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Temperature-triggered gelation and controlled drug release via NIPAAm/NVP-based hydrogels

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Abstract Novel physically crosslinked hydrogels based on *N*-isopropylacrylamide and 1-vinyl-2-pyrrolidinone were synthesised using photopolymerisation. The gelation behaviour of the copolymers was investigated using modulated differential scanning calorimetry, oscillatory rheological analysis and the test tube inversion method. A number of the samples gelled spontaneously under physiological conditions and importantly did not undergo syneresis within the desired temperature window. Two active pharmaceutical ingredients (APIs), diclofenac sodium and procaine hydrochloride, were entrapped within the thermogelling materials by increasing external test temperature. The temperature-triggered gelation of the copolymer gels was used as a means of controlling the release of the APIs, and was found to retard the dissolution rate significantly.

Introduction

Aqueous polymer solutions that are transformed into gels by changes in environmental conditions, thus resulting in in situ hydrogel formation, have attracted the attention of many investigators for scientific interest and for practical biomedical or pharmaceutical applications [1]. For example, when selected aqueous thermoresponsive polymer solutions of high polymer concentration (usually about 5 wt% or over) are elevated above their lower critical solution temperature (LCST), the solutions do not only precipitate but may form a gel upon further heating. As the phenomenon is totally reversible upon cooling, it is known as thermoreversible gelation [2].

In situ forming systems have been reported in the literature for biomedical applications including drug delivery, cell encapsulation and tissue repair. These systems are injectable fluids that can be introduced into the body in a minimally invasive manner before solidifying or gelling within the desired tissue, organ or body cavity. Moreover, such injectable gel-forming matrices offer several advantages over systems shaped into their final form before implantation [3]. For example, injectable materials do not require a surgical procedure for placement (and withdrawal if biodegradable), and various therapeutic agents can be incorporated by simple mixing. When they are used to fill a cavity or a defect, their flowing nature enables a good fit. In situ implant formation can occur as a result of either a physical or chemical change of the system [4]. Indeed, numerous review articles have been dedicated to this topic [2, 4–6].

Despite polyethylene oxide (PEO)–polypropylene (PPO) block copolymers being the most extensively investigated negative thermogelling materials, controlled release of active agents via thermogelling PNIPAAm-based copolymers continues to receive attention in the literature [7–10]. Fang et al. [7] developed novel injectable, thermoreversible hydrogels by chemically grafting PNIPAAm onto chitosan and hyaluronic acid (HA) chains. In vitro drug release analysis showed that the gels significantly prolonged release of the active agents nalbuphine and indomethacin at physiological temperature [7]. Wei et al. [8] designed and synthesised new intelligent sol–gel drug delivery systems. The block copolymer gels consisting of PNIPAAm/PMMA exhibited delayed release of prednisone acetate when test temperature was raised above their LCST [8].

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The thermosensitive in situ gel-forming properties of novel PNIPAAm/chitosan copolymer gels was investigated by Cao et al. [9], who highlighted their potential for ocular drug delivery. In a related study, Hsiue et al. [10] designed thermosensitive PNIPAAm-*g*-PHEMA drug vehicles for glaucoma therapy. The thermoresponsive ophthalmic drop was prepared by mixing linear gel particles and the anti-glaucoma drug, epinephrine, which produced a clear solution at room temperature and transformed into a soft film after contacting the surface of the cornea. In vivo studies showed that the polymeric system extended the release of the eyedrop by approximately 18 h, when compared with the traditional delivery route [10].

One of the main aims of this study was to retard the release rate of two active pharmaceutical ingredients (API's), namely diclofenac sodium and procaine hydrochloride, using novel thermogelling materials. Procaine hydrochloride is a local anaesthetic, used in the treatment of arthritis and pain conditions. It has a stability problem and rapidly undergoes hydrolysis in the body and remains active only for a comparatively short time [11]. Diclofenac sodium is a non-steroid-type anti-inflammatory agent and is widely used clinically because of its strong analgesic, antipyretic and anti-inflammatory effects. It is extensively metabolised in the liver and because of its short biological half-life, the drug has to be given frequently [12]. These drawbacks are common in the parenteral administration of numerous APIs. Poly(*N*-isopropylacrylamide-co-*N*-vinylpyrrolidone) has been previously proposed as a potential injectable thermogelling implant material [13]. The novel photopolymerised NIPAAm/NVP hydrogels developed herein differ from those in the aforementioned study owing to the alternative formulation and method of fabrication. The temperature-dependent gelation behaviour was used as a means of delaying the drug release and the devices show potential for therapeutic transdermal delivery.

