Removable Colored Coatings Based on Calcium Alginate Hydrogels

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Received April 7, 2006; Revised Manuscript Received June 1, 2006

This article describes the creation of a nontoxic, biodegradable coating using calcium alginate and FD&C approved dyes. The coating is robust but is rapidly removed upon treatment with disodium ethylenediamine tetraacetate (EDTA). Dye leaching from calcium alginate films was studied, and it was determined that the efficiency of dye retention is proportional to the degree of cross-linking. Degradation rates were studied on calcium alginate beads serving as a model for a coating. We determined that degradation rates depend on the gel's cross-linking and on the amount of EDTA used. Bead size also influenced the degradation rates; smaller beads degraded faster than larger beads. We show that the coating can be used as an easily removable and environmentally friendly logotype on an artificial turf surface. Applications of these coatings can be extended to food, cosmetic, medicinal, and textile uses and to wherever nontoxic, easily removable colored coating is desired.

Introduction

Sodium alginate is an anionic linear polysaccharide composed of (1−4)-linked D-mannuronic and L-glucuronic acid repeat units (Figure 1).[1−3] These sugar residues can either be arranged in blocks or they can be randomly distributed. Calcium and other divalent cations can chelate carboxylate groups and make cross-links between chains, forming an insoluble, nonthermoreversible hydrogel.[4] Calcium alginate is a firm, clear, and quick-setting gel that is tough but flexible when dried. The stability of calcium alginate hydrogel depends on the stability of calcium complexes within the gel. Thus, chelating agents that strongly bind calcium can quickly solubilize the hydrogel. Compounds capable of such quick calcium removal include sodium citrate and disodium ethylenediamine tetraacetate (EDTA).

Calcium alginate is a valuable material (sales of sodium alginate exceed $100 million/year) used to make microcapsules for drug delivery and immobilization of biocatalysts, coatings for medical implants, food thickeners and coatings, textile print thickeners, and a wide variety of other applications where its unique barrier, ion chelating, and biocompatible properties are useful.[5−11] We have not, however, encountered any examples where dyed calcium alginate films were used to create colored coatings that can be applied and removed on demand. Temporary field lines and logos for sports complexes and roadway markings, as well as coatings for plants, fruit, and the body are just a few examples that underscore where removable coatings could be applied. Colored removable coatings of a biodegradable nature would certainly be a nice addition to the white removable coatings developed here at Cornell University.[12] Herein, we demonstrate that thin films composed of dyed calcium alginate make excellent coatings that are readily removed by application of an aqueous EDTA solution. We support these results with leaching and degradation rate studies. To our knowledge, this is the first example of a removable coating produced from environmentally benign materials.

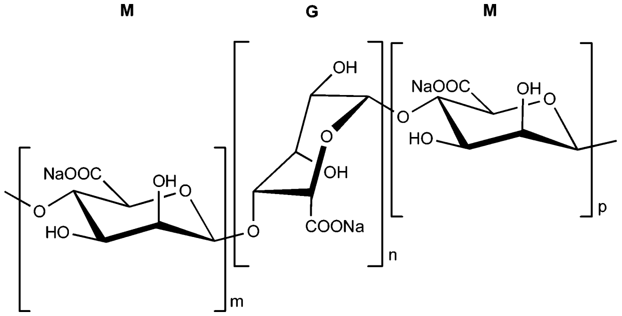

Figure 1. Sodium alginate structural units. M, mannuronate residue; G, glucuronate residue.

Experimental Section

Materials. All materials were used as received. Sodium alginate (from *Macrocystis pyrifera* (Kelp), low viscosity, viscosity of 2% solution at 25 °C: 250 cps), and Reactive Green 19 (practical grade) were obtained from Sigma-Aldrich. Red food color (containing water, propylene glycol, FD&C Reds 40 and 3, and propylparaben as a preservative) was a McCormick product purchased in a local supermarket. Calcium chloride (anhydrous, 4−20 mesh pellets) and disodium ethylenediamine tetraacetate (EDTA) were obtained from Fisher Scientific. A piece of artificial turf was a generous gift from Brian Hunt, Re-Markable Paint Company. To prevent different extents of gelation between different samples, one solution of sodium alginate was used during the whole study.

