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Development of a novel porous cryo-foam for potential wound healing applications

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Abstract This body of work describes the development of a porous hydrogel for wound healing applications. In the present study poly (vinyl alcohol) (PVA) and poly (acrylic acid) (PAA) based hydrogels were prepared, and their properties were examined. Varying concentrations of the polymers and distilled water were used to prepare the hydrogels. The use of a high shear mixer, for foaming the PVA and PVA/PAA gels, and how this physical change can affect the structure and porosity of the hydrogel in question, represents a key feature of this work. The mechanical and thermal properties were determined by parallel plate rheometry and modulated differential scanning calorimetry (MDSC) respectively. The results indicated that the hydrogels containing low concentration of PVA and high volume of H_2O are significantly weaker than those synthesised with higher concentrations of PVA. The thermal analysis shows distinct endotherms and provides evidence of crystallisation. The chemical structure of the hydrogels was confirmed by means of attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR).

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1 Introduction

The term hydrogel is used to describe materials that are three-dimensional, hydrophilic polymeric networks capable of imbibing large amounts of water. These hydrogels exhibit a thermodynamic compatibility with water allowing them to swell in aqueous media. Hydrogels are prepared by synthetic chemical reactions from a range of speciality vinyl and acrylic monomers and are becoming increasingly important materials for pharmaceutical applications. They are used in a variety of applications including diagnostic, therapeutic, and implantable devices. Hydrogels can also be found as wound dressing materials and under this application they have been extensively investigated [1–6].

Hydrogels combine the features of moist wound healing with good fluid absorbance and are transparent to allow the monitoring of healing. In situ forming hydrogels which forms the shape of wound defect will have advantages over the use of preformed hydrogel scaffolds since it enables conformability of the dressing on wounds without wrinkling. Most commercially available dressings in the form of membranes and sheets are problematic as far as the conformability is concerned and in situ formed dressings are therefore superior to preformed dressings.

Hydrogels have been widely used in such applications because of their biocompatibility with the human body and because they resemble natural living tissue more than any other class of synthetic biomaterials. This is due to their high water content and soft consistency that is similar to natural tissue. Hydrogels may be composed of homopolymers or copolymers and are insoluble due to the presence of chemical crosslinks or physical crosslinks, such as entanglements or crystallites.

The polymers studied in this research are PVA and PAA, a neutral and an ionic polymer, respectively. The polymers were selected because the combination of their individual applications helps to create a hydrogel that is desirable for pharmaceutical and biomedical processes. PVA is well known for its processability, strength, and long-term temperature and pH stability. The characteristics which make it ideal for biomedical use are its biocompatibility, non-toxicity, and minimal cell and protein adhesion. PAA is known to be a model hydrophilic system and is very fragile and breaks easily. To add strength to this hydrophilic system, PAA is polymerised and crosslinked with other polymers, in particular PVA. Extensive work has been done on the structure, physical characterisation and diffusion of PVA by Reinhart and Peppas [7]. Am Ende [8] has investigated the characteristics of PAA and showed that the diffusion increased as the pH increased. Gudeman [9] studied solute diffusion through PVA/PAA membranes as a function of temperature, ionic strength, and pH of the swelling agent. The novel aspect of the research was the differing functionality created by foaming the PVA and the PVA/PAA gels using a high shear mixing apparatus prior to the freeze/thawing stage. Research was also carried out to determine how different concentrations of PVA/PAA affect the physical and mechanical properties of the resulting cryogels/cryofoams.

2 Experimental

2.1 Preparation of poly (acrylic acid) and poly (vinyl alcohol) by means of freeze thawing

PVA (weight average molecular weight 146,000–186,000) and PAA (weight average molecular weight 3,000,000) used in this study were supplied by Aldrich. Solutions were prepared by mixing various amounts of PVA and PAA in distilled water using the quantities shown in Table 1. Dissolution was achieved by heating the PVA mixture to 80°C for 1 h, while slowly stirring until the polymer was no longer apparent, after which time PAA is slowly added for 30 min. The solutions were mixed at a high shear rate until foam was formed. The foam was then poured into polystyrene cups. The polystyrene cups were placed in approximately 300 ml of liquid nitrogen for a period of 10 min. The solidified solutions were then placed in a fridge at 3°C.

