International Journal of Pharmaceutics 423 (2012) 326–334

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Physical characterisation and component release of poly(vinyl alcohol)–tetrahydroxyborate hydrogels and their applicability as potential topical drug delivery systems

Diarmaid J. Murphy[a], Mayur G. Sankalia[a], Ryan G. Loughlin[a], Ryan F. Donnelly[a], Mark G. Jenkins[b], Paul A. McCarron[c,*]

[a] School of Pharmacy, Queen's University Belfast, Belfast, UK
[b] Antrim Area Hospital, Antrim, UK
[c] Department of Pharmacy and Pharmaceutical Sciences, Saad Centre for Pharmacy and Diabetes, University of Ulster, Coleraine Campus, Cromore Road, Coleraine, Co. Londonderry BT52 1SA, UK

ARTICLE INFO

Article history:
Received 24 May 2011
Received in revised form 4 November 2011
Accepted 5 November 2011
Available online 15 November 2011

Keywords:
Local anaesthesia
Topical
Hydrogels
Lacerations
Wound care

ABSTRACT

Poly(vinyl alcohol)–tetrahydroxyborate (PVA–THB) hydrogels are dilatant formulations with potential for topical wound management. To support this contention, the physical properties, rheological behaviour and component release of candidate formulations were investigated. Oscillatory rheometry and texture profile analysis were used at room temperature and 37 °C. Results showed that it was possible to control the rheological and textural properties by altering component concentration and modifying the type of PVA polymer used. Hydrogels made using PVA grades with higher degrees of hydrolysis displayed favourable characteristics from a wound healing perspective. *In vitro* release of borate and PVA were assessed in order to evaluate potential clinical dosing of free species originating from the hydrogel structure. Component diffusion was influenced by both concentration and molecular weight, where relevant, with up to 5% free PVA cumulative release observed after 30 min. The results of this study demonstrated the importance of poly(vinyl alcohol) selection for ensuring appropriate gel formation in PVA–THB hydrogels. The benefits of higher degrees of hydrolysis, in particular, included lower excipient release and reduced bioadhesion. The unique physical characteristics of these hydrogels make them an appealing delivery vehicle for chronic and acute wound management purposes.

1. Introduction

Acute laceration and chronic ulceration are traumatic sites of impairment to the normal barrier function and structure of skin. Excellent accessibility means that localised drug delivery intended to alleviate pain and improve wound healing are relatively straightforward. Typical drug candidates include local anaesthetics, antibiotics and growth factors. Despite obvious advantages of the topical drug delivery approach within wound management, proprietary formulations are lacking. For example, local anaesthesia of lacerations is rarely induced topically, with infiltration being the established method (Capellan and Hollander, 2003; Singer and Dagum, 2008).

An effective topical formulation for wound management must accommodate the irregularities of the site and achieve intimate contact with exposed tissue. The ideal formulation should be sufficiently fluid in nature to fill the shape of the wound and have sufficient cohesive properties to allow it to be removed intact. In practice, few pharmaceutical materials display these attributes. Chemically cross-linked gels generally display good elasticity and sufficient cohesive integrity, but do not flow appreciably into the wound bed. In contrast, physically bonded gels, while displaying the necessary flow, have poor cohesive integrity and are difficult to remove unless washed or cleaned away.

Hydrogels are of particular interest in wound management because of their low toxicity and potential for extended drug release (Nanjawade et al., 2007; Peppas et al., 2000a,b, 2006). In addition, many hydrogels act to absorb wound exudate preventing maceration in cases where this is excessive, or can hydrate wounds that are otherwise dry, maintaining a moist wound environment. Control of wound exudate is accepted as an important aspect of overall wound management. One promising family of hydrogels are those based on poly(vinyl alcohol) (PVA), complexed with one of a range of cross-linkers, such as tetrahydroxyborate (THB) anions (Beltman and Lyklema, 1974; Eliseev et al., 2000; Roy et al., 1957; Shibayama et al., 1993). Complexation of PVA with THB anions at sufficient

* Corresponding author. Tel.: +44 28 7012 3285; fax: +44 28 7032 3509.
E-mail address: p.mccarron@ulster.ac.uk (P.A. McCarron).

0378-5173/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2011.11.018

Nomenclature

PVA	poly(vinyl alcohol)
THB	tetrahydroxyborate anion
G'	storage modulus
G''	loss modulus
13–23;	98 PVA poly(vinyl alcohol), average molecular weight 13,000–23,000; degree of hydrolysis 98%
31–50;	88 PVA poly(vinyl alcohol), average molecular weight 31,000–50,000, degree of hydrolysis 88%
31–50;	98 PVA poly(vinyl alcohol), average molecular weight 31,000–50,000, degree of hydrolysis 98%
M_W	average molecular weight

concentration leads to hydrogel formation, a process studied by a number of groups (Ide et al., 1998; Keita et al., 1995; Koike et al., 1995; Nemoto et al., 1996; Pezron et al., 1988a,b, 1989a,b; Takada et al., 1998). The mechanism of interaction has been elucidated using magnetic resonance studies (Bowcher and Dawber, 1989; Dawber and Green, 1986) and results reveal that a THB anion can interact with two distinct *cis*-diol groups on PVA. First, THB anions interact with available *cis*-diol groups, leading to mono-diol complexation and formation of a charged poly(electrolyte) structure. Intra- and inter-chain electrostatic repulsion causes an expansion in polymeric volume. This leads to a more favourable conformation and formation of the second *cis*-diol interaction, leading to a di-diol complex. It is this (*cis*-diol)–TBH–(*cis*-diol) complexation, occurring both intramolecularly and intermolecularly, that gives rise to the formation of a hydrogel system.

Results from formulation studies have shown that the concentration of THB anions has a larger effect on hydrogel formation than the PVA concentration (Pezron et al., 1988a, 1989a). Sodium ions formed from the dissociation of sodium tetraborate, which is the common source of aqueous THB, assist the second step of the complexation reaction by attenuating the overall negative charge on the polyelectrolyte chain. Cross-links form closer together and the cross-link density increases (Keita et al., 1995).

The physical properties of PVA–THB hydrogels can be attributed to the reversible nature of the TBA-mediated cross-links. Light scattering observations show that they have a finite life-time (t_{life}) and the length of observation determines the type of response (Lin et al., 2005). If observation is long (low frequency), then cross-links have sufficient time to dissociate and the system behaves like a viscous fluid ($t > t_{life}$, $G'' > G'$; where G' represents the storage modulus, or solid like response, and G'' the loss modulus, or liquid like response). In contrast, if observation is short (high frequency), then they do not have enough time to dissociate and the system behaves like an elastic solid ($t < t_{life}$, $G' > G''$). It has been suggested that the frequency over which fluid-like structure exists decreases with increasing concentration of PVA (Lin et al., 2005).

