# Tunable Transport of Glucose Through Ionically-Crosslinked Alginate Gels: Effect of Alginate and Calcium Concentration

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**ABSTRACT:** Alginate beads have numerous biomedical applications, ranging from cell encapsulation to drug release. The present study focuses on the controlled release of glucose from calcium-alginate beads. The effects of alginate concentrations (1–6 wt %) and calcium chloride concentrations (0.1–1.0*M*) on glucose release from beads were examined. It was found that the time required for complete glucose release from beads could be tuned from 15 min to over 2 h, simply by varying alginate and calcium chloride concentrations in beads. For calcium-alginate beads with sodium alginate concentrations of 1–4 wt %, higher sodium alginate concentrations lead to more prolonged release of glucose and thus a smaller value of a rate constant *k*, a parameter shown to be proportional to the diffusion coefficient of glucose in the alginate gel. For beads with sodium alginate concentrations of 4–6 wt %, there was no statistically significant difference in *k* values, indicating a lower limit for glucose release from calcium-alginate beads. Similarly, higher calcium chloride concentrations appear to extend glucose release, however, no conclusive trend can be drawn from the data. In a 50 : 50 mixture of calcium-alginate beads of two different alginate concentrations (1 and 4 wt %), glucose release showed a two-step profile over the time range of 20–50 min, indicating that the pattern and time of glucose release from beads can be tuned by making combinations of beads with varying alginate and/or calcium chloride concentrations.  J Appl Polym Sci 107: 2956–2962, 2008

**Key words:** diffusion; hydrogels; polysaccharides; biopolymers; biomaterials

## INTRODUCTION

Alginic acid, a linear block copolymer polysaccharide derived from algae, consists of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues joined by 1,3-glycosidic linkages, and forms in a non-regular, block-wise pattern along the chain. The block-wise pattern contains three types of polymer segments: one consisting essentially of D-mannuronic acid units, the second of L-guluronic acid units, and the third of alternating D-mannuronic acid and L-guluronic acid residues.[1] The proportion of each block and the arrangement of blocks along the molecules vary depending on the algal source. When a solution of sodium alginate is added drop-wise to a solution containing divalent metal ions such as $Ca^{2+}$ or $Cu^{2+}$, water-insoluble cation-alginate gel beads are formed in aqueous solution.[2] Depending on the composition of the two residues and their sequential distribution within the molecules, ionically crosslinked complexes form either precipitants or hydrogels.[3] Guluronic acid blocks are known to form a rigid buckled structure, the so-called ''egg-box'' array, in which chelating calcium ions are nestled in the aqueous environment of an ordered gel structure due to the spatial arrangements of the oxygen atoms of carboxyl and hydroxyl groups in the guluronic block.[3] This interaction is not only based upon electrostatic interactions, which neutralize acidic groups, but also on the coordinating function of the calcium ions as the chelating center.[3]

Alginate gel beads are used in many applications as matrices for cell immobilization and drug delivery. Alginate has long been investigated as a material for transplantable cell encapsulation systems because it protects transported cells from the host's immune system, promotes normal cell function, and does not produce a fibrotic response *in vivo*.[4–6] Rat islets were first encapsulated in alginate to create a bioartificial endocrine pancreas.[7] Hepatocytes have also been encapsulated in alginate to create extracorporeal liver assist

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devices.[5] Also, alginate has been used to encapsulate microsomes or whole cells for the production of glucuronides in response to drug dosages and insulin.[8,9] Alginate has also been utilized to create tissue engineered thyroid tissue and parathyroid tissue.[10,11]

