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Research paper

Characterisation and controlled drug release from novel drug-loaded hydrogels

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Abstract

Hydrogel based devices belong to the group of swelling controlled drug delivery systems. Temperature responsive poly(*N*-isopropylacrylamide)–poly(vinylpyrrolidinone) random copolymers were produced by free radical polymerisation, using 1-hydroxycyclohexylphenyketone as an ultraviolet-light sensitive initiator, and poly(ethylene glycol) dimethacrylate as the crosslinking agent (where appropriate). The hydrogels were synthesised to have lower critical solution temperatures (LCST) near body temperature, which is favourable particularly for 'smart' drug delivery applications. Two model drugs (diclofenac sodium and procaine HCl) were entrapped within these xerogels, by incorporating the active agents prior to photopolymerisation. The properties of the placebo samples were contrasted with the drug-loaded copolymers at low levels of drug integration. Modulated differential scanning calorimetry (MDSC), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and atomic force microscopy (AFM) were used to investigate the influence of the drugs incorporated on the solid-state properties of the xerogels. MDSC and swelling studies were carried out to ascertain their effects on the LCST and swelling behaviour of the hydrated samples. In all cases, drug dissolution analysis showed that the active agent was released at a slower rate at temperatures above the phase transition temperature. Finally, preliminary *in vitro* cytotoxicity evaluations were performed to establish the toxicological pattern of the gels.

Keywords: Hydrogels; Photopolymerisation; Lower critical solution temperature; Temperature sensitive; Drug delivery

1. Introduction

Polymers have gained in importance in the pharmaceutical industry as both drug encapsulants and vehicles of drug carriage. Polymers employed to delay drug dissolution aim to slow the rate at which drug molecules are exposed to water from the aqueous environment surrounding the drug delivery system [1]. Hydrogels formed by chemical or physical crosslinking are a special class of polymers that imbibe a considerable amount of water while maintaining their overall shape. The research on hydrogels with respect to drug delivery and biomedical devices has been extensive over the last few decades because of their biocompatibility properties and easy control of solute transport [2–4]. More recently, many investigators have prepared hydrogels with additional functions, such as the ability to swell or shrink in response to a signal. These hydrogels with additional functions are often called 'intelligent' or 'smart' hydrogels. The most widely known smart hydrogels are those which respond (i.e., swell, shrink, bend, or degrade) to changes in environmental conditions. For this reason, they are

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usually known as environmentally sensitive hydrogels [3–5]. These types of stimuli-responsive polymers can undergo relatively large and abrupt, physical or chemical changes in response to small external changes in the environmental conditions. Of particular interest to this work is the use of intelligent polymers, which are capable of undergoing sharp physical or chemical modifications in response to external temperature change [6–13].

Negative temperature sensitive gels are the most commonly used of the temperature sensitive hydrogels and have a lower critical solution temperature (LCST). These hydrogels swell below the phase transition temperature and shrink as the temperature increases above it. Hence, this type of swelling behaviour is known as negative (or inverse) temperature dependence [3,4,14]. The temperature which induces gel collapse corresponds to the LCST of the uncrosslinked polymer. Most physically crosslinked polymers increase their water-solubility as the temperature increases. Polymers with LCST, however, decrease their water-solubility as the temperature increases [14]. Poly(*N*-isopropylacrylamide) is the most popular of the temperature sensitive hydrogels and is quite unique with respect to the sharpness of its almost discontinuous transition at approximately 32 °C in distilled water [2,12,13,15,16]. Adjustment of the LCST to near body temperature is essential particularly for 'smart' drug delivery applications [3,5]. Physical entrapment is one of the simplest methods used for incorporating active agent into hydrogels that are intended for controlled drug delivery applications. With physical entrapment, the active agent is contained within the hydrogel, which has a tight enough structure to inhibit diffusion of the drug into the surrounding environment, i.e. there must be sufficient crosslinking or entanglements to ensure the solute remains in the hydrogel [17]. In one such approach, the hydrogel monomer(s) are mixed with drug, an initiator, with or without a crosslinker, and allowed to polymerise, thus trapping the drug within the matrix [18].

In previous works by our research group, both physically and chemically crosslinked hydrogels based on *N*-isopropylacrylamide (NIPAAm) and 1-vinyl-2-pyrrolidinone (NVP), with phase transition temperatures in the region of 37 °C, have been synthesised and characterised [19–22]. In this contribution, active agents such as diclofenac sodium and procaine HCl are incorporated into these hydrogels, and the role that the LCST plays on the rate of drug release from both the physically and chemically crosslinked copolymers is investigated. Despite the number of recent studies incorporating active agent into hydrogels via the above technique, the properties of the drug-loaded samples are often overlooked [17,23–27]. It is therefore another primary objective of this study to contrast the characteristic copolymer properties with and without incorporated drug (for both xerogels and hydrated samples), as this area continues to receive limited attention in the literature. Finally, preliminary *in vitro* cytotoxicity evaluations are performed to establish the toxicological pattern of the gels.

2. Experimental details

2.1. Synthesis

The hydrogels investigated in this work were prepared by free-radical polymerisation using ultraviolet (UV) light. The monomers used were 1-vinyl-2-pyrrolidinone (NVP, Lancaster synthesis) and *N*-isopropylacrylamide (NIPAAm, TCI Europe). 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 NVP/NIPAAm monomeric mixtures containing an appropriate amount of distilled water (if required) and stirred continuously until completely dissolved. The polymers were chemically crosslinked using poly(ethylene glycol) dimethacrylate with molecular weight 600 (PEG600DMA) (Sigma–Aldrich) at 0.1 wt% of the total monomer content. The mould was positioned horizontally to the gravity direction under two UVA 340 UV lamps (Q-panel products) and the solution was cured in an enclosed environment at ambient temperature. Once cured, the samples were dried in a vacuum oven at 40 °C, 500 mm Hg for 24 h prior to use. The preparation of the linear samples is similar to that of the crosslinked polymer as described above, but in the absence of crosslinker. Further information on the synthesis of these gels has been reported previously [19–21]. As NIPAAm monomer is a solid, PNIPAAm could not be synthesised by UV polymerisation using this procedure. Therefore, all tests on PNIPAAm homopolymer were carried out on poly(*N*-isopropylacrylamide) (Polysciences Inc.).

For drug incorporation, diclofenac sodium and procaine HCl (generously donated by Pharmaplaz Ltd.) were added to the monomeric mixtures at 25 wt%, 10 wt%, 5 wt%, 2.5 wt% and 1 wt% of the total monomer content. The active agent was added to the aqueous monomeric solutions and stirred continuously for 1 h until dissolved. The photoinitiator and crosslinker (where necessary) was added to the solution as before and the samples photopolymerised as previously described. The compositions of the samples used throughout this investigation are listed in Table 1.

