In vitro release modulation from crosslinked pellets for site-specific drug delivery to the gastrointestinal tract

I. Comparison of pH-responsive drug release and associated kinetics

Viness Pillay, Reza Fassihi*

Temple University, School of Pharmacy, Department of Pharmaceutical Sciences, 3307 North Broad Street, Philadelphia, PA 19140, USA

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Abstract

Multiple unit dosage forms for oral delivery of bioactive agents offer many advantages over single unit products (e.g., site-specific delivery, predictable gastrointestinal transit time and less localized adverse effects). In view of such benefits, this paper investigates the crosslinking of sodium alginate, low methoxylated pectin and their novel binary mixture with calcium ions through ionotropic gelation to pelletize the model drug, diclofenac sodium, using “environmentally benign” solvents and processing techniques. Crosslinked pellets of the above polymers in 2% (w/v) aqueous calcium chloride solution were prepared and evaluated for their structural and release behavior. The average size of the different pellets was 1.3 mm and drug entrapment capacity was optimized by reducing the calcium chloride solution pH to 1.6. Three types of pellet formulations were subjected to dissolution studies using the USP 23 Apparatus 2 and 3 over a pH range simulating the human gastrointestinal tract. Negligible drug release occurred in pH 1–4. However, rate of drug release in pH 6.6 ranged from rapid to slow (i.e., 100% drug release in 4 to 10 h, respectively) but always in a controlled manner. Weight change/erosion studies and swelling measurements were used to provide experimental correlation of kinetic model analysis for each of the three pellet systems. From model fitting studies and statistical treatment, the modified Hopfenberg equation \[ \frac{M_t}{M_{\text{in}}} = 1 - \left[1 - k_i (t - t_{\text{lag}})\right]^{\frac{1}{n}} \] best described the release kinetics for calcium–pectinate pellets. The model assumes heterogeneous erosion with kinetic constant \( k_i = k_o / C_o r_o \), in which \( k_o \) is the erosion rate constant, \( C_o \) is the uniform initial concentration of drug in the matrix, \( r_o \) is the initial radius and \( t_{\text{lag}} \) is the lag time. The \( n \) values of 1, 2 and 3 apply to a slab, cylinder and sphere, respectively. In addition, the exponential models, namely the Power Law \( \frac{M_t}{M_{\text{in}}} = k_i t^n \) and its derivative containing the lag time \( \frac{M_t}{M_{\text{in}}} = k_i (t - t_{\text{lag}})^n \), employed in the statistical treatment of data provided \( n \) values of \( n = 0.8 \)–1 in the case of the calcium–alginate and calcium–alginate–pectinate release kinetics. It is concluded that the proper selection of rate-controlling polymers and their interactive potential for crosslinking is important, and will determine the overall size and shape of pellets, the duration and pattern of dissolution profiles, pH sensitivity, drug loading capacity and mechanism of drug release. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Crosslinked pellets; Calcium–alginate; Calcium–pectinate; Site-specific delivery; Multiple unit dosage form; Swelling; Erosion; Weight change dynamics; Release modulation; Diclofenac sodium

*Corresponding author. Tel.: +1-215-707-7670; fax: +1-215-707-3678; e-mail: afassihi@vm.temple.edu

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1. Introduction

Oral modified release systems are popular and can be formulated as single or multiple unit dosage forms. The relative merits of multiple unit dosage forms (e.g., in terms of bioavailability, more consistent blood levels, predictable gastrointestinal transit time, less localized gastrointestinal disturbances and greater product safety) over single unit products are well established. In modified release systems the design of the dosage form allows for a specific drug delivery pattern so that the release rate becomes the rate-limiting step. This should be viewed in the context of other existing parameters within the gastrointestinal tract. For example, the two major rate limiting factors to drug absorption are gastrointestinal environment (e.g., pH, site and efficiency of absorption, gut metabolism, gastrointestinal content) and transit rate of the dosage form. From a manufacturing point of view, irrespective of the types of the dosage form (single or multiple unit), currently the utilization of hydrophilic swellable ingredients in the design of modified release systems are common and offer significant flexibility in pharmaceutical technology [1–6]. In view of the many benefits offered by multiple unit dosage forms, it is speculated that such systems are particularly useful: (i) for delivering highly-irritant drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) [7,8]; (ii) for site-specific targeting within the gastrointestinal tract [9]; and (iii) for delivery of enzymes, peptides/proteins and vaccines [10,11].

In the present study, crosslinking through ionotropic gelation of sodium alginate and pectin with calcium ions was employed to encapsulate (pelletize) the model drug, diclofenac sodium, into swellable multiple unit calcium–alginate and calcium–pectinate matrices. In addition, a 1:1 combination of drug–sodium alginate and drug–pectin dispersions was made and crosslinked to form a novel binary crosslinked polymeric system i.e., calcium–alginate–pectinate matrices. Applying a similar argument of Kim and Fassihi [5], it may be postulated that the most crucial factors regulating drug release and the associated kinetics from such crosslinked pellets are degree of polymer relaxation, maintenance of gel layer boundary, gel erosion and polymer dissolution. Consequently, in order to logically identify these regulating mechanisms, part I of this paper will deal with dissolution studies of pellets; while part II will focus on evaluating and characterizing those physicochemical parameters which affect pellet formation and its swelling behavior.

