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Rheological and thermal characteristics of a two phase hydrogel system for potential wound healing applications

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Abstract Hydrogels fabricated from single polymers have been extensively investigated for wound healing applications. However, in many cases a single polymer cannot meet divergent demands in terms of both properties and performance. In this work, a two phase hydrogel was prepared by physically imbedding a xerogel in the core of a freeze thawed hydrogel. The outer hydrogel was prepared by freeze thawing poly (vinyl alcohol) (PVA) and poly (acrylic acid) (PAA) while the xerogels were prepared by UV polymerisation of 1-vinyl-2-pyrrolidinone (NVP). The rheological results indicated that the two phase hydrogels over a period of 2 weeks formed a strong interface and demonstrated greater physical strength. This suggested that the inner gel containing PVP diffused into the PVA/PAA hydrogel, which in turn increased hydrogen bonding, resulting in the overall increase in the stiffness of the gel. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) confirmed hydrogen bonding had occurred between the constituents of the two phase hydrogels. Thermal analysis suggested that T_g of each of the samples was above 80 °C, which indicated no impact on the behaviour of the gel at body temperature, but did however, give an indication of the stiffness of the dry polymer.

Introduction

Wound healing is a dynamic process and the performance requirements of a dressing can change as healing progresses. It is widely accepted that a warm, moist environment encourages rapid healing and most modern wound care products are designed to provide these conditions [1, 2]. Fluid balance in burn injury is very important since heavy loss of water from the body by exudation and evaporation may lead to a fall in body temperature and increase in the metabolic rate. Besides this, dressings should have certain other properties like ease of application and removal, and proper adherence so that there will not be any area of non-adherence left to create fluid-filled pockets for the proliferation of bacteria [2]. Numerous wound dressing materials are commercially available and others have been extensively investigated [3].

The term hydrogel is used to describe materials that are three-dimensional, hydrophilic polymeric networks capable of imbibing large amounts of water. Hydrogels are becoming increasingly important materials for pharmaceutical and biomedical applications. They are used in a variety of applications including diagnostic, therapeutic and implantable devices. 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 biomaterial. 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 can be insoluble due to the presence of chemical crosslinks or physical crosslinks, such as entanglements or crystallites [4–6]. A xerogel is a solid formed from a gel by drying with unhindered shrinkage. Xerogels usually retain high porosity (25%) and enormous surface area (150–900 m^2/g),

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along with very small pore size (1–10 nm). Heat treatment of a xerogel at elevated temperature produces viscous sintering (shrinkage of the xerogel due to a small amount of viscous flow) and effectively transforms the porous gel into a dense glass.

The polymers used in this study were poly (vinyl alcohol) (PVA) and poly (acrylic acid) (PAA), a neutral and an ionic polymer, respectively. The two polymers were selected because the combination of their individual characteristics helps to create a hydrogel that is desirable for pharmaceutical and biomedical processes. Also, these polymers are miscible with each other and can be prepared in aqueous solution. Poly (1-vinyl-2-pyrrolidinone) (PVP) was used as a solid dispersion to enhance physical strength of the hydrogel. The toxicity of PVP has been extensively studied in a variety of species, including humans and other primates and has been proved to be a very low order [7].

One method of producing a hydrogel is freeze/thawing processing [8]. PVA/PAA hydrogels prepared using freeze/thaw cycling are excellent wound healing candidates, simply because they exhibit a high degree of swelling in water, a rubbery elastic nature, are non-toxic, non-carcinogenic and can be readily accepted in the body. Hassan and Peppas [8] discussed the use of PVA with particular interest in physically crosslinking hydrogels that were prepared by cycles of freezing and thawing. This method of preparing hydrogels addresses the toxicity issues because it does not require the presence of a crosslinking agent. It was also noted that such physically crosslinked materials also exhibit improved properties, most notably higher mechanical strength than PVA gels crosslinked by chemical or irradiative techniques because the mechanical load can be distributed along the crystallites of the three-dimensional structures [8].

In this work, a two phase hydrogel was investigated, whereby the inner gel poly (1-vinyl-2-pyrrolidinone) (PVP) was prepared by UV polymerisation and the outer (PVA/PAA) gel was prepared by freeze thawing. The primary objective for using a two phase hydrogel was to improve the mechanical properties of the hydrogel, which will lead to better adhesion to the skin for the development of a wound healing application. All the materials were characterised by conventional spectroscopic methods and properties were correlated to their structures.

