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ORIGINAL PAPER

# Cation Effect on Slow Release from Alginate Beads: A Fluorescence Study

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**Abstract** In this study, spherical alginate beads containing pyranine ($P_y$) as a fluorescence probe were prepared by ionotropic gelation of a sodium alginate solution. The steady state fluorescence technique was used to study pyranine release from the alginate beads crosslinked with calcium, barium and aluminum ions, respectively. The slow release of $P_y$ was observed with the time drive mode of the spectrophotometer at 512 nm. Fluorescence emission intensity ($I_p$) from $P_y$ was monitored during the release process, and the encapsulation efficiency (EE) of pyranine from the alginate beads was calculated. The Fickian Diffusion model was used to measure the release coefficients, $D_{sl}$. It was seen that the slow release coefficients of pyranine from the alginate beads crosslinked with $Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$ ions increased in the following order: $D_{sl}$ ($Al^{3+}$)> $D_{sl}$ ($Ca^{2+}$)> $D_{sl}$ ($Ba^{2+}$). In contrast, the initial amount of pyranine and EE into the beads showed the reverse behavior.

**Keywords** Slow release · Metal ions · Fluorescence · Encapsulation efficiency · Pyranine

## Introduction

Alginic acid, a polysaccharide of homopolymeric blocks of (1–4)-linked β-D-mannuronate and α-L-guluronate, is extracted from the cell walls of brown algae. The ionized form of alginic acid is called alginate, which is an anionic polymer. One of the most important properties of alginate is that it forms a viscous gel when it comes in contact with the most divalent or trivalent metal cations. Such gelation is caused by crosslinking between the metal cation and anionic centers on the chain. These structures have been extensively studied and are well-known as "egg-box structures" [1, 2].

As alginate is a non-toxic material, alginate gels have broad applications and are used in controlled release systems [3, 4], the food industry [5], water purification agents [6], composite films [7] and many others. The physical characteristics of alginate gels are dependent on the gelling cation. The effects of changing the gelling cation on the mechanical properties of micro beads have been discussed in the literature [8, 9]. The most commonly used cations are $Ca^{2+}$, $Al^{3+}$, and $Ba^{2+}$. There are numerous ways that the controlled release systems of calcium alginate gels are used, including the release of antibiotics [10], bacteria [11], macromolecular drugs [12], proteins [13], spermatozoa [14] and vitamins [15]. Although barium alginate gels have better chemical and physical stability than calcium alginate [16], it has been reported that drug release systems with this matrix are similar but demonstrate lower levels of intensity, and have been used for delivery of insecticides [17] and spermatozoa [18]. It has been found that the presence of barium decreases the size of alginate gel beads and reduces permeability [9]. Controlled release studies of aluminum alginates have also been carried out, such as for the release of theophylline [19].

Pyranine is a water-soluble and pH-sensitive fluorescent compound. Studies on pyranine have covered many interesting areas, such as intracellular pH determination [20] and proton transfer [21]. Pyranine has been used as a probe for monitoring the gelation of polyacrylamide-sodium alginate composite gels and gel-to-sol transition properties of the κ-carrageenan polymer [22, 23]. The cation effect on the thermal transition of iota carrageenan has been investigated via the

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photon transmission technique [24], and activation energies were found to be strongly correlated to the $CaCl_2$ content in the system. The monovalent and divalent cation effects have also been studied as regards the phase transitions of iota carrageenan [25]. Hysteresis has been observed between the rod like to helix to coil and coil to rod like helix transitions and dimer groups were seen to transform into dimers.

The mechanisms of drug release from alginate matrices are generally studied by fitting various isothermal and kinetic models to the experimental data, which are typically obtained from discrete measurements in a dissolution apparatus. Recently, steady state fluorescence technique has been employed to study small molecule diffusion into polyacrylamide at various temperatures [26] and iota carrageenan gels [27], and studies have also been made of the sorption and desorption of polyacrylamide [28] and PVA-pyrene chains in and out of agarose gels [29]. In this study, a Fickian diffusion model was used to quantify the experimental findings for each sample and it was found that agarose gel possesses two distinct diffusion regions.

