Confocal fluorescence mapping of pH profile inside hydrogel beads: Regulation of the internal pH of alginate beads using an alkali insoluble/acid soluble basic buffer (magnesium hydroxide)

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Abstract

Hydrogel beads are particularly promising vehicles for the encapsulation, protection, and release of bioactive agents in foods, supplements, and pharmaceuticals. However, most hydrogel beads are highly porous so that their internal pH is typically fairly similar to that of the surrounding solution, which can lead to degradation of encapsulated pH-sensitive bioactives. In this study, hydrogel beads were fabricated with self-regulating internal pH microclimates by encapsulating $\text{Mg}(\text{OH})_2$ inside them. This buffer is insoluble at neutral pH, but dissolves under acidic conditions thereby releasing OH^- ions that neutralize H^+ ions. A quantitative fluorescence confocal laser scanning microscopy (CLSM) method was developed to map the local pH inside the hydrogel beads, which was based on trapping a pH-sensitive dye (fluorescein tetramethylrhodamine dextran, FRD) inside the beads. Hydrogel beads were prepared by injecting a solution containing alginate (gelling agent), $\text{Mg}(\text{OH})_2$ (buffer) and FRD (pH probe) into another solution containing calcium chloride (cross-linking agent) using a commercial encapsulation unit. The fluorescence CLSM method was then used to detect the pH profile inside the beads after they were exposed to highly acidic simulated gastric fluids (SGF). Before exposure to SGF, the pH profile inside buffer-free beads was fairly uniform (pH 6.8-7.0), whereas the pH at the center of the hydrogel beads was slightly higher than that at the edge for $\text{Mg}(\text{OH})_2$ -loaded beads (pH 7.6-7.2). After exposure to SGF, the pH inside buffer-free beads rapidly became highly acidic (around pH 3), whereas the pH inside $\text{Mg}(\text{OH})_2$ -loaded beads remained fairly similar to the initial value (around pH 7.5). The CLSM method developed in this work may be useful for quantifying the local pH in other systems, while the buffer-loaded hydrogel beads may be useful for encapsulation of acid-labile bioactives.

Keywords: alginate; hydrogel beads; buffer; fluorescence imaging; gastric conditions.

1. Introduction

There is growing interest in the design and fabrication of particle-based delivery systems to load, protect and release bioactive agents in the food, supplement, and pharmaceutical industries (Badrudodoza, Godfrin, Myerson, Trout, & Doyle, 2016;

Janes, Calvo, & Alonso, 2001). A diverse range of bioactive agents with differing molecular, physicochemical and biological properties need to be encapsulated, including proteins, peptides, probiotics, prebiotics, vitamins, minerals, nutraceuticals, triacylglycerol oils, flavors, colors, antimicrobials, and preservatives (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012; Zhu, Mallery, & Schwendeman, 2000). For example, many bioactive proteins and peptides need to be encapsulated because of their propensity to cause bitterness or astringency in the mouth, or their susceptibility to denaturation or degradation in the stomach and small intestine (Liu & Schwendeman, 2012). Similarly, probiotic bacteria often need to be encapsulated to maintain their viability when exposed to highly acidic gastric conditions, but then released in the colon where they exhibit their beneficial actions on colonic microflora (Li, et al., 2017). Many hydrophobic bioactive agents (such as carotenoids, curcumin and polyphenols) need to be encapsulated to prevent their chemical degradation within foods and the gastrointestinal tract (GIT) (Altunbas, Lee, Rajasekaran, Schneider, & Pochan, 2011; Zhang, Zhang, & McClements, 2016).

Numerous studies have shown that particle-based delivery systems can be designed to improve the handling, dispersibility, stability, and bioavailability of certain bioactive agents (Keen, Slater, & Routh, 2012; McClements, 2016). However, to be effective these delivery systems must be carefully designed based on the molecular and physicochemical properties of the bioactives, the processing and storage conditions they are exposed to (such as pH, ionic strength, and temperature), and the desired quality attributes of the final products (such as optical, rheological, organoleptic, and stability properties). For certain applications, hydrogel beads are particularly suitable for encapsulation and delivery of bioactive agents (McClements, 2016). These hydrogel beads are typically spherical particles whose interiors are comprised of a porous network of cross-linked hydrophilic polymers. Hydrogel beads are often formed from natural polymers (such as polysaccharides and proteins) using relatively mild preparation conditions, and so they usually have little adverse effects on the properties of the encapsulated bioactives (Kim, Bae, & Okano, 1992; Zhang, Zhang, Zou, & McClements, 2016).

