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Tough Hybrid Hydrogels Adapted to the Undergraduate Laboratory

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ABSTRACT

Polymer materials are indispensable in our daily lives. This makes polymer technology of critical importance in higher education. In particular, hands-on experiment-based practicals/laboratories with a focus on polymer science are of tremendous value in the undergraduate curriculum. Along these lines, hydrogels are highly crosslinked polymer networks which show some unique properties such as water absorbance and large extensibility, making them particularly well-suited in various biomedical applications. The properties of hydrogels can be systematically varied via changes in composition. In this practical laboratory, we use hybrid hydrogel formulations containing alginate and polyacrylamide to explore the consequences of compositional changes on mechanical behavior. Mechanical properties are determined using a simplified tensile test that is amenable to large groups of students using standard laboratory equipment. We used marbles to induce an extensional force and a ruler to measure the elongation of the gel as a function of the attached weight. Hereby, stress–strain curves can be obtained and students are able to compare the difference between single and double network hydrogels as well as quantify the influence of network composition. This practical combines the use of chemical synthesis (i.e., reactant calculations) with practical skills which makes it interesting to use in a third year chemical/biomedical course. Furthermore, students can learn how to deal with chemicals and gain insight in polymer chemistry and its wide applicability, particularly well-suited for students coming from outside the traditional chemical science background.

KEYWORDS

Undergraduate laboratory; hydrogel synthesis; biomaterials synthesis; polymer chemistry; materials science; network formation; hybrid hydrogels; tensile testing; biomedical science

INTRODUCTION

Hydrogels have properties that are ideal in a wide variety of biomedical applications, showing great promise in emerging therapies and regenerative medicine.[1-3] They consist of a three dimensional structural scaffold that is highly swollen with water. Hydrogels for biomaterials are often composed of water compatible polymers owing to the physical/mechanical attributes associated with this material class. They can be constructed from a diverse range of components, both synthetically or naturally derived.[4-5] Biocompatibility, high water content, permeability for metabolites, variable composition and structure similar to the biological extracellular matrix make hydrogels appealing candidates in a wide range of applications from tissue engineering to drug delivery to bio(chemical)sensors.[6-10] However, implementation in some of the most mechanically demanding applications has typically been limited by inadequate strength/resilience/toughness. Several innovative design strategies have been developed to address this shortcoming. Of particular interest are dual network and hybrid gels containing complementary networks due to (1) the astounding mechanical properties (2) high optical clarity and (3) straightforward, practical preparation.[11-15]

Owing to the wide-ranging applicability, complex molecular makeup, and unusual mechanics of contemporary hydrogels, exposure to the topic in a hands-on, experimental setting is valuable for students in various disciplines including chemistry, physics, materials science, and biomedical sciences.[16-19] A basic understanding of hydrogels and visualization of their properties would serve students across multiple disciplines and as such we introduce with this work an adaptable protocol intended for the undergraduate laboratory.[20] This is complementary to a recent laboratory experimental procedure related to self-healing hydrogels for high school students and undergraduates.[21] Likewise, several explorations into the preparation of simple hydrogels have appeared in this journal, targeting different students at various educational levels.[22-25]

We were particularly intrigued by a seminal report describing simple protocols and formulations that led to gels with astonishing tensile and compressive properties.[26] The concept of the work was inspired by many preceding dual network examples, and acted as a springboard for several adaptations to hybrid gels with alginate.[27-32] The original work from Suo and coworkers describes the combination of alginate with polyacrylamide (PAAm), wherein the alginate (Alg) is crosslinked by ionic bonding between guluronate residues and bivalent Ca^{+2} ions (Figure 1). The polyacrylamide network is covalently crosslinked from the UV-activated curing with methylene bis-acrylamide (MBAA). Furthermore, the two types of networks are covalently joined to each other through reaction between carboxylates from alginate and the amine groups of acrylamide (Figure 1A; a more detailed description of the formation of the networks can be found in the lab text included in the supplementary information, page S7).

Despite being composed of 86% water, the combination of networks gave rise to remarkably tough materials, far surpassing the properties associated with the individual components. This enhanced toughness is due to the energy dissipation created by the unzipping of the alginate network during deformation. Extensive tensile testing was performed with detailed analysis for a series of gels with variable [Alg]:[PAAm] composition.