In the past, conventional crosslinked calcium–alginate beads have been investigated for the development of a multiple unit controlled drug delivery system [12–17]. However, it has suffered much criticism due to its highly pH-dependent characteristics of swelling and drug release. The present study employs these so-called “flaws” to design an oral multiple unit drug delivery system for targeting either the proximal or distal small intestinal tract and controlling the release process of highly ulcerogenic drugs (i.e., NSAIDs) such as diclofenac sodium. The conventional calcium–alginate and newly formulated calcium–pectinate or calcium–alginate–pectinate systems will be evaluated for their release properties in simulated gastric and intestinal environments using both USP 23 Apparatus 2 and 3. Furthermore, various mathematical models will be employed to determine the drug release mechanism.

2. Materials and methods

A medium viscosity sodium alginate (200cP for 1% aqueous solution at 20°C) obtained from TIC Gums (MD, USA) was used. Based on its source (Laminaria digitata), the alginate has the following approximate mannuronic (M) and guluronic (G) configuration: M/G=1.22, % M=55, % G=45, % MM=39, % MG+GM=32 and % GG=29. Citrus Coloids (Hereford, UK) provided the citrus low methoxyl pectin, with degree of methylolation of approximately 35%. Calcium chloride (anhydrous) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Diclofenac sodium was obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and were used without further purification.


Separate solutions comprising 2.5% (w/v) sodium
alginate and 2.5% (w/v) pectin were individually prepared by initially dissolving the polymer in 150 ml deionized water. On complete solution, an accurately weighed quantity of diclofenac sodium powder was added in increments to each solution (drug:polymer=1:2) to afford homogeneous dispersions that were made up to 200 ml volumes with deionized water (see Table 1). These dispersions were sonicated for 30 min to remove any air bubbles that may have been formed during the stirring process (solid-state ultrasonic FS-9, Fisher Scientific).

Each dispersion was added dropwise via a flat-tip 19-gauge needle into gently agitated acidified solutions of 2% (w/v) calcium chloride (1000 ml, pH 1.6 unless otherwise stated) by employing an eight-channel peristaltic pump (HP 89092A) set at a flow-rate of 2 ml/min. The droplets from each dispersion instantaneously gelled into discrete diclofenac–calcium–alginate or diclofenac–calcium–pectinate matrices upon contact with the calcium chloride solution. The calcium–alginate and calcium–pectinate matrices were further allowed to stir in their calcium chloride solutions for an additional 30 min. Thereafter the matrices were allowed to cure in a dark room for a period of 24 h. On expiration of this period the calcium chloride solutions were decanted and the pellets were washed with 3×500ml volumes of deionized water. The pellets were thereafter vacuum dried at 21°C for 48 h (vacuum oven Model 5830-4, National Appliance). An additional batch of a 1:1 mixture of the sodium alginate and pectinate dispersion was also prepared. This secondary dispersion was crosslinked and treated in an identical manner as described above to form discrete pellets of calcium–alginate–pectinate. Due to the pH-dependent solubility of diclofenac, additional batches of pellets were prepared using calcium chloride solution of pH 6.2.

2.2. Determination of drug entrapment capacity

Two batches of each formulation were crosslinked in either plain calcium chloride solutions (pH 6.2) or acidified calcium chloride solutions (pH 1.6). Two hundred mg each of drug-loaded calcium–alginate, calcium–pectinate and calcium–alginate–pectinate pellets was placed in separate 1000-ml conical flasks containing 500 ml each of phosphate buffer, pH 6.2. The matrices were magnetically stirred to promote swelling and break up of the crosslinked structure. This afforded liberation and subsequent dissolution of diclofenac. These solutions were vacuum filtered through a 0.45-μm membrane filter. The filtrates were then made up to 900 ml volumes with phosphate buffer, pH 6.2. Aliquots of these solutions were subjected in triplicate to ultraviolet spectrosocopy (HP 8452A diode array spectrophotometer) at 276 nm. The entrapment capacity was determined by the following empirical relationship:

\[
\text{Drug entrapment capacity } (\%) = \frac{\text{AQ}}{\text{TQ}} \times 100
\]

where AQ is the actual quantity of drug present in the matrices and TQ is the 100% theoretical quantity of drug present in the matrices (i.e., actual initial loading dose).

2.3. Characterization of pellet erosion/weight changes

Studies were conducted in triplicate on each of the three formulations in phosphate buffer, pH 4 and 6.6. For each formulation, pellets equivalent to 50 mg diclofenac, were exposed to dissolution media at an agitation rate of 50 rpm using the USP 23 rotating paddle apparatus. At specific time intervals the entire quantity of pellets were removed from the dissolution medium and vacuum dried until no further weight changes were noted. These studies were conducted over a time period up to 24 h for each of the three formulations. The fractional weight change was transformed to a percentage using the following empirical relationship:

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
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<tbody>
<tr>
<td><strong>Quantities of ingredients employed in the crosslinking reactions and pellet formation</strong></td>
</tr>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
</tr>
<tr>
<td>Sodium alginate/pectin</td>
</tr>
<tr>
<td>Deionized water</td>
</tr>
<tr>
<td>Calcium chloride (2% w/v)</td>
</tr>
</tbody>
</table>

Note: For production of calcium–alginate–pectinate pellets 1:1 proportions of sodium alginate and pectin were used.
Dynamic weight change (%) = \[1 - (\text{initial weight} - \text{final weight}/\text{initial weight}) \] \cdot 100 (2)

where initial and final weights represent the pre-test and dried post-test weights of the pellets, respectively.