This paper examines the effect of crosslinking metal ions on release behavior via online monitoring of fluorescence intensity. Pyranine was selected as a model drug and fluoroprobe. We discuss the influence of different metal ions ($Ca^{2+}$, $Al^{3+}$, and $Ba^{2+}$) in the formation of alginate beads, and present our findings about pyranine release kinetics from the beads using the Fickian Diffusion model. Additionally, we examined the initial amount of pyranine and encapsulation efficiency (EE) for each case.

## Experiment

### Materials and Methods

Alginic acid sodium salt (Viscosity of ~250 cps) was purchased from the Sigma-Aldrich Company (St. Louis, Missouri), and a calcium chloride dehydrate (Analytical grade) was obtained from J.T. Baker (Deventer, Holland). Barium chloride dehydrate and aluminum chloride hexahydrate were of analytical grade and purchased from Merck (Darmstadt, Germany). Fluorescence measurements were carried out with the use of a Shimadzu RF-5301PC spectrophotometer (Kyoto, Japan) at room temperature.

### Preparation of the Beads

A homogeneous solution of sodium alginate (2 % w/v) was prepared by mixing an appropriate amount of sodium alginate and 5 mL deionized water. After complete dissolution, pyranine was added to produce a pyranine solution of $10^{-4}$ mol/L. The solution was mixed, and left for several hours to eliminate air bubbles. The mixture was added drop wise through a syringe with an inner diameter of 0.8 cm into a metal ion solution, where the metal ions correspond to $Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$ respectively. The addition of the alginate/pyranine solution was conducted 20 cm over the metal chloride solution vessel in order to produced perfect spherical beads. The beads were then filtered, washed with 10 mL of deionized water and placed on a Petri dish, and then placed in a spectrometric cuvette without drying. The details of the alginate beads are given in Fig. 1 and all formulations are listed in Table 1. During the preparations of the beads, the same amount of pyranine was added to the alginate solutions. However, due to the different gelation characteristics of barium, calcium and aluminum cations, the encapsulation efficiencies differ. Moreover, additional pyranine on the surface of the beads diffuses into the water during the washing step. Hence, the amount of pyranine left in the gelling solution and washing water was measured using a Shimadzu UV-1800 (Kyoto, Japan) spectrophotometer and these values were subtracted. Hereafter the "initial amount of pyranine" refers to the initial amount of pyranine before the release experiments carried out for each sample.

### Encapsulation

The amount of pyranine that escaped into the metal chloride solution and washing water was determined to be 445 nm via UV–VIS spectrophotometer. The encapsulation efficiency (EE) of pyranine into the alginate beads was calculated using the following equation:

$$Encapsulation\% = \frac{n_0 - (n_m + n_w)}{n_0} \times 100 \quad (1)$$

where $n_o$ is the initial amount of pyranine, and $n_m$ and $n_w$ are the amount of pyranine in the metal solution and washing water, respectively. The units are in moles.

### Slow Release Studies

Specified amounts of wet alginate beads were placed on the bottom of a quartz fluorescence cuvette in order to create a single layer structure. After 1 mL of deionized water was added without disturbing this single layer, the cuvette was immediately placed into the spectrophotometer. The position of the alginate beads in the cuvette is given in Fig. 2. The excitation wavelength for pyranine was set to 340 nm and the fluorescence emission intensity at 512 nm was monitored with the time-drive mode of the spectrophotometer. At the end of the measurement, the cuvette was taken out and shaken well to let the pyranine diffuse completely into the water, and the final fluorescence intensity ($I_f$) of the solution was measured.