2.2 Preparation of samples

Table 1 Polymeric and H_2O feed ratios used in the preparation of the freeze-thawed gels

Gel	PVA (g)	PAA (g)	H_2O (ml)	Mix time (min)
1A	4	0.4	100	5
2A	5	0.4	100	5
3A	3	0.4	100	5
4A	1	0.4	100	5
5A	2	0.4	100	5
6A	6	0.4	100	5
1B	3	0.1	100	5
2B	3	0.2	100	5
3B	3	0.3	100	5
4B	3	0.4	100	5
5B	3	0.5	100	5
6B	3	0.6	100	5
1C	2	0.4	100	0
2C	3	0.4	100	0
3C	4	0.4	100	0
4C	1	0	100	0
1D	10	0	0	5
1E	0	1	100	5

Four gel types were prepared: A, B, C, D and E (see Table 1). The notation A was used when the concentration of PVA was modified (quantities of PAA and H_2O stay constant). Notation B was used when the concentration of PAA was modified (quantities of PVA and H_2O stay constant). Notation C was used when the concentration of PVA/PAA varied. Notation D was used for PVA homopolymer and notation E was used for PAA homopolymer. High shear mixing was carried out on the hydrogels before freezing, so that comparisons could be made from the gel being high shear mixed as apposed to the gel being freeze/thawed. Table 1 illustrates the polymeric and H_2O feed ratios. From carrying out various tests it was found that 1B, 6B, 1C, 4C, and 1D formed the best gel. From this, it was decided to carry out testing on these gels.

2.3 Digital optical microscope

An Olympus optical microscope was used with a magnification of 10× to analyse visual characteristics of the samples. In order to calculate the distribution of the pores within the hydrogel, images were taken in triplicate and a sample range of 100 μm^2 was evaluated. The dots per square inch (DPI) were enhanced so a visual approximation of pores could be counted.

2.4 Modulated differential scanning calorimetry (MDSC)

A Modulated DSC (2920 TA Instruments) containing a refrigerator cooling system was used to evaluate the gels. Samples of between 8 and 12 mg were weighed out using a Sartorius scales capable of being read to five decimal places. Aluminium pans were crimped before testing, with an empty crimped aluminium pan being used as the reference cell. Calorimetry scans were carried out from 30 to 220°C for each of the samples. All MDSC measurements were carried out at a scanning rate of 2°C/min. Samples were taken from PVA/PAA gels that had being dried in an oven at 37°C for 24 h. The instrument was calibrated using indium as standard.

2.5 Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR was carried out on the samples which had being exposed to atmospheric conditions for a minimum of 7 days, using a Nicolet Avator 360 Ftir, with a 32 scan per sample cycle and a resolution of 8 cm^{-1}. The samples were scanned between the following wavelengths 400–4000 cm^{-1}.

2.6 Rheometry

Rheological measurements were performed using an Advanced Rheometer AR1000 (TA instruments) fitted with a Peltier temperature control. The samples were tested using a 40 mm diameter steel plate. The cryogels (diameter 40 mm and thickness 20 mm) were placed on the Peltier plate, and tests were carried out using temperatures of 30–80°C at a 2° interval. The tests were in an oscillation form with a strain sweep of 1, 5 and 10 Hz. In all experiments, a normal force of 0.3 N was applied to the surface of the samples in order to avoid the slipping of the gel from the Peltier plate. The force resulted in a slight compression of the samples.