Equipment, Characterization. A. In turf tests, a TLC sprayer (Kontes, part #422501−0125) was used to disperse sodium alginate solution colored with red food color. Air-pressure was provided by a pump.

B. A syringe pump was used in the preparation of beads.

C. Scanning electron microscopy images were obtained on a Leica 440 SEM at the Cornell Center for Materials Research. Both film and beads were characterized at 25 kV after sputter coating with palladium−gold.

D. Optical microscopy images of hydrated beads were obtained on a Leica DMIL with a mounted Sony DSC−F717 digital camera.

E. ImageJ software (NIH, http://rsb.info.nih.gov/ij/) was used to measure perimeters (px) of calcium alginate beads. Using Microsoft

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10.1021/bm060341q CCC: $33.50

Figure 2. Removal of the logotype (colored with Red Food Color) from the artificial turf surface: (A) Dried logotype before the treatment, (B) letter "C" sprayed with 0.1M EDTA solution (using a hand-held spray bottle) and letter "U" sprayed the same way with water, and (C) total removal of "C" with a water wash while "U" remains visible on the surface.

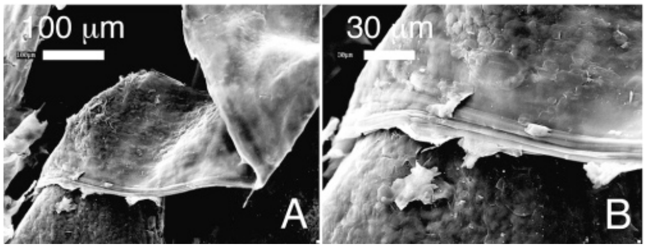


Figure 3. (A) SEM micrograph of an alginate film lifted off of the artificial turf surface after drying. (B) Higher magnification of the same micrograph.

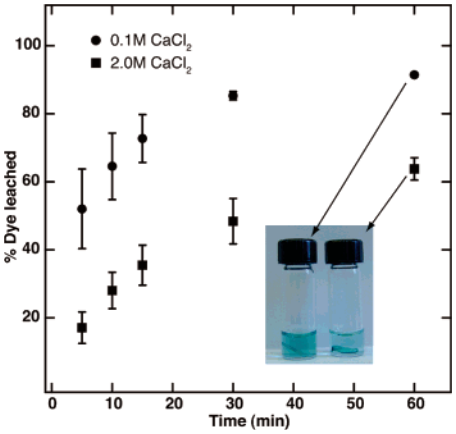


Figure 4. Relative rate of leaching of the dye out of the films lifted off the artificial turf as a function of $CaCl_2$ concentration.

Excel, perimeters were converted to diameters (μm), and the mean size and standard deviation were calculated.

F. Electronic absorption (UV) spectra were recorded on a Cary 50 Bio UV/Vis spectrophotometer.

Methods. A. Preparation of Films for the Leaching Study. Sodium alginate (1% aqueous solution w/v) containing reactive green 19 (1 g/L) was dispersed onto a piece of artificial turf previously sprayed with an aqueous solution of calcium chloride (0.1 or 2.0 M). The calcium chloride solution was applied using a simple hand-held spray bottle, whereas the alginate solution was sprayed using a TLC sprayer air-pressured by the pump. Upon drying, pieces of the film were removed and used in the leaching study.

B. Preparation of Beads for the Degradation Study. To a calcium chloride solution (0.1M, 1.0M, or 2.0M) stirring at 200 rpm in a crystallization dish (Kimax, 90 × 50), an aqueous sodium alginate solution (1% w/v) containing Reactive Green 19 (1 g/L) was extruded through a 27 or 30 gauge needle (at 1.0 mL/min or 3.0 mL/min pumping rate) using a syringe pump from a distance of 20 cm (see Figure 5). The amount of beads produced was controlled by regulating the amount of time the pump was operating (i.e., at 1.0 mL/min and 30 s of extrusion the amount of beads corresponded to 0.5 mL of sodium alginate used). The resulting beads were allowed to harden for 30 min before being used in the degradation study.

