Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 289 (2005) 51–62
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Lyophilised wafers as a drug delivery system for wound healing containing methylcellulose as a viscosity modifier

K.H. Matthews[a,*], H.N.E. Stevens[a], A.D. Auffret[b], M.J. Humphrey[b], G.M. Eccleston[a]

[a] *Department of Pharmaceutical Sciences, University of Strathclyde, Strathclyde Institute for Biomedical Sciences, 27 Taylor Street, Glasgow G4 ONR, UK*
[b] *Pfizer Central Research, Sandwich, Kent CT13 9NJ, UK*

Received 2 July 2004; received in revised form 4 October 2004; accepted 15 October 2004

Abstract

Lyophilised wafers have potential as drug delivery systems for suppurating wounds. A dual series of wafers made from low molecular weight sodium alginate (SA) and xanthan gum (XG) respectively, modified with high molecular weight methylcellulose (MC) were produced. The swelling and flow properties of these wafers on model suppurating surfaces were both qualitatively and quantitatively investigated. The wafers instantaneously adhered to the surfaces, absorbing water and transforming from glassy, porous solids to highly viscous gels. The rate at which this occurred varied for the series studied with clear distinctions between the behaviour of SA and XG systems. For SA wafers there was a distinct relationship between the flow-rate and MC content. Increased amounts of MC decreased the rate at which the SA wafers flowed across a model gelatine surface. Flow rheometry was used to quantify the effect of increased MC content on both series of wafers and for the SA series, highlighted a substantial increase in apparent viscosity as a function of incremental increases in MC content. These results reflected those from the gelatine model. Observations of the reluctance of a swollen, unmodified XG wafer to flow compared with the relative ease of unmodified, low molecular weight SA was attributed to the yield stress characteristic of xanthan gels. XG is known to exhibit complex, loosely bound network structures in solution via the association of helical backbone structures. The inclusion of sodium fluorescein as a visible model for a soluble drug highlighted the potential of lyophilised wafers as useful drug delivery systems for suppurating wounds.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Lyophilised wafer; Wound healing; Sodium alginate; Methylcellulose; Xanthan; Model surface

* Corresponding author. Present address: School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, Scotland, AB10 1FR, UK Tel.: +44 1224 262548; fax: +44 1224 262555.
E-mail address: k.h.matthews@rgu.ac.uk (K.H. Matthews).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.10.022

1. Introduction

Chronic wounds, including diabetic and venous ulcers, are estimated to affect more than twelve million people globally (Technology Catalysts International, 1998). Management of such wounds varies among patients depending on the aetiology and severity of the ulcerated area (Mulder, 1991), the particular stage of the healing process and the amount of exudate present (Thomas, 1990). For open, granulating wounds left to heal naturally it is generally accepted that a dressing in contact with the wound surface should maintain a moist environment at the interface of wound and dressing (Winter, 1962; Hinmann et al., 1963; Eaglstein, 1985) be conformable and mouldable with the contours of the damaged area (Morgan, 1999) and have low adherence (Thomas, 1990). The latter requirement minimises the risk of damage to newly formed epithelium upon removal of the dressing.

Conventional dressings are prepared either from fibrous material such as lint or from non-fibrous materials such as hydrogels (Peppas, 1987) or hydrocolloids (Lawrence and Lilley, 1987; Thomas, 1990; Morgan, 1999). They are specifically designed for either dry to lightly suppurating wounds, or medium to heavily suppurating wounds. The former often have an occlusive backing to re-hydrate dead tissue whereas the latter are designed to either absorb substantial amounts of exudate, or release it as water vapour.

Drugs, including antibiotics such as neomycin, gentamycin and framycetin (BNF, 1999), iodine (Hansson, 1997; Mertz et al., 1999), silver salts (Furr et al., 1994; Cho et al., 2002), chlorhexidine (Thomas, 1990; Fumal et al., 2002) and metronidazole (Thomas and Hay, 1987) have long been used in the anti-infective treatment of wounds. Although they may be incorporated in wound dressings, they are more usually applied directly to the wound surface in the form of powders, pastes, ointments, creams, foams or gels. A disadvantage of such traditional vehicles is that it is difficult to apply an exact known amount of the medicament to the wound. Furthermore, the medicament is not always applied evenly to the area of the wound so that local concentrations will vary across the wound, especially if it is suppurating. Such modes of application do not lend themselves to controlled or extended delivery.

With the more recent interest in cytokines in wound healing, as accelerants of cell function, the need for predictable delivery systems becomes essential. Growth factors, such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and granulocyte-macrophage colony-stimulating factor (GM-CSGF), intended to give target cells a metabolic boost (de Riel, 1984) and stimulate granulation and epithelialisation processes in chronic leg ulcers (Thomas, 1990) should be present in controlled and sustained amounts and accurately target the wound site (Emming et al., 2002). Vehicles for delivery in human and animal studies have included, polymer gels (Guzman-Gardearzabal et al., 2000), crosslinked hydrogels (Yamamoto et al., 2001; Draye et al., 1998; Gombotz and Wee, 1998; Hubbell, 1996), protein matrices (Yamaguchi and Yoshikawa, 2001; Sakiyama-Elbert and Hubbell, 2000; Gryzybowski et al., 1999; Ono et al., 1999) and lyophilised fleeces (Berscht et al., 1994).

However, the ideal method for the administration of a growth factor to the wound site has not been established and data are sometimes contradictory. In many studies, suppuration rates are not considered despite the importance of fluid production on the choice of delivery system. Puolakkainen and coworkers (Puolakkainen et al., 1995) using a rat model showed that a transforming growth factor (TGF) was released at different rates from four formulation types that included a phosphate-buffered saline solution, a poloxomer gel, a hydroactive paste (*DuoDERM*™) and a poly(ethylene oxide) hydrogel. It was concluded that the enhancement in healing was significantly dependent on the carrier used and that the poloxomer gel in this comparison provided the most sustained release of TGF.