2.4. Determination of swelling properties of the pellets

The swelling behavior of the three formulations was each evaluated in pH 1.5, 4 and 6.6 using the USP 23 Apparatus 2. At specific time intervals a triplicate sample of each formulation was removed and measured with a manually operated Vernier caliper (25 \times 0.01 \text{ mm capacity, Germany}). Dried and swollen calcium–alginate pellets were of spherical geometry and therefore diametral and axial measurements were identical. In contrast, the calcium–pectinate and calcium–alginate–pectinate pellets had a disc-like geometry; thus both diametral and axial changes were measured.

2.5. Drug release studies

One set of dissolution studies were performed in a fully calibrated six station dissolution test apparatus (VK 7000, Vankel Industries, Edison, NJ, USA) (37±0.5°C, 50 rpm) using the USP 23 rotating paddle method in buffer media (pH 1.5, 4 and 6.6; 900 ml) for calcium–alginate, calcium–pectinate and calcium–alginate–pectinate pellets. A quantity of pellets equivalent to 100 mg diclofenac for each formulation was employed in all dissolution studies. All studies were conducted in triplicate using an automated sampling procedure.

To determine the effect of continuous pH changes with time (i.e., simulated gastrointestinal pH variation) the second set of dissolution studies were performed at 37±0.5°C using the USP 23 Apparatus 3 (Bio Dis II Release Rate Tester, Vankel Industries,) and buffers of different pH values (220 ml per vessel). Each formulation was subjected in duplicate to a continuous run for 1 h at pH 1.5, 1 h at pH 3.1 h at pH 5.4, 3 h at pH 6.0, 3 h at pH 6.8 and 3 h at pH 7.4. The standard oscillation rate of 10 dips per minute (dpm) was employed throughout the studies.

2.6. Dissolution data analysis and model fitting

In order to precisely determine the nature of release mechanism from different pellets, an in-depth application of kinetic modeling and analysis of release profiles becomes apparent. The kinetics of drug release (phosphate buffer, pH 6.6) were analyzed using WinNonlin, Version 1.0 (SCI Software), which is based on non-linear regression. In all least-squares analyses, the Gaussian–Newton (Leverberg–Hartley) approach was adopted.

The monoeXponential decline in pellet drug content was analyzed by first-order kinetics (Eq. (3)) [18]. The power law expression (Eq. (4)) together with its modified form to accommodate the lag time (Eq. (5)) and geometry-independent form (Eq. (6)) were considered for data analysis [19–21]. In addition, the Hopfenberg model, a geometry-dependent equation (Eq. (7)), was also employed [22]. As a result of the lag time in drug release, slight modification was also made to the Hopfenberg model (Eq. (8)). The theoretical background and application of the above models is presented in Section 3.

3. Results and discussion

3.1. Drug entrapment capacity

During the preformulation stages, each of the three formulations were made in two batches. One batch was formulated by crosslinking the respective dispersion in plain 2% (w/v) calcium chloride (inherent pH = 6.2). Another batch was formed by crosslinking the respective dispersion in acidified 2% (w/v) calcium chloride (adjusted pH = 1.6). It was found that as the pH of the calcium chloride solution was lowered, there was an increase in the drug entrapment capacity of the respective pellets (Table 2).

This trend in drug entrapment capacity may be attributed to the reduction in solubility of diclofenac in stronger acid medium, since diclofenac being a weak acid (pK_a = 4), inherently has a negligible solubility in acid [6]. The most marked effect between crosslinking solution pH and entrapment capacity was observed in the case of the calcium–alginate formulation. This may be related to higher degree of crosslinking and consequently higher
Table 2

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Diclofenac sodium entrapment capacity(\pm)S.D. (%)</th>
<th>Calcium chloride, pH 6.2</th>
<th>Calcium chloride, pH 1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium–alginate</td>
<td>49.94(\pm)0.03</td>
<td>87.74(\pm)0.89</td>
<td></td>
</tr>
<tr>
<td>Calcium–pectinate</td>
<td>98.80(\pm)0.05</td>
<td>102.56(\pm)0.24</td>
<td></td>
</tr>
<tr>
<td>Calcium–alginate–pectinate</td>
<td>84.94(\pm)0.11</td>
<td>97.56(\pm)0.31</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{pK}_a = 4\), drug solubility is therefore pH-dependent.

crosslink density. It appears that the formation of a firm matrix structure allows for more extensive embedment of drug substance.

3.2. Dynamics of weight changes for crosslinked alginate, pectin and their binary mixture in dissolution studies

As early as 1963, Huag et al. [23] recognized the pH-sensitive nature of pectin since it was rapidly degraded both in alkali and in neutral solutions. In addition to pH-dependent degradation, pectin and alginic acid were also shown to undergo enzymatic hydrolysis. The ability of these polymers to undergo such reactions may have an impact on their degradation/erosion rates in vivo, which in turn will influence the drug release rate. As a means of tailoring/metering this erosion rate, it is possible to employ such polymers of different molecular weights to enable a compromise between rates of drug release and erosion [24].