Table 1
Name and composition of hydrogels synthesised

Hydrogel name	NVP (wt%)	NIPAAm (wt%)	Distilled water (wt%)
PVP homopolymer (100–0)	100	–	–
A1 (L1)	15	65	20
90 wt% NVP-10 wt% NIPAAm (90–10)	90	10	–
80 wt% NVP-20 wt% NIPAAm (80–20)	80	20	–

2.2. Glass transition temperature determination

Differential scanning calorimetric measurements were carried out using a TA Instruments DSC 2920 Modulated DSC (AGB Scientific Ltd.). Xerogels of between 8 and 10 mg were weighed out using a Sartorius scales capable of being read to five decimal places. All measurements were conducted in sealed non-hermetic aluminium pans by heating the samples at a rate of 10 °C/min from 20 to 200 °C, with an empty crimped aluminium pan being used as the reference cell. Prior to this, samples were heated from 20 to 200 °C at 10 °C/min to remove all the residual moisture and erase the effect of previous thermal history. The glass transition temperature (T_g) was considered at the mid-point temperature of the endothermic drift in the heating curves. All DSC tests were carried out under a 20 ml/min flow of nitrogen to prevent oxidation. High purity indium was used to calibrate the temperature value.

2.3. Attenuated total reflectance Fourier transform infrared spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was carried out on the rectangular samples that 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.

2.4. Atomic force microscopy

Atomic force microscopy (AFM) studies were carried out on 50μm and 5μm samples using a Topometrix Explorer™ scanning probe microscope (SPM). Samples were mounted onto a metal stub using an instant cyanoacrylate based adhesive, such that the area of interest of each sample was face up to be analysed. A calibration scan was carried out on a standard calibration grid provided by Veeco Instruments Ltd., prior to sample analysis. Topographical data was obtained on sample regions in contact mode, using standard SPM probe tips.

2.5. Phase transition determination

Homogeneous solutions of the xerogels (which incorporated drug) were prepared, by weighing appropriate amounts of the xerogel and distilled water, leaving these mixtures at room temperature for a period of hours/days. Once dissolved, further amounts of distilled water were added, until solutions of appropriate concentration were achieved. For PNIPAAm homopolymer analysis, the drug was simply added to aqueous polymeric solutions and allowed to dissolve. Modulated DSC was then used for examination of the phase transition phenomenon characteristic of these thermosensitive gels. The analyses were performed using a DSC 2920 Modulated DSC (TA Instruments) containing a refrigerator cooling system. Samples of between 8 and 10 mg were transferred by syringe and 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 20 to 55 °C for each of the aqueous solutions. The DSC measurements were carried out at a scanning rate of 1 °C/min under nitrogen atmosphere. Calibration was performed using indium as standard. It is noted that for the study of phase separation and phase separation kinetics, not the absolute value, but the changes of the specific heat capacity as a function of temperature and time are important.

2.6. Swelling studies

The swelling characteristics of the gels were investigated in triplicate at temperatures ranging from ambient temperature to 44 °C. Samples of the cured polymer (with drug incorporated) with a mass of 1.1 ± 0.35 g were placed in a petri dish; the petri dish was filled with distilled water and placed in a fan oven at the required temperature. Petri dish lids and Petri Seal (Diversified Biotech Ltd.) were placed on the petri dishes while in the oven to prevent evaporation. Periodically, excess polymer solution was removed after predetermined time intervals by pouring the solution through a Buchner funnel. The samples were then blotted free of surface water with filter paper, and the wet weight of the gel sample was measured using a Sartorius scales at room temperature. The samples were re-submerged in fresh distilled water and returned to the oven. The percentage that the hydrogels swelled was calculated using formula (1):

$$\text{Swelling}\,(\%) = W_t/W_0 \times 100 \tag{1}$$

where W_t is the mass of the gel at a predetermined time and W_0 is the dry mass of the gel. In order to provide a clear visual demonstration of the swelling behaviour of the gels and for comparative reasons, pictures of the swollen samples were taken after the removal of the distilled water solution. This process was continued until the sample appeared to have dissolved or for up to 120 h. The gel fraction (%) was calculated using the following Eq. (2):

$$\text{Gel fraction}\,(\%) = \frac{W_{\text{ex}}}{W_0} \times 100 \tag{2}$$

where W_o and W_{ex} are the weight of the dried gel after photopolymerisation, and the dried weight of the sample after extraction of soluble parts, respectively.

2.7. Drug dissolution

Drug dissolution studies were conducted on polymers using a Sotax® on-line dissolution system. The test was carried out in triplicate using the Paddle method (USP XXV method II) at a number of different temperatures at 100 rpm. At predetermined time intervals, samples were withdrawn automatically, filtered, passed through a Perkin

Elmer Lambda 20 UV/vis spectrometer, before being returned to the dissolution vessel. The wavelength and absorption of a 100% drug concentration in each test media was determined in triplicate using a Perkin Elmer Lambda 40 UV/vis spectrometer. The average values were entered into software calculations prior to commencement of testing to form a reference standard.

2.8. Cytotoxicity testing

2.8.1. Cell culture

Cell culture vessels and media components were sourced from Sarstedt and Sigma–Aldrich respectively. Human hepatoma (HepG2) cells were grown in Dulbecco's modified Eagles medium-Nutrient mixture Ham's F-12 (1:1), supplemented with 5% fetal bovine serum, 200 mM L-glutamine, and penicillin 10,000 IUml^{-1}, streptomycin 10 mg ml^{-1} and amphotericin B solution 250 μg ml^{-1}, in a humidified atmosphere of 5% CO_2, 95% air at 37 °C. The cells were cultured in 75 cm^2 cell flasks and allowed to attach for 24 h prior to treatment.

2.8.2. Sample preparation

Prior to cytotoxicity testing, individual xerogels were weighed on an analytical balance and placed in a 50 ml capped tube and sterilised under UV light for 15 min. Twenty millilitres of complete media was added and the tubes were placed in a 37 °C incubator until the polymers/copolymers had fully dissolved. Xerogels containing higher concentrations of PNIPAAm took longer to dissolve (6 days). Once dissolved, all hydrogel media were serially diluted in the range of 0.025–25 mg ml^{-1} in complete media.

2.8.3. MTT assay

Methyl thiazoyl tetrazolium bromide (MTT) is a yellow salt that is reduced in the mitochondria of viable cells to a blue formazan product, which absorbs maximally at 540 nm. HepG2 cells were seeded at 1×10^5 ml^{-1} in 96-well plates and following overnight incubation were exposed to hydrogel containing media for 3 or 24 h. Subsequently wells were washed with 100 μl warm PBS and 100 μl of MTT containing media (0.5 mg ml^{-1}). Following 4hrs incubation at 37 °C, the MTT containing medium was carefully aspirated to avoid disturbing any formazan crystals formed and 100 μl of DMSO added to each well. Plates were shaken for 15 s, incubated at room temperature for 10 min and optical densities recorded at 540 nm using a BioTek plate reader. Cell viability was expressed as a percentage of the untreated control cells.

