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Protein Diffusion Profiles Studied for Collagen-Alginate Composite Hydrogels

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Protein Diffusion Profiles Studied for Collagen-Alginate Composite Hydrogels

by

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Student Name (printed)

Student Signature

_____May 9, 2021_____

Date

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Dedication:

Thank you to my Mom and Dad for supporting me through this journey, both in academia and in life.

Immense gratitude is also extended to Dr. Roh and all in Roh Lab for allowing the completion of this Capstone Project.

Abstract

Hydrogels have been extensively researched as synthetic extracellular matrices that are used to encapsulate various proteins and target cells and aid in scaffolding for proliferation and diffusion. They are especially interesting in that they have been proven to be successful in simulating *in vivo* conditions while allowing for observation, analysis, and manipulation of factors that contribute to important properties that affect the biomolecules they are able to hold, such as diffusivity and rheology, which can then seek to be improved. This paper explores specifically the effects on diffusion when alginate, a renown biosynthetic material already commonly used, and collagen, the most abundant protein in the human body, are combined to form a composite hydrogel. Using bovine serum albumin (BSA) as a model biomolecule, diffusion release profiles were constructed for various formulations of collagen-alginate composite hydrogels. Mathematical analysis for a kinetic diffusion model was employed to calculate the effective diffusion coefficient of the model biomolecule from each hydrogel formulation. We illustrated and concluded that the diffusion is more rapid from the collagen-alginate gels than from the corresponding alginate-only gels.

Background

Alginate has been used in a myriad of tissue engineering applications, including those involving the formation of hydrogels. Alginate is a polysaccharide that is harvested from seaweed and certain bacteria, and is especially of interest due to its remarkable compatibility with the human body in that it is biodegradable and nontoxic (Sun and Tan, 2013).Its chemical structure allows for it to be a sought-after material for hydrogelation. Its anionic and hydrophilic properties enable it to – when in contact with divalent cations – form a gel through crosslinking.

Collagen has been increasingly popular in fulfilling the same purpose of hydrogel formation. Specifically, Type I collagen has been more researched out of all the different types. It is different from alginate in that it is considered a native protein; in fact, it is the most prevalent in humans. Collagen gelation occurs with correct temperature, pH, and presence of a water-based solvent (Antoine et al, 2014).

Combining alginate and collagen as a hydrogel has been shown to be quite effective in that it is able to form desired properties. Such characteristics include porosity, permeability, and stiffness that resemble those of tissues and organs. This allows for *in vitro* studies and conceptualization of a myriad of different postulations. In an experiment conducted by Liu and his fellow researchers, he uses a collagen-alginate composite hydrogel to both emulate and examine how tumors developed. The hydrogels were formed to replicate breast tissues, of which they injected tumor spheroids, and were consequently analyzed as a platform for tumor invasion (Liu et al, 2018.) Another study, conducted by Zhou and his team, took advantage of the same properties presented by the collagen-alginate hydrogels and went to a slightly different direction; they sought to create an environment that would facilitate the proliferation of stem cells (Zhou et al, 2019). They prepared different ratios of alginate and collagen and – with the help of various

analytical tools, including x-ray diffraction and electron microscopy – determined which pair would yield the most effective results (in their case, a 2:1 ratio of alginate and collagen).

Nonetheless, for both types of hydrogels, encapsulation of target biomaterials is possible and have been studied for regenerative purposes. Exploration of a blended composite hydrogel consisting of the two materials have also been studied and have been proven effective in scaffolding the production and diffusion of various cells and biomaterials. An additional comparison between the composite hydrogel and the alginate-only hydrogel, particularly the rate at which the biomaterials are diffused out of the gel, is detailed in this paper.

Materials and Methods

Protein Assay

Pierce™ BCA Protein Assay Kit was used to determine the concentration of bovine serum albumin, the chosen protein to study in the paper, in the samples collected to construct a diffusion release profile of the BSA in the hydrogel beads. Standard curves were created according to the manufacturer's instructions. The samples were read in a 96-well plate using a spectrophotometer at 562 nm.