In addition to promising applications in cell encapsulation and tissue engineering, alginate gels also show potential for delivery structures because gel beads can be formed very easily in aqueous solutions at room temperature, without the use of any organic solvents. Calcium-alginate beads have a wide range of applications, including delivery of insulin, pesticides, proteins, drugs, and preservatives.[5] Some encapsulated proteins appear to crosslink with the alginate themselves, further extending the sustained release.[12] Alginate gels are not thermoreversible, but they will dissolve in the presence of a cation-sequestering agent such as EDTA.[13] Moreover, calcium-alginate gel beads shrink at acidic pH and erode in an alkaline environment such as the intestine, so they therefore serve as a potential oral delivery system.[14] Alginate is also a mucoadhesive and is likely to stick to intestinal mucosa for prolonged periods of time.[15] Alginate gel beads have been studied for the development of oral drug delivery systems for entrapment in both *in vitro* and *in vivo* studies of controlled release of proteins and drugs.[16]

Despite widespread interest in alginate-based biomaterials, there are relatively few studies on controlling the transport of small molecules such as glucose through alginate matrices. Such information is important in designing alginate gels for cell encapsulation and tissue engineering.

## MATERIALS AND METHODS

### Materials

Sodium alginate was obtained as a dry powder from Sigma Chemical Company (St. Louis, MO). The molecular weight of this product has been reported as 269 kDa.[17] Additional materials were obtained from Sigma-Aldrich.

### Preparation of glucose-loaded calcium-alginate gel beads

Sodium alginate was dissolved in deionized and distilled water at the following concentrations: 1, 2, 3, 4, and 6 wt %. D-glucose was then dissolved completely in the alginate solution to achieve a concentration of 100 g/L. Calcium chloride solutions were made at concentrations of 1.0, 0.5, and 0.1*M* in distilled water with a glucose concentration of 100 g/L. The same concentration of glucose was used in both the alginate and calcium chloride to prevent pre-experimental mass transfer. The glucose-alginate solution (10 mL) was added dropwise into 150 mL of mildly agitated $CaCl_2$ solution using a syringe pump through a 20-gauge needle at a drop rate of 1.5 mL/min. A peristaltic pump was used to ensure reproducibility. The drops of alginate solution began crosslinking with $Ca^{2+}$ ions upon contact to form calcium alginate gel beads. The beads were cured in the $CaCl_2$ solution at room temperature for 16–18 h, protected from light. After the gelling time was complete, the calcium-alginate beads were collected. The average bead diameter was 2 mm.

### Glucose release studies from calcium-alginate beads

The following procedure was used to determine the glucose release profile for the alginate gel beads. Once cured and collected, sixty alginate beads were washed three times with nano-pure water to remove residual, nonencapsulated glucose from bead surfaces. The beads were added into 150 mL PBS solution (pH = 7.4) at room temperature (25°C) with a magnetic stirrer. Samples were collected every 30 s for the first 5 min, then every 5 min until the experiment reached 30 min, and every 15 min thereafter. The release experiment was carried for 2–3 h with mild agitation. Once the run was complete, the glucose concentration for each sample was measured to determine the release profile.

### Glucose assay

Glucose concentrations were determined with an enzymatic assay, using a uQuant – Universal Microplate Spectrophotometer with a 96-well plate assay format. Samples from release studies were diluted 1 : 11 with distilled water. A 30 μL aliquot of diluted sample or glucose standard was added to each well of a 96-well plate. A 200 μL quantity of color reagent, PGO enzymes (glucose oxide and peroxide mixture), was then added to each sample on the plate. Protected from light, the plate was incubated at 37°C for 30 min to activate the color reagent and absorbance was then read on the uQuant spectrophotometer at 450 nm.

## RESULTS AND DISCUSSION

### Diffusion analysis

The following mechanism was fitted to the measured glucose concentrations for each release experiment:

$$\% \text{ Release} = 100 \cdot (1 - e^{-k \cdot t}) \quad (1)$$

where $t$ is time, and $k$ is a fitting parameter (time$^{-1}$).

