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# Student explorations of calcium alginate bead formation by varying pH and concentration of acidic beverage juices

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**Abstract:** Teaching experiments involving edible,biodegradable calcium alginatebeadsserve asan attractive model system to introduce upper secondary age students to core chemistry topics through innovations in sustainable consumer products. A teaching experiment isdescribed that engages students with the synthesis of calcium alginate hydrogel beads from sodium alginate and calcium lactate, two food-safe and renewable materials. The beads' outer membranes are a result of ionic interactions between carboxylate groups from alginate strands and the divalent calcium cations between them, thus forming cross-linked polymers. Protonation of the carboxylate groups on the alginate strands decreases crosslinldng density affecting bead formation. First, various concentrations of citricacid areused to lower the pH of the sodium alginate solution and the effect on the calcium alginate bead fonnation is observed. A correlationbetween pH and bead shape and firmness is derived. This information is then used to explore juices with varying natural acidities. The experiment is amenable to implementation in the classroom or as an at-home activity. Learning outcomes include acid-base reactions, chemical bonding, polymer structures, and green chemistry concepts. Students consider the environmental challenges of traditional plastics used in packaging and how innovative new commercial products are allempting to provide solutions.

**Keywords:** acid-base chemistry; green chemistry; hydrogels; sustainable polymers.

## Introduction

Plastics are ubiquitous materials weuse in our daily lives. These materials remain an essential component in the packagingindustry helpingprolong theshelflifeof foodandother consumer products.Thedurablequality of plasticsunfortunately contributes to the damage they causeto the environment (Schneiderman & HiUmyer, 2017). Traditional plastics derived from fossil fuels take centuries to biodegrade, accumulating in landfills and poUuting thenatural environment(Geyer,Jambeck, & Law, 2017).Solutions to combat this global issueinvolve thedevelopment of sustainable and environmentally friendly alternatives to petroleum-based plastics. These materials are designed to be biodegradable or compostable that break down into benign subunits such as carbon dioxide and water. Biodegradable materials are degraded in natural environments such as soil or aquatic systems. Compostable plastics are broken down under controlled industrial conditions (Wissinger et al., 2020). Polylactic acid (PLA), derived fromcom, is an example of a compostable bioplastic.

An example of a biodegradable product is the edible, hydrogel pods sourced from seaweed derivatives produced by The Sldpping Rocks Laboratory Notpla Limited (2022). The gel-like water spheres begin to biodegrade in approximately four to six weeks and areenvisioned asan alternative to single-useplastic water

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botUes as illustrated by their use in the 2019 London Marathon (OohoWater Admin, 2019; Patel, 2019). The Skipping Rocks Laboratory has applied this technology to other innovative products such as condiment sachets and food containers all aiming to reduce the plastic footprint through degradable packaging. Showcasing examples of these current innovations in sustainability and green chemistry through teaching experiments is an effective tool in engaging students. These guided-inquiry experiments connect students to global issues addressed by the UN Sustainable Development Goals (United Nations Department of Economic andSocial Affairs, 2022) and providea framework to develop problem-solving skills. Goals 3(Good Health and Well-being), 12 (Responsible Consumption and Production), and 14 (Life Below Water) are direcUy Lied to plastics. Herein we reporta teaching experiment that introduces students at the uppersecondary age group to the chemistry behind the formation of edible water beads derived from alginate, a linear anionic polysaccharide that can be extracted from algae.

## Background

Alginate is an attractive model system to explore in a teaching labdue its tunable properties that are desired in various industries ranging from food, medicine, and construction. Asa hydrogel, alginate can absorb a large amount of water molecules. Alginate can be woven into a fiber forwound dressings (Aderibigbe & Buyana, 2018). Formation of alginate gel beads through spherification have been incorporated into imitation food and encapsulation of biomolecules 0eong, Kirn, Lee, Cho, & Kirn, 2020). Thin film and coatings derived from alginate are used in food packaging to improve shelf life(Parreidt, Millier, &Schmid, 2018). Alginate capsules containing sunflower oil were utilized for self-healing properties to rejuvenate asphalt (AI-Mansoori, Norambuena-Contreras, Micaelo, & Garcia, 2018).