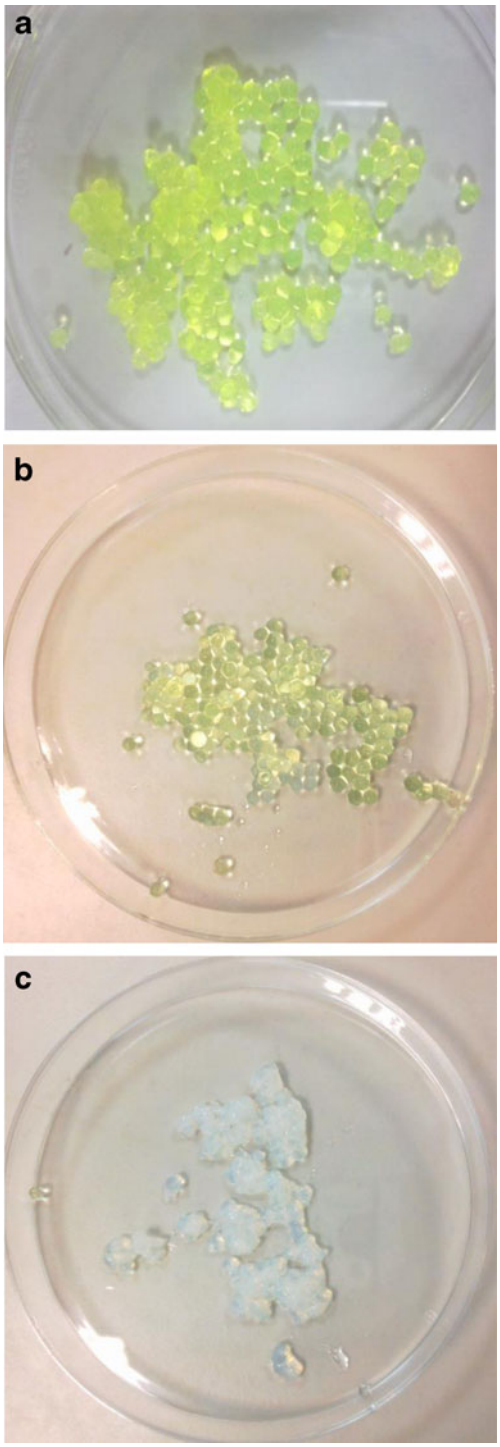


**Fig. 1** Single layer alginate beads with **a** $Ca^{2+}$, **b** $Ba^{2+}$, and **c** $Al^{3+}$ ions, respectively

## Results and Discussion

The fluorescence spectra of pyranine intensity from the alginate beads crosslinked with $Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$ ions prepared from their salts given in the experimental section with 3 % (w/v) concentrations for each case were monitored.

**Table 1** Experimentally measured and calculated parameters for thealginate beads crosslinked with different metal ($Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$) ions, respectively

| Metal | Initial amount of pyranine*$10^{-8}$ (mol) | Encapsulation efficiency of pyranine (%) | Release coefficient, $D_{sl}$*$10^{-12}$ ($m^2$/s) |
|---|---|---|---|
| $Al^{3+}$ | 1,14 | 56,51 | 122,65 |
| $Ca^{2+}$ | 1,49 | 59,27 | 69,36 |
| $Ba^{2+}$ | 2,09 | 92,02 | 42,98 |

Figure 3 gives the typical fluorescence spectra of released pyranine at 50, 100 and 200 min during the release experiments, where one should expect an increase in fluorescence intensity, $I$ at 512 nm due to the increasing amount of free $P_y$ molecules in the water.

The normalized fluorescence intensities at 512 nm as measured versus time are shown in Fig. 4 for the beads crosslinked with different metal ions ($Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$). $P_y$ molecules released from the beads crosslinked with $Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$ ions increased as the slow release time increased. The results, as depicted in Fig. 4a, b, and c, indicate that the amount of pyranine released from the beads increases for all alginate samples. In such a situation, the release process can then be treated using the Fickian Diffusion Model [30, 31].

