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Gamma-irradiation of lyophilised wound healing wafers

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Abstract

Lyophilised wafers are being developed as drug delivery systems that can be applied directly to the surface of suppurating wounds. They are produced by the freeze-drying of polymer solutions and gels. This study investigates the possibility of sterilising these glassy, solid dosage forms with gamma-irradiation and determining the rheological properties of rehydrated wafers post-irradiation. One series of wafers was formulated using sodium alginate (SA) modified with increasing amounts of methylcellulose (MC), the other being composed of xanthan gum (XG) and MC. Batches were divided into three lots, two of which were exposed to 25 and 40 kGrays (kGy) of Cobalt-60 gamma-irradiation, respectively, the third being retained as a non-irradiated control. Apparent viscosities of solutions/gels resulting from the volumetric addition of distilled water to individual wafers were determined using continuous shear, flow-rheometry. Flow behaviour on proprietary suppurating surfaces was also determined. Large reductions in viscosity were apparent for irradiated SA samples while those of XG appeared to be largely unaffected. In addition, an increase in the yield stress of xanthan formulations was observed. Xanthan wafers appeared to withstand large doses of irradiation with no detrimental effect on the rheology of reconstituted gels. This offers the possibility of manufacturing sterilisable delivery systems for wounds.

Keywords: Lyophilised wafer; Wound healing; Sodium alginate; Methylcellulose; Xanthan; Gamma-irradiation

1. Introduction

Chronic wounds, such as diabetic and venous ulcers, display a relatively long healing process and present a difficult physical environment for the targeted application of antibacterials, growth hormones, polypeptide growth factors and other therapeutic agents (Loots et al., 2002; Thomas et al., 1996; Puolakkainen et al., 1995; Thomas, 1990). Large variations in the rate at which exudate is produced suggest that there is no single topical delivery system suited to all wound types. In the absence of applied therapeutic agents, containment of a moist environment in the immediate wound area, left to heal by secondary intention, is generally recognised to be beneficial whether the suppuration is high or low. Relatively dry wounds require the application of hydrated or pre-swollen substrates whereas wet wound beds require materials that can maintain a balance between the absorption and retention of wound fluid, and the release of water vapour to the atmosphere.

Moisture-retentive dressings such as hydrogels and hydrocolloids can be used to maintain the ideal conditions for the complex and highly regulated healing cycle but the inevitable colonisation of the wound site by potentially pathogenic bacteria can compromise this process. As both endogenous and exogenous bacteria can cause wound infection the risk is minimised if proper care is taken. For endogenous bacteria this normally involves surgical debridement of devitalised tissue and control of bacterial load and inflammation (Bowler, 2002). Sterilisation of applied treatments may be expected to minimise the risk of contamination by exogenous foreign bodies and the dressings themselves can retain harmful bacteria within their swollen structures, minimising dispersion when removed (Walker et al., 2003).

The production of lyophilised wafers as matrices for the direct delivery of therapeutic agents to chronic wounds has been

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described (Matthews et al., 2003, 2005). A wide range of viscous behaviour was demonstrated for wafers composed of sodium alginate (SA) and xanthan gum (XG) modified with varying amounts of high molecular weight methylcellulose (MC). XG wafers were of particular interest due to the existence of a yield stress that retarded the viscous flow of rehydrated wafers, permitting a longer residence time on the target surface. Use of a non-animal model for medium to heavily suppurating wounds (Matthews et al., 2005) demonstrated the potential of these systems as stable vehicles for the storage and delivery of both soluble and insoluble wound healing drugs.

In recent years, the exposure to gamma-irradiation has been increasingly used to sterilise or reduce bacterial charge in drug-delivery devices (Maggi et al., 2003). Autoclaving procedures can compromise the stability of thermally labile antibacterial agents (Traub and Leonard, 1995) and the temperatures involved may be expected to degrade proteinaceous growth factors (Bare et al., 1994). Alternatively, ethylene oxide can be used but concerns over its safety to operators and patients, and the environmental impact of its diluents (CFCs) has resulted in a trend towards the use of gamma-irradiation, especially for the sterilisation of disposable medical devices (Woolston and Davis, 1994). Gamma-rays, generated by a Cobalt-60 source in a specially designed irradiation cell, should be powerful enough to completely destroy biological systems but not to damage the material being sterilised. In practice, the sterilisation of pharmaceutical preparations such as, powders, liquids, creams, ointments, tablets and capsules is undertaken, however, the effects of ionising radiation on the novel systems described in our previous paper (Matthews et al., 2005) is unknown.

