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Glucose Oxidase-Mediated Gelation: A Simple Test To Detect Glucose in Food Products

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ABSTRACT: This paper reports a simple, rapid, and sugar-selective method to induce gelation from glucose-containing samples. This method employs glucose oxidase (GOx) to selectively "recognize" and oxidize glucose to generate gluconic acid, which acts to solubilize calcium carbonate and release calcium ions. The release of calcium ions triggers gelation of the calcium-responsive polysaccharide alginate to form a calcium-alginate hydrogel. Rheological measurements confirm that gel formation is triggered by glucose but not fructose or sucrose (consistent with GOx's selectivity). Vial inversion tests demonstrate that gel formation can be readily observed without the need for instrumentation. Proof-of-concept studies demonstrate that this gel-forming method can detect glucose in food/beverage products sweetened with glucose or high-fructose corn syrups. These results indicate that the enzyme-induced gelation of alginate may provide a simple means to test for sweeteners using components that are safe for use on-site or in the home.

KEYWORDS: *alginate, glucose oxidase, high-fructose corn syrup (HFCS), hydrogel, stimuli responsive biopolymer*

INTRODUCTION

Economic, political, and climatic instabilities affect the global sugar market, and historically the U.S. price for table sugar (sucrose) has been substantially higher than world prices.[1] The abundance of starch and thus glucose syrup was an attractive alternative, but glucose is less sweet than sucrose. The discovery that the enzyme xylose isomerase could catalyze the conversion of glucose to the sweeter monosaccharide fructose[2] coupled with advances in enzyme immobilization enabled the creation of large-volume processes to generate high-fructose corn syrup (HFCS).[3] HFCS emerged as a less expensive sweetener (compared to sucrose) and was broadly accepted into the marketplace (e.g., soft drinks) beginning in the early 1970s.[4−6] At the same time that HFCS emerged as a major sweetener, there was an increase in the incidences of obesity and diabetes in affluent countries (e.g., the United States) leading to hypotheses and controversies over whether the timing of these observations reflects a coincidental correlation or a causal relationship.[6−9]

Whereas the relative merits of sugar, glucose, and HFCS may be unresolved scientifically,[10] many consumers have formed preferences and purchase products on the basis of the labeled ingredient statements.[11] Unfortunately, ingredient statements may not be entirely trustworthy when a product is manufactured from many ingredients, each of which may have a supply chain that spans continents, cultures, and languages. Furthermore, strong cost incentives may encourage adulteration by substituting lower cost sweeteners (e.g., HFCS) for more expensive sugar. The existence of glucose in HFCS and other artificial sweeteners makes it a potential marker for the detection of non-sucrose sweeteners. Thus, a simple method to detect glucose on-site or in the home may allow buyers or consumers to make more informed decisions.

Analysis of glucose in foods and beverages is challenging because of the complexity of the matrix and the structural similarities of various components (e.g., other sugars). A similar challenge was faced in the development of in-home tests for blood glucose, which became integral to the individualized management of diabetes.[12−15] In this case, enzymes such as glucose oxidase (GOx) were enlisted to "recognize" glucose and generate an electrochemically active species (e.g., H_2O_2) that allows transduction into an electrical output.[16−18] Here, we also enlist glucose oxidase to recognize glucose, but we transduce this recognition into a visually observable mechanical output.

Figure 1 schematically illustrates the recognition−transduction approach used to detect glucose by inducing gelation of the polysaccharide alginate. First, GOx catalyzes the

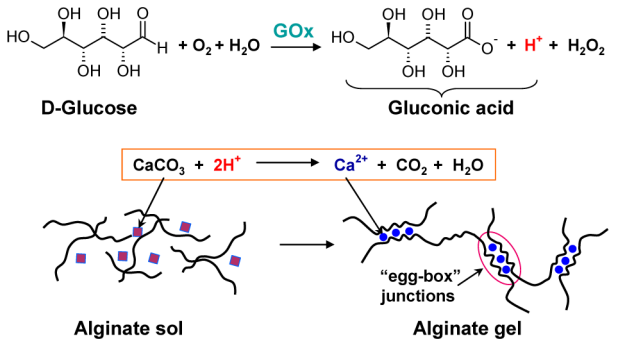

Figure 1. Schematic illustration of glucose oxidase (GOx)-mediated gelation of alginate. GOx-catalyzed oxidation of glucose generates protons, which solubilize the Ca^{2+} ions that trigger alginate's hydrogel formation.