The importance of sustained release and the maintenance of steady-state concentrations of growth factors in the wound environment (Emming et al., 2002; Prats et al., 2002) are recognised as guiding principles. To achieve these requirements, a suitable topical delivery system must be designed to cope with wounds with varying degrees of suppuration. Currently there is no single delivery system suitable for both lightly and heavily suppurating wounds, nor does there appear to be an established non-animal model with which to test the performance of such delivery systems. The present work reports initial studies on the formulation of topical, bioadhesive, drug delivery wafers modified to deal with both extremes of wound type. The polymers

sodium alginate (SA) and xanthan gum (XG) were chosen, from preliminary screening of a wide range of water-soluble polymer excipients, for their inherent ability to form coherent and stable freeze-dried forms (wafers). The ability to alter the swelling and flow properties of wafers after application to the wound by incorporation of a viscosity modifying agent such as high molecular weight methylcellulose (MC) was assessed with a view to their use in wounds with different degrees of suppuration. The rheological properties of the wafer-forming gels before lyophilisation were also investigated to determine whether these properties correlate with the behaviour of the wafers after re-hydration. The eventual aim of this work is to use such modified wafers to deliver the new generation of wound healing drugs to wound surfaces.

2. Materials and methods

2.1. Materials

Sodium alginate (low viscosity grade) and methylcellulose (Methocel™ A4M) were used as received from Hopkins & Williams, UK and the Dow Chemical Company, USA respectively. Pharmaceutical grade (USP/EP) xanthan gum (Xantural™ 180) was obtained from CP Kelco US, Inc., USA and both gelatine powder (approx. 150 bloom, from pig skin) and sodium fluorescein were purchased from Sigma, UK. Saline containing commercial xanthan gels (*Normylgel*™ and *Hypergel*™), were used as received from *SCA* Mölnlyke (Sweden). Ripe melons, ('Honeydew', *Cucumis melo* L.) were obtained commercially and distilled water was used throughout.

2.2. Thermogravimetric Analysis (TGA)

The water contents of sodium alginate (SA), methylcellulose (MC) and xanthan gum (XG) as received were determined using TGA. A Mettler-Toledo TG50 instrument with an MT5 balance, TC15 controller and ESTAR™ software was programmed to heat approximately 10 mg samples from 25 to 200 °C at a rate of 10 °C min^{-1}. The total weight loss to 175 °C was equated with the water content.

2.3. Preparation of gels and wafers

SA was dissolved in distilled water (25.00 g in 500 mL) to produce a stock solution of concentration 5% (w/v). Increasing quantities of MC were dispersed in weighed amounts of this stock solution to produce both gels and the corresponding wafers (cast from the gels). For each batch, 50.0 g of the SA stock solution (equivalent to 2.381 g of SA) was poured into a stainless steel beaker (200 ml) and placed on a hot-plate with mechanical, overhead stirring (500 rpm). 5 mL of a volumetric solution of sodium fluorescein (0.76 g dissolved in 100 ml deionised water) was added at room temperature and stirring continued until the SA solution was uniformly dyed. Solutions were subsequently heated to between 60 and 70 °C and the appropriate quantity of MC added (0.000, 0.274, 0.625, 1.075, 1.675 and 2.491 g for batches 1–6, respectively). Stirring was then increased (1000 rpm) to produce a homogeneous slurry of insoluble MC particles at the elevated temperature. Although the first batch did not contain MC, the SA/sodium fluorescein solution was heated in exactly the same manner to all MC modified formulations to maintain an equivalent thermal history.

While still hot, slurries were swiftly poured in aliquots of 5.0 g to the individual compartments of '6-well' polystyrene microplates (Costar) and allowed to cool to room temperature to form the cast gels (total number of castings limited to 36 by the capacity of the freeze-dryer). The remaining material from each batch was also cooled to form a gel and then stored in sealed vessels for rheological measurement at 25 °C. The fine particles of MC remained in uniform suspension from the time the stirrer was removed until gelling of MC had taken place at approximately 36–40 °C. Initially opaque, MC modified gels became transparent on further cooling.

Lyophilisation of the cast gels was undertaken in a laboratory-scale freeze-drier (Virtis-Advantage EL) with shelf cooling. Initially, the gels were cooled to −50 °C and then heated in a series of thermal ramps to room temperature under reduced pressure. The bulk of the frozen water was removed by sublimation (primary drying) and the residual unfrozen water contained within the freeze concentrate was removed by desorption to the gas phase (secondary drying). Twenty-six hours was allowed to complete the whole process. Lyophilised wafers were then removed from

their moulds, weighed, placed in polyethylene bags and stored in glass desiccators containing silica gel.

For the XG/MC wafer formulations, xanthan gum (6.00 g) was dissolved in cold distilled water (300 ml) overnight to produce a stock gel of concentration 2% (w/v). For each batch of wafers, 50.0 g of this stock gel was placed in a stainless-steel beaker and 5 mL of the sodium fluorescein solution added until the XG gel was uniformly dyed. The dyed XG gel was heated to between 60 and 70 °C and MC added in the appropriate quantity (0.000, 0.113 and 0.254 g). Manual stirring with a glass rod was effective at forming a uniform suspension of the finely powdered MC prior to casting. As for SA/MC formulations, gelling occurred in the same temperature range (36–40 °C) within the polystyrene moulds. Table 1 shows the solid contents of the gels (including sodium fluorescein) corrected to account for the water content of the raw materials (see Section 3.1).

2.4. Qualitative and quantitative models for a suppurating wound

A novel qualitative model for a suppurating wound involved mounting over-ripe, sliced melon segments (Melon, 'Honeydew', *Cucumis melo* L.) to a vertical surface and applying the commercial saline-containing xanthan gels (*Normylgel*™ and *Hypergel*™), the wafer preparations and the corresponding gels before lyophilisation to the inner flesh. The behaviour of individual formulations on the surface was observed.

A simple laboratory, non-animal model was also developed to quantify the hydration and flow properties of lyophilised wafers after application to moist surfaces. This essentially consisted of glass Petri dishes containing a 4% (w/v) gelatine medium made by heating distilled water (300 mL) to 60 °C in a stainless steel beaker (500 mL) and adding powdered gelatine (12.0 g) with mechanical stirring (500 rpm) until all of the protein was in solution. The clear solution was removed from the heat and equal amounts poured into individual glass Petri dishes, covered and allowed to cool to room temperature (25 °C) overnight.

Wafers of known diameter (D_o = 34 mm) were placed in the centre of the Petri dish on top of the gelatine surface. The wafers absorbed water from the medium and slowly converted to viscous liquids and gels. As this occurred the wafers expanded outwards and the increase in diameter (D_t) as a function of time was recorded. By placing an accurately ruled card beneath the Petri dish, it was possible to gauge the diameter of the discs to within ±0.25 mm. The swelling properties of respective samples were compared from the expansion ratios calculated from the measured increases in diameter (Eq. (1)).

$$E = \frac{D_t}{D_o} \tag{1}$$

where E = 'expansion ratio', D_t = diameter of sample at time, t, D_o = diameter of sample at time zero.