3 Results and discussion

3.1 Surface topography of high shear mixed hydrogels

An example of a typical freeze/thawed cryogel is shown in Fig. 1a. This gel was used as a reference to evaluate the porosity of the high shear gels. As illustrated in Fig. 1b, the gel containing 10 g PVA with 100 ml H_2O mixed at a high shear rate (250 rpm for 5 min) and subsequently freeze-thawed, demonstrates an increase in surface porosity when compared to the non high shear mix gels (Fig. 1e, f). When evaluating Fig. 1c, d for the PVA/PAA hydrogel with the

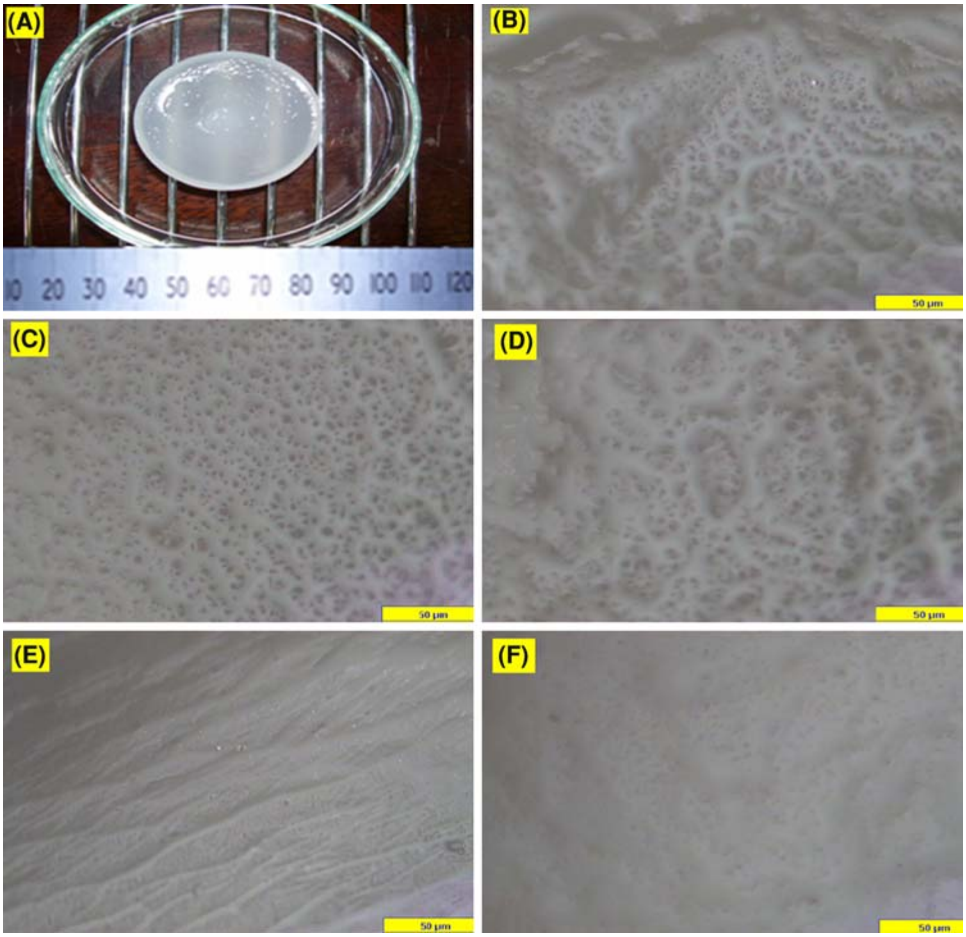


Fig. 1 Visual representation of PVA and PVA/PAA gels using an Olympus microscope (10×) where **a** is a typical hydrogel formed by the freezing process, **b** gel 1D containing 10 g PVA with 100 ml H_2O, **c** gel 1B containing 3 g PVA + 0.1 g PAA with 100 ml H_2O mixed at high shear rate, **d** gel 6B containing 3 g PVA + 0.6 g PAA with 100 ml H_2O mixed at high shear rate, **e** gel 1C containing 2 g PVA + 0.4 g PAA with 100 ml H_2O, not mixed at a high shear rate and **f** gel 4C containing 1 g PVA + 0.4 g PAA with 100 ml H_2O, not mixed at a high shear rate

same composition of PVA, the porosity is more evident especially when higher concentrations of PVA were used. In order to calculate the distribution of the pores within the hydrogel, images were taken in triplicate and a sample range of 100 µm^2 was evaluated. The dots per square inch (DPI) were enhanced so a visual approximation of pores could be counted It was found that 300 pores per sample range were achieved.