In the present study, we use the term “weight changes” since gravimetric determinations showed both negative and positive weight deviations to occur. In their dried state, the mean weight of a single calcium–alginate, calcium–pectinate and calcium–alginate–pectinate pellet corresponded to 3.62\(\pm\)0.15 mg, 6.32\(\pm\)0.56 mg and 4.49\(\pm\)0.87 mg, respectively (determined on 10 individual pellets for each formulation). No measurements were performed at pH 1.5 since minimal swelling and negligible drug release was observed. However, at pH 4 and 6.6, a sudden sharp decrease in weight of the calcium–alginate and calcium–alginate–pectinate formulations was observed during the first two hours (Fig. 1a,b). This may be attributed to the initial rapid swelling and loss of free calcium ions. At both pH values (4 and 6.6) calcium–pectinate shows a rapid

Fig. 1. Dynamic weight change measurements performed on calcium–alginate (○), calcium–pectinate (△) and calcium–alginate–pectinate (□) pellets after exposure to buffer media of (a) pH 4 and (b) pH 6.6 using the USP 23 Apparatus 2 \((n = 3)\). S.D.s were within \(\pm\)0.91 in all cases.
and extensive weight loss (Fig. 1a, b) which may also explain the rapid drug release behavior of calcium–pectinate pellets at these two pH values as shown in Fig. 3b, c. In addition, this can also be seen from attainment of the highest erosion rate constant for calcium–pectinate ($k_5 = 16.51\%$/h) derived from the modified Hopfenberg model as shown in Table 4 and discussed in Section 3.6. The prominent increase in weight of calcium–alginate–pectinate at pH 4 and 6.6 and calcium–alginate at pH 4 beyond two hours may be attributed to the significant uptake of phosphate species from the dissolution medium (Fig. 1a). Once all calcium ions have reacted within the matrix, a plateau weight is reached since further sequestration/interaction by phosphate species may not be possible. This interaction, however, was not macroscopic in this case (i.e., no precipitation) since sink and possibly dilution effect were maintained in 900 ml. This phenomenon is further elaborated in Section 3.5.

3.3. Swelling properties of the crosslinked alginate, pectin and binary mixture

Fig. 2a–f demonstrate the nature of swelling behavior associated with the pellets. At pH 1.5 the calcium–pectinate formulation has a higher capacity to expand both diametrically and axially compared to the calcium–alginate and calcium–alginate–pectinate formulations (Fig. 2a, b). The increase in size changes (due to swelling) for calcium–alginate, calcium–pectinate and calcium–alginate–pectinate systems were in the order of 24%, 55% and 45% of their original size, respectively. This was due to the insignificant relaxation of the three polymers in acidic environment. Both the drug and crosslinked polymers display low solubility and low swellability in the acidic environment and consequently contributed to negligible drug release ($\approx 3.3\%$ after 12 h in all three cases), as shown in Fig. 3a.

At pH 4, a dramatic change in the swelling capacity was observed for all three formulations. Diametrically, calcium–alginate swelled to $>2.6$-times its original size while at pH 1.5 it swelled to only 0.24-times its original size (Fig. 2a, c). Axial swelling of the calcium–alginate–pectinate formulation was approximately three-fold higher than diametral swelling (Fig. 2c, d), i.e., 4.9-times axially compared to 1.7-times diametrically. It was observed that the calcium–pectinate pellets swelled to a maximum of 97.77% of their original size in 3 h, while in that same period their weight reduction corresponded to 54.61% of the original weight. This illustrates that matrix structure by itself is highly susceptible to the pH environment. Furthermore, matrix weight loss may be attributed to both drug and the low degree of crosslinking (i.e., greater pellet erosion), since degree of pectin methoxylation was $\approx 35\%$, which would limit extensive crosslink formation. High methoxylated pectin ($\approx 75\%$) was also subjected to crosslinking reactions, but failed to produce any pellets. This may be attributed to the lack of free carboxy and hydroxy residues necessary for formation of “calcium entrapment cavities”. At pH 4 and 6.6 (Fig. 2c–f) the calcium–pectinate formulation demonstrated the lowest capacity to swell compared to calcium–alginate and calcium–alginate–pectinate. Yotsuyanagi et al. [25] reported that pH-dependent swelling of calcium–alginate beads occurred in a sigmoidal manner with an inflexion point representing the $pK_a$ value of the carboxyl residues. However, in our study we did not observe similar swelling behavior.

Based on the maximum mean diametral swelling value in the “terminal phase”, the maximum diametral change (at pH 6.6) attained for each of the three formulations occurred after 4, 7 and 8 h for calcium–pectinate, calcium–alginate–pectinate and calcium–alginate, respectively and corresponded to 0.80 (80%), 1.96 (196%) and 2.97 (297%) times of their original size (from actual calculated values). No distinct plateau or terminal phase could be observed in the case of calcium–pectinate pellets. The application of dynamic weight changes of the three systems appears to be a more distinct marker to explain the nature of drug release behavior. Within 6 h the overall weight for calcium–pectinate dropped to 0.99% of its original weight, while in the case of calcium–alginate and calcium–alginate–pectinate, the weight dropped to 95.55% and 56.48% of their initial weight in 24 and 12 h, respectively.

