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An examination of the thermorheological and drug release properties of zinc tetraphenylporphyrin-containing thermoresponsive hydrogels, designed as light activated antimicrobial implants

David S. Jones*, Colin J. Lorimer, Gavin P. Andrews, Colin P. McCoy, Sean P. Gorman

School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

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Abstract

This study describes the thermorheological, mechanical and drug release properties of novel, light-activated antimicrobial implants. Hydrogels, based on *N*-isopropylacrylamide (NIPAA) and hydroxyethylmethacrylate (HEMA) and either devoid of or containing zinc tetraphenylporphyrin, were prepared by free radical polymerisation and characterised using oscillatory rheometry and texture profile analysis. Drug release was studied at both 20 and 37 °C. Hydrogels containing NIPAA exhibited a sol–gel temperature (*Tm*), which increased as the proportion of HEMA increased and was < 37 °C. The viscoelastic properties (storage modulus G', loss modulus G'', loss tangent and dynamic viscosity η') were affected by hydrogel composition and temperature, with the copolymers exhibiting lower values of G', G'' and η' values than either homopolymer. Similar relationships between the composition of the hydrogels and the textural/mechanical properties were observed. At 37 °C rheological structuring (increased G'', G'', η' and reduced loss tangent) occurred for all NIPAA-containing polymers and increased as the NIPAA content increased. At 20 °C drug release was diffusion controlled, the rate of which was similar for all NIPAA-containing polymers and was lower than drug release from *p*(HEMA), despite the greater elasticity of this homopolymer. At 37 °C drug release from the NIPAA-containing hydrogels was initially non-diffusion controlled, following which drug release levelled. Drug release decreased as the NIPAA content increased and correlated to hydrogel elasticity. It is suggested that the ability to engineer the release of Zn-TPP from these hydrogels, in conjunction with their acceptable mechanical properties, may be clinically advantageous for the treatment of infection.

Keywords: Thermally reponsive polymers; Viscoelastic; Rheology; Drug release

1. Introduction

Hydrogels based on poly(*N*-isopropylacrylamide, NIPAA) have attracted scientific interest in light of their known thermally induced alteration in polymer morphology (Dinarvand and D'Emanuele, 1995; Chytry et al., 1997). These systems exhibit negative temperature dependent swelling in water with a dramatic deswelling transition occurring at temperatures corresponding to the lower critical solution temperature (LCST) (Gutowska et al., 1997). This has been suggested to be a result of entropy-driven release of water molecules (Gutowska et al., 1992) and by dissociation of the hydrophobic interaction between NIPAA segments and water (Shibayama et al., 1996). The phase transition of *p*(NIPAA) based hydrogels occurs close to physiological temperatures and has enabled the application of such systems to a number of disciplines including bioengineering, pharmaceutics and biotechnology. For example, the use of copolymers of *N*,*N*-diethylacrylamide and *N*,*N*-dimethylacrylamide for the separation of DNA fragments in capillary electrophoresis has been reported (Kan et al., 2004), whereas Ohya et al. (Ohya et al., 2001, 2005) described the use of thermoresponsive hydrogel copolymers composed of *p*(NIPAA-*co*-hyaluronic acid) and *p*(NIPAA-*co*-gelatin) as non-cell adhesive structures for tissue engineering. Similarly Tsuda et al. (2004) described the attachment and growth of bovine endothelial cells on co-polymers of NIPAA and *n*-butylmethacrylate at temperatures above the LCST. The authors reported that by alteration of the ratio of the two monomers,

* Corresponding author. Tel.: +44 2890 272011; fax: +44 2890 247794.
E-mail address: d.jones@qub.ac.uk (D.S. Jones).

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cell attachment and detachment could be controlled, thereby offering opportunities for tissue engineering.

One application that has received considerable attention is the use of thermoresponsive hydrogels to enable stimulus-responsive drug delivery for pharmaceutical and medical device applications. Below the LCST, drug release from *p*(NIPAA) based hydrogels occurs by diffusion, however above this temperature, rapid drug release, due to polymer deswelling, and then controlled drug delivery through the collapsed network has been reported (Afrassiabi et al., 1987; Bae et al., 1987). Moreover, several authors have reported the formation of a dense surface layer on hydrophobic copolymers of *p*(NIPAA) following thermally induced polymer deswelling that was able to effectively block the release of drug (Mukae et al., 1990; Okano et al., 1990). More recently, the application of thermoresponsive polymers to control drug delivery from medical devices has been reported. One such application is as a polymeric matrix for the controlled delivery of therapeutic agents to prevent restenosis and to reduce vascular injury resulting from this condition (Kavanagh et al., 2004). In this regard it has been shown that copolymers based on *p*(NIPAA) can offer controlled release of colchicines for an extended period (Doorty et al., 2003; Wilson et al., 2003).

The mechanical and rheological properties of bioactive medical implants are important determinants of their clinical and non-clinical efficacy, influencing key parameters such as, drug release, resistance to deformation following exposure to applied stresses, retention at the site of application and ease of insertion (Jones et al., 2003c, 2005b). In spite of the ability of *p*(NIPAA) and copolymers of *p*(NIPAA) to undergo thermally induced changes in polymer morphology, little attention has been paid to the rheological characterisation of thermoresponsive gels based on *p*(NIPAA) and its copolymers and the relationship of such properties to subsequent drug release. The provision of this information would be advantageous to enable a relationship between the structural rheological properties of hydrogels based on *p*(NIPAA) and drug release, at defined temperatures, to be defined. Furthermore (and uniquely) this study also presents physicochemical information on a novel strategy for the treatment of infection. In this approach alteration in hydrogel chemistry and the local temperature may be employed to control the release of a model drug. Specifically, thermoresponsive polymers offer possibilities for the on–off control of drug release, which will both optimise drug usage and reduce toxicity. For this purpose zinc tetraphenylporphyrin, an agent that is employed for the treatment of topical infection and carcinomas (Dougherty, 1996), was employed as a model therapeutic agent. Following exposure to light the released porphyrin facilitates the production of singlet oxygen, a highly potent antimicrobial species.