3.2 Modulated differential scanning calorimeter (MDSC)

Due to the high water content (95–97 wt.%) in the hydrogel samples, MDSC scans failed to detect any thermal transitions of the polymer present in the swollen samples. Therefore, all samples were dried at 37°C for 48 h before testing. The water content in the two-phase hydrogel was noted to be 5% (±0.5%) after drying. As illustrated in Fig. 2b, two transitions were evident for the hydrogel containing 10 g of PVA with 100 ml H_2O mixed at a high shear rate. The peak observed at 136.30°C exhibited the glass transition temperature (T_g) of PVA. This corresponds to values reported by López using PVA with a weight average molecular weight of 94,000 [10]. The peak at 222.01°C gives the melting temperature (T_m) of PVA.

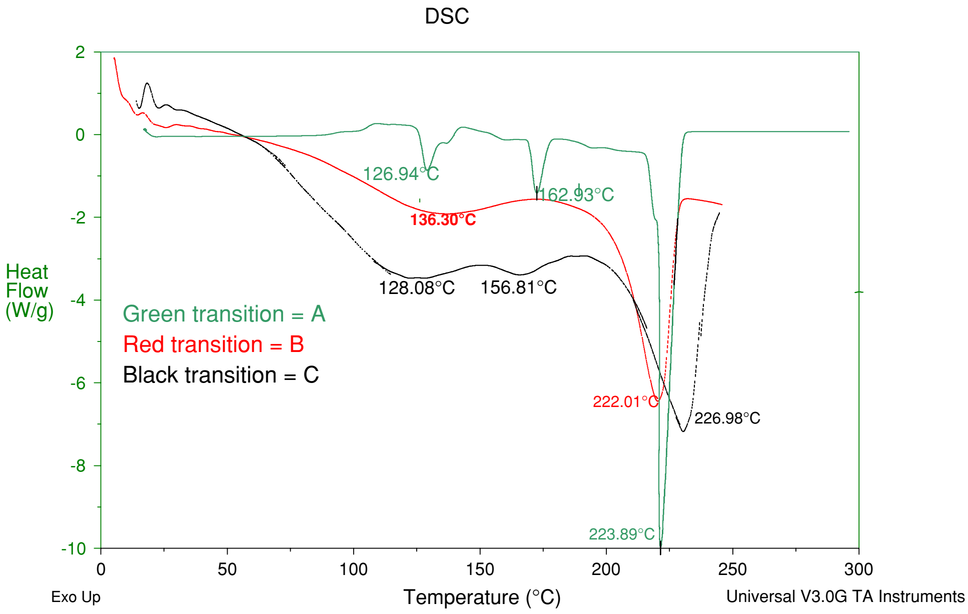
When evaluating the thermograph in Fig. 2c the peak at 128.08°C is designated as the *α* relaxation which represents the glass transition of PVA. The relaxation observed at 156.81°C is designated as the *β* relaxation, which is due to the relaxation in the PVA crystalline domains. The third relaxation occurs at a temperature of 226.58°C and is caused by the melting point of the crystalline domains of PVA. PAA has been known to have a T_g of 106°C, however, this peak was not observed throughout the testing carried out. The reason as to why the T_g of PAA is not observed is due to an endothermic reaction occurring between 70 and 110°C which cause the PVA molecules to align masking the transition of the PAA [11]. With reference to Fig. 2a, the transition observed at 126.94°C exhibits the glass transition temperature of PVA. The peak at 162.93°C is assigned as the *β* relaxation, which is due to the relaxation in the PVA crystalline domains. The trends observed for the two *β* relaxations in Fig. 2a, c are due to hydrogen bonding between the O–H group of the PVA and the carboxylic acid group of the PAA. These hydrogen bonds act as physical cross-links and give rise to an increase in molecular chain stiffness of PVA in crystalline regions. The peak at 223.89°C gives the melting temperature of PVA. The extent of crystallinity in a PVA hydrogel has an important effect on the mechanical properties of the gels. Gels with a high crystallinity have reduced elasticity and are fragile, whereas if the crystallinity is too low, gels are poorly coherent [12].