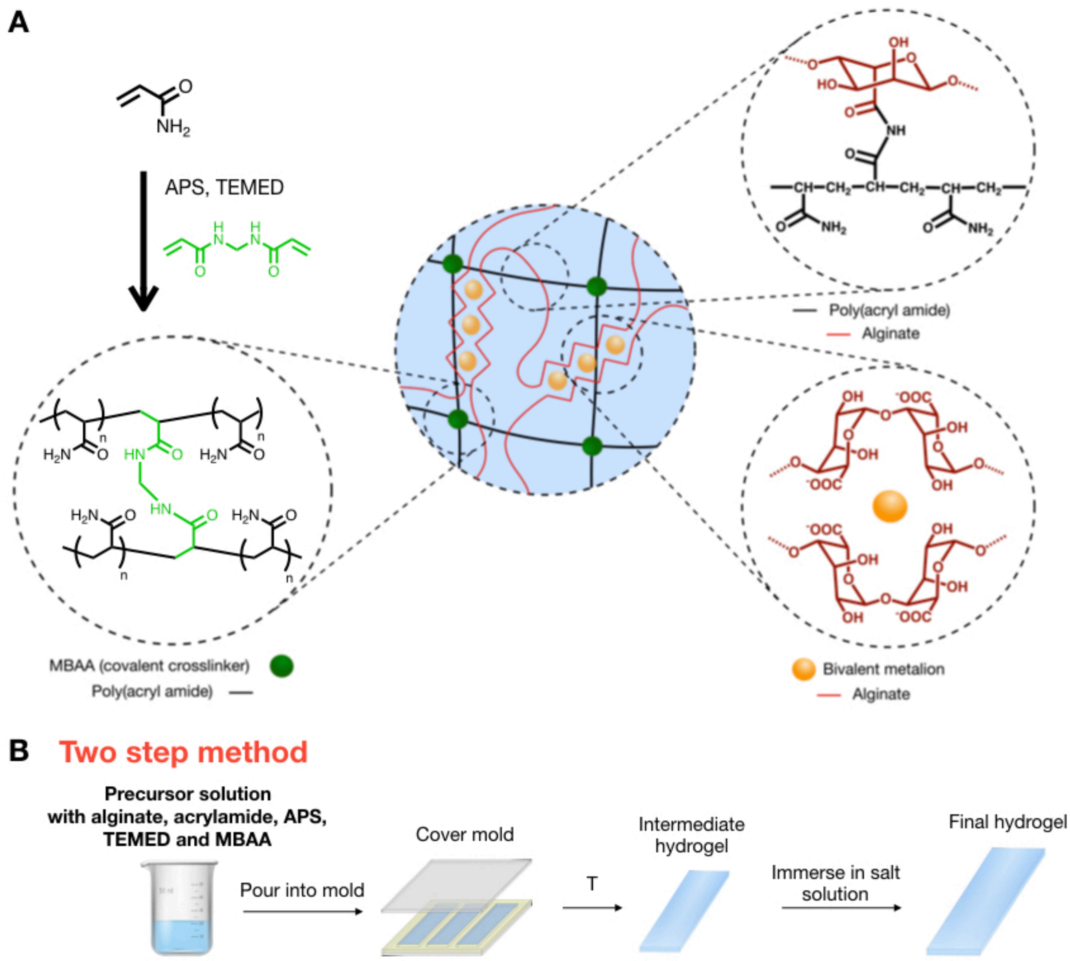


Figure 1. (A) graphical illustration of the chemical structures and synthetic step to make the hybrid hydrogels; (B) illustration of the procedure for preparing the gels using standard laboratory protocols.

Hybrid alginate–polyacrylamide gels can be made via a facile two-step method using routine laboratory equipment (Figure 1B). The first network is created via free radical polymerization of acrylamide in the presence of the bifunctional crosslinker methylene bis-acrylamide (MBAA) using a well-established thermal initiator ammonium persulfate (APS). Alginate is obtained commercially in polymeric form and can be used without further modification. It is ionically crosslinked in the last step of the procedure. The synthetic protocol starts with making a solution of acrylamide, MBAA, APS, TEMED and alginate, which is heated to initiate the radical polymerization. The poly(acrylamide) network is formed in the presence of alginate, generating an entangled scaffold from the two different polymers. An intermediate hydrogel is formed during this first stage, whereby the hybrid polymer scaffold is fortified upon introduction of a bivalent ion. Immersing the intermediate hydrogel in an aqueous solution of calcium chloride serves this purpose. The calcium ions displace the sodium ions initially present, thereby crosslinking the alginate by forming interchain ionic bonds between the carboxyl groups on the guluronate repeating units.

We were inspired by the original report and identified an opportunity to adapt the experimental procedure to an undergraduate laboratory experiment. Here we describe in detail the protocol that we implemented, where the actual laboratory guidelines can be found in the supporting information. Furthermore, we provide several suggestions on additional adaptations that could be made to tailor the lab for various subjects/majors/disciplines and for different durations (e.g. 1-day, 2-day labs). For example, we executed the experimental protocol with a group of ca. 80 students in their 3rd (final) year of Bachelor's studies in the Biomedical Sciences Department in Hasselt University. Students in the Biomedical Sciences department at Hasselt University have a limited education in traditional chemical sciences. However, tissue engineering forms an important part of their curriculum, and as such this lab serves to introduce the students to one particular strategy for addressing biomaterials synthesis aimed at applications in regenerative medicine/tissue engineering. This lab serves as an introduction to materials synthesis and some common mechanical performance benchmarking tools. We feel that this laboratory experiment offers several opportunities for tailoring to a diverse range of subjects in Universities operating under a variety of structures.

The following section describes the sequence of experimental activities that we followed, including calculations used for the synthetic protocol and ultimately property evaluation using a benchtop tensile testing protocol. Within each section the detailed protocol is provided, followed by suggested adaptations that could be made based on the desired emphasis.

EXPERIMENTAL PROCEDURE

Materials: Sodium alginate (NaAlg), acrylamide (AAm), ammonium persulfate (APS), N,N'-methylenebisacrylamide (MBAA), tetramethylethylenediamine (TEMED), $CaCl_2$, demineralized water, acetone, mold + glass plates, clamps, grip seal bags, marbles, a long ruler, ring stand, pipets, pipet tips, 500 mL beaker, stirring bar, weighing boats, spatula, gloves, 5 mL volumetric flasks, syringe, large gauge needles (diameter 1.2 mm), stirring plate, lab coat, safety glasses, gloves (see Supporting Information for further details on specific items used).

Synthetic Procedure: The experimental procedure conducted in our laboratories was carried out by students working in groups of two. Each group was assigned two gel compositions; one single network gel (see Figure 2D; gel #1 or #8) and one dual network gel (#2–7). For each gel composition, three individual samples were prepared to provide statistical comparison, giving a total of six gels to be tested for each group, provided they had enough time. An overview of the different steps of the practical and the time distribution are provided for a 1-day (i.e. 8 h) experiment (Figure 2A). The students start the practical by calculating how much of each of the components they need to make stock solutions and the precursor solutions of the hydrogels they were assigned. For these calculations the students should consider the total mass of monomer/polymer and the water content (*e.g.,* 86 wt %). (see Supporting Information for an example calculation sheet). Before starting with the preparation of the hydrogels the students prepared stock solutions in 5 mL volumetric flasks for the following chemicals: MBAA (0.01 g/mL), APS (0.03 g/mL), TEMED (0.02 g/mL). A stock solution of alginate (0.075 g/mL) was made by the lab supervisors in advance, owing to the long dissolution time required (~24 h) for the particular source of starting material used (Sigma-Aldrich product number W201502). Ultimately, the final material properties will depend acutely on the source and purification protocols of the alginate. However, the trends in tensile property variation with composition will likely be consistent, regardless of the source.