3. Results and discussion

3.1. Synthesis and incorporation of active agent

The synthesis and properties of hydrogel A1(L1) (both in the physically and chemically crosslinked form) has been previously investigated by our research group [19–22], and these copolymers were consequently chosen for drug incorporation analysis. As polyvinylpyrrolidinone (abbreviated to PVP) homopolymer has been extensively researched and does not have negative temperature sensitive capabilities [28], it was used as a control where appropriate throughout this work. The active pharmaceutical ingredients were integrated using the method outlined in the introduction. Diclofenac sodium and procaine HCl were the active agents used. Drug loadings of 25 wt%, 10 wt%, 5 wt%, 2.5 wt% and 1 wt% of the total monomer content were examined and its effect on the curing time and integrity of the resulting xerogels is discussed. At higher drug loadings, the negative temperature sensitive copolymers exhibited similar curing behaviour. For example, at 5 wt% drug and above, the xerogels were discarded, as they had not cured satisfactorily even after 48 h. There was a marked improvement with samples containing 2.5 wt% drug, and at 1 wt% drug incorporation the copolymers cured to a standard comparable to that of the original samples synthesised in the absence of drug (typically transparent and glass like in appearance), as illustrated in Fig. 1. The samples however were slightly sticky on the surface, took approximately four times longer to cure and appeared a shade darker in colour than the original xerogels (this is not uncommon as yellowing is often exhibited by polymers produced by UV curing and the darker colour is likely due to the longer exposure to the UV light). Longer curing times remains a common drawback when integrating drug into xerogels using this technique [17,23]. Scott and Peppas suggest that active agent may act as a retarding agent over the course of the radical polymerisation, by scavenging free radicals available for the polymerisation [23].

In this study, given that solid NIPAAm monomer must be dissolved in liquid NVP/distilled water solutions during the preparation of the copolymer samples, it is possible that the mixture reached saturation point thus hindering

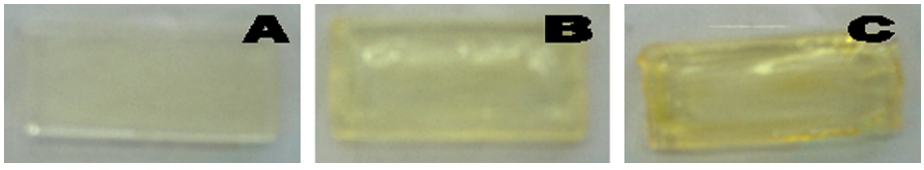

Fig. 1. Rectangular samples used for FTIR analysis; (A) PVP homopolymer, (B) PVP homopolymer incorporating 1 wt% procaine HCl and (C) A1(L1) incorporating 1 wt% diclofenac sodium.

polymerisation, especially when high loadings of drug were then added. Devine et al. synthesised PVP/acrylic acid copolymers (both liquid monomer precursors) using a similar method to the one described herein and established that aspirin and paracetamol could be successfully incorporated at 25 wt% of the total monomer content [29]. The samples were found to maintain similar properties to the xerogels produced without drug integration, but again the curing rate was retarded. These findings suggest that the use of liquid monomers might allow higher drug loadings. This theory is further supported as 100 wt% NVP (liquid monomer) cured to a similar standard with drug loadings of up to 25 wt% diclofenac sodium as it did with no incorporated drug (again a change in shade and curing rate was documented). However, procaine HCl fell out of solution using higher concentrations of drug, so this behaviour is directly related to the solubility of the drug in the monomeric mixture. It is worth taking into consideration that much of the recent literature on incorporating active agent using this technique, tend to use relatively low drug loadings, usually less than 10wt% of the total monomer content [17,23–27]. Also, please note that it was not within the scope of this analysis to investigate the effect of photopolymerisation on the potency of the active agent. For the continuation of this investigation, samples integrating diclofenac sodium and procaine HCl at 1wt% are employed in characterisation and drug release studies.

3.2. Solid-state properties of xerogels incorporating active agent

Three of the most commonly used techniques for analysing xerogels include differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM). For calorimetric studies, given the importance of the plasticising effect of water on these types of polymer, all samples were initially heated to 200 °C at a rate of 10 °C/min to remove any residual moisture that may obscure interpretation of the results. A second scan was then run on the same sample under identical conditions and evaluated. As reported in literature, the glass transition temperature of linear PNIPAAm is between 85 and 130 °C, probably depending on molecular weight [30], while PVP is described as a highly amorphous polymer with a T_g ranging between 100 and 180 °C, again depending on molecular weight [28]. If a copolymer is random, it will exhibit a T_g that approximates the weighted average of the two T_gs of the homopolymers. Block copolymers of sufficient size and incompatible block types will exhibit T_gs characteristic of each homopolymer but slightly shifted owing to incomplete phase separation [31]. Throughout this work, a single distinctive endotherm is exhibited by the analysed samples representing the T_g, thus A1(L1) can be characterised as a random copolymer. Also, the xerogel is most likely predominantly amorphous in nature, as semi-crystalline materials are not known to exhibit such distinctive T_gs. This opinion is strengthened as in general, fully amorphous polymers are stiff, brittle and clear in the virgin state.

All of the xerogels synthesised were found to have T_gs of over 100 °C using the modulated DSC reversing heat flow signal, and this accounts for the stiffness of the dry polymers at room and body temperature. For controlled drug delivery applications, the selection of polymer systems with glass transition temperatures well above storage and/or release temperatures is advantageous, as glassy matrices often offer improved stability over rubbery matrices of similar composition [32]. Wu and McGinity found that interactions between the drug and polymer lowered the T_g of their samples, thus affecting mechanical properties such as tensile strength, albeit with blends fashioned using solvent casting [33]. In this work, incorporation of the drugs into the copolymers resulted in a distinct decrease in T_g as can be seen in Fig. 2, with transition temperature decreases of approximately 53 and 75 °C recorded for A1(L1) samples incorporating diclofenac sodium and procaine HCl, respectively. A similar plasticising trend was observed for PVP homopolymer drug samples, but the decreases were not so pronounced. This trend may explain the rubbery nature of some of the copolymer gels, which were discarded due to insufficiently curing at higher drug loadings. The presence of a single T_g in the drug-loaded samples was used as criterion for establishing drug–polymer miscibility, thus suggesting complete miscibility at the concentration studied. Nair et al. carried out an extensive study on drug-polymer blends, created by solvent casting PVP homopolymer with a number of active agents, reporting an almost linear decrease in T_g with increasing drug concentration [34]. The plasticising effect caused by drug incorporation was subsequently examined at the molecular level using infrared spectroscopy.