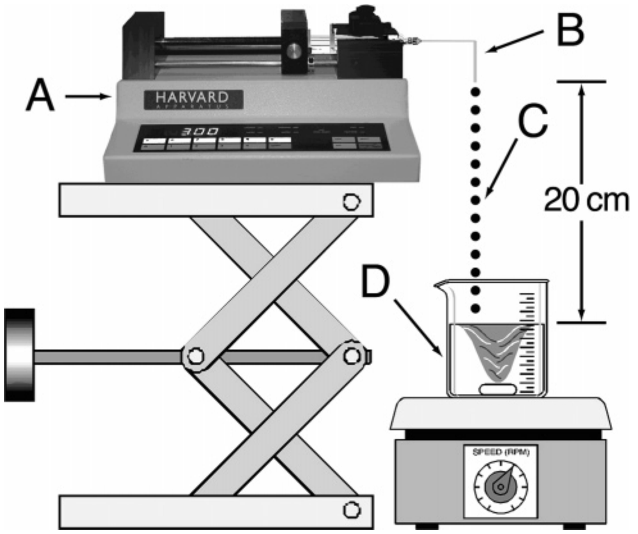


Figure 5. Method of alginate bead preparation: (A) Syringe pump at flow rates of 1−3 mL/min, (B) 27 or 30 gauge needle bent at 90° angle, (C) extruded 1% sodium alginate/dye aqueous solution, and (D) receveing bath of 0.1M to 2.0M $CaCl_2$ stirring at 200 rpm.

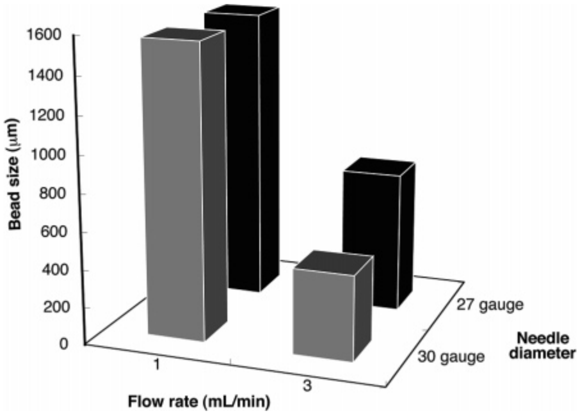


Figure 6. Bead size as a function of flow rate and on extrusion needle diameter.

C. Leaching Study. A piece of film (average area 56 mm^2) was placed in a vial and filled with water (1 mL). Absorbance at 624 nm was measured at 5, 10, 15, 30, and 60 min time marks. At 1 h time mark, EDTA solution (1 mL, 0.5M, made in basic water to promote dissolution) was added, and the film was dissolved. Final absorbance (multiplied by a factor of 2 to adjust for volume change) was considered to be 100% dye leaching. All previous measurements were normalized to this value. In a separate experiment, it was determined that calcium cations, when present in high concentration (∼3.0 M), can lower the absorbance of the dye to about 95% of its value. This was not of a concern since there was never this concentration of calcium upon degradation of even the highest cross-linked beads. All other components of the assays (alginate, EDTA, basic water) were found to have no effects on the absorbance of the reactive green 19.