3.3 Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR has found widespread application for the characterisation of hydrogels and has been used to characterise both the polymerisation systems used to produce hydrogels, as well as the resulting hydrogel polymers. ATR-FTIR was used to confirm the structure of the PVA and PVA/PAA hydrogels.

With reference to Fig. 3a, the gel (1C) containing 2 g PVA + 0.4 g PAA with 100 ml H_2O which was not high shear mixed yielded peaks between 2100 and 2457 cm^{-1}

Fig. 2 A DSC thermograph representing PVA and PVA/PAA gels where (a) gel 1C containing 2 g PVA + 0.4 g PAA with 100 ml H_2O, not mixed at a high shear rate, (b) gel 1D containing 10 g PVA with 100 ml H_2O mixed at a high shear rate, and (c) gel 1B containing 3 g PVA + 0.1 g PAA with 100 ml H_2O, mixed at a high shear rate



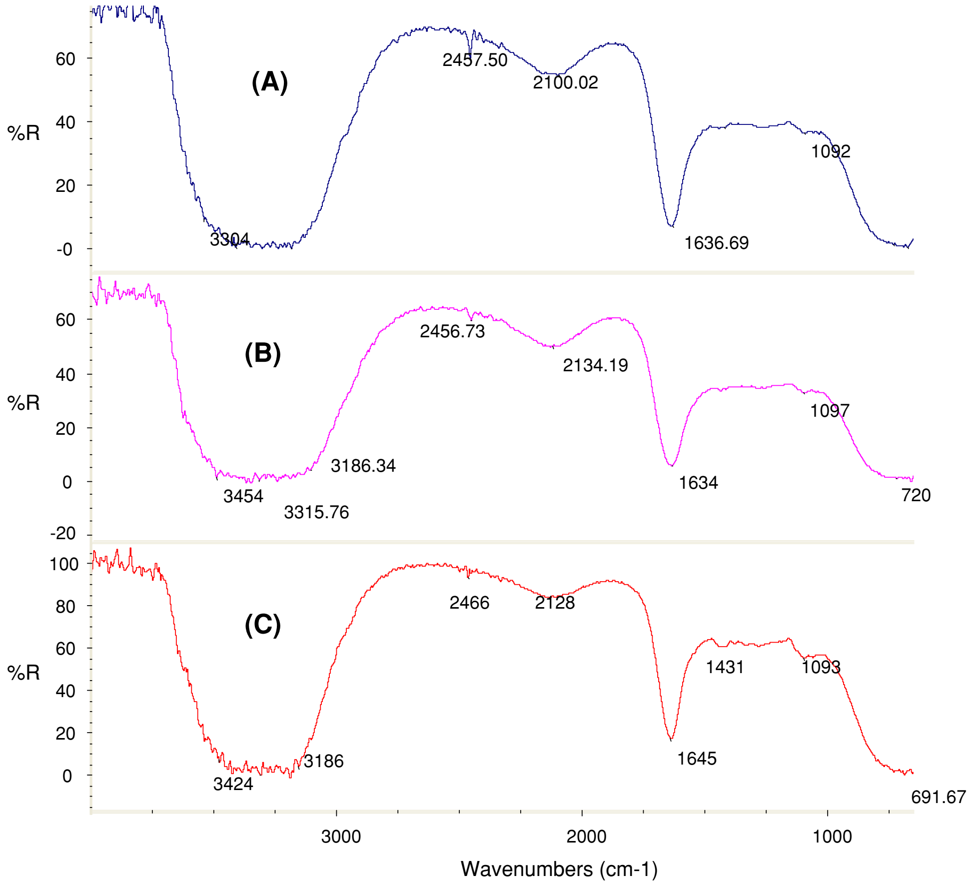


Fig. 3 Shows the ATR-FTIR spectra for (a) gel 1C containing 2 g PVA + 0.4 g PAA with 100 ml H_2O, not mixed at a high shear rate, (b) gel 1B containing 3 g PVA + 0.1 g PAA with 100 ml H_2O, mixed at a high shear rate, and (c) gel 1D containing 10 g PVA with 100 ml H_2O mixed at a high shear rate

and these are indicative of the O–H overtone vibrations of PAA. The peak at 3304 cm^{-1} is characteristic of O–H bonds of the PVA stretching. This band also appears at 3575 cm^{-1} when higher concentrations of PVA are used [13]. The band at 1636 cm^{-1} shows the C=O bonds of PAA. As outlined in Fig. 3, the