The parameter $k$, with units of inverse time, is a ''rate constant'' for glucose release and is related to the diffusion coefficient of glucose in the alginate gel

**TABLE I**
**High and Low Extreme Values Chosen for Each Variable Studied in a Design of Experiments ($2^{5-2}$ Factorial Design)**

| Alginate concentration | $CaCl_2$ concentration ($M$) | Glucose concentration (g/L) | Gelling time (h) | Sodium citrate concentration |
|---|---|---|---|---|
| 1% (w/v) | 0.5 | 100 | 0.5 | 0% (w/v) |
| 3% (w/v) | 0.1 | 80 | 5 | 0.25% (w/v) |

beads. To obtain eq. (1), a diffusion model is applied to the transport of glucose from beads into a well-stirred solution of limited volume. The increase in solute concentration in the surrounding liquid is measured over time, and the diffusion coefficient can be calculated using an unsteady-state diffusion model,[18] which yields:

$$\frac{C_t}{C_\infty} = 1 - \sum_{n=1}^{\infty} \frac{6\alpha(\alpha+1)}{9+9\alpha+q_n^2} \exp\left(-D\frac{q_n^2}{r^2}t\right) \tag{2}$$

where $r$ is the radius of calcium-alginate beads in the PBS solution and $q_n^2$ are the positive, nonzero roots of the equation:

$$\tan(q_n) = \frac{3q_n}{3+\alpha q_n^2} \tag{3}$$

$$\alpha = K_p \frac{V}{(4/3)\pi r^3} \tag{4}$$

$$K_p = \frac{\text{Concentration of glucose in alginate beads at equilibrium}}{\text{Concentration of glucose in bulk (PBS) at equilibrium}} \tag{5}$$

$K_p$ is a partition coefficient for equilibrium between glucose in the calcium-alginate gel beads and glucose in the PBS solution with a total volume $V$ (mL). Assuming there is no partitioning of glucose between alginate beads and the PBS solution, $K_p$ is 1. Therefore, both $\alpha$ and $q_n^2$ are functions of the total bulk volume, $V$, and the radius of the beads, $r$:

$$\alpha = \frac{V}{(4/3)\pi r^3} \tag{6}$$

If $V$ and $r$ are kept constant for all runs, $k$ is proportional to the diffusion coefficient:

$$k = \frac{q_n^2}{r^2} D \tag{7}$$

where $D$ is the diffusion coefficient (in this case a function of sodium alginate and calcium chloride concentration), and $C_t/C_\infty$ is the percentage of release. Therefore, eq. (1) can be rewritten as:

$$\%\ \text{Release} = A_1 + A_2 \cdot \exp(-k \cdot t) \tag{8}$$

where parameter $A_1$ is function of final concentration of glucose in PBS and parameter $A_2$ is function of $q_n^2$ and $\alpha$. Under all of the above assumptions, eq. (1) can be correlated to eq. (2), making $k$ a correlative diffusion coefficient.

In experiments run with multiple varied parameters, data was analyzed using one-way ANOVA followed by Tukey's comparison test. Differences were considered statistically significant when $P < 0.05$. Reported data results are the average of three samples, with the standard deviation taken to be the error.

### Preliminary screening by design of experiments

In a preliminary study, design of experiments was utilized to identify the most relevant physical parameters for glucose release. Previous studies have shown that the gel microstructure and mechanical properties depend on calcium chloride concentration, gelling time, intrinsic viscosity and molecular weight of the alginate, and alginate concentration.[19] Also, sodium citrate has been reported to aid calcium chloride in supplying calcium ions for effective gelation.[20] Therefore, a $2^{5-2}$ fractional factorial design of experiments was initially conducted to determine which factors most significantly affect the release rate of glucose from the calcium-alginate gel beads, varying the following: sodium alginate weight concentration, calcium chloride concentration, glucose