Presumably many excipients, which include water-soluble polymers, are not unduly damaged at the expense of their function. It is known that cellulose and its commonly encountered cellulose *esters* will tolerate sterilisation doses and that sterilisation by radiation is frequently the preferred method for dressings (Woolston and Davis, 1994) however, there does not appear to be any directly relevant information on the effect of irradiation on lyophilised cellulose ethers, alginates or xanthan, preferred polymers for the fabrication of wafers.

Generally for polymers, free radicals and ionic species produced by the radiation either result in crosslinking or chain scission, both serving to alter the molecular weight with associated changes in physical properties. Although both mechanisms will take place, one will usually predominate. In the case of sodium alginate it is well established that the overall effect of gamma-irradiation is to degrade the polymer (Hartman et al., 1975; Kume and Takehisa, 1983; King, 1994). The three studies cited used irradiation levels of 25, 50 and 10 kiloGrays (kGy), respectively, the lowest level of 10 kGy being the recommended limit for food products. Another study (Grant and D'Appolonia, 1991) concluded that a lower dose of 300 kRads (3 kGy) actually increased the gel viscosities of highly branched, low molecular weight polysaccharides (pentosans) by the relocation of branch-points or increased branching. Similarly, for starch/xanthan mixtures it has been reported that irradiation increased the apparent viscosity at the higher dosages of 10–30 kGy (Hanna et al., 1997). For modified cellulosic materials, gamma-irradiation of carboxymethylcellulose (CMC) in the solid-state resulted in degradation irrespective of the degree of substitution (DS) (Fei et al., 2000). In solution, however, crosslinking was favoured with high DS grades and increased polymer concentration. The study used doses of irradiation up to an excessive 100 kGy. A more recent study on the stability of prolonged release matrix tablets of hydroxypropyl methylcellulose (HPMC) after gamma-irradiation in the range of 7.5–50 kGy (Maggi et al., 2003) concluded that chemical modifications in the hydrophilic polymer caused a "progressive decrease of the average molecular weight with increasing radiation dose".

Other than the studies cited in this paper, there does not appear to be any reference to the effects of gamma-irradiation on methylcellulose, MC or indeed, lyophilised blends of MC with both SA and xanthan gum, XG. This work therefore aimed to investigate the effect of sterilising doses of gamma-irradiation on the rheological properties of reconstituted wafers composed of these polymers of interest. Lyophilised wafers are being developed as sterile, drug delivery systems for the treatment of chronic wounds.

2. Materials and methods

2.1. Materials

All materials were of the same source and batch as those used and described in our previous paper (Matthews et al., 2005). Sodium alginate was a low viscosity grade of unknown molecular weight from Hopkins and Williams, UK. Methylcellulose (Methocel™ A4M) was supplied by the Dow Chemical Company, USA and pharmaceutical grade (USP/EP) xanthan gum (Xantural™ 180) was obtained from CP Kelco US, Inc., USA. Gelatine powder (approximately 150 bloom from pig skin) and agar, used in the wound models, were purchased from Sigma. Water contents of sodium alginate, methylcellulose and xanthan gum were determined prior to formulation using a Mettler-Toledo TG50 thermogravimetric analyser (TGA) and the results used when calculating the solids content of wafers (Table 1).

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

Batch no.	Wafer composition	Calculated solids in gel (50 g)[a] (g)	Calculated solids in each wafer[a] (mg)	Mean weight of wafers (mg)
	SA:MC			
1	100:0	1.983	198	235 (±9)
2	90:10	2.246	225	265 (±10)
3	80:20	2.580	258	301 (±9)
4	70:30	2.944	294	341 (±12)
5	60:40	3.572	357	394 (±13)
	XG:MC			
6	100:0	0.980	98	97 (±3)
7	90:10	1.088	109	114 (±1)
8	80:20	1.222	122	128 (±3)

Wafer compositions are approximate and based on apparent material weights uncorrected for water content.

[a] Corrected to account for the water content of raw materials.

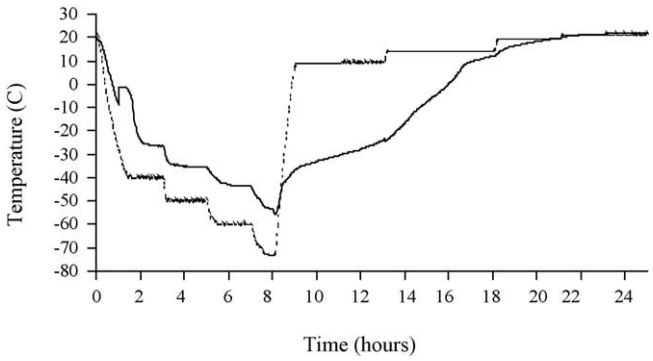

Fig. 1. Temperature profile of the freeze-drying process used to produce lyophilised wafers. Mean shelf temperature (- - -); mean sample temperature (—). Note the exotherm for the sample at 1 h and the relatively fast ramping of the shelf temperature after 8 h.