Many bioactive agents are highly susceptible to physical, chemical, or biological degradation over a certain pH range, but stable at other pH values. For example, some natural colors fade rapidly under highly acidic pH conditions, but slowly under neutral conditions (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Similarly, many probiotics are rapidly inactivated under highly acidic conditions, but viable under neutral conditions (Anselmo, McHugh, Webster, Langer, & Jaklenec, 2016; Li, et al., 2016). A major drawback associated with the utilization of conventional hydrogel beads for encapsulation of pH-labile bioactives is that they are highly porous (Zhang, Zhang, Chen, Tong, & McClements, 2015), and so small acidic or basic ions (such as H^+ or OH^-) can easily diffuse in or out of them, thereby causing the internal pH to be fairly similar to the external pH. Consequently, pH-labile substances may be rapidly degraded when they are encapsulated in hydrogel beads that are exposed to destabilizing pH conditions. The pH sensitivity of conventional hydrogel beads therefore limits their utilization for certain applications in the food, supplement, and pharmaceutical industries. Attempts have been made to improve the pH-stability of bioactive agents in hydrogel beads by delaying the diffusion of acidic or basic ions into them, *e.g.*, by reducing the pore size of the internal core or the outer shell (Mei, et al., 2014). Nevertheless, these approaches have had limited success because the pH-determining ions involved are so small (< 1 nm) that they can rapidly diffuse through even small pores.

In the current study, hydrogel beads were fabricated with self-regulating internal pH microclimates. This was achieved by trapping a basic buffer (magnesium hydroxide) that was insoluble under neutral conditions but soluble under acidic conditions inside the beads. Initially, the hydrogel beads are formed under neutral conditions so that the buffer remains in an insoluble form. However, when the beads are submerged in an acidic environment some of the magnesium hydroxide dissolves, which releases OH^- ions that can neutralize the H^+ ions, thereby maintaining a neutral internal pH (until all the buffer has dissolved). A quantitative fluorescent method was developed to map the local pH inside the hydrogel beads when they were exposed to different pH conditions. This method involved encapsulating a pH-sensitive fluorescent probe inside of the hydrogel beads. The probe used has two functional

groups, one that is pH sensitive and one that is pH insensitive, so that the pH can be determined quantitatively from the ratio of the fluorescence intensity of these two groups. Moreover, the probe is attached to a high molecular weight polymer (dextran) so that it can be trapped successfully trapped within the biopolymer (alginate) network that makes up the interior of the hydrogel beads.

2. Materials and methods

2.1. Materials

Fluorescein tetramethylrhodamine dextran (FRD, average M_r 70,000) was purchased from Molecular Probes (Eugene, OR). Alginic acid (sodium salt), calcium chloride, and magnesium hydrate were purchased from the Sigma Chemical Company (St. Louis, MO). Sodium phosphate was purchased from Fisher Chemical Company (Pittsburgh, PA). All chemicals used were analytical grade. Double distilled water was used to make all solutions.

2.2. Hydrogel beads preparation

Aqueous alginate solutions were prepared by dispersing powdered alginic acid (0.8%, w/w) into phosphate buffer solution (5 mM, pH 7.0) and then continuously stirring at 60 °C for an hour. The temperature was then reduced to 35 °C and the samples were continually stirred until the solution became clear indicating that the alginate had fully dissolved. 10 mg/mL FRD in phosphate buffer (5 mM, pH 7.0) was then added to the alginate solution (at a volume ratio of 1:200) with or without 0.3% $Mg(OH)_2$. After ensuring the sample was homogeneous by stirring, the mixture was injected into a 10% w/w calcium chloride solution using a commercial encapsulation unit (Encapsulator B-390, BUCHI, Switzerland) with a 120 μm vibrating nozzle to prepare the hydrogel beads. The encapsulation device was operated under fixed conditions: frequency = 800 Hz; electrode potential = 800 V; and operating pressure = 500 mbar. The alginate beads formed were kept in the Ca^{2+} solution for 1 h at ambient temperature to promote cross-linking and bead hardening.

