QUANTITATIVE DETERMINATION OF MECHANICAL STABILITY OF A MICROCAPSULE

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Abstract
Synthetic reactive polyelectrolytes based on methacrylic acid are used to form covalently reinforced cross-linked networks within calcium-gelled Alginate-Poly-L-lysine-Alginate (APA) microcapsules. The cross-linked nature of the capsule was demonstrated by its resistance to citrate and sodium chloride. The internal morphology of the capsules was tested by optical microscopy. The mechanical strength of individual capsule was determined by a micro compression tester. The encapsulation processes and properties of the microcapsules were discussed in some detail.

Introduction
One of the most common microencapsulation systems involves alginate/poly-L-lysine/alginate (APA) microcapsules derived from the original protocol of Lim and Sun.[1] These capsules are primarily composed of alginate, a naturally produced polysaccharide composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. Calcium ions are used to cross-link G-rich regions of the alginate chains, and the resulting calcium alginate (CaAlg) hydrogel beads are over coated with poly-L-lysine (PLL) and alginate to strengthen and make the capsules biocompatible. We previously described polyelectrolytes bearing complementary reactive groups that underwent a covalent cross-linking reaction to produce a covalent cross-linked polyelectrolyte complex coating on CaAlg beads.[2] Another approach has been used to construct alternate hydrogel cores by the reinforcement of the alginate core through the formation of an interpenetrating network or composite may lead to improved mechanical properties of the capsules. A number of alginate composite materials have been explored.[3] Compounds added to the alginate forming the bead core were designed to be thermally (agarose[4]) ionically (carrageenan[5]) or photochemically gelled,[6] or designed to modify viscosity or water content (carboxymethylcellulose[3]), act as wall forming materials (cellulose sulphate,[7] caprolactone[8]). This paper describes the use of self-cross-linking polyelectrolytes to covalently reinforce the core and surface of CaAlg beads. The internal morphology and quantitative determination of mechanical strength of the capsules will also be discussed.

Experimental
Materials
Sodium alginate (Keltone LV, M_n = 428 kDa) was a gift of the Nutrasweet Kelco Company. Methacrylic acid (MAA), 2-(methacryloyloxy)ethyl acetoacetate (MOEAA), and PLL (M_n = 15-30 kDa), were purchased from Sigma-Aldrich, and used as received. 2, 2'-Azobis (isobutyronitrile) (AIBN) was purchased from Dupont and used as received.

Poly(methacrylic acid, sodium salt-*co*-2-[methacryloyloxy] ethyl acetoacetate) (p(MAA-*co*-MOEAA), 70:30 (A70)
p(MAA-*co*-MOEAA) 70:30 was prepared by free radical polymerization following the procedure published earlier in the literature.[9] Briefly, MAA (4.84 g; 56.2 mmol), MOEAA (5.16 g; 24.1 mmol) and AIBN (132 mg; 0.80 mmol, 1 mol% relative to monomer) were dissolved in 100 ml of ethanol in a 125 mL HDPE bottle and then heated at 60 °C for 24 h. The polymer was isolated by precipitation in diethyl ether and then dried to a constant weight in a vacuum oven at 50 ºC. Yield: 8.74 g (87%). This polymer was neutralized with 1M NaOH to form the sodium salt of A70 and then diluting to the desired polymer concentration.

Instrumentation
The composition of p(MAA-*co*-MOEAA) was determined by ^{1}H NMR spectroscopy in DMSO-d_6 using a Bruker AV 200 spectrometer. The pK_a value of A70 was determined by potentiometric titration (PC Titrator, Man Tech Associates Inc.) of the hydrochloride salts with 0.100 M NaOH. Molecular weight of A70 was determined using a Waters 515 gel permeation chromatography (GPC) system described previously, [2] calibrated with narrow-dispersed PEG standards. Samples were eluted at a flow rate of 0.80 mL/min with a mobile phase consisting of 0.3 M sodium nitrate in phosphate buffer (pH 7). The pH of 1wt% A70 was adjusted to 7 with 1M NaOH.