A

Calculations
Stock solutions & hydrogel composition
45 min

Prepare of stock solutions
30 min

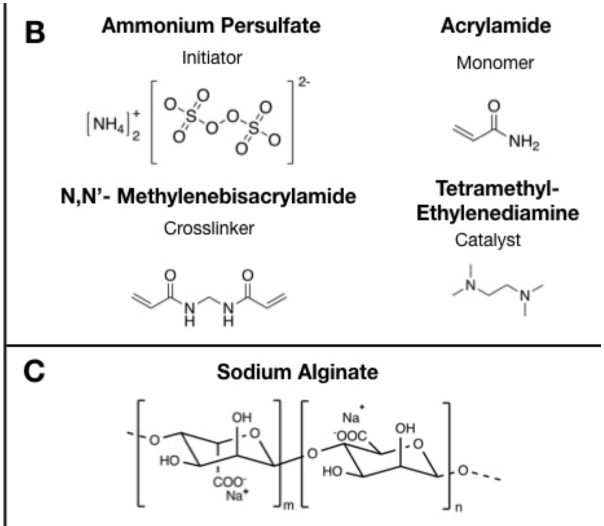
Prepare hydrogels
Prepare hydrogel precursor solution
1 hour
Heat hydrogel solution in mold
1 hour (inactive)
Prepare $CaCl_2$ solution
10 min (during 1 hour heating time)

- - - Break - - -

Soak gels in $CaCl_2$ solution
30 min (inactive)
Cleaning of glassware
15 min (during soaking time)

Tensile Testing
Prepare set-up
15 min (during soaking time)
Measure tensile strength
1.5 hours

Data processing
Making stress-strain curves
2 hours (at home)



D

Gel#	%AAm	% CaAlginate
1	0	100
2	65	35
3	75	25
4	80	20
5	85	15
6	90	10
7	95	5
8	100	0

Figure 1*.* A) Timeline of the activities performed during a one-day lab. B) Chemical structures of the components used to synthesize the poly(acrylamide) network. C) Chemical structure of sodium alginate, the major constituent of the second network. D) Compositions of the gels.

To start the preparation of the hydrogel precursor solution for gels #2–7, acrylamide is dissolved in water, as calculated according to the concentrations provided. For safety reasons the acrylamide is first weighed in a 50 mL bottle under a separate lab hood and is transported to the students own hood while sealed with a septum or secure cap. Acrylamide is a highly toxic monomer, and extreme caution should be taken in handling this chemical. This should be highlighted and emphasized, particularly for students who may be less familiar with precautions associated with handling toxic materials (i.e. Biomedical students, Materials Science students, etc.). Next the students add the water directly to the acrylamide in the 50 mL bottle. A stirring bar is added and the solution is stirred until the monomer dissolved. The alginate is added to the AAm solution using a syringe with a wide-gauge needle (diameter 1.2 mm) because of the high viscosity of the alginate stock solution. The required volume of stock solutions of MBAA and TEMED are added using micropipets. Thereafter the bottle is sealed with a septum and flushed with nitrogen gas for 5 minutes using a balloon. The required volume of APS is added with a micropipet while minimizing exposure to air as much as possible. It is important to add APS *after* deoxygenation, as it can cause premature gelation in the beaker during the deoxygenation step. To make the gels more visible, food coloring can also be added. To obtain dog bone shaped samples the hydrogel precursor solution is poured into a PMMA mold (Figure 3). This procedure should be carried out inside a fume hood with the bench covered with paper to absorb possible spillage of the hydrogel precursor solution. A glass plate is put on top of the paper and some drops of water are placed on top. Next, the PMMA mold is placed on top of the wet glass. The water ensures good contact between the mold and the glass plate and thus reduces spillage of the hydrogel precursor solution. The monomer/polymer precursor solution can then be poured into the mold. The mold is covered by a second glass plate while ensuring as little air as possible remains inside the mold. The plates are clamped together using foldback clips and placed in an oven at 50 °C for 1.5 hours. Meanwhile, a 0.3 M $CaCl_2$ solution can already be prepared by the students. After the break, the molds are taken out of the oven and allowed to cool down for 10 min. The glass covers are removed and the mold is placed inside the 0.3M $CaCl_2$ solution for 15 min. The gels are removed from the mold and equilibrated for another 15 minutes inside an aqueous solution of $CaCl_2$. Thereafter the excess water is removed from the gels by gently dabbing with a paper towel.

The preparation of the hydrogel precursor solution for gel #8 (100% acrylamide) is the same as for the other gels, except that alginate excluded. Therefore, gel #8 is not submerged in the $CaCl_2$ solution. The gel can be measured directly after thermal curing. The preparation of the hydrogel precursor solution for gel #1 (100% alginate) is comparatively straightforward. This sample requires that the 0.075 g/mL stock solutions are poured directly into a mold and placed inside a 0.3M $CaCl_2$ solution without cover. After one hour the gels are removed from the mold and allowed to equilibrate for another 20 minutes to ensure homogeneous crosslinking. Excess water is removed from the gels by dabbing with a paper towel.