Therefore, this study describes the thermorheological and mechanical properties of hydrogel copolymers based on *p*(NIPAA) and the release of porphyrin from these polymers following a thermally-induced alteration in polymer rheology. The use of the polymeric platforms described in this study would be particularly suited to areas in which light is readily accessible. It is therefore proposed that these formulations may have applications as light-activated, bioactive implants for the treatment of periodontal disease, an infective and inflammatory condition affecting the supportive structures of the tooth (Jones et al., 2000) or as bioactive wound dressings.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAA) 2-hydroxyethyl methacrylate (HEMA), N, N', N'', N'''-tetramethylethylenediamine (TEMED), potassium persulphate and zinc tetraphenylporphyrin were purchased from Sigma Chemical. Co. (Poole, Dorset, UK).

All other chemicals were AnalaR, or equivalent, grade and were purchased from BDH Laboratory Supplies, Poole, England.

2.2. Preparation of thermoresponsive hydrogels based on p(NIPAA)

The thermoresponsive gels were prepared by free radical polymerisation using a modification of the method described by Park and Hoffman (1992). In this, the required masses of monomer (2 g in total), namely NIPAA or mixtures of NIPAA and HEMA were dissolved in deionised water (8 g) with stirring. Following this, potassium persulfate (12.5 mg) and then TEMED (0.125 ml) were added and stirred until dissolution had occurred. Polymerisation was then allowed to occur for 16 h at 20 °C. When required, zinc tetraphenylporphyrin (Zn-TPP, 0.04% w/w) was dissolved in the monomer solution prior to polymerisation.

2.3. Characterisation of the mechanical properties of the thermoresponsive hydrogels

The mechanical properties of the hydrogels were determined using a Stable Micro Systems TA-XT2 texture analyser in texture profile analysis mode, as previously reported by the authors (Jones et al., 2002a, 2003b). In this an analytical probe was twice depressed into each sample (20 mL contained within a McCartney bottle) to a defined depth (10 mm), at a defined rate (1 mm s^{-1}), with a defined delay period (10 s), between the beginning of the second and the end of the first compression. At least five replicate analyses of each sample were performed at ambient temperature and, from the resultant force–distance plot the following parameters were obtained:

hardness	the force required to achieve maximum deformation,
compressibility	the work required to deform the product during the first compression of the probe,
cohesiveness	the ratio of the work required to deform the product during the second compression of the probe to that during the first compression of the probe.

2.4. Isothermal oscillatory rheometry of the thermoresponsive hydrogels

Oscillatory rheometry of the various gel formulations was performed at 20.0 ± 0.1 °C and 37.0 ± 0.1 °C using a AR5000 controlled stress rheometer (T.A. Instruments, Surrey, England) in conjunction with a 2 cm parallel plate geometry and a sample gap of 1 mm, as previously reported (Jones et al., 2001; Andrews et al., 2005). Samples of each formulation were applied to the lower plate of the rheometer and allowed to equilibrate for 30 min prior to analysis. Initially the linear viscoelastic region for each formulation was determined following a torque sweep from 0.1–100 Pa at frequencies of 0.01 and 1.0 Hz and was defined as the region where stress was directly proportional to strain, and, the storage modulus (G') remained constant. All frequency sweep analyses were investigated over the frequency range of 0.01–1.0 Hz following application of a constant strain with the linear viscoelastic region (1.7×10^{-3}–1.5×10^{-2}, depending on the temperature and the nature of the formulation). The storage modulus (G'), loss modulus (G''), dynamic viscosity $(\eta)'$ and the loss tangent ($\tan\delta$) were then determined using Rheology Solutions software provided by T.A. Instruments. In each case the rheological properties of at least five replicates were determined.

2.5. Thermorheological properties of the hydrogels

The thermorheological properties of the various hydrogel formulations were characterised using a AR2000 controlled stress rheometer (T.A. Instruments, Surrey, England) in conjunction with a 2 cm parallel plate geometry and a sample gap of 1 mm, as previously reported (Jones et al., 2003a). In this method samples were applied to the lower plate of the rheometer and allowed to equilibrate for 30 min as before. The samples were exposed to a defined oscillatory frequency (1 Hz) and strain (within the linear viscoelastic region) and concurrently, the temperature of the sample was incrementally ramped from 20–50 °C. As before, the viscoelastic parameters were determined at each temperature using the Rheology Solutions software. Using this technique, the lower critical solution temperature (LCST) was identified as the temperature at which a sharp increase in the modulus and dynamic viscosity was observed (Jeon et al., 2000; Jones et al., 2003a). In all cases, the thermorheological properties of five replicate samples were examined.

2.6. Drug release

The release of Zn-TPP from the various hydrogels was performed as previously described (Jones et al., 2002b, 2005a). In this, sections of the various hydrogels, contained within a circular mould (volume 10 cm^3), were immersed into and anchored to the surface of beakers containing deionised water that had been prewarmed to either 20 or 37 °C and the beakers incubated at 20 or 37 °C in a shaking water bath (100 osc min^{-1}). The volume of dissolution fluid was chosen to ensure that sink conditions were maintained throughout the period of drug release. At pre-determined intervals, samples of dissolution fluid (5 mL) were removed and replaced with an equal volume of fresh, pre-warmed dissolution fluid. The mass of Zn-TPP in the samples of dissolution fluid were analysed by visible spectroscopy (λ_{max}440 nm) with reference to a previously constructed calibration curve ($r > 0.99$).