2.3. Simulated gastric conditions

The impact of exposure of the hydrogel beads to simulated gastric fluid (SGF) on their internal pH was determined using a method described previously (Zhang, Zhang, Zhang, Decker, & McClements, 2015). Hydrogel beads (with or without encapsulated $\text{Mg}(\text{OH})_2$) were added at a ratio of 1:4 (w/w) to SGF that had been preheated to 37 °C and adjusted to pH 2.5. This mixture was then incubated in an incubated shaker for 2 h at 37 °C to mimic stomach conditions.

2.4. Confocal Laser Scanning Microscopy

Images were obtained using confocal laser scanning microscopy with a 20× objective lens (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, USA). The images of the ratiometric fluorescence dye were obtained using emission wavelengths of 543 and 488 nm, and detection wavelengths/bandwidths of 650 nm/LP and 590 nm/50 nm, respectively. All samples were imaged using an exposure time of 0.5 s and a 12.5% excitation power level for both channels. The complete images of each sample were typically acquired in less than 2 min with at least eight measurements. The microstructural images for confocal microscopy were analyzed using image analysis software (NIS-Elements, Nikon, Melville, NY, USA).

2.5. Standard curve preparation

A stock FRD solution (10 mg/mL) was prepared by dissolving the powdered FRD dye in phosphate buffer (5 mM, pH 7) solution. 5 $\mu\text{L}/\text{ml}$ FRD stock solution at pH 2.5–7 (adjusted by hydrochloric acid) was imaged using confocal microscopy to determine the linear range of the pH response. Solutions containing different concentrations of FRD (2.5, 5 and 10 $\mu\text{L}/\text{ml}$) were also used to prepare a standard curve using a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) to assess the impact of dye concentration on the fluorescence intensity ratio used to calculate the local pH.

2.6. Image processing for pH measurement

The images were analyzed using Image J Software (1.50I, imagej.nih.gov). The ratio of pixel intensities of two images obtained from two wavelengths (488 and 543

nm) were calculated and correlated with the pH obtained from the standard curve. The images were processed by repeated scans with frame averaging from at least eight measurements. The pH gradient within the beads was determined by dividing their circular images into a series of same-pixel thickness concentric rings, and the mean pixel intensity was calculated for each ring from the number of pixels and individual pixel intensities in the ring.

3. Results and Discussion

3.1. Development of pH standard curve

In this study, relatively small hydrogel beads (around 200 μm) were fabricated using a specialized extrusion device (Encapsulator) with a vibrating nozzle (**Figure 1**). The small dimensions and viscoelastic nature of these beads meant that it was not possible to measure their internal pH using traditional methods, such as pH electrodes. It was therefore necessary to develop an alternative method to measure the pH within the beads so as to confirm the potential of the encapsulated $\text{Mg}(\text{OH})_2$ to control their internal pH profile. A fluorescent probe method was therefore developed to determine the local pH profile inside the beads. A dextran conjugated fluorescent probe, Fluorescein and Tetramethylrhodamine (FRD), was used as the pH indicator. This probe was selected because it has a relatively high molecular weight (around 70 kDa), which ensures that it remains trapped inside the porous biopolymer network that makes up the bead interior. Moreover, this probe is a ratiometric dye that has both pH-dependent (FITC) and pH-independent (TMR) fluorescence groups. For this type of dye, the pH-independent channel can be used as an internal standard for quantitative pH evaluation (Chen & Arriaga, 2006; Deriy, et al., 2009). This ratiometric method also eliminates problems associated with photo-bleaching, variable bead thickness, and non-uniform dye distribution within the bead (Cope, Hibberd, Whetstone, MacRae, & Melia, 2002; Li & Schwendeman, 2005).