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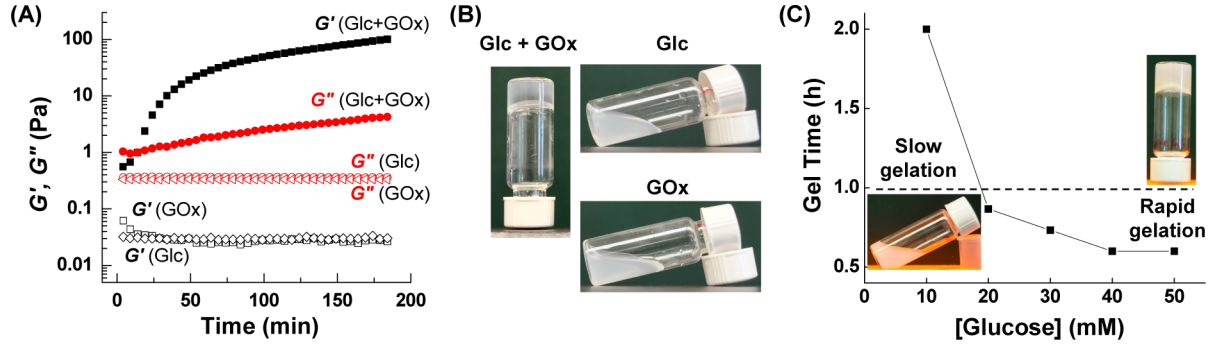

Figure 2. Demonstration that the GOx-catalyzed oxidation of glucose (Glc) triggers alginate gelation. (A) Time sweeps of rheological measurements show that both GOx (10 U/mL) and Glc (40 mM) are required to induce gelation of a mixture of alginate (1%) and $CaCO_3$ (20 mM). (B) Vial inversion tests provide visual evidence that both GOx and Glc are required to form a self-supporting gel in 1 h. (C) Vial inversion tests show that gels are formed within 1 h for solutions containing >20 mM glucose.

oxidation of glucose in the presence of oxygen to generate gluconic acid and hydrogen peroxide.[19−21] The in situ-generated gluconic acid dissociates, and the protons react to solubilize $CaCO_3$ and release Ca^{2+} ions.[22] The released Ca^{2+} interacts with alginate to form the "egg-box" network junctions that serve as the physical cross-links responsible for the gelation of calcium alginate hydrogels.[23−27]

Here, we demonstrate that the GOx-mediated oxidation of glucose can trigger gelation of the common food biopolymer alginate. GOx confers sugar selectivity to this process, whereas gel formation is a readily observable measure that requires no specialized instrumentation and is insensitive to color in the sample (compared to color-based tests).

MATERIALS AND METHODS

Materials. The following materials were purchased from Sigma-Aldrich: alginic acid sodium salt from brown algae (medium viscosity, molecular weight 80−120 kDa), D-(+)-glucose (≥99.5%), D-(−)-fructose (≥99%), sucrose (≥99.5%), and GOx from *Aspergillus niger* (138800 U/g). Precipitated calcium carbonate ($CaCO_3$) particles (70 ± 21 nm as reported by the manufacturer) were obtained from Specialty Minerals, Birmingham, UK. Syrup, table sugars, and beverages were purchased from local grocery stores.

Sample Preparation. Alginate solutions (1−1.5%) were prepared by dissolving sodium alginate powder in distilled water, followed by stirring overnight; then $CaCO_3$ (20−30 mM) particles was dispersed into sodium alginate solution, followed by ultrasonication for 30 min. These alginate and $CaCO_3$ levels are in excess of those required to form strong self-supporting gels. The alginate/$CaCO_3$ thus prepared (pH ∼8.0) was stirred before use to ensure the particles remained homogeneously dispersed in the alginate solution. No aggregation or settling of the particles was observed over a period of several hours, during which time the gel-forming experiments were conducted. GOx solution was prepared by dissolving GOx (1000 U/mL) in phosphate-buffered saline (20 mM, pH 7.4). Purified sugars (glucose, fructose, or sucrose) were dissolved in water to a concentration of 0.5 M before use. Food products (e.g., beverages) containing different sugar-based sweeteners were diluted in water to levels of 30 mg sugars/mL on the basis of information provided on the ingredient statement.