D. Degradation Study. Each sample contained an amount of beads corresponding to 0.5 mL alginate (30 s of extrusion at 1 mL/min) except for the samples in the assay investigating degradation rate as a function of size. In that case, the amount of beads corresponding to 1.5 mL of alginate was used (30 s of extrusion at 3 mL/min or 90 s of extrusion at 1 mL/min). Hardened, hydradated beads were placed in a filter syringe and strained. At time zero, EDTA (5 mL, solution in basic water, see Figure 8 for concentration) was drawn into the syringe, and an absorbance measurement was taken at regular time intervals. The highest absorbance (corresponding to the total dissolution of all beads) was considered to be 100% bead degradation. All measurements within the same sample were normalized to this value. The final absorbance

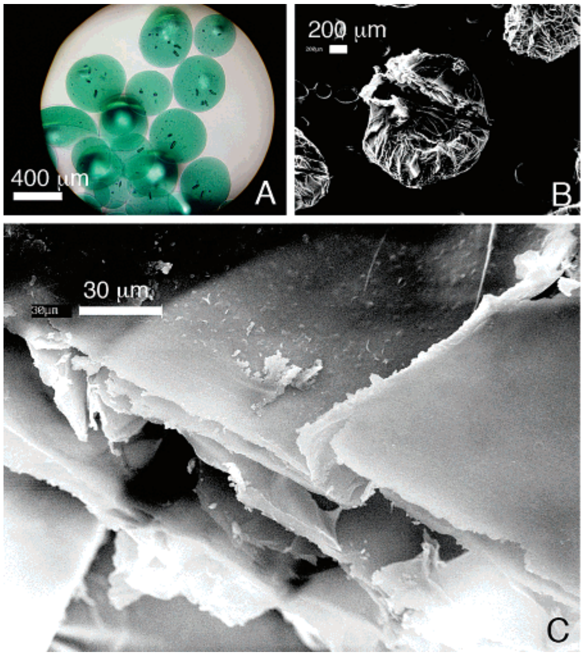


Figure 7. (A) Optical micrograph of alginate beads. (B) SEM micrograph of lyophilized beads, and (C) a detail of a bead's cross-section made by a razor blade.

was not significantly affected by the presence of EDTA and calcium cations, as discussed in section C.

Results and Discussion

The main focus of our study was to make and remove a colored coating from an artificial turf surface. As outlined in Scheme 1, our coating approach is very simple. A surface coated with $CaCl_2$ (gelling agent) was sprayed with an aqueous sodium alginate solution containing dye. An air-pressurized atomizer was used to disperse the alginate solution into very fine droplets of gel (<10 μm as determined by scanning electron microscopy). These droplets then coalesced into a thin film. The dried image composed of a colored alginate thin film was stable to a water jet and would not come off until the image was treated with an aqueous solution of EDTA.

To remove the colored alginate film from the artificial turf, one needs to remove calcium ions from the gel's interior using EDTA and wash away the soluble residue with water (Figure 2). Upon incubation with EDTA and a short wait (a couple of minutes), a hand-held spray bottle filled with water provided enough pressure to quickly remove the film and wash away any traces of color and/or alginate from the surface (letter "C"). As evident from Figure 2, the portion of the logotype treated only with water wash was not removed (letter "U"). Simple water spray treatment only slightly faded the letter "U".

We examined the calcium alginate film that was spray-cast using scanning electron microscopy (SEM) in order to gain more insight into its formation and properties. SEM micrographs at low magnification revealed a smooth featureless surface (Figure 3). At higher magnification, the image exposed outlines of spheres on the surface. This suggested that the film was comprised of coalesced droplets. As previously hypothesized, the calcium alginate film consists of intergelated micron-sized beads that form from the spray cast by the atomizer (TLC sprayer).

We decided to study the influence that cross-linking has on dye leaching from the alginate films. Two alginate coatings containing the dye reactive green 19 were spray cast onto artificial turf already spray-coated with two different $CaCl_2$ concentrations, 0.1 and 2.0 M. The resulting thin films, upon drying, were scraped from the turf and used in the leaching assay. The leaching assay subjected these films to a situation comparable to 5/16 in. (0.8 cm) of rain falling onto a field without drainage. The results (Figure 4) confirmed our hypothesis that the films with a higher degree of cross-linking would leach more slowly. Within 1 h 91% of the dye had leached out of the 0.1 M $CaCl_2$ cross-linked film, whereas 64% of the dye had leached out of the 2.0 M $CaCl_2$ cross-linked film. This difference was visible even to the naked eye, as shown by the image inset within Figure 4. The amount of cross-linking had an influence not only on the leaching rate but also on the morphology of the formed films. Upon drying on the turf, the 0.1 M $CaCl_2$ cross-linked film was brittle and could be easily removed, whereas the 2.0 M $CaCl_2$ cross-linked film adhered well and was difficult to remove from the turf. This is important