In preliminary confocal microscopy studies, it was observed that the fluorescence emission intensity was pH-independent when an excitation wavelength of 543 nm was used, but pH-dependent when an excitation wavelength of 488 nm was used (data not

shown). The pH of a sample could therefore be obtained independent of dye concentration by taking the ratio of the emission intensities at these two excitation wavelengths (543 nm/ 488 nm). Standard curves of fluorescence intensity ratio versus pH were determined using a series of buffer solutions (pH 2.5-7.0) with dye concentrations of 2.5, 5 and 10 $\mu\text{L}/\text{mL}$ of stock solution (10 mg/mL). The fluorescence intensity ratio was exponentially related to pH from pH 2.5 to 7.0 when determined from the confocal microscopy images (**Fig. 2a**), but linearly related when measured using a microplate reader (**Fig. 2b**). Moreover, the fluorescence intensity ratio (TMR/FITC) was largely independent of overall dye concentration across the entire pH range as determined using the microplate reader. These results suggest that the pH inside the beads can be successfully mapped even though there may be some local variations in dye concentration within the beads *e.g.*, due to diffusion of dye outwards. The major advantage of the confocal microscopy approach is that the local pH can be obtained from the images, whereas only the overall pH of a sample can be obtained using the microplate reader.

3.2 Mapping the pH inside the beads

The highly acidic pH of human gastric juices causes an unwanted reduction in the activity of many orally delivered bioactive agents, such as many enzymes, probiotics, and nutraceuticals. Consequently, an antacid buffering agent ($\text{Mg}(\text{OH})_2$) was encapsulated inside the hydrogel beads to create an internal pH that was considerably higher than the external pH, thereby protecting any co-encapsulated acid-sensitive bioactives. Under neutral pH conditions, $\text{Mg}(\text{OH})_2$ forms insoluble solid particles that are retained within the hydrogel beads. However, when the beads are exposed to acid conditions some of the $\text{Mg}(\text{OH})_2$ particles will dissolve, thereby releasing OH^- ions that will neutralize any H^+ ions that diffuse into the hydrogel interior, thereby helping to maintain a high internal pH.

Hydrogel beads were formed by injecting a solution containing gelling agent (alginate) and fluorescence dye (FRD) in the absence or presence of insoluble buffer particles (magnesium oxide) into another solution containing a cross-linking agent (calcium chloride). The initial beads formed by this method had mean diameters of

around 200 μm as determined by confocal fluorescence and optical microscopy (**Fig. 3 and 4**). Initially, the average internal pH values of the hydrogel beads determined from the CLSM images were around pH 6.5 for buffer-free beads and around pH 7.5 for buffer-loaded beads. The presence of $\text{Mg}(\text{OH})_2$ slightly increased the internal pH of the buffer-loaded hydrogel beads, which can be attributed to the fact that it is a weak base and some dissolution may have occurred. The confocal images also indicated that the pH values inside the beads were not uniformly distributed after $\text{Mg}(\text{OH})_2$ encapsulation (**Fig. 3b**). There appeared to be microenvironments around the insoluble $\text{Mg}(\text{OH})_2$ particles that had a higher pH than the surrounding solution. The pH gradient distribution analysis indicated that the pH was relatively uniform throughout the buffer-free hydrogel beads, being in the range from pH 6.8-7 (**Fig. 4c**). Conversely, the pH was slightly lower at the edge of the buffer-loaded hydrogel beads than in their interiors, with the local pH values increasing from around pH 7.2 at the edge to pH 7.6 at the center (**Fig. 5c**). Presumably, most of the insoluble buffer particles were located in the center rather than at the edge of the hydrogel beads.

3.3 Monitoring pH changes after exposure to gastric conditions

Many types of bioactive agents are degraded when exposed to the highly acidic conditions found in the human stomach, and therefore it would be valuable to have delivery systems that protected them within the gastric fluids. In this section, we therefore applied the confocal fluorescence pH mapping method to monitor changes in the internal pH of the hydrogel beads in the absence and presence of encapsulated magnesium hydroxide. After incubation in simulated gastric fluids (SGF), the fluorescence intensity inside the buffer-free hydrogel beads rapidly decreased to around pH 3 (**Figs. 6 and 7**). Presumably, the small H^+ ions rapidly diffused through the porous alginate network within the hydrogel beads driven by the concentration gradient between the inside and outside of the beads. Consequently, the internal pH of the buffer-free beads was fairly similar to that of the external pH of the SGF after 2 h incubation in the gastric phase. Conversely, the pH value within the $\text{Mg}(\text{OH})_2$ -loaded hydrogel beads remained fairly similar to that of the original value ($\approx$ pH 7.5) after 2 hours of incubation in the acidic SGF (**Figs. 6 and 7**). This result suggests that