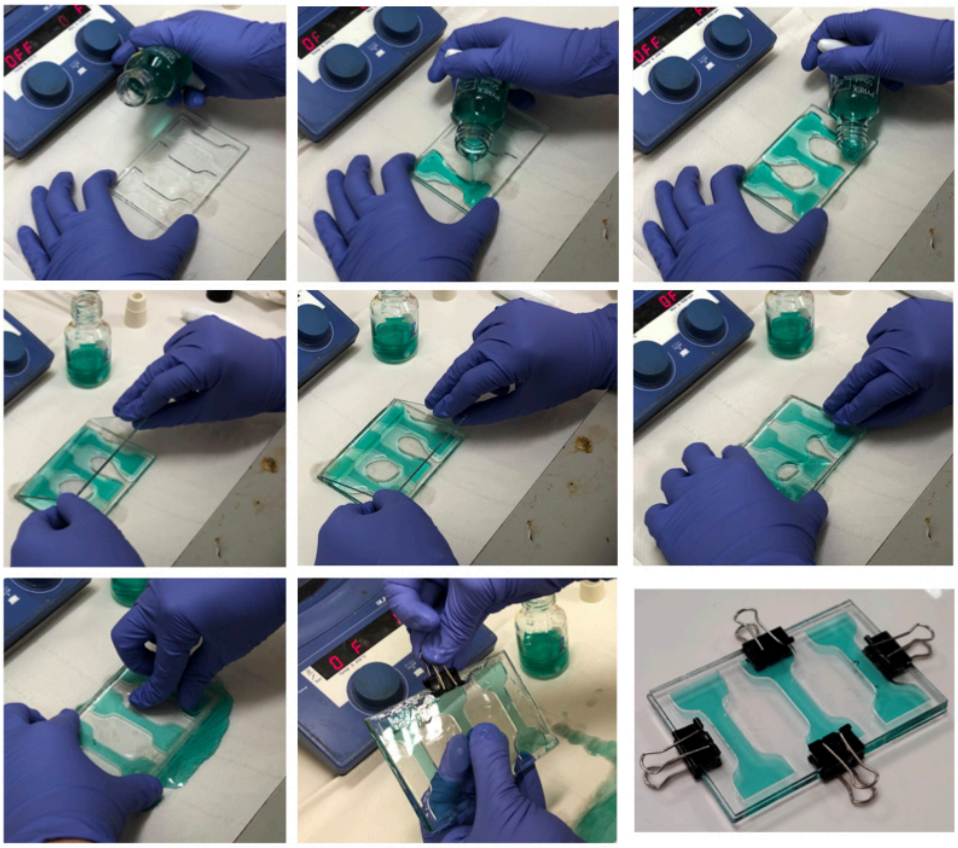


Figure 2. Protocol for preparing hydrogels in preformed dog-bone shaped molds for consistent sample quality. The gels precursors in the photo array have been artificially colored for visual clarity. Alternatives to these home-made molds can be found in supporting information.

Tensile testing: The procedure for deoxygenation procedure and tensile testing is summarized in Figure 4. The tensile properties of the gels are measured with a simple setup, which is amenable to large groups (Figure 4E). Other innovative alternatives can be found in literature.[33-35] Students measure the thickness (t) and width (w) of the sample to obtain cross-sectional area (Figure 4D). These values are subsequently used to calculate the stress (σ = F/A, where A is the cross-sectional area). The grip sections of the dog bone sample are clamped with foldback clips, in which one end is hung from a ring stand. The students place marbles into a plastic bag, measuring the mass of each sequential marble addition (the lower foldback clip and paperclip included), and attach it to the lower end of the hydrogel using a paperclip. The weight will be increased gradually and the elongation of the hydrogel sample is recorded for each iterative marble addition. Alternatively, if the practical can be conducted in small groups of up to 10 students the tensile tests can be demonstrated to the students using a standard tensile tester. Depending on the limits of the tensile testing equipment the gels can be stretched until they break. However, this is not necessary for the students to complete their assessment. The student can use the data they obtain from this experiment to calculate the stress and strain at each point measured and make a corresponding stress-strain curve. Optionally, the students can compare the mechanical properties of different compositions using the collective data obtained by other groups.