Preparation of Alginate Beads

As a comparative model for the study of the alginate-collagen composite hydrogel beads, alginate-only hydrogel beads were constructed. Four solutions of varying concentrations, 0.5%, 1%, 2%, and 4% w/v, were made. 200 µg/ml of BSA were added to each solution. The hydrogel beads were made by pipetting ten 10 µl beads into vials containing 500 ml of 25 mM calcium

chloride solution, which also contained 200 µg/ml of BSA. The BSA in calcium chloride allowed

for the extinguishment of the concentration gradient when the beads were formed, preventing the

protein from significantly diffusing out of the beads before the first sample was taken.

Preparation Alginate-Collagen Beads

      3 mg/ml collagen solution was prepared over ice using FibriCol® collagen and following

the manufacturer's directions for use. Alginate solutions of concentrations 0.5%, 1%, and 2%

w/v were used for the composite beads, which were also chilled over ice when added to the

collagen to prevent gelation. 200 µg/ml of BSA was added to the combined collagen-alginate

solution. Ten beads 10 µlwere formed in vials containing 500 ml of 37°C $CaCl_2$ solution, to

trigger gelation of both the alginate and collagen, in the same manner as described in the

alginate-only beads.

Sampling of Release Media

      Samples of the $CaCl_2$ loading solutions containing both the alginate-only and

collagen-alginate beads were taken one minute after the beads were formed. Equivalent volume

of DI water, 500 ml, was then added to each of the vials containing ten beads in order to create

sink conditions. The vials were also rotated until the next sample was collected. Samples of the

releasing media were then taken at the 1, 6, 16, 36 minute mark to create the diffusion release

profile. For the collagen-alginate beads, additional sampling at the 66, 126, and 1806 minute

marks were taken. After each sample collected, another 500 ml of DI water was added to

replenish the sink conditions. The first six diffusion sample vials were placed in a 4°C freezer

overnight until the last sample was taken the next day and subsequently read using the spectrophotometer.

The collected spectrophotometer readings reporting the amount of BSA diffused out the beads at the specified time intervals were plotted using Microsoft Excel for depiction of diffusion profile. Desmos (https://www.desmos.com/calculator) was then used to determine the diffusion coefficient by inputting the mathematical model equation for radial diffusion (1), and fitting the resulting curve to best fit with the obtained experimental data. This was accomplished by adjusting the diffusion coefficient, D, which was made into a variable parameter on the website.

Results

Figure 1 below outlines the procedure completed for hydrogel formation. The alginate only beads were made in the same manner, but without the added collagen were prepared at room temperature. The radius of the beads was obtained and kept consistent by keeping each bead's volume at 10 ul, made possible by micropipetting the solutions into the calcium chloride solution. This is essential in calculating the effective diffusion coefficient of the BSA.
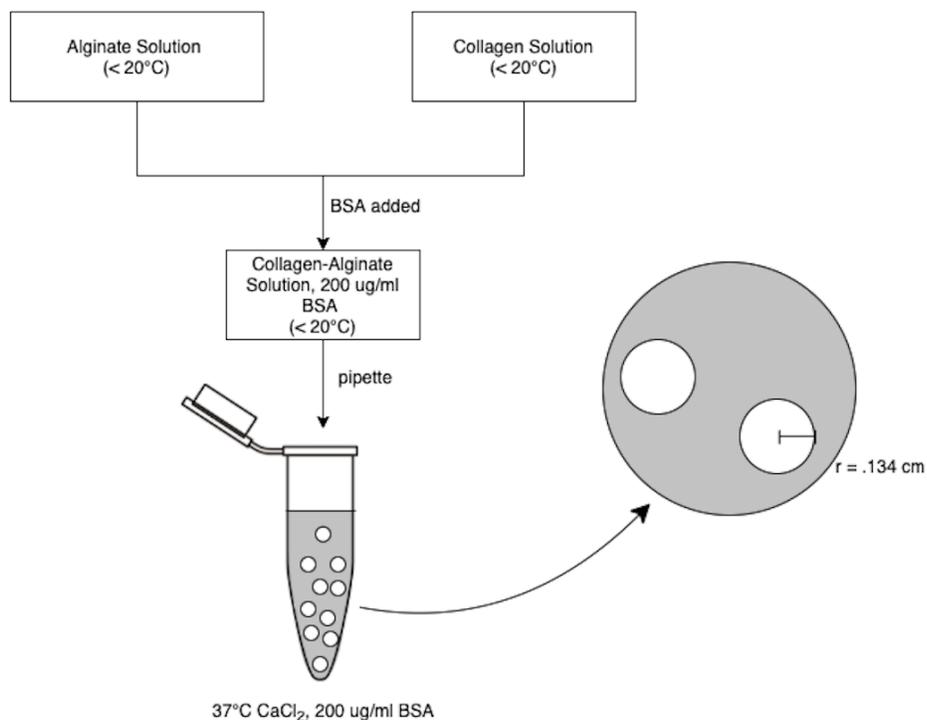
Figure 1. Schematic of collagen-alginate gel formation and radius of resulting beads