2.2. Preparation of gels and wafers

The preparation of lyophilised wafers has already been described in detail (Matthews et al., 2005). Briefly, SA (25.00 g) was dissolved in distilled water and made up to 500 mL to produce a 5% (w/v) stock solution. For each SA/MC wafer batch, a precise amount (50.0 g) of stock solution was placed in a stainless steel beaker and heated to between 60 and 70 °C with overhead stirring (500 rpm). MC (0.000, 0.276, 0.627, 1.009 and 1.669 g for Batch Nos. 1–5) was added and the stirring increased to produce a homogeneous slurry of MC in SA. While still hot, slurries were swiftly poured in aliquots of 5.0 g to individual compartments of six-well polystyrene plates and allowed to cool to room temperature. Gelling of MC occurred during this stage. Cast samples were subsequently freeze-dried. Thermal profiling of the lyophilisation process as obtained from constant monitoring of both sample and shelf temperatures using thermocouples, was undertaken for the duration of the freezing and drying process (Fig. 1). It should be noted that the cycle depicted is not optimised for the systems studied but merely reflects a general type of cycle that has been used successfully to produce shaped, lyophilised products (Thapa et al., 2003).

For XG/MC wafers, xanthan gum (6.00 g) was dissolved at refrigerated temperatures overnight in distilled water (300 mL) to produce a weak gel. As for SA/MC samples, proportions of XG gel (50.00 g) were also heated to between 60 and 70 °C and MC (0.000, 0.113 and 0.254 g for Batch Nos. 6–8) were added and dispersed with manual stirring prior to casting and cooling. Gelling of MC occurred in the same temperature range, 36–40 °C, as SA/MC samples.

2.3. Irradiation of lyophilised wafers

To investigate the effect of sterilising doses of gamma-irradiation on lyophilised wafers, each batch of wafers was separated into three lots. One lot from each batch was irradiated with 25 kGy, another with 40 kGy (*Isotron Plc*) using a Cobalt-60 source with an approximate dose rate of 5 kGy/h. The third lot was kept as a non-irradiated control. These prescribed doses of irradiation are typically used for sterilising medical devices.

2.4. Continuous shear

Continuous shear measurements (shear rate sweep cycle) were undertaken on reconstituted gels resulting from the addition of distilled water (5 mL) to both irradiated and non-irradiated (control) wafer samples contained in 50 mL glass beakers. Manual stirring for 5–10 min was used to aid dissolution of the wafers and ensure formation of a homogeneous phase. Measurements were undertaken on a *Carrimed CSL100* rheometer at 25 °C with cone and plate geometry. The shear rate was increased from 0 to 1200 s^{-1} in 5 min followed by a constant shear rate decrease to 0 in the same time interval. Three cones of diameter, 2, 4 and 6 cm were used depending on the apparent viscosities 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 (Eqs. (2) and (3), Section 3.1).

2.5. Gelatine and agar surfaces as models for suppurating wounds

As described in our previous paper (Matthews et al., 2005) a gelatine medium serves as a useful non-animal model with which to test the performance of wafers. Placement of a disc-shaped wafer on the centre of the upper surface of a 4% (w/v) gelatine medium contained within a Petri dish results in the absorption of water from the medium to the wafer which consequently, in the case of SA forms a viscous liquid or, for XG, a transparent gel. The extent to which the rehydrated wafer flows uniformly across the gelatine surface can be equated with the rheological properties quantified by continuous shear measurements. Consequently, it was of interest to assess the performance of irradiated and non-irradiated wafers using this model. Furthermore, in an attempt to better understand this model, a second set of measurements were conducted using agar in place of gelatine. For this purpose, a stock solution of agar (1%, w/v) was prepared by heating distilled water (300 mL) to 60 °C in a stainless steel beaker and adding powdered agar (3.00 g) with mechanical stirring until completely dissolved. Equal amounts of the hot agar solution were poured to Petri dishes and allowed to cool to room temperature overnight. As for the gelatine medium, the agar medium was expected to provide a suitable model surface on which to assess wafer performance. It was also of interest to compare the relative performance of both gelatine and agar surfaces. Measurement of the increase in diameter of the disc-shaped wafers was used to gauge the relative performance according to the following equation:

$$E = \frac{D_t}{D_0} \quad (1)$$

where E is the 'expansion ratio', D_t the diameter at time, t and D_0 is the diameter of sample at time zero.