ATR-FTIR was the spectroscopic method employed to study the physiochemical interactions between the drug and polymer in loaded A1(L1) xerogels. In all cases, the disappearance of the characteristic monomeric peaks

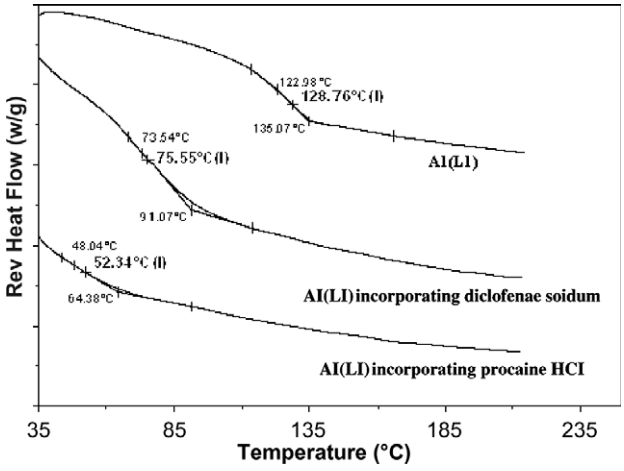

Fig. 2. DSC plots for copolymer A1(L1) both with and without active agent incorporated.

(NVP: C=C and C=O stretching vibrations both found in the region of 1680–1630 cm^{-1}, and strong vinyl double bonds peaks in the range 800–1000 cm^{-1} [35,36]/NIPAAm: 1617 cm^{-1} (C=C), 1409 cm^{-1} (CH_2=) and vinyl groups at 995–905 cm^{-1} [19,37,38]) in the drug-loaded and placebo copolymer spectra indicate that the polymerisation reaction has taken place. Furthermore, the characteristic peaks due to C=O stretching and NH bending for secondary amides in PNIPAAm were exhibited at 1641–1638 cm^{-1} (Amide I) and 1538–1534 cm^{-1} (Amide II), respectively, while a double band representing the isopropyl group was observed at 1386–1385 cm^{-1} and 1366–1365 cm^{-1}. These values are in good agreement with work reported in the literature [39,40].

If the drug and polymer interact then the functional groups in the FTIR spectrum will show the emergence of additional bands or alternations in wavenumber position or broadening compared to the spectrum of the pure drug and polymer [41,42]. However in this work, the IR spectra of the copolymers (with and without incorporated drug) are almost indistinguishable, as illustrated in Fig. 3. The characteristic peaks appear nearly identical in frequency and bandwidth, and surprisingly no new peaks are observed within the 1800–1000 cm^{-1} region with incorporation of the active agents, using this technique. This is particularly unexpected given that the characteristic peaks of diclofenac sodium, for example, are displayed between 1620–1386 cm^{-1}, with intense bands in the frequency range 1620–1560 cm^{-1} attributed to aromatic ring stretching [43,44]. It is therefore possible that the drugs do not interact with the copolymer on the molecular level but are instead simply dispersed within the polymeric matrix. Given such a scenario, one would still expect to observe characteristic active agent peaks in the drug–polymer spectrum. Hence it is likely that the broad copolymer bands mask the characteristic drug peaks within this region. The negligible effect on the bandwidth with integration of the drug is still somewhat surprising, but may be a consequence of the active agents being incorporated at such low concentrations. Nair et al. reported similar behaviour for solvent cast blends with characteristic polymeric peaks sometimes masking those of the drugs, with changes in the IR spectra becoming more apparent at higher drug loadings [34].

The microscopic morphology of the xerogels synthesised can be imaged using atomic force microscopy (AFM). Lyons et al. investigated the exfoliation of nanoclay particles, incorporated into hot melt extruded monolithic polymer matrices for oral drug delivery, using contact mode AFM [46]. A similar procedure to the one described in the aforementioned study was employed herein to determine the surface topography of the placebo and loaded xerogels, as represented in Figs. 4 and 5, respectively. Throughout this work, the surface roughness of the samples was measured using the RMS extracted from the AFM pictures. Firstly, a large scan area (100 × 100 μm^2 scan size) was utilised to investigate the regularity and lack of impurities on the surface of the homopolymer. PVP homopolymer exhibits a smooth surface and this may be one of the reasons why it has achieved considerable success in coating applications [48]. Conversely, as the drug particles were not fully visible in this range, further magnification (5×5 μm^2 scan size) was required. At this setting, a number of globular structures of relatively uniform size

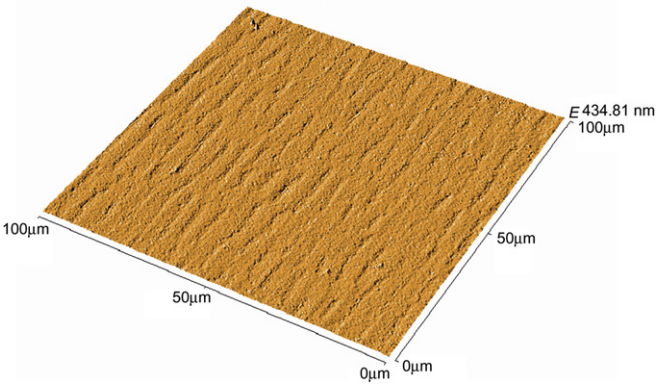

Fig. 4. Contact mode AFM image of PVP homopolymer.

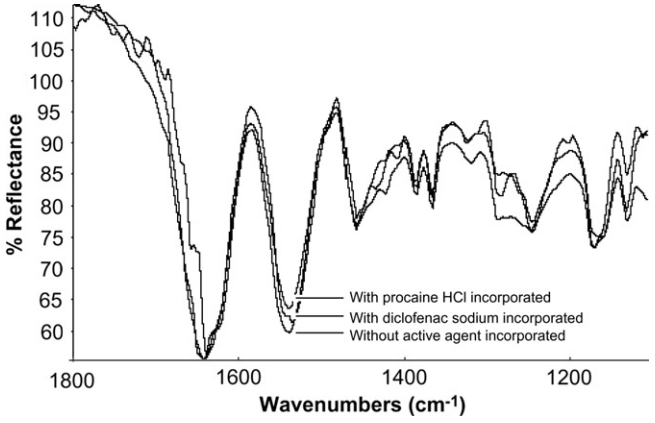

Fig. 3. ATR-FTIR spectra of A1(L1) both with and without active agent incorporated.