**TABLE II**
**Rate Constant, *k*, Values for a Full Factorial Design ($min^{-1}$)**

| Alginate conc. (wt %) | $CaCl_2$ conc. (*M*) | | |
|---|---|---|---|
| | 0.1 | 0.5 | 1.0 |
| 1 | 0.134 ± 0.008 | 0.105 ± 0.010 | 0.332 ± 0.041 |
| 2 | 0.106 ± 0.008 | 0.084 ± 0.007 | 0.201 ± 0.019 |
| 3 | 0.087 ± 0.007 | 0.050 ± 0.006 | 0.057 ± 0.006 |
| 4 | 0.074 ± 0.010 | 0.032 ± 0.004 | 0.011 ± 0.007 |

content, gelling time, and effect of sodium citrate. The high and low extremes for each parameter tested are given in Table I. The value of *k*, the rate constant, was analyzed as the response variable. Results from these preliminary experiments indicated that increasing the concentration of alginate slows down the release rate of glucose. Increasing the concentration of calcium chloride has the same effect, but less significantly than alginate. The rate of glucose release also increased with higher glucose contents, but this result was expected due to a larger concentration gradient driving mass transfer. No significant differences in the release rates were found upon varying gelling time or the presence of sodium citrate (data not shown). Therefore, a more detailed study focused solely on the effects of alginate concentration and calcium chloride concentration on the rate of glucose release.

### Effect of sodium alginate concentration and calcium chloride concentration

Alginate concentrations of 1, 2, 3, 4, and 6 wt % and calcium chloride concentrations of 0.1, 0.5, and 1.0*M* were investigated in glucose release experiments. All runs were performed in triplicate with 100 g/L glucose content in alginate beads and 17.5 h gelling time. Table II summarizes the value of *k* obtained in these experiments.

The release data for beads with a constant $CaCl_2$ concentration of 0.1*M* and varying sodium alginate concentrations are shown in Figure 1. For these systems, we obtained complete release of glucose from the beads in roughly 30–50 min. As seen in Table II, the rate constant, *k*, is a function of alginate concentration and decreases from 0.13 to 0.07 $min^{-1}$ as the