Preparation of Core cross-linked Microcapsules
APA and Ca(Alg/A70)PA microcapsules were prepared using CaAlg and Ca(Alg/A70), respectively as a base hydrogel following a literature procedure described by Ross et al.[10] To prepare a Ca(Alg/A70) composite beads, aqueous solutions of 1.5 wt% sodium alginate (NaAlg) and 0.5 wt% A70 at pH 7 were filtered through sterile 0.45 μm Acrodisc syringe filters (Pall Corporation, USA). A modified syringe pump (Rassel Mechanical Inc.) was used to extrude the alginate-A70 solution at a rate of 30.1 mL/hr through a 27-gauge blunt needle (Popper & Sons, New York), with a concentric airflow (4 L/min) passing by the needle tip to induce droplet formation. The droplets were collected in a 1.1 wt% $CaCl_2$, 0.45 wt% NaCl gelling bath causing the formation of Ca(Alg/A70) composite beads. After beads formation was complete, the supernatant was removed, and the resulting concentrated Ca(Alg/A70) composite bead suspension was washed in sequence with four-fold volumes of a) 1.1 wt% $CaCl_2$, 0.45 wt% NaCl for 2 minutes; b) 0.55 wt% $CaCl_2$, 0.68 wt% NaCl for 2 minutes; c) 0.28 wt% $CaCl_2$, 0.78 wt% NaCl for 2 minutes; d) 0.1 wt% CHES, 1.1 wt% $CaCl_2$, 0.45 wt% NaCl for 3 minutes.; and then e) 0.9 wt% NaCl for 2 minutes and stored in saline. These beads (3 mL) were subsequently exposed to 0.05 wt% PLL (10 mL) for 6 minutes and then

washed with 15 ml of a) 0.1% CHES, 1.1% $CaCl_2$, 0.45% NaCl for 3 minutes; b) 1.1% $CaCl_2$, 0.45% NaCl for 2 minutes; and c) 0.9% saline for 2 minutes. Finally the beads were coated with 0.03 wt% Na alginate (10 mL) in saline for 4 minutes followed by three washes with 0.9% saline (12 mL).

Chemical and Mechanical Stress Test

The mechanical stability of capsules was tested by agitating the capsules while they were exposed to hypotonic solution (the osmotic pressure test (OPT),[11] or by using a Ca chelation test.[2] Briefly, in the OPT, 100 µL of capsules and 10 ml of distilled water were placed in a 15 mL polypropylene conical tube and then agitated for 3 h on an orbital mixer (REAX 3, Wiarton, ON) at 30 rpm. The capsules were then stained by adding 0.12 mL of a 0.4% solution of trypan blue in 0.85% saline. The percentage of intact capsules was determined from an image taken with a digital camera. In the calcium chelation test, 100 µL of capsules were exposed to 5% w/v (170 mM, 5 mL) sodium citrate solution in distilled water while being agitated for 15 minutes on an orbital mixer at 30 rpm, before analysis, as described for the OPT. The samples were then examined by optical microscopy (Olympus BX51) fitted with Q-Imaging Retiga EXi digital camera and ImagePro software.

Mechanical Stabilities by Compression Test

The mechanical properties of individual capsules were measured using a micro compression tester. Single capsule was compressed between a 4 mm^2 silicon wafer attached to a piezo-electric transducer, and a glass microscopy slide mounted on an inverse microscope. The wafer was positioned over one capsule at a time and moved down vertically at a constant speed of 10 µm/s with the help of a stepper motor while plotting the force registered against vertical displacement at room temperature. Compression data were corrected both for the buoyancy of the silicon wafer, and for the elastic give of the experimental set up.