The life cycle of calcium alginate beads meets many of the criteria described in the Sustainable Polymer framework from the National Science foundation (NSF) Center for Sustainable Polymers (Wissinger et al., 2020) andsummarized in figurel. The reagents usedin thisexperiment areorcan bepotentiallyobtainedfrom natural feedstocks. The process to synthesize calcium alginate can be completed at room temperature. The reagents are non-toxic and inexpensive and food-grade quality of the materials can be purchased online enabling the chemistry to be safely conducted at home in the kitchen. for end-of-use the alginate beads are biodegradable. The aforementioned features make studying alginate very accessible for lower and upper secondary age groups. While there are several demos found online showcasing the basics of synthesizing calcium alginate, the teaching experiments described here are translated into curricula that meet learning standards set for upper secondary age students.

The structure of alginate is comprised of two uronic acid blocks, a P-D-mannuronate (M) and a-L-guluronate (G) residue linked through a 1,4-glycosidic bond.Thealcohol functionality at the C6position is fuUy oxidized to a carboxylic acid. The polymer has three different arrangements that provide a unique

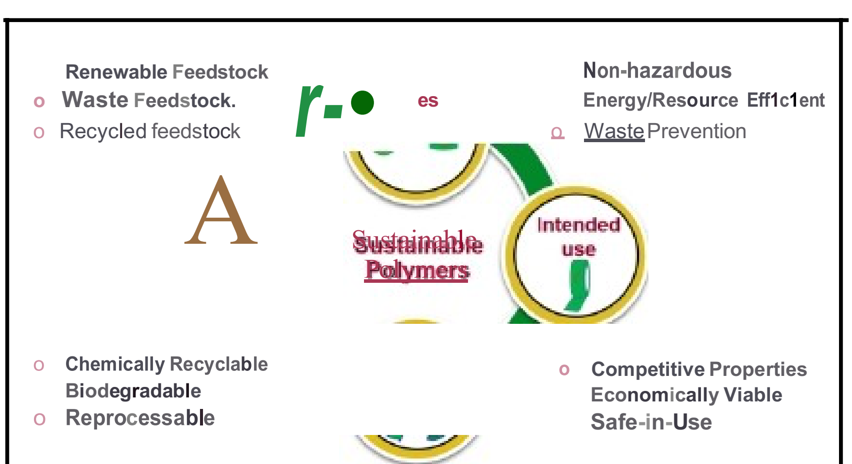



**Figure 1:- The criteria addressed by the alginate experiment curriculumthat align with the sustainablepolymer** framework(adapted from Wissinger et at. 2020).

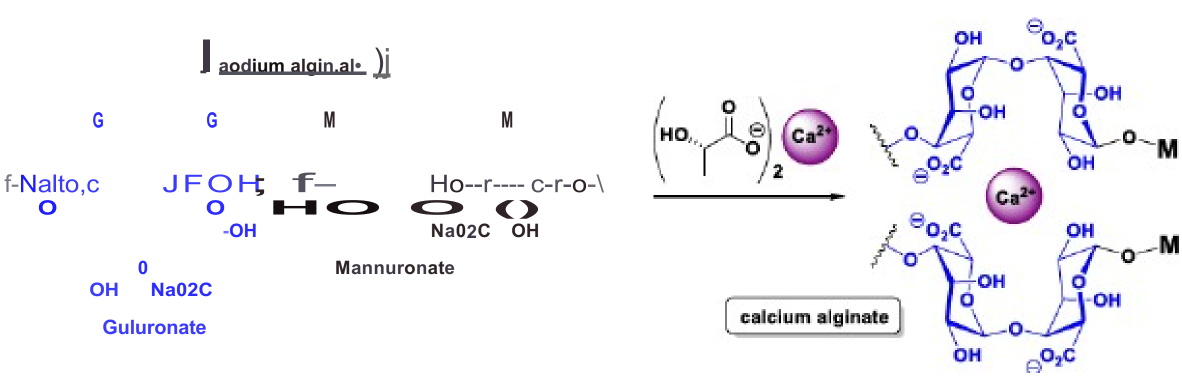
structure: A ftat ribbonlike block of repeating MM residues, an egg-box block of repealing GGresidues, and a helical shaped region of alternating MG residues (Lee & Mooney, 2012; Zhang, Cheng, & Ao, 2021). The conformational shape of the GG block allows for the carboxylate anions to strongly coordinate to multivalent cations connecting two separatepolymer chains. Alginatebeads can be synthesized by reactingits sodium salt form withcalcium lactate viaa double displacement reaction (Scheme I). The crosslinking coordination of the divalent cation to the carboxylate groups is the key focus of this teaching lab.