Experimental

Preparation of the xerogels

The hydrogels investigated in this work were prepared by free-radical polymerisation using ultra violet light. The monomer used was 1-vinyl-2-pyrrolidinone (NVP, Lancaster synthesis). To initiate the reactions, 1-hydroxycyclohexylphenylketone (Irgacure® 184, Ciba speciality chemicals) was used as a UV-light sensitive initiator at 3 wt% of the total monomer weight. This was added to the 20 mL NVP mixture and stirred continuously until completely dissolved. The solution was then pipetted into a silicone mould (W.P. Notcutt, Middlesex) that contained 20 disk impressions (1 mL of the monomer was put into each impression). The mould was then positioned horizontally to the gravity direction under two UVA 340 UV lamps (Q-panel products) and the solution was cured for 1 h in an enclosed environment. The samples were dried in a vacuum oven at 40 °C, 500 mmHg for 24 h prior to use.

Preparation of the outer hydrogel

The outer hydrogels were prepared by heating a known quantity of PVA (weight average molecular weight 146,000–186,000) mixture in 40 mL of H_2O to 80 °C for 1 h, while slowly stirring until the polymer is no longer apparent, after which time PAA (weight average molecular weight 3,000,000) was slowly added for 30 min. Both polymers used in this study were supplied by Aldrich. From previous studies the ratio for viable hydrogels was found to be 85% PVA and 15% PAA, where 85:15 equates to 1 g PVA + 0.2 g PAA. All outer hydrogels in this current study were synthesised in this ratio. To remove air bubbles the solution was placed in an ultra sonic bath for 5–10 min.

Preparation of the two phase hydrogel

The two phase hydrogels system was prepared by placing 20 mL of the PVA/PAA solution into a polystyrene mould. The polystyrene mould was placed in approximately 300 mL of liquid nitrogen for a period of 10 min. The xerogel was then placed on top of the frozen hydrogel and the remaining 20 mL of the PVA/PAA solution was poured into the mould. The polystyrene mould was returned to the liquid nitrogen bath, again for a period of 10 min. Solidified solutions were then placed in a fridge to thaw at 3 °C for 24 h.

Differential scanning calorimetry

A Differential scanning calorimetry (DSC) (2920 TA Instruments) containing a refrigerator cooling system was used to evaluate the gels. Approximately 8–12 mg samples were weighed out using a Sartorius scale. 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 at a scanning rate of 2 °C/min. Samples were taken from the two phase hydrogel that had being dried in an oven at 37 °C for 24 h. The instrument was calibrated using indium as standard.

Rotational 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 swollen hydrogels containing 98% water (diameter 40 mm and thickness 20 mm) were placed on the Peltier plate, and tests carried out using temperatures of 30–80 °C at a two-degree interval. The tests were carried out on a weekly basis ranging from 1 to 2 weeks. The tests were performed in an oscillation mode with a strain sweep of 1, 5 and 10 Hz. 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.

Attenuated total reflectance Fourier transform infrared spectroscopy

The Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was carried out using the attenuated total reflectance (ATR) mode on a Nicolet Avator 360 (FTIR) with a 32 scan per sample cycle and a resolution of 8. The samples were scanned from 400 to 4,000 cm^{-1}.

Results and discussion

Rheological analysis for the hydrogels and the two phase hydrogel system

In examining the stress–strain relationship for the hydrogels, they were subjected to a strain, while the stress was measured. For viscoelastic behaviour, when equilibrium is reached, both the stress and strain will vary, but the strain lags behind the stress. These relationships are shown in Eqs. 1, 2

$$\text{Strain } e = e_{\mathrm{o}} \sin \omega t \tag{1}$$

$$\text{Stress } \sigma = \sigma_{\mathrm{o}} \sin(\omega t + \delta), \tag{2}$$

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. The storage modulus (G') which is in phase with the strain is defined as the amount of energy stored in the specimen due to the applied strain. The loss modulus (G'') which is $\pi/2$ out of phase with the strain can be defined as the dissipation of energy [9]. In this study, we were only concerned with the energy stored in the sample, and to see how this energy affects the stiffness of the hydrogels.

Hydrogels are commonly known as viscoelastic materials, however, from carrying out rheometry testing it was noted that the temperature affects the physical structure of the hydrogels for both the storage modulus (G') and loss modulus (G'') therefore, the hydrogels in question are temperature dependent [10]. The gel containing the ratio 85:15% PVA/PAA as shown in Fig. 1, confirmed G' is much greater than G'', when analysed between 30 and 75 °C. The gels showed that the G' value, which corresponds to the stiffness of the hydrogel ranging between 150 and 200 Pa at a frequency of 1, 5 and 10 Hz. It is evident that as the temperature is increased the gels weaken as reported by Ricciardi et al. [10]. PVA/PAA properties depend upon the degrees of polymerisation and hydrolysis. The solubility of PVA/PAA in water increases greatly as its degree of hydrolysis increases [11]. Properties such as water solubility and high elasticity make these hydrogels useful as skin adhesives for the development of a wound healing device.