2.7. Statistical analysis

The effect of hydrogel composition and temperature (20 and 37 °C) on the mass of drug released at defined periods and on the viscoelastic properties, namely storage modulus (G'), loss modulus (G''), loss tangent ($\tan\delta$) and dynamic viscosity $(\eta)'$ were statistically examined using a two way Analysis of Variance (ANOVA). Conversely, the effect of hydrogel composition on the lower critical solution temperature and on the mechanical properties (hardness, compressibility and cohesiveness) of the various polymers was determined using a one-way ANOVA. Post hoc analysis of the effects of the individual hydrogel compositions and temperature on the various measured parameters was performed using Tukey's HSD test. In all cases $p < 0.05$ was accepted to denote significance and therefore individual significance values are not included in the text.

3. Results

The effect of composition and oscillatory frequency on the viscoelastic properties, namely the storage modulus (G'), loss modulus (G''), loss tangent ($\tan\delta$) and dynamic viscosity $(\eta)'$ of the various hydrogels at 20 °C, are presented in Figs. 1a–d, respectively. Furthermore, the viscoelastic properties of the hydrogels at three specific frequencies (0.2, 0.5 and 0.7 Hz) are summarised in Tables 1–4. As may be observed, increasing the oscillatory frequency significantly increased G' and G'' (albeit marginally), reduced η' but had no effect on the loss tangent. Conversely, the inclusion of the porphyrin did not significantly affect the viscoelastic properties of the various hydrogels. The chemical composition of the hydrogels significantly affected polymer viscoelasticity. The greatest values of G', G'' and η' were associated with *p*(HEMA), whereas the lowest observed values were observed with *p*(HEMA-*co*-NIPPA) (30:70). For example, at 1 Hz the G', G'' and η' for *p*(HEMA) containing Zn-TPP were 41.83 ± 1.98 kPa, 10.63 ± 0.78 kPa and 2.01 ± 0.90 kPa s, respectively, whereas the comparator values for the drug-containing *p*(NIPAA-*co*-HEMA, 70:30) at 1 Hz were 0.40 ± 0.02 kPa, 0.34 ± 0.01 kPa and 0.04 ± 0.00 kPa s, respectively. Conversely, the lowest and largest values of the loss tangent were associated with *p*HEMA (0.25 ± 0.01)and *p*(NIPAA-*co*-HEMA, 70:30) (0.85±0.06), both at 1 Hz. Within the range of frequencies under investigation the loss tangent was always less than unity and therefore calculation of the relaxation time of the hydrogels was not performed. Non-linear relationships between the composition of the hydrogels and G', G'' and η' were apparent. Sequentially increasing the content of NIPAA from 0% to 30% to 50% to 70% significantly decreased the G', G'' and η' of the hydrogels in comparison