Classroom experiments involving alginate have been successfully incorporated for lower, upper and freshman/sophomore age groups. These past experiments demonstrate the versatile properties of alginate polymers as they areprepared in a variety of different forms:

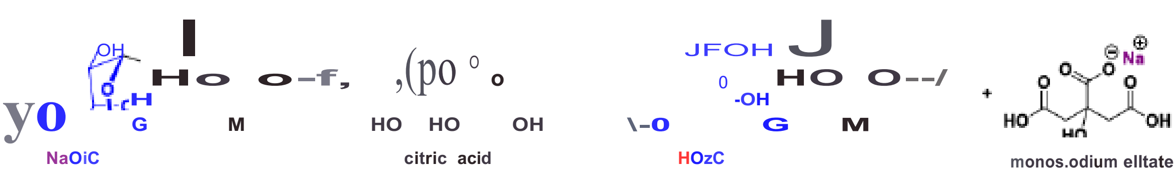
- Pods (Corcoran & Wissinger, 2020; Corcoran et al., 2022)
- Worms (Waldman et al., 1998)
- Spaghetti (Erda!, Hakkarainen, & Blomqvist, 2019)
- Chilosan alginate discs (Ward & WylUe, 2019)
- Slime (Boyd, 2021)

Onenotableparameter that wasnot studied in theseteaching experiments wastheinvestigation of pH on bead formation. This phenomenon presents as a great opportunity to use in a classroom teaching experiment. Alginate polymers are known for being pHresponsive and the direct impact of pH effects on bead shape and the calcium interactions has been well studied (Chuang et al., 2017; Liang et al., 2004; Zheng et al., 2017). Studentsare exploring this effectusing citricacid, which is derived from natural resourcesand friendly to use.

This new addition of calcium alginate bead investigation is designed for upper secondary students to explore the effect of pH on calcium alginate bead formation using varying concentrations of citric acid (Scheme 2). Similar to calcium lactate and sodium alginate, food grade citric acid is readily available for purchase and can also be safely used in the experiment. When citric acid reacts with sodium alginate, the carboxylate groups on the polymer canbe protonated depending on the pH. The pKa values of the carboxyl groups on the mannuronate and guluronate blocks are 3.38 for and 3.65 respectlvely (Francis et al., 2013). Recall that the G-blocks of alginate are the site of coordinations with divalent cations. When pH is above the pKa of the carboxylic acid there will beahigher concentration of itsdeprotonated form. When pH is below the




Scheme 1: Synthesis of calciumalginate with calciumcoordinated to the a··l•guturonate (G) residues.




Scheme 2: Acid-base reactionbetween sodiumalginate and citric acid.

pKa, U,ecarboxylic acid will beprimarily in ils protonatedstate. Lowering the pHof the alginate below U,epKa of the guluronate residuehas a majorimpact on bead shape. Ala lower pH,calcium alginate beads form a more oblate, less spherical shape(Chuang etal., 2017).The protonation of U,e carboxylale anions on the G residues disrupts the coordination with U,e calcium cations reducing the available sites for crosslinldng.This weakens the crosslinldng network lowering U,e resistance to deformation under the bead's own weight (Figure 2). A second effect that contributes to U,e oblateness of the bead shape is caused by the reduction of inlerfacial tension at a lower pH (Yoo & Mitragotri, 2010).