The rheology of PVA–THB hydrogels indicates significant potential as a drug delivery platform in topical wound care. Although there has been much basic rheological study undertaken (Koga et al., 1999; Koike et al., 1995; Lin et al., 2005; Nemoto et al., 1996; Takada et al., 1998), there is relatively little information on how these systems behave in a topical context. Our group have considered the possibility of using a PVA–THB hydrogel as a potential topical local anaesthetic hydrogel (Loughlin et al., 2008). To investigate the factors that influence the physical and rheological characteristics of PVA–THB hydrogels, texture profile analysis and oscillatory rheometry were used to characterise formulations containing various quantities of PVA and sodium tetraborate at concentrations above the gelation point. A further aim of this study was to evaluate the effect of excipient concentration and PVA grade. Tests were conducted at room temperature and 37 °C, as the PVA–THB interaction is known to be temperature sensitive (Koga et al., 1999). The adhesiveness of candidate hydrogel formulations and commercially available alternatives were compared. A concern during the therapeutic use of any topical formulation applied to a site where the barrier function of the *stratum corneum* is comprised, is absorption of free excipients. Therefore, excipient release was evaluated as a function of polymer grade and excipient concentration.

2. Materials and methods

2.1. Materials

Three grades of poly(vinyl alcohol) (PVA) were used in this study, namely 13,000–23,000 M_W, 98% hydrolysed (13–23; 98 PVA); 31,000–50,000 M_W, 87–89% hydrolysed (31–50; 88 PVA) and 31,000–50,000 M_W, 98% hydrolysed (31–50; 98 PVA). All were obtained from Sigma–Aldrich, Dorset, UK. Sodium tetrahydroxyborate decahydrate (borax), sodium chloride and newborn calf serum (USA origin, sterile filtered, cell culture grade) were also obtained from Sigma–Aldrich, UK. Pharmaceutical grade PVA was obtained from Merck KGaA, Darmstadt, Germany and comprised 4–88 (31,000), 5–88 (37,000), 8–88 (67,000), 26–88 (160,000), 40–88 (205,000) and 28–99 (145,000). The first identifier in the notation refers to the viscosity of a 4% solution, the second refers to the degree of hydrolysis, and approximate molecular weight is shown in parenthesis. Hydrosorb®, Aquaflo®, Intrasite®, Intrasite-C® and Aquaform® were purchased from AAH Pharmaceuticals, Belfast, UK. Porcine skin was obtained from a local abattoir and either used immediately or stored frozen at −20 °C until use. All other reagents and solvents were of appropriate laboratory standard, obtained from commercial sources and used without further purification.

2.2. Preparation and texture analysis

PVA (20%, w/w) and sodium tetrahydroxyborate (5%, w/v) stock solutions were prepared in deionised water. Hydrogels were formed by mixing appropriate proportions of both solutions for approximately 30 min, with periodic stirring. Corrections for mass loss due to evaporation were made using deionised water to bring the formulation back to its original weight. Hydrogels were stored in sealed poly(propylene) containers (44 mm diameter, 55 mm depth; Sarstedt, Wexford, Ireland) at room temperature for 48 h. This permitted thermal equilibrium throughout the hydrogel and elimination of air bubbles prior to testing.

Textural properties of PVA–THB hydrogels were evaluated using a TA-XT2 Texture Analyser (Stable Micro Systems, Halsmere, UK) in texture profile analysis (TPA) mode. A tubular probe (10 mm diameter, 40 mm in length) was compressed twice into each sample to a depth of 15 mm at a rate of 10 mm s^{-1} with a 15 s delay between compressions. Hardness and compressibility were derived from the force–time plots produced using texture profile analysis. Hardness was defined as the force necessary to produce a given deformation and determined by the force maximum of the first positive curve of the force–time plot. Compressibility was defined as the work required to deform the product during the first compression of the probe and determined by the area under the first positive curve of the force–time plot.

2.3. Adhesiveness testing

Dermal adhesive properties of hydrogels were evaluated at room temperature (25 °C) using the Texture Analyser in adhesive mode. Excised porcine skin was cut along the subcutaneous–dermal interface to separate the subcutaneous fat. The epidermal side of the skin was then attached to a 1.0 cm × 1.0 cm Perspex®

face with cyanoacrylate adhesive, dermal side uppermost, and the assembly secured to the upper part of the instrument. Approximately 30 g of hydrogel was loaded into a lower Perspex® well (diameter 60 mm, depth 15 mm). The tissue was lowered to the hydrogel surface and the interface maintained for 30 s under 0.1 N. After 30 s, the tissue was moved upwards at 10 mm s^{-1}. Adhesion was defined as the force maxima of the force–time plots produced via detachment of the skin from the hydrogel surface. Commercially available sheet and amorphous hydrogels were analysed using the same procedure by way of comparison.

2.4. Rheological analysis

Oscillatory rheometry was performed on an AR1500 Rheometer (TA Instruments, Crawley, West Sussex, UK) using parallel plate geometry. All measurements were performed at 25 °C or 37 °C using a 60 mm geometry, unless otherwise stated. A constant gap of 1000 μm was maintained throughout testing without the requirement for a normal force. At 37 °C, a solvent trap was used to reduce water loss from the formulation during testing. The linear viscoelastic region was initially determined using oscillatory stress sweeps at stresses between 0 and 100 Pa. Subsequent frequency sweeps were conducted at a stress within the linear viscoelastic region over a frequency range 0.1–100 Hz and used to determine the storage modulus, loss modulus and loss tangent.

2.5. Component release

Component release (boron species and free PVA) from hydrogels was assessed over 2 h into phosphate buffer (pH 6.8; BP 1999) at 37 °C. At defined time points (5, 10, 15, 30, 60 and 120 min) release medium (5 ml) was removed for analysis and fresh buffer added to maintain constant volume. Given the relative solubilities of the components and volumes of release media used, sink conditions were assumed for the study. The maximum amount of PVA and borax present in a 4 g sample was 0.4 g and 0.1 g, respectively. The solubility of PVA and sodium tetrahydroxyborate is at least 10 times these amounts at 37 °C. Although the solubility of both components may be reduced in phosphate buffer, neither is likely to approach 10% of their solubility limit under the conditions of the assay.

Boron species release was assessed using an enhancer cell arrangement with Cuprophan® membrane (nominal molecular weight cut-off 10,000) separating 4.0 g of hydrogel from 500 ml phosphate buffer (Caleva 7ST dissolution apparatus). The concentration of boron-containing species was quantified using a modification of the method of Lapid et al., which measures the enhancement of fluorescence seen with chromotropic acid in the presence of boric acid. The excitation wavelength was modified (330 nm) to allow the analysis to be run in a 96-well format (Lapid et al., 1976). A linear relationship between fluorescence intensity and boric acid concentration was obtained over a concentration range from 0.5 to 3.0×10^{-5} M (R^2 0.9929). Calibration curves were run with new standards on each day of analysis and the concentration of boron species was calculated using the calibration plot run on that day.

PVA release from hydrogel samples was assessed using methods described previously (Joshi et al., 1979; Loughlin et al., 2008). Briefly, 4.0 g of hydrogel was loaded into tissue culture inserts that comprised a lower membrane perforated with 8.0 μm pores. Inserts were modified to be free standing and suspended in a stirred receiver phase of 100 ml phosphate buffer at 37 °C (pH 6.8). PVA concentration in the receiver phase was determined by absorption of its complex with boric acid and iodine at 690 nm. The analytical method was calibrated against PVA standards over a concentration range of 5–50 μg ml^{-1} (R^2 0.9996). At least 5 standards were run on five separate days (limit of detection 2.2 μg ml^{-1}, limit of quantitation 6.7 μg ml^{-1}).

