ENGINEERING TRIPOS PART IIA

BIOENGINEERING TEACHING LAB ISG-84 (INGLIS BUILDING) EXPERIMENT 3G5

HYDROGELS: Fabrication and Characterisation

LEARNING OBJECTIVES

- To gain experimental experience in production and characterisation of hydrogels
- To develop an understanding of the factors that affect their properties
- To analyse the viscoelastic behaviour of hydrogels

1 INTRODUCTION

Hydrogels consist of a three-dimensional viscoelastic network, supporting a large amount of excess water or biological fluids, which permits the diffusion and attachment of molecules and cells. They have received a lot of attention for use in a wide variety of biomedical applications including tissue regeneration, cell therapeutics, wound healing and drug release. Dissolution (e.g. in an acidic environment), swelling, degradation, mechanical strength and biological performance (e.g. cell attachment, proliferation, and secretion) are important for optimal functioning.

They can be classified into natural and synthetic hydrogels. Natural hydrogels include protein-based (e.g. collagen, fibrin, silk fibroin) and polysaccharide-based (e.g. chitosan, alginate). The source of proteins is mainly animal extraction. For example, collagen is mainly extracted from porcine tissue or murine tails, whereas silk fibroin is extracted from insects. Polysaccharides are widely distributed in nature in various forms. They include cellulose which is the most predominant polymeric material in nature.

This laboratory exercise focuses on agarose hydrogels. Agarose is a naturally occurring polysaccharide normally extracted from the walls of a certain red seaweed (*Rhodophyceae*). It has been used extensively in cartilage tissue engineering as a scaffolding material as it can yield constructs with functional properties approaching those of native articular cartilage (a non-vascular type of supporting connective tissue that is found throughout the body). It is also commonly used in biochemistry and molecular biology techniques such as gel electrophoresis. Agarose gel formation appears to occur by cooling an agarose homogeneous solution below the coil-helix transition temperature. Upon cooling, non-covalent hydrogen bonds connect long chain agarose molecules to each other, forming double helical structures that aggregate into thick bundles, which in turn cross-link to form a 3D hydrogel network. As both the initial helices and the aggregates are formed with hydrogen bonds, the presence of ions in solution is thought to disrupt the process of gel formation. This exercise uses a solution of Sodium Nitrate ($NaNO_3$) to investigate the effects of ionic co-solutes on the gel properties.

2 SAFETY

A number of substances and processes that are potentially dangerous are involved in this laboratory excersise. It is good experimental practice to ensure that you are familiar with these risks and know how to avoid harm by reading the attached risk assessment, asking for clarification where necessary. Lab coats, safety goggles and gloves are provided.

3 EXPERIMENTAL PROCEDURE

In this experiment you will investigate agarose hydrogels in terms of their gelling temperature, mechanical properties and swelling behaviour. Plain gels and also gels made with chemical additives such as added salts will be examined.

In each session, students will split into groups of two to conduct the investigation activities. Each group will conduct their investigation using plain and salted gels. Each student will conduct their own analysis and prepare a report.

The experiment comprises one whole-group activity **(G)** and four sub-group **(S)** activities. The whole-group activity of making the gels is undertaken first, after which the sub-groups undertake the investigative activities on rotation.

An agarose aqueous solution is required for Thermal (S1) and Optical (S2) Measurement of Gelling Point whereas an agarose gel is required for Mechanical Measurements (S3) and Swelling Studies (S4). For S3 and S4 studies, the gels have been pre-made.

3.1 Making Gels (G)

Expected time to complete - 10 minutes

A 3% w/v agarose gel will be used. Gel concentrations are specified by $\%weight/volume$. The gel is subdivided into two categories: Plain and Salted. Plain gels are made with no additives. Salted gels are made with a co-solute of sodium nitrate ($NaNO_3$, 1%). Most of the gels will be pre-made to help with repeatability of testing, but you will need to make two each. You can weigh these all out together, and then heat them when you are ready to run the tests.

Figure 1: Equipment used for fabrication of agarose hydrogels.