Experimental details

Preparation of samples

The hydrogels investigated in this study were prepared by UV-initiated free-radical polymerisation. 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 and NIPAAm/NVP monomeric mixtures containing an appropriate amount of distilled water (where necessary) and stirred continuously until completely dissolved. Sample A1(L1), having a monomeric feed ratio of 15 wt% NVP, 65 wt% NIPAAm and 20 wt% water, was specifically chosen for this investigation. As NIPAAm monomer is a solid, PNIPAAm homopolymer could not be synthesised by UV-initiated polymerisation using this procedure. Therefore, all the tests on PNIPAAm homopolymer were carried out on poly (*N*-isopropylacrylamide) purchased from Polysciences Inc.

Homogeneous solutions of the physically crosslinked gels were prepared, by weighing suitable amounts of xerogel (dehydrated form of gel) and an appropriate solvent (i.e., distilled water or pH 6.8 buffer solution), leaving these mixtures at room temperature for a period of hours/days, whilst applying gentle stirring with the use of magnetic stirrers where necessary. Once completely dissolved, further amounts of appropriate solvent were added, until solutions of desired concentration were achieved.

Gel permeation chromatography (GPC)

The GPC studies were performed using an integrated GPC system comprising an isocratic LC pump, LC-30 RI detector and a 900 series interface (all Perkin Elmer). A Mixed B column with a 50 Å column in series was used for all analysis using a mixture of hexafluoroisopropanol (HFIP) and chloroform as the solvent carrier. The molecular weight recorded for each sample was estimated using narrow range molecular weight standards (Polymer Laboratories).

Rheological investigation of solution viscosity

The Advanced Rheometer AR1000 (TA Instruments) parallel plate rheometer was used to investigate the solution viscosity of selected physically crosslinked samples. The geometry used was a 6 cm diameter steel parallel plate, and the tests were performed in flow mode using a sample gap of 0.8 mm. Aqueous copolymer solutions of 1.5 mL were pipetted onto the Peltier plate, and the solution viscosity was measured at 20 °C over a shear rate range of between 1 and 1000/s. Samples for this analysis were prepared as described in 'Preparation of samples'.

Thermoreversible gelation analysis

Modulated differential scanning calorimetry (MDSC)

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 calibrated 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.

Rheological analysis

The AR1000 Advanced Rheometer (TA Instruments) was also used to investigate the gelation behaviour of selected physically crosslinked samples. For this analysis, samples were prepared as previously described in 'Preparation of samples'. The geometry used was a 6 cm diameter steel parallel plate and the tests were performed using a sample gap of 0.8 mm. For determination of the gelation temperature, oscillatory measurements were performed over a temperature range of between 25 and 50 °C at a ramp rate of 1 °C/min.

Test tube inversion method

The gelation temperatures were taken in a thermostable bath by immersing the polymer solutions in sealed glass test tubes (100 mm length, 14 mm diameter). To guard against leakage, the test tubes were weighed before and after gelation measurement. These experiments were conducted at 1 °C intervals, while, the polymer solutions were allowed to equilibrate at the desired analysis temperature for 5 min before testing. The gelation temperature was determined visually when the polymer solutions did not flow for a period of 5 min following inversion of the test tubes in the water bath. All the tests were carried out in triplicate using a thermometer with an accuracy of ±0.2 °C and pictures were taken to show the appearance of the samples above and below gelation temperature.