Gel Formation. Typically, we prepared a stock suspension containing GOx (10 U/mL)/alginate (1.5%)/$CaCO_3$ (30 mM) and then mixed this stock suspension with a diluted solution containing the sugar-based product (30 mg sugars/mL). The mixed suspension (pH ∼7.5) was briefly vortexed and exposed to air for 1−3 h. Photographs were typically taken 1 h after the mixing.

Rheology. Rheological measurements were performed on a Rheometrics AR2000 stress-controlled rheometer (TA Instruments). A cone-and-plate geometry of 40 mm diameter with a 2° cone angle was used with a solvent trap to prevent drying. Time sweeps were typically performed at 10 rad/s with strains of 25−30% for liquid-like samples and 1% for gel-like samples. Dynamic stress sweep experiments were first performed on a sample to identify its linear viscoelastic (LVE) region, and dynamic frequency sweeps were then performed within the LVE region. All rheological experiments were conducted at 25 °C.

RESULTS AND DISCUSSION

In our initial study, we used rheology to demonstrate GOx-mediated gelation of an alginate/$CaCO_3$ suspension. In the first experiment, a mixture of alginate (1%), $CaCO_3$ (20 mM), and glucose (40 mM) was prepared, and GOx (10 U/mL) was added to initiate the reaction. This sample was loaded onto the rheometer stage that had been set to 25 °C, and data were recorded 4 min after initiation of the reaction. Figure 2A shows that when GOx was added to the glucose/alginate/$CaCO_3$ suspension, the elastic modulus (G') and viscous modulus (G'') increased during the 180 min experiment. The increase in G' was faster than that in G'', and after 14 min G' exceeded G'', indicating that the solution was transitioning into a gel.[28] Figure 2A also shows results for two controls lacking either glucose or GOx. For both controls, the moduli remained small and nearly constant over the course of the experiment, and G'' exceeded G'. These observations indicate that the controls remained solutions throughout the 180 min experiment. Thus, the results in Figure 2 provide a rheological demonstration that the GOx-mediated oxidation of glucose can trigger the gelation of alginate as proposed in Figure 1.

Gel formation is a simple end-point measurement as it can be detected visually without the need for instrumentation.[29] For instance, the results in Figure 2A can be reproduced qualitatively using a vial inversion test. For this test, we mixed alginate (1%), $CaCO_3$ (20 mM), GOx (10 U/mL), and glucose (40 mM) in a 4 mL vial and exposed this mixture to air. Figure 2B shows that after 1 h of incubation, the vial could be inverted and the hydrogel that had formed could support its own weight. Controls lacking either Glc or GOx did not form gels as evidenced by the fact that they remained liquids as shown in Figure 2B.

The limit for detecting glucose by a vial inversion method was estimated by observing gel formation at various glucose concentrations. In this experiment, we mixed alginate (1%), $CaCO_3$ (20 mM), and GOx (10 U/mL) with various amounts of glucose and periodically observed whether the vial's contents had undergone gel formation. Figure 2C shows that the gel time (the time required to form a self-supporting gel)

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decreased with increasing glucose concentration. Solutions prepared with >20 mM glucose formed gels within 1 h, whereas solutions prepared with 10 mM glucose formed weak gels only after 2 h of incubation. Solutions prepared with 5 mM glucose did not form gels even after overnight incubation. The photographs inserted in Figure 2C show the vials for 10 and 50 mM samples after being incubated for 1 h. If we use 1 h as a reasonable incubation period, Figure 2C indicates that 20 mM is the limit for glucose detection by this vial inversion method. This detection limit is an order-of-magnitude lower than glucose levels typically present in beverages sweetened with HFCS.[30]

Next we examined the sugar specificity of the GOx-mediated gel formation. In this study, 40 mM glucose, fructose, or sucrose was added into a suspension of alginate (1%), $CaCO_3$ (20 mM), and GOx (10 U/mL), and these mixtures were exposed to air for 3 h. After incubation, the samples were loaded onto the rheometer stage and dynamic frequency sweeps were performed. Figure 3A shows that when glucose

