = R \quad C = C_{eq} \) \quad (surface of beads)
In the above equations, \( R \) is radius of the sphere and \( r \) is radius of the small concentric shell within the spherical bead. It may be noted that the analytical solution for the above problem does not exist, essentially because of the double transport (i.e., simultaneous transport of liquid and drug) as well as the much-complicated problem of concentration dependence of \( D \).

\[
\frac{M_t}{M_\infty} = 6 \left( \frac{Dt}{R^2} \right) - 3D t \quad (IV.4)
\]

Equation (IV.4) is thus valid for \( M_t/M_\infty < 0.4 \), but at longer times i.e., for sorption \( M_t/M_\infty > 0.6 \), the liquid release profile is given by:

\[
\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \exp \left( -\frac{\pi^2 D t}{R^2} \right) \quad (IV.5)
\]

At intermediate time intervals: \( 0.4 < M_t/M_\infty < 0.6 \), both Eqs. (IV.4) and (IV.5) do not give a good approximation within \( \pm 5 \% \) of the theoretical profile given by Eq. (IV.3). Thus, for a constant diffusivity i.e., at short-term sorption, when the amount of liquid or drug transported is low (i.e., \( M_t/M_\infty < 0.4 \)), \( M_t \) is proportional to square root of time, \( t^{1/2} \) and hence,

\[
\frac{M_t}{M_\infty} = \frac{6}{R} \left( \frac{Dt}{\pi} \right)^{1/2} \quad (IV.6)
\]

The above equation is valid for the initial drug release (i.e., \( M_t/M_\infty \leq 0.4 \)) and it gives the well-known \( t^{1/2} \) dependence of the release profile. Diffusion coefficient can then be calculated for water sorption or drug release by the beads using,

\[
D = \left( \frac{R \theta}{6M_\infty} \right)^2 \pi \quad (IV.7)
\]
Here, \( \theta \) is slope of the linear portion of the plot of \( M_t/M_\infty \) vs. \( t^{1/2} \) when there is no lag-time for diffusion, \( R \) is radius of the beads and \( M_\infty \) is the maximum sorption value. The value of \( \theta \) was computed from the least squares procedure at 95% confidence level. Only those values giving the correlation coefficient of \( r > 0.99 \) were selected. The plots of \( M_t/M_\infty \) vs. \( t^{1/2} \) for liquid sorption from the beads during sorption process are displayed in Figure IV.7. It is observed that initial portions of the sorption curves in all the formulations exhibited slight sigmoidal trends, suggesting a deviation in transport from Fickian behavior to anomalous case. However, equilibrium sorption occurred for all formulations at around \( t^{1/2} = 15 \) h\(^{1/2}\).

Drying results of the beads presented in Figure IV.6 were converted into desorption rate i.e., \( (1 - M_t/M_\infty) \) and these data are now displayed in Figure IV.8 for the same formulations as presented in Figure IV.7. We observed a slight lag time initially, but later, the desorption curves increased considerably up to 10 h and then leveled off at about 20 h for all formulations. Since the curves are overcrowded within a short interval values of \( \ln(1 - M_t/M_\infty) \) and hence, it was difficult to comment on these data for the individual formulations. Values of \( D \) were calculated by following the same procedure as before for sorption data i.e., from the initial slopes of the linear portion of \( \ln(1 - M_t/M_\infty) \) vs. \( t \) as shown in Figure IV.8.
Figure IV.7. Plot of \( \frac{M}{M_\infty} \) as a function of square root of time for liquid sorption from the beads during sorption process.

Figure IV.8. Plot of \( \ln(1 - \frac{M}{M_\infty}) \) as a function of time for liquid desorption from beads during desorption process.
Values of $D$ calculated for sorption and desorption processes are included in Table IV.1. It is observed that the values of $D$ for sorption are higher than those observed for desorption by nearly three orders of magnitude. This trend is in good agreement with the earlier literature [9]. This is due to slower drying of the beads compared to rapid liquid sorption of the aqueous media by the beads. Sorption of liquid occurs due to diffusion, whereas desorption is a diffusion-controlled evaporation of the liquid. However, the process of evaporation is controlled by diffusion in the sense that the rate of evaporation depends largely on the rate at which solvent is supplied to the evaporating surface as a result of diffusion of the liquid. During sorption, when the sample is in contact with the liquid, the concentration of liquid on the surface reaches equilibrium as soon as the sorption starts. However, in case of desorption, the rate of evaporation is proportional to the difference in liquid concentration on the surface to a point at the center of the bead [29].