Drug dissolution

Drug dissolution studies were conducted on samples using a Sotax® on-line dissolution system. The tests were 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, and 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 before commencement of testing to form a reference standard. For the drug release studies, appropriate amounts of aqueous copolymer drug solutions were pipetted into the dissolution vessel before testing. The active agents (diclofenac sodium and procaine hydrochloride—both generously donated by Pharmaplaz Ltd.) were incorporated individually at 1 wt% of the total aqueous copolymer solution and stirred gently for 1 h before the dissolution analysis.

Results and discussion

Preparation and initial analysis of samples

Our research group has previously synthesised and documented the properties of a number of physically crosslinked negative temperature sensitive PNIPAAm-based hydrogels. By alternating the feed ratio, using the hydrophobic NIPAAm monomer and hydrophilic NVP monomer, copolymers were designed to have appropriate phase transition temperatures [14]. Analysis has as yet to be conducted to establish whether the aforementioned copolymers undergo thermoreversible gelation. Accordingly, the gelation phenomenon was studied herein using A1(L1) copolymers in distilled water and pH 6.8 buffer environments. For polymers capable of undergoing thermoreversible gelation, at the appropriate temperature and above the critical gel concentration, the gel phase appears. The critical gel concentration is most often inversely related to the molecular weight of the polymer employed [1]; however, this is not always the case, as detailed by Wang and Li [15]. In this study, molecular weight analysis was carried out using GPC, and the results from this examination are presented in Table 1.

Hydrogel A1(L1) was chosen for the current investigation, primarily because this copolymer has a LCST of approximately 37 °C in distilled water, as determined using cloud-point measurement and MDSC [14]. Note that PNIPAAm homopolymer was also investigated where appropriate throughout. Aqueous PNIPAAm and A1(L1) copolymer solutions were prepared using polymer/copolymer concentrations of between 1 and 6 wt%. At copolymer concentrations above 6 wt%, the xerogel was insoluble at room temperature. Active agents were added individually at 1 wt% of the total aqueous copolymer solution, and where possible the properties of the samples with and without drug incorporated are contrasted. The active agents used for the drug dissolution analysis were procaine hydrochloride and

Table 1 Molecular weight data as determined using gel permeation chromatography

Polymers	M_n	M_w	M_p	M_w/M_n
PVP	54,523	128,773	77,466	2.36
PNIPAAm	12,020	31,180	16,900	2.59
A1(L1)	15,293	79,423	39,390	5.22

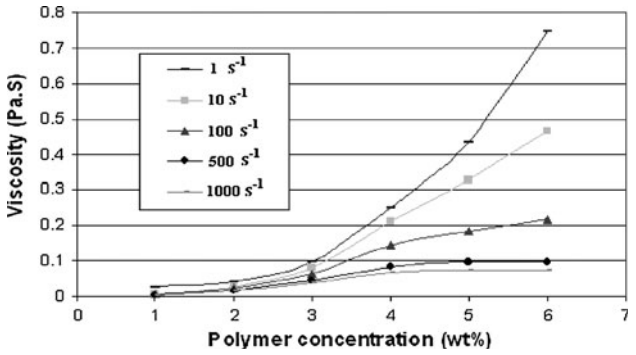

Fig. 1 Viscosity as a function of concentration and shear rate for aqueous A1(L1) samples analysed at 20 °C using parallel plate rheometry

diclofenac sodium, which are freely soluble in water and pH 6.8 buffer media [16, 17].

Solution viscosity is an important consideration when administering formulations containing thermoreversible gels (e.g., injection through a needle). The rheological analysis was undertaken using a procedure similar to that described in the literature [18, 19]. Flow experiments on the A1(L1) pseudogels were carried out in the dilute regime (1–6 wt% in distilled water) at 20 °C, which is a number of degrees Celsius below the predicted gelling temperature of the samples. As expected, the viscosity increased in a concentration-dependent manner as illustrated in Fig. 1.