2.6. Statistical analysis

A three-way analysis of variance with a $3 \times 3 \times 4$ factorial design was used to determine the effects of PVA, borax concentration and PVA grade on hardness, compressibility and dermal adhesion. Hardness and compressibility data were analysed at room temperature and 37 °C, while the adhesion data was analysed at room temperature only. Post hoc analysis using Tukey's HSD test compared the means of individual groups for the textural analysis data. Commercially available hydrogels were compared to a PVA–THB hydrogel using Student's *t*-test. A three-way analysis of variance with a $3 \times 3 \times 4$ factorial design was used to evaluate the effects of PVA and THB concentrations, and PVA type on rheological parameters (storage modulus, loss modulus and loss tangent). A value of $p < 0.05$ was considered significant in all statistical comparisons.

3. Results

3.1. Texture analysis

The effects of PVA and THB concentrations on the mechanical properties of PVA–THB hydrogels, produced using three different grades of PVA are displayed in Table 1. Increasing either PVA or THB concentration resulted in an increase in compressibility. Furthermore, the range of compressibility data seen in moving from 1.0% to 2.5% THB for each PVA concentration was seen to widen when its concentration was increased from 6% to 10%. The hardest hydrogels were formed from 31 to 50; 98 PVA grades, while the opposite was true with the 31–50; 88 PVA variant. The data in Table 1 demonstrated that increasing PVA molecular weight increased compressibility. Comparing 31–50; 98 PVA hydrogels with 31–50; 88 PVA counterparts revealed that the degree of hydrolysis also affected compressibility. The 98% hydrolysed types gave significantly higher compressibility when compared to counterparts made using the 88% hydrolysed PVA.

A significant difference was observed between all of the groups in the factorial analysis. In addition, there was a large interaction noted between the independent variables. For the hardness and compressibility data, this level of significance was noted at both room temperature and 37 °C. There was a significant reduction in both hardness and compressibility when the analysis was repeated at 37 °C. This reduction occurred in all formulations and was independent of the type of PVA used. At 37 °C, the hydrogels retained approximately 30% of their mechanical strength compared to the analysis at room temperature.

The pH of each hydrogel formulation is shown in Table 1. There are two trends in the data. The first shows a rise in pH across each 1.0–2.5% THB range interval. The second is a superimposed reduction in pH as the PVA concentration is increased. This can be seen by comparing the pH change where the THB concentrations are held constant.

3.2. Adhesiveness testing

The relative dermal adhesiveness of different PVA–THB formulations is presented in Fig. 1. The adhesiveness was affected primarily by the THB concentration. Increasing THB concentration from 1.0% (w/w) to 2.5% (w/w) produced a significant reduction in adhesion at each concentration of PVA (Fig. 1(A)). For example, increasing the THB concentration from 1.0% to 2.5% (w/w) in a 10% (w/w) 31–50; 98 PVA hydrogel (Fig. 1(B)) reduced adhesiveness by approximately 60%. Although some differences were observed in certain cases, the

Table 1
Composition, pH and mechanical properties of PVA–THB hydrogels at room temperature (RT) and 37 °C.

PVA grade	PVA (%, w/w)	Borax (%, w/w)	pH	Hardness (N)		Compressibility (N s)	
				RT	37 °C	RT	37 °C
13–23; 98	6	1.0	8.11 ± 0.02	0.69 ± 0.11	0.18 ± 0.02	0.64 ± 0.11	0.17 ± 0.01
		1.5	8.32 ± 0.02	1.56 ± 0.15	0.29 ± 0.03	1.48 ± 0.16	0.27 ± 0.03
		2.0	8.48 ± 0.04	2.07 ± 0.24	0.36 ± 0.06	1.95 ± 0.26	0.34 ± 0.06
		2.5	8.58 ± 0.06	2.28 ± 0.28	0.39 ± 0.06	2.14 ± 0.28	0.37 ± 0.06
	8	1.0	7.94 ± 0.14	1.65 ± 0.13	0.33 ± 0.02	1.54 ± 0.12	0.3 ± 0.02
		1.5	8.22 ± 0.19	3.71 ± 0.18	0.91 ± 0.08	3.48 ± 0.17	0.86 ± 0.08
		2.0	8.37 ± 0.18	5.19 ± 0.08	1.27 ± 0.04	4.92 ± 0.04	1.2 ± 0.04
		2.5	8.47 ± 0.16	6.24 ± 0.18	1.55 ± 0.03	5.87 ± 0.23	1.45 ± 0.04
	10	1.0	7.91 ± 0.19	2.07 ± 0.38	0.52 ± 0.1	1.94 ± 0.37	0.48 ± 0.09
		1.5	8.09 ± 0.16	5.39 ± 0.58	1.6 ± 0.24	5.13 ± 0.52	1.51 ± 0.22
		2.0	8.23 ± 0.13	7.94 ± 0.61	2.59 ± 0.42	7.75 ± 0.54	2.45 ± 0.4
		2.5	8.35 ± 0.13	10.61 ± 0.26	3.39 ± 0.22	10.26 ± 0.26	3.2 ± 0.22
31–50; 88	6	1.0	7.92 ± 0.08	0.12 ± 0.01	0.14 ± 0.02	0.42 ± 0.11	0.14 ± 0.02
		1.5	8.20 ± 0.03	1.12 ± 0.3	0.26 ± 0.04	1.02 ± 0.27	0.24 ± 0.04
		2.0	8.37 ± 0.01	1.53 ± 0.4	0.33 ± 0.05	1.38 ± 0.35	0.3 ± 0.05
		2.5	8.51 ± 0.01	1.79 ± 0.56	0.37 ± 0.11	1.61 ± 0.49	0.34 ± 0.1
	8	1.0	7.70 ± 0.04	0.76 ± 0.11	0.16 ± 0.06	0.71 ± 0.1	0.23 ± 0.02
		1.5	8.00 ± 0.04	2.32 ± 0.41	0.61 ± 0.15	2.13 ± 0.4	0.57 ± 0.14
		2.0	8.22 ± 0.08	3.91 ± 0.98	1.03 ± 0.3	3.53 ± 0.91	0.95 ± 0.26
		2.5	8.31 ± 0.05	4.74 ± 1.21	1.33 ± 0.22	4.28 ± 1.12	1.23 ± 0.2
	10	1.0	7.52 ± 0.16	0.79 ± 0.11	0.21 ± 0.08	0.74 ± 0.1	0.23 ± 0.03
		1.5	7.74 ± 0.09	3.02 ± 0.09	0.95 ± 0.06	2.78 ± 0.09	0.89 ± 0.05
		2.0	7.87 ± 0.06	5.85 ± 0.57	1.9 ± 0.39	5.31 ± 0.49	1.77 ± 0.38
		2.5	7.99 ± 0.04	9.89 ± 1.92	3.59 ± 1.24	8.87 ± 1.63	3.26 ± 1.1
31–50; 98	6	1	8.07 ± 0.07	1.88 ± 0.6	0.5 ± 0.27	1.75 ± 0.55	0.46 ± 0.25
		1.5	8.35 ± 0.08	3.75 ± 0.64	0.92 ± 0.58	3.6 ± 0.5	0.75 ± 0.64
		2	8.53 ± 0.06	4.79 ± 0.45	1.48 ± 0.27	4.56 ± 0.28	1.35 ± 0.25
		2.5	8.62 ± 0.03	5.96 ± 0.41	1.52 ± 0.26	5.6 ± 0.46	1.42 ± 0.2
	8	1	7.98 ± 0.12	4.64 ± 0.79	1.34 ± 0.13	4.22 ± 0.74	1.24 ± 0.12
		1.5	8.20 ± 0.05	8.03 ± 0.53	2.82 ± 0.07	7.75 ± 0.81	2.59 ± 0.07
		2	8.36 ± 0.03	9.77 ± 1.3	3.59 ± 0.18	9.91 ± 1	3.28 ± 0.2
		2.5	8.48 ± 0.03	13.69 ± 2.33	4.26 ± 0.26	12.75 ± 1.77	3.87 ± 0.23
	10	1	7.77 ± 0.16	5.01 ± 0.91	1.67 ± 0.18	4.65 ± 0.85	1.56 ± 0.17
		1.5	7.99 ± 0.12	11.32 ± 1.83	4.07 ± 0.5	10.41 ± 1.67	3.75 ± 0.4
		2	8.16 ± 0.09	14.48 ± 3.11	5.71 ± 0.58	14.53 ± 1.93	5.34 ± 0.42
		2.5	8.30 ± 0.09	18.63 ± 1.58	7.08 ± 0.17	17.1 ± 1.78	6.45 ± 0.22