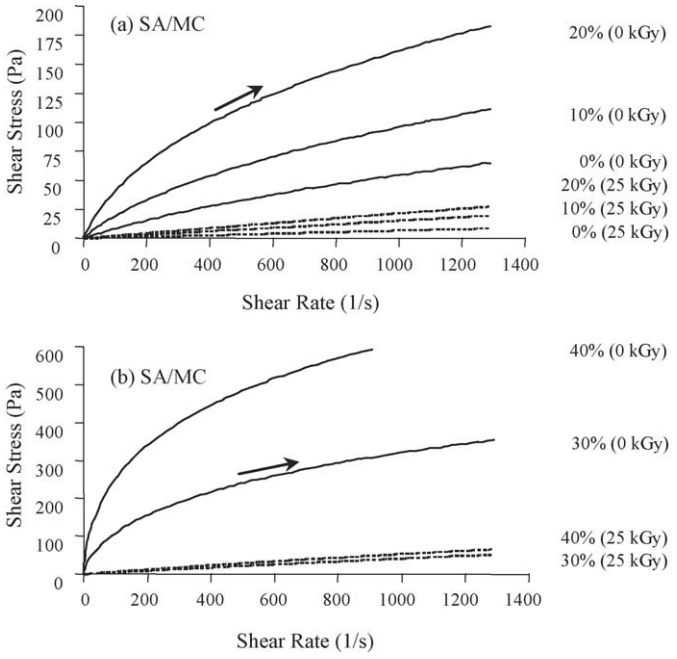

Fig. 2. Flow curves of gels resulting from the rehydration of irradiated and non-irradiated lyophilised wafer formulations. (a) Sodium alginate (SA) formulations modified with 0–20% (w/w) methylcellulose (MC) and irradiated with 25 kGy; (b) SA formulations modified with 30–50% (w/w) MC and irradiated with 25 kGy.

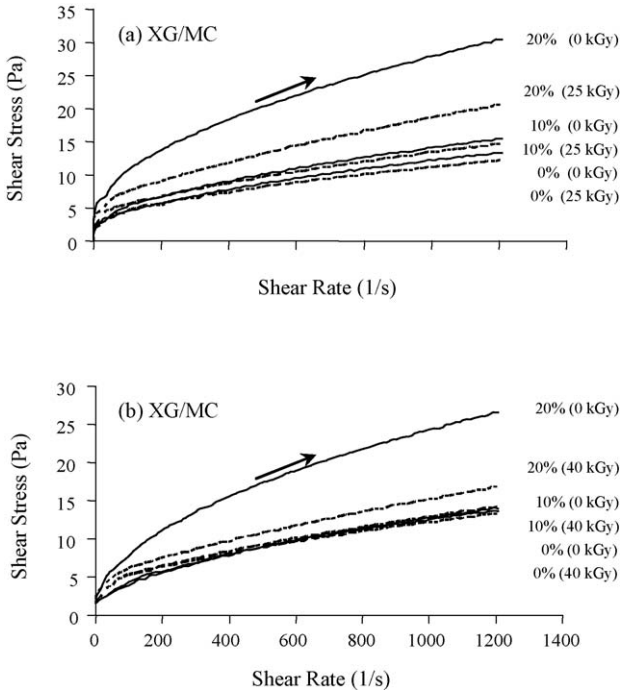

Fig. 3. Flow curves of gels resulting from the rehydration of irradiated and non-irradiated lyophilised wafer formulations. (a) Xanthan gum (XG) formulations modified with 0–20 % (w/w) methylcellulose (MC) and irradiated with 25 kGy; (b) XG formulations modified with 0–20 % (w/w) MC and irradiated with 40 kGy.

3. Results

3.1. Rheological measurement

As previously outlined (Matthews et al., 2005) all gels reconstituted from lyophilised wafers demonstrated shear thinning with pseudoplastic type flow curves (Figs. 2 and 3). All SA/MC curves (Fig. 2(a and b)) intersect the origin whereas the XG/MC curves (Fig. 3(a and b)) show a small yield value (yield stress). Irradiation of SA/MC wafers, subsequently rehydrated by the addition of a volumetric amount (5 mL) of distilled water, produced solutions with drastically reduced apparent viscosities (η_{app} = shear stress, σ/shear rate, γ) at all points on the shear rate sweep cycle (Fig. 2(a and b)). These reductions in apparent viscosity were especially notable for wafers modified with increasing quantities of MC, even at the lower irradiation dose of 25 kGy. Results obtained for SA/MC samples irradiated at the higher dose of 40 kGy were not included due to difficulties encountered in accurately measuring the resultant 'water-like' viscosities with the available equipment.