3.4. Evaluation of drug release from calcium–alginate, calcium–pectinate and calcium–alginate–pectinate pellet formulations at different pH conditions using the USP 23 Apparatus 2

Fig. 3a–c show the relative increase in drug
release rates with an increase in media pH. It is evident that the three pellet formulations (i.e., calcium–alginate, calcium–pectinate and calcium–alginate–pectinate) demonstrate pH-dependent release characteristics. At pH 1.5 and 4 insignificant drug release was observed. Based on the drug chemistry, this was expected since diclofenac is a weak acid. In addition to low drug solubility in the acid media, it was observed that no significant swelling of pellets occurred at these pH values. However, release behavior at pH 6.6, where diclofenac is freely soluble, is strongly influenced by the nature of
drug release from the calcium–alginate, calcium–pectinate and calcium–alginate–pectinate systems would be markedly hindered if optimum swelling were not attained. At pH 1.5, the three systems do not demonstrate significant swelling and consequently negligible drug release. At pH 4 (Fig. 3b) the extent of drug release was not dramatically changed compared to that in pH 1.5 (Fig. 3a). The calcium–alginate and calcium–alginate–pectinate systems released 3.77% and 4.24% of their drug content over a 12-h period, whereas the calcium–pectinate pellets demonstrated higher drug release rate (i.e., from ≈3.3% in pH 1.5 to 16% in pH 4). This nevertheless still represents poor release over a 12-h dissolution period. At pH 6.6 (Fig. 3c), each formulation released >97% of its drug content at varying rates. This may be attributed to both matrix structure and drug solubility at this pH and thus a greater degree of matrix relaxation/swelling in all three cases. These processes are multidimensional, as swelling involves diametral and axial aspects, while erosion may occur on the superficial outer surfaces as well as within the bulk structure of the pellet. The anticipated pH release profile derived from Fig. 3a–c for time

Fig. 3. Release of diclofenac sodium from calcium–alginate (○), calcium–pectinate (△) and calcium–alginate–pectinate (□) pellets in different pH media using the USP 23 Apparatus 2: (a) pH 1.5; (b) pH 4; and (c) pH 6.6 (n = 3). S.D.s were within ±0.025 in all cases.

Fig. 4. Composite depiction of release of diclofenac sodium from calcium–alginate (○), calcium–pectinate (△) and calcium–alginate–pectinate (□) pellets in a changing pH environment (derived using data from Fig. 3) (n = 3). S.D.s were within ±0.01 in all cases.
periods and pH variation equivalent to the actual in vivo conditions is presented in composite Fig. 4. Verification of the true in vivo performance for such pellet systems require additional in vivo studies.

3.5. Influence of continuous pH changes on drug release from pellets using the USP 23 Apparatus 3

Coupe et al. [26] reported that after a light breakfast, emptying from the stomach essentially occurs in a randomized manner. Most subjects demonstrated a lag phase in gastric emptying. A mean gastric emptying time of 105±45 min was reported. Based on this data, we therefore opted to expose the pellets for a maximum of 120 min at pH 1.5 and 3, which realistically represent the gastric pH environment followed by an additional 10 h in the higher pH range (see Section 2.5).

In spite of the meticulous engineering of the instrument, a simple solution for the maintenance of sink conditions within each vessel could not be easily established. For all three formulations partial disintegration/erosion of the pellets was observed, producing an opaque solution in the vessels. Hence the partial drug particle detachment from the pellets was assumed to be incompletely dissolved. The problem of sink condition maintenance was further complicated by the fact that the ionic ratio of the pellet component to the concentration of phosphate species in the dissolution media was sufficiently high to possibly lead to the sequestration reaction. Osberg et al. [2] reported a similar phenomenon with calcium–alginate beads and phosphate buffer. Hence, the calcium ions in the crosslinked polymer are likely to react with the phosphate species to yield insoluble phosphated calcium precipitates. This phenomenon was not observed during tests conducted in the 900 ml dissolution vessels since the calcium:phosphate ionic species ratio may have been below the threshold for sequestration to occur.

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In general, when a 0.2 M monobasic potassium phosphate buffer (i.e., USP 23-recommended concentration) was prepared, the concentrations of the individual ionic species existing in solution was calculated by predicting a proton balance equation from the ionization of the parent acid (in salt form). For example, at the lower pH of 5.4, the following equilibrium concentrations were calculated:

\[
\begin{align*}
[H_3PO_4] &= 0.197 \text{ mol/l (or 0.177 mol/900 ml and 0.043 mol/220 ml)}; \\
[HPO_4^{2-}] &= 0.0031 \text{ mol/l (or 0.0028 mol/900 ml and 0.0007 mol/220 ml)}; \\
[PO_4^{3-}] &= 1.64 \times 10^{-10} \text{ mol/l (or 1.48 \times 10^{-10} mol/900 ml and 3.61 \times 10^{-11} mol/220 ml).}
\end{align*}
\]

It can be assumed that the concentrations of these species necessary for calcium sequestration are already in effect. In addition, for greater confidence in accuracy of assay, the same quantity of pellets (equivalent to 100 mg diclofenac sodium) employed in 900 ml of dissolution media, was also tested in 220 ml. Although on a molar level the quantity of phosphate species is lower in 220 ml, the maintenance of sink and dilution effect does not exist as in the case of 900 ml. The dilution effect is considered equally important for its role in maintaining large inter-ionic distance which may reduce interaction between calcium ions and phosphate species. In this case, reducing the concentration of electrolytes in the buffer system (i.e., amount of phosphate salt used) to <0.2 M may be questionable in terms of providing adequate buffer capacity, and hence was not adopted.