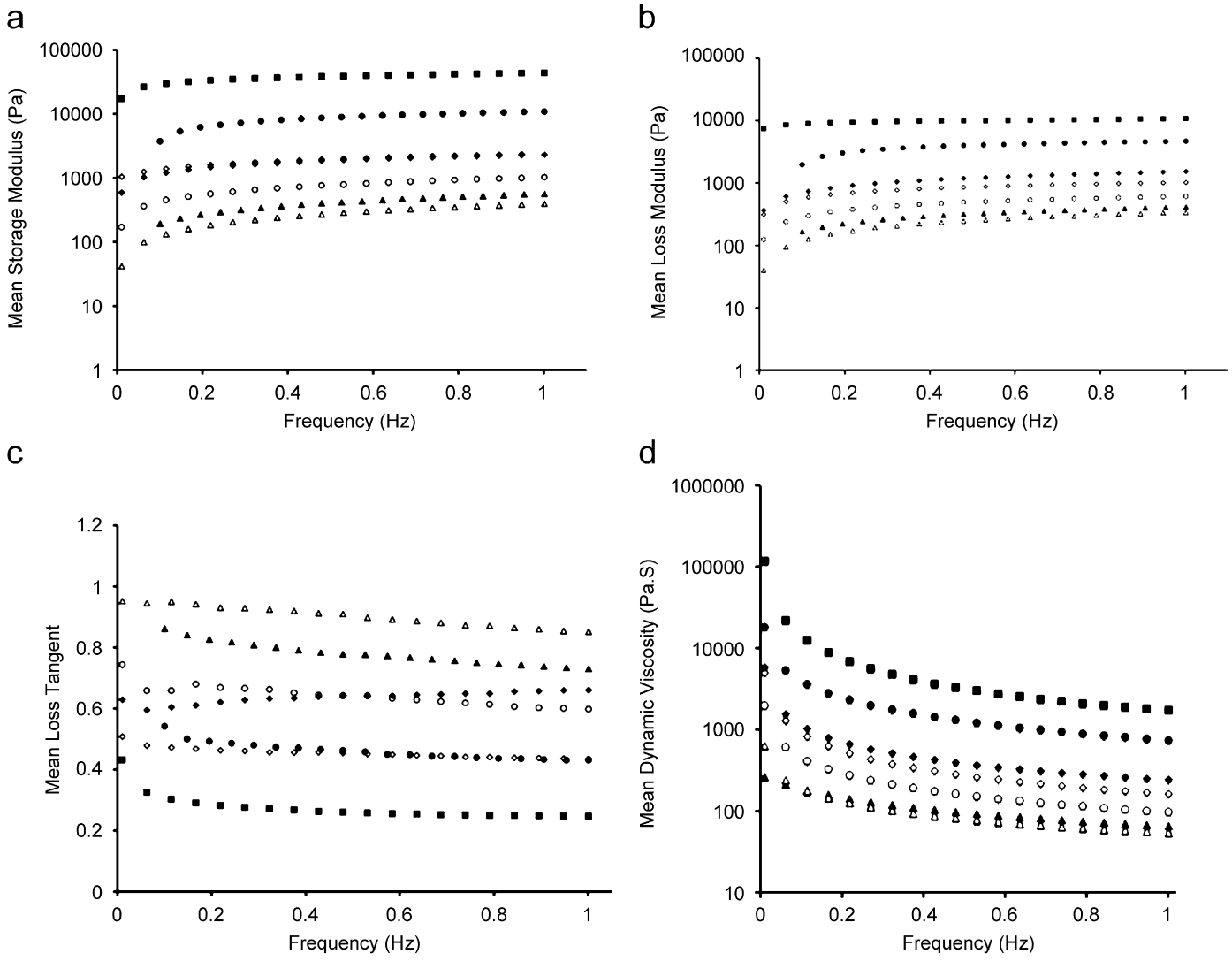

Fig. 1. The effect of hydrogel composition on the mean viscoelastic properties at 20 °C. (a–d) refer to the storage modulus, loss modulus, the loss tangent and the dynamic viscosity, respectively. Symbols: open diamonds refer to *p*(NIPAA), open circles refer to *p*(NIPAA-*co*-HEMA, 90:10), closed triangles refer to *p*(NIPAA-*co*-HEMA, 80:20), open triangles refer to *p*(NIPAA-*co*-HEMA, 70:30), closed diamonds refer to *p*(NIPAA-*co*-HEMA, 50:50), closed circles refer to *p*(NIPAA-*co*-HEMA, 30:70) and closed squares refer to *p*(HEMA). Standard deviation values have been omitted to enhance clarity; however, in all cases, the coefficient of variation was less than 8%.

to *p*(HEMA). Further increases in the NIPAA content (from 70% to 80% to 90% to 100%) resulted in increased moduli and dynamic viscosity. Conversely, increasing the concentration of NIPAA produced hydrogels with sequentially greater loss tangents, which peaked at 70% NIPAA. Further increases in the concentration of NIPAA significantly lowered the loss tangent.

The mechanical properties of the hydrogels following the exposure to compressional stresses are presented in Table 5. The hydrogels exhibited a wide range of properties in which the greatest hardness (maximum force of compression) and work required to compress the formulations (compressibility) were exhibited by *p*(HEMA) whereas the lowest observed values of these parameters were associated with the hydrogel composed of *p*(NIPAA-*co*-HEMA) (50:50). These parameters were unaffected by the incorporation of Zn-TPP. Similarly the hydrogels exhibited a wide range of cohesiveness values (0.87–0.98), which were unaffected by the presence of the antimicrobial agent.

The effects of hydrogel composition and temperature on the release of zinc TPP are presented in Figs. 2a (referring to drug release at 20 °C) and 2b (referring to drug release at 37 °C) and Table 6. Modelling of the release of drug from the hydrogels was performed using the generalised linear model (Peppas, 1985) in conjunction with logarithmic transformations and least squares linear regression analysis (Jones, 2002), as follows:

$$\log \frac{M_t}{M_\infty} = \log k + n \log t, \tag{1}$$

where, M_t is the mass of drug released at time t, M_∞ is the total drug content, k is a constant that refers to the fraction of drug released at unit time and n is the release exponent from which the mechanism of drug release may be elucidated.

Table 1
The effects of polymer composition and drug loading on the storage modulus of candidate hydrogels

Hydrogel components (% w/w)[a]		Mean (± s.d.) storage modulus (kPa) of formulations at selected oscillatory frequencies (20 ± 0.1 °C)		
Ratio of NIPPA[b] to HEMA[c]	Drug loading (% w/w)	0.2 Hz	0.4 Hz	0.7 Hz
100:0	0	1.51 ± 0.06	1.83 ± 0.08	2.10 ± 0.12
100:0	0.04	1.42 ± 0.05	1.73 ± 0.03	2.12 ± 0.03
90:10	0	0.57 ± 0.04	0.73 ± 0.03	0.89 ± 0.04
90:10	0.04	0.69 ± 0.09	0.87 ± 0.08	1.03 ± 0.09
80:20	0	0.27 ± 0.01	0.33 ± 0.05	0.43 ± 0.04
80:20	0.04	0.32 ± 0.05	0.40 ± 0.06	0.48 ± 0.07
70:30	0	0.20 ± 0.01	0.28 ± 0.02	0.35 ± 0.05
70:30	0.04	0.19 ± 0.02	0.29 ± 0.03	0.38 ± 0.02
50:50	0	1.78 ± 0.11	1.99 ± 0.11	2.17 ± 0.18
50:50	0.04	1.81 ± 0.04	2.13 ± 0.04	2.22 ± 0.05
30:70	0	7.00 ± 0.16	8.49 ± 0.12	9.87 ± 0.24
30:70	0.04	7.23 ± 0.21	8.59 ± 0.37	9.81 ± 0.45
0:100	0	33.48 ± 1.33	37.46 ± 1.62	40.70 ± 2.25
0:100	0.04	36.45 ± 1.23	37.06 ± 1.37	37.49 ± 1.93

[a]All formulations contained 0.5% w/w EGDMA.
[b]*n*-Isopropylacrylamide.
[c]Hydroxyethylmethacrylate.

Table 2
The effects of polymer composition and drug loading on the loss modulus of candidate hydrogels

Hydrogel components (% w/w)[a]		Mean (± s.d.) loss modulus (kPa) of formulations at selected oscillatory frequencies (20 ± 0.1 °C)		
Ratio of NIPPA[b] to HEMA[c]	Drug loading (% w/w)	0.2 Hz	0.4 Hz	0.7 Hz
100:0	0	0.92 ± 0.08	1.11 ± 0.08	1.30 ± 0.08
100:0	0.04	0.93 ± 0.05	1.05 ± 0.07	1.16 ± 0.07
90:10	0	0.38 ± 0.01	0.47 ± 0.01	0.55 ± 0.02
90:10	0.04	0.37 ± 0.01	0.46 ± 0.01	0.53 ± 0.02
80:20	0	0.22 ± 0.01	0.29 ± 0.01	0.35 ± 0.01
80:20	0.04	0.21 ± 0.02	0.26 ± 0.02	0.31 ± 0.02
70:30	0	0.17 ± 0.01	0.23 ± 0.01	0.29 ± 0.03
70:30	0.04	0.16 ± 0.01	0.21 ± 0.01	0.24 ± 0.03
50:50	0	0.92 ± 0.05	1.15 ± 0.07	1.30 ± 0.12
50:50	0.04	0.88 ± 0.04	1.05 ± 0.08	1.16 ± 0.13
30:70	0	3.40 ± 0.14	3.95 ± 0.16	4.38 ± 0.17
30:70	0.04	3.32 ± 0.11	8.82 ± 0.19	4.20 ± 0.16
0:100	0	9.48 ± 0.30	9.89 ± 0.25	10.30 ± 0.25
0:100	0.04	9.25 ± 0.15	9.64 ± 0.15	10.00 ± 0.34