ATR-FTIR spectrum for gel 2B (3 g PVA + 0.2 g PAA with 100 ml H_2O which was mixed at a high shear), demonstrated peaks between 3186 and 3454 cm^{-1} which are indicative of the O–H stretching bonds of the carboxylic acids of PAA while the band observed between 2134 cm^{-1} and 2456 cm^{-1} is due to the O–H overtone vibrations of PAA [14]. The band at 1634 cm^{-1} represents the C=O bond of PAA. When characterising the ATR-FTIR spectrum for the gel containing 10 g PVA + 100 ml H_2O, the most characteristic bands for PVA are located between 3186 and 3424 cm^{-1} and can be attributed to the O–H stretching frequency of PVA. The band at 1645 cm^{-1} shows CH2 bonds of PVA. Hassan and Peppas [15] have found that PVA gels referred to an absorption peak at 1141 cm^{-1}, which is indicative of PVA crystallinity. This is due to the C–C stretching mode which increases with an increase in the degree of crystallinity. The peak observed at 1093 cm^{-1} in the hydrogels tested, indicates that a crystalline effect is occurring.

3.4 Rheometry

To examine the stress–strain relationship for the hydrogels, they were subjected to an alternating strain, while simultaneously measuring the stress. For viscoelastic behaviour, the stress and strain will both vary sinusoidally when equilibrium is reached but the strain lags behind the stress. These relationships are shown in Eqs. 1 and 2 [16].

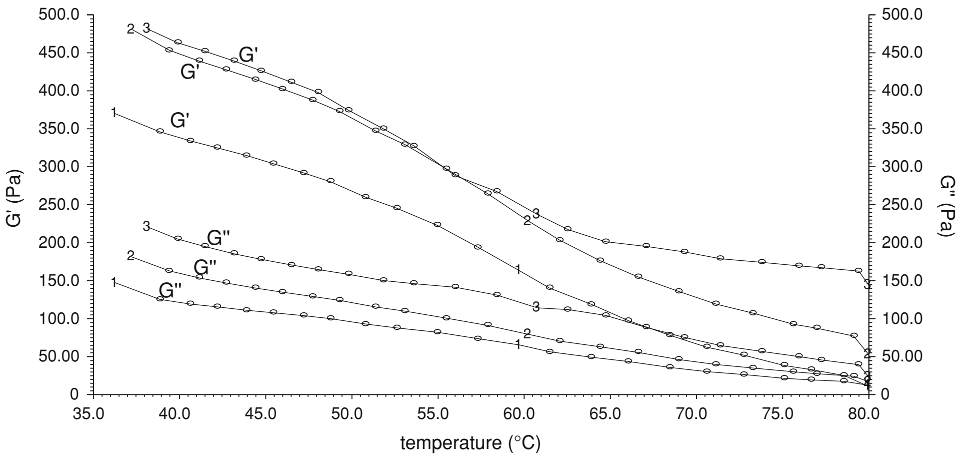
$$\text{Strain } e = e_0 \sin \omega t \tag{1}$$