Therefore, in order to obtain the true amount of drug released in each vessel, a pH-adjustment technique was adopted. Upon completion of the simulation test, the medium pH in each vessel was adjusted to 8. At this pH, both drug and polymer was totally solubilized, affording clear solutions, which could be subject to UV spectrophotometry. When such measures were not adopted, a false value of ≈27% drug release was obtained for all three formulations. However, once the pH-adjustment approach was employed it became evident that >99% drug content of pellets was released from each of the three formulations (Fig. 5). It was found that the calcium–pectinate formulation provided more rapid drug release (not shown) in comparison to that of the calcium–alginate and calcium–alginate–pectinate formulations. The calcium–alginate and calcium–alginate–pectinate formulations appeared more responsive at a pH≈5.4. The calcium–alginate formulation provided slowest release over a 9-h period. In addition, drug release from calcium–alginate–pectinate formulation appears to be more susceptible to changes in the media pH with greater pellet swelling/relaxation and release compared to calcium–alginate pellets. The practically insoluble nature of alginate in the acidic environment was most probably
is presented as a monoeXponential decline in drug content from the dosage form and this follows the equation:

\[ A = A_0(e^{kt} - 1) \]  

where \( A \) is the amount of drug remaining in the core matrix and \( A_0 \) is the initial loading dose.

Furthermore, drug release from simple swellable systems may be described by the well-known power law expression [19]:

\[ M_1/M_\infty = k_1t^n \]  

where \( M_1 \) and \( M_\infty \) are the amounts of drug released at time \( t \) and the overall amount released, respectively, \( k_1 \) is a release constant and \( n \) is a release exponent indicative of the release mechanism. In the case of highly crosslinked materials, swelling of the polymer may initially be slow and hence create a lag phase in drug release. Consequently, release data from such systems may be more appropriately analyzed, as also discussed by Kim and Fassihi [20], by using the modified form of the power law expression (Eq. (5)):

\[ M_1/M_\infty = k_1(t - t_{L,\text{min}})^n \]  

where \( t_{L,\text{min}} \) represents the delay in release or minimum lag time in the present case. We allude to the use of “minimum lag time” since data treatment is performed on pellets directly exposed to pH 6.6 where the lag time is 0.35 h in all formulations. However, in vivo this will not be the case since the pellets must initially pass the gastric region where negligible drug release occurs, while the transit time is in excess of 0.35 h.

Classically, in both of the above cases, \( n = 0.5, 0.5 < n < 1 \), or \( n = 1 \) for a slab, is indicative of Fickian release, anomalous transport, or Case II transport kinetics, respectively. However, the \( n \) values may change with the matrix geometry.

Irrespective of dosage form geometry, Peppas and Sahlin [21] reported on the evaluation of contributions provided by Fickian diffusion and matrix relaxation/dissolution through the use of the following equation:

\[ M_1/M_\infty = k_1t^n + k_2t^{2n} \]  

where \( k_1 \) is the Fickian kinetic constant and \( k_2 \) is the
relaxational/dissolution rate constant (i.e., anomalous transport).

Drug release from systems with surface erosion and varying geometries also have been analyzed by Hopfenberg [22]. Hopfenberg proposed a model applicable to either a slab, cylinder or sphere showing heterogeneous erosion (Eq. (7)):

\[ M_t/M_\infty = 1 - (1 - k_1 t)^n \]  

where \( k_1 \) is equal to \( k_o/C_o r_o \), \( k_o \) is the erosion rate constant, \( C_o \) is the uniform initial concentration of drug in the matrix, and \( r_o \) is the initial radius for a sphere or cylinder or the half-thickness for a slab. In Eq. (7) the \( n \) values are as follows: \( n = 1 \) for a slab, \( n = 2 \) for a cylinder, and \( n = 3 \) for a sphere. The model assumes that time-dependent diffusional resistances internal or external to the eroding matrix do not influence the release kinetics. Furthermore, the contribution of the secondary surfaces to the release process is not considered, as discussed by Katzhen-dler et al. [27].

Since the pellet systems showed a lag time during dissolution studies, we attempted to further highlight the utility of the Hopfenberg model, with modification, i.e., introduction of a minimum lag time component to the model:

\[ M_t/M_\infty = 1 - \left[ 1 - k_1 (t - t_{L,min}) \right]^n \]  

where \( t_L \) is equal to the lag time (0.35 h for all present formulations).

Dissolution data derived for calcium–alginate, calcium–pectinate and calcium–alginate–pectinate formulations were subjected to model analysis using all of the above equations. Table 3 illustrates the data generated by use of the above mentioned kinetic models. As a measure of the goodness of fit of a particular model based on the maximum likelihood, the Akaike Information Criterion (AIC) was used (Eq. (9)):

\[ \text{AIC} = N_d \ln \text{SSR} + 2P \]  