According to Fick's law, the equation for diffusion in one dimension is expressed [30, 31] as

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D(c)\frac{\partial c}{\partial x}\right) = D\frac{\partial^2 c}{\partial x^2} \tag{2}$$

where $D$ is the diffusion coefficient and $c$ is the concentration of diffusing species at time $t$. For plane sheet geometry and to keep the initial concentration of water constant, the solution of Fick's equation is given by the following equation

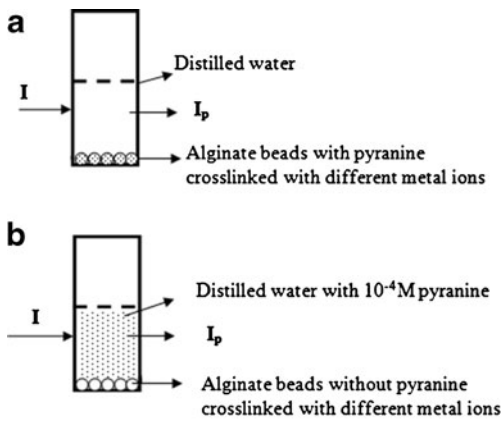


**Fig. 2** Position of Alginate beads **a** before and **b** after the slow release process is started, respectively

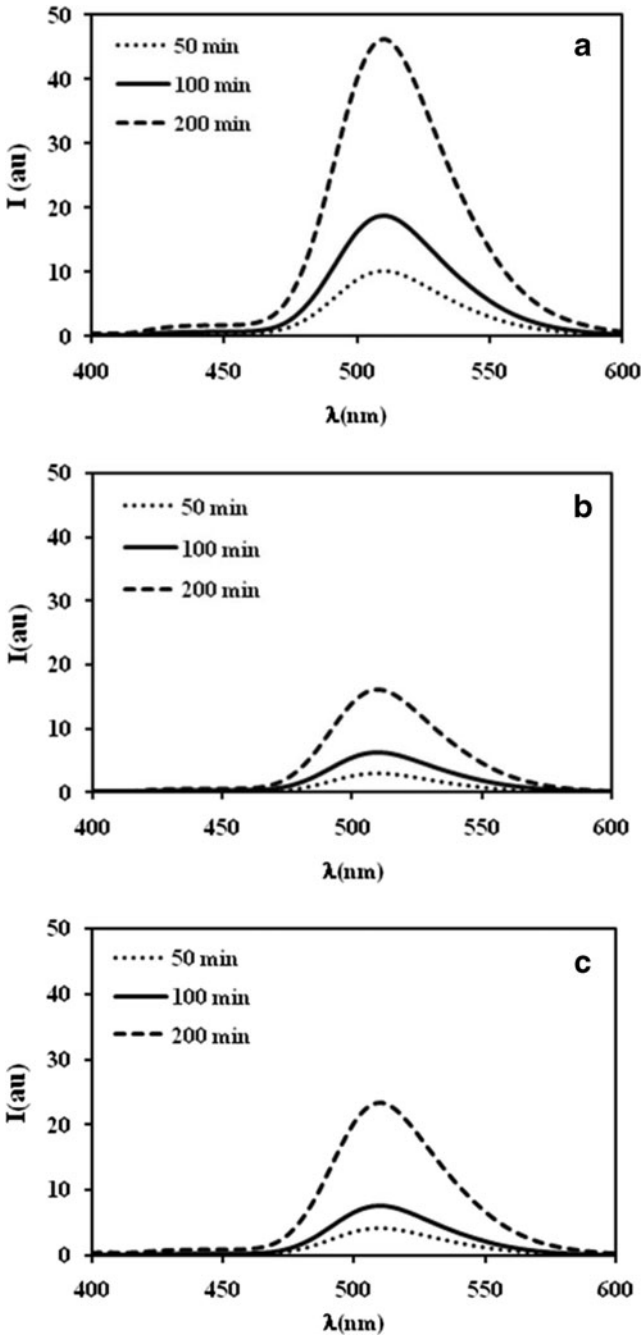


**Fig. 3** Fluorescence spectra of pyranine released from the alginate beads crosslinked with 3 (w/v) % **a** $Ca^{2+}$, **b** $Ba^{2+}$, and **c** $Al^{3+}$ ions at 50, 100, and 200 min, respectively

$$\frac{M}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left(-\frac{D(2n+1)^2\pi^2 t}{d^2}\right) \qquad (3)$$

where $d$ is the thickness of the specimen and $M$ and $M_\infty$ are the amount of material sorbed or desorbed at times $t$ and $\infty$, respectively.