Diffusion coefficients for sorption decreased systematically with increasing pH from 5 to 9 of the counterion media. The $D$ values for sorption of the beads prepared in pH 5 media varied from $5.80 \times 10^{-3}$ to $6.01 \times 10^{-5}$ cm$^2$/s, whereas for beads prepared in pH 9 media, these ranged between $6.29 \times 10^{-6}$ and $6.88 \times 10^{-6}$ cm$^2$/s. Such lower $D$ values confirmed the more rigid nature of the beads formed in pH 9 media. However, for beads prepared in pH 5 media, the network structure is somewhat loose, which would create a high hydrodynamic free volume in the matrix to accommodate more of solvent molecules, thus inducing the matrix swelling. Lower water uptake and diffusion values observed in beads prepared in pH 9 media further confirmed the formation of a rigid matrix. The increase in $D$ values with increasing drug loading may be due to a slight decrease in the rigidity of the polymer matrix in the presence of dispersion of the drug.
IV.2.5. Numerical Analysis of Transport Phenomenon - Calculation of Concentration Profiles

The complex problem of double transport in bead geometry discussed earlier was further investigated by using an explicit numerical method with the finite difference method as suggested originally by Vergnaud [29]. Here, the polymeric bead was divided into concentric spheres of varying radii, \( r+\Delta r, r, r-\Delta r \). The number of concentric spheres is related to the radial thickness, \( \Delta r \) of each spherical part. Each position in the radial direction within the sphere is defined by an integer, \( n \):

\[
r = n \Delta r
\]  

(IV.8)

The concentrations of drug and liquid are constant within each spherical part of thickness, \( \Delta r \) located between two adjacent spheres at the same time, \( t \). The matter balance can then be calculated for the short lapse of time, \( \Delta t \) within the spherical part of thickness, \( \Delta r \) and centered on the position, \( r \) defined by the integer, \( n \) using Fick’s first law as per the matrix space-time diagram shown in Figure IV.9.

New concentration \( (CN_n) \) within the bead at position, \( r \) (integer, \( n \)) after lapse of time, \( \Delta t \) is expressed in terms of concentration at previous times \( (C_n) \) in the same place and in the two adjacent planes.

\[
CN_n = C_n + \frac{\Delta t}{n^2 (\Delta r)^2} \left[ J_{n+1/2} - J_{n-1/2} \right]
\]  

(IV.9)

Here, \( J \) is a function of diffusivity and concentration, which may be written as:

\[
J_{n+1/2} = \left( n - 1/2 \right)^2 \cdot D_{n+1/2} \cdot (C_{n+1} - C_n)
\]  

(IV.10)
Figure IV.9. Scheme used for spherical geometry in numerical analysis.
Thus, diffusivity at the intermediate position \((n - \frac{1}{2})\) can be computed from the diffusivities at positions, \(n\) and \(n + 1\) by either way. In the program, we have assumed zero concentration \((C_0)\) at the center of the sphere and then calculated the matter balance for the sphere of radius, \(\Delta r/2\) located in the middle of the bead. The concentration of the drug and the liquid on the surface of the beads represents the concentration at equilibrium \((C_{eq})\) following the second assumption. Thus, the concentration, \(C_0\) at the center of the sphere is considered to be equal that in the bead (as a whole) at time zero i.e.,

\[
C_0 = C_{eq}\quad \text{liquid, drug}\quad \text{(IV.11)}
\]

The amount of drug remaining (or liquid entered) in the beads at time, \(t\) was then obtained by integrating the concentration of the drug at this time with respect to space:

\[
M_t = 4\pi \int_0^r r^2 \cdot C_{eq} \cdot dr
\quad \text{(IV.12)}
\]

This expression was rewritten using the finite difference method as:

\[
M_t = 4\pi (\Delta r)^2 \left[ \frac{C_2}{24} + \sum_{n=1}^{\infty} \frac{n^2}{2} C_n + \frac{9}{8} (n-1)^2 C_{eq} + \frac{3}{8} n^2 \cdot C_n \right]
\quad \text{(IV.13)}
\]

The preceding equations were used to compute the concentration at any place and at any time, either for liquid or drug, since their diffusivities are known. Then, one can obtain the plot of \(M_t\) (as calculated from Eq. (IV.13)) vs. \(t^{1/2}\) for 25 \%, 50 \%, 75 \% and 100 \% drug loading or liquid entering as shown in Figure IV.10. It was observed that the theoretical curves generated for the different drug loadings even though originate from zero, but they all exhibited some lag-time in the beginning. However, \(M_t\) values calculated followed the trend that at higher loading of the drug, higher release was occurred.

Concentration profiles of the migrating liquid developed inside the dosage form during the diffusion process are displayed in Figure IV.11. It is the attainment of equilibrium that will eventually exist between the concentration...
in the gel bead and the concentration in the bulk that encourages the remainder of the drug to remain in the bead. Thus, the steep gradients of concentration of liquid and drug were developed in the dosage form, especially next to the surface. This gave the concentration profile of the migrating liquid as minimum at the center of the sphere, but extended to large values near the surface. Each curve in Figure IV.11 refers to the concentration of solvent at different time intervals at every 30 min interval. Since it is possible that transport can also take place in one-dimension and the contribution of each spherical slice to the transport will not be the same, and therefore, the volume of each slice could be proportional to the radius squared. Under these conditions, the behavior of the external slice was more effective than that of the internal slice, which has a smaller radius. Therefore, the concentration at position \( n-1/2 \) would give the mean value of those in adjacent places i.e., \( n \) and \( n+1 \).