The learning objectives of this teaching experiment for upper secondary age students involve discussing the need for findingallernatives to traditional plastics and introduction Lo green chemistry and sustainability. This Leaching experiment invokes and brings awareness Lo the plastics problem at a level suitable for its audience. The topics of polymer structure, chemical bonding, solutions and solubility, and acid-base chemistry are incorporated into the experiment. In U,e first part of this multi-day experimen students are required Lo consider how pH relates Lo protonation and how Ulis can affect the structure of an ionizable polymer. Students will mix solutions of varying concentrations and directly observe that more solute in a solution resulls in a higher concentration. Furthermore, the relationship between concentration and pH is observed when an acidic solution diluted wiU, water resulls in a higher pH. In the second part, students undertake an exploratory investigation taldng the knowledge obtained from U,e previousstudies and predict which juice beverage can form beads. Since Ulis experiment uses food safe materials, students explore juices which can be tied to pH as they naturally contain organic acids. Students are asked to determine whether juices could substitute for water and create new environmentally-friendly ways to deliver thirst quenching beverages without U,e use of plastic bollles.

## Experimental overview

### Materials and design (see Supplementary Material for details regarding procedures and setup)

Thischemistry experiment explores the effectof pHon calcium alginate beads and their practical application to hold liquids such as juices or condiments. This two-part experiment can take place in two50 min class sessions. Part Iexamines the effect ofpHon calcium alginate beads filled with water. This knowledge is then applied to predict and test the viability of beads filled with juice.

To synthesize calcium alginate beads, a solution of sodium alginate and the contents of the bead is dropped or placedinto calcium lactatesolutionand allowed to react for15 minor more.1\vo methods are given to preparethebeads.Thefirst utilizes a standard plastic pipette thatiscut to createa larger opening fordelivery of the sodiumalginate solutions.Thesecond uses a teaspoon that is gently placed liquidside up just below the surfaceof thecalcium lactatefor5-10stoallowinitiation oftheoutershell membraneformation. Theteaspoon is then gentlyinverted to emptythe contentsinto the calcium lactatesolution. Food coloring is used to improve visibility of beadswhen recovered from the lactate baths.

**Figure 2: Left: calciumalginate** formed at pH 3.45. Right: **calciumalginate formed at pH** 7.2.

### Part I - pH control with citric acid

Students workingroups of five and begin byprepalingfive sodiumalginate+citricacid solutionsinwater. The pHof each solution is measured and recorded. Eachsolution, ranging in pH between6and 3areadded toa beaker containing 0.06%w/vcalciumlactate viaa cutpipette or teaspoon.The beadssit insolution forat least 15 min while the calcium alginate outer membrane forms. After 15 min, observations of the beads in solution are made. After removal fromsolution, the texture, shape, and firmness of the beads are analyzed at eachpH and reported in a table.

### Part II - Investigation of juice-beverage beads

Part II of the experiment encourages students lo begin thinking about the practical applications of sodium alginate beads and to share and compile data. From Part I, a relationship between pH and bead formation is drawn.Students consider the tartnessof various juices such ascranberry, apple, andlemon juiceand correlate with the order of pH and bead formation and shape. Pairs are assigned one type of juice and work with two sodium alginate solutions, a non-diluted juice solution and a diluted juice solution. Again, the pH of each solution is determined and the same protocol as that used for water is followed lo prepare calcium alginate juice filled beads. Students examine thebeads and share data with other groupstocollect data on each type of juice.

### Potential extension - contact angle analysis

Instructors can choose to include an optional extension that involves measuring the contact angle of the calcium alginate beads. JmageJ software, which can be downloaded online for *free* through the National Institutes of Health (https://imagej.nih.gov/ii/download.html), allowsquantification of contact anglethrough theContactAngle Plug-in (https://imagej.nih.gov/ij/plugins/contact-angle.html). Photographs of thecalcium alginate beads can beused todetermine contact angles using a reported literature procedure (Buahom, 2018) and as described in theSupplementary material.