Data represent the mean ± standard deviation ($n = 4$).

concentration of PVA did not significantly affect the adhesion over the range investigated. Changing the grade of PVA used did not significantly alter the adhesiveness of the formulations. Data for 1% (w/w) THB and 31–50; 88 PVA was not recorded as these hydrogels displayed poor cohesive properties and did not detach cleanly from the tissue surface during testing (Fig. 1(C)).

The dermal adhesion of a candidate PVA–THB formulation was compared to that of several commercially available wound management formulations and the results are presented in Table 2. These formulations comprise two forms; an amorphous gel and a sheet form. The PVA–THB hydrogel displayed noticeable adhesive strength, which was significantly higher than, for example, Intrasite® ($p = 0.008$), the next most adhesive formulation. The results confirm that amorphous formulations are more adhesive to dermal structures than their sheet-like counterparts.

Table 2
Comparison of the dermal adhesiveness of a selected PVA–THB hydrogel with several commercially available hydrogel formulations.

Hydrogel	Physical form	Mean adhesiveness ($N\,cm^{-2}$)	95% confidence interval
Aquaflo®	Sheet	0.051	0.007
Hydrosorb®	Sheet	0.108	0.006
Intrasite-C®	Sheet	0.055	0.009
Intrasite®	Amorphous	0.356	0.017
Aquaform®	Amorphous	0.325	0.030
PVA–THB hydrogel[a]	Amorphous	0.595	0.099

$n = 6$.
[a] 10% PVA (31–50; 98) 2.5% borax.

3.3. Rheological testing

Representative plots from oscillatory rheometry of hydrogels at 25 °C are displayed in Fig. 2. The mean storage modulus variation increased with frequency in all hydrogels tested. Increasing the concentration of THB increased the storage modulus in all cases. However, at the higher concentration range, the change in the storage modulus became less pronounced. In every instance where the concentration of THB was held constant, increasing the PVA concentration increased the storage modulus, with a greater increase seen upon changing the concentration of PVA from 6.0 to 8.0% (w/w) than from 8.0 to 10.0% (w/w).

A representative plot of variations in the mean loss modulus with increasing concentration of THB is displayed in Fig. 2(B). Loss modulus increased with frequency until around 1–2 Hz. In every case, increasing the concentration of either PVA or THB increased the loss modulus. The increase in loss modulus was more pronounced moving from 6.0 to 8.0% (w/w) PVA compared to moving from 8.0 to 10.0% (w/w) PVA. As the THB concentration approached 2.5% (w/w), the relative increase in the loss modulus seen upon further THB addition was reduced.

A representative plot of variations in the mean loss tangent with increasing frequency is displayed in Fig. 2(C). The loss tangent was dependent upon the frequency, with values reducing as frequency increased. In every case, the loss tangent was reduced as the concentration of THB and/or PVA increased. At higher concentrations of

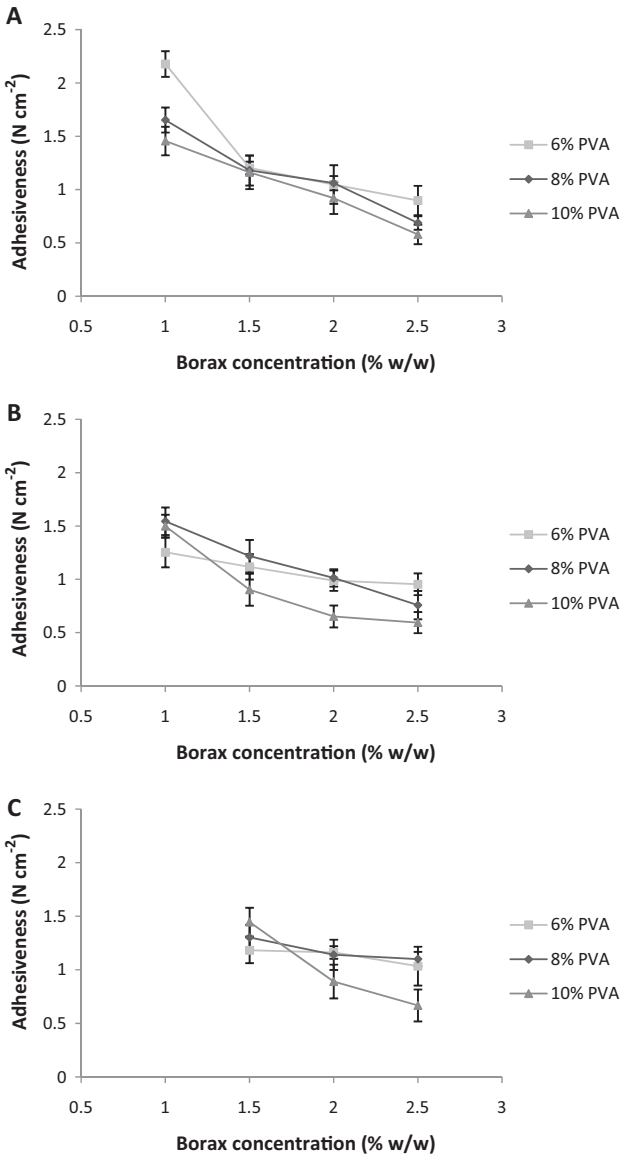

Fig. 1. Mean dermal adhesiveness with 95% confidence intervals ($n = 5$) of PVA–THB hydrogels composed of various concentrations of PVA and borax ((A) 13–23; 98 PVA, (B) 31–50; 98 PVA, (C) 31–50; 88 PVA). The legend shows the PVA concentration in each case. All experiments were conducted at room temperature (25 °C).