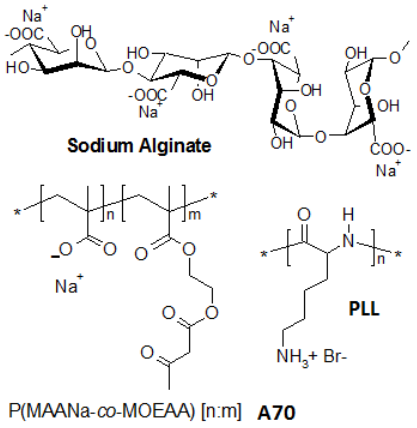
Results and Discussion

The aim of this work is to explore covalent reinforcement of CaAlg beads with a cross-linked network inside and outside the core formed by reaction between a polyanion present in the core, and a polyamine diffusing in from the outside. Here we explore the ability of these polyelectrolytes to form cross-linked networks, leading to increase the mechanical strength of the capsules, and study their morphology and strength in quantitative manner. The polyelectrolytes used in this study are described in Table 1 and Scheme 1.

Table 1: Polyelectrolyte properties.

Polyelectrolyte	Composition	MW Mn (kDa)	pK_a
NaAlg (G:M)	40:60[a]	428[a]	3.20/3.38 (G/M)[a]
A70 (MAA:MOEAA)	70:30 (± 4)[b]	42[c]	7.1[d]
PLL	--	15-30[a]	10.5[e]

a) given by supplier, b) determined by ^1H NMR, c) from GPC, d) from titration in 50% methanol, e) ref[12].



Scheme 1: Chemical structure of polyelectrolytes.

CaAlg beads containing the synthetic polyanions were prepared by dripping mixtures of NaAlg and the polyanion into a $CaCl_2$ bath.[10] The composite beads formed from a solution containing 1.5 wt% NaAlg and 0.5 wt% A70 had an average diameter of 650 µm determined by optical microscopy (OM), and appeared identical to those formed from alginate alone (Figure 1). The Ca(Alg/A70) composite or CaAlg beads were exposed to 0.05% PLL, washed with saline and then coated with a 0.03% NaAlg solution. When examined by OM, the capsules looked similar to the uncoated bead but the capsule surface was easily stained by trypan blue indicating the presence of the polycation (image not shown).

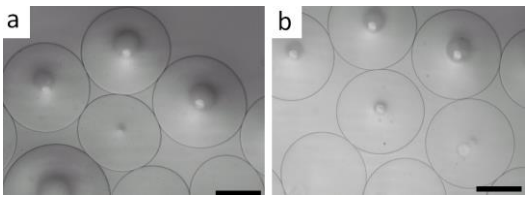


Figure 1: Optical Microscope image of a) CaAlg beads, b) Ca(Alg/A70) composite beads. The scale bar is 300 µm.

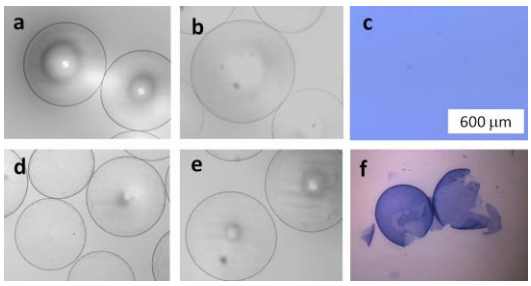


Figure 2: Optical microscope image of a) APA and d) Ca(Alg-A70)P)A microcapsules; b) e), hollow microcapsules after treatment with 170 mM Na citrate, and c, f) after further treatment with 2M NaCl and staining with trypan blue.