## Hazards

Sodium alginate, calcium lactate, and citric acid can be purchased in food grade. Juice beverages can be obtainedatlocal markets. Even though juice beveragesand foodgrade reagents will be used in theexperiment, studentsshould wear properlabattire, goggles, and gloves accordingly toinstillsafety practices.Citricacid isa minor skin irritant. Exposure toskin requires washing with water. Beads prepared in the lab usingequipment and glassware should not be consumed. Consumption of beads is only allowed when kitchen equipment is used loconduct the experiment. Possible food allergies must betakenin consideration for ingesting beads that **contain juices.**

## Discussion/ results

### Part I - pH control with citric acid

Figure 3 illustrates the beads formed from the dropper and spoon methods giving different sized beads. The spoon method gives beads of approximately 2.5-3.5 cm in diameter whereas the dropper affords beads with diameters ofapproximately 0.5-0.6 cm.Each method hasvarying degrees of technique involved.Thedropper

**Figure 3: leftimage: beads fonned with spoon.Right image: beads fonned w;1h** pipette.

method providesbetter controlin delivery resulting in more preciseand consistent shapes.Thespoon method is less consistent with size and shape, however the larger appearance of the beads makes it easier toidentify significant changes in bead shapewhen changing the pH. Turkey basters and boba strawswere alsoshown to effectively transfer the alginate solutions into the calcium lactate baths.

Representative student data expected forPart I are shown in Tables 1 and 2(seeSupplementary material for more information). Beads with no citric acid and the highest pH are the firmest and roundest As pH is lowered, thebeadsstartlosing theirstructural integrity, becoming Jessspheroid inshapeandareeasily broken when gently squeezed. The oblateness of the alginate beads is more apparent as the pH nearsand eventually becomes lower than the pKaof thecarboxyl groups(3.38) of the Gblock. Usingfoodcoloring *dye* is essential to helpobserve the results of the experiment.

## Part II - **Investigation of juice-beverage beads**

Table 3 illustrates the data and observations expected for juices. When analyzing the non-diluted alginate-juice solutions, students will notice that alginate-apple juice readily forms a bead with a pH above four ensuring that the carboxyl groupson the G-block remain deprotonated. Cranberry juice and lemon juice are relativelymore acidiccompared toapple juice. Particularlyforlemon juice, no bead is formedat all.Dilution of lemon andcranberry juices raises thepH high enough toallowforsuccessful formation of alginate-juice beads.

**Table 1: Example of data and observations using:the pipette dropping:method.**

| Sodiumalginate+ citric acid%w/v | pH | Photo | Observations |
|---|---|---|---|
| 0.00 | 6.00 |  | Round, finn. smooth. retains shape |
| 0.40 | 3.77 |  | Round and smooth, squishy. does not fultyretain shape |
| 0.80 | 3.45 |  | Spread ou oblate, squishy |
| 1.20 | 3.25 |  | Very oblate, does not retainshape |
| 1.60 | 3.13 |  | Flat. squishy, not a bead |

Table 2: Example of data and observations using the teaspoon method.

| Sodium alginate+ dtrk add°4 w/v | pH | Photo | Observations |
|---|---|---|---|
| 0.00 | 6.00 |  | Round. firm, smooth, retains shape |
| 0.40 | 3.77 |  | Round and smooth, squishy, does not fully retain shape |
| 0.80 | |  | Spread out. oblate, squishy |
| 1.20 | 3.25 |  | Veryoblate, does not retain shape |
| 1.60 | 3.13 |  | Fla squishy, *not* abead |

Table 3: Example of data and observations for alginate.juicepedment using teaspoon method.

| Sodium alginate+ juice solution | pH | Observations (in solution) | Photo | Observations(out of solution) |
|---|---|---|---|---|
| Apple juice: non-diluted | lf.17 | formed beads |  | Round, butvery squishy bead |
| Apple juice: diluted | 4.94 | formed beads |  | Round, firmbead |
| Cranberry juice: non-diluted | 3.55 | Dispersed, very clumpy, stayed at the top ofsolt.tion | | Clumpy, flat, not abead |
| Cranberry juice, diluted | 4.41 | Drops stayed togethe<, formed beads |  | Round, squishy bead |
| lemon juice: non-diluted | 3.15 | Drops dispersed Immediately |  | No beads formed |
| lemon juice: diluted | 3.83 | Drops spread out. but did not disperse completely |  | Oblate,""Y squishy bead |