- Prepare four falcon tubes, label them with your CRDid, two marked as "Plain" and two as "Salted".
- For each tube weigh out 0.6 g of agarose with a spatula onto a weighing boat, and for the salted sample weigh out 0.2 g $NaNO_3$ onto a separate weighing boat. Pour in the tube marked *plain* or *salted*.
- Measure out 20 ml distilled water from the distilled water wash bottle into a measuring cylinder. Rinse the weighing boats into the tube with the water to ensure all the agarose (and $NaNO_3$) are transferred into the tube. Repeat for all samples.
- The solutions can now be kept until you are ready to heat them and form gels.
- To form the gel, place the falcon tube into a small beaker. Loosen the top of the falcon tube, as when it heats pressure can build and cause an explosion if the lid is kept on tight.
- Place the beaker into the microwave. Set it to 50% power for 1 minute. Keep a close eye on the solutions, and stop the microwave if it starts to boil. Take the tube out and stir with a plastic spatula, and heat for longer if there is undissolved material in the solution.
- The sample is now ready for test S1 or S2.

3.2 Thermal Measurement of Gelling Point (S1)

Expected time to complete - 20 minutes

This experiment measures the temperature at which a gel solution changes phase (the gelling point) by detecting the rate at which energy is released from a hot solution to the environment. Newton's Law of Cooling dictates that the rate of change of a hot body's temperature (due to heat loss via convection) is proportional to the difference of its temperature T and the ambient temperature T_a;

$$\frac{dT}{dt} \propto T - T_a \; (= \Delta T(t)) \tag{1}$$

where the constant of proportionality depends on the body's geometry, and the fluid that the body is immersed in. Assuming conditions where these factors remain constant, deviations from this law can be interpreted as changes in the body's internal energy via enthalpy associated with phase changes. The phase change we are interested in is the liquid to solid transition, which occurs at the gelling point.

For this experiment you will use plain and salted agarose solutions prepared in 3.1. Once the solution is heated, carefully remove from the microwave, and place the thermocouple which is attached to a falcon tube lid into your solution and screw the lid shut as shown in Figure 2. Place the falcon tube into the chilled water bath, being careful not to tangle the cables from the thermocouple. After 30 minutes, take your tube out of the bath, and remove the thermocouple. Repeat for the salted agarose solution.

Before starting the experiment, complete the sheet which is next to the water bath providing your name/group identifier, the sample type and the thermocouple probe you are using, the data logger it is connected to and the time you are starting this experiment. This is to ensure that you receive the correct data from your run. Make sure the datalogger is running (data will be logged every second).

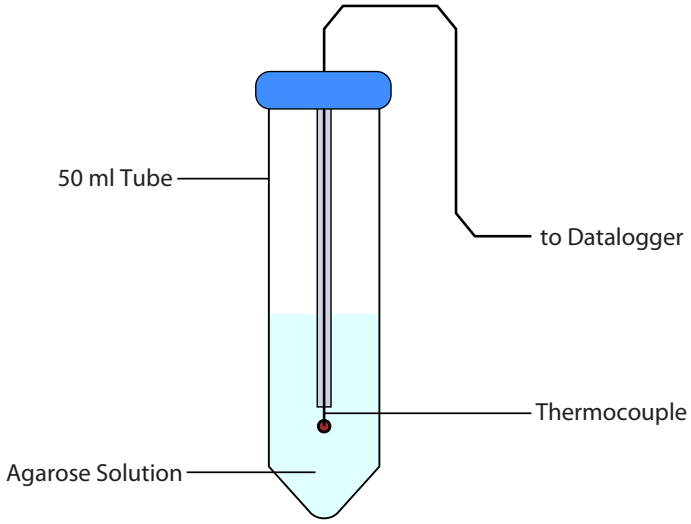

Figure 2: Schematic illustration of the setup used for thermal measurement of the gelling point.

3.3 Optical Measurement of Gelling Point (S2)

Expected time to complete - 35 minutes

This experiment uses an optical method to determine the gelling point of the agarose solution. In particular, a UV/Vis scanning spectrophotometer (Figure 3) is used to measure the amount of light absorbed by the sample over time. As agarose solution cools, the molecules undergo conformational changes known as the *coil to helix* transition. This transition results in a molecular arrangement that interacts much more strongly with the incident light. The absorbance data will be combined with a temperature log, from which you can identify the gelling point.