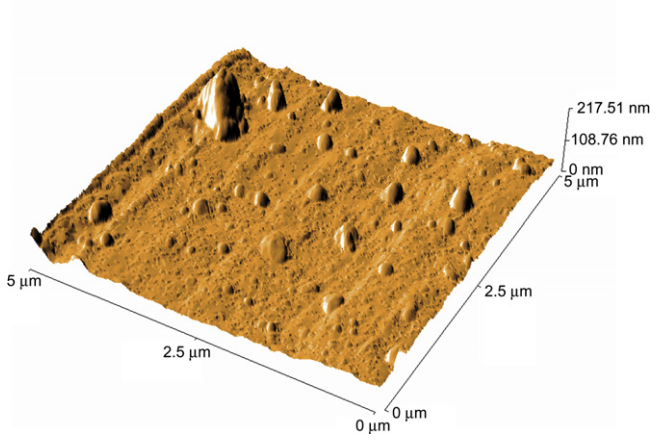

Fig. 5. Contact mode AFM image of PVP homopolymer incorporating 1 wt% diclofenac sodium.

appear embedded in the polymeric surface of the PVP sample incorporating diclofenac sodium. These particles are believed to be surface drug and appear well dispersed, which is advantageous for controlled drug delivery applications. It is thought that the migration of drug to the surface occurred during the synthesis process, although Nair et al. has commented that migration can also occur over a period of time, depending on the storage conditions of drug–polymer blends fashioned using different techniques [34]. Pan et al. carried out AFM studies on curcumin-eluting stents, and proposed that the absence of drug particles on the stent surfaces was a consequence of the drug and polymer (PLGA) mixing at the molecular level [47]. This would again bring into question whether our polymer-drug samples mixed on the molecular level during the photopolymerisation process. However, this may not necessarily be an entirely negative occurrence, as such an event would no doubt affect the properties of the active agents. Comparable behaviour was recorded throughout for the homopolymer integrating procaine HCl. A similar trend was also documented for A1(L1) samples when compared with the homopolymer, however the slightly sticky surface typical of the drug-loaded copolymers obscured the clarity of these scans somewhat. Non-contact mode AFM may be more suitable for investigating such samples. As near identical trends were exhibited by both the physically and chemically crosslinked xerogels with incorporation of drug, only the physically crosslinked samples have been discussed throughout this section for clarity sake.

3.3. Properties of hydrogels incorporating active agent

The properties of hydrated gels (with and without drug incorporated during the synthesis) were analysed using modulated DSC and swelling studies. The effect of incorporation on both physically and chemically crosslinked samples is discussed throughout this section, as the behaviour of the two types of polymers in aqueous media is known to differ considerably. It is noted that adjustment of the LCST to near body temperature is essential particularly for 'smart' drug delivery applications [3,5]. With this in mind, our previous studies have focused on the thermosensitive properties of PNIPAAm based copolymers with LCSTs around 37 °C in distilled water and pH buffer environments [19–22]. For calorimetric analysis, the LCST can be identified as an endothermic transition peak, believed to be mainly related to the breaking of hydrogen bonds between water molecules and the polymer [2]. The greater sensitivity of modulated DSC in phase transition temperature analysis, when compared with conventional DSC, is detailed in the abovementioned works by our research group, with particular emphasis on the role of the reversing heat flow signal. Throughout this and said calorimetric studies, all aqueous polymer and copolymer solutions were made up at 3 wt% for comparative examinations, as it is important to keep polymer concentration constant as the transition temperature and transition enthalpy (ΔH) are known to be concentration dependent. Please note that volume phase transition temperatures of the chemically crosslinked copolymers were approximately 2.5 °C lower than the LCSTs of the corresponding physically crosslinked gels. Schild et al. states that the two transition temperatures may vary slightly depending on the nature of the crosslinking agent [2].

The effect of various cosolutes, including common salts, surfactants, etc., on the LCST of thermosensitive polymers, continues to be the subject of widespread attention [2]. Despite this, a more limited number of studies have been reported on the effects of active agent on the phase transition temperature. This is surprising, as many investigators have proposed their negative temperature dependent polymeric systems for potential drug delivery applications, and also given the importance of the phase transition temperature in such delivery devices. Coughlan and Corrigan recently carried out calorimetric studies on the LCST of PNIPAAm homopolymer in a variety of aqueous drug solutions [49]. They concluded that the active agents had an effect on the phase transition temperature in all cases, the magnitude of which was dependent on the nature of the drug and concentration. At the concentration tested in this study, the drugs were found to have a negligible effect on the LCST and transition enthalpy of the negative temperature sensitive copolymers, as demonstrated in Fig. 6. This was also the case for the chemically crosslinked samples. For PNIPAAm homopolymer analysis, the drug was simply added to aqueous polymeric solutions at the corresponding concentration, and again little change in LCST was recorded. Therefore for the continuation of this investigation, the phase transition temperature of placebo A1(L1) is quoted throughout, as the transition temperature of both the loaded and unloaded samples is almost identical. It would be important to investigate this effect further if higher drug loadings were incorporated.

Physically crosslinked A1(L1) exhibited interesting swelling behaviour related to its phase transition temperature, below which it was water-soluble and above which it

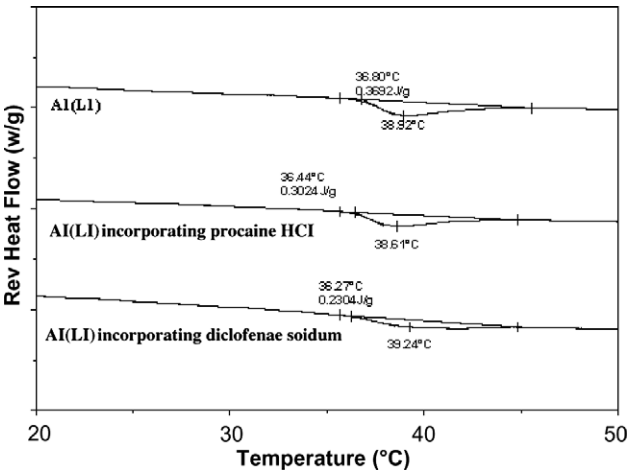

Fig. 6. Effect of drug incorporation on the LCST of physically crosslinked A1(L1) in distilled water.

become slightly less water soluble, significantly less water soluble, or water insoluble, depending on how high the test temperature was above the LCST [20]. In this work, swelling studies were carried out on the physically and chemically crosslinked samples incorporating drug, as it is believed that the presence of the active agents may affect the degree of swelling, thus influencing the rate of drug release from these polymeric materials. Analyses on the physically crosslinked copolymers were carried out in distilled water over a range of temperatures for 120 h, as illustrated in Fig. 7.