Standard Curve

  A standard curve was constructed in order to determine concentrations of BSA in the diffusion experiment. The curve was checked and compared against for consistency at each day of reading. The coefficient of determination for the constructed standard curve was calculated to be 0.99903.
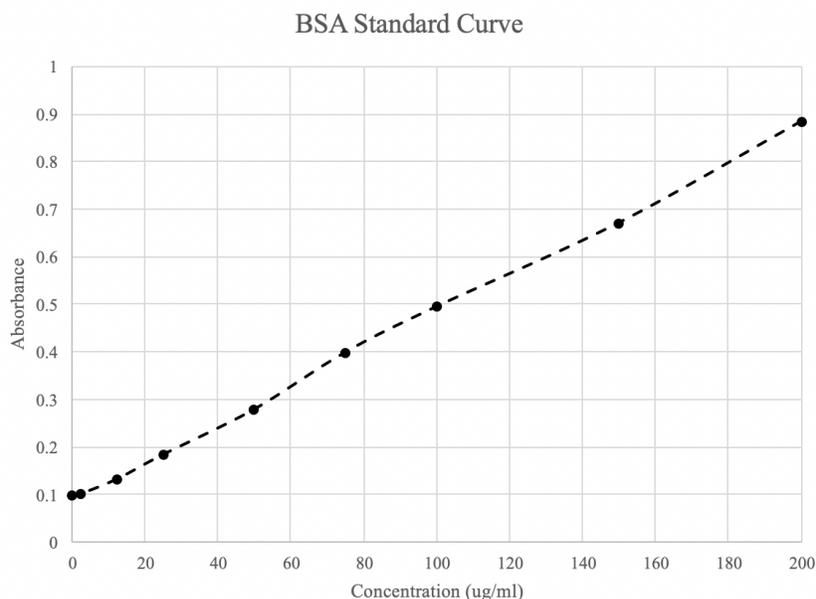
BSA Standard Curve

Figure 2. Standard curve of 200 µg/ml BSA using Pierce™ BCA Protein Assay Kit, read using a spectrophotometer at 562 nm.