- Agarose solutions will be prepared as described in 3.1. Before heating the solution in the microwave, place a cuvette and the thermocouple holder into the oven at 90°C, for no more than 5 minutes. This is to prevent the solution cooling too fast when it comes into contact with the cuvette.
- Carefully remove the agarose solution from the microwave, and pour into the warm cuvette up to the fill line. Quickly put the warm thermocouple holder in the sample.
- Place the cuvette into the spectrophotometer with the flat/clear sides facing front to back and close the lid. "Blank" the spectrophotometer, and then press "Start" at the same time you are connecting the thermocouple probe to the dock. This is to make a reference point on both data sets to enable you to link up the data. If you cannot see the Blank and Start options, press the three-dot button to get back to them.
- Note start time and sample type (salted or unsalted). Set a timer for 30 minutes, after which remove your sample from the spectrophotometer.
- Remember to save the data to a USB. This is important, as after the spectrophotometer is blanked for the next run, the data is lost.

  Data from this experiment will be shared between groups.

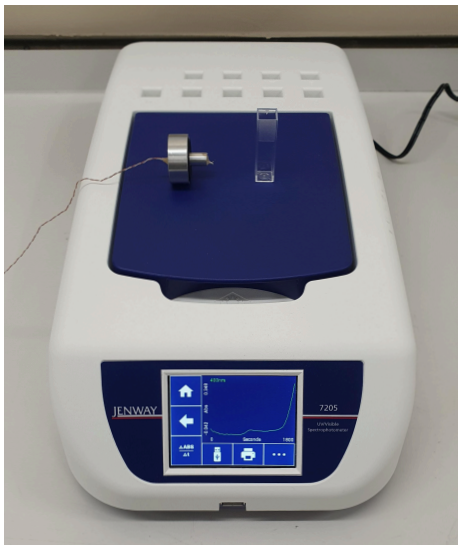

Figure 3: A UV/Vis spectrophotometer is used for measurement of the gelling point. Also shown are the thermocouple holder and the cuvette.

3.4 Mechanical Measurements (S3)

Expected time to complete - 35 minutes

In contrast to many engineering materials, hydrogels tend to have very low stiffnesses – typically ranging from 1 to 1000 kPa. Efforts need to be made to minimise the sample strain during measurements, since excessive straining occurs easily with such highly compliant materials and can produce misleading results. The inertia of the testing equipment is determined by the mechanical linkage system that is to be moved. In conventional testing machines, such effects can be significant. A customised set-up allowing such effects to be minimised has been designed here (Figure 4). In this experiment, you will apply a constant stress and measure the strain response in uniaxial compression.

Recall from part IA that time dependent-deformation due to an applied stress $\sigma(t)$:

$$\sigma(t) = \sigma_0 H(t) \tag{2}$$

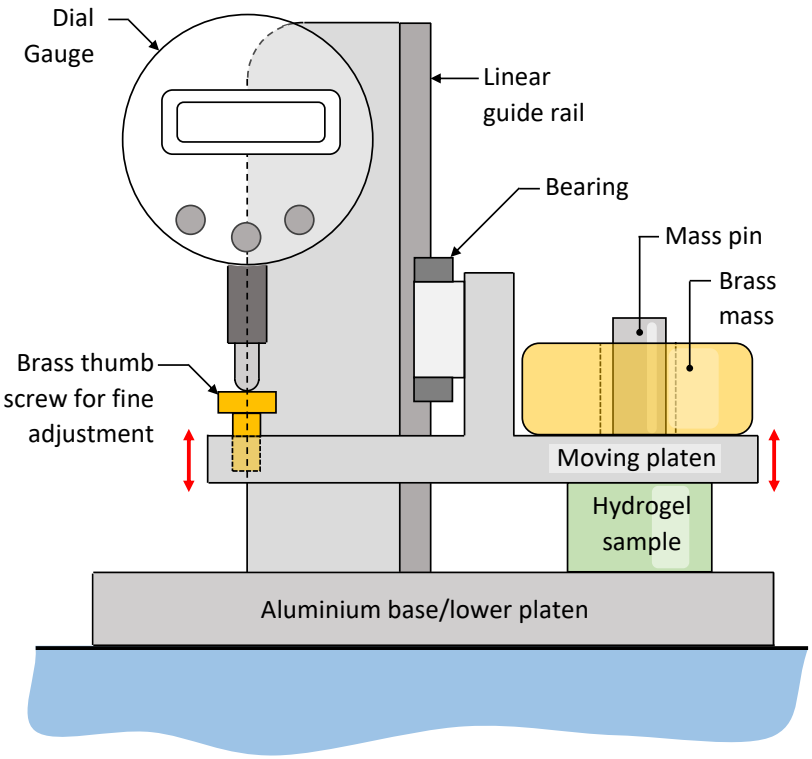

Figure 4: Schematic illustration of the compression set-up used for measuring the mechanical properties of agarose hydrogel.

is given by the *creep compliance function* $J(t)$:

$$J(t) = \frac{\epsilon(t)}{\sigma_0} \tag{3}$$

which governs both the material's response to both step loads and cyclic loading.

- You will be provided with four samples of plain and salted agarose gels (two of each). The samples are 16 mm in height and 16 mm in diameter .The salted agarose has a small amount of food colouring in it to help identify. This has no effect on mechanical strength. The test will run once on each sample type, i.e. four runs in total. The variable will be the weight being added each time. There are five weights to choose from which are all labelled, each group should choose two weights. In your calculations remember to include the weight of the moving platen (Figure 4) - for values see label on your set-up.

- Raise the upper platen on the test rig (which slides up and down along a linear bearing), and place the sample centrally beneath the round pin in the platen on which the masses locate - see Figure 5. Place a metal shunt of 16 mm under the edge of the platen, and lower it to ensure it just makes contact with the sample. Once the sample is in position, turn on the gauge by pressing on/off, and ensure that it is set to mm.

- Place the weight on the pin above the sample (Figure 5). Ensure that you have noted the weight and which sample you are running, and zero the gauge by pressing "Zero". The main changes to the gel happen in the first 20 seconds of the test. To be able to capture this, it is best to record the first 40 seconds on a phone. Have one person record while the other starts the experiment. You will need to log change at 1, 5, 10, 15, 20, 30 and 40 seconds, then every 20 seconds after that for a total of 6 minutes. Set up a timer next to the gauge and start it. As it hits 10 seconds, remove the shunt to start the test. Remember that all times will be 10 seconds off from the clock.

- Whilst the load is being applied, the sample will deform, and may undergo structural changes. Observe these changes and make notes on any that you believe are significant.

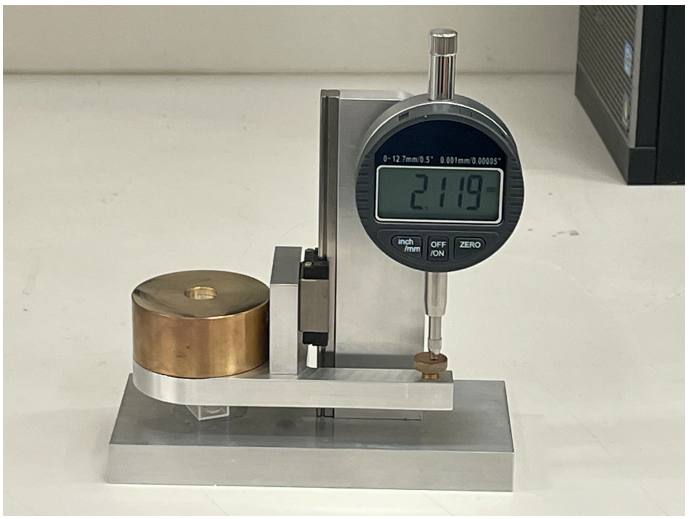

Figure 5: Illustration of the compression set-up used for measuring the mechanical properties of agarose hydrogel.

3.5 Swelling Studies (S4)

Expected time to complete - 25 minutes

This experiment investigates the ability of hydrogel materials to absorb large amounts of water, and the effect that co-solutes (such as $NaNO_3$) have on this process. When a hydrogel is in a dry state, the polymer chains are in close proximity and may interact with each other. As a fluid enters the hydrogel, the polymer chains undergo hydration and other interactions such as hydrophobic or electrostatic interactions.