In addition, the flow curves for the aqueous copolymer solutions at each concentration exhibited shear-thinning behaviour. Billmeyer [20] states that pseudoplastic or shear-thinning fluids have a lower apparent viscosity at higher shear rates. Fowers et al. [6] found that ReGel® samples with a viscosity of <1 poise (0.1 Pa s) below their gelling temperature could easily be injected through a 25-gauge needle, while Yamasaki et al. [21] used a 18-gauge needle to inject sodium carboxymethylcellulose with a viscosity of roughly 0.3 Pa s (note that the needle gauge is inversely proportional to its diameter, so the larger the gauge number, the narrower the diameter). Within the shear rate range analysed in this study, samples at concentrations of 3 wt% copolymer and below exhibited viscosities of approximately 0.1 Pa s or less, thus show potential for injection using both of the hypodermic needles referenced. Once more using the aforementioned studies as indicators, it is estimated that A1(L1) samples at the three highest copolymer concentrations would necessitate use of lower gauge needles owing to their higher viscosities.

Thermoreversible gelation

Temperature-induced sol–gel reversible hydrogels have gained the attention of many investigators for biomedical applications, as administration is convenient, and no organic solvents or toxic crosslinkers are involved during the gelation process [1, 22]. The sol phase is defined as a flowing fluid, whereas the gel phase is non-flowing on an experimental time frame, while maintaining its integrity [1]. The boundary between the sol and gel phases was determined by a number of experimental methods.

MDSC

Adjustment of the LCST to near body temperature is essential particularly for smart drug delivery applications. For aqueous A1(L1) solutions, the LCSTs at concentrations ranging between 3 and 6 wt% were found to be nearly identical. Similar behaviour has been documented for PNIPAAm homopolymer over a concentration range of 3–5 wt%, with the LCST peak maximum values particularly, exhibiting negligible variation [23]. Consequently throughout this study, the phase transition temperature recorded at 3 wt% is quoted as the LCST for each of the aqueous polymeric solutions (1–6 wt%), in an attempt to avoid needless ambiguity. Thus, physically crosslinked A1(L1) has a transition temperature onset value of 36.93 °C and peak maximum value of 38.92 °C in distilled water, while the copolymer exhibited LCST onset and peak maximum values of 33.05 and 35.24 °C, respectively, in pH 6.8 buffer media. At the concentration analysed in this study, the drugs incorporated were found to have little effect on the LCST of the negative temperature sensitive polymers, as illustrated in Fig. 2. Therefore, for the continuation of this investigation, the phase transition temperature of A1(L1) without drug incorporated is quoted throughout.

Test tube inversion method

When a test tube containing a solution is tilted, it is defined as a sol phase if the solution deforms by flow or as a gel

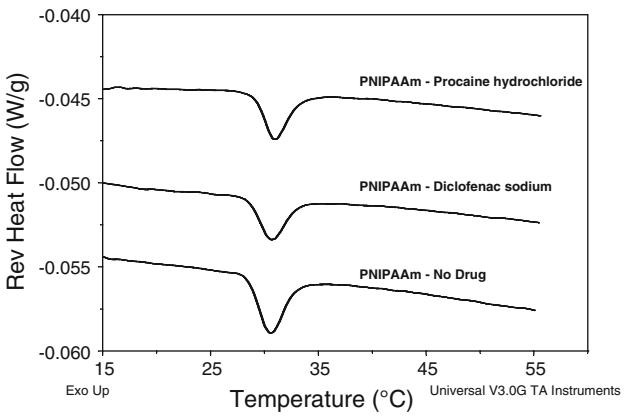

Fig. 2 LCST endotherms of 3 wt% PNIPAAm homopolymer before and after drug incorporation