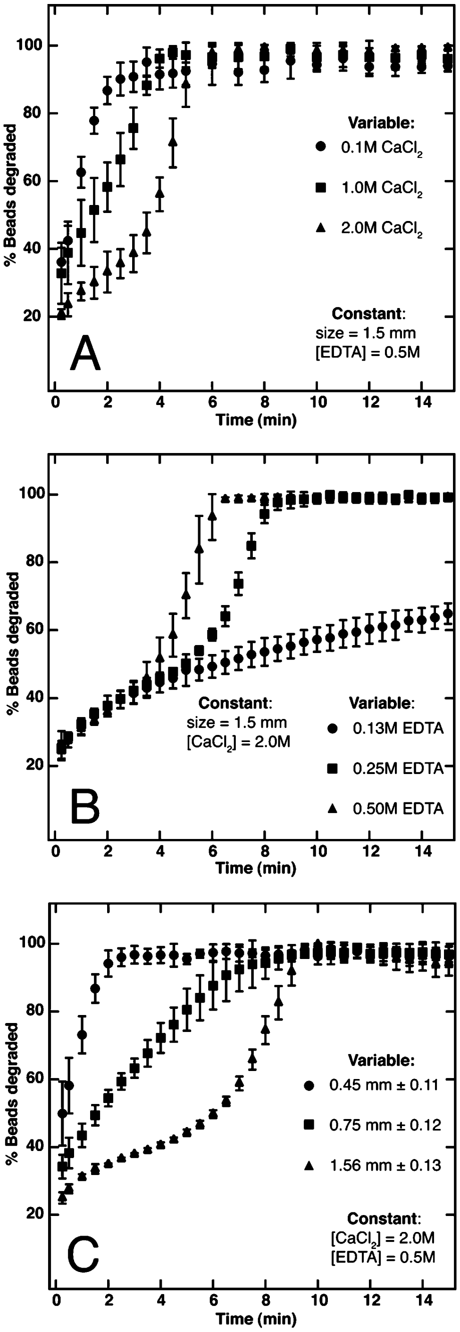
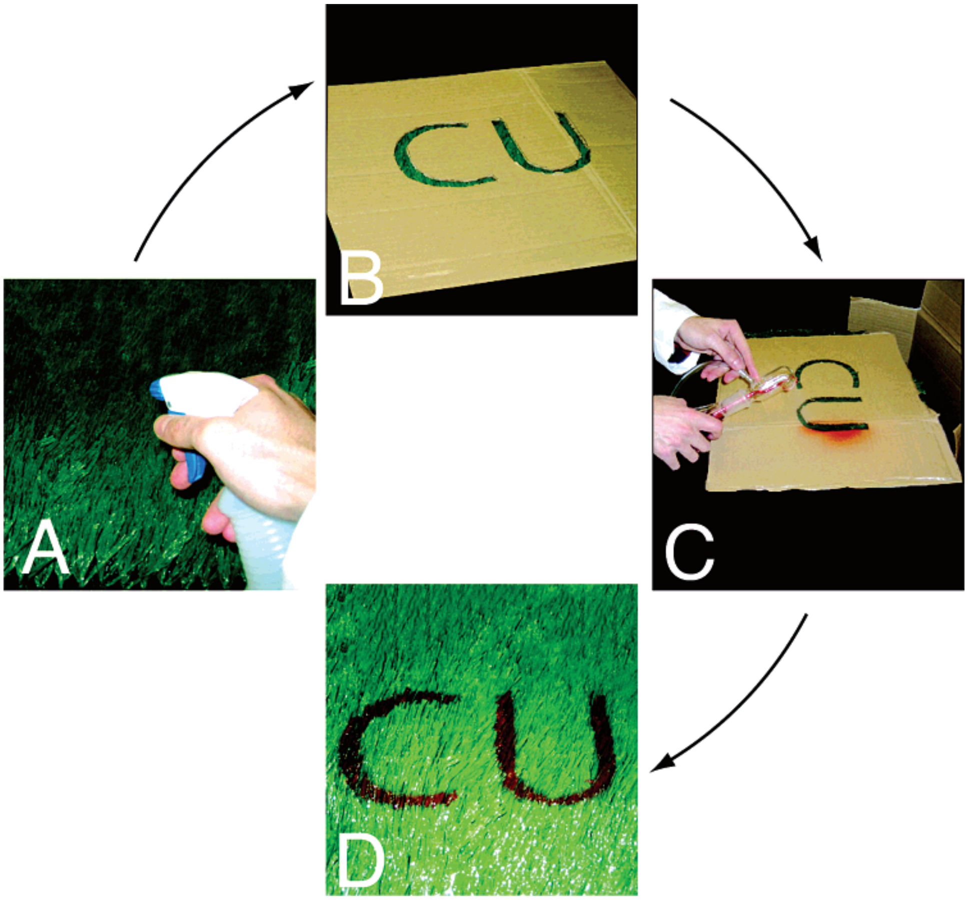