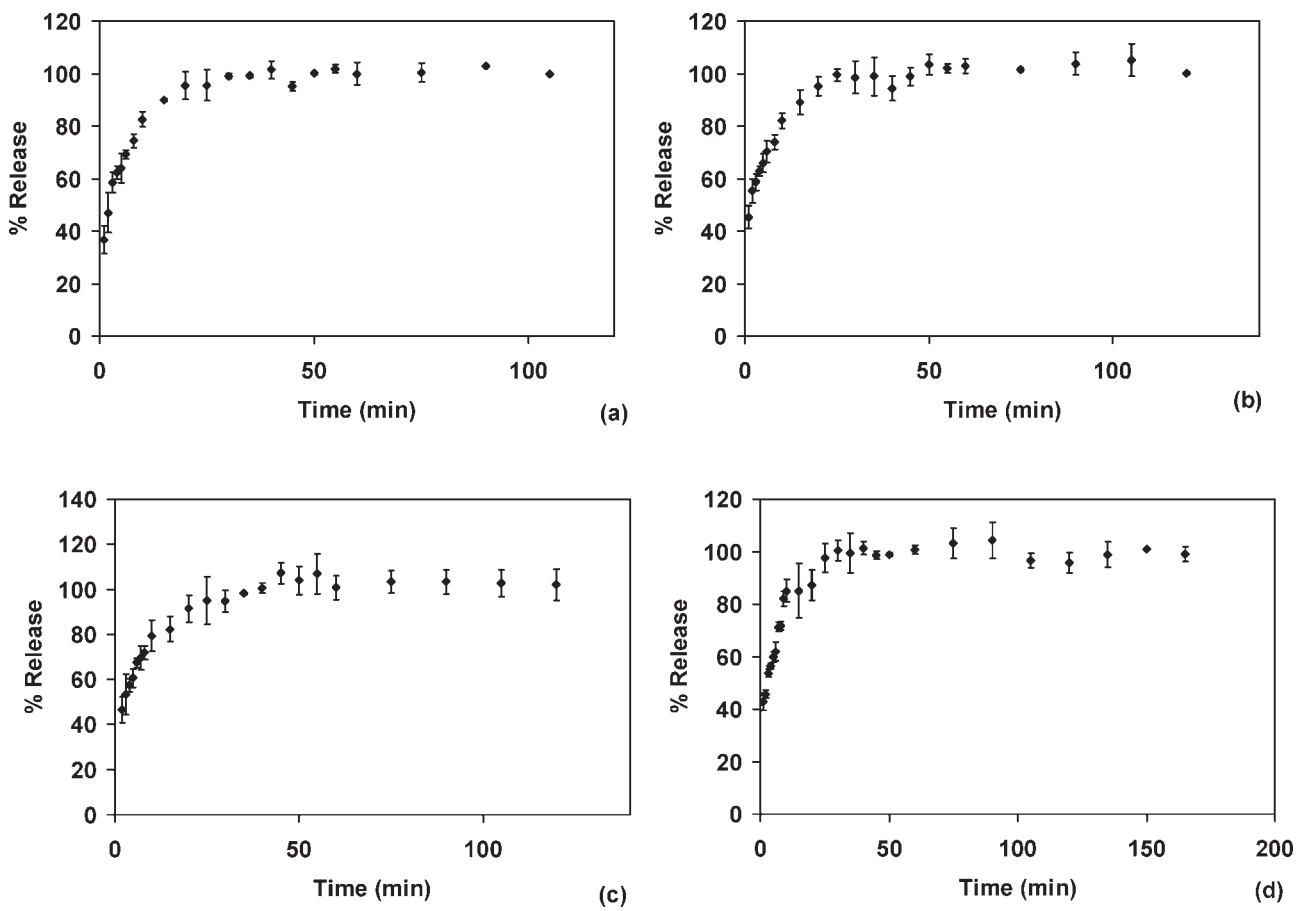


**Figure 1** Percent glucose release from calcium-alginate gel beads prepared with 0.1*M* $CaCl_2$ and (a) 1 wt % alginate, (b) 2 wt % alginate, (c) 3 wt % alginate, and (d) 4 wt % sodium alginate.