Note that analysis carried out to represent room temperature was undertaken in a fan-assisted oven at 20 °C throughout this study. The placebo chemically crosslinked hydrogel exhibited a swelling ratio of over 18 at 20 °C, thus falling marginally short of being characterised as superabsorbent, and showed noteworthy deswelling and reswelling characteristics owing to its phase transition temperature. Despite having such a high swelling ratio, the hydrogel maintained good gel integrity and yielded a constant G' value of approximately 1900 Pa, under an oscillating torque of up to 4000 micro N m using parallel plate rheometry [21]. When the samples incorporating drug were examined at the equivalent temperature, they were found to have a swelling ratio of roughly eleven, thus imbibing less water, a trend also exhibited by the physically crosslinked copolymers. Therefore, it was deemed unnecessary to perform comparative rheological analysis, as the degrees of swelling typical of the loaded and placebo samples were so clearly different. El-Sherbiny et al. also incorporated active agent into their copolymers during the photopolymerisation process, and stated that percent equilibrium swelling had obvious dependence on the selection of drug incorporated [25]. Shantha and Harding reported similar findings for systems integrating active agent during a solution polymerisation process [50].

The obvious temperature sensitive capabilities of the chemically crosslinked hydrogels incorporating active agent are highlighted in Figs. 8 and 9. A phase transition temperature peak maximum value of 36.56 °C was recorded for the hydrogel, using modulated DSC [21]. As

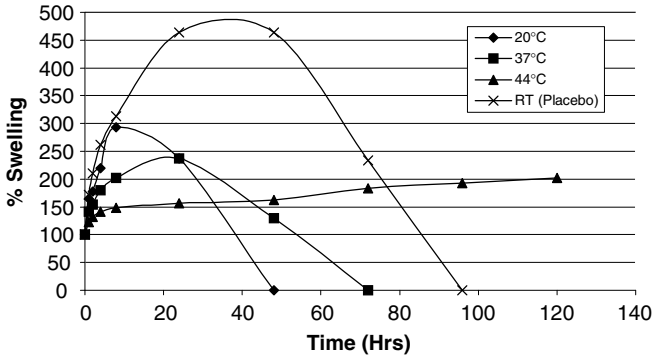

Fig. 7. Swelling behaviour at different temperatures of physically crosslinked A1(L1) hydrogels incorporating procaine HCl, unless stated otherwise.

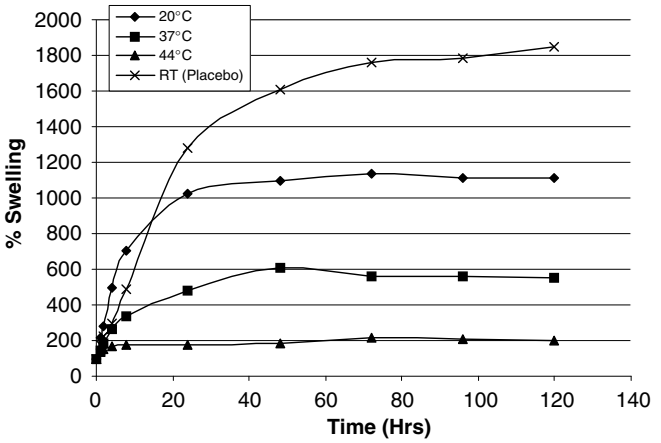

Fig. 8. Swelling behaviour at different temperatures of chemically crosslinked A1(L1) hydrogels incorporating procaine HCl, unless stated otherwise.

can be seen at 37 °C, the negative temperature dependent potential of the hydrogel is apparent, as the sample imbibed approximately half the volume of water it did at 20 °C. At 44 °C, the chemically crosslinked copolymer incorporating procaine HCl exhibits very similar hydration behaviour when compared with the pseudogel sample, at the corresponding temperature. As these samples hydrate at a similar rate and to a comparable volume over a set range of temperatures, it is predicted that the drug release profiles within this temperature window will be somewhat similar. Please note that the crosslinked samples polymerised in the presence of diclofenac sodium exhibited almost identical trends.

The hydrophilic and hydrophobic balance of polymer side groups in PNIPAAm hydrogels, i.e., —CONH— is hydrophilic and —$CH(CH_3)_2$— is hydrophobic [8], are responsible for these interesting negative temperature sensitive properties. Any molecules that can form hydrogen bonds to each other can alternatively form hydrogen bonds to water molecules. Because of this competition with water molecules, the hydrogen bonds formed between two molecules dissolved in water are relatively weak. It is generally believed that the phase transition behaviour of PNIPAAm based hydrogels in aqueous solutions is strongly related to the destabilisation of hydrogen bonds between water molecules and amide groups with increasing temperature, probably induced by the presence of the hydrophobic isopropyl group and backbone chain [16].

3.4. Drug dissolution

As diclofenac sodium and procaine HCl are reported to be freely water soluble [51,52], they are ideal for the current drug delivery investigation. Physically crosslinked A1(L1) has a phase transition temperature onset value of 36.80 °C and peak maximum value of 38.90 °C in distilled water, as detected using modulated DSC [20]. In this study, the drug release behaviour of the negative temperature sen-

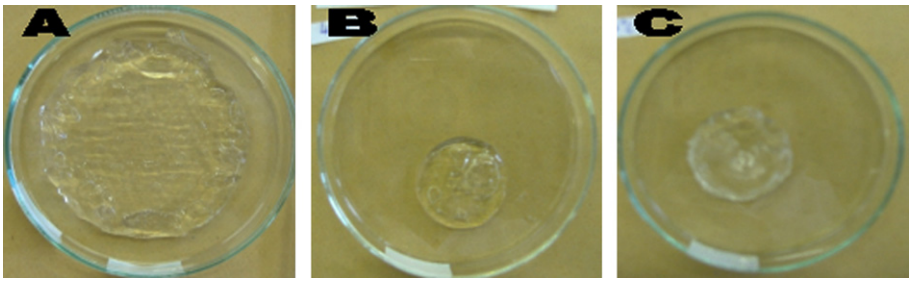

Fig. 9. Typical appearance of A1(L1) after 120 h in distilled water; (A) chemically crosslinked placebo gel swollen at 20 °C, (B) physically crosslinked gel incorporating procaine HCl swollen at 44 °C, and (C) chemically crosslinked gel incorporating procaine HCl swollen at 44 °C.

sitive pseudogels, containing diclofenac sodium and procaine HCl, was investigated at a number of different temperatures in distilled water. Diclofenac sodium loaded samples were first analysed and found to completely release all incorporated drug after approximately 12 h at 30 °C, as can be seen in Fig. 10. Surprisingly, the release rate was almost identical at 37 °C, which corresponds to the phase transition temperature onset value of the copolymer in distilled water. This was most likely because the temperature was not high enough for the hydrophobic interactions to dominate sufficiently, and significantly slow the rate of water sorption. With negative thermosensitive polymers, it is believed that a small fraction of the gel begins to undergo its phase transition at the onset temperature, while the bulk undergoes the transition at the peak maximum value. At both lower test temperatures, it was noted that the gels had disintegrated but not fully dissolved after the release of the drug. Hydration studies carried out at corresponding temperatures show that these gels swelled 2–3 times their initial mass, attained maximum swollen weight between 8 and 24 h and took between 48 and 72 h to fully dissolve. Taking into consideration the greater volume of dissolution media and the aggressive environment, the accelerated break down of the gels is not unexpected.