[a]All formulations contained 0.5% w/w EGDMA.
[b]*n*-Isopropylacrylamide.
[c]Hydroxyethylmethacrylate.

No differences were observed in the release of Zn-TPP from the various NIPAA containing hydrogels at 20 °C; however, drug release from *p*(HEMA) hydrogels was greater than the NIPAA-based polymers. The time required for the release of 15% w/w of the original loading of drug from *p*(HEMA) at 20 °C was 35.75 ± 2.04 min whereas for the *p*(NIPAA-*co*-HEMA) hydrogels this value increased to circa 47 min. Increasing the temperature of the release medium to 37 °C did not affect drug release from *p*(HEMA). Conversely, the rate of release of drug from the NIPAA-based copolymers was both lower than from *p*(HEMA) and decreased as the proportion of NIPAA within the various hydrogels increased. Importantly, the *p*(NIPAA)-based polymers exhibited a lower critical solution temperature (LCST) that ranged from 31.21 ± 0.4 °C for *p*(NIPAA) to 34.79 ± 0.25 °C for the 1:1 *p*(NIPAA-*co*-HEMA) copolymer. The sequential incorporation of HEMA within the copolymer significantly increased the LCST. At a temperature that exceeded the LCST (37 °C), the viscoelastic properties of the *p*(NIPAA)-based hydrogels dramatically increased and were dependent on the composition of the hydrogels. For example, *p*(NIPAA) exhibited the greatest G'' and η' (12.99 ± 1.25 kPa and 3.54 ± 0.54 kPa s, respectively, whereas the lowest values of G'' and η' were 1.02 ± 0.21 kPa and 0.55 ± 0.02 kPa s, respectively, and were associated with the hydrogel composed of

Table 3
The effects of polymer composition and drug loading on the loss tangent of candidate hydrogels

Hydrogel components (% w/w)[a]		Mean (± s.d.) loss tangent of formulations at selected oscillatory frequencies (20 ± 0.1 °C)		
Ratio of NIPPA[b] to HEMA[c]	Drug loading (% w/w)	0.2 Hz	0.4 Hz	0.7 Hz
100:0	0	0.61 ± 0.01	0.62 ± 0.01	0.64 ± 0.01
100:0	0.04	0.52 ± 0.04	0.53 ± 0.01	0.54 ± 0.02
90:10	0	0.68 ± 0.02	0.64 ± 0.04	0.62 ± 0.04
90:10	0.04	0.66 ± 0.02	0.63 ± 0.02	0.61 ± 0.01
80:20	0	0.83 ± 0.03	0.78 ± 0.02	0.76 ± 0.02
80:20	0.04	0.64 ± 0.02	0.26 ± 0.02	0.31 ± 0.02
70:30	0	0.93 ± 0.01	0.91 ± 0.02	0.88 ± 0.01
70:30	0.04	0.64 ± 0.02	0.63 ± 0.01	0.61 ± 0.02
50:50	0	0.62 ± 0.08	0.64 ± 0.07	0.64 ± 0.12
50:50	0.04	0.49 ± 0.04	0.50 ± 0.02	0.51 ± 0.02
30:70	0	0.49 ± 0.02	0.47 ± 0.02	0.44 ± 0.02
30:70	0.04	0.46 ± 0.02	0.45 ± 0.02	0.43 ± 0.02
0:100	0	0.28 ± 0.01	0.26 ± 0.01	0.25 ± 0.21
0:100	0.04	0.30 ± 0.02	0.28 ± 0.02	0.27 ± 0.02

[a]All formulations contained 0.5% w/w EGDMA.
[b]*n*-Isopropylacrylamide.
[c]Hydroxyethylmethacrylate.

Table 4
The effects of polymer composition and drug loading on the dynamic viscosity of candidate hydrogels

Ratio of NIPPA[a] to HEMA[b]	Drug loading (% w/w)	Mean (± s.d.) dynamic viscosity (kPa s) of formulations at selected oscillatory frequencies (20 ± 0.1 °C)			Mean (± s.d.) zero shear viscosity (Pa s)
		0.2 Hz	0.4 Hz	0.7 Hz	
100:0	0	0.51 ± 0.02	0.31 ± 0.01	0.22 ± 0.01	8.39 ± 0.64
100:0	0.04	0.53 ± 0.04	0.33 ± 0.01	0.25 ± 0.02	7.93 ± 0.76
90:10	0	0.28 ± 0.02	0.18 ± 0.01	0.13 ± 0.01	4.34 ± 0.32
90:10	0.04	0.26 ± 0.01	0.16 ± 0.02	0.12 ± 0.01	4.56 ± 0.40
80:20	0	0.18 ± 0.01	0.11 ± 0.00	0.08 ± 0.00	1.60 ± 0.14
80:20	0.04	0.15 ± 0.02	0.10 ± 0.02	0.07 ± 0.01	1.41 ± 0.08
70:30	0	0.13 ± 0.01	0.09 ± 0.01	0.05 ± 0.00	1.32 ± 0.11
70:30	0.04	0.11 ± 0.02	0.08 ± 0.01	0.04 ± 0.00	1.46 ± 0.14
50:50	0	0.67 ± 0.04	0.43 ± 0.03	0.31 ± 0.02	10.08 ± 0.76
50:50	0.04	0.62 ± 0.02	0.42 ± 0.02	0.33 ± 0.01	9.98 ± 0.53
30:70	0	3.62 ± 0.21	1.77 ± 0.15	1.20 ± 0.05	30.90 ± 2.15
30:70	0.04	3.56 ± 0.14	1.89 ± 0.08	1.21 ± 0.05	28.89 ± 1.95
0:100	0	6.91 ± 0.22	3.69 ± 0.10	2.34 ± 0.13	210.27 ± 16.52
0:100	0.04	6.79 ± 0.20	3.63 ± 0.20	2.20 ± 0.17	199.33 ± 16.58