For XG/MC samples, the viscosity degrading effects of gamma-rays are clearly discernable for XG wafers modified with 20% (w/w) MC at both 25 and 40 kGy (Fig. 3(a and b)). Less clear are the differences for the other two xanthan containing formulations. A small decrease for unmodified XG and XG modified with 10% (w/w) (Fig. 3(a)) following an exposure of 25 kGy was even less noticeable at the higher dose of 40 kGy (Fig. 3(b)). Plots of 'log shear stress against log shear rate' at four different shear rates (200, 400, 600 and 800 s^{-1}) for both the SA/MC (Fig. 4) and XG/MC series of formulations (Fig. 5) were linear, the gradient of the straight lines equating to the rate index of pseudoplasticity according to the power law equations (Eqs. (2) and (3)),

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

and

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

where σ is the shear stress (Pa), η' the 'viscosity coefficient', γ the shear rate (s^{-1}), c the 'rate index' of pseudoplasticity and σ_o is the yield stress (Pa). The latter equation (Eq. (3)) being a simple extension of the former (Eq. (2)) to include the yield

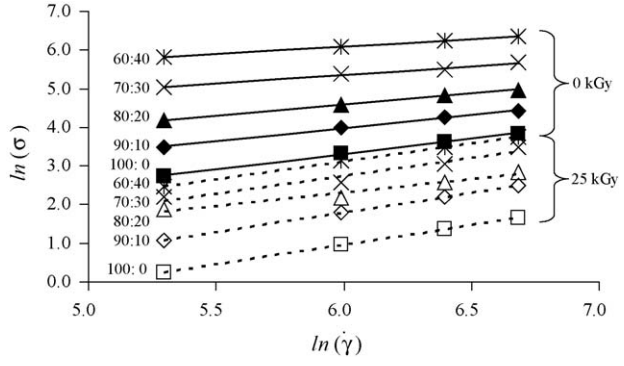

Fig. 4. Plot of the natural logarithm of shear stress, ln(σ), as a function of the natural logarithm of shear rate, ln(γ), for sodium alginate (SA) gels modified with 0–40% (w/w) methylcellulose (MC) resulting from the rehydration of irradiated (- - -) and non-irradiated (—) lyophilised wafer formulations.

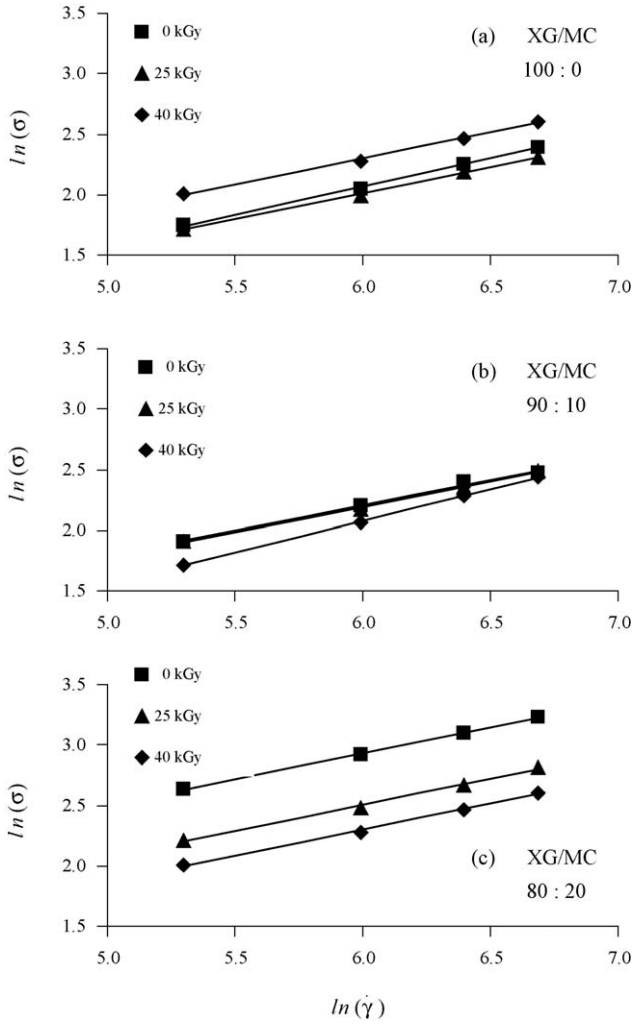

Fig. 5. Plots of ln(σ) as a function of ln(γ) for (a) unmodified xanthan (XG/MC, 100:0); (b) XG/MC, 90:10; (c) XG/MC, 80:20 at 0, 25 and 40 kGy irradiation, respectively.

stress evident with XG/MC formulations and commonly known as the Herschel–Bulkley equation (Eq. (3)).

Apparent viscosities ($\eta_{app} = \sigma/\gamma$) at 400 and 800 s^{-1} for both irradiated and non-irradiated samples of SA/MC and XG/MC are presented (Table 2) and a summary of the computed values of η', c and yield stress (σ_o), where relevant (Table 3).