Each diameter was measured four times at 45° intervals and the mean and standard deviation calculated. Standard deviations are displayed as error bars (Fig. 3). The experiment was then duplicated with fresh gelatine media.

Table 1
Formulation details for gels and wafers produced from sodium alginate (SA) and xanthan gum (XG) modified with methylcellulose (MC)

Batch No.	Component ratio in gel SA:MC	Calculated solids in gel (50 g)[a] (g)	Calculated solids in each wafer[a] (mg)	Mean wt. of wafers (mg)
1	100:0	2.021	183.7	261.8 (±2.7)
2	90:10	2.282	207.3	296.2 (±6.9)
3	80:20	2.616	237.8	337.5 (±9.2)
4	70:30	3.044	276.7	369.8 (±8.3)
5	60:40	3.616	328.7	430.8 (±7.5)
6	50:50	4.392	399.3	465.0 (±6.9)
	XG:MC			
7	100:0	0.920	83.6	106.6 (±1.5)
8	90:10	1.022	92.9	125.3 (±3.8)
9	80:20	1.149	104.5	145.9 (±3.0)

Component ratios are approximate and based on apparent material weights uncorrected for water content.
[a] Corrected to account for the water content of raw materials.

2.5. Rheological characterisation

The gels before lyophilisation were analysed using continuous shear rheology and the rupture strength of the corresponding wafers formed after lyophilisation were measured and compared using texture analysis.

2.5.1. Continuous shear

Continuous shear measurements (shear rate sweep cycle) were undertaken on freshly prepared gels before lyophilisation using a *Carri-med CSL100* rheometer (TA Instruments, UK) at 25 °C with cone and plate geometry. The shear rate was increased from zero to 1200 s^{-1} in 5 min followed by a constant shear rate decrease to zero in the same time interval. Three cones of diameter, 2, 4 and 6 cm were used depending on the apparent viscosity of the samples at the shear rates used. All measurements were repeated at least once and data analysed using the system software according to simple 'power law' relationships.

2.5.2. Texture analysis

The relative strengths of the lyophilised wafers were compared using a Texture Analyser TAXT21 (Stable Microsystems, UK) with a 5 kg load cell and fitted with a 6 mm cylindrical, stainless steel probe. Wafer samples were supported by a hollow, cylindrical perspex holder, positioned at the centre of the instrument base plate, and secured around the edge with adhesive (Fig. 1). The experiment was undertaken in compressive mode at a test speed of 10 mm s^{-1} for 4.5 s. The probe was driven through the wafer from an initial stationary position 2–3 mm above the disc-shaped wafer (diameter, 34 mm; thickness, 5 mm) until it ruptured. Net forces on the load cell were monitored in real-time and the peak force just before rupture recorded as the 'rupture strength'.

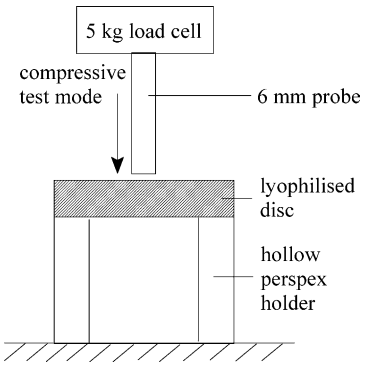

Fig. 1. Cross-sectional representation of the test-rig mounted on the Texture Analyser used to determine the rupture strength of lyophilised wafers. (Test speed = 10 mm s^{-1}; test time = 4.5 s).

3. Results

3.1. Thermogravimetric analysis (TGA)

The water contents of the SA, XG and MC polymers were 16.7, 10.0 and 4.8% respectively. These values were used to correct the actual quantities of polymer weighed for water content (Table 1).

3.2. Suppurating wound models

Both the commercial xanthan gels and the pre-lyophilised gels very quickly absorbed fluid and were diluted to the extent that they rapidly flowed off the vertical surface of the melon segments. In contrast, when lyophilized wafers were placed on the wet surface of the melon, immediate adhesion occurred. This was followed by a variable absorption of fluid to form a gel, which on further dilution became more mobile. The time taken for this process was dependent on the specific formulation. For example, unmodified SA wafers absorbed fluid most rapidly and only retained their shape for a few hours. The rate of absorption of fluid was decreased as a function of increased MC content, so that wafers of high MC content maintained their shape much longer. In contrast, xanthan wafers did not absorb much fluid and maintained their cast shape for the total duration of the experiment (5 days). Sodium fluorescein, contained within all wafers as a visible model for a soluble drug, was observed to diffuse into the melon pulp in the immediate environment of the wafers. Diffusion of dye continued in all directions during the experiment.

Similar trends were shown with the gelatine model that was used to quantify the observations of the qualitative melon model. Fig. 2 shows representative photographs of the expansion experiments on the gelatine medium. After placement of lyophilised discs on the gelatine surface at time zero, the wafers hydrated slowly to form gels, which on further hydration thinned and spread uniformly in all directions over

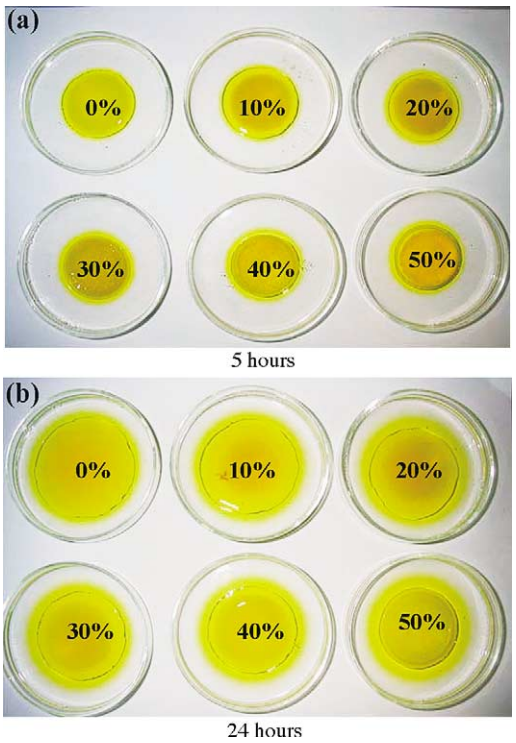

Fig. 2. Photographic representation of wafers on a gelatine (4%, w/w) surface at (a) 5 and (b) 24 h. Wafers modified with increased amounts of methyl cellulose (MC) can be observed to form more viscous gels and flow outwards at increasingly slower rates.

the surface. Diffusion of sodium fluorescein, radially outwards through the gelatine medium preceded the edge of the expanding gels in all cases. The expansion ratios as a function of time for all wafers tabulated in Table 2 provide a measure of the extent and rate of hydration of the wafers.