$$\text{Stress } \sigma = \sigma_0 \sin(\omega t + \delta) \tag{2}$$

where ω is angular frequency and δ is the phase lag. The stress–strain relationship can be defined by quantities G' and G'' which are 90° out of phase with the strain. G' is in phase with the solid and is called the storage modulus because it defines the energy stored in the specimen due to the applied strain. G'' which is $\pi/2$ out of phase with the strain defines the dissipation of energy and is called the loss modulus [17].

As presented in Fig. 4, the rheological results show that G' is much larger than G'', when analysed between 30 and 45°C. This suggests that the elastic response dominates, which is typical for gels and solid like materials. The gel containing 10 g PVA with 100 ml H_2O mixed at a high

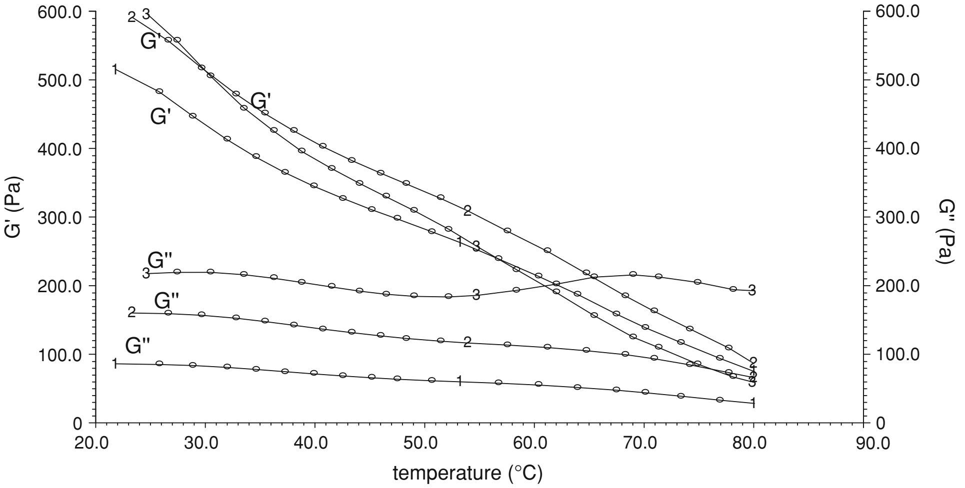
Fig. 4 Rheological results for the gel containing 10 g PVA with 100 ml H_2O where the storage modulus (G′) 1, 2, and 3 represents 1, 5 and 10 Hz respectively



shear rate shows the G′ value which corresponds to the stiffness of the hydrogel ranging between 350 and 500 Pa at frequencies of 1, 5, and 10 Hz respectively. It is evident that as the temperature is increased the gels weaken as reported by Ricciardi [16]. The result shown in Fig. 5, for the gel containing 3 g PVA + 0.2 g PAA with 100 ml H_2O which was mixed at a high shear rate shows that with the incorporation of PAA, the gels held their structural shape for a longer period of time when compared to the gel in Fig. 4. The stiffness of the gel was found to be between 500 and 600 Pa after being ramped from 25 to 80°C at a rate of 2°C/min. A decrease in stiffness is observed between 35 and 45°C. This decrease is due to the uncoiling of crystalline regions within the polymer matrix [18]. This happens at such low temperatures due to the high quantity of water and its plasticising effect on the polymers. At 55°C a much sharper decrease in stiffness is observed. The predominant factor in the gelation of these hydrogels is hydrogen bonds acting as physical cross-links. These hydrogen bonds are formed between the carboxylic acid groups of the PAA and the O–H group of PVA.