As illustrated in Fig. 2, an increase in stiffness ranging between 400 and 500 Pa was observed for the gel containing the ratio 85:15% PVA/PAA and the inner gel containing PVP after week 1. The stiffness of the gel gradually decreased as the temperature increased from 40 to 80 °C. At this point, the gel had completely broken down into an aqueous solution. This decrease is due to the uncoiling of crystalline regions within the polymer matrix [12]. 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 the gel containing the ratio 85:15% PVA/PAA and the inner gel containing PVP after 2 weeks as shown in Fig. 3, the results illustrate that the initial stiffness of the hydrogels increased by 300 Pa when compared to the samples tested in week 1. As the storage modulus (G') is higher than the loss modulus (G'') at test temperatures (30–80 °C) the elastic response of the sample dominates over the viscous behaviour and this is reflected by the increase in stiffness. In addition, with increasing temperatures the sample flexibility is diminished and the swollen sample becomes less rigid. This result suggests that the inner gel containing PVP breaks down and diffuses out onto the PVA/PAA hydrogel which leads to more hydrogen bonding, therefore, increasing the stiffness of the two phase hydrogel. Razzak et al. [13] investigated a PVP/PVA copolymer prepared for biomedical applications. The PVA/PVP blended hydrogels showed some of the requirements of an ideal wound dressing. For example, it effectively absorbed extrudate from the wound, it was

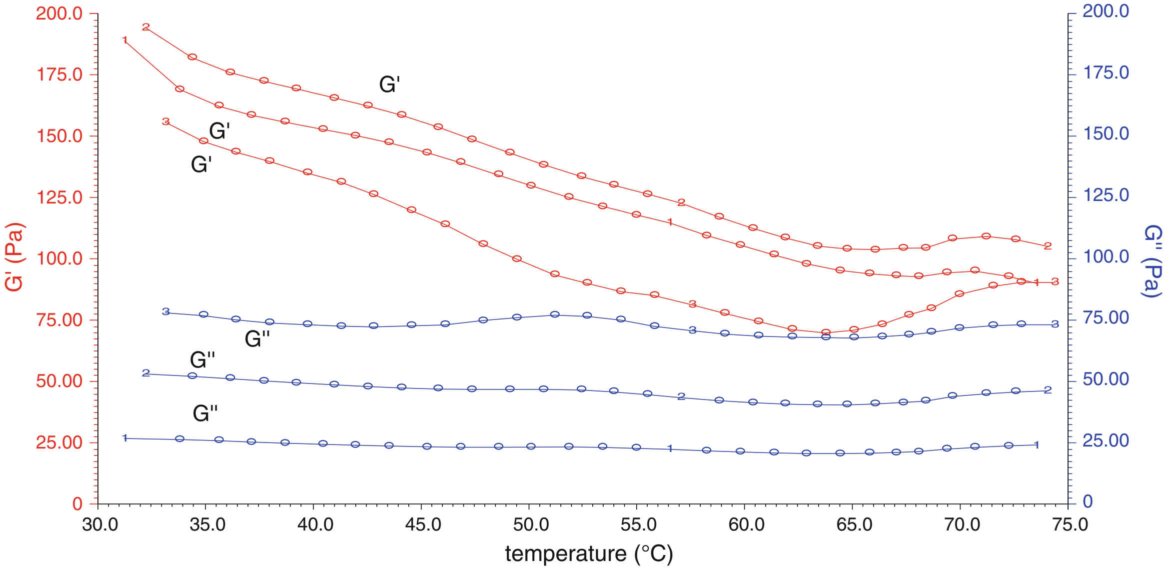


Fig. 1 Rheological results for the hydrogel containing the ratio 85:15% PVA/PAA, where the storage modulus (G') 1, 2 and 3 represents 1, 5 and 10 Hz, respectively