Protein Release Profiles

The first set of experiments consisted of obtaining the release profiles of the alginate-only hydrogels. Although both higher and lower concentrations of BSA were investigated, it was concluded that the 200 µg/ml concentration used throughout the rest of this experiment gave the most reliable results for analysis and were compatible with the BCA assay kit that was used. The release profiles constructed were based on accumulated mass of BSA collected in the releasing media. By the 30 minute mark, the value recorded from the spectrophotometer (at 562 nm) was approximately the value recorded in the blank sample. It was concluded that all of the BSA had been diffused, and total protein content was thereafter calculated. The results can be seen in Figure 2. It is shown that approximately 35-45% of BSA was released within the first minute, and over 80% released within 40 minutes.
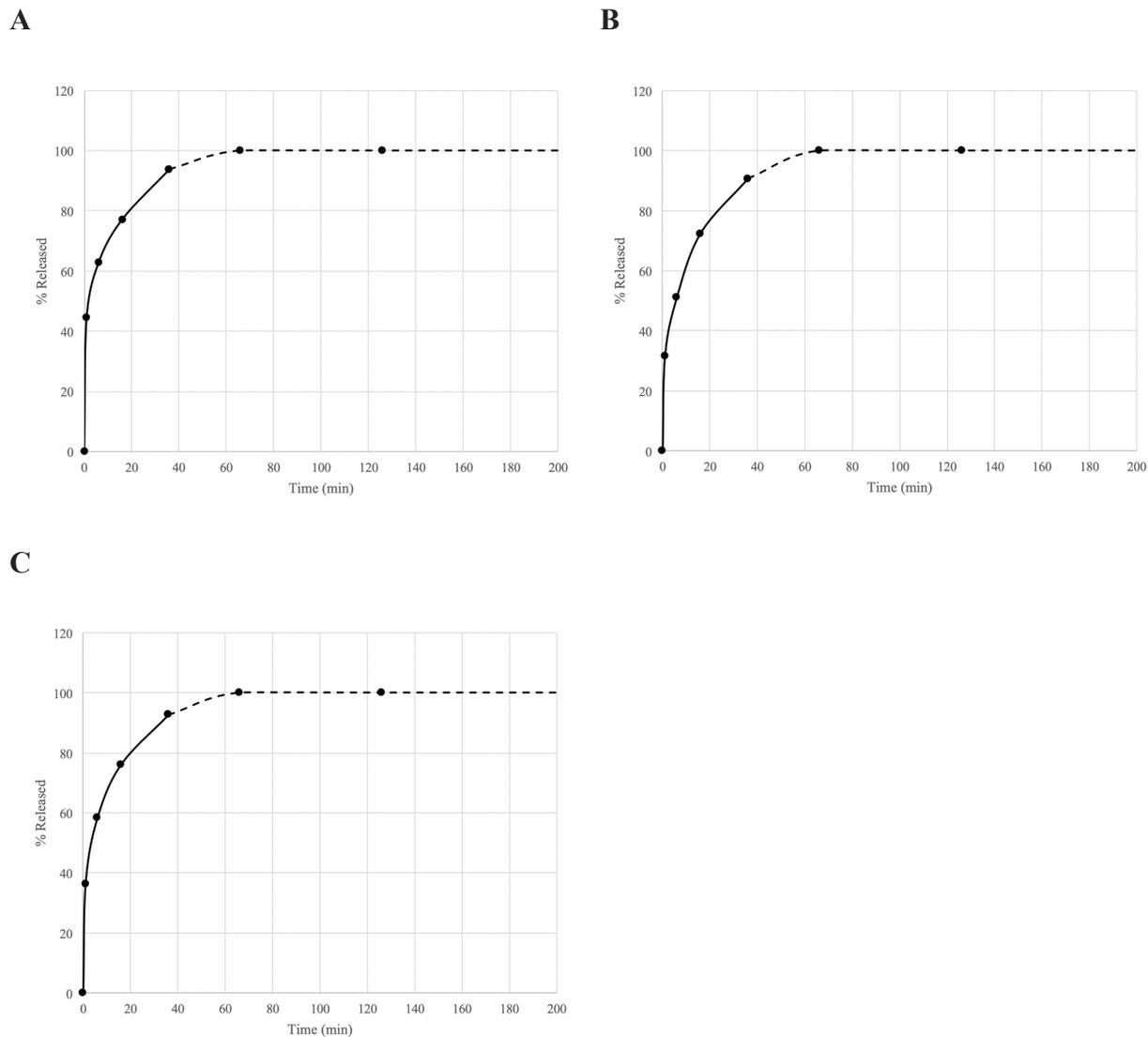
A

B

C

Figure 3. Constructed 200 µg/ml BSA release profiles for 0.5% (A), 1% (B), and 2% alginate

(C). Dotted lines connecting markers represent the theoretical value of percent release, projected

values were used to compare the release profile to the collected data from the collagen-alginate

beads.