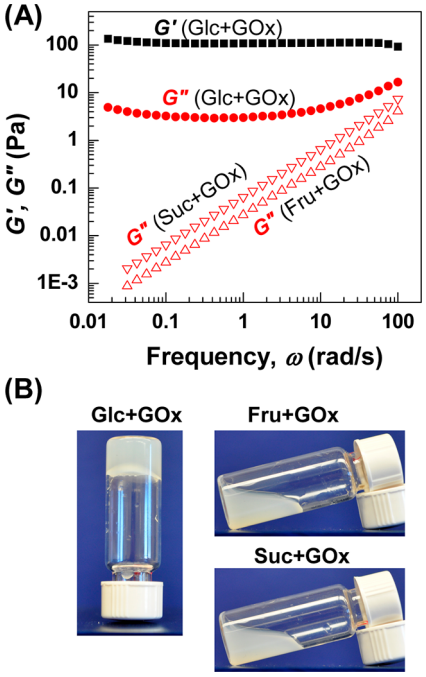

Figure 3. GOx confers sugar selectivity to alginate gelation. (A) Dynamic frequency sweeps demonstrate that a suspension of GOx (10 U/mL)/alginate (1%)/$CaCO_3$ (20 mM) forms a hydrogel in the presence of 40 mM glucose (Glc) but not fructose (Fru) or sucrose (Suc) (samples incubated in air for 3 h; G' values for controls were too low to measure accurately). (B) Vial inversion tests demonstrate sugar selectivity of GOx-mediated gel formation.

was present, the sample behaved as a soft solid with G' exceeding G'' and both moduli being nearly independent of frequency. This behavior is consistent with the formation of a gel from the glucose-containing solution. Figure 3A also shows that the sucrose and fructose controls behave as viscous liquids with the viscous modulus (G'') varying strongly with frequency (note: the elastic modulus G' is not shown for these controls because it was too low to be measured accurately). The rheological behavior for these controls confirms that neither fructose nor sucrose can induce a gel formation in the presence of glucose.

The sugar selectivity for GOx-mediated alginate gelation is also apparent using a vial inversion approach. Using the same experimental conditions as for the above rheology experiment, Figure 3B shows that glucose (but not fructose or sucrose) triggers gel formation for the GOx/alginate/$CaCO_3$ mixture. Thus, Figure 3 demonstrates that GOx confers sugar selectivity to gel formation, and this gel formation can be observed without the need for complex instrumentation.

In subsequent studies, we examined the ability of this GOx-mediated gelation method to detect glucose in food products. For this, we prepared a stock suspension containing GOx (10 U/mL)/alginate (1.5%)/$CaCO_3$ (30 mM). Two parts of this stock suspension were mixed with 1 part of a sugar-containing product that had been appropriately diluted (30 mg sugars/mL) to obtain a reaction suspension containing 10 mg/mL sugar.

Our first sample was store-bought syrup containing HFCS. After mixing the diluted syrup with the stock suspension, the vial was left in air over 1 h. As shown in Figure 4A, a self-

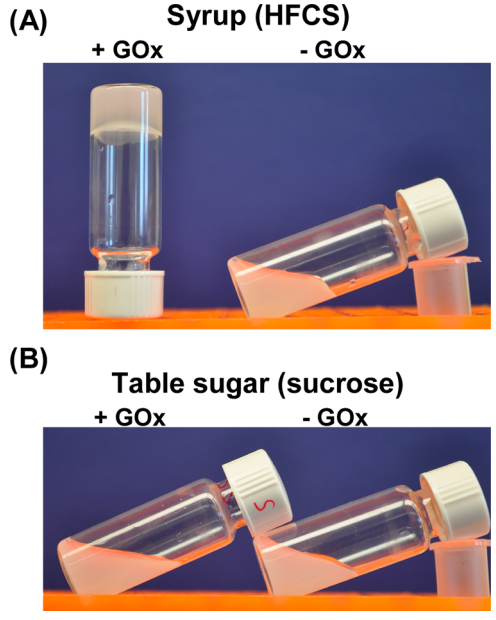

Figure 4. GOx-mediated gel formation for samples containing high-fructose corn syrup (HFCS) but not table sugar (sucrose). (A) Vial inversion tests show that HFCS induces gelation, whereas results from the control lacking GOx indicate that other ingredients in the syrup do not induce gelation. (B) Vial inversion tests show that table sugar does not induce gel formation. Samples were prepared by mixing 1 part of a solution containing sugar product (30 mg sugars/mL) with 2 parts of a stock suspension containing GOx (10 U/mL)/alginate (1.5%)/$CaCO_3$ (30 mM).