**IV.2.6. In-vitro Drug Release**

*In-vitro* drug release studies were performed in 0.1 N HCl or pH 7.4 phosphate buffer for 6 h. Since there was no significant difference (\( P < 0.01 \)) of the drug release in both the media, only the data obtained in pH 7.4 phosphate buffer are presented in Figure IV.12 for different loadings of cephalexin in the beads. The effect of drug loading on the release rates was investigated for formulations F9, F10, F11 and F12. The release rates were slower for formulations containing lower amount of the drug, while the release rate increased with increasing amount of drug in the beads.

The release rate can be correlated with the diffusion coefficient (see Table IV.1), which indicates that as the diffusion coefficient increases (for formulations F9, F10, F11 and F12), the release rate also has increased. Drug in the beads might act as the inert filler by occupying the free volume of the swollen hydrogel. This could have created a tortuous path for water molecules to permeate, but the degree of tortuosity depends upon the volume fraction of the filler [34]. However, for all formulations, the release rate was extended up to 6 h.
The results of % cumulative release vs. time for beads prepared in counterion media having three different pH values, loaded with 25 % of cephalexin, are presented in Figure IV.13. Drug release rates were higher for beads prepared in the counterion media having pH 5 compared to those prepared in pH 9 media. This could be attributed to the formation of a rigid matrix in higher pH media. At lower pH, possibly less number of carboxylates are available for matrix crosslinking than at higher pH. SEM images indicated that beads prepared in the counterion media having pH 5 have a porous structure, while those prepared in pH 9 media have the smooth surfaces. This could be another reason for the fast drug release rates, since the porous matrices allowed faster diffusion of the drug into the dissolution media than the rigid beads. Similarly, here also a correlation between diffusion coefficient and release rate can be established. It can be observed that for formulations F1, F5 and F9, the diffusion coefficient decreased from $5.8 \times 10^{-5}$ to $0.629 \times 10^{-5}$ and the release rates also showed a drastic decrease (see Figure IV.13). The results of control formulations (C1, C2 and C3) are also compared in Figure IV.13.

The initial 60 % drug release data were analyzed using the empirical equation proposed by Ritger and Peppas [28] by fitting to Eq. (IV.14).

$$\frac{M_t}{M_\infty} = k t^n$$  \hspace{1cm} (IV.14)

A least-squares method at 95 % confidence level was used to estimate the values of the parameter, $k$ and diffusional exponent, $n$. These data along with the values of the correlation coefficient, $r$ are included in Table IV.1. The values of $k$ increase with increasing % loading of cephalexin in the beads, but the values of $n$ decrease with increasing % loading of cephalexin. This indicates the smaller level interactions between the beads and the swelling media with increasing size of the beads. The values of $n$ are in the range of 0.62 to 0.76. These indicated that the drug release was deviated only slightly from the Fickian trend and followed the anomalous trend.
Figure IV.10. Plot of $M_t$ calculated from Eq. (IV.13) as a function of $t^{1/2}$ for 25 %, 50 %, 75 % and 100 % drug loadings.

Figure IV.11. Penetrant concentration profiles developed inside the drug-loaded beads.
Figure IV.12. Effect of % drug loading on *in-vitro* release profiles.

Figure IV.13. Effect of pH of counterion media on *in-vitro* release profiles.
IV.3. Conclusions

Gellan gum beads of the narrow size distribution with volume mean particle size ranging between 925 and 1183 µm were prepared by the cation-induced ionotropic gelation method. Beads prepared in acidic pH media showed a porous structure, while those prepared in basic media have the smooth surfaces. Different % entrapment efficiency of cephalexin was observed by varying the % drug loading as well as the pH of the counterion media. Release rates were higher when the % loading of cephalexin was higher. Similarly, the beads prepared in acidic pH media gave higher release rates. However, no significant difference in the release rates could be seen in 0.1 N HCl or pH 7.4 phosphate buffer media. The n values indicated the release mechanism to be slightly deviated from the Fickian trend.

The present work demonstrates that by using a combination of calcium and zinc as the counterions, one could produce the more sustained release formulations using gellan gum beads compared with use of calcium ions alone. Thus, it is necessary to use the combination of calcium and zinc cations to produce the uniform size gellan gum beads having sustained drug release properties. The simulation procedure successfully computed the concentration profiles of drug transport through the beads as well as it helped in explaining the double transport processes taking place while studying the drug release characteristics of the beads.
IV.4. Literature Cited