Plots of the logarithm of viscosity coefficient, ln(η') as a function of methylcellulose content (Fig. 6) and rate index as a function of methylcellulose content (Fig. 7) for all SA/MC and XG/MC samples, both irradiated and non-irradiated, are also presented. As previously mentioned, the viscosities of SA/MC samples following irradiation at 40 kGy are not included due to the uniformly low viscosities of the reconstituted solutions.

3.2. Performance of wafers on model gelatine and agar surfaces

Changes in the expansion ratio of irradiated XG/MC wafers are presented (Table 4). The performance of non-irradiated SA/MC wafers on a gelatine surface was reported in our pre-

Table 2
Apparent viscosities at two shear rates (ascending) for gels reconstituted from irradiated and non-irradiated wafers

Batch no.	Wafer composition	Irradiation dose (kGy)	η_{app} (Pa s)	
			Shear rate, $\gamma = 400\ s^{-1}$	Shear rate, $\gamma = 800\ s^{-1}$
	SA:MC[b]			
1	100:0	0	0.069	0.058
		25	0.007	0.007
2	90:10	0	0.134	0.105
		25	0.015	0.015
3	80:20	0	0.247	0.180
		25	0.015	0.015
4	70:30	0	0.542	0.366
		25	0.043	0.041
5	60:40	0	1.115	0.712
		25	0.057	0.054
	XG:MC			
6	100:0	0	0.015	0.014
		25	0.018	0.013
		40	0.024	0.017
7	90:10	0	0.023	0.016
		25	0.022	0.015
		40	0.020	0.014
8	80:20	0	0.055	0.032
		25	0.030	0.021
		40	0.024	0.017

[b] SA/MC gels from wafers irradiated at 40 kGy could not be accurately measured due to their low, 'water-like' viscosities.

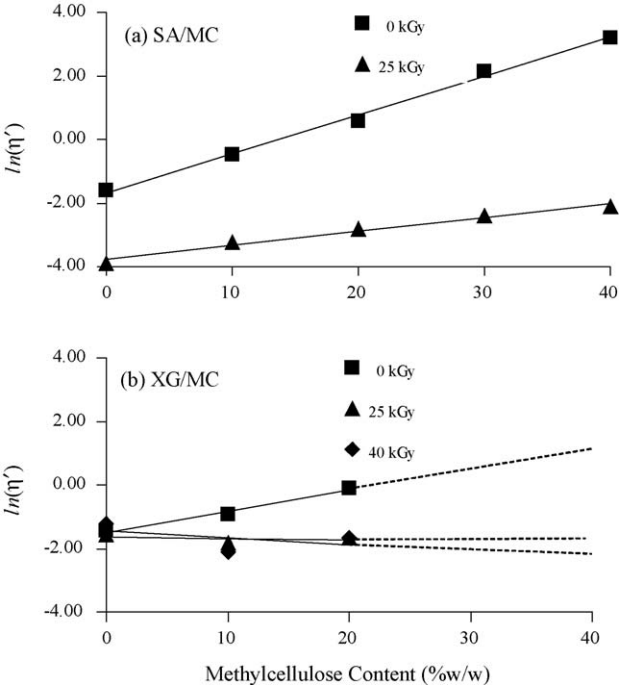

Fig. 6. Plots of the natural logarithm of the viscosity coefficient (consistency), ln(η') as a function of methylcellulose content (% w/w) for (a) sodium alginate (SA) gels modified with 0–40% (w/w) methylcellulose (MC) and (b) xanthan gum (XG) gels modified with 0–20% (w/w) MC, both irradiated (25 and 40 kGy) and non-irradiated (0 kGy).

Table 3
Calculated values of viscosity coefficient (η') and rate index (c) for gels reconstituted from irradiated and non-irradiated wafers of SA and XG

Batch no.	Wafer composition	Irradiation dose (kGy)	Viscosity coefficient (η')	Rate index (c)	Yield stress (σ_o)
	SA:MC				
1	100:0	0	0.20	0.82	–
		25	0.02	0.80	–
2	90:10	0	0.61	0.74	–
		25	0.04	0.86	–
3	80:20	0	1.80	0.66	–
		25	0.06	0.85	–
4	70:30	0	8.46	0.53	–
		25	0.09	0.88	–
5	60:40	0	24.87	0.48	–
		25	0.12	0.88	–
	XG:MC				
6	100:0	0	0.24	0.54	1.12
		25	0.21	0.55	1.27
		40	0.30	0.52	1.76
7	90:10	0	0.39	0.51	1.28
		25	0.16	0.61	2.32
		40	0.12	0.64	2.02
8	80:20	0	0.91	0.49	2.60
		25	0.19	0.63	3.20
		40	0.19	0.61	2.06

Calculated values of yield stress (σ_o) for xanthan containing wafers are also included. SA/MC samples irradiated at 40 kGy were not measured due to their 'water-like' viscosities.