Unmodified SA hydrated the quickest and lost its overall moulded shape within hours. At all time points after zero, the swollen diameter of lyophilised discs was greatest for unmodified SA; after 24 h, the diameter of the viscous and spreading solution had increased by 60% ($D_t/D_o = 1.60$) and the area covered by 146%. In contrast, the SA wafer containing 50% MC did not hydrate very quickly and was able to maintain its moulded shape, particularly at the top surface not in direct contact with the gelatine, for several hours. After 24 h it had increased its diameter by 24% ($D_t/D_o = 1.24$) and its area by 53%. Modification of SA with progressively increased amounts of MC was reflected by a proportional reduction in the expansion ratio at all time points over a period of 24 h (Table 2, Fig. 3). Calculated error based on the accuracy of measurement of diameters (±0.25 mm) is included and equivalent to 2% of the expansion ratio.

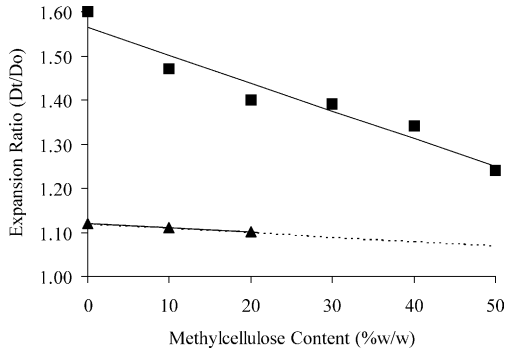

Fig. 3. Plot of expansion ratios, D_t/D_o, of (■) sodium alginate (SA) and (▲) xanthan (XG) wafers after 24 h on a gelatine (4%, w/v) surface as a function of methyl cellulose (MC) content. D_t = mean diameter at time, t; D_o = initial diameter of lyophilised wafer (D_o = 34 mm). Error in expansion ratios = ±0.01.

In contrast, the XG/MC series of lyophilised wafers showed relatively low expansion ratios even after 24 h, i.e. ratios of 1.12, 1.11 and 1.10 for unmodified XG and XG modified with 10 and 20% MC respectively.

3.3. Rheological measurements

All the freshly prepared gels before lyophilisation demonstrated shear thinning with pseudoplastic type

Table 2
Expansion ratio as a function of time for SA and XG wafers modified with varying quantities of MC on a gelatine surface (4%, w/v)

Time (h)	Expansion ratio (D_t/D_o)								
	SA (%MC)						XG (%MC)		
	0	10	20	30	40	50	0	10	20
0.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.0	1.06	1.03	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.0	1.11	1.10	1.04	1.03	1.03	1.02	1.06	1.05	1.02
3.0	1.15	1.13	1.08	1.05	1.05	1.03	1.06	1.06	1.02
4.0	1.19	1.15	1.11	1.08	1.07	1.06	1.06	1.06	1.02
5.0	1.23	1.19	1.15	1.10	1.10	1.08	1.07	1.06	1.02
6.0	1.25	1.22	1.17	1.11	1.11	1.10	1.07	1.06	1.03
24.0	1.60	1.47	1.40	1.39	1.34	1.24	1.12	1.11	1.10

Individual wafer diameters were recorded as the mean of four measurements (±0.25 mm). Error = ±0.01.

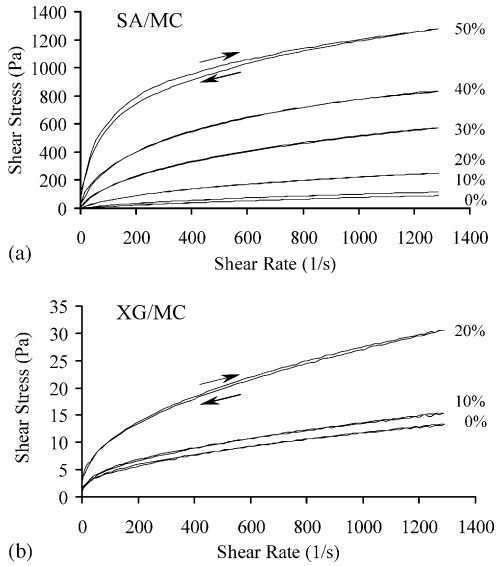

Fig. 4. Flow curves of pre-lyophilised gel formulations. (a) Sodium alginate (SA) formulations modified with 0–50% methyl cellulose (MC); (b) Xanthan (XG) formulations modified with 0–20% MC. Arrows denote ascending and descending cycles.

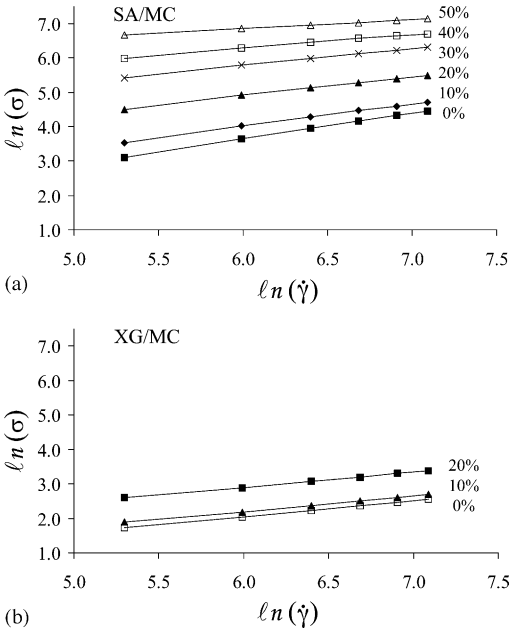

Fig. 5. Plot of the natural logarithm of shear stress, $\ell n(\sigma)$ vs. the natural logarithm of shear rate, $\ell n\ (\dot{\gamma})$ for pre-lyophilised gel formulations. (a) Sodium alginate (SA) modified with 0–50% methyl cellulose (MC); (b) Xanthan (XG) modified with 0–20% MC.