With reference to Fig. 6, the gel containing 3 g PVA + 0.2 g PAA with 100 ml H_2O which was not mixed at a high shear rate shows a decrease in gel stiffness when compared to Fig. 5. As the temperature was increased, the chain mobility increases which aligns the molecules back to their original form which inevitably decreases the stiffness of the hydrogel. The result in Fig. 6 shows a gel stiffness in the order of 200 Pa, and as the temperature is increased the gel decreases in stiffness. This result indicates that by high shear mixing the hydrogels at a speed of 250 rpm for a minimum of 5 min a stiffer hydrogel is produced. The hydrogels are viscoelastic with both the storage modulus (G′) and loss modulus (G″) being temperature dependent. The different concentrations of PVA show different responses to the temperature ramp [14]. Ramazani-Harandi [19] notes that it is practically preferred in many cases to qualitatively assess the strength of hydrogels by observation and feeling the swollen gels. However, this method is highly dependent on the experience of the test operator. Therefore, in this work the elastic component (G′) portion of the curves were used to evaluate the comparative stiffness of the hydrogels.

Fig. 5 Rheological results for the gel 2B containing 3 g PVA + 0.2 g PAA with 100 ml H_2O, mixed at a high shear rate where the storage modulus (G′) 1, 2, and 3 represents 1, 5 and 10 Hz respectively



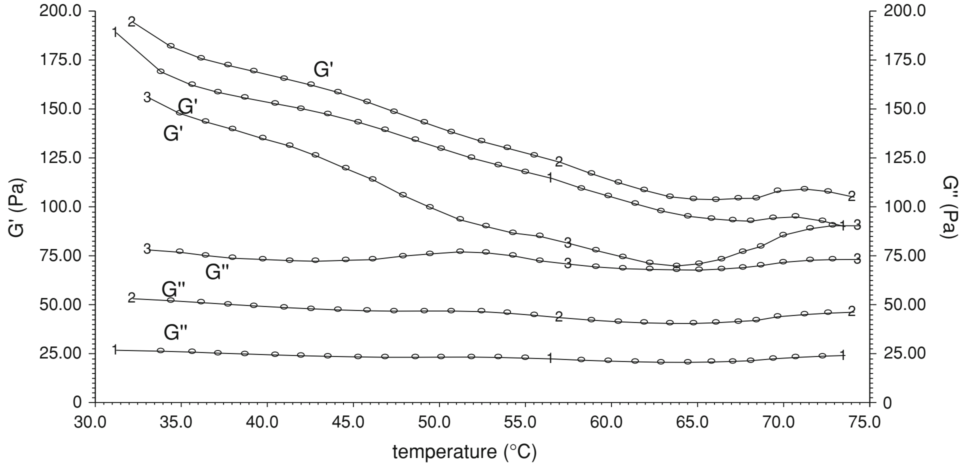


Fig. 6 Rheological results for the gel 2B containing 3 g PVA + 0.2 g PAA with 100 ml H_2O, not mixed at a high shear rate where the storage modulus (G′) 1, 2, and 3 represents 1, 5 and 10 Hz respectively

4 Conclusion

From the preliminary results obtained using the different analytical techniques such as surface topography, ATR-FTIR, rheology and MDSC, it was possible to conclude that the concentrations of the different blends of PVA and PVA/PAA effects the porosity and the strength of the cryogel/cryo-foam. The rheometry results show that hydrogels are viscoelastic with both the storage modulus (G′) and loss modulus (G″) being temperature dependent. The ATR-FTIR results confirmed the structure of the PVA/PAA hydrogels.

The combination of both high shear mixing and various concentrations of PVA and PVA/PAA, gives good results in the synthesis of new porous cryo-foam. In addition the work has indicated that the thermal transitions which are present are in the region of interest to drug delivery systems and may be modified by careful blending. It is possible to vary many parameters to change or optimise the overall properties of the material for a specific application. It is, however, interesting to observe that there can be considerable overlap in the structure, behaviour, and stability of the gels by the careful manipulation of reaction conditions.

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