supporting gel was formed, indicating the presence of glucose (from HFCS). Concurrently, a control mixture lacking GOx was prepared, and Figure 4A shows that no gel formation was observed. Results from this control indicate that other ingredients in the syrup do not induce gel formation.

Next we tested table sugar (pure cane sugar) by mixing a solution of this sugar with the stock suspension and exposing the sample to air for 1 h. As expected, Figure 4B shows gels were not formed for samples containing table sugar either with or without GOx. Thus, Figure 4 shows that GOx-mediated alginate gelation can detect glucose in the syrup and distinguish it from table sugar.

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As a proof-of-concept we examined the capability of GOx-mediated gelation to detect glucose in beverage products. Our first example was a comparison of two brands of ice tea: the label for ice tea I reports "HFCS", whereas the label for ice tea II reports "real sugar". First, the ice tea was diluted in water to a sugars concentration of 30 mg/mL, and then 1 part of this diluted ice tea was mixed with 2 parts of the stock suspension. After mixing, the vial was left in air for 1 h. As shown in Figure 5A, ice tea I induced gel formation consistent with the presence of glucose in the HFCS. Ice tea II was unable to induce gel formation, as expected for this sucrose-containing beverage. The controls (without GOx) did not gel, indicating no contribution of other ingredients in the ice teas to the gel formation.

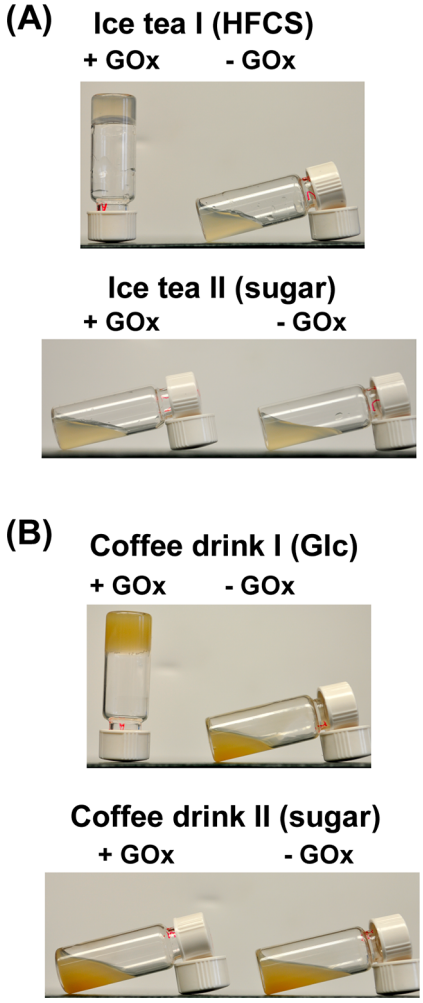

Figure 5. GOx-mediated gel formation to "detect" non-table sugar sweeteners in selected beverages. (A) Vial inversion tests show gelation with an ice tea containing HFCS (ice tea I), but not with an ice tea containing "sugar" (ice tea II). (B) Vial inversion tests show gelation with a coffee drink containing glucose (coffee drink I) but not with a coffee drink containing "sugar" (coffee drink II). Samples were prepared by mixing 1 part of diluted beverage (30 mg sugars/mL) with 2 parts of a stock suspension containing GOx (10 U/mL)/alginate (1.5%)/$CaCO_3$ (30 mM).