flow curves (Fig. 4). The SA/MC curves intersected the origin whereas the XG/MC samples showed a small yield value, σ_o Plots of 'log shear stress against log shear rate' for both SA/MC and XG/MC systems were linear (Fig. 5a,b) showing that under the shear rates experienced the behaviour of the SA/MC gels can be represented by a power law equation (Eq. (2)) and the XG/MC gels by a simple extension of this equation, the Herschel–Bulkley equation (Eq. (3)) as follows,

$$\sigma = \eta' \dot{\gamma}^c \tag{2}$$

and

$$\sigma = \eta' \dot{\gamma}^c + \sigma_o \tag{3}$$

where σ = shear stress (Pa), η' = 'viscosity coefficient', $\dot{\gamma}$ = shear rate (s^{-1}), c = 'rate index' of pseudoplasticity, and σ_o = yield stress (Pa).

Plots of (a) the viscosity coefficient (η') and (b) the pseudoplastic rate index (c) with MC content are shown (Fig. 6). The apparent viscosities ($\eta_{app} = \sigma/\dot{\gamma}$) at 200 s^{-1} and 1200 s^{-1} plus yield values (for the XG gels) are given in Table 3.

3.3.1. *Texture analysis*

The mean values of the peak forces at the point of rupture of the wafer, i.e. the rupture strength, ($n = 6$) against MC content of the wafers are shown (Fig. 7). Standard deviations were less than 1.0 N for all samples.

4. Discussion

The desire to develop a new method for the direct application of precise amounts of wound healing agents to a wide range of wound types forms the basis of this work. From consideration of existing technologies for the delivery of drugs to wounds, it appears that there is no universal treatment for the wide variety of wound environments encountered. Variations in the degree of suppuration, wound type and healing-stage require a flexible approach to the practice of wound management and a consideration of all the appropriate treatments available. Therefore, a single wound treatment capable of delivering therapeutic compounds accurately and in a contained manner to wound sites of variable

Table 3
Apparent viscosity (η_{app}) at 200 and 1200 s^{-1} for SA and XG wafers modified with MC

Composition SA:MC	η_{app} (Pa s) at 200 s^{-1}	η_{app} (Pa s) at 1200 s^{-1}	Yield stress (σ_o) (Pa)
100:0	0.110	0.072	0.00
90:10	0.171	0.092	0.00
80:20	0.448	0.202	0.00
70:30	1.130	0.462	0.00
60:40	1.993	0.678	0.00
50:50	3.967	1.047	0.00
XG: MC			
100: 0	0.029	0.011	1.12
90: 10	0.034	0.013	1.28
80: 20	0.069	0.025	2.60

Yield stresses (σ_o) for XG wafers are also included.

suppuration does not appear to be feasible. However, we considered that it might be possible to develop a drug delivery system with inherent properties that can be readily varied to compensate for different levels of suppuration.

The choice of high molecular weight MC as a suitable viscosity modifier for SA and XG wafers was based on a requirement to produce high viscosity increases with a minimum amount of material and to exploit its characteristic gelling properties. A solution of MC at room temperature, upon heating, will initially display a decrease in viscosity followed by a sudden increase corresponding to gel formation. This particular gel formation has a different mechanism from the classic sol–gel process typical of gelatine, say, and is a result of the formation of an infinite and reversible network structure of hydrophobic, polymer-polymer interactions that form due to loss of water of solvation (Sarker, 1979). Conversely, solid MC particles are insoluble in water above a critical temperature, the 'incipient gelation temperature' (IGT) but will swell gradually on cooling until the gelling temperature is reached ('hot-cold method'). When preparing the wafer formulations with MC it was noted that the IGT appeared to occur at 36–40 °C with the particular

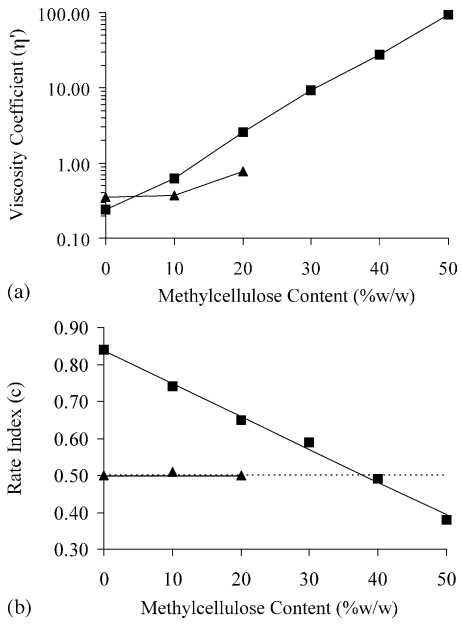

Fig. 6. Plot of (a) viscosity coefficient (η') and (b) rate index (c) as a function of methylcellulose (MC) content for SA/MC (■) and XG/MC (▲) formulations pre-lyophilisation. Viscosity coefficients are plotted on a logarithmic scale.

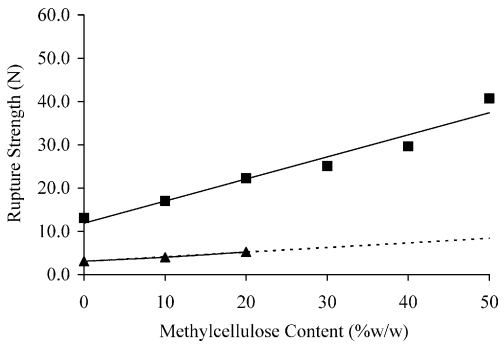

Fig. 7. Rupture strength as a function of methylcellulose (MC) content for SA/MC (■) and XG/MC (▲) lyophilised wafers. Errors are contained within markers (<1 N).

grade of MC used (*Methocel*^{TM}*A4M*). According to Sarker (1979) the IGT for *Methocel*^{TM}*A* grades is in the range 35–49 °C for polymer concentrations of 2–6% (w/w). However, as these values only concern aqueous MC gels, no direct comparison with the gels outlined in this work could be made. Despite these differences, the proximity to body temperatures observed when preparing the SA/MC and XG/MC gels was considered advantageous for maintaining optimised gelling conditions in a real wound environment.