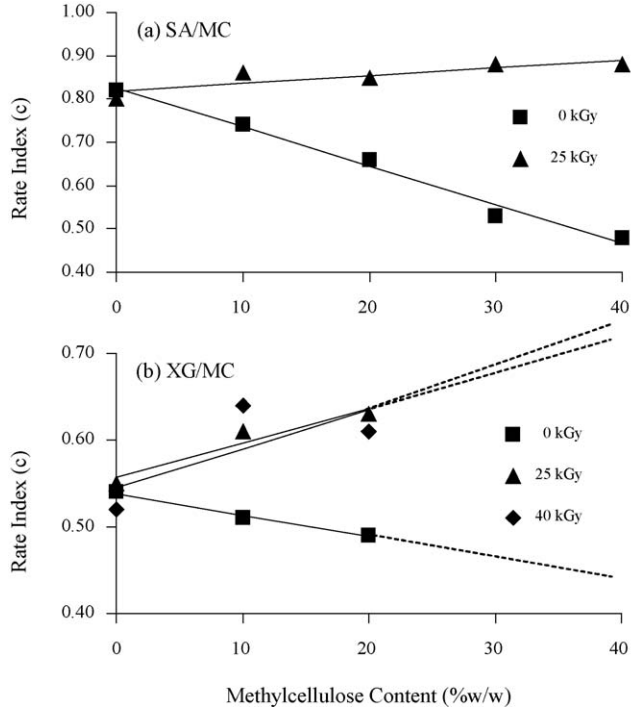

Fig. 7. Plots of the rate index (degree of pseudoplasticity) as a function of methylcellulose (MC) content (%, w/w) for gels resulting from the rehydration of irradiated and non-irradiated lyophilised wafer formulations. (a) Sodium alginate (SA) modified with 0–40% (w/w) MC at 0 and 25 kGy, respectively, (b) xanthan gum (XG) modified with 0–20% (w/w) MC at 0, 25 and 40 kGy, respectively.

Table 4
Expansion ratio as a function of time for XG wafers modified with 0, 10 and 20% (w/v) MC on gelatine (4%, w/v) and agar (1%, w/v) surfaces following irradiation at 25 and 40 kGy

Time (h)	XG/MC irradiated at 25 kGy			XG/MC irradiated at 40 kGy		
	0	10	20	0	10	20
Expansion ratio (D_t/D_o) on model gelatine surface						
0.00	1.00	1.00	1.00	1.00	1.00	1.00
2.50	1.06	1.04	1.02	1.06	1.06	1.02
3.50	1.07	1.05	1.02	1.06	1.07	1.02
26.50	1.11	1.10	1.11	1.11	1.13	1.10
94.50	1.12	1.12	1.13	1.12	1.15	1.11
Expansion ratio (D_t/D_o) on model agar surface						
0.00	1.00	1.00	1.00	1.00	1.00	1.00
2.50	1.14	1.12	1.10	1.11	1.11	1.08
3.50	1.18	1.17	1.15	1.15	1.16	1.13
26.50	1.64	1.63	1.59	1.58	1.61	1.66
94.50	2.03	2.05	2.01	2.04	2.08	2.10

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

vious paper (Matthews et al., 2005) as were non-irradiated XG/MC wafers. The time points chosen were randomly selected to demonstrate the scale of expansion of wafers over the first few hours, after 1 day and at 4 days. Errors incurred in the measurements were dictated by the calibrated scale used and these translate as an error of ±0.01 in the values of expansion ratio quoted.

4. Discussion

As highlighted in our previous paper (Matthews et al., 2005) it was clear from the rheological measurements on non-irradiated wafers containing SA/MC and XG/MC that MC was an effective viscosity modifier for both systems studied. The ability to produce lyophilised wafers with such a wide range of gel viscosities upon rehydration was thought to be one solution to the design of delivery systems capable of exercising controlled flow properties on wounds with different rates of suppuration. Unmodified, low molecular weight SA wafers may be expected to form a viscous liquid covering on lightly suppurating wounds whereas those modified with increasing amounts of high molecular weight MC would be suited to wounds with a higher rate of suppuration. For these wafers, the molecular weight and concentration of SA and the absolute amount of MC in the lyophilised product would determine the gel viscosities on absorption of wound fluid. In principle, a suitable wafer would be able to absorb a predetermined quantity of fluid and maintain a sufficiently high viscosity to inhibit flow for as long as possible. XG gels in particular, exhibit a yield stress which inhibits/delays viscous flow and increases the likelihood of confinement to a target area. Addition of incremental quantities of MC to XG gels would further enhance the gel effect. In order to produce sterilised wafers with the appropriate viscosity/flow characteristics, it was therefore of interest to investigate the gel characteristics following sterilising doses of gamma-irradiation. Production of sterile, solid dosage forms using gamma-rays, post-lyophilisation, would provide an

alternative to the expensive aseptic conditions of sterile filtration normally employed in the production of lyophilised products (FDA, 1993).