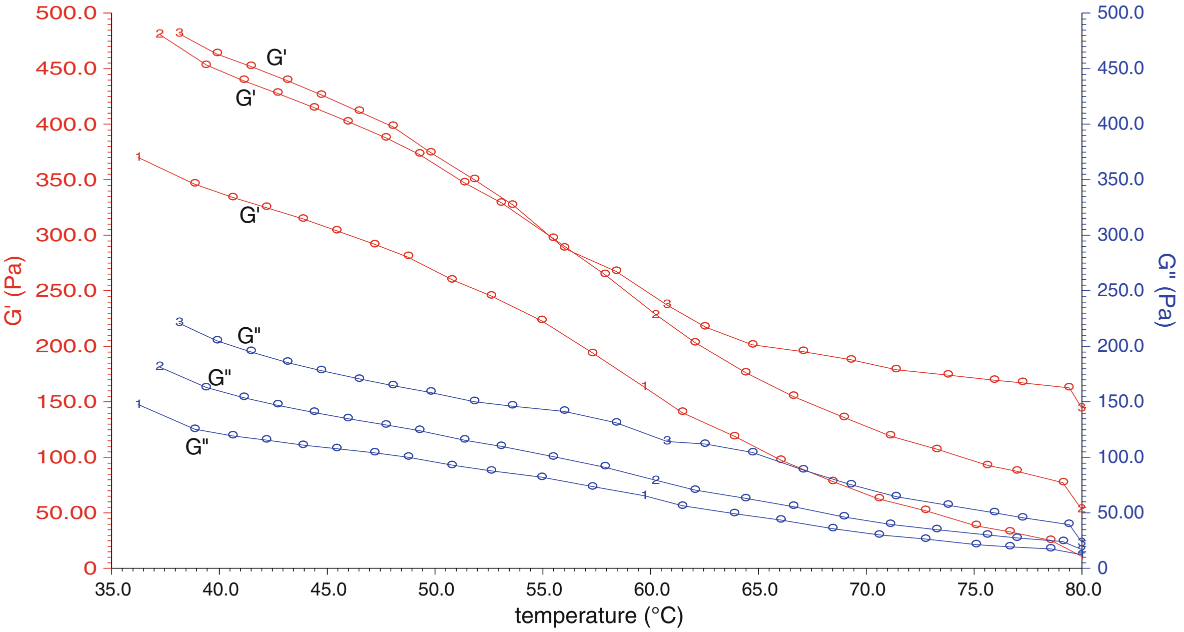


Fig. 2 Rheological results for the two phase hydrogel after 1 week. The outer gel contains the ratio 85:15% PVA/PAA and the inner gel contains PVP, where the storage modulus (G') 1, 2 and 3 represents 1, 5 and 10 Hz, respectively

pleasant to touch and painless to remove. It exhibited high elasticity and good mechanical strength. It had good transparency and could act as a barrier against microbes. It was found that the hydrogel wound dressing had potential for use in a tropical environment.

In order to provide a clear and visual demonstration of the swelling behaviour of the xerogels, pictures of the two phase samples are shown in Figs. 4, 5, 6. Figure 4 shows a pure PVA/PAA hydrogel, while Figs. 5, 6 illustrate the two PVA/PAA hydrogels with a UV polymerised PVP xerogel incorporated into the core of the hydrogel. From these pictures it was possible to view that physical PVP hydrogel diffused out of the hydrogel by an extra 4 mm in diameter from week 1 to week 2. Ramazani-Harandi et al. [14] 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.