these antacid hydrogel beads could be used to protect acid-sensitive bioactives during passage through the stomach. The confocal fluorescence images suggest that the pH was higher in the center of the beads than at their edges (**Fig. 6**). This effect is probably because the insoluble $\text{Mg}(\text{OH})_2$ particles in the exterior regions of the beads dissolve first as hydrogen ions from the SGF diffuse into the beads. To verify this dissolution process, DIC optical images of the $\text{Mg}(\text{OH})_2$ -loaded hydrogel beads were taken throughout the simulated stomach phase (**Fig. 8**). These images clearly indicated that $\text{Mg}(\text{OH})_2$ particles slowly dissolved during the stomach phase incubation, which can be attributed to their neutralization reaction with hydrogen ions arising from the gastric fluids.

3.4 Dynamic pH change during incubation

The acid degradation of many bioactives is a rapid process, and so it is important to determine how long the hydrogel beads can maintain their high internal pH values after exposure to gastric fluids. Therefore, the internal pH of buffer-free and buffer-loaded hydrogel beads was periodically determined throughout the stomach phase (**Fig. 8**). In the presence of buffer, the average pH inside the beads remained fairly constant (pH 6.6 to 7.5) throughout the entire 2 hour incubation period within the SGF (**Fig. 7**). This result suggested that the amount of $\text{Mg}(\text{OH})_2$ inside the beads was sufficient to act as a local antacid throughout the duration of the gastric phase. On the contrary, the pH value inside the buffer-free hydrogel beads fell sharply from pH 6.5 to 3.3 within the first 5 minutes, followed by a more gradual decrease at longer incubation times. As mentioned earlier, small H^+ ions can rapidly diffuse into highly porous hydrogel beads, thereby causing the pH inside the beads to be fairly similar to that outside.

The amount of time required for hydrogen ions to diffuse into the hydrogel beads can be estimated from the following expression (Crank, 1975):

$$\Phi = \frac{M(t)}{M(\infty)} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{D_{gel} n^2 \pi^2 t}{a^2}\right) \quad (1)$$

Here, Φ is the fraction of hydrogen ions that have diffused into the hydrogel beads at time t , $M(t)$ and $M(\infty)$ are the hydrogen ion concentrations within the hydrogel beads at time t and at equilibrium, n is an integer, a is the radius of the hydrogel beads, and D_{gel} is the diffusion coefficient of the hydrogen ions through the biopolymer network inside the beads, which can be estimated using the following expression (Chan & Neufeld, 2009):

$$D_{gel} = D_w \exp\left(-\pi\left(\frac{r_H - r_f}{\xi + 2r_f}\right)^2\right) \quad (2)$$

Here, D_w is the diffusion coefficient of the hydrogen ions through pure water, r_H is the hydrodynamic radius of the hydrogen ions, r_f is the cross-sectional radius of the biopolymer chains (alginate) in the hydrogel network, and ξ is the mesh pore diameter of the hydrogel network. The following structural parameters have been reported for alginate hydrogels: pore diameter (ξ) = 4 to 400 nm; alginate chain radius (r_f) = 0.83 nm (Chan, et al., 2009). The radius of hydration (r_H) of the hydrogen ion (actually H_3O^+) has been estimated to be 0.1 nm (Marcus, 2012). The diffusion coefficient of hydrogen ions in water can be estimated from the expression: $D_w = k_B T / 6\pi\eta r_H$, where k_B is Boltzmann's constant, T is absolute temperature, and η is the viscosity of the solvent. The above equations were used to estimate the time-dependence of the fraction of hydrogen ions diffusing into hydrogel beads with different pore sizes after submerging in a more acidic environment (**Figure 9**). For these calculations, it was assumed that the pore diameter (ξ) of the alginate beads was 10 nm. Even assuming this relatively small pore size, the theoretical predictions suggest that the hydrogen ions would rapidly diffuse into the hydrogel beads in a few seconds, which supports our experimental results. These predictions suggest that any acid-sensitive bioactives encapsulated within the hydrogels beads would be rapidly exposed to highly acid conditions in the stomach in the absence of a co-encapsulated insoluble buffering agent.