where \( N_d \) represents the number of data points, SSR

Table 3
Drug release kinetic data derived from various mathematical models

<table>
<thead>
<tr>
<th>Model</th>
<th>Pellet formulations</th>
<th>( k_1 )</th>
<th>( k_2 )</th>
<th>( n )</th>
<th>AIC</th>
<th>Condition No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 t^n )</td>
<td>Calcium–alginate</td>
<td>0.119</td>
<td>–</td>
<td>1.102</td>
<td>–62.6</td>
<td>27.59</td>
</tr>
<tr>
<td></td>
<td>Calcium–pectinate</td>
<td>0.095</td>
<td>–</td>
<td>1.770</td>
<td>–35.25</td>
<td>49.36</td>
</tr>
<tr>
<td></td>
<td>Calcium–alginate–pectinate</td>
<td>0.124</td>
<td>–</td>
<td>0.968</td>
<td>–60.95</td>
<td>24.50</td>
</tr>
<tr>
<td>( k_1 (t - t_{L,min})^n )</td>
<td>Calcium–alginate</td>
<td>0.165</td>
<td>–</td>
<td>0.925</td>
<td>–64.70</td>
<td>17.88</td>
</tr>
<tr>
<td></td>
<td>Calcium–pectinate</td>
<td>0.160</td>
<td>–</td>
<td>1.447</td>
<td>–39.48</td>
<td>25.25</td>
</tr>
<tr>
<td></td>
<td>Calcium–alginate–pectinate</td>
<td>0.164</td>
<td>–</td>
<td>0.829</td>
<td>–76.18</td>
<td>16.97</td>
</tr>
<tr>
<td>( k_1 t^n + k_2 t^2 )</td>
<td>Calcium–alginate</td>
<td>3 \cdot 10^{-6}</td>
<td>0.140</td>
<td>0.475</td>
<td>–59.47</td>
<td>118.8</td>
</tr>
<tr>
<td></td>
<td>Calcium–pectinate</td>
<td>3 \cdot 10^{-6}</td>
<td>0.128</td>
<td>0.692</td>
<td>–30.61</td>
<td>106.7</td>
</tr>
<tr>
<td></td>
<td>Calcium–alginate–pectinate</td>
<td>2.6 \cdot 10^{-6}</td>
<td>0.142</td>
<td>0.425</td>
<td>–71.83</td>
<td>127.4</td>
</tr>
<tr>
<td>( 1 - (1 - k_1 t)^n )</td>
<td>Calcium–alginate</td>
<td>0.111</td>
<td>–</td>
<td>1.282</td>
<td>–65.80</td>
<td>97.03</td>
</tr>
<tr>
<td></td>
<td>Calcium–pectinate</td>
<td>0.234</td>
<td>–</td>
<td>0.702</td>
<td>–26.52</td>
<td>16.65</td>
</tr>
<tr>
<td></td>
<td>Calcium–alginate–pectinate</td>
<td>0.075</td>
<td>–</td>
<td>1.819</td>
<td>–88.02</td>
<td>268</td>
</tr>
<tr>
<td>( 1 - [1 - k_1 (t - t_{L,min})]^n )</td>
<td>Calcium–alginate</td>
<td>0.095</td>
<td>–</td>
<td>1.821 (2)</td>
<td>–71.39</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>Calcium–pectinate</td>
<td>0.245</td>
<td>–</td>
<td>0.843 (3)</td>
<td>–30.67</td>
<td>19.63</td>
</tr>
<tr>
<td></td>
<td>Calcium–alginate–pectinate</td>
<td>0.055</td>
<td>–</td>
<td>2.972 (3)</td>
<td>–91.01</td>
<td>931.2</td>
</tr>
<tr>
<td>( A_o \exp(-k_1 t) )</td>
<td>Calcium–alginate</td>
<td>0.226</td>
<td>–</td>
<td>–</td>
<td>–52.11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Calcium–pectinate</td>
<td>0.336</td>
<td>–</td>
<td>–</td>
<td>–27.22</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Calcium–alginate–pectinate</td>
<td>0.189</td>
<td>–</td>
<td>–</td>
<td>–63.41</td>
<td>–</td>
</tr>
</tbody>
</table>

* \( n \) value in parentheses indicates the theoretical value provided by the modified Hopfenberg model.
is the residual sum of squares and $P$ denotes the number of parameters used in the model. Generally, the lower the AIC value the better is the model fit [28,29]. In addition, as one of the other Win Nonlin parameters, the condition number of the matrix of partial derivatives (i.e., the square root of the ratio of the largest to smallest Eigenvalue for the partial matrices) [29] was used as a confirmatory indicator of the soundness of statistical and experimental interpretation. Essentially the Eigenvalues and their associated Eigen vectors can be thought of as building blocks for matrices. Hence very large values of the condition number may be indicative of instability in the model fitting process. In non-linear regression analysis, the $r^2$ and mean square error (MSE) values offer weaker data interpretation due to their calculation being based on linearization of such data. Hence $r^2$ and MSE may only be considered as wide estimates and were not adopted in this work.

From model fitting, using WinNonlin, it became evident that choice of the most suitable model based on the AIC values were not conclusive and robust enough, due to the closeness of the values and discordance with release, swelling and weight change/erosion data. The condition numbers derived from the Eigenvalues of the variance–covariance matrices appeared to correlate well with experimental observations. Statistically, the large value of $k_2$ (0.128, indicating substantial matrix relaxation) for the calcium–pectinate formulation as compared to the $k_1$ value ($3 \times 10^{-6}$) (derived from Eq. (6)) only confirms that erosion is not the only operating release mechanism for this particular formulation, and may not necessarily be due to the lack of model suitability. Furthermore, based on the higher solubility of the crosslinked pectinate polymer, one may expect the higher dissolution rate constant. It is also shown that both diametral and axial swelling are not of the same degree and consequently, the basic initial geometry of the system is not maintained.