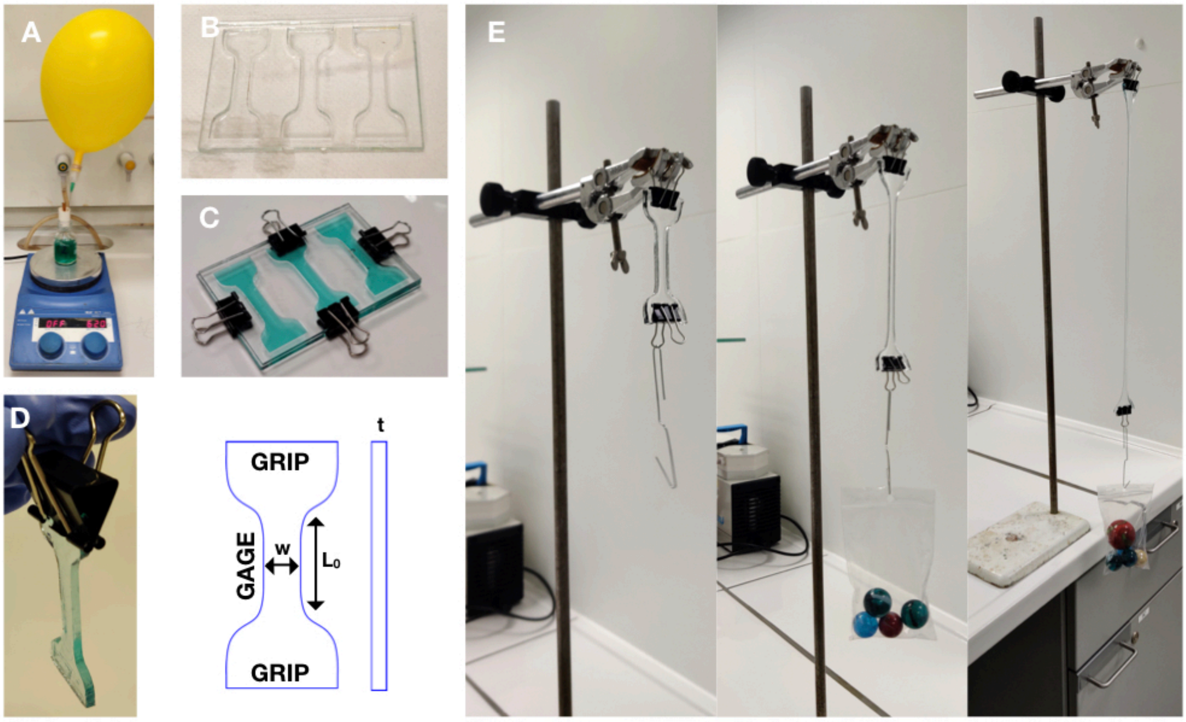


Figure 4. A) Deoxygenation step using a nitrogen-filled balloon. B) Dogbone shaped mold cut out of a PMMA sheet (3mm thick) using a laser cutter. C) Mold filled with precursor solution (artificially colored) and clamped in between two glass plates. D) Left: example of end product for gel #2. Right: dimensions of the dog-bone shape. E) Custom mechanical tensile testing setup.

Assessment: With the collected data the student should be able to make the stress-strain curves of the different gels and plot the results in a graph. The students are also asked to calculate the Young's modulus for the various samples. A comparison between samples with different compositions can be made, depending on the desired complexity of the analysis and the targeted learning emphasis. Additional calculations for advanced materials science students could include the work of extension (i.e., toughness).

HAZARDS

The use of chemicals always involves risks. Therefore, it is important that the students are aware of the safety precautions of each chemical compound that will be used. The most hazardous chemical of the practical is acrylamide because it is a carcinogenic powder which can cause gene mutations and organ damage. To prevent exposure, this compound is weighed on an enclosed balance placed under a fume hood. Alternatively, an aqueous stock solution of acrylamide monomer could be provided to the students instead of the powdered product, prepared by the lab instructor prior to the lab. This would mitigate some of the risk associated with handling this highly toxic substance. In any case, the potential hazards should be emphasized repeatedly to students. Also, TEMED should be handled with great care due to its flammability and corrosive properties; it is also kept inside the fume hood at all times. While MBAA is also toxic if swallowed or inhaled, alginate and $CaCl_2$ don't have special precautions. Since the required chemicals can have some harmful effects in contact with skin or eyes, students should wear personal protective equipment (gloves, eye protection and a lab coat) at all times. In addition, all synthetic procedures were performed under a fume hood to prevent inhalation of potential hazardous fumes. After the gels have solidified, they were allowed outside the fume hood but students should still wear gloves while performing the tensile tests. Gloves should be switched regularly if needed. The acrylamide waste, including the hydrogels which can contain acrylamide monomer, are disposed in a separate waste container for hazardous chemicals. All other waste is collected in the correct container: aqueous, non-halogenated, halogenated or solid waste. The primary risks during this practical are related to the chemicals, which are minimized by the protective clothing, fume hood, recommended glassware and awareness. The students are also informed once more of the safety precautions before they are allowed to start. Examples of safety sheets are provided in the supporting information.

RESULTS AND DISCUSSION

Results: With the collected data the student should be able to make the stress-strain curves of the different gels and compare the results in a graph. Figure 5 illustrates an example of the stress-strain curves and mechanical properties associated with the different compositions explored in this experiment. The data displayed in Figure 5 were obtained from a standard tensile tester. Using this procedure gels with a tensile strength of 10-500 kPa and stretch of up to 1000% and higher can be prepared. A clear correlation between the modulus and extension of the gels and the amount of alginate present in the gels is shown. The work of extension, calculated as the area under the stress-strain curve, shows the composition of 85 wt % acrylamide / 15 wt % alginate as being the toughest hydrogel.

These results demonstrate the strong contrast in mechanical properties of single network compared with double network hydrogels. PAAm gels are known to be very soft and elastic, typically exhibiting very low Young's modulus (particularly at low crosslink densities). The 100% alginate network is stronger yet very brittle, breaking at low strain but higher stress compared to PAAm (Figure 5). These properties contrast sharply and can also be seen in the double network hydrogels (#2-7). Increasing alginate content leads to increasing modulus and decreasing strain. The hybrid gel thus becomes stronger, but also more brittle. By adjusting the ratio of the two networks, an optimal combination can be found in terms of toughness (i.e., a gel that is both strong and soft). This is nicely displayed in the work of extension (i.e., toughness) graph in Figure 5. As mentioned earlier, this shows that gel #5 is the toughest hydrogel amongst the compositions explored in this exercise.