phase if there is no flow. The flow is a function of time, tilting rate, amount of solution and the diameter of the test tube. Considering the time temperature superposition principle in polymer deformation, the test parameters should be fixed before determining the sol–gel boundary [1]. It was found that PNIPAAm homopolymer did not form satisfactory gels at any of the concentrations tested, irrespective of the experimentation temperature. In distilled water, the aqueous solutions turned cloudy/white at the LCST onset (recorded previously using MDSC and rheometry as between 27.5 and 29.5 °C depending on concentration) [23] but remained completely free flowing, even at temperatures up to 60 °C. In the buffer media at each concentration, the polymer solutions again clouded at a temperature corresponding to the LCST onset. However, in all cases using this media, the phase separation behaviour was more drastic and at roughly 5 °C above peak maximum transition temperature, consisted of a shrunken gel phase and a free flowing clear solution phase. Schild [24] states that co-solutes may interfere and perturb the LCST if they bind to the polymer or substantially change the water structure. This behaviour is a result of salts incorporated in preparation of the buffer media exerting a salting out effect. Liu et al. [22] carried out a similar vial inversion study on aqueous PNIPAAm solutions and stated that the samples appeared dehydrated to some extent, at temperatures above the LCST. This is also in agreement with study by Han and Bae [25], who state that the aqueous homopolymer solutions result in a shrunken mass at higher concentrations, once the temperature is elevated above phase transition temperature.

All aqueous copolymer solutions exhibited similar thermoresponsive behaviour with increasing temperature, at concentrations lower than 5 wt%. In each case, the samples clouded at a temperature roughly corresponding to the phase transition temperature onset value recorded using MDSC. At temperatures parallel to LCST peak maximum values, the aqueous copolymer solutions continued to be completely cloudy and free flowing. Thereafter, separate shrunken gel-clear solution phases emerged comparable to the trend exhibited by PNIPAAm solutions in buffer media. Once again, this trend occurred at temperatures a number of degrees Celsius above the MDSC peak maximum value. This behaviour is known as syneresis (gel shrinking by expelling water), and is an undesirable characteristic of many thermogelling materials based on PNIPAAm. It is worth noting that at the two highest copolymer concentrations, the aqueous solutions appeared white and opaque at room temperature. This clouding behaviour is not a consequence of the LCST being reached, as confirmed using MDSC analysis, but is simply due to the high copolymer concentration in the solution. At 5 wt%, encouraging results were obtained as some of the samples

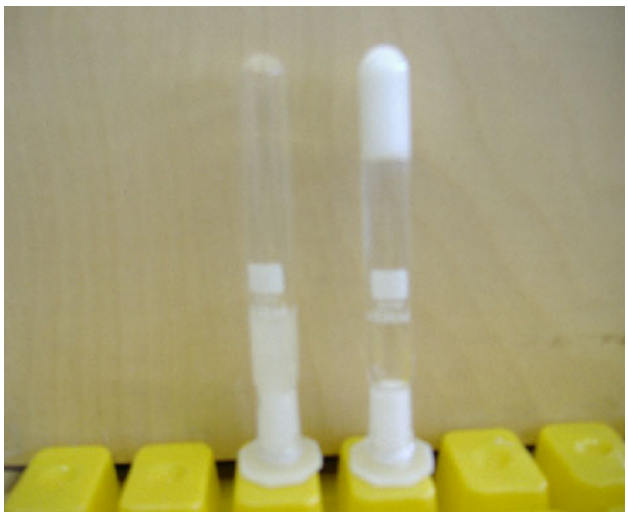

Fig. 3 Visual illustration of thermoreversible A1(L1) copolymer at 6 wt% in pH 6.8 buffer media: sol at room temperature (*left*) and gel having been submerged in a water bath at 44 °C (*right*)

prepared were found to form adequate gels. For the copolymers at concentrations of 6 wt%, improved quality gels were attained using both distilled water and the buffer media. These gels formed at temperatures roughly 3 °C above their LCST peak maximum values. In all cases, the temperature at which the gelation occurred was within the area covered by the endothermic transition peak detected using MDSC. Little or no syneresis was a distinguishing feature of these gels at least up to 6 °C above their gelling temperature. Typical gelling behaviour displayed using the test tube inversion method is illustrated in Fig. 3. Thereafter, a shrunken gel phase followed at higher test temperatures. It is characteristic of negative temperature sensitive polymers to undergo this development following the gel phase. Han and Bae [25] recorded similar gelling trends for PNIPAAm/PAA, which consisted of good quality gels over a 6 °C range but underwent a shrunken gel phase upon further increases in temperature. Diclofenac sodium and procaine hydrochloride incorporated at 1 wt% were found to have little impact on the gelation behaviour of the copolymers. It would be important to investigate this effect further if higher drug loadings were incorporated or other active agents were used.