As a second proof-of-concept, we examined two coffee drinks: the label for coffee drink I reports "glucose", whereas the label for coffee drink II reports "sugar". The gel-forming experiments were performed as described above. Figure 5B shows that glucose-containing coffee drink I induced gel formation, whereas sugar-containing coffee drink II did not induce gel formation. In sum, the test results in Figure 5 demonstrate that GOx-mediated alginate gel formation allows a simple and rapid detection of glucose, which may serve as a marker for the presence of a non-sucrose sweetener.

In conclusion, we demonstrate a simple and rapid test for glucose-containing sweeteners. This method offers sugar selectivity due to the molecular recognition of the GOx enzyme and transduces recognition into a readily observable mechanical response (i.e., gel formation) that is independent of color in the sample. Furthermore, this method employs common components (GOx and alginate) that should be safe in the kitchen. Thus, we envision this test could be used on-site by ingredient buyers or in homes, stores, or restaurants. More broadly, this work suggests that emerging research on enzyme-induced gel formation for biomedicine[31−34] may find broader applications in the food industry.

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Author Contributions

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) *Sugar and Sweeteners Yearbook Tables*, Tables 3 and 4; USDA Economic Research Service: Washington, DC, updated March 12, 2012.

(2) Marshall, R. O.; Kooi, E. R. Enzymatic conversion of D-glucose to D-fructose. *Science* **1957**, *125*, 648−649.

(3) Bhosale, S. H.; Rao, M. B.; Deshpande, V. V. Molecular and industrial aspects of glucose isomerase. *Microbiol. Rev.* **1996**, *60*, 280−300.

(4) Hanover, L. M.; White, J. S. Manufacturing, composition, and applications of fructose. *Am. J. Clin. Nutr.* **1993**, *58*, S724−S732.

(5) Marriott, B. P.; Cole, N.; Lee, E. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *J. Nutr.* **2009**, *139*, S1228−S1235.

(6) White, J. S. Straight talk about high-fructose corn syrup: what it is and what it ain't. *Am. J. Clin. Nutr.* **2008**, *88*, 1716S−1721S.

(7) Bray, G. A.; Nielsen, S. J.; Popkin, B. M. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am. J. Clin. Nutr.* **2004**, *79*, 537−543.

(8) Tappy, L.; Le, K. A. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol. Rev.* **2010**, *90*, 23−46.

(9) Johnson, R. J.; Segal, M. S.; Sautin, Y.; Nakagawa, T.; Feig, D. I.; Kang, D. H.; Gersch, M. S.; Benner, S.; Sanchez-Lozada, L. G. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular diseased. *Am. J. Clin. Nutr.* **2007**, *86*, 899−906.

(10) Moeller, S. M.; Fryhofer, S. A.; Osbahr, A. J.; Robinowitz, C. B. The effects of high fructose syrup. *J. Am. Coll. Nutr.* **2009**, *28*, 619−626.

(11) Borra, S. Consumer perspectives on food labels. *Am. J. Clin. Nutr.* **2006**, *83*, 1235S−1235S.