Swelling will be studied using a conventional gravimetric method on pre-prepared samples. In this test you will use three samples. Plain agarose (3% w/v) which is white and opaque, salted agarose (1% $NaNO_3$) which has a small amount of red dye added to identify it, and 25% w/v gelatin which is slightly brown.

First weigh the samples on the balance making a note of the values (W_d) in the table at the end of the lab script. Immerse in separate beakers with 150 mL distilled water. Every 5 minutes take the samples out carefully with tweezers. To remove excess water, roll samples onto a tissue paper and tap both flat sides once without putting any pressure and weigh them immediately before putting back into the water. Record their weight (W_s), making visual observations about their condition. Continue for 20 minutes in total. For best use of time, put one sample in as you start the timer, the second sample at 1 minute, and the third sample at 2 minutes. It should take less than 1 minute to weigh each sample so weigh the first one again at 5 minutes, the second at 6 minutes, and the third at 7 minutes, etc.

4 ANALYSIS

4.1 Thermal Measurement of Gelling Point (S1)

At high temperatures, the agarose solution is in the *sol* state and the solution remains a liquid. During this time, the solution loses heat according to Newton's Law of Cooling (Eqn.1). As the gel cools and the agarose undergoes the *coil to helix* transition, thermal energy is absorbed in the conformational change, resulting in a temperature drop that exceeds the Newton rate.

This method produces a time series of the gel temperature $T(t)$. The temperature difference variable $\Delta T(t)$ can be estimated by subtracting the ambient temperature T_a of the surrounding "thermal reservoir" from $T(t)$. T_a can be taken to be the temperature measured at 30 minutes. This time series can therefore be used to determine the gelling point T_g by plotting the $\ln\Delta T$ as a function of t and noting the temperature at which the linear relationship ceases to hold.

4.2 Optical Measurement of Gelling Point (S2)

In addition to inducing temperature changes, the *coil to helix* transition causes changes in the agarose solution's interactions with light. The most obvious of these is that the solution goes from clear to cloudy, which can be detected by passing a light beam through the sample and measuring how much of the incident light is absorbed. Plot the absorbance reading at 400 nm as a function of time. Find the baseline and then work out the point when there is a set change in absorbance from that baseline.

4.3 Mechanical Measurements (S3)

To measure the *creep compliance function* $J(t)$, copy the time data and height change data ΔH of your table into an excel or similar spreadsheet. Create two more columns – one for the strain $\epsilon(t)$, which is $\frac{\Delta H}{H_0}$, and another for $\epsilon(t)/\sigma_0$ $(= J(t))$. Plot $J(t)$ as a function of t.

Fitting the measured $J(t)$ to a proposed constitutive equation helps to understand the behaviour of the material. If $J(t)$ is invariant to σ_0, then it exhibits a linear viscoelastic behaviour. The response may be predicted using the principal of superposition (i.e. the response due to sum of two or more inputs is identical to the sum of responses due to each input applied separately).

In this exercise, you will use a four-component Burgers model, which is essentially a Maxwell and Kelvin-Voigt model connected in series (Figure 6). Under this model, $J(t)$ can be expressed as an instantaneous elastic jump, followed by a delayed elastic response and finally a steady state (linear) viscous response:

$$J(t) = \frac{1}{E_1} + \frac{1}{E_2}(1 - e^{-\frac{E_2}{\eta_2}t}) + \frac{t}{\eta_1}$$

This relationship contains four parameters (E_1, E_2, η_1, η_2) which can be estimated by separating the contributions that the different material parameters make to the overall curve as illustrated in Figure 6.