Rheological analysis

Using this technique, gelation temperature is usually defined as the sol/gel transition temperature, at which the storage modulus G' is equal to the loss modulus G'' [15, 26]. A1(L1) copolymers were again studied at concentrations ranging from 1 to 6 wt%, but on this occasion using distilled water only, as some buffer media are known to be corrosive to the Peltier plate surface, especially at

elevated test temperatures. All compositions were investigated at a scan rate of 1 °C/min over a temperature range of 25–50 °C and exhibited crossover points averaging 38.3 °C, which appeared to have no obvious leaning on concentration within the limits analysed. These findings correspond reasonably well with peak maximum LCST results recorded using MDSC. Figure 4 illustrates the changes in storage modulus and loss modulus as a function of temperature, using 6 wt% aqueous solution. For each of the aqueous copolymer solutions, G′ was found to be lower than G″ before the gelling point (data not shown). This is characteristic behaviour indicating the common viscoelastic behaviour of a liquid. At temperatures above the crossover point, there was an abrupt increase in G′, thus the elastic response dominates and this is typical for gels and solid like materials, while G″ also increases but not to the same magnitude. Wang and Li [15] and Nisbet et al. [27] reported similar developments for aqueous methylcellulose and xyloglucan polymeric solutions, respectively. In this study, it was also noted that the maximum G′ values attained using 3 and 4 wt% aqueous solutions were much higher than the maximum figure recorded for 6 wt% copolymer solution, within the temperature range studied. Initially, this finding may appear surprising. However, when one considers the syneresis effect characteristic at lower polymeric concentrations above LCST, this coagulation cannot be directly contrasted with the fully formed gel. This may also explain the undulation in Fig. 4 at approximately 48.5 °C, a temperature in the region where syneresis first became apparent for 6 wt% aqueous solutions using the vial inversion method.

Using the standard rheological means of characterising gel formation (i.e., when G′ is equal G″), each of the aqueous solutions exhibited gel like traits on increasing temperature. Despite this, only 6 wt% aqueous A1(L1) solution was deemed appropriate for drug release experiments, as it proved the most promising gel when scrutinised using rheological and vial inversion analysis. It alone was chosen considering that it is desirable that the gel maintains its set structure in the dissolution apparatus whilst undergoing constant agitation.

Drug release from sol–gel copolymers

Ruel-Gariépy and Leroux [4] suggest that an ideal system for drug delivery would be a free flowing, injectable liquid at ambient temperature. It should then gel at body temperature with minimal syneresis. Moreover, loading with drugs or cells should be easily achieved by mixing [4]. The two dissolution media used in this study included distilled water and pH 6.8 buffer solution. Throughout the drug dissolution analysis, it is noted that the sol–gel drug samples were tested in the same media as from which they were prepared. For the drug release examination, 1.5 mL aqueous A1(L1) drug loaded samples were pipetted into the dissolution vessel, which contained 900 mL of the