The integrity of uncoated and PLL coated Ca(Alg/A70) composite beads in the presence of Na citrate and NaCl was examined by OM and compared with that of classical APA microcapsules. Uncoated beads composed of Ca(Alg/A70) or

CaAlg are stable at physiological salt concentrations (0.9% NaCl) but they dissolve when exposed to 170 mM (5% w/v) Na citrate, which extracts the calcium from the gel. In contrast, addition of Na citrate to PLL coated capsules such as APA (Figure 2a) or Ca(Alg/A70)PA (Figure 2d) caused the core of the capsules to dissolve, while the shell survived (Figure 2b, e). However, upon addition of 2M NaCl, the ionically cross-linked APA shells were dissolved completely (Figure 2c) while the covalently cross-linked shells of the Ca-(Alg/A70)PA capsules remained (Figure 2f).

The mechanical stability of APA and core cross-linked Ca(Alg/A70)PA microcapsule was determined using a high strain rate micro compression tester. It was used to measure the maximum load before rupture, applied through a stepper motor at a constant speed of 10 μm/s to the maximum load while plotting the force registered against vertical displacement. Figure 3 shows a typical force versus displacement curve, for compressing a single APA and core cross-linked Ca(Alg/A70)PA capsules. During the experiments, the force transducer probe started to move downward at point 1 and touched the capsule at point 2. The compression commenced immediately and the force started to increase until the capsule underwent rupture (point 3). Upon compression, the capsules deformed to between 2 and 2.5 times their original diameter, and about 20 to 25% of their original height, before cracking. It was noted that the present core-cross linked Ca(Alg/A70)PA capsules, upon exceeding their maximum compressive loading, do not undergo catastrophic failure, but rather undergo progressive cracking that still provides some matrix isolation for the biological species embedded in the fragments. This is in contrast to APA core-shell capsules that fail by a catastrophic bursting mechanism, which exposes all of the capsule content to the host.

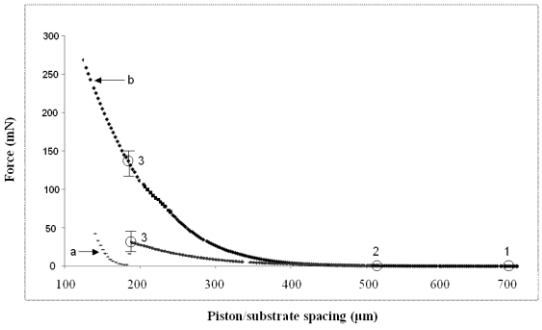


Figure 3: Force versus displacement curve obtained from compression of a) APA, b) core cross-linked Ca(A/A70)PA capsule. The diameter of the microcapsule was about 500 μm.

Typically, core cross-linked capsule exhibited a first crack about 120-150 mNewtons of compressive force (Figure 3). Compression of the same type of capsule after extraction of the calcium in the core with Na citrate shows a slight reduction (data not shown) in load at failure, indicating that most of the capsule strength derives from the synthetic polymer network, rather than from the CaAlg matrix. Extraction with citrate is designed to mimic the slow exchange of calcium for sodium known to take place in biological species. The results indicate that the capsules strength of these covalently cross-linked capsules should not suffer from such ion exchange. In contrast, typical force at failure for non-cross linked APA capsules described here is between 20 and 40 mNewtons, with the failure mechanism resembling the sudden bursting of a balloon, rather than progressive cracking (Figure 3).

Conclusion

We studied the synthesis and characterization of reactive polyelectrolytes, and their association behavior to improve the mechanical stability by the formation of covalent cross-linked network within the conventional alginate/poly-L-lysine/alginate (APA) microcapsules. The internal and external cross-linked network can be formed in CaAlg capsules by inclusion of A70 in conventional APA microcapsule cores. These microcapsules have greater resistance to chemical and mechanical stress tests, compared to control APA capsules. A micro compression tester equipped with an inverse microscope was developed to measure the bursting force (e.g.; mechanical strength) of single microcapsule. This technique makes it possible to compare the mechanical strength of microcapsules made of different morphologies, and to infer information about microcapsule mechanical properties.

Acknowledgment

The author would like to gratefully acknowledge King Fahd University of Petroleum and Minerals, Saudi Arabia for providing excellent research facilities.

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