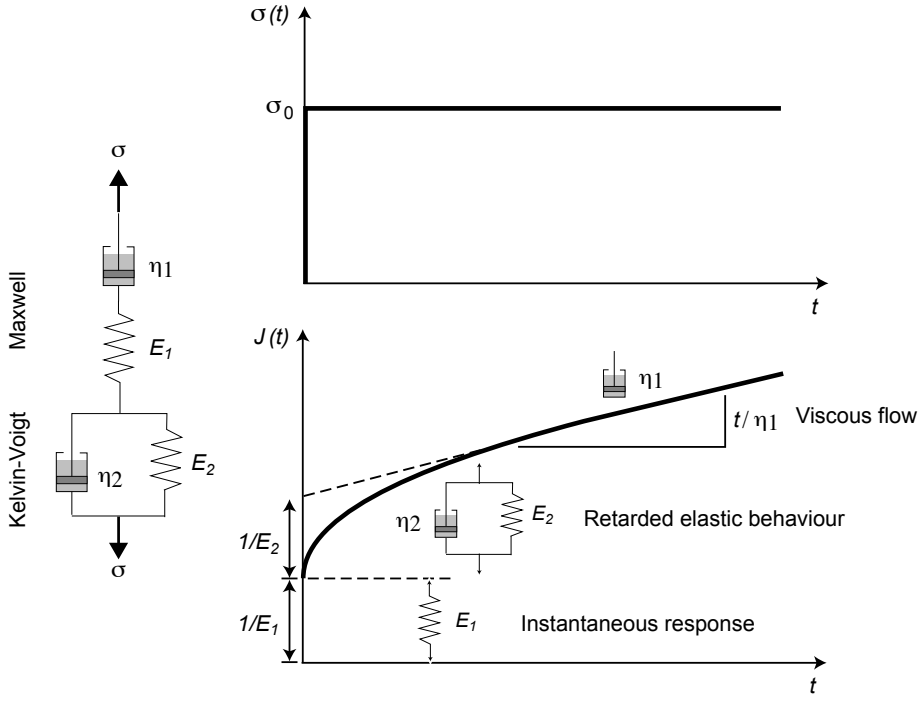

Figure 6: Schematic illustrations of the four-element Burgers model consisting of Maxwell and Kelvin–Voigt models in series, and the compliance function *J(t)* for the Burgers model.

4.4 Swelling Studies (S4)

Calculate the percentage of swelling using the following formula:

$$\%\ Swelling = [(W_s - W_d)/W_d \times 100] \tag{4}$$

Plot the percentage of swelling as a function of immersion time. Describe what is happening when a hydrogel is immersed in a fluid using a simple schematic. Discuss the rate of change of mass of *plain* and *salted* agarose gels and compare with the corresponding values of the gelatin hydrogel.

5 REPORT

Your report should examine the evidence that has been collected during the laboratory exercise. Specifically, the report must contain the following elements:

[See also **http://teaching.eng.cam.ac.uk/content/part-iia-coursework-labs-overview**.]

1. An abstract, summarising the scope of the experiment, as well as your findings.
2. A short introduction to the topic of hydrogels, outlining their uses in bio-engineering, and the context of the investigation you have undertaken.
3. Clearly present results of each (S1-4) of the sub-group investigations, commenting on the properties measured and the effect of added salt. There is no need to provide a detailed description of the experimental methods employed but the reader should be able to understand what you did without referring to the handout.
4. A discussion of the results of the investigations as a whole in order to explain the effects that you have observed. If you have observed results that are not explained by your theoretical understanding, consider where these discrepancies may have arisen.
5. A short conclusion, summarising the theoretical and experimental insights you have made into agarose gel charactersation in this experiment.

Notes on Marking

The coursework is marked out of 5. One mark is reserved for "effort" in the lab, this is awarded by the demonstrator and is normally only withheld where participants do not take an active part in the practical activity. You are being marked on an assumption of 2 hours spent in the lab and 2-3 hours on the write up. Please note the advice on workload balance http://teaching.eng.cam.ac.uk/node/32. Reports will most commonly be awarded 4/5 overall. Marks will be deducted for significant errors in interpretation and/or sloppy presentation. 5/5 marks are rare and reflect the demonstration of further insight. An example of such insight would be a brief reflection on an appropriate external reference which illuminates a point from the coursework.

RJ Macleod, F Riche, JC Stallard, AE Markaki
October 2025

Results Tables: Mechanical Measurements (S3)

Sample type:

Weight:

Time (s)	ΔH **(mm)**	**Time (s)**	ΔH **(mm)**

Sample type:

Weight:

Time (s)	ΔH **(mm)**	**Time (s)**	ΔH **(mm)**

Sample type:

Weight:

Time (s)	ΔH **(mm)**	**Time (s)**	ΔH **(mm)**

Results Tables: Swelling Studies (S4)

Time (s)	W**(g) plain agarose**	W**(g) salted agarose**	W**(g) gelatin**