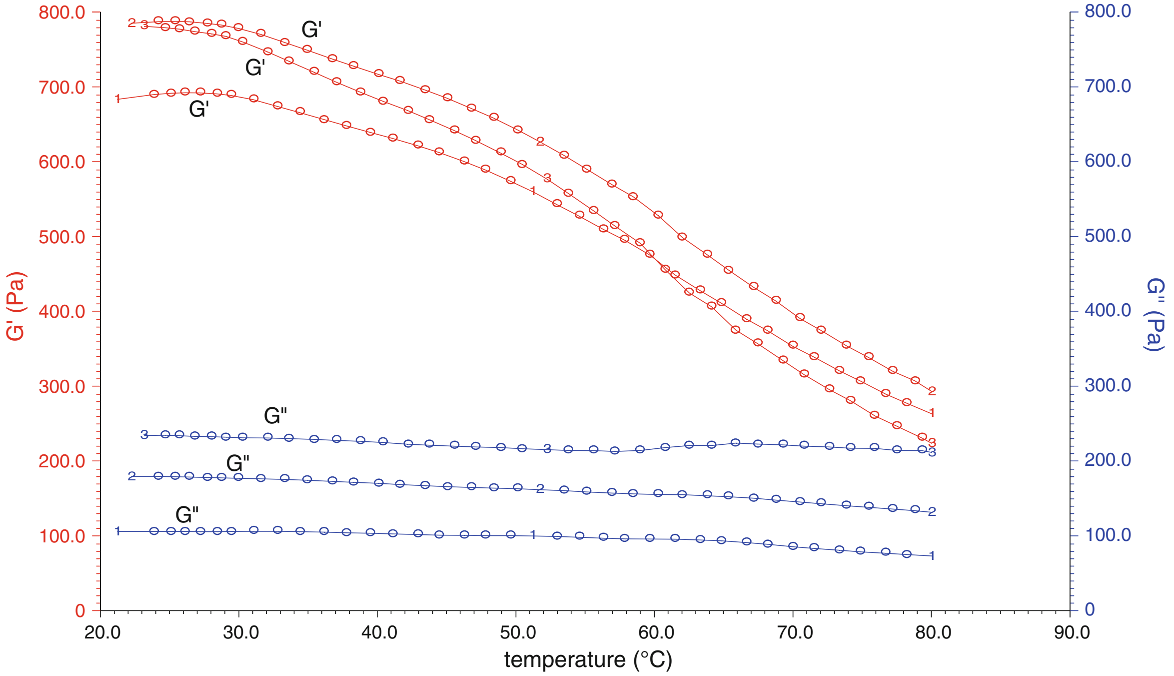


Fig. 3 Rheological results for the two phase hydrogel after 2 weeks. The outer gel contains the ratio 85:15% PVA/PAA and the inner gel contains PVP, where the storage modulus (G') 1, 2, and 3 represents 1, 5 and 10 Hz, respectively

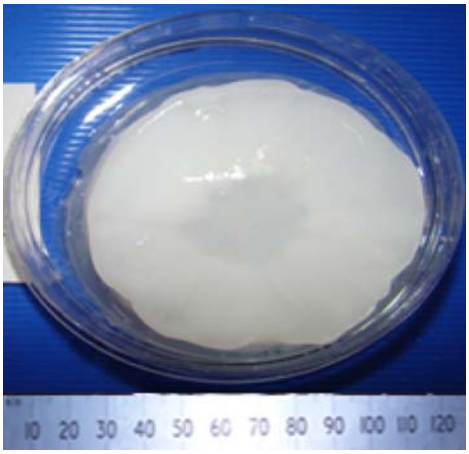


Fig. 4 85:15% PVA/PAA

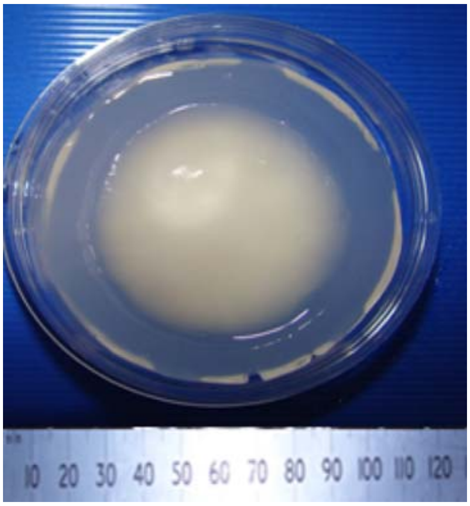


Fig. 5 85:15% PVA/PAA with 100% PVP week 1

Thermal analysis of the hydrogel and the two phase hydrogel system

Due to the high water content (94–98 wt%) in the hydrogel samples, the T_g values of the polymers was masked by the presence of water. Therefore, all samples were dried at 37 °C for 48 h before testing. Moisture testing confirmed the water content in the hydrogels was 5% (±0.5%) after drying. As shown in Fig. 7, two transitions were evident for the dried hydrogel containing the ratio 85:15% PVA/PAA. The peak observed at 88.90 °C exhibits the glass transition temperature (T_g) of PVA. This corresponds to values reported by Hernández using PVA with a weight average molecular weight of 94,000 [15]. The peak at 212.21 °C illustrates the melting temperature (T_m) of PVA. The extent of crystallinity in a PVA hydrogel has an important effect on the mechanical properties of the hydrogels. Hydrogels with a high crystallinity will have