[+]All formulations contained 0.5% w/w EGDMA.
[a]*n*-Isopropylacrylamide.
[b]Hydroxyethylmethacrylate.

p(NIPAA-*co*-HEMA, 1:1 ratio) (all values at 1 Hz). Sequentially increasing the HEMA content of the hydrogels significantly decreased G'' and η'.

4. Discussion

In this study, the drug release and rheological properties of poly(*N*-isopropylacrylamide) and copolymers with hydroxyethylmethacrylate were examined. The drug selected for inclusion within the polymer matrix was zinc tetraphenylporphyrin, a photosensitiser which upon exposure to visible light releases singlet oxygen thereby eliciting an antimicrobial effect (Maisch et al., 2005). Accordingly, the implants described in this study are designed for location within a body cavity in which the temperature may be readily regulated and which is readily accessible to suitable light source (e.g. a fibre-optic). Alteration of the local temperature of the implanted bioactive system, e.g. due to the application of warm/cold air or liquid, may be employed to regulate drug release within the local environment. Furthermore, replenishment of the bioactive component may be also performed by immersion of the hydrogel in a solution of bioactive agent below the LCST (under which conditions swelling is greater). Two suggested areas in which these hydrogels may find clinical usefulness due to the ability to externally

Table 5
The effects of polymer composition and drug loading on the mechanical (textural) properties of candidate hydrogels

Ratio of NIPPA[a] to HEMA[b]	Drug loading (% w/w)	Hardness (N)	Compressibility (N mm)	Cohesiveness
100:0	0	0.15 ± 0.02	0.60 ± 0.06	0.97 ± 0.03
100:0	0.04	0.15 ± 0.01	0.62 ± 0.09	0.98 ± 0.01
90:10	0	0.16 ± 0.01	0.66 ± 0.05	0.96 ± 0.06
90:10	0.04	0.17 ± 0.01	0.66 ± 0.02	0.94 ± 0.04
50:50	0	0.04 ± 0.01	0.18 ± 0.01	0.87 ± 0.02
50:50	0.04	0.05 ± 0.01	0.20 ± 0.03	0.85 ± 0.03
30:70	0	0.15 ± 0.01	0.63 ± 0.01	0.91 ± 0.04
30:70	0.04	0.16 ± 0.01	0.64 ± 0.04	0.93 ± 0.04
0:100	0	0.24 ± 0.01	0.85 ± 0.04	0.85 ± 0.01
0:100	0.04	0.23 ± 0.04	0.88 ± 0.07	0.86 ± 0.03

[a]*n*-Isopropylacrylamide.
[b]Hydroxyethylmethacrylate.

control the temperature of the implant are the treatment of infection within the oral cavity, e.g. periodontal disease in which the delivery system is implanted into and is retained within the periodontal pocket, and/or for the treatment of skin and wound infections (e.g. diabetic leg ulcers). It is important to note that this is the first such report of the concurrent use of thermally responsive hydrogels and photosensitisers as bioactive implants for the treatment of infection and represents a novel strategy for the prevention/treatment of infection.

Specifically, the rheological/mechanical and drug release properties of the various candidate thermoresponsive gel systems were examined due to the importance of these properties to their clinical efficacy and to gain information regarding the relationship between polymer rheology and drug release. The rheological/mechanical properties were determined using two methods, namely texture profile analysis and oscillatory rheometry. The former provides information regarding sample deformation and recovery following exposure to compressional stresses, whereas oscillatory rheometry, a non-destructive technique, provides information concerning the viscoelastic properties of the sample as a function of temperature (Jones et al., 1997a, 1998). The LCST of gels composed of *p*(NIPAA) was 31.2 ± 0.6 °C, which is in good agreement with literature values of circa 32 °C (Vernon et al., 1996). Copolymerisation of NIPAA with HEMA, a hydrophilic monomer, significantly raised the LCST. This phenomenon has been previously observed by Yoshida et al. (1994) for copolymers of NIPAA and acrylamide, a more hydrophilic monomer. With respect to the potential biomedical applications of these systems, the LCST occurred at temperatures lower than physiological temperature (37 °C), thereby enabling thermal switching of the rheological state of the polymers under clinical conditions.

Whilst there have been studies that have described the physical changes of thermoresponsive gels, both below and above the LCST (Durand and Hourdet, 2000; Koh et al., 2002), few studies have characterised the viscoelastic properties of both physical states, especially within the context of drug diffusion, and the possible clinical importance of this property. Below the LCST (20 °C) all formulations exhibited predominantly elastic properties consistent with those of chemically cross-linked polymers ($G' > G''$ at all frequencies) (Barnes et al., 1996). Differences in the viscoelastic properties of the hydrogels (below the LCST) were observed. In particular, the storage and loss moduli and the dynamic viscosity of hydrogels composed of *p*(HEMA) were greater than those of *p*(NIPAA) and the various copolymers, which, in light of the standardisation of the concentration of cross-linker employed, reflect material differences and, in particular, differences in (adjacent) polymer chain interactions. In particular, it may be suggested that there was more limited interaction between HEMA and NIPAA than between adjacent groupings on the homopolymers. Consequently, substitution of HEMA–HEMA or NIPAA–NIPAA interactions with HEMA–NIPAA interactions affected the elastic nature of the hydrogels. Despite the observed differences in the viscoelastic properties of the hydrogels at 20 °C, a correlation between these properties and drug release (at 20 °C) was not observed. The release of Zn-TPP from the various hydrogels was controlled by diffusion through the swollen polymer matrix (in which the cumulative mass of drug release was proportional to $t^{0.5}$). The rate of drug release was greatest from *p*(HEMA), despite the greater elasticity of this polymer. Therefore, it may be suggested that rheological properties of the hydrogels is not a primary determinant of drug release, due primarily to the highly swollen nature (and hence low cross-link density) of the hydrogels. This is in contrast to more heavily cross-linked hydrogels in which drug release is controlled by the network structure (Jones et al., 2005a).