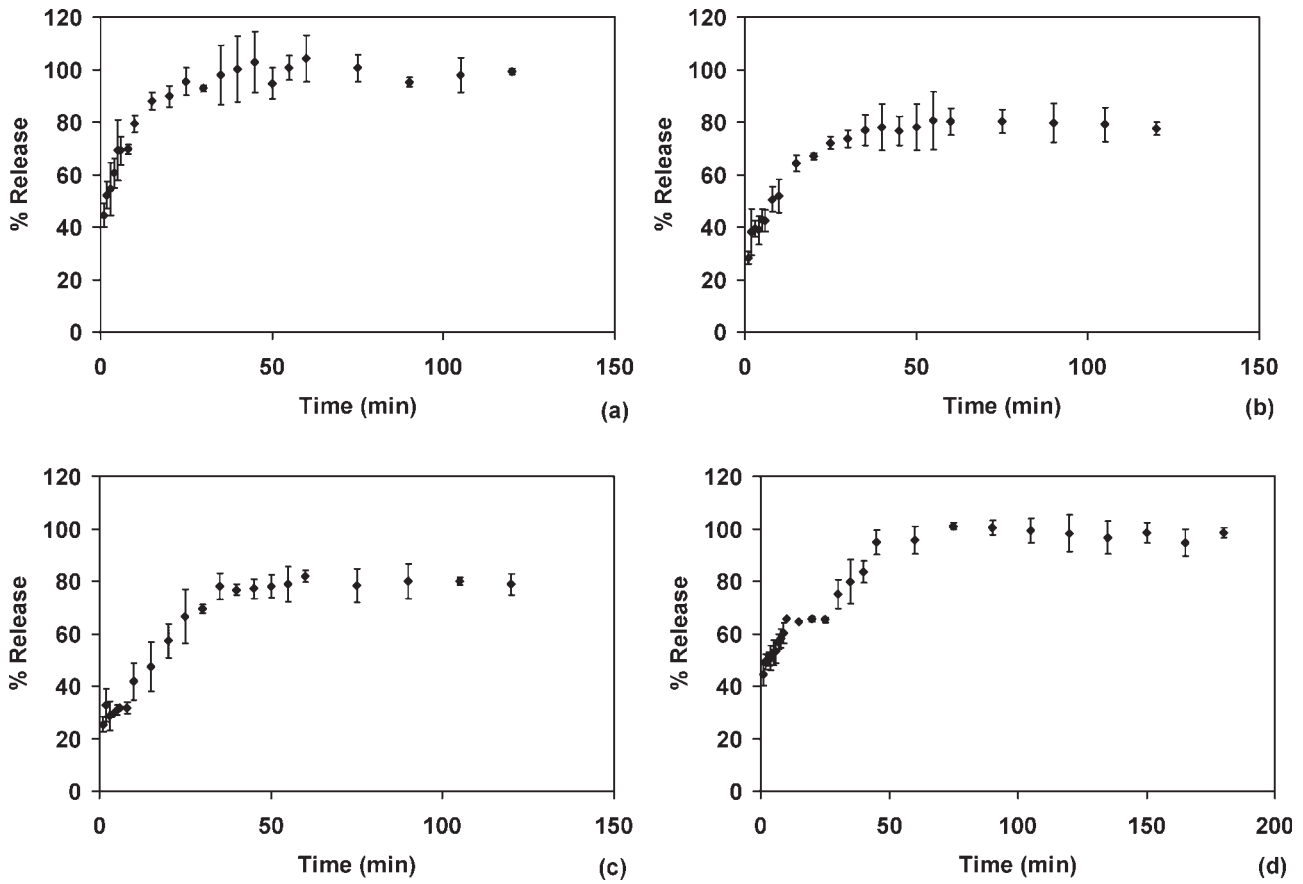


**Figure 2** Percent glucose release from calcium-alginate gel beads prepared with 0.5*M* $CaCl_2$ and (a) 1 wt % alginate, (b) 2 wt % alginate, (c) 3 wt % alginate, and (d) 4 wt % sodium alginate.

sodium alginate concentration increases from 1 to 4 wt %. Extended release can thus be obtained by increasing the sodium alginate concentration, while faster release can be obtained using a lower concentration of alginate. Similar results are obtained using 0.5*M* $CaCl_2$ (Fig. 2), and 1*M* $CaCl_2$ (Fig. 3), although in these cases, release occurred over longer time scales of 100–180 min. Alginate beads prepared with 6 wt % sodium alginate were also explored in this study, but the difference in release rate using these beads compared to the 4 wt % alginate beads was not statistically significant (data not shown). Figure 4 shows the rate constant $k$ as a function of alginate and $CaCl_2$ concentration. As expected, $k$ decreases as the alginate concentration increases, corresponding to a slower release. The dependence of $k$ on $CaCl_2$ concentration, however, is nonmontonic. This may be due to difficulties in forming beads with a homogenous microstructure at high $CaCl_2$ concentration.

### Multiple step release profiles

By mixing calcium-alginate gel beads prepared with different concentrations of alginate and/or $CaCl_2$, release patterns can be manipulated to accommodate the needs of different applications. In the case where a ''cascade'' type of release pattern is desired, it can now be attained by designing a system using a mixture of different types of beads. In this study, such a two-step system was created by mixing (50 : 50) calcium-alginate beads prepared with 1 wt % alginate in 0.5*M* $CaCl_2$ with an equal number of beads prepared with 4 wt % alginate in 0.5*M* $CaCl_2$. The resulting release pattern is shown in Figure 5, and portrays a cascade, or pulsed, controlled release pattern. Such a release pattern may be useful in delivering glucose to diabetic individuals and/or endurance athletes.