Figure 8. Degradation studies of alginate beads as a function of (A) $CaCl_2$ concentration, (B) EDTA concentration, and (C) average bead size.

Scheme 1. Application of the Logotype onto the Artificial Turf Surface[a]



[a] (A) An aqueous $CaCl_2$ solution is sprayed onto the surface, (B) a mask (template) is placed on the turf, (C) a colored alginate solution (red food color, 1% v/v) is sprayed over the template, and (D) a logotype is revealed upon mask removal.

for removable coating applications because the quality of the coating is equally as important as rapid degradation. The large error bars at early time points in Figure 4 indicate that the consistency of the film depended not only on the operator of the atomizer but on the cross-linking as well. The variability of film thickness plays a large role in the relative rates of leaching. Dye located within the middle of the thicker portion of the film would be expected to leach more slowly, as shown by Milagre et al. in their double layer calcium alginate capsules.[9]

To better understand the calcium alginate properties relevant to removable colored coatings, we wanted to address the degradation rate and its dependence on the amount of cross-linking, the amount of chelating agent used to degrade the coating, and the relative morphology of the coating. Films are inherently more difficult to study relative to beads, as seen in the relatively large error associated with the leaching studies; therefore, we chose to use well-defined beads as a model system.

We prepared calcium-alginate beads using a dripping method.[13,14] A syringe filled with an aqueous sodium alginate/dye solution was fitted with a needle bent at 90°. The syringe and needle were loaded onto a syringe pump, and the contents of the syringe were pumped through air and allowed to drop into a bath containing calcium chloride (Figure 5). The height was adjusted to a distance that allowed reproducible droplets to form. As shown in Figure 6, at a flow rate of 1 mL/min, the calcium-alginate bead size is constant as a function of needle inner diameter (ID). At a slow flow rate (1 mL/min), we obtained beads with an 8% coefficient of variation (CV). At a higher flow rate (3 mL/min), the bead size was dependent on the needle ID. The size of the extruded droplet (and subsequently the size of the bead formed) was smaller in the case of the smaller needle diameter. The ability to decrease bead size at a higher flow rate was offset by the higher CVs of 16% and 23% observed for 27 and 30 gauge needles, respectively.

We examined the beads by light and scanning electron microscopy (Figure 7). Light microscopy of the freshly prepared, hydrated beads revealed a generally spherical shape and smooth surface. Insoluble debris that was visible inside the beads was due to the encapsulated dye (reactive green 19), which was obtained as practical reagent grade. The SEM image of the lyophilized beads also exposed a smooth but wrinkled surface. Cross-sectioned beads, however, revealed a complex onion-like layered structure. The complexity of this structure suggests that both the formation and drying (lyophilization) processes happen in stages.