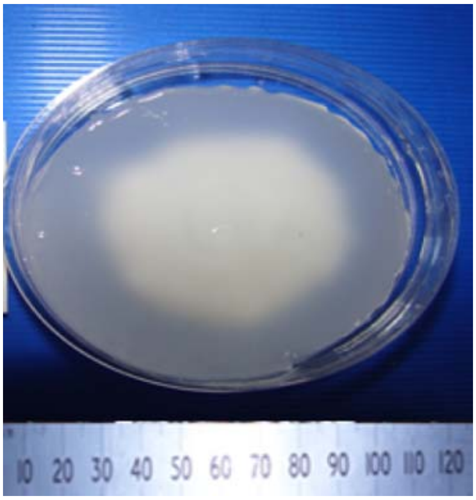


Fig. 6 85:15% PVA/PAA with 100% PVP week 2

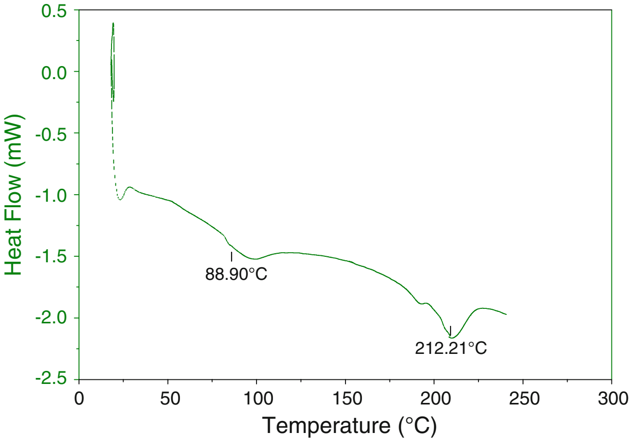


Fig. 7 Thermal transitions for the hydrogel containing the ratio 85:15% PVA/PAA

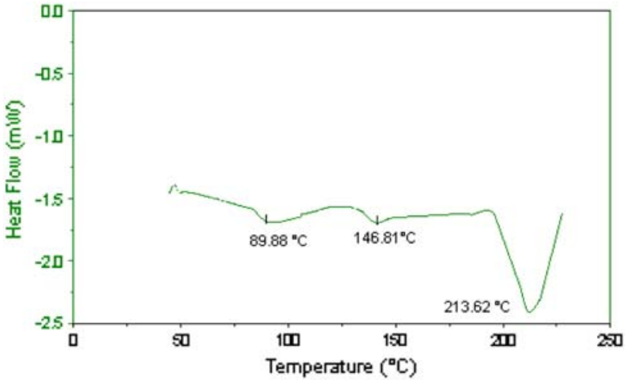


Fig. 8 Thermal transitions for the two phase hydrogel after 1 week. The outer gel contains the ratio 85:15% PVA/PAA and the inner gel contains PVP

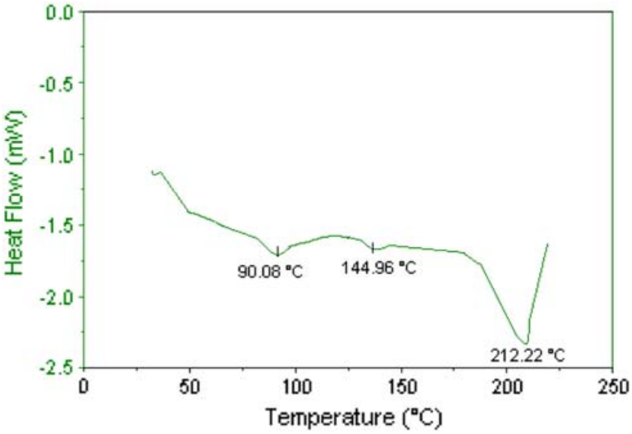


Fig. 9 Thermal transitions for the two phase hydrogel after 2 weeks. The outer gel contains the ratio 85:15% PVA/PAA and the inner gel contains PVP

reduced flexibility, however, if the crystallinity is below its expected value, hydrogels will tend to have poor consistency [10].

DSC analysis was also performed in order to determine the effect of the addition of PVP had on the properties of the PVA/PAA hydrogel. Yaung and Kwei [16] performed DSC analysis on PVP hydrogels and found that the T_g was 146 °C. In this work, the T_g of the PVP two phase samples was found to be 146.81 °C at week 1 and 144.96 °C at week 2. As shown in Figs. 8, 9 the decrease in T_g correlates to the diffusion of the PVP throughout the PVA/PAA hydrogel, therefore, the water present within the hydrogel acts as a plasticiser and reduces the T_g of the PVP polymer. The transitions observed at 89.88 and 90.08 °C are the glass transition temperatures of the PVA, while the transitions at 213.62 and 212.22°C are that of the melting temperatures of PVA. It can be seen from the thermograms obtained that the T_g of each of the samples was above 80 °C, which would have no impact on the behaviour of the gel at body temperature, but did however, give an indication of the stiffness of the dry polymer.