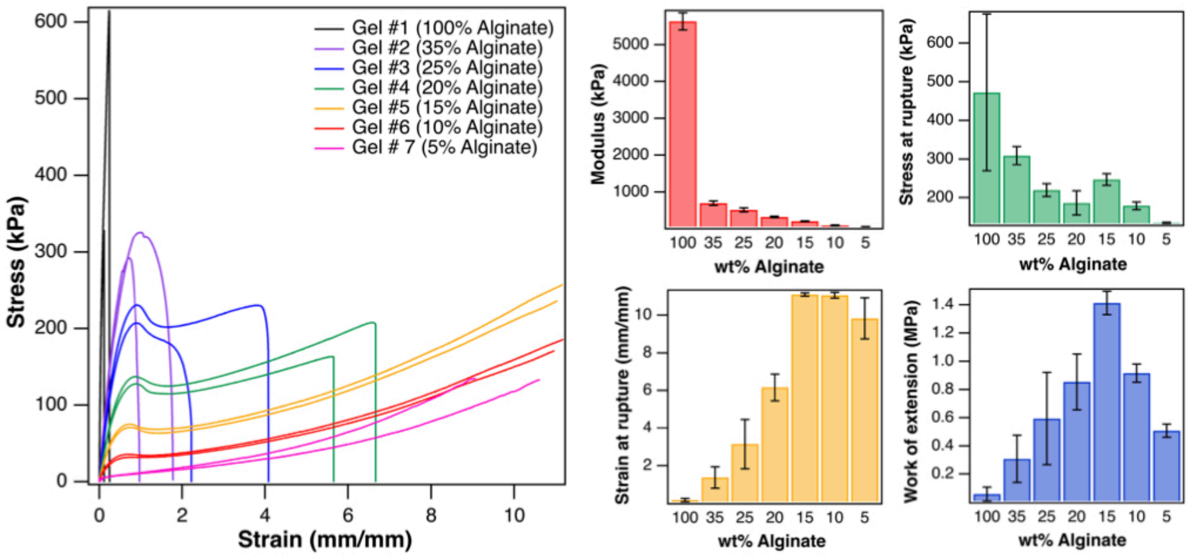


Figure 5. Tensile testing/stress-strain curves for various gel compositions. Measurements were made on a standard tensile tester (500 N load cell, 100 mm/min load rate). Evaluation of several key parameters are provided from the stress-strain measurements, including modulus, stress at break, strain at break and work of extension. Percentages of alginate are expressed in terms of total solids concentration. 100% alginate means 100% of the total solids are comprised of alginate. The solids concentration in water were held constant at 86 wt %.

Depending on the level of the course, the toughening mechanism can be explored/discussed in depth. There are several key reports that extensively describe the toughening mechanism in these systems, as well as the trade-off between strength and toughness.[16-17, 36-38] In general double network hydrogels are composed of a brittle/stiff polyelectrolyte network and a soft/stretchable neutral network. The toughness results from the internal fracture of the polyelectrolyte network. Disrupting the ionic crosslinks in this instance dissipates energy during deformation. In the meantime, the elasticity of the neutral 2nd network maintains the general shape and endows the material with some degree of recoverability. The mechanical properties are therefore dependent on the ratio of the two networks as well as the crosslink density of the respective networks. Exploring these parameters in detail is a natural extension of this laboratory experiment for more advanced students. On the other hand, simply investigating the composition–mechanics relationships with the provided protocol is ideal for introductory laboratory courses.

During deformation of the hybrid hydrogels the alginate chains remain intact. However, the dynamic reversible nature of the ionic crosslinking enables this network to partially recover its strength upon release. The ionic bonds can reform after deformation, while the breaking of the ionic bonds provides an energy dissipation mechanism. The materials exhibit mechanical hysteresis properties as reported by Sun et al[26] and this offers an appealing avenue for further exploration in a more advanced version of the lab, tailored toward students with an emphasis on mechanics and/or gels. This would require performing repeated elongations, and would be best performed with a standard tensile testing instrument to capture the real nuanced hysteresis and demonstrate its dependence on gel composition.

Adaptations: The emphasis and protocol of this lab are highly adaptable, depending on the target student discipline. For example, chemistry students could benefit from systematically altering the chemical makeup. This could be done by introducing alternate comonomers (e.g. acrylic acid[39]) and monitoring the influence on the mechanical properties. Likewise, crosslinking density could by systematically varied via the MBAA concentration[26] and $CaCl_2$ concentration. Network density is clearly correlated with extensibility and modulus, and could be investigated in this manner. The effect of different ions on the double network properties could be explored.[27] A more in depth explanation of the toughening mechanism of these double network hydrogels could be provided in an adapted version of the lab text, individually tailored to students with a different background. Additional hysteresis measurements[26] could also be performed to test the influence of the ionic crosslinks on the mechanical properties. As part of the learning goals, students could be asked to speculate as to the origin of hysteresis and provide a summary of potential strategies to curb this phenomenon.

The procedure can readily be adapted to a multi-day lab. Examples of layouts can be found in supporting information (page S25). However, some attention must be paid to storage of the gels prepared in a multi-day format. The hydrogels should always be stored in a humid environment in a well-sealed container to prevent extensive water from evaporating. This can be done with parafilm-sealed petri-dishes stored in a refrigerator or a sealed desiccator.

Learning goals: We present here a list of several learning goals that were set for students in their 3rd (final) year of Bachelor's studies in the Biomedical Sciences Department. Students were graded through observation during the lab practical (correct calculations, safety, independence) and a post-lab assessment. Bear in mind that these students have very little experience performing chemical lab protocols and mechanical testing. More experienced students with a strong background in chemical sciences may benefit from additional learning goals and their knowledge can be tested in the post lab assessment or in a full lab report. This is open to adaptation, and should be tailored to fit the format of the curriculum in which it is used. However, we provide some suggestions here.

Learning Goals: 1. Looking up safety sheet of the chemicals used and adjust the handling of the chemicals accordingly; 2. Perform calculations to obtain the values needed during the experiment; 3. Accurately making stock solutions; 4. Follow the lab instruction to prepare double network hydrogels; 5. Performing simple tensile tests; 6. Calculating stress and strain, making a stress-strain graph; 7. Interpretation the obtained data (calculation of the elastic modulus, …); 8. Demonstrate understanding of the formation of the two networks.

Observations: We executed the experimental protocol with a group of ca. 80 students in their 3rd (final) year of Bachelor's studies in the Biomedical Sciences Department at Hasselt University in Belgium. Students successfully prepared double network hydrogels using this procedure. Careful reading of the instructions turned out to be essential (as usual!), and the laboratory manual was modified for the second year to highlight some important points. For example, the procedure had to be repeated by 4 of the 80 students because gelation occurred inside the beaker during the deoxygenation step. This indicated how important it is to add APS (initiator) at the right time perform subsequent steps in a timely manner. A few students (2 out of 80) had no gelation after heating, possibly due to a missing component such as initiator or crosslinker. These students joined another group to perform the tensile tests, and as such still managed to participate in a key activity. This also highlights the crucial role of each reactant, and the acute dependence of performance on chemical reactions. Which components are responsible for which chemical processes? This provides key learning points, particularly for students without a traditional chemical education/background.