(12) Heller, A.; Feldman, B. Electrochemical glucose sensors and their applications in diabetes management. *Chem. Rev.* **2008**, *108*, 2482−2505.
(13) Raba, J.; Mottola, H. A. Glucose-oxidase as an analytical reagent. *Crit. Rev. Anal. Chem.* **1995**, *25*, 1−42.
(14) Vashist, S. K.; Zheng, D.; Al-Rubeaan, K.; Luong, J. H. T.; Sheu, F. S. Technology behind commercial devices for blood glucose monitoring in diabetes management: a review. *Anal. Chim. Acta* **2011**, *703*, 124−136.
(15) Wu, Q.; Wang, L.; Yu, H. J.; Wang, J. J.; Chen, Z. F. Organization of glucose-responsive systems and their properties. *Chem. Rev.* **2011**, *111*, 7855−7875.
(16) Newman, J. D.; Turner, A. P. F. Home blood glucose biosensors: a commercial perspective. *Biosens. Bioelectron.* **2005**, *20*, 2435−2453.
(17) Heller, A.; Feldman, B. Electrochemistry in diabetes management. *Acc. Chem. Res.* **2010**, *43*, 963−973.
(18) Meyer, W. L.; Liu, Y.; Shi, X. W.; Yang, X. H.; Bentley, W. E.; Payne, G. F. Chitosan-coated wires: conferring electrical properties to chitosan fibers. *Biomacromolecules* **2009**, *10*, 858−864.
(19) Wang, J. Electrochemical glucose biosensors. *Chem. Rev.* **2008**, *108*, 814−825.
(20) Johnson, L. M.; DeForest, C. A.; Pendurti, A.; Anseth, K. S.; Bowman, C. N. Formation of three-dimensional hydrogel multilayers using enzyme-mediated redox chain initiation. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1963−1972.
(21) Ikeda, M.; Tanida, T.; Yoshii, T.; Hamachi, I. Rational molecular design of stimulus-responsive supramolecular hydrogels based on dipeptides. *Adv. Mater.* **2011**, *23*, 2819−2822.
(22) Wang, C. Y.; Liu, H. X.; Gao, Q. X.; Liu, X. X.; Tong, Z. Alginate-calcium carbonate porous microparticle hybrid hydrogels with versatile drug loading capabilities and variable mechanical strengths. *Carbohydr. Polym.* **2008**, *71*, 476−480.
(23) Lee, K. Y.; Mooney, D. J. Alginate: properties and biomedical applications. *Prog. Polym. Sci.* **2010**, *37*, 106−126.
(24) Astete, C. E.; Sabliov, C. M.; Watanabe, F.; Biris, A. Ca^{2+} cross-linked alginic acid nanoparticles for solubilization of lipophilic natural colorants. *J. Agric. Food Chem.* **2009**, *57*, 7505−7512.
(25) Shi, X. W.; Tsao, C. Y.; Yang, X. H.; Liu, Y.; Dykstra, P.; Rubloff, G. W.; Ghodssi, R.; Bentley, W. E.; Payne, G. F. Electroaddressing of cell populations by co-deposition with calcium alginate hydrogels. *Adv. Funct. Mater.* **2009**, *19*, 2074−2080.
(26) Cheng, Y.; Luo, X. L.; Betz, J.; Payne, G. F.; Bentley, W. E.; Rubloff, G. W. Mechanism of anodic electrodeposition of calcium alginate. *Soft Matter* **2011**, *7*, 5677−5684.
(27) Javvaji, V.; Baradwaj, A. G.; Payne, G. F.; Raghavan, S. R. Light-activated ionic gelation of common biopolymers. *Langmuir* **2010**, *27*, 12591−12596.
(28) Stokke, B. T.; Draget, K. I.; Smidsrod, O.; Yuguchi, Y.; Urakawa, H.; Kajiwara, K. Small-angle X-ray scattering and rheological characterization of alginate gels. 1. Ca-alginate gels. *Macromolecules* **2000**, *33*, 1853−1863.
(29) Raghavan, S. R.; Cipriano, B. H. Gel formation: phase diagrams using tabletop rheology and calorimetry. In *Molecular Gels: Materials with Self-Assembled Fibrillar Networks*; Weiss, R. G., Terech, P., Eds.; Springer: Dordrecht, The Netherlands, 2005; pp 233−244.
(30) Ventura, E. E.; Davis, J. N.; Goran, M. I. Sugar content of popular sweetened beverages based on objective laboratory analysis: focus on fructose content. *Obesity* **2011**, *19*, 868−874.
(31) Thornton, P. D.; Mart, R. J.; Ulijn, R. V. Enzyme-responsive polymer hydrogel particles for controlled release. *Adv. Mater.* **2007**, *19*, 1252−1256.
(32) Thornton, P. D.; Mart, R. J.; Webb, S. J.; Ulijn, R. V. Enzyme-responsive hydrogel particles for the controlled release of proteins: designing peptide actuators to match payload. *Soft Matter* **2008**, *4*, 821−827.
(33) Yang, Z.; Liang, G.; Xu, B. Enzymatic hydrogelation of small molecules. *Acc. Chem. Res.* **2008**, *41*, 315−326.
(34) Berron, B. J.; Johnson, L. M.; Ba, X.; McCall, J. D.; Alvey, N. J.; Anseth, K. S.; Bowman, C. N. Glucose oxidase-mediated polymerization as a platform for dual-mode signal amplification and biodetection. *Biotechnol. Bioeng.* **2011**, *108*, 1521−1528.