Attenuated total reflectance Fourier transform infrared spectroscopy for the PVA/PAA hydrogel and the two phase hydrogel system

IR spectroscopy has been used to characterise both the polymerisation systems used to produce hydrogels, as well as the resulting hydrogel polymers [16]. In this study, ATR-FTIR was used to confirm the hydrogen bonding in the two phase hydrogel system. With reference to Fig. 10, for the hydrogel containing the ratio 85:15% PVA/PAA, the absorption band between 3,339 and 3,166 cm^{-1} is

Fig. 10 ATR-FTIR spectrum for the hydrogel containing the ratio 85:15% PVA/PAA

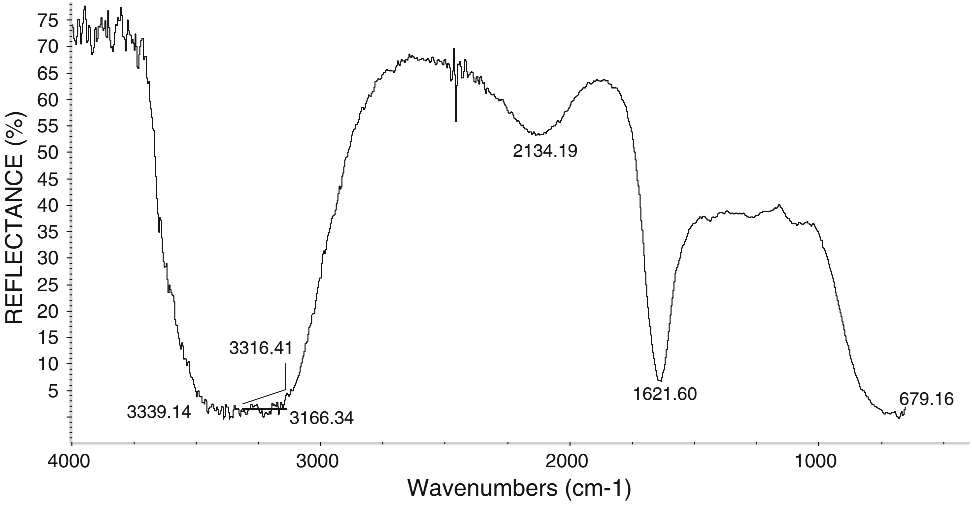
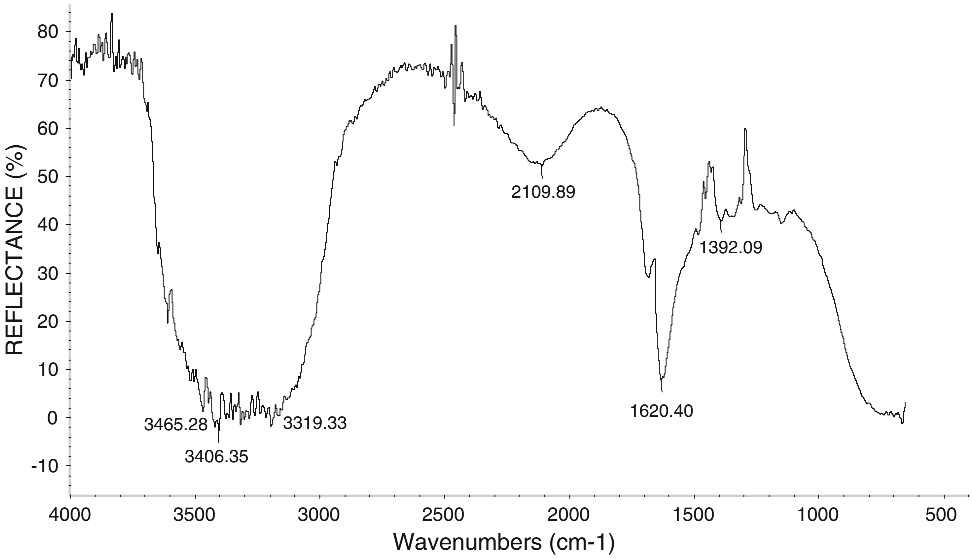


Fig. 11 ATR-FTIR spectrum for the two phase hydrogel after 1 week. The outer gel contains the ratio 85:15% PVA/PAA and the inner gel contains PVP



indicative of the O–H bonds of the PVA while the band observed at 2,134 cm^{-1} is due to the O–H overtone vibrations of PAA. The strong peak at 1,621 cm^{-1} shows the C=O stretching frequency of PAA [17].