At 37 °C (above the LCST) the viscoelastic properties of the *p*(NIPAA)-based hydrogels were dramatically enhanced; polymer–polymer interactions replacing polymer–water interactions expelling the solvent and enhancing rheological structure (Durand and Hourdet, 2000; Koh et al., 2002). The increases in the viscoelastic properties of the formed networks were substantial and, in some cases, were ten-fold greater than the corresponding network at 20 °C. The lower elasticity of the network of the hydrogel copolymers (due to increasing proportions of HEMA) may be suggested to be due to reduced secondary bonding between the polymer chains, the interactions between NIPAA molecules being greater than between NIPAA and HEMA. The incorporation of drug into the polymer

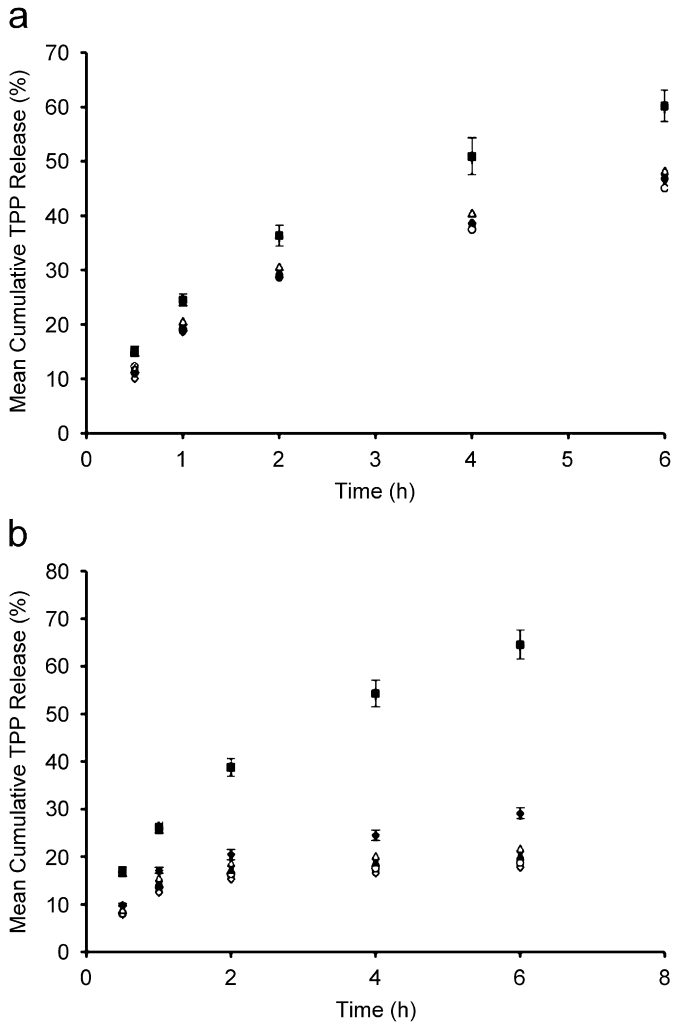

Fig. 2. The effect of hydrogel composition on the mean (± s.d.) release of Zn-TPP at 20 °C (a) and 37 °C (b). Symbols: open diamonds refer to *p*(NIPAA), open circles refer to *p*(NIPAA-*co*-HEMA, 90:10), closed triangles refer to *p*(NIPAA-*co*-HEMA, 80:20), open triangles refer to *p*(NIPAA-*co*-HEMA, 70:30), closed diamonds refer to *p*(NIPAA-*co*-HEMA, 50:50) and closed squares refer to *p*(HEMA).

systems did not affect the viscoelastic properties of the hydrogels. Therefore, the interaction between the drug and the polymers may be assumed to be negligible. Unique to this study is the observed relationship between the viscoelastic properties (above the LCST) and the subsequent release of Zn-TPP from the *p*(NIPAA)-based hydrogels. As the (visco)elastic properties of the collapsed hydrogel network increased, the initial rate of release of Zn-TPP decreased. Therefore, the increased elastic properties retarded drug diffusion due to enhanced tortuosity/diffusional path length resulting from enhanced polymer chain interactions and the expulsion of free water. In all cases, the rate of drug release at 20 °C was greater from the *p*(NIPAA)-based hydrogels than at 37 °C. The mechanism of drug release from these systems at the higher temperature was anomalous ($n < 0.5$) (Peppas, 1985). The observed drug release above the LCST may be ascribed to the concurrent thermorheological structuring of, and drug release from the hydrogels. In these systems, the rate of release was greatest at the early stages of hydrogel contact with the dissolution fluid, however, as the contact time increased, the increasing elasticity of the hydrogel network acted to reduce drug diffusion. Of particular interest is the apparent cessation of drug release from hydrogels composed of at least 70% NIPAA and the dramatic reduction in drug release from hydrogels containing 50% NIPAA. Therefore, in hydrogel systems in which the NIPAA content was 70% or greater, temperature may be employed to effectively switch drug release off (and on). In this second period (where drug release was inhibited) no correlation was observed between hydrogel viscoelasticity and drug release. The formation of a surface layer that impedes drug diffusion has been acknowledged by previous authors (Yoshida et al., 1992; Makino et al., 2000); however, this study has uniquely related this effect to the thermorheological structuring of NIPAA-based polymers. Consequently, above the LCST the rheological structuring of the hydrogel network increased as the composition of HEMA decreased, resulting in decreased initial release of Zn-TPP. However, the lack of correlation between NIPAA content (> 70%) and drug release (in the second phase of drug release) infers that a critical elasticity exists at the hydrogel–fluid interface that is responsible for the inhibition of drug release. Further increases in elastic character (associated with increased NIPAA content) may reflect bulk and not interfacial effects.