4. Conclusions

Certain types of bioactive agents are degraded when exposed to highly acidic conditions, such as probiotics, enzymes, and some nutraceuticals. The development of

microparticle-based delivery systems that are able to control the pH in the immediate vicinity of these bioactive agents may therefore be useful for improving their stability. In this study, edible hydrogel beads were fabricated using a simple injection method that were able to maintain an internal pH that was appreciably higher than the pH of the surrounding solution. A fluorescence method to map the pH profile inside the hydrogel beads was also developed based on ratiometric imaging with a confocal laser scanning microscopy. This method showed that the average internal pH of the buffer-free hydrogel beads was around 6.5, while that of the buffer-loaded beads was around 7.5. In addition, the method showed that there were some local variations in pH inside the hydrogel beads. After exposure to simulated gastric fluids, the average pH inside the buffer-loaded beads remained fairly constant (around pH 6.6 to 7.5), whereas that in the buffer-free beads fell sharply within the first few minutes (from pH 6.5 to 3.3). The confocal microscopy method also indicated that the pH was higher in the center of the buffer-loaded beads than at their edges. These results show that the encapsulated $\text{Mg}(\text{OH})_2$ particles acted as effective buffering agents that inhibited excessive acidification within the beads during the gastric phase. Overall, the novel hydrogel beads developed in this study may be suitable for encapsulation and delivery of acid-sensitive bioactives, which may be advantageous for certain food, supplement, and pharmaceutical applications.

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6. References

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Figure 1. Schematic diagram showing the fabrication of self-adjusting microclimate pH hydrogel beads. The beads are formed by injecting alginate and insoluble buffer into a cross-linking calcium ion solution.

(a)

(b)

Figure 2. Standard curve of pH versus fluorescence intensity ratio (TMR/FITC) taken by (a) microplate at concentrations of 2.5, 5 and 10 $\mu\text{L}/\text{ml}$ stock solution (10 mg/mL) and (b) confocal microscopy at 5 $\mu\text{L}/\text{ml}$ stock solution (10 mg/mL).

(a)

(b)

Figure 3. Differential interference contrast (DIC) and fluorescent microscopy images of (a) hydrogel beads without $\text{Mg}(\text{OH})_2$ encapsulation and (b) with 0.3% $\text{Mg}(\text{OH})_2$ encapsulation. These images were taken after the beads were prepared. The images of the ratio channel were generated using Image J software (imagej.nih.gov) and the color strip shown on the top right represents the pseudocolor change with pH (intensity of TMR signal was enhanced using Image J to improve contrast).

Figure 4. An illustration of the image processing approach used to generate the pH gradient profile within buffer-free hydrogel beads. (a) The fluorescence intensity ratio image with pseudocolor color; (b) concentric rings of similar pixel thickness obtained from the images of the beads; and (c) the average pH value within each ring as a function of distance from the bead edge.

Figure 5. An illustration of the image processing approach used to generate the pH gradient profile within $\text{Mg}(\text{OH})_2$ -loaded hydrogel beads. (a) The fluorescence intensity ratio image with pseudocolor color; (b) concentric rings of similar pixel thickness obtained from the images of the beads; and (c) the average pH value within each ring as a function of distance from the bead edge.

Figure 6. Confocal fluorescence microscopy images of hydrogel beads without $\text{Mg}(\text{OH})_2$ encapsulation (top) and with 0.3% $\text{Mg}(\text{OH})_2$ encapsulation (bottom) after passage through the stomach phase. The images of the ratio channel were generated using Image J software and the color strip (top right of images) represents the pseudo-color change with pH. The intensity of the TMR signal was enhanced using Image J to improve the contrast.

Figure 7. The pH value of hydrogel beads without and with $\text{Mg}(\text{OH})_2$ encapsulation during 2h stomach digestion process.

Figure 8. Differential interference contrast (DIC) images of hydrogel beads containing encapsulated $\text{Mg}(\text{OH})_2$ taken during incubation in the simulated stomach phase.

Figure 9. Predicted time dependence of the fraction of hydrogen ions (H_3O^+) absorbed by alginate beads with a diameter of $200\text{ }\mu\text{m}$ and a pore size of 10 nm after submersion in an acidic environment.

“Confocal fluorescence mapping of pH profile inside hydrogel beads (microgels) with controllable internal pH values”

Zhang et al

Food Hydrocolloids

Graphical Abstract

The fluorescent images of hydrogel beads with and without buffer encapsulation after going through stomach phase (Intensity ratio image was taken based on a pH molecule probe).