The second set of experiments involved adding 3 mg/ml of collagen to the varying

concentrations of alginate solutions. As stated, preparation of samples was done over ice to

prevent premature gelation of the collagen, and beads were formed by dropping the

collagen-alginate solution into warm calcium chloride. The release profile of this set was

calculated in the same manner as the first set on alginate-only beads, and the results are shown in

Figure 3. Initial release of the BSA from these beads ranged from about 50-70% within the first
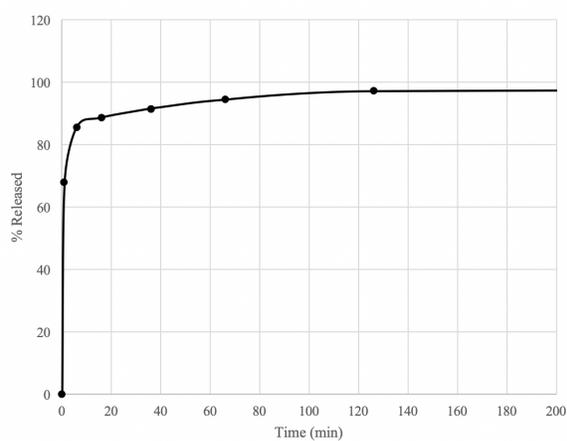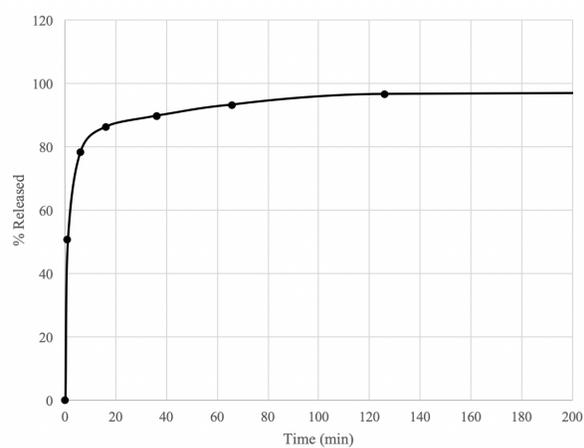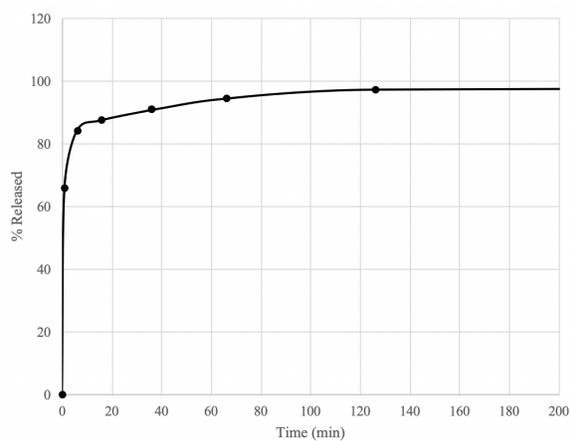
minute.

A

B

C

Figure 4. Constructed 200 µg/ml BSA release profiles for 3 mg/ml collagen with 0.5% (A), 1% (B), and 2% alginate (C). Sample collected at the 30 hour mark (100% release) not shown for clearer depiction of the initial and early release profile.

Mathematical Model of Diffusion Release Through Spheres

A mathematical model of radial diffusion, that is, diffusing substances leaving or entering a sphere, was used in attempt to calculate the diffusion coefficient of BSA through the varying compositions and concentrations of the beads (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-Dn^2\pi^2 t/a^2\right).$$

(1)

where M_t is the amount of solute that has been diffused out of the sphere at time t, M_∞ is amount of solute that has been diffused as time approaches infinity (the total amount of solute loaded into the sphere), a is the radius of the sphere, and D is the effective diffusion coefficient.