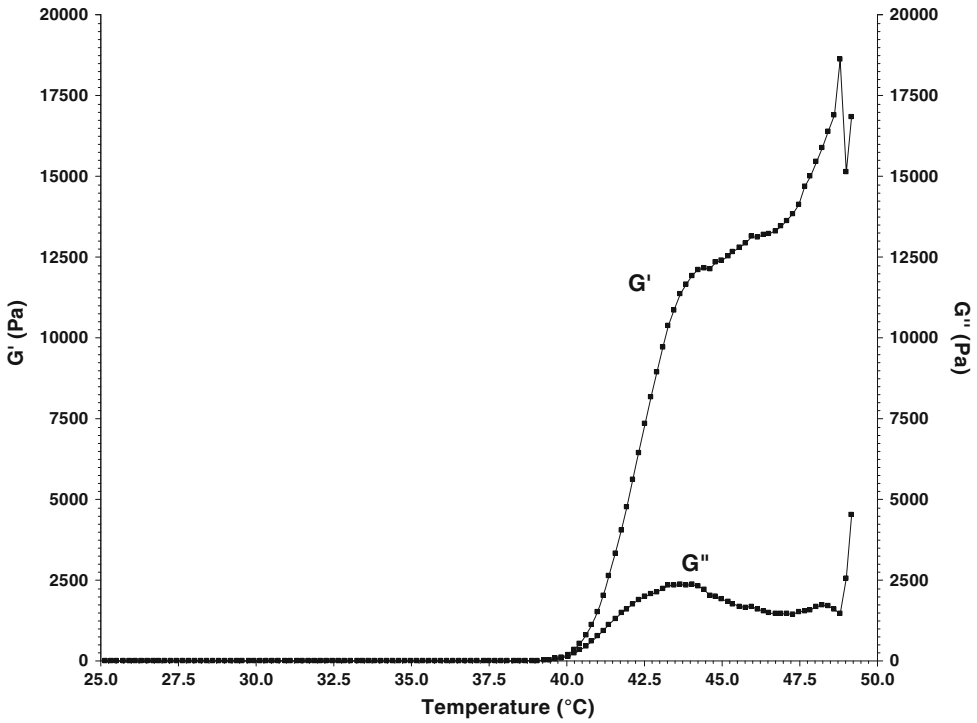

Fig. 4 Temperature dependence of storage modulus and loss modulus of 6 wt% A1(L1) aqueous solution at a heating rate of 1 °C/min

appropriate media. The ability of the aqueous solutions to first form 'rigid' gel structures, and second to retain these structures on a permanent or semi permanent basis under constant agitation, showed obvious dependence on the test temperature and dissolution media used. For example, aqueous copolymer solutions prepared using pH 6.8 buffer media and incorporating diclofenac sodium produced excellent gels, as illustrated in Fig. 5. Said gels formed almost instantly on contact with the buffer media at a test temperature of 44 °C.

The incorporated drug was fully released from these gels after approximately 15 h at this temperature as shown in Fig. 6, but the gel showed no signs of breaking down even when left in the aggressive dissolution environment for over 120 h, thus the drug was likely released through diffusion. At 37 and 40 °C, the gels formed with a slightly more rugged appearance but maintained their shape throughout the duration of the test (24 h), though releasing the integrated drug after about one and half hours. At the lowest test temperature, the samples did not form rigid structures; in line with test tube inversion analysis carried out at the corresponding temperature, and consequently released the drug almost instantaneously. Very similar thermogelling and drug release trends were recorded for the A1(L1) samples containing procaine hydrochloride in the buffer media environment at related dissolution analysis temperatures.

In distilled water, the thermogelling behaviour was not so distinguished, as also established using the test tube inversion method. The drug release behaviour was similar for both active agents in distilled water, with only tests carried out at the highest test temperature resulting in a delay in the release time as can be seen in Figs. 7 and 8. Procaine hydrochloride was released at a slightly quicker rate than diclofenac sodium in all cases, most likely due to its lower molecular weight [16, 17], a trend constant throughout this study. Overall, the results detailed herein are encouraging, as the release of both bioactive agents can be delayed for a number of hours, depending on the test temperature and dissolution media used.

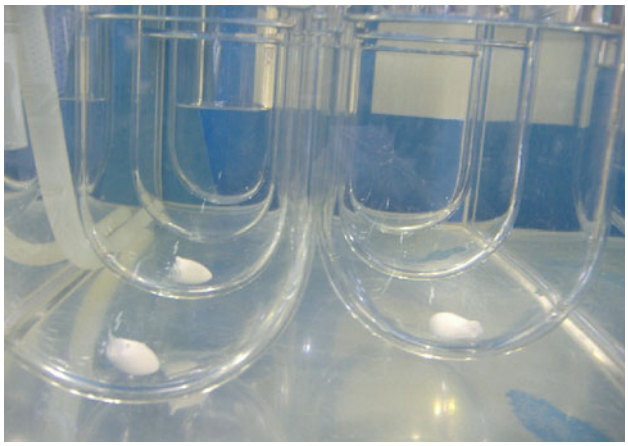

Fig. 5 Appearance of thermogelling diclofenac sodium drug carriers in pH 6.8 buffer media at a temperature of 44 °C