Texture profile analysis is a rheological technique in which the sample is exposed to successive compressional stresses and the resistance of the sample to deformation (and the resulting sample recovery) determined. This use of this technique to characterise physical gels has been widely reported (Ferrari et al., 1995; Jones et al., 1997b,c, 2004); however, this is one of the few studies that have employed texture profile analysis to characterise the compressive rheological properties of cross-linked hydrogels. Whilst the authors have employed texture profile analysis for the determination of the viscosity of gel systems using dimensional analysis (Jones et al., 2002a), the rheological properties of the hydrogels described in this study were unsuitable for dimensional analysis due to their cross-linked properties. In texture profile analysis, the hardness and compressibility refer specifically to the resistance of the gel to compression, the former being the maximum resistance to compression and the latter referring to the work required to compress the sample. The differences in the hardness and compressibility refer to material differences, once again reflecting differences in the physical interactions between the adjacent polymer chains. The cohesiveness describes the effect of successive compressive stresses on the compressibility of the formulations following a defined delay period. The high cohesiveness of the various NIPAA-based hydrogels (in association with the comparatively low values of the hardness and compressibility) illustrates the ability of these formulations to recover the rheological structure within a short period following deformation. As before, the incorporation of drug into the polymer systems did not affect the mechanical properties.

It is important to relate the rheological and drug release properties to the proposed use of the systems under investigation. Typically, formulations designed for implantation into

Table 6
The effect of temperature and hydrogel composition on the viscoelastic properties and the lower critical solution temperatures of poly(*n*-isopropylacrylamide)-based hydrogels

	Mean (± s.d.) viscoelastic properties at 37 °C			Mean (± s.d.) time required for the release of 15% w/w of the original drug loading ($t_{15\%}$, min) at	
Hydrogel composition (NIPAA:HEMA)	G'' (kPa)	η' (kPa s)	Mean (± s.d.) LCST (°C)	20 °C	37 °C
100:0	12.99 ± 1.25	3.54 ± 0.54	31.21 ± 0.40	47.81 ± 2.69	142.81 ± 3.45
90:10	9.95 ± 1.33	2.58 ± 0.23	32.49 ± 0.51	47.09 ± 3.19	129.81 ± 7.62
80:20	7.81 ± 1.36	1.25 ± 0.15	32.62 ± 0.35	46.62 ± 2.48	105.21 ± 7.25
70:30	4.41 ± 0.60	0.68 ± 0.08	33.88 ± 0.50	47.32 ± 2.38	87.67 ± 3.05
50:50	1.02 ± 0.21	0.55 ± 0.02	34.79 ± 0.25	48.28 ± 2.67	62.49 ± 3.04

the periodontal pocket are administered using a syringe, compressive stresses facilitating product administration (Jones et al., 2000). Increasing the hardness and compressibility would result in a reduction in the syringeability of the formulations and this may in turn limit their ability to flow into the periodontal pocket. However, the relatively low values of hardness and compressibility associated with the NIPAA-based hydrogels would be expected to facilitate flow into the periodontal pocket (Jones et al., 2000) or indeed into a wound. Furthermore, whilst thixotropy was observed (denoted by cohesiveness values less than unity), the relative recovery of the NIPAA-based hydrogels was acceptable. Therefore, it may be suggested that the flow properties of these hydrogels are clinically suitable. Two other (advantageous) features of the hydrogels described in this study are the antimicrobial activity of the chosen drug and the hydrogel and temperature responsive release of the said drug. The drug chosen in this study (Zn-TPP) is a porphyrin, whose antimicrobial properties result from the release of singlet oxygen following exposure to light (de Rosa and Bentley, 2000; Lambrechts et al., 2005), e.g. daylight. One problem associated with the use of porphyrins as an antimicrobial strategy is the possible epithelial cell cytotoxicity due to the indiscriminate release of porphyrin. Therefore, the strategy described in this study firstly ensures local release at the site of application that may be engineered by the choice of hydrogel composition and temperature at the site. Of particular interest is the ability of these hydrogels to release Zn-TPP below the LCST, which may be achieved by washing the area with cold water to achieve a reduction in the local temperature by circa 3 °C. Raising the local temperature will result in rheological structuring, which then may be employed to effectively "switch off" drug release. This "off–on" drug release ensures that optimisation of drug release occurs and that local toxicity is minimised.

5. Conclusions

This study described the synthesis and characterisation of thermally responsive hydrogels based on NIPAA containing the antimicrobial agent Zn-TPP that have been designed as thermoresponsive bioactive implants for the treatment of infective oral disorders and wounds. In combination with a suitable light source, e.g. daylight, the released porphyrin is rendered antimicrobial. Control of drug release may be obtained by manipulation of the composition of the hydrogel and by alteration of temperature of the hydrogel. Importantly, below the LCST release occurs by diffusion whereas, above this temperature, the release is biphasic and is dramatically reduced, due to temperature-induced enhancement of the viscoelastic properties of the NIPAA-based hydrogels. Interestingly, dependent on the composition of the hydrogels, drug release may be effectively switched off. It is suggested that these hydrogels may be clinical advantageous for the treatment of infection, due to the ability to engineer the release of Zn-TPP, in conjunction with their acceptable mechanical properties.

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