Qualitatively, the cut melon segments offered an unusual and novel model for a medium to heavily-suppurating wound surface while the gelatine model provided a water-producing substrate on which the swelling and flow of adhered wafers could be quantitatively compared. Initial observations of the behaviour of SA and XG gels before lyophilisation when applied to the vertically-mounted, inner pulp surface of melon segments revealed that the gels quickly absorbed fluid and became dilute to the extent that they flowed readily from the target area of surface. Adhesion was minimal and a very short residence time was observed. This behaviour was in complete contrast to wafers, formed by lyophilisation of the gels, for these exhibited sustained adhesion with restricted flow sometimes for several days (c.f. XG wafers and SA wafers containing higher quantities of MC). Furthermore, when sodium fluorescein was incorporated in the wafers as a visible model for a soluble drug, staining of the fruit pulp in the environment of the applied formulation for the duration of the experiment was apparent.

The differences in hydration and swelling of the SA and XG wafers were quantified using the gelatine model. The expansion and flow of wafers containing SA and MC on re-hydration was clearly dependent on the MC content. As the amount of MC increased, the expansion ratio at each time-point decreased. XG wafers, on the other hand, maintained a relatively constant expansion ratio after the initial two hours in which they were observed to swell slightly with an associated increase in diameter but did not appreciably flow. Although bearing little resemblance to a suppurating wound, the gelatine surface appeared to serve as a quick and reliable quality control method for estimating the flow properties, and therefore potential performance, of lyophilised wafers in a suppurating environment.

The aqueous solutions of SA and SA/MC exhibited the classic shear-thinning, pseudoplastic type flow behaviour typical of entangled macromolecular systems. Characteristics of such behaviour, which can be expressed by the simple power law relationship (Eq. (2)) include, a decrease in apparent viscosity as a function of increased shear rate and the absence of a yield stress, i.e. the plot of shear stress versus shear rate (apparent viscosity) passes through the origin. The degree of pseudoplasticity exhibited by the systems, represented by the rate index, sometimes referred to as the 'flow behaviour index' (Togrul and Arslan, 2003) or 'flow index' (Attwood et al., 1991), decreased with increasing MC content, indicating increasing non-Newtonian behaviour with increasing quantities of MC (a Newtonian fluid has a rate index of 1). The logarithmic increase in calculated values of the viscosity coefficient, *V*, suggest that there might be a synergistic effect between SA and MC. Such effects have been reported for mixed gels of XG and locust bean gum (Copetti et al., 1997) and are thought to be a consequence of polymer chain interactions and formation of mixed junction zones between the two materials.

In contrast, the XG gels were of a much lower consistency than the SA systems and displayed much lower apparent viscosities. Although MC increased the consistency of the XG systems, no significant synergistic effects were apparent. Very small increases in the apparent viscosities at all shear rates, on addition of MC to XG, compared with larger increases for the SA/MC systems. This was accompanied by a relatively small log increase in the rate index. Despite the occurrence of a yield stress with the XG formulations that increased slightly with increased MC content, the degree of pseudoplasticity exhibited by these systems was constant with a rate index value of 0.5. This precise value effectively represents a perfect, inverse-square relationship between shear stress and shear rate for the XG/MC gels, i.e. shear stress is equal to the square root of shear rate for all MC contents, and qualifies the model proposed by Casson (Casson, 1959). This model has been applied to the rheology of xanthan gum and considers the existence of disperse rod-like structures within a continuous phase (Jansson et al., 1975; Melton et al., 1976; Holzwarth and Prestridge, 1977; Whitcomb and Macosko, 1978; Norton et al., 1984; Talukdar et al., 1996; Hanna et al., 1997). These rigid rods are formed from the alignment of adjacent sets of helices that underpin the network structure of xanthan gels and produce both its yield stress and pseudoplastic properties.

Although there is no general agreement on the precise multiplicity of the helical domains or the added effects of tri-saccharide pendant groups attached to the cellulose-like backbone of XG, one author has reported xanthan gels to exist as a nematic liquid crystalline phase dispersed in an isotropic phase (Carnali, 1991).

In our studies, only XG modified with up to 20% MC was produced. If the Casson relationship also holds for XG gels with higher MC contents of up to 40% (w/w), then the extrapolated intersect with the negatively sloping line associated with the rate index/MC content of SA/MC formulations, at approximately 38% MC content (Fig. 6b) indicates equivalent degrees of pseudoplasticity for both gel systems. It should be stressed here, however, that these equivalent values do not relate to the apparent viscosities measured for both systems and characterised by the viscosity coefficient (Fig. 6a). Many rheologists, especially in the food industry where there is much interest in the rheology of natural gums and starches, refer to the viscosity coefficient as the 'consistency coefficient'. In these terms, the addition of MC has a measurably greater effect on the consistency of SA than it does on XG.

Comparative testing of the rupture strength of both series of wafers indicated the improvements to mechanical strength resulting from an increased MC content (Fig. 7).

This effect was more marked for the SA/MC wafers than the XG/MC series. Substantially weaker mechanical strengths for the XG/MC series most probably reflected the density of the wafers produced which was dependent on the concentration of polymers in the prelyophilised gels and reflected in the calculated solids content of wafers (Table 1). There is the possibility, however, that the increased slope of rupture strength as a function of MC content for the SA series, compared to the XG series, may again reflect a degree of synergism between SA and MC that is not apparent with XG and MC.

Different rheological behaviours attributed to inherent differences in the molecular configuration of polymer chains provide an explanation for the differences observed on the hydration and swelling of wafers. The curves of shear stress as a function of shear rate for all SA samples intersected with the origin of the graph unlike those of XG which intersected the shear stress axis at values greater than zero (Table 3). Despite the fact that the XG gels have a lower pseudoplasticity index than SA gels, wafers formed from XG gels take much longer to hydrate with both types of model for suppurating wounds.

As discussed above, at very low shear stresses, the inability of XG gels to flow (yield value) has been attributed to the formation of high molecular weight aggregates of stiff rod-like molecules via hydrogen bonding. These attractive forces are disrupted when shear stress is applied. In the gelatine model, gravitational forces are only significant when the wafer is swollen and adhesive forces operate at the interface of wafer and medium. Swelling forces, however, may be expected to act on the gel structure and disrupt it when a critical dilution is obtained. This did not appear to happen for XG wafers with the gelatine model and it is proposed that the loss of water to the atmosphere, upon full saturation of the XG wafer samples, was balanced by further uptake of water from the medium. The net internal forces within the gel did not exceed those necessary to disrupt the rigid gel structure (i.e. yield stress) and hence no flow was observed for these samples.