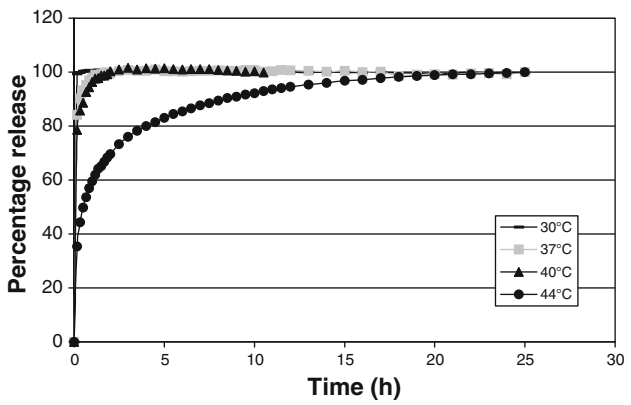

Fig. 6 Effect of temperature on the release rate from thermogelling A1(L1) diclofenac sodium drug carriers in pH 6.8 buffer media

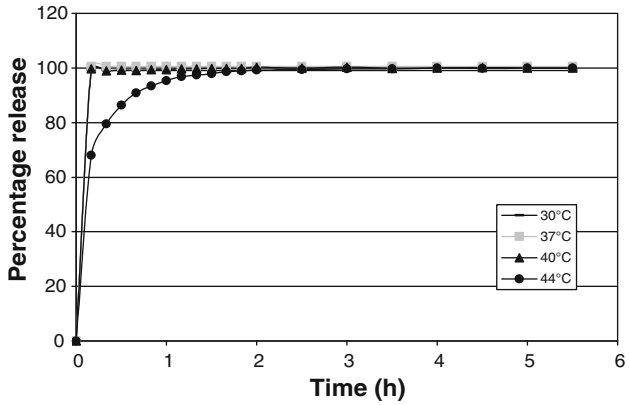

Fig. 7 Effect of temperature on the release rate from thermogelling A1(L1) procaine hydrochloride drug carriers in distilled water

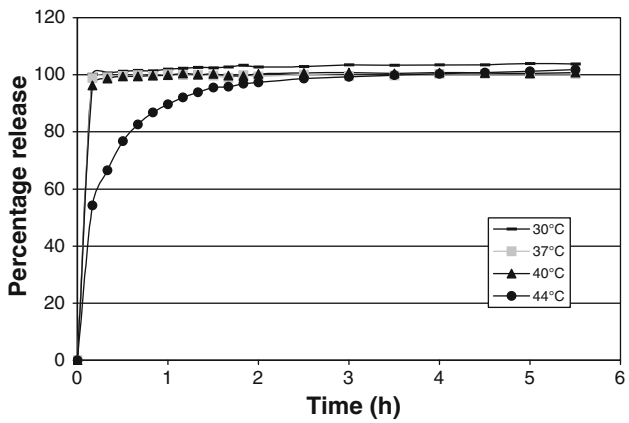

Fig. 8 Effect of temperature on the release rate from thermogelling A1(L1) diclofenac sodium drug carriers in distilled water

Conclusion

The novel copolymers prepared underwent thermoreversible gelation without syneresis at appropriate concentrations and temperatures. Two highly water-soluble drugs, diclofenac sodium and procaine hydrochloride, were uniformly integrated into the aqueous copolymer solutions at room temperature. The in situ temperature-triggered gelation was used as a means of controlling the dissolution of the drugs, and was found to retard the release rate significantly. In vitro cytotoxicity and genotoxicity tests on the copolymers are currently underway and preliminary methyl thiazoyl tetrazolium bromide (MTT) and neutral red (NR) assays indicate no cytotoxicity and very low levels of DNA damage, following a simple purification step. Subsequent study will include characterisation using analytical techniques such as GC–MS and the battery of toxicity tests will be extended to cater for additional cytotoxicity, mutagenicity and immuno-toxicity, results of which will be presented in a future study.

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