With reference to Figs. 11 (week 1) and 12 (week 2) for the two phase hydrogel containing the ratio 85:15% PVA/PAA and the inner gel containing PVP the range of OH stretching vibration (3,000–3,700 cm^{-1}) was masked by the quantity of water residue. However, a broad peak existed in all samples that are characteristic of the stretching vibration associated with hydroxyl groups as shown in Figs. 10, 11, 12.

As shown in Fig. 11, for the gel containing the ratio 85:15% PVA/PAA and the inner gel containing PVP after 1 week, the peaks between 3,406 and 3,465 cm^{-1} are characteristic of the stretching frequency of O–H bonds of PVA, these peak appears at 3,475 cm^{-1} for pure PVA [15]. The peak observed at 2,109 cm^{-1} indicates the O–H overtone of PAA. Formation of hydrogen bonding is exhibited in the IR spectrums as a negative shift of the stretching vibration of the functional group involved in the hydrogen bond, which is typically a carbonyl group. Yaung and Kwei [16] describes how the frequency of the PVP carbonyl group shifts from 1,640 to 1,610 cm^{-1} to 1,630 to 1,620 cm^{-1} when it forms hydrogen bonds to the carboxyl group of the PAA [16]. In this work, the carboxyl group for PVP was found to be at 1,620 cm^{-1} after week 1 and shifted to 1,610 cm^{-1} on week 2 thus giving evidence of

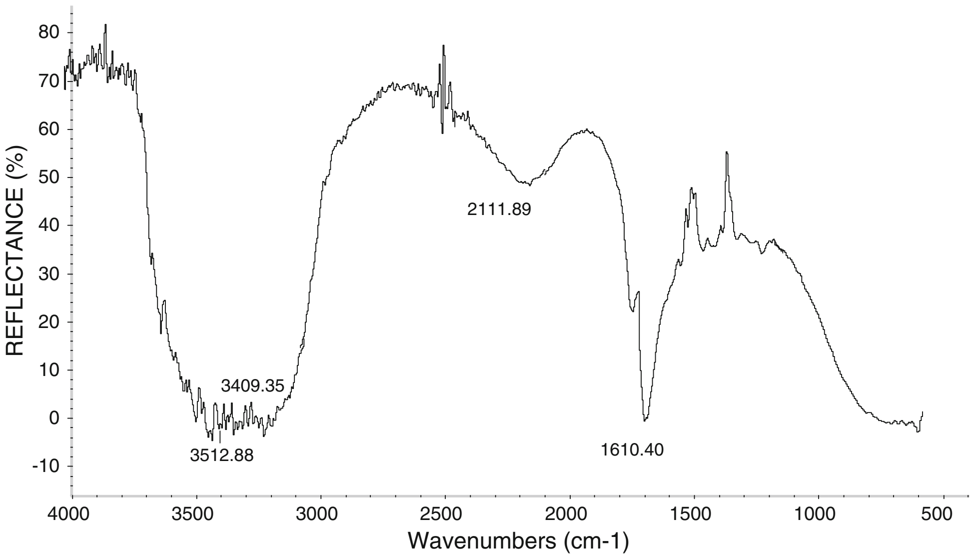


Fig. 12 ATR-FTIR spectrum for the two phase hydrogel after 2 weeks. The outer gel contains the ratio 85:15% PVA/PAA and the inner gel contains PVP

hydrogen bonding in the copolymer complex. This confirms the rheological results which also showed an increase in hydrogen bonding from week 1 to week 2.

Conclusion

In this study, the novel two phase hydrogels prepared by UV polymerisation and freeze thawing techniques showed increased mechanical properties due to the presence of crystalline regions that serve as the physical crosslinks. The rheological results confirmed the stiffness of the two phase hydrogels increased from 500 to 800 Pa over a period of 2 weeks. However, at temperatures above 35 °C, under the same nominal force, the hydrogels were observed to lose their physical structure.

The ATR-FTIR spectroscopy results confirmed hydrogen bonding had occurred between the two phase hydrogel systems which resulted in a negative shift of the carbonyl group from 1,620 cm^{-1} after week 1 to 1,610 cm^{-1} after week 2. The thermal analysis results suggested that the T_g of each of the samples was above 80°C, which indicated no impact on the behaviour of the gel at body temperature, but did however, give an indication of the stiffness of the dry polymer.

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. Therefore, such hydrogels with enhanced mechanical properties show promise for a variety of applications in the biomedical and pharmaceutical fields such as wound dressings, diagnostic, therapeutic and implantable devices.

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