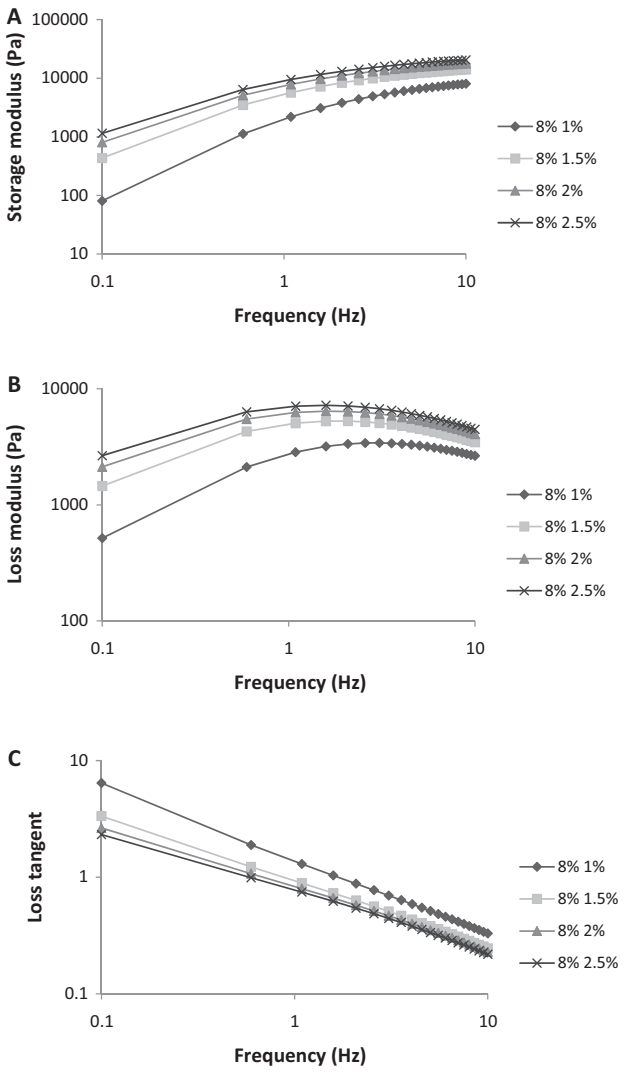

Fig. 2. Rheological data showing variation in (A) mean storage modulus, (B) mean loss modulus and (C) mean loss tangent with frequency for 8% 31–50; 88 PVA–THB hydrogels composed of varying concentrations of borax ($n = 5$). The legend shows the PVA and borax concentration used in each case. All experiments were conducted at room temperature (25 °C).

THB, values for the different concentrations of PVA started to overlap. Similarly, at higher frequencies, the loss tangent values start to merge. Both the molecular weight of the polymer and the degree of hydrolysis influenced the rheological characteristics. Increasing the molecular weight of the polymer increased the storage and loss modulus and reduced the loss tangent. It was also clear that the degree of hydrolysis also has a significant impact on the relative rheological parameters. Hydrogels composed of PVA of a higher degree of hydrolysis displayed higher values for both the storage and loss moduli and lower values for the loss tangent at higher concentrations of polymer.

The observed cross-over frequency (the frequency where the storage modulus and loss modulus are equal, representing the change from a predominantly viscous, liquid-like response to a predominantly elastic, solid-like response) for each of the concentrations of PVA and THB, and grades of PVA tested are displayed in Table 3. At constant PVA concentration, the cross-over frequency fell as the concentration of THB was increased for all concentrations of PVA. At lower concentrations of THB, increasing the PVA concentration increased the frequency of the cross-over point. At higher concentrations of THB, increasing the concentration of PVA produced more variable effects. At 37 °C, the trend of cross-over point reduction with increasing THB concentration was maintained. At constant THB concentration, increasing the concentration of PVA tended to reduce the cross-over frequency at higher concentrations of THB while having more variable effects at lower concentrations. Between the different grades of PVA, hydrogels composed of 31–50; 98 PVA generally provided the lowest frequency cross-over point at equivalent PVA–THB concentrations.

3.4. Component release

A representative plot of boron species release, quantified as boric acid, from PVA–THB hydrogels is displayed in Fig. 3(A). There is a detectable release of boric acid over 2 h from all of the formulations. In all cases, the release of boric acid rose with increasing concentration of THB within the formulation. For hydrogels composed of either of the two 98% hydrolysed PVA grades there was a general trend of a reduction in percentage borate release as PVA concentration increased from 6.0 to 10.0% (w/w).

Table 3
Cross-over frequency changes according to hydrogel composition and temperature.

PVA grade	PVA (%, w/w)	Borax (%, w/w)	Frequency (Hz)	
			25 °C cross-over point	37 °C cross-over point
13–23; 98	6	1.0	1.95	5.09
		1.5	1.32	3.50
		2.0	1.18	3.27
		2.5	1.16	3.30
	8	1.0	2.09	4.65
		1.5	1.27	3.01
		2.0	1.01	2.60
		2.5	0.93	2.44
	10	1.0	3.20	5.20
		1.5	1.64	2.98
		2.0	1.17	2.42
		2.5	0.98	2.18
31–50; 88	6	1.0	2.61	6.11
		1.5	1.39	3.57
		2.0	1.15	3.16
		2.5	1.01	3.11
	8	1.0	2.9	6.36
		1.5	1.25	3.28
		2.0	0.89	2.51
		2.5	0.83	2.32
	10	1.0	5.68	7.52
		1.5	2.12	3.65
		2.0	1.40	2.51
		2.5	0.99	2.04
31–50; 98	6	1.0	0.9	3.54
		1.5	0.6	1.83
		2.0	0.54	1.64
		2.5	0.54	1.61
	8	1.0	1.11	2.10
		1.5	0.63	1.44
		2.0	0.52	1.27
		2.5	0.46	1.20
	10	1.0	1.53	2.60
		1.5	0.79	1.53
		2.0	0.53	1.23
		2.5	0.43	1.09

Data represent mean values ($n = 5$).

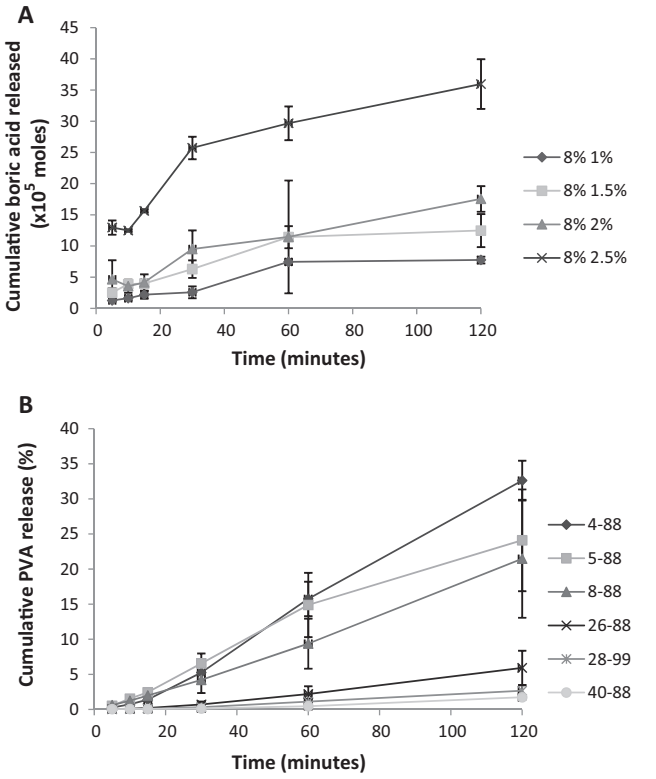

Fig. 3. (A) Boron species release, measured as mean boric acid with standard deviation ($n = 3$), from PVA–THB hydrogels composed of varying concentrations of borax at fixed PVA concentration (8% 31–50; 88 PVA). The legend shows PVA and borax concentration in each case. (B) Mean PVA release with standard deviation ($n = 3$) from 10% to 2.5% PVA–THB hydrogels composed of different pharmaceutical grades of PVA. The legend shows the grade of PVA used in each case. All experiments were conducted at 37 °C.