## Extension - contact angle analysis

Contact angle analysis can provide further infonnation on the effects of pH on bead shape. Usage of contact angle is commonly applied for water droplets·where their relativewettability, how the material spreadsover a solid surface,is determined. Droplets thatpossesslowwettability havecontact anglesin between180"and 90°, have hydrophobic surfaces wherethereis lessaffinity to maintain contactwith thesurface. Materialswith high wettability, havea strong affinity for the solid and spreads out and contact angles less than 90°.Table 4 and figure 4 represent an example of contact angles study on the beverage-alginate beads. The dilutions of alginate-cranberry juice show a decreasing trend in contact angle as pH lowered. This aligns with the oblateness of the beads as they become less spheroid in shape.

## Implementation

Due to the ongoing Covid-19 Pandemic, classroom implementation of thefull experiment was delayed due to the challenges with handling social distancing guidelines and the change from in-person to online virtual classes. The general experiment involving the synthesis of calcium alginate was successfully conducted virtually in fall 2020 with a group of upper secondary agestudents. Avirtual lecture was provided discussing the plastics problem, basic polymer structure, reaction chemistry, and overview of the experimental procedure. Premade kits containing all the essential reagents were provided to students beforehand. In place

**Table 4: Anatyslsof contact angles of beverage.alginatebeads.**

| Entry | Beverage | Beverage:H20 | pHof juice+ alginate | Contact angte• |
|---|---|---|---|---|
| 1 | Apple | | 4.12 | 127.1 |
| 2 | Cranberry | 1,3 | 3.33 | SS.3 |
| 3 | Cranberry | 1,4 | 3.S3 | 109.9 |
| 4 | Cranberry | 1,9 | 3.76 | 140.9 |
| S | Lemon | 1,9 | 3.44 | 78.2 |
| 6 | Water | | 6.92 | 146.8 |

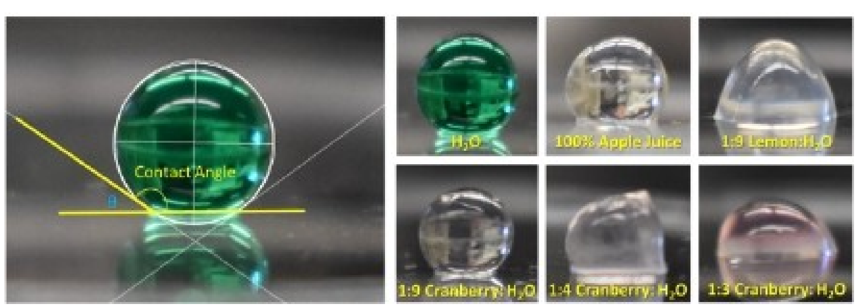



**Figure 4: Leftimage:phototakenfromlmag:eJsoftwarew;th contactang1eplug-in.Yellowlineswereaddedto aid invisualization oflocatlonof contact angle. Angle *8*, calculated from tmaget ls used10 determine contact angle. Right Image:photosof beads used to determine contact angle.**

of glassware, studentsused containers they had available in I.he kitchen of their homes. Weplan to implement this teaching experiment in I.he upcoming academic year assuming classes are in-person or provide inexpensive pH probes for at-home learning.

## Conclusions

Wereport a teaching experiment I.hat brings awareness to sustainable solutions to single-use plastic bottles and packaging.Applications of green chemistry focuson the derivation of products fromrenewable feedstocks (Principle #7) and design for degradation (Principle #10) while also exploring new sustainable commercial products in lab. The investigation into I.he pH effect on calcium alginate beads provides an engaging and versatile experiment for upper secondary age groups with appropriate level science learning outcomes that cover acid/base chemistry and bonding. Students are exposed to solutions to real-world problems associated with petroleum-based plastics through the curricula with a vision of how a career in a Science, Technology, Math, and Engineering (STEM) fieldcan contribute to a sustainable future.

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## Supplementary material

Infonnation regarding experimental procedures and lab worksheets are available here: https://doi.org/10.1515/c-ti 2021-0027.

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