Using the calcium alginate beads, we investigated the influence of $CaCl_2$ concentration, EDTA concentration, and bead size on degradation rate (Figure 8). In the first experiment, the concentration of $CaCl_2$ used to cross-link the beads was varied, while the size of the beads and concentration of EDTA was held constant (Figure 8A). Each sample contained an amount of beads corresponding to 0.5 mL of alginate extruded. Dye released from the interior of the beads due to degradation was detected in the supernatant as a function of time using UV/vis spectroscopy. As expected, beads with higher cross-linking took longer to dissolve and release the contained dye. Time of dissolution was very short, and in all three cases, EDTA was used in excess. Absorbance maxima, corresponding to total dissolution of dye-impregnated beads, correlated with the time where no more beads could be visually observed. This absorbance maximum, as evident from the plot, was stable from that point on, indicating that the degradation process was complete.

It was hypothesized that the EDTA concentration would greatly influence the degradation rate of calcium alginate beads. In the second degradation experiment, the concentration of EDTA was varied while all other variables were held constant and the rate of degradation of calcium alginate beads was measured (Figure 8B). As in the first degradation experiment (Figure 8A), the amount of beads used in each sample corresponded to 0.5 mL of alginate extruded. As expected, higher concentrations of EDTA promoted faster bead degradation. In the case of 0.13 M EDTA (Figure 8B), the EDTA concentration was too low and the beads did not disassemble and dissolve completely in 15 min. The rise in absorbance over time was due to diffusion and partial dye release.

Another important parameter for the degradation rate is the size of the beads that are being degraded. In the third assay of the degradation rate, the size of the beads was varied while all other parameters were held constant (Figure 8C). As shown by Kikuchi et al., smaller beads degrade faster than larger beads.[15] This was certainly the case in our system as well, as was evident from the slower degradation rate of large (1.5 mm) beads in comparison to the medium (0.7 mm) and small-sized (0.5 mm) beads. For this assay, three times the amount of beads were used than for A or B (corresponding to 1.5 mL of alginate extruded); hence, the comparable degradation curve was slower than in A or B. This is further evidence that the degradation rate depends not only on the accessibility of the calcium ions to EDTA molecules but on the relative amounts of reagents as well. Another interesting feature of the degradation curves in this study was their shape. Although small and large beads had degradation curves with sharp transitions just before total dissolution, medium-sized beads showed gradual degradation without the sharp transition. We attribute this to the pulsatile nature of release as shown by Kikuchi et al.[15] For large beads, the majority of calcium ions were located in the outer shell whereas the majority of the dye was located in the middle of the bead in a semigel, semiliquid environment. Once EDTA chelated most of the outer shell calcium ions, this interior spilled out immediately. In the case of the medium-sized beads, this pulsatile release was absent due to the high coefficient of variation of the size, which prevented uniform degradation and therefore prevented this sharp transition to occur. Small-sized beads degraded too quickly; thus, nothing can be said about the pulsatile nature of the dye release.

Conclusions

In conclusion, we report an easy protocol for the preparation of a colored, nontoxic, biodegradable calcium alginate coating. We show that both the leaching rate of the dye from the coating as well as the degradation rate of the coating depend on the amount of cross-linking and the amount of calcium chelator used. We also show that calcium alginate coatings can be effectively used as a convenient removable coating for an artificial turf surface. Colored calcium alginate coatings can find their application in food, cosmetic, medical, textile, and other industries due to the ease of application and removal as well as their biocompatibility. All of the materials used in preparation and removal of such coatings are cheap, readily available, and environmentally friendly.

Acknowledgment. We thank Dreyfus and Beckman Foundation, NSF (Sensors), NSF-MRSEC (CCMR), ARO MAP-MURI, NYSTAR, Tri-Institutional Training Program in Chemical Biology, Rohm and Haas, and 3M for support.

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BM060341Q