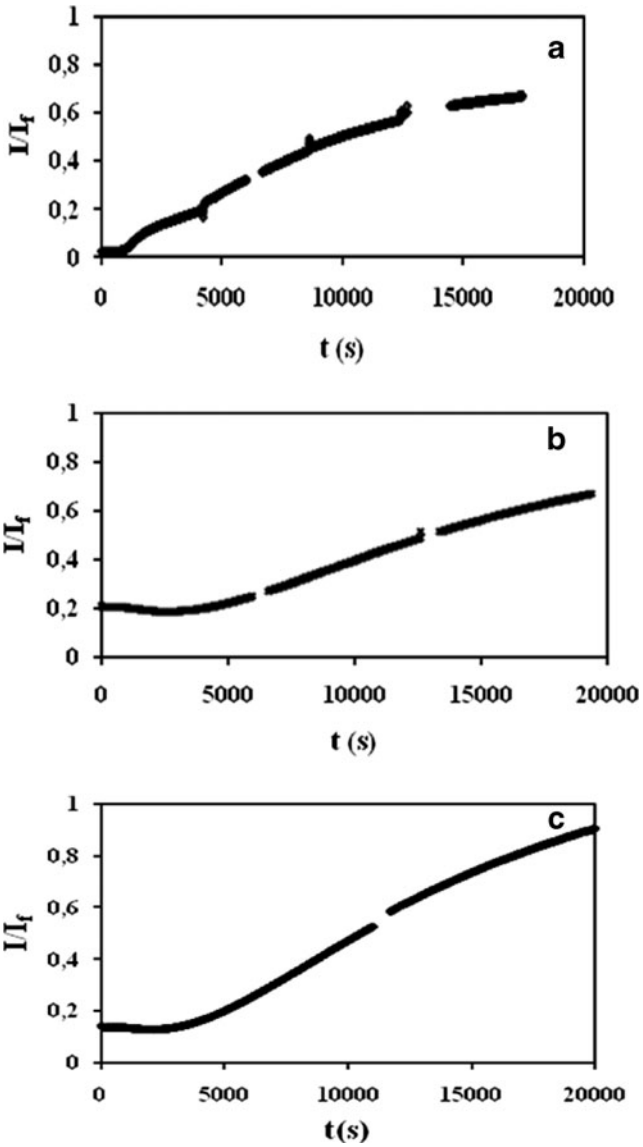


**Fig. 4** The plots of normalized fluorescence intensities versus time during slow release from the beads crosslinked with **a** $Ca^{2+}$, **b** $Ba^{2+}$, and **c** $Al^{3+}$ ions, respectively

In the desorption experiments, if the alginate beads crosslinked with various cations at the bottom of the cell are assumed to be thin slabs, then the corresponding solution of Eq. (3) for short times and $n=0$ can be given as follows (30,31)

$$\frac{I}{I_f} = 4\left(\frac{D_{sl}}{\pi d^2} t\right)^{1/2} \qquad (4)$$

where, $D_{sl}$ is the slow release coefficient and $d$ is chosen as the thickness of the single layer structure. Here it is assumed that the fluorescence intensities $I$ and $I_f$ of the pyranine released from the alginate beads are proportional to the amount of material desorbing at time $t$ and $\infty$. The fit of Eq. (4) to the

data in Fig. 4 is given in Fig. 5 where $D_{sl}$ values were produced from the slope of linear lines. The behavior of $D_{sl}$, EE and the initial amount of pyranine for the beads are given in Fig. 6 and Table 1. The results predict that slow release coefficients follow an order of $Al^{3+} > Ca^{2+} > Ba^{2+}$, while the EE and the initial amount of pyranine, however, follow the reverse order i.e. $Al^{3+} < Ca^{2+} < Ba^{2+}$. The results can be explained in terms of the extent of crosslinking and the size of the cations involved in the beads. Since $Ba^{2+}$ and $Ca^{2+}$ ions are divalent, their bonding to the alginate is expected to occur in a planar two dimensional manner inside the beads [32]. However, since $Ba^{2+}$ ions has the largest radius (1.74 A°) as