## CONCLUSION

In this study, the effect of $CaCl_2$ concentration and alginate concentration on the transport of glucose from alginate beads was investigated. Results show that it is possible to tune the transport of glucose and create systems with controlled glucose release over nearly 3 h. Release time can be extended by increasing either the sodium alginate concentration or calcium chloride concentration used in bead

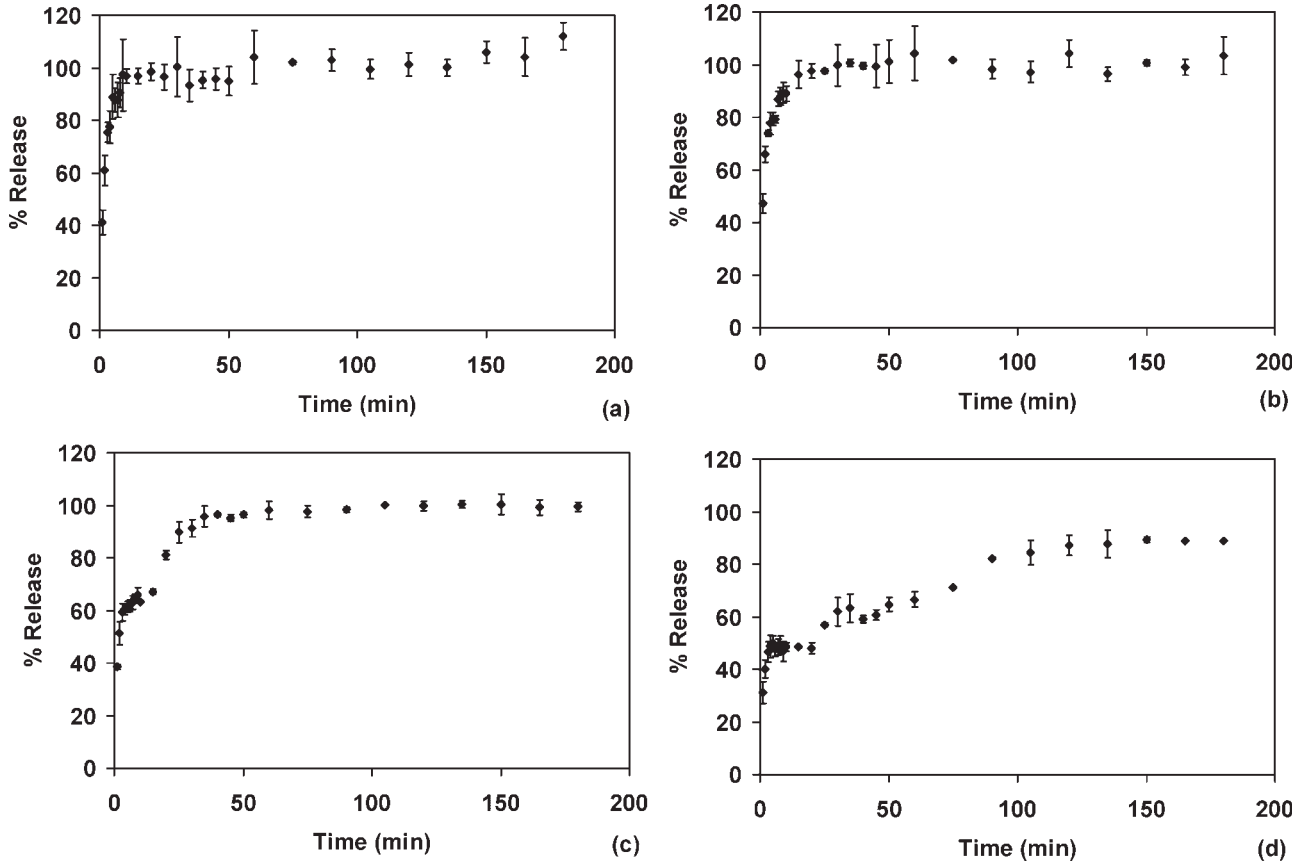


**Figure 3** Percent glucose release from calcium-alginate gel beads prepared with 1.0*M* $CaCl_2$ and (a) 1 wt % alginate, (b) 2 wt % alginate, (c) 3 wt % alginate, and (d) 4 wt % sodium alginate.

formation. This study has also shown that beads with different individual profiles can be mixed heterogeneously to result in a more complex release profile that contains multiple steps.