From weight change/erosion data, it is apparent that calcium–pectinate pellets demonstrated the highest erosion rate constant (Table 4) with minimum swellability (Fig. 2e,f). This finding is further evident by attainment of the lowest condition number when the Hopfenberg (Eq. (7)) and modified Hopfenberg (Eq. (8)) models were applied to the calcium–pectinate release data. The corresponding high condition numbers for the same two models in the case of calcium–alginate and calcium–alginate–pectinate pellets, is also reflected by the high swellability (Fig. 2e,f) and low erosion rate constants (Table 4). Although the classic Power law expression (Eqs. (4) and (5)) is designated for moderately swellable systems (i.e., equilibrium swelling ratio of not greater than 1.33 or 25% water content by volume), the model reasonably describes the release kinetics for calcium–alginate and calcium–alginate–pectinate pellets. This is discerned in the light of statistically lower condition numbers and $n$ values that approach $n=1$ for release rate up to 80% ($k_{calcium-pectinate}=0.9823$), as opposed to the classically reported limit of 60% value. Based on these findings, it is apparent that the notion of a maximum of 25% hydration of the swellable system (i.e., moderately swelling matrix) inherent in the Power law expression used [19], must be viewed in relation to structural configuration.

![Table 4](image)

Calculated and predicted kinetic constants based on the modified Hopfenberg model

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$k_0^a$</th>
<th>$C_0^b$</th>
<th>$r_0^c$</th>
<th>$k_{i,calc}^d$</th>
<th>$n_{i,end}^e$</th>
<th>$k_{i,pred}^f$</th>
<th>$n_{i,pred}^g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium–alginate</td>
<td>0.65</td>
<td>87.74</td>
<td>0.85</td>
<td>0.009</td>
<td>2</td>
<td>0.095</td>
<td>1.821</td>
</tr>
<tr>
<td>Calcium–pectinate</td>
<td>16.51</td>
<td>102.56</td>
<td>0.69</td>
<td>0.233</td>
<td>1</td>
<td>0.245</td>
<td>0.843</td>
</tr>
<tr>
<td>Calcium–alginate–pectinate</td>
<td>3.06</td>
<td>97.56</td>
<td>0.49</td>
<td>0.065</td>
<td>3</td>
<td>0.055</td>
<td>2.972</td>
</tr>
</tbody>
</table>

1 Linear regression was performed on the dynamic weight change data to determine $k_0$ (%/h): calcium–alginate: $y = -0.65x + 109.35$, $r^2 = 0.87$; calcium–pectinate: $y = -16.51x + 91.11$, $r^2 = 0.96$; calcium–alginate–pectinate: $y = -3.06x + 92.67$, $r^2 = 0.95$.

2 $C_0$ corresponds to the drug loading entrapment capacity (%).

3 For a sphere or cylinder $r_x = \text{initial radius}$; for a slab $r_x = \text{half-thickness (mm)}$.

4 Calculated $k$ value: $k_{calc}=k/C_{o,0}$ (mm$^{-1}$ h$^{-1}$).

5 $n$ value obtained by geometrical approximation in swollen state.

6 $k_i$ value predicted from modeling actual data.

7 $n$ value predicted from modeling actual data.
of the matrix (polymer) and its resilience to chain disentanglement beyond the equilibrium ratio. Based on the results obtained in this study, it is apparent that further work and development of appropriate mathematical models may be necessary for polymeric devices that: (i) exhibit heterogeneous structure; (ii) display both time-dependent geometrical transformation and superswelling properties; and (iii) behave in a pH-responsive manner.

Macroscopically as evidenced in this work, at the maximal swollen state calcium–alginate, calcium–pectinate and calcium–alginate–pectinate are typically cylindrical, disc-like (slab) and spherical respectively. Table 3 shows the corresponding \( n \) values for the modified Hopfenberg model when the maximal swollen state of the matrices is considered. In order to compare the kinetic constants predicted by the modified Hopfenberg model, these parameters were also calculated from actual pellet size (for \( r_n \)) and geometry (for \( n \) value). These constants are depicted in Table 4.

From Table 4 it is evident that the \( k \) values calculated from the actual size and geometrical data are very similar to the modeled (i.e., predicted) \( k \) values except for calcium–alginate. The low \( k \) value (erosion rate constant) for calcium–alginate may be attributed to the fact that it undergoes negligible erosion.

### 4. Conclusions

It is shown that the drug entrapment capacity of a weak acidic drug, such as diclofenac sodium, was significantly enhanced by pH drop in the crosslinking solution. Dissolution studies over a pH range similar to the human gastrointestinal tract demonstrated that the rate of drug release from the different pellets in simulated small intestinal environments ranged from rapid to slow (i.e., 100% drug release ranged from 4 to 10 h) but always in a controlled manner, depending on the type of the formulation employed. From model fitting studies and statistical treatment of the data, it became apparent that the modified version of the Hopfenberg equation best describes the release kinetics from the pellets made of pectin; while the exponential models better describe the release kinetics for calcium–alginate and its binary mixture with pectin. Based on the provided methodologies and depending on the site of drug absorption within the gastrointestinal tract, desirable pellets can be easily and reproducibly manufactured. The application of a single polymer (as used traditionally) or use of binary mixture (i.e., new approach) for the formation of pellets allows the formulator to predict and produce pH-sensitive pellets of different geometries, strengths and release characteristics. More specifically, some of the formulation problems encountered for the delivery of gastric-irritating or acid-labile drugs can be overcome by the application of multiple unit crosslinked pH-responsive pellets. The results of this study may be of value to those pharmaceutical scientists who are engaged in site-specific delivery, as well as the oral delivery of enzymes, peptides, proteins and ulcerogenic agents.

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### References