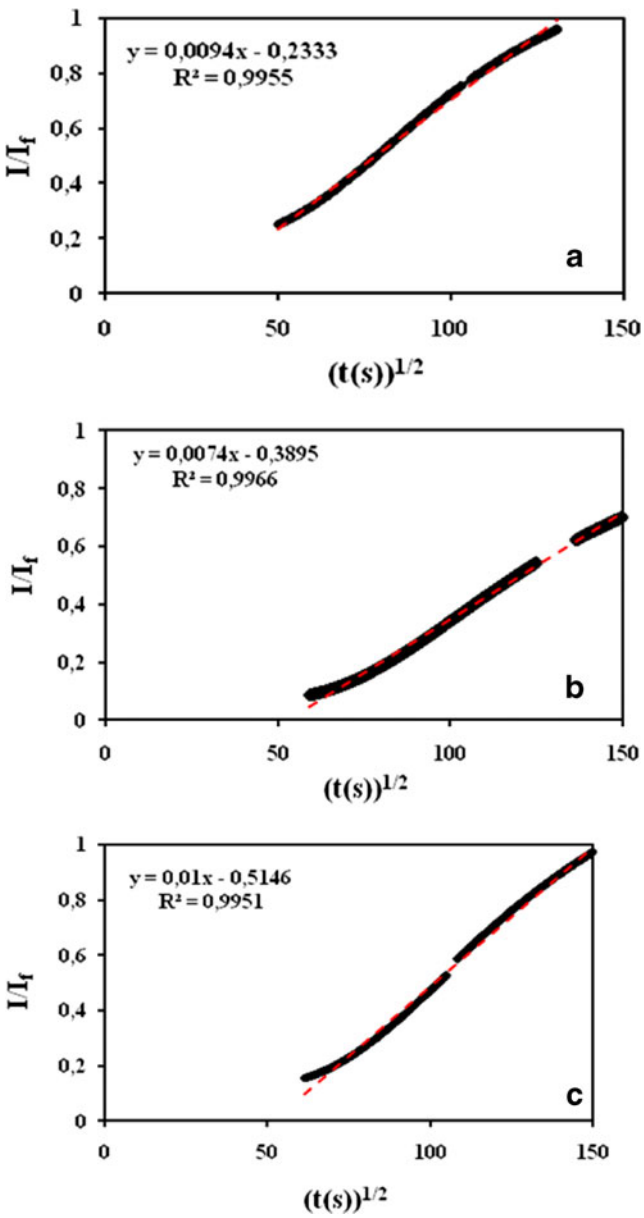


**Fig. 5** Linear regression of the data in Fig. 4 according to Eq. (4). The slope of the straight line produced the slow release coefficient $D_{sl}$ for alginate beads **a** $Ca^{2+}$, **b** $Ba^{2+}$, and **c** $Al^{3+}$ ions, respectively