An example of data sets obtained by students is available in the supporting information. During tensile testing it is important that the students gradually increase the mass of the marbles. Increasing the mass (force) by large increments may lead to rupture with very few data points along the curve. Inaccurate evaluation of modulus is thus exacerbated. The most common error made by the students in the post-lab assessment was failures in unit conversions or wrong calculations. Mistakes during the synthesis of the hydrogels can also lead to divergent data. Despite these minor deviations, most students were able to deduce the general trend and used the correct terminology (soft, tough, brittle) for each of the measured gels. The students were typically capable of describing relationships between composition and mechanics, and were importantly able to calculate the Young's modulus and work of extension (toughness), despite this being their first exposure to mechanics/materials science generally.

Two additional data sets were also added to the supporting information. These datasets were obtained using hydrogels that were synthesized using a slightly different protocol. For these gels a petri dish was used as a mold. The circular intermediate hydrogel was soaked for only 5 minutes in a $CaCl_2$ solution. Rectangular shapes were cut out of the circular gel for tensile testing. This gives rise to slightly different mechanical properties, but the general trend is still observed.

CONCLUSION

The aim of this laboratory is to prepare a double network hydrogel by free radical polymerization and explore the influence of the composition on the mechanical properties. This lab procedure gives the students an opportunity to gain experience in both polymer chemistry and material science. The hydrogels are prepared using an easy procedure and the students are made aware of the safety precautions associated with handling hazardous chemicals. A simple setup allows the students to explore tensile testing in a fun and hands-on manner. The adjustable procedure makes the experiment interesting for an undergraduate laboratory in a diverse range of subjects from chemistry, to polymer and materials sciences, to biomedical sciences.

ASSOCIATED CONTENT

Supporting information

Detailed list of materials and equipment used, student handouts, safety rapport, example calculation sheet, student results, description of adaptations to the procedure.

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Notes

The authors declare no competing financial interest.

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REFERENCES

1. Lee, J.-H.; Kim, H.-W., Emerging properties of hydrogels in tissue engineering. *J. Tissue Eng.* **2018,** *9* 2041731418768285-2041731418768285.
2. Li, L. Q.; Stiadle, J. M.; Lau, H. K.; Zerdoum, A. B.; Jia, X. Q.; Thibeault, S. L.; Kiick, K. L., Tissue engineering-based therapeutic strategies for vocal fold repair and regeneration. *Biomaterials* **2016,** *108* 91-110.
3. Liu, S.; Maheshwari, R.; Kiick, K. L., Polymer-Based Therapeutics. *Macromolecules* **2009,** *42* (1), 3-13.
4. Place, E. S.; George, J. H.; Williams, C. K.; Stevens, M. M., Synthetic polymer scaffolds for tissue engineering. *Chem. Soc. Rev.* **2009,** *38* (4), 1139-1151.
5. Lee, K. Y.; Mooney, D. J., Alginate: Properties and biomedical applications. *Prog. Polym. Sci.* **2012,** *37* (1), 106-126.
6. Lee, K. Y.; Mooney, D. J., Hydrogels for Tissue Engineering. *Chem. Rev.* **2001,** *101* (7), 1869-1880.
7. Jia, X. Q.; Kiick, K. L., Hybrid Multicomponent Hydrogels for Tissue Engineering. *Macromol. Biosci.* **2009,** *9* (2), 140-156.
8. Bouten, C. V. C.; Dankers, P. Y. W.; Driessen-Mol, A.; Pedron, S.; Brizard, A. M. A.; Baaijens, F. P. T., Substrates for cardiovascular tissue engineering. *Adv. Drug Deliv. Rev.* **2011,** *63* (4-5), 221-241.
9. Kim, I. L.; Mauck, R. L.; Burdick, J. A., Hydrogel design for cartilage tissue engineering: A case study with hyaluronic acid. *Biomaterials* **2011,** *32* (34), 8771-8782.
10. Li, J. Y.; Mooney, D. J., Designing hydrogels for controlled drug delivery. *Nature Reviews Materials* **2016,** *1* (12).
11. Palmese, L. L.; Thapa, R. K.; Sullivan, M. O.; Kiick, K. L., Hybrid hydrogels for biomedical applications. *Curr. Opin. Chem. Eng.* **2019,** *24* 143-157.
12. Tanaka, Y.; Gong, J. P.; Osada, Y., Novel hydrogels with excellent mechanical performance. *Prog. Polym. Sci.* **2005,** *30* (1), 1-9.
13. Yang, J.; Li, Y.; Zhu, L.; Qin, G.; Chen, Q., Double network hydrogels with controlled shape deformation: A mini review. *J. Polym. Sci., Part B: Polym. Phys.* **2018,** *56* (19), 1351-1362.
14. Takahashi, R.; Sun, T. L.; Saruwatari, Y.; Kurokawa, T.; King, D. R.; Gong, J. P., Creating Stiff, Tough, and Functional Hydrogel Composites with Low-Melting-Point Alloys. *Adv. Mater.* **2018,** *30* (16).
15. Sun, T. L.; Kurokawa, T.; Kuroda, S.; Bin Ihsan, A.; Akasaki, T.; Sato, K.; Haque, M. A.; Nakajima, T.; Gong, J. P., Physical hydrogels composed of polyampholytes demonstrate high toughness and viscoelasticity. *Nat. Mater.* **2013,** *12* (10), 932-937.
16. Matsuda, T.; Nakajima, T.; Fukuda, Y.; Hong, W.; Sakai, T.; Kurokawa, T.; Chung, U.; Gong, J. P., Yielding Criteria of Double Network Hydrogels. *Macromolecules* **2016,** *49* (5), 1865-1872.
17. Gong, J. P., Why are double network hydrogels so tough? *Soft Matter* **2010,** *6* (12), 2583-2590.
18. Nakajima, T.; Furukawa, H.; Tanaka, Y.; Kurokawa, T.; Osada, Y.; Gong, J. P., True Chemical Structure of Double Network Hydrogels. *Macromolecules* **2009,** *42* (6), 2184-2189.
19. Sereda, G.; Hawkins, B., Introducing Students to the Medical Applications of Cross-Linked Hydrogels Using Nontoxic Materials and Experiments Suitable for Many Settings. *J. Chem. Educ.* **2018,** *95* (11), 2068-2070.
20. Kosbar, L. L.; Wenzel, T. J., Inclusion of Synthetic Polymers within the Curriculum of the ACS Certified Undergraduate Degree. *J. Chem. Educ.* **2017,** *94* (11), 1599-1602.
21. Morris, R. K.; Hilker, A. P.; Mattice, T. M.; Donovan, S. M.; Wentzel, M. T.; Willoughby, P. H., Simple and Versatile Protocol for Preparing Self-Healing Poly(vinyl alcohol) Hydrogels. *J. Chem. Educ.* **2019,** *96* (10), 2247-2252.
22. Bowles, R. D.; Saroka, J. M.; Archer, S. D.; Bonassar, L. J., Novel Model-Based Inquiry of Ionic Bonding in Alginate Hydrogels Used in Tissue Engineering for High School Students. *J. Chem. Educ.* **2012,** *89* (10), 1308-1311.