Using this model, the release profiles depicted in Figures 2 and 3 were graphed in Desmos (desmos.com/calculator) and fitted accordingly. Estimations of the diffusion coefficient for each hydrogel formulation is given in Table 1.

Diffusion Coefficients and Comparisons

Table 1. Diffusion coefficients of BSA through varying gel compositions and concentrations

Material and Concentration	Diffusion Coefficient (cm^2/s x 10^6)
0.5% Alginate Only	4.67
1% Alginate Only	1.83
2% Alginate Only	3.08
3mg/ml Collagen, 0.5% Alginate	13.2
3mg/ml Collagen, 1% Alginate	7.67
3mg/ml Collagen, 2% Alginate	11.7

The calculated diffusion coefficients of BSA were approximately within the range of some values reported in the previous literatures (Dhanya and Sasikumar, 2018), recorded within the range of 4-5x10^{-6} cm^2/s, depending on pH and temperature. Other recorded literature values for BSA indicate that the majority of the calculated diffusion coefficients in this paper is about a magnitude off; 2.67x10^{-7} cm^2/s was reported as the BSA diffusion coefficient in Sheth's *Predicting Drug Release From Degradable Hydrogels* paper.

Comparisons of the alginate-only and collagen-alginate hydrogels, nonetheless, can be observed from the data presented. When inspecting the conjugates (0.5% alginate with and without collagen, and so forth), it is found that the diffusion coefficient is about 3-4 times greater

from the hydrogel beads with collagen than those without, indicating that collagen facilitates more rapid diffusion. This can also be illustrated in Figure 4.
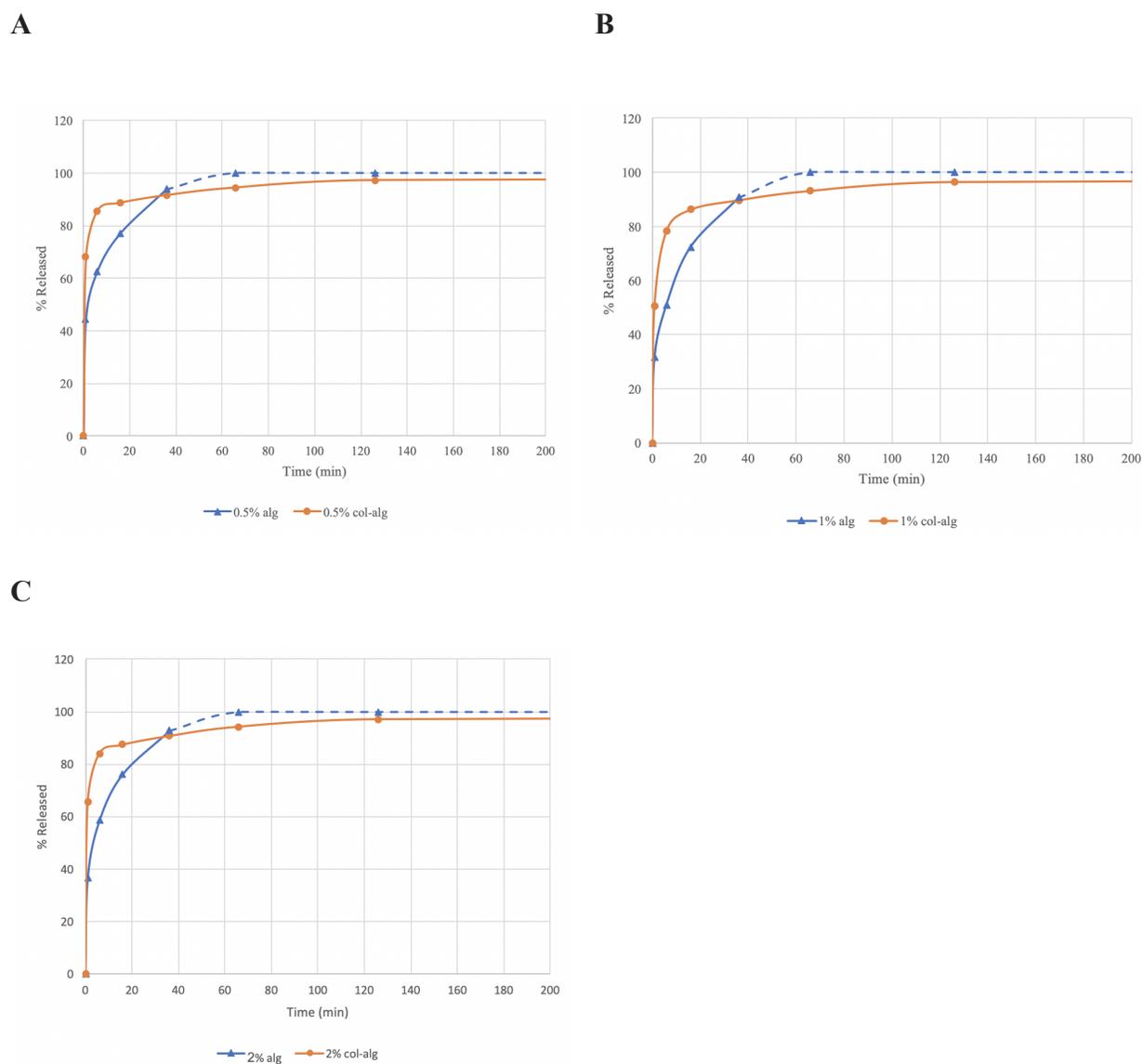
A