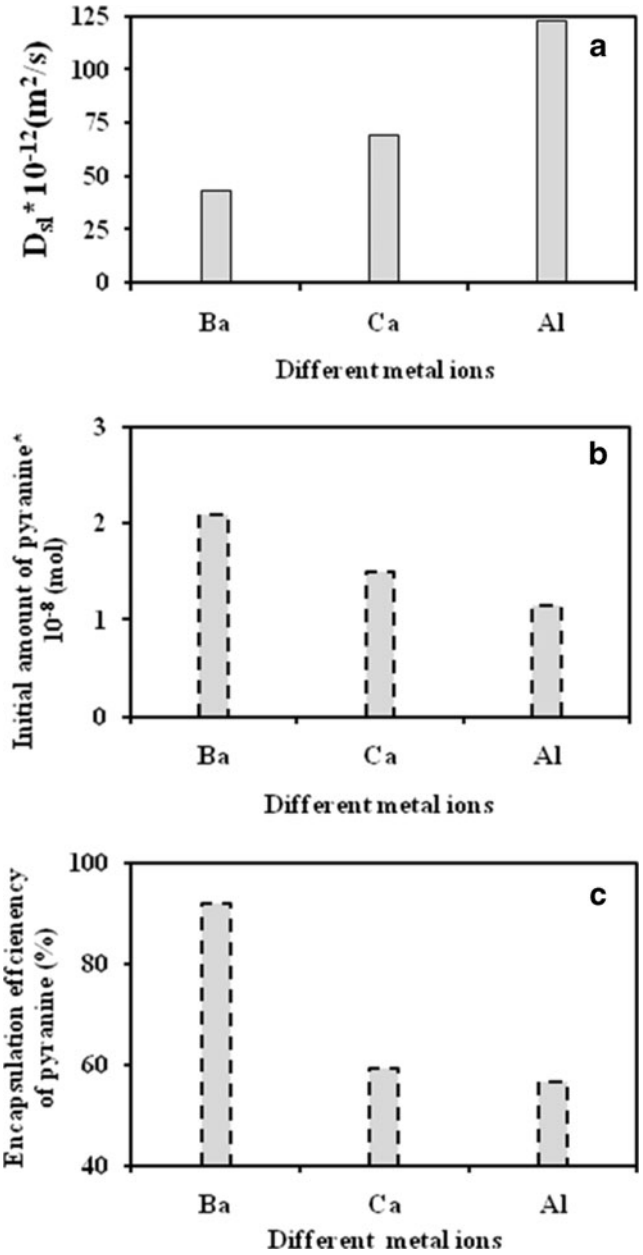


**Fig. 6** Graphical presentation of **a** Slow release coefficient, **b** the initial amount of pyranine and (**c**) encapsulation efficiency of the beads crosslinked with different metal ($Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$) ions, respectively

compared to the other two cations (i.e.1.14 A° for $Ca^{2+}$ and 0.68 A° for $Al^{3+}$ ions), it is supposed to fill a larger space between the alginate and pyranine molecules, producing a tight arrangement with smaller voids. Therefore, the removal of pyranine molecules from the barium alginate beads is hindered, resulting in the lowest release coefficient and the highest EE, and the initial amount of pyranine. In the case of calcium alginate beads, although two dimensional crosslinking occurs, due to the relatively smaller size of $Ca^{2+}$ ions compared to the $Ba^{2+}$ ions the pyranine release from $Ca^{2+}$ beads is much faster, leading to a greater release coefficient and smaller EE and

initial amount of pyranine compared to the release from the $Ba^{2+}$ beads.

The trivalent $Al^{3+}$ ions are expected to form a three-dimensional valent bonding structure with the sodium alginate. This three-dimensional bonding results in extended crosslinking throughout the whole bead. This is because the crosslinking occurs in two different planes at the same time resulting in a compacting of the alginate molecules [32]. Because $Al^{3+}$ ions have the smallest size among the three crosslinking cations (i.e. 0.68 A°), its diffusion from the beads into the outer solution is relatively faster compared to that of $Ba^{2+}$ ions, which consequently results in less water uptake than by the beads crosslinked with $Ba^{2+}$ ions. In other words, in spite of the three-dimensional crosslinking, it exhibits greater water uptake than $Ba^{2+}$ ion-crosslinked beads. Moreover, because of the extended three-dimensional crosslinking, the beads exhibit greater stability. Hence it can be concluded that the nature of crosslinker cations exerts a great influence on the swelling and degradation behavior of beads. Moreover, the beads crosslinked with $Ba^{2+}$ ions exhibit fair stability with minimum water uptake. From the above discussion, it is clear that $Al^{3+}$ ion-crosslinked alginate beads are sufficiently strong and have low water uptake.

## Conclusion

In this study, we compared the slow release coefficients, the encapsulation efficiencies and the initial amount of pyranine in alginate beads crosslinked with different metal ($Ca^{2+}$, $Ba^{2+}$, and $Al^{3+}$) ions, respectively. The steady state fluorescence method was used to produce emission intensities from pyranine to monitor the slow release processes, and Fick's diffusion model was employed to produce slow release coefficients. It was found that the slow release coefficients of different cationic beads follow the order $Al^{3+}>Ca^{2+}>Ba^{2+}$. The encapsulation efficiency and initial amount of pyranine shows the reverse order with respect to the slow release coefficients. The results obtained can be explained in terms of the extent of crosslinking and the size of the cations involved in the beads.

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