The results lend insight into physicochemical properties of solutes in alginate gels. While diffusion coefficients of glucose in alginate have been reported, there is little information in the literature on the effect of $Ca^{2+}$ concentration and alginate concentration on these coefficients.[17,21–23] The findings of this study may have relevance for other solutes in alginate gels, as well as other hydrogel systems.

These calcium-alginate gel controlled release systems are biocompatible and thus have potential uses in tissue engineering, cell encapsulation, and drug

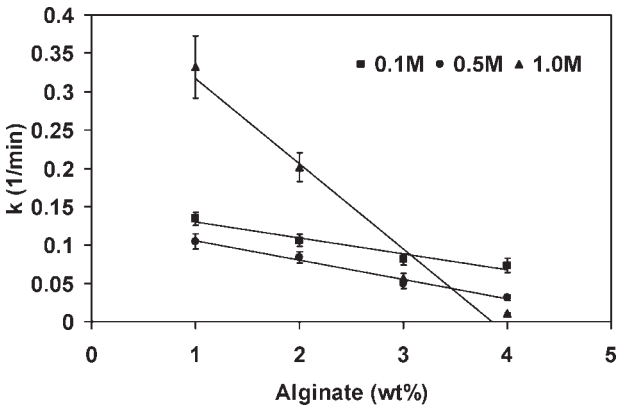


**Figure 4** Comparison of the *k* value trends with alginate concentration among different calcium chloride concentrations.

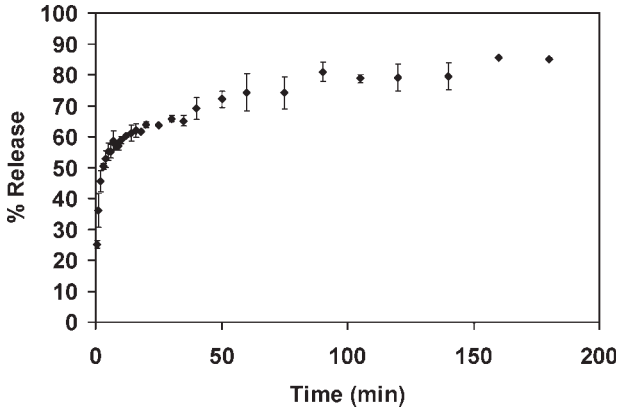


**Figure 5** Glucose release profile of a mixed system: 50 : 50 mixture of calcium-alginate gel beads prepared with 1 wt % alginate and 0.5*M* $CaCl_2$ and calcium-alginate gel beads prepared with 4 wt % alginate and 0.5*M* $CaCl_2$.

delivery. An alginate-based gel could also potentially be used for controlled delivery of glucose to diabetic patients and endurance athletes over extended periods of time. The present study is significant for the design of such glucose delivery systems. For clinical use of alginate gels, it is essential that glucose delivery be finely tuned, to avoid adverse effects of hypoglycemia and hyperglycemia. The present study demonstrates that delivery can be tuned based on calcium and alginate concentrations. In addition, because combinations of different beads can be mixed to provide a multistep release profile, one can envision creating a cocktail of beads for specific clinical targets. Future work on this subject should include *in vivo* studies, to determine the effects of alginate and calcium chloride concentrations in beads on *in vivo* glucose release, and provide a correlation between *in vitro* findings and *in vivo* findings.

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