In order to mimic the anticipated *in vivo* release of PVA more closely, a series of hydrogels composed of 10.0% (w/w) PVA and 2.5% (w/w) THB were made with different pharmaceutical grades of PVA. The release profile for these hydrogels is displayed in Fig. 3(B). Interestingly, 5–88 PVA exhibited approximately 6% cumulative release at 30 min compared to 40–88 PVA which exhibited approximately 0.1% release at the same time point. PVA with a higher degree of hydrolysis exhibited a lower extent of release compared to a similar molecular weight grade with a lower degree of hydrolysis. Thus, 28–99 exhibited 0.3% cumulative release at 30 min, which compared to 0.7% for 26–88 PVA.

4. Discussion

The physical characteristics of PVA–THB hydrogels investigated in this study confirmed their potential for topical application to sites of traumatic wounds, and the acute laceration in particular. Success is dependent on sufficient fluidity to enable flow within the wound space and achieve intimate contact with the intended absorptive surface for drug delivery purposes. At the same time, sufficient cohesive strength is necessary for intact removal from the wound cavity. As shown in this work, PVA–THB hydrogels display these flow properties due to an equilibrium cross-linking reaction that is responsive to applied stresses depending upon its length of application. When the stress is applied over a long time (low frequency), cross-links have sufficient time to re-orientate in the direction of the stress and the material behaves like a viscous liquid. If the stress is applied over a shorter timescale than the average lifetime of the PVA–THB bond, cross-links have insufficient time to re-orientate and the material behaves like an elastic solid.

The data in Table 1 show the significant differences in hardness and compressibility as concentrations of PVA and THB changed. Increasing PVA concentration caused a significant increase in the hardness and compressibility of the hydrogel in each case. This is due to both an increased cross-link density at a given THB concentration and also an increased number of non-THB polymer–polymer contacts. Similarly, increasing THB concentration caused a significant increase in hardness and compressibility as PVA concentration was held constant. This increase was due primarily to the greater number of PVA–THB interchain links, which effectively increases the density of the three dimensional network formed. Furthermore, the grade of PVA used introduced molecular weight and degree of hydrolysis dependent effects. Increasing the former elevated polymer density and the probability of further di-diol inter-chain contacts. Increasing the degree of hydrolysis also increased the likelihood of the cross-linking interaction occurring and may increase the possibility for hydrophilic interactions between adjacent hydroxyl groups.

The reversibility of the PVA–THB interaction with temperature is also easily discerned from Table 1. This is important as wound temperature may vary considerably and affect the performance of the hydrogel. The hardness and compressibility are reduced by over 50% upon increasing the temperature from room temperature 25 °C to 37 °C. This decrease in structural integrity is mainly due to a reduction in PVA–THB cross-linking as the temperature increases (Koga et al., 1999; te Nijenhuis, 2007).

The data in Fig. 1 showed that THB concentrations, rather than PVA concentrations, were more important in influencing

adhesion. The reduction seen in Fig. 1 is explained by formation of a more structured network due to more THB. This results in fewer free hydroxyl groups to make appropriate electrostatic interactions with the tissue substrate. In addition, polymer chain flexibility is curtailed as the equilibrium reaction shifts to greater amounts of the di-diol reaction. Reduced polymer flexibility discourages interpenetration with native substrate components, such as cellular structures, which leads to a reduction in bioadhesion. This is consistent with the theory of bioadhesion of polymeric systems, which predicts that adhesion is related to polymer chain flexibility and availability of suitable functional groups to promote physical interactions with the biological surface (Huang et al., 2000; Peppas and Sahlin, 1996).

In this work, dermal adhesion was studied. This was chosen as it models the exposed surface area of the acute laceration, the majority of which will not have an intact *stratum corneum*. An ideal formulation would have limited or no adhesion, to minimise trauma to tissue upon removal. In reality, some adhesion is inevitable. This poses a dilemma for the clinical development of these systems. Reducing adhesion is achieved by increasing THB concentration and, as a consequence, the hardness and compressibility of the formulation. Therefore, optimising the adhesiveness may have deleterious effects on flow properties. Some measure of the optimum dermal adhesiveness can be seen by comparing commercially available hydrogels with a candidate PVA–THB hydrogel (Table 2). This shows that PVA–THB hydrogels display a significantly higher dermal adhesion than commercial counterparts. Although this level of adhesion exceeds that of other amorphous hydrogel counterparts, it is not considered to hinder clinical utilisation. From a wound care perspective, optimum adhesiveness will need to be accessed clinically. It has been demonstrated previously that the optimum clinical formulations of PVA–THB hydrogels strike the appropriate balance between material fluidity and adhesiveness and that the adhesiveness of these formulations is not an issue (McCarron et al., 2011). In particular, the relative cohesive to adhesive strength may prove to be more indicative as to the ease of removal of a formulation rather than simply its adhesiveness.

The importance of optimising rheological flow of PVA–THB hydrogels for topical use has been emphasised above. Consequently, oscillatory rheometry was used to observe structural effects following systematic variation in both PVA and THB concentrations. ANOVA on storage modulus, loss modulus and loss tangent revealed significant differences between all of the formulations. However, interactions between some of the variables were observed, but it was possible to define clear trends. Increasing the concentration of PVA increased the storage modulus in nearly all cases, brought about by increases in both the probability of polymer–polymer interactions and the equilibrium concentration of PVA–THB di-diol cross-links. This will increase the elastic nature of the formulation by increasing cross-link density and shifting the equilibrium away from mono-diol formation and towards di-diol cross-links. As the concentration of PVA chains increases, it is also more likely that inter-chain cross-links will be formed as opposed to intra-chain cross-links.

Increasing the concentration of THB increased the storage modulus at all concentrations of PVA (Fig. 2(A)). This is due to a resultant increase in di-diol cross-linking and inter-chain attachment. The addition of further THB may also cause an increase in cross-link density through the attenuating effect of sodium cations shielding the potential mono-diol electrostatic repulsion.

The loss modulus follows a similar trend to that observed for the storage modulus. In general, increasing the concentration of either PVA or THB led to an increase in the loss modulus (for the impact of increasing THB, see Fig. 2(B)). For both the storage and loss moduli, increasing the frequency leads to an increase in the recorded moduli as expected. This is due to the cross-links having insufficient time to reorganise at the applied frequency ($t < t_{life}$) and as a consequence, a more elastic response is seen. At lower frequencies, the cross-links have sufficient time to re-orientate and the loss modulus predominates.