B

C

Figure 5. Comparison of release profiles of 0.5% alginate with and without collagen (A), 1% alginate with and without collagen (B), and 2% alginate with and without collagen (C). Initial release of BSA from the beads can be seen to be higher in the beads containing collagen.

Discussion

It is in general expected that the hydrogels made of higher concentrations of polymers would result in slower diffusion of encapsulated biomolecules, thus smaller diffusion coefficients. However, in our results, there was a slight discrepancy concerning the 1% alginate concentration, where it yielded slower diffusion rates than both the 0.5% and the 2% alginate concentrations in both the sets with and without collagen. Although consistent, it should be checked for further analysis. It was presumed that the concentrations of the alginate gels were in small enough increments that errors may have occurred either during preparation or reading.

Nevertheless, the overall trend shows that the addition of collagen into the composition of gel formation causes the diffusion rate to increase. In order to better understand this phenomena, we studied the rheological properties of alginate-only and collagen-alginate composite hydrogels.
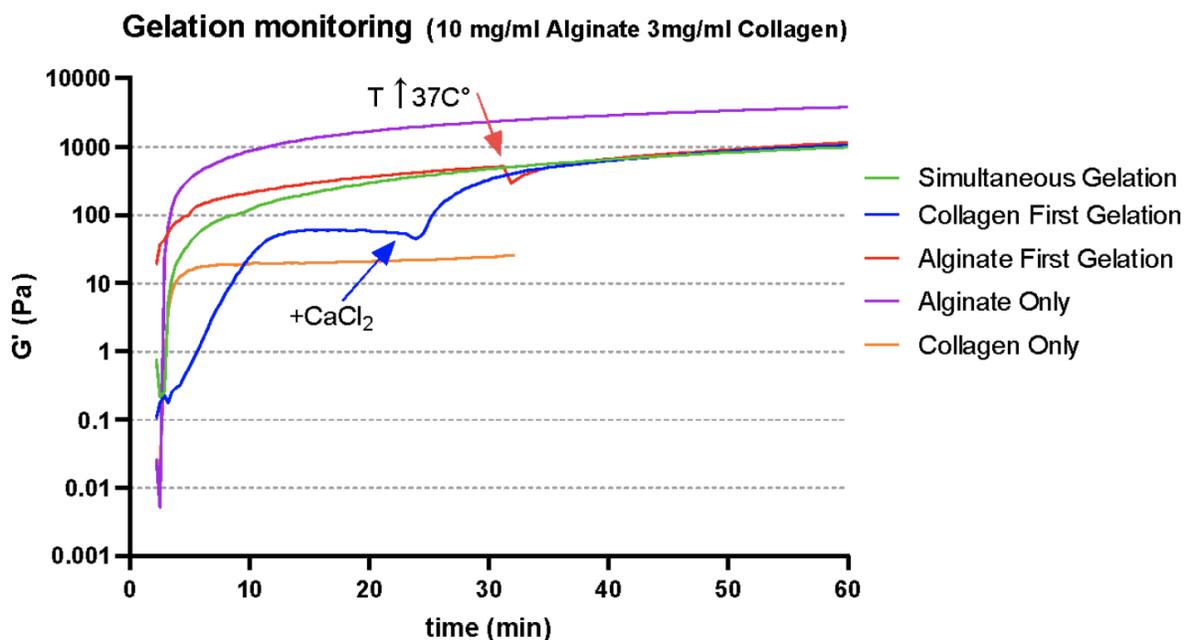