SA samples, on the other hand, did not exhibit a yield stress and quickly transformed from glassy solids to highly viscous liquids upon the uptake of water. In the absence of a yield stress, viscous flow was immediate and continued at a rate appropriate with the apparent viscosity of the individual sample. This viscosity increased disproportionately as a function of increased MC content. Alternatively, as XG wafers did not appreciably flow in the gelatine model, the small differences in expansion ratio between unmodified XG wafers and those modified with 10 and 20% MC respectively (Table 2 and Fig. 2) may be indicative of reductions in the swelling rate with increasing MC content.

Other notable results from the work outlined concerned the apparent stability of lyophilised wafers under normal environmental conditions. Differences between the calculated solids content and the actual mean weights of the lyophilised wafers can be attributed to the presence of significant amounts of water in the wafers as expected for the choice of polymers. Determination of the water contents of the starting materials by TGA indicated values of 16.7, 10.0 and 4.8%w/w respectively for SA, XG and MC. These differences, referred to as 'calculated total solids' (Table 1) give an appreciation of the hydrophilic nature of polysaccharides in their pure form. The fact that the

lyophilised wafers maintain their shape and structure indefinitely under normal atmospheric conditions indicates the elevated (above room-temperature) values of the glass transition temperatures (T_g) of these light and porous, polymer glasses. This inherent stability results in an extended shelf-life and supports their proposed application as stable matrices for the storage and topically targeted delivery of wound healing agents.

Regarding the overall quality of the lyophilised wafers, as MC was insoluble in hot water at a temperature above the IGT, uniform distribution of MC particles in the SA solutions or XG gels was readily achieved. This resulted in a more homogeneous gel upon cooling and this homogeneity was reflected in the lyophilised wafer. Although only water-soluble therapeutic agents were modelled with sodium fluorescein, it was envisaged that insoluble compounds could be readily suspended in wafer formulations providing they were stable in a temperature range above the IGT of the grade of MC used and the maximum process temperature (70 °C in this study). Overall, the ability of MC to form viscous gel systems in combination with other water-soluble polymers, such as SA and XG, permitted formulation of a wide variety of wafers with a different range of effective viscosities suited to a variety of wound environments. The ability to incorporate both soluble (and insoluble) compounds that may be released on rehydration of the lyophilised matrix, indicates the potential usefulness of these novel drug delivery systems in the therapeutic treatment of chronic wounds.

5. Conclusions

This initial study on the swelling and flow properties of a dual series of lyophilised wafers has demonstrated major differences between the rheological properties of SA and XG. Incorporation of increased amounts of high molecular weight MC modified the viscosity of hydrated SA resulting in an exponential decrease in the flow rate across a model gelatine surface. Its effect on the flow behaviour of XG wafers was less clear due to the presence of a yield stress with the XG formulations. However, there was some evidence that the inclusion of MC varied the hydration rate of XG wafers.

The simplicity and effectiveness of both the qualitative fruit and quantitative gelatine models used to demonstrate the effectiveness of MC as a viscosity modifier for low molecular weight SA was evident. In addition, the wide range of viscosity behaviour apparent with these two distinct formulation types suggested that it was possible to design a lyophilised wafer capable of self-adhering to a suppurating wound surface, absorbing fluid and flowing at a controlled rate commensurate with the degree of suppuration. Visible diffusion of sodium fluorescein, as a model water-soluble drug, from the wafer to the contact media, indicated the potential of these lyophilised formulations as drug delivery systems for the treatment of chronic wounds.

In future work, it is intended to further develop lyophilised wafers as drug delivery systems for chronic wounds. This will include studies to quantify the rate of water uptake and subsequent release rates for a range of suitable model drugs. In particular, it is of interest to assess the performance of wafers and the stability of drugs following sterilisation with gamma-irradiation. Development of sterile dressings is desirable.

Acknowledgement

The authors wish to thank Pfizer Ltd., for the funding with which these studies were possible.

References

Attwood, D., Collett, J.H., O'Connor, C.A., 1991. Influence of gamma-irradiation on the rheological properties of gels of the poloxamine, synperonic-T908. Int. J. Pharmaceut. 70, 147–152.

Berscht, P.C., Nies, B., Liebendorfer, A., Kreuter, J., 1994. Incorporation of basic fibroblast growth-factor into methyl-pyrrolidinone chitosan fleeces and determination of the in-vitro release characteristics. Biomaterials 15, 593–600.

Carnali, J.O., 1991. A dispersed anisotropic phase as the origin of the weak-gel properties of aqueous xanthan gum. J. App. Pol. Sci. 43, 929–941.

Casson, N., 1959. In: Mills, C.C. (Ed.), Rheology of Dispersion Systems. Pergamon Press, New York, p. 84.

Cho, Y.S., Lee, J.W., Lee, J.S., Lee, J.H., Yoon, T.R., Kuroyanagi, Y., Park, M.H., Kim, H.J., 2002. Hyaluronic acid and silver sulfadiazine-impregnated polyurethane foams for wound dressing application. J. Mater. Sci.-Mater. Med. 13, 861–865.

Copetti, G., Grassi, M., Lapasin, R., Pricl, S., 1997. Synergistic gelation of xanthan gum with locust bean gum: a rheological investigation. Glycoconjug. J. 14, 951–961.

Draye, J.P., Delaey, B., Van de Voorde, A., Van Den Bulcke, A., Bogdanov, B., Schacht, E., 1998. In vitro release characteristics of

bioactive molecules from dextran dialdehyde cross-linked gelatin hydrogel films. Biomaterials 19, 99–107.

Eaglstein, W.H., 1985. The effect of occlusive dressings on collagen synthesis and re-epithelialization in superficial wounds. In: Ryan, T.J. (Ed.), An Environment for Healing: the Role of Occlusion International Congress and Symposium Series, 88. Royal Society of Medicine, London, pp. 31–38.

British National Formulary, 1999. Antibacter. preparat. 38, 517–518.