A representative plot of changes in the loss tangent (the ratio of the viscous modulus to the elastic modulus) showed a reduction with increasing THB concentration (Fig. 2(C)) demonstrating an increasing dominance of the elastic like response as the concentration of THB increases. As with the storage and loss moduli, the magnitude of effect reduced as the concentration of THB was increased. In addition, there was a greater variation in values at lower frequencies than that seen at higher frequencies. Higher concentrations of PVA produced a much more detectable reduction in the loss tangent at lower frequency. These results are in line with the mechanical analysis results where increasing the concentration of either component results in a more rigid hydrogel which displays greater solid-like character and, hence, a lower loss tangent value. In addition, as the frequency of the stimulus becomes greater the relative solid-like response increases, hence the reduction in loss tangent values at high frequency. At higher frequencies, the predominantly solid-like response tends to overshadow the variability seen at lower frequencies.

The cross-over point at both 25 °C and 37 °C for each formulation is displayed in Table 3. The reduction in the cross-over frequency with increasing THB concentration is due to the increasing network formation as a result of higher THB anion concentrations. This tends to create a denser three dimensional network of bonds, which increases viscosity and produces a greater elastic response at lower frequencies. This trend is also observed at 37 °C, despite the thermo-reversible nature of the PVA–THB interaction. At a fixed THB concentration, increasing the concentration of PVA has a more variable effect on the cross-over frequency. For example, at 25 °C and 1% (w/w) THB concentration, increasing the concentration of PVA increases the cross-over frequency for each type of PVA. This would seem counterintuitive given that an increase in network structure should reduce the cross-over frequency. At low THB concentrations, increases in polymer concentration may encourage mono-diol complexation with a resultant expansion in polymer chain volume and alignment due to electrostatic effects. This may reduce the storage modulus through a decrease in the polymer–polymer interactions which is not sufficiently offset through the PVA–THB interaction due to the low concentration of the THB anion. Above 2% (w/w) THB, increasing the PVA concentration reduces the cross-over frequency for each PVA type as expected. At 37 °C, the trends observed are similar although the individual cross-over frequencies are all higher. The increase in the cross-over frequency is due to the reduction in the equilibrium concentration of PVA–THB di-diol interactions due to the raised temperature. This reduces the cross-link density and allows the hydrogel to display an increased viscous-to-elastic response at a given frequency of observation.

The use of a PVA–THB hydrogel on broken skin may lead to improved drug delivery. When a combination of ineffective cutaneous barrier function, an aqueous hydrogel structure and reversible cross-linking are considered together, then the release and potential absorption of formulation excipients are a concern. The extent of PVA and boron species release was assessed over 2 h to establish potential clinical exposure. Formulation development for topical anaesthesia in particular requires a relatively fast onset of action. Preliminary data from our group suggest that an application time of approximately 30 min should be sufficient to provide anaesthesia of topical lacerations. Therefore, it was not considered necessary to examine component release much beyond the likely application time of the formulation. Release of boron containing species, measured as boric acid, increased as the concentration of THB incorporated was increased. However,

increasing the concentration of THB in the system will increase the number of cross-links and this should act to constrict the network and reduce the ability of free species to diffuse away. This may be more important in reducing the likelihood of free PVA chains diffusing away from the surface of the hydrogel if anchoring cross-links are severed. Finally, poly(borate) species may start to form above borate concentrations of 0.025 M (~1%) (Salentine, 1983; Smith and Wiersema, 1972). This may serve to reduce the rate of boric acid release further as the larger poly(borate) species should exhibit slower diffusion. Although hydrolysis of these species does occur upon dilution and temperature increase, it is not clear how much this will affect component release from the hydrogel structure. There was no clear trend to how increasing the PVA concentration affects the release of boron containing species. This may be due to the presence of an excess of available boron containing species, from the boric acid–tetrahydroxyborate equilibrium, which are relatively unperturbed by the available *cis*-diol groups, assuming the concentration of di-diol cross-links is small compared to the concentration of free boric acid.

At fixed concentrations of PVA and THB, PVA release is governed by the molecular weight and the degree of hydrolysis of the polymer. In particular, the cumulative PVA released over 2 h is substantially reduced as the molecular weight of the polymer used increases. For example, the cumulative release of 40–88 PVA (approximate molecular weight 205,000) is under 1% at 30 min, whereas the cumulative release of 5–88 PVA (approximate molecular weight 37,000) is over 5% at the same time point (Fig. 3(B)). This is due to both a reduction in the diffusion coefficient due to the increasing size of the polymer chains and a reduction in the free volume available for diffusion as greater amounts of the solvent are taken up by the larger polymer chains. In addition, it is also clear that increasing the degree of hydrolysis of the polymer slowed its release from the hydrogel (Fig. 3(C)). Comparing the release of 26–88 PVA (approximate molecular weight 160,000) and 28–99 PVA (approximate molecular weight 145,000) shows a reduced cumulative release for the more hydrolysed 28–99 PVA grade. Although the molecular weight of the 28–99 PVA is lower than the 28–99 PVA counterpart, the former's higher degree of hydrolysis will induce more extensive network formation and reduce its apparent diffusivity.

5. Conclusion

The mechanical and rheological properties of PVA–THB hydrogels are dependent upon the initial concentrations of PVA and THB. This determines the equilibrium cross-link density and the physical properties of the resultant formulation. Hardness, compressibility and adhesiveness are all readily controlled through systematic variation in concentration. The grade of PVA was also found to be important in determining the final properties of the hydrogel. The use of higher molecular weight polymers with a higher degree of hydrolysis lead to the formation of a more rigid hydrogel. With regard to adhesion, a high concentration of THB was more important than the PVA concentration within the range of concentrations examined in this study.

The rheological data corroborated the mechanical observations. Both the storage modulus and loss modulus increased with increasing concentrations of PVA and THB, and as expected, the relative component of the storage modulus increased with increasing frequency. The cross-over frequency dropped as the concentration of THB increased at all concentrations of PVA in line with expectations, as the material became more rigid through increased network formation. Surprisingly, at low concentrations of THB, increasing the PVA concentration increased the cross-over frequency. This was attributed to an equilibrium shift towards mono-diol complexation and away from di-diol complexation as the concentration of PVA was increased and the relative concentration of di-diol cross-links fell. There may also be an effect due a reduction in pH and a greater demand for electrostatic shielding, which reduced further the number of di-diol cross-links. In all cases, increasing the temperature reduced the number of THB–PVA bonds, which reduced network formation and made the hydrogel less mechanically rigid, reducing the storage modulus and loss modulus.

Component release from PVA–THB hydrogels was dependent upon both the concentrations of PVA and THB, and the grade of polymer. Larger molecular weight PVA with a high degree of hydrolysis restricted the release of free polymeric species through increased network formation and reduced free volume for diffusion to occur, as well as a reduced overall diffusivity. Boron release increased with the concentration of THB, but in a non-linear fashion, due to increased network formation and the potential for poly(borate) formation.