23. Chen, Y.-H.; He, Y.-C.; Yaung, J.-F., Exploring pH-Sensitive Hydrogels Using an Ionic Soft Contact Lens: An Activity Using Common Household Materials. *J. Chem. Educ.* **2014,** *91* (10), 1671-1674.
24. Hurst, G. A., Green and Smart: Hydrogels To Facilitate Independent Practical Learning. *J. Chem. Educ.* **2017,** *94* (11), 1766-1771.
25. Warren, D. S.; Sutherland, S. P. H.; Kao, J. Y.; Weal, G. R.; Mackay, S. M., The Preparation and Simple Analysis of a Clay Nanoparticle Composite Hydrogel. *J. Chem. Educ.* **2017,** *94* (11), 1772-1779.
26. Sun, J. Y.; Zhao, X. H.; Illeperuma, W. R. K.; Chaudhuri, O.; Oh, K. H.; Mooney, D. J.; Vlassak, J. J.; Suo, Z. G., Highly stretchable and tough hydrogels. *Nature* **2012,** *489* (7414), 133-136.
27. Yang, C. H.; Wang, M. X.; Haider, H.; Yang, J. H.; Sun, J. Y.; Chen, Y. M.; Zhou, J. X.; Suo, Z. G., Strengthening Alginate/Polyacrylamide Hydrogels Using Various Multivalent Cations. *ACS Appl. Mater. Interfaces* **2013,** *5* (21), 10418-10422.
28. Morelle, X. P.; Illeperuma, W. R.; Tian, K.; Bai, R. B.; Suo, Z. G.; Vlassak, J. J., Highly Stretchable and Tough Hydrogels below Water Freezing Temperature. *Adv. Mater.* **2018,** *30* (35).
29. Zhang, W. L.; Hu, J.; Tang, J. D.; Wang, Z. T.; Wang, J. K.; Lu, T. Q.; Suo, Z. G., Fracture Toughness and Fatigue Threshold of Tough Hydrogels. *ACS Macro Lett.* **2019,** *8* (1), 17-23.
30. Haider, H.; Yang, C. H.; Zheng, W. J.; Yang, J. H.; Wang, M. X.; Yang, S.; Zrinyi, M.; Osada, Y.; Suo, Z. G.; Zhang, Q. Q.; Zhou, J. X.; Chen, Y. M., Exceptionally tough and notch-insensitive magnetic hydrogels. *Soft Matter* **2015,** *11* (42), 8253-8261.
31. Li, J. Y.; Illeperuma, W. B. K.; Suo, Z. G.; Vlassak, J. J., Hybrid Hydrogels with Extremely High Stiffness and Toughness. *ACS Macro Lett.* **2014,** *3* (6), 520-523.
32. Darnell, M. C.; Sun, J. Y.; Mehta, M.; Johnson, C.; Arany, P. R.; Suo, Z. G.; Mooney, D. J., Performance and biocompatibility of extremely tough alginate/polyacrylamide hydrogels. *Biomaterials* **2013,** *34* (33), 8042-8048.
33. Arrizabalaga, J. H.; Simmons, A. D.; Nollert, M. U., Fabrication of an Economical Arduino-Based Uniaxial Tensile Tester. *J. Chem. Educ.* **2017,** *94* (4), 530-533.
34. Gilmer, T. C.; Williams, M., Polymer mechanical properties via a new laboratory tensile tester. *J. Chem. Educ.* **1996,** *73* (11), 1062.
35. Moser, R.; Kettlgruber, G.; Siket, C. M.; Drack, M.; Graz, I. M.; Cakmak, U.; Major, Z.; Kaltenbrunner, M.; Bauer, S., From Playroom to Lab: Tough Stretchable Electronics Analyzed with a Tabletop Tensile Tester Made from Toy-Bricks. *Adv. Sci.* **2016,** *3* (4), 1500396.
36. Ahmed, S.; Nakajima, T.; Kurokawa, T.; Haque, M. A.; Gong, J. P., Brittle-ductile transition of double network hydrogels: Mechanical balance of two networks as the key factor. *Polymer* **2014,** *55* (3), 914-923.
37. Kolvin, I.; Kolinski, J. M.; Gong, J. P.; Fineberg, J., How Supertough Gels Break. *Phys. Rev. Lett.* **2018,** *121* (13).
38. Ritchie, R. O., The conflicts between strength and toughness. *Nat. Mater.* **2011,** *10* (11), 817-822.
39. Li, X.; Wang, H.; Li, D.; Long, S.; Zhang, G.; Wu, Z., Dual ionically cross-linked double-network hydrogels with high strength, toughness, swelling resistance, and improved 3D printing Processability. *ACS Appl. Mater. Interfaces* **2018,** *10* (37), 31198-31207.

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