Emming, S.A., Smola, H., Krieg, T., 2002. Treatment of chronic wounds: state of the art and future concepts. Cells. Tissues Organs 172, 105–117.

Fumal, I., Braham, C., Paquet, P., Pierard-Franchimont, C., Pierard, G.E., 2002. The beneficial toxicity paradox of antimicrobials in leg ulcer healing impaired by a polymicrobial flora: a proof-of-concept study. Dermatology 204, 70–74.

Furr, J.R., Russel, A.D., Turner, T.D., Andrews, A., 1994. Antibacterial activity of Actisorb-Plus™ Actisorb™ and silver nitrate. J. Hosp. Infect. 27, 201–208.

Gombotz, W.R., Wee, S.F., 1998. Protein release from alginate matrices. Adv. Drug. Deliv. Rev. 31, 267–285.

Gryzybowski, J., Oldak, E., Antos-Bielska, M., Janiak, M.K., Pojda, Z., 1999. New cytokine dressing. I. Kinetics of the in vitro rhG-CSF, rhGM-CSF and rhEGF release from the dressings. Int. J. Pharmaceut. 184, 173–178.

Guzman-Gardearzabal, E., Leyva-Bohorquez, G., Salas-Coiln, S., Paz-Janeiro, J.L., Alvarado-Ruiz, R., Garcia-Salazar, R., 2000. Treatment of chronic ulcers in the lower extremities with topical becaplermin gel 0.01%: a multicenter open-label study. Adv. Therap. 17, 184–189.

Hanna, M.A., Chinnaswamy, R., Gray, D.R., Miladinov, V.D., 1997. Extrudates of starch-xanthan gum mixtures as affected by chemical agents and irradiation. J. Food Sci. 62, 816–820.

Hansson, C., 1997. Interactive wound dressings—a practical guide to their use in older patients. Drugs Aging 11, 271–284.

Hinmann, C.C., et al., 1963. Effect of air exposure and occlusion on experimental human skin wounds. Nature 200, 377–379.

Holzwarth, G., Prestridge, E.B., 1977. Multistranded helix in xanthan polysaccharide. Science 197, 757–759.

Hubbell, J.A., 1996. Hydrogel systems for barriers and local drug delivery in the control of wound healing. J. Contr. Release 39, 305–313.

Jansson, P.E., Kenne, L., Lindberg, B., 1975. Structure of extracellular polysaccharide from *Xanthemonas campestris*. Carbohydrates 45, 275–282.

Kirton, V., Munro-Ashman, D., 1965. Contact dermatitis from neomycin and framycetin. Lancet 2, 138–139.

Lawrence, J.C., Lilley, H.A., 1987. Are hydrocolloid dressings bacteria proof? Pharm. J. 239, 184.

Melton, L.D., Mindt, L., Rees, D.A., Sanderson, G.R., 1976. Covalent structure of the polysaccharide from *Xanthemonas campestris*: evidence from partial hydrolysis studies. Cabohydr. Res. 46, 245–257.

Mertz, P.M., Oliveira-Gandia, M.F., Davis, S.C., 1999. The evaluation of a cadexomer iodine wound dressing on methicillin resistant staphylococcus aureus (MRSA) in acute wounds. Dermatologic. Surg. 25, 89–93.

Morgan, D.A., 1999. Wound management products in the drug tariff. Pharm. J. 263, 820–825.

Mulder, G.D., 1991. If wounds could talk. In: Clinical and Experimental Approaches to Dermal and Epidermal Repair: Normal and Chronic Wounds. Wiley-Liss, New York, pp. 55–66.

Norton, I.T., Goodall, D.M., Franggou, S.A., Morris, E.R., Rees, D.A., 1984. Mechanism and dynamics of conformational ordering in xanthan polysaccharide. J. Mol. Bio. 175, 371–394.

Ono, I., Tateshita, T., Inoue, M., 1999. Effects of collagen matrix containing basic fibroblast growth factor on wound contraction. J. Biomed. Mat. Res. 48, 621–630.

Peppas, N.A. (Ed.), 1987. Hydrogels in Medicine and Pharmacy. CRC Press, Florida.

Prats, P.A., Duconge, J., Valenzuela, C., Berlanga, J., Edrosa, D.R., Fernandez-Sanchez, E., 2002. Disposition and receptor site binding of 1–125–EGF after topical administration to skin wounds. Biopharm. Drug Dispos. 23, 67–76.

Puolakkainen, P.A., Twardzik, D.R., Ranchalis, J.E., 1995. The enhancement in wound healing by transforming growth factor-beta (1) (TGF-beta(1)) depends on the topical delivery system. J. Surg. Res. 58, 321–329.

Sakiyama-Elbert, S.E., Hubbell, J.A., 2000. Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. J. Contr. Release 69, 149–158.

Sarker, N., 1979. Thermal gelation properties of methyl and hydroxypropylmethyl cellulose. J. Appl. Pol. Sci. 24, 1073–1087.

Talukdar, M.M., Vinckier, I., Moldenaers, P., Kinget, R., 1996. Rheological characterisation of xanthan gum and hydroxypropylmethyl cellulose with respect to controlled-release drug delivery. J. Pharm. Sci. 85, 537–540.

Technology Catalysts International Corporation, 1998. Wound Healing – Technological Innovations and Market Overview, Second ed., pp. 1.

Thomas, S., Hay, N.P., 1987. Are hydrocolloid dressings bacteria proof? Pharm. J. 239, 184.

Thomas, S., 1990. Wound Management and Dressings. The Pharmaceutical Press, London, pp. 1–87.

Togrul, H., Arslan, N., 2003. Production of carboxymethyl cellulose from sugar beet pulp cellulose and rheological behaviour of carboxymethyl cellulose. Carbohydr. Polym. 54, 73–82.

Whitcomb, P.J., Macosko, C.W., 1978. Rheology of xanthan gum. J. Rheol. 22, 493–505.

Winter, G.D., 1962. Formation of the scab and the rate of epithelialization of superficial wounds in the skin of the young domestic pig. Nature 193, 293–294.

Yamaguchi, Y., Yoshikawa, K., 2001. Cutaneous wound healing—an update. J. Derm. 28, 521–534.

Yamamoto, M., Ikada, Y., Tabata, Y., 2001. Controlled release of growth factors based on biodegradation of gelatin hydrogel. J. Biomat. Sci. – Poly. Ed. 12, 77–88.