Figure 6. Comparison of gelation profiles, elastic modulus versus time (Ahmadi, 2020).

The G' modulus describes the elastic behavior of a material and its values is directly resulting from the crosslinking mechanisms and the resulting structures. In more simplified terms, it can also show how solid-like the properties of a material of interest, such as complex viscosity, exhibit (Ahmed, 2014), which is often directly proportional to the crosslinking densities. It can be observed from Figure 5 above that the G' values are the highest for the alginate-only hydrogels. When collagen is added to the alginate, the resulting values decrease, and are lowest for the collagen only hydrogels. It can be inferred that addition of collagen decreased the crosslinking density of the alginate-only hydrogel by forming the interpenetrating network (IPN). This change in the final structure of the hydrogels by mixing multiple crosslinking components with different crosslinking mechanisms should be responsible for the increased diffusion of BSA from the alginate-collagen composite gel. We expect that similar characteristics would be found in many microenvironments of human tissues and organs. Thus there is a great potential of this composite gel as a model for research purposes, such as those aforementioned earlier in the paper.

Further Insights and Potential Directions

In order to confirm this property-structure relationship discussed above, the microstructures of each hydrogel formulation should be characterized by employing microscopy techniques such as scanning electron microscopy.

A multitude of mathematical models and equations have been engendered for release kinetics, and while Crank's presented model is an accurate representation of the profile of

interest, other analytical methods should also be considered. For example, Fickian models for solute release can be employed (Ritger and Peppas, 1986). However, to do so, the experiment should be modified such that the initial release of BSA in all of the gels does not exceed 60%, which is the stipulation in using the Fickian release equation. Parameters such as time of sampling, concentration of protein, and even geometry of the gel may be manipulated as needed to obtain the correct data to employ the correct analysis.

It is also important to note that while Desmos was used and is able to give an adequate approximation of the diffusion coefficient for the analytical purposes of this experiment, more robust applications may be necessary to give the most accurate value if exact examinations, specifically regarding the diffusion coefficient, are to be scrutinized.

Other proteins, such as insulin and lysozyme, biomolecules, and cells can also be used for experimentation and analysis of effective diffusion. In addition, viability of cells within the collagen-alginate beads can also be tested in conjunction with the outlined experiment to research how or whether the composition of the gel allows for growth and development of target cells. Other factors that may influence this could include ratio of alginate and collagen, as well as the pH of the components.

Conclusions

The observed effect of collagen in alginate hydrogels is that it promotes diffusion of protein, specifically BSA, out of its encapsulation. This could be affected by collagen's effect on the elastic modulus of the hydrogels, in comparison to that of the alginate only hydrogel. Factors

such as the crosslinking mechanisms of both types of gelation, as well as individual mechanical properties of the two different materials, could greatly contribute to the resulting structure of the hydrogel. While many studies have already explored the use of collagen alginate in biomedical and bioengineering applications, understanding the effect on release of encapsulated biomaterials could be essential in exploring and researching the potential of many more innovative implementations. All in all, collagen-alginate hydrogels could very well be applicable, perhaps not only in a laboratory setting, in the very near future.

Acknowledgements

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