From consideration of previous studies on the effect of ionising radiation on the polymers of choice, it was unclear what the net effect of gamma-rays on lyophilised matrix structures and their reconstituted gels would be. From the results detailed in this study, it was clear that unmodified SA formulations and those modified with MC readily degraded, even at the lower sterilising dose of 25 kGy. The large decrease in apparent viscosity at all shear rates, highlighted by the decrease in the value of η' (Table 3) from 0.20 to 0.02 for unmodified SA and 24.87 to 0.12 for SA containing 50% MC, was evident. This reduction in the apparent viscosity, or consistency, was attributed to gamma-rays causing scission of polymer chains far in excess of any branching effect. These conclusions were in agreement with the work of previous authors who reported the degradation of SA upon exposure to gamma-rays (Hartman et al., 1975; Kume and Takehisa, 1983; King, 1994).

In surprising contrast, the effects of gamma-ray induced degradation was less apparent with unmodified XG wafers (Fig. 3(a and b)). The small decreases in apparent viscosity for unmodified XG and XG modified with 10% MC, following irradiation at 25 kGy, are just discernable in the shear range from 400 to 1200 s^{-1} (Fig. 3(a)) but become indeterminable in the same range of shear rates following irradiation at 40 kGy (Fig. 3(b)). From 0 to 200 s^{-1}, the shear stresses recorded for the same two irradiated samples (broken lines) are significantly greater than the non-irradiated equivalents (unbroken lines) and unmodified XG appears to show an overall increase in apparent viscosity. This observation is in agreement with the conclusions of earlier work (Hanna et al., 1997) that suggested xanthan was able to tolerate sterilising doses of radiation. This particular piece of research went further to suggest that some evidence of an increased yield stress was also apparent following gamma-irradiation. Referring to the calculated values of σ_o (Table 3) there does appear to be a slight increase in the computer calculated yield stress for unmodified XG. Although the absolute accuracy of these values are largely unknown and related to the inertial forces generated by the probe as it begins to rotate from an initially static position, there was no doubt that the yield stress inherent with XG samples was still present after irradiation.

The rheological measurements undertaken for rehydrated XG/MC wafers should be compared with those of the SA/MC series of samples (Fig. 2) where the differences between irradiated and non-irradiated samples are beyond doubt and characterised by large decreases in apparent viscosity following irradiation. The $\ln(\sigma)$ versus $\ln(\gamma)$ plot (Fig. 4), produced by sampling the apparent viscosity curves at four different shear rates, further illustrate the overall decrease in viscosity caused by exposure of the SA/MC series of wafers to gamma-irradiation. The gradients of the series of straight lines obtained are equivalent to the rate indices, c (Eqs. (2) and (3)), and do not vary to any great extent unlike the magnitudes of the lines, relative to shear stress, which are much reduced following irradiation of 25 kGy. The same plots for the XG/MC series (Fig. 5(a–c)) portray a completely different outcome to sterilising doses of gamma-rays. An increase in the magnitude of the 40 kGy line for unmodified XG (Fig. 5(a)) is in contrast to the decreases apparent for both 10 and 20% MC modified samples, the latter being more evident (Fig. 5(c)).

The natural logarithms of computer generated values of η' (Table 3) are illustrated as a function of MC content (Fig. 6(a and b)). The divergent relationship of the lines plotted for SA/MC samples (Fig. 6(a)) indicate that an increase in the proportion of MC, relative to SA, results in a more dramatic decrease in gel consistency following irradiation at 25 kGy. This was clear evidence that lyophilised MC also degrades (in addition to SA) when exposed to gamma-rays. Degradation of MC was considered responsible for the decrease in consistency of modified xanthan formulations although the similarity in values (Fig. 5(b)) indicated that the higher dose of 40 kGy did not appreciably affect the gel rheology any more than the lower dose of 25 kGy. Convergence of all three lines at 0% MC content (Fig. 6(b)) highlighted the lack of change in the consistency of unmodified XG gels following rehydration of the irradiated wafers. These results suggested that it was possible to expose XG wafers to sterilising doses of gamma-rays up to 40 kGy with no adverse effect on the gel rheology.

Reference to the rate index of pseudoplasticity (c) as a function of MC content (Fig. 7(a and b)) indicates that irradiation increases this parameter for all samples containing MC. There are no significant changes to the rate index for unmodified SA and XG despite SA showing a considerable decrease in consistency. As a Newtonian fluid has a rate index of 1.00, increased MC