The adhesiveness of PVA–THB hydrogels, when compared to those of similar proprietary wound management products, represents a challenge for the topical used of this type of formulation. A balance is needed between the requirement for the formulation to be fluid enough to flow into a wound and allow sufficient drug release within the appropriate period of time, and the desire for it to be cohesive enough to be removed in one piece following an extended residence time.

Acknowledgements

The authors would like to acknowledge financial support from the Research and Development Office (Northern Ireland) Trauma and Rehabilitation Recognised Research Group (RRG 8.46 RRG/3273/06).

References

Beltman, H., Lyklema, J., 1974. Rheological monitoring of the formation of polyvinyl alcohol–Congo Red gels. Faraday Discuss. Chem. Soc. 57, 92–100.
Bowcher, T.L., Dawber, J.G., 1989. C-13 and B-11 Nuclear Magnetic-Resonance Study of the reaction of polyvinyl-alcohol with the tetrahydroxyborate ion. Polym. Commun. 30, 215–217.
Capellan, O., Hollander, J.E., 2003. Management of lacerations in the emergency department. Emerg. Med. Clin. N. Am. 21, 205–231.
Dawber, J.G., Green, S.I.E., 1986. An B-11 nuclear-magnetic-resonance study of the reaction of the tetrahydroxyborate ion with polyhydroxy compounds. J. Chem. Soc. Faraday Trans. I 82, 3407–3413.
Eliseev, A.A., Lukashin, A.V., Vertegel, A.A., Heifets, L.I., Zhirov, A.I., Tretyakov, Y.D., 2000. Complexes of Cu (II) with polyvinyl alcohol as precursors for the preparation of CuO/SiO_2 nanocomposites. Mater. Res. Innovat. 3, 308–312.
Huang, Y., Leobandung, W., Foss, A., Peppas, N.A., 2000. Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces. J. Control. Release 65, 63–71.
Ide, N., Sato, T., Miyamoto, T., Fukuda, T., 1998. Thermoreversible hydrogel of short-chain O-(2,3-dihydroxypropyl)cellulose. Macromolecules 31, 8878–8885.
Joshi, D.P., Lanchunfung, Y.L., Pritchard, J.G., 1979. Determination of poly(vinyl alcohol) via its complex with boric-acid and iodine. Anal. Chim. Acta 104, 153–160.
Keita, G., Ricard, A., Audebert, R., Pezron, E., Leibler, L., 1995. The poly(vinyl alcohol) borate system – influence of polyelectrolyte effects on phase-diagrams. Polymer 36, 49–54.
Koga, K., Takada, A., Nemoto, N., 1999. Dynamic light scattering and dynamic viscoelasticity of poly(vinyl alcohol) in aqueous borax solutions. 5. Temperature effects. Macromolecules 32, 8872–8879.
Koike, A., Nemoto, N., Inoue, T., Osaki, K., 1995. Dynamic light-scattering and dynamic viscoelasticity of poly(vinyl alcohol) in aqueous borax solutions. 1. Concentration-effect. Macromolecules 28, 2339–2344.
Lapid, J., Farhi, S., Koresh, Y., 1976. Spectrofluorometric determination of boron with chromotropic-acid. Anal. Lett. 9, 355–360.
Lin, H.L., Liu, Y.F., Yu, T.L., Liu, W.H., Rwei, S.P., 2005. Light scattering and viscoelasticity study of poly(vinyl alcohol)–borax aqueous solutions and gels. Polymer 46, 5541–5549.
Loughlin, R.G., Tunney, M.M., Donnelly, R.F., Murphy, D.J., Jenkins, M., McCarron, P.A., 2008. Modulation of gel formation and drug-release characteristics of lidocaine-loaded poly(vinyl alcohol)–tetraborate hydrogel systems using scavenger polyol sugars. Eur. J. Pharm. Biopharm. 69, 1135–1146.
McCarron, P.A., Murphy, D.J., Little, C., McDonald, J., Kelly, O.J., Jenkins, M.G., 2011. Preliminary clinical assessment of polyvinyl alcohol–tetrahydroxyborate

hydrogels as potential topical formulations for local anesthesia of lacerations. Acad. Emerg. Med. 18, 333–339.
Nanjawade, B.K., Manvi, F.V., Manjappa, A.S., 2007. In situ-forming hydrogels for sustained ophthalmic drug delivery. J. Control. Release 122, 119–134.
Nemoto, N., Koike, A., Osaki, K., 1996. Dynamic light scattering and dynamic viscoelasticity of poly(vinyl alcohol) in aqueous borax solutions. 2. Polymer concentration and molecular weight effects. Macromolecules 29, 1445–1451.
Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000a. Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm. 50, 27–46.
Peppas, N.A., Hilt, J.Z., Khademhosseini, A., Langer, R., 2006. Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Adv. Mater. 18, 1345–1360.
Peppas, N.A., Huang, Y., Torres-Lugo, M., Ward, J.H., Zhang, J., 2000b. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annu. Rev. Biomed. Eng. 2, 9–29, doi:10.1146/annurev.bioeng.2.1.9.
Peppas, N.A., Sahlin, J.J., 1996. Hydrogels as mucoadhesive and bioadhesive materials: a review. Biomaterials 17, 1553–1561.
Pezron, E., Leibler, L., Lafuma, F., 1989a. Complex-formation in polymer-ion solutions. 2. Poly-electrolyte effects. Macromolecules 22, 2656–2662.
Pezron, E., Leibler, L., Ricard, A., Audebert, R., 1988a. Reversible gel formation induced by ion complexation. 2. Phase-diagrams. Macromolecules 21, 1126–1131.
Pezron, E., Leibler, L., Ricard, A., Lafuma, F., Audebert, R., 1989b. Complex-formation in polymer ion solutions. 1. Polymer concentration effects. Macromolecules 22, 1169–1174.
Pezron, E., Ricard, A., Lafuma, F., Audebert, R., 1988b. Reversible gel formation induced by ion complexation. 1. Borax galactomannan interactions. Macromolecules 21, 1121–1125.
Roy, G.L., Laferriere, A.L., Edwards, J.O., 1957. A comparative study of polyol complexes of arsenite, borate, and tellurate ions. J. Inorg. Nucl. Chem. 4, 106–114.
Salentine, C.G., 1983. High-field B-11 NMR of alkali borates – aqueous polyborate equilibria. Inorg. Chem. 22, 3920–3924.
Shibayama, M., Adachi, M., Ikkai, F., Kurokawa, H., Sakurai, S., Nomura, S., 1993. Gelation of poly(vinyl alcohol) vanadate aqueous-solutions. Macromolecules 26, 623–627.
Singer, A.J., Dagum, A.B., 2008. Current management of acute cutaneous wounds. N. Engl. J. Med. 359, 1037–1046.
Smith, H.D., Wiersema, R.J., 1972. Boron-11 Nuclear Magnetic-Resonance Study of polyborate ions in solution. Inorg. Chem. 11, 1152.
Takada, A., Nishimura, P., Koike, A., Nemoto, N., 1998. Dynamic light scattering and dynamic viscoelasticity of poly(vinyl alcohol) in aqueous borax solutions. 4. Further investigation on polymer concentration and molecular weight dependencies. Macromolecules 31, 436–443.
te Nijenhuis, K., 2007. On the nature of crosslinks in thermoreversible gels. Polym. Bull. 58, 27–42.