At 40 °C, the negative temperature dependant capabilities of the copolymers became clearer, as the drug took an extra 7 h to release with a 3 °C increase in test temperature. When the temperature was further elevated to 44 °C, the active agent was still not completely released even after 24 h. Also when examined after the analysis, the samples were observed not to have disintegrated to the same degree as they had at the lower temperatures. In fact, after inspection at the highest test temperature, samples were found to have excellent stability while imbibing roughly twice their initial weight in water, and when dried had a gel fraction of approximately 73%. For this reason, the diffusion process is believed to have played a predominant role in the release of drug from these samples particularly at this temperature. As the dissolution environment is approximately 5 °C higher than the LCST peak maximum value of the copolymer, the water sorption rate is lessened considerably, and thus the samples take longer to release the active agent and to disintegrate. The thermosensitive pseudogels incorporating procaine HCl exhibit an almost identical release tendency when compared with the diclofenac sodium samples, owing to the phase transition temperature behaviour, as can be seen in Fig. 11.

The only disparity is that procaine HCl appears be completely released at a marginally faster rate, at all test temperatures. Given that the molecular weights of procaine HCl and diclofenac sodium are 272.8 [51] and 318.1 [52], respectively, the solute size of the diclofenac sodium is likely to be slightly larger than that of procaine HCl, thus

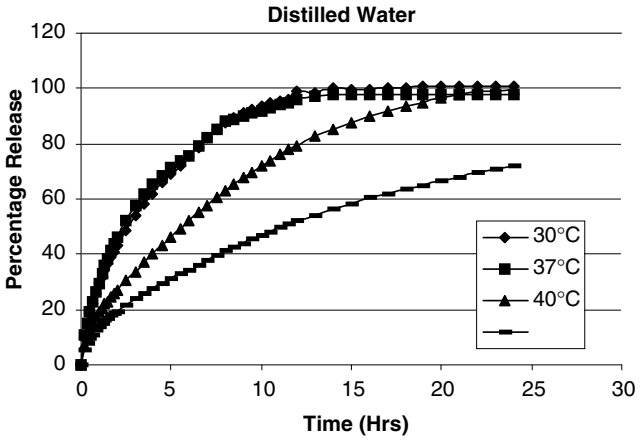

Fig. 10. Effect of temperature on the release rate from physically crosslinked A1(L1) diclofenac sodium drug carriers in distilled water.

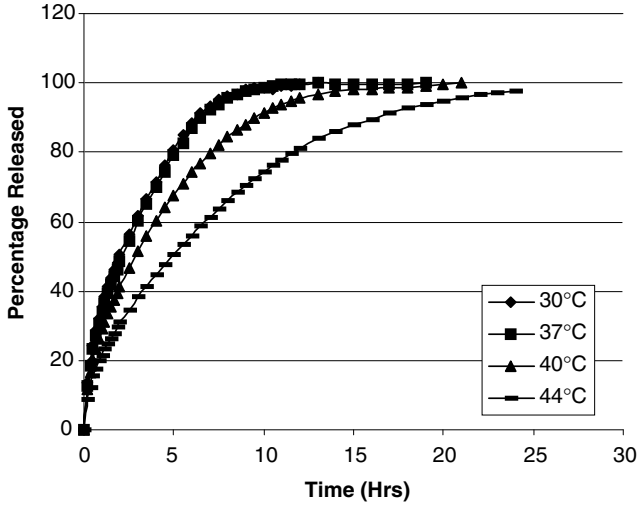

Fig. 11. Effect of temperature on the release rate from physically crosslinked A1(L1) procaine HCl drug carriers in distilled water.

accounting for its slower diffusion through the polymer chains. This theory is further strengthened, as at the highest test temperature, the trend is most obvious. As the samples swell to a much lesser degree at this temperature, the mesh size is therefore the smallest, thus making it more difficult for the diclofenac sodium molecules to diffuse out freely. The negative temperature dependent release trends exhibited by the chemically crosslinked copolymers are again very similar using both incorporated drugs, thus this behaviour is discussed herein using only procaine HCl, for simplicity sake. The volume phase transition temperature of chemically crosslinked A1(L1) was recorded at 33.86 °C (peak onset) and 36.56 °C (peak maximum) in distilled water, which is around 2.5 °C lower than the LCST values exhibited by the physically crosslinked copolymer [21]. The two transition temperatures have been reported to vary slightly depending on the nature of the crosslinking agent [2]. In general, similar drug release behaviour was displayed by the chemically crosslinked hydrogels, when compared with the trends documented for the pseudogels tested in distilled water. The primary difference observed was a consequence of crosslinking agent incorporated shifting the temperature at which the copolymers underwent their negative temperature dependent transition. In Fig. 12, the drug release profiles for chemically crosslinked A1(L1), incorporating procaine HCl are plotted over a range of temperatures, for analyses carried out in distilled water.

As with the physically crosslinked samples there was an initial burst release of drug over the first number of hours, followed by a period of more sustained released, at each test temperature. This behaviour had been predicted, as what is believed to have been drug particles has been imaged on the surface of the xerogel samples using AFM, and this active agent would therefore be released more quickly. Release profiles for both the physically and chemically crosslinked copolymers are almost identical at 30 °C. This is the case throughout this investigation, as the test temperature is below the LCST of the samples. At first glance, this may appear strange given the diverse swelling ratios typical of both these types of crosslinked copolymer, especially below their phase transition temperature. However, one should take into consideration that the drug still diffuses out through both types of crosslinked network, even at the higher test temperatures despite the copolymers not having reached their maximum swollen weights (in fact only hydrating one to two times their initial weight at temperatures sufficiently above LCST). In other words, the samples do not have to be fully hydrated in order to release the active agent. For larger drug molecules, it may be necessary for the copolymers to hydrate further/fully in order to release incorporated active agent.

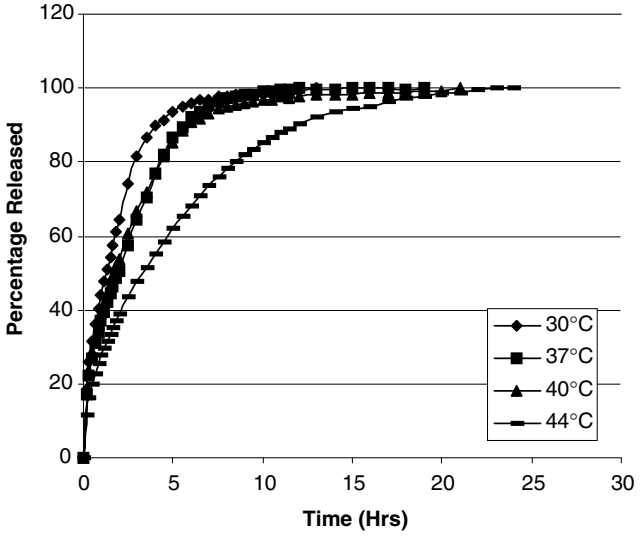

Fig. 12. Effect of temperature on the release rate from chemically crosslinked A1(L1) procaine HCl drug carriers in distilled water.

3.5. Cytotoxicity testing

HepG2 cells were chosen as the *in vitro* test system, as the gels prepared are intended for oral drug delivery application. Anything that's ingested and absorbed in the gut/duodenum finds its way to the liver via hepatic portal vein where further metabolism may take place (i.e. first pass metabolism), thus accounting for the use of said cell line in this study. Preliminary results for physically crosslinked PVP/PNIPAAm hydrogels are encouraging. Data generated for the MTT assay following short-term 3hr exposure shows no toxicity for 90–10 hydrogel media when compared to healthy untreated control cells. Cell viability remains at or above 100%, which may suggest a proliferative effect. Proliferative effects of hydrogels in direct contact with cells for short periods of time has previously been reported for crosslinked PVP by Devine et al. [29] and Smith et al. [53]. Further testing of crosslinked PVP hydrogels at various time intervals is required to confirm such proliferation. Possible tests include the Sulphorhodamine B (SRB) protein assay and the ^{3}H-thymidine incorporation assay. It is noted that a decrease in cell viability was observed for 100–0 and 80–20 hydrogel media at concentrations of 12.5–25 mg ml^{-1} after 3 h as shown in Fig. 13. Also, cell morphology appeared normal in all hydrogel media tested for 3 h.

Following 24 h of exposure a decrease in cellular viability was observed for all hydrogel media at each concentration. The drop in viability is most pronounced at concentrations > 2.5 mg ml^{-1}. It could be speculated that the observed toxicity after long periods of exposure when in direct contact with cells is the result of an interaction of the cells with unreacted monomer, unreacted photoinitiator or both. However, this would require further investigation using HPLC analysis. Cell morphology was significantly altered at 25 mg ml^{-1} for all hydrogel media. Cells took on the typical necrotic appearance indicative of large cellular damage with cellular debris

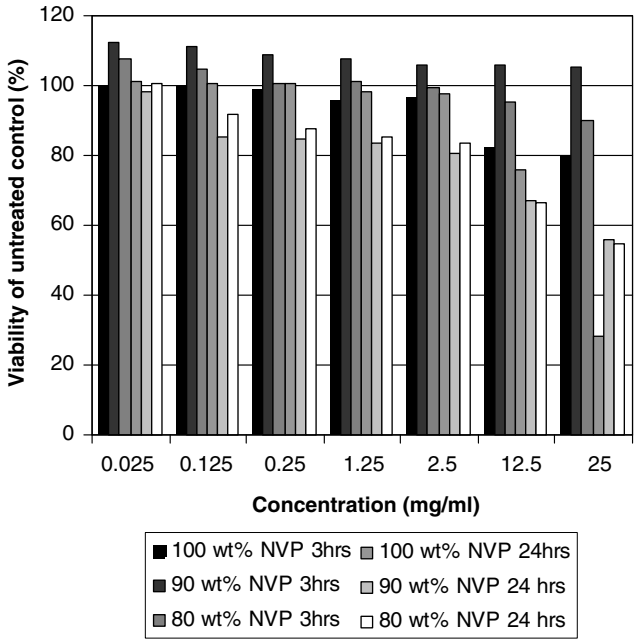

Fig. 13. Effect of PVP and PVP/PNIPAAm hydrogels in direct contact with hepatoma cells for short and long periods of time using the MTT cell viability assay as a measure of toxicity. Each column is the average of three separate experiments where $n = 6$.

distributed across test wells and a large reduction in cell number.

4. Conclusion

The potential of novel physically and chemically crosslinked PVP/PNIPAAm copolymers as controlled release oral drug delivery devices was investigated. The placebo xerogels appeared stiff, brittle and clear after the UV photopolymerisation process. It was found that the samples did not cure satisfactorily when higher percentages of active agent was incorporated. However, the drug-loaded systems attained a similar appearance as the placebo samples at lower levels of drug integration, though were a shade darker in colour as a result of the longer curing times necessary. The disappearance of the characteristic monomeric peaks in the drug-loaded and placebo copolymer spectrum suggest that the polymerisation reaction had taken place. While integration of the drug had a negligible effect on the IR spectrum, this may be a consequence of the active agents being incorporated at such low loadings. The synthesised copolymers exhibited a single distinctive endotherm representing the glass transition temperature, thus being characterised as random copolymers, and are likely amorphous in nature as semi-crystalline materials are not known to exhibit such distinctive T_gs. Incorporation of the active agents was found to have a plasticising effect, decreasing the T_g in all cases. However, the T_g of the samples remain above storage and/or release temperatures at the level of drug integrated, which is beneficial as glassy matrices offer improved stability over rubbery matrices of similar composition. A number of globular structures of relatively uniform size were observed embedded in the polymeric surface of the drug-loaded samples using AFM. These particles are believed to be surface drug and appear well dispersed, which is advantageous for controlled drug delivery applications. At the concentration incorporated, the drugs were found to have a negligible effect on the LCST and transition enthalpy of the negative temperature sensitive copolymers. Finally, the swelling results indicate that a swollen plateau is reached at a set of temperatures above the LCST, where the hydrophilic–hydrophobic interactions are balanced, thus the gels attain near identical swelling ratios within this temperature window. It was also established that the physically and chemically crosslinked hydrogels imbibed less water with incorporation of the active agents.

In summary, the hydrogels examined were designed to have phase transition temperatures near body temperature, which is important especially for drug delivery applications. Drug dissolution analysis in distilled water showed that changes in test temperature had obvious effect on the rate of drug release from the copolymers. In all cases, the active agents were released at a slower rate at temperatures above the LCST. It is noted that transition of the copolymer from glassy to rubbery is required to attain diffusion of the drug. Therefore, drug release is delayed for the period of time required for hydration of the matrices. As the hydrophobic interactions become more dominant above the LCST, this significantly slows the rate of water sorption and thus the drug release time. Given that the molecular weight of diclofenac sodium is slightly higher than that of procaine HCl, its solute size is likely to be slightly larger, thus accounting for its marginally slower release throughout. Further assessment on the biocompatibility of these hydrogels is currently underway and includes direct and indirect contact studies and elution testing in line with ISO 10993–5 *in vitro* cytotoxicity guidelines. Furthermore, as the drug release pattern is nearly identical for both the physically and chemically crosslinked copolymers, use of the chemical crosslinking agent is not essential for these drug delivery systems. This is advantageous, as crosslinkers used in the synthesis of the hydrogels are not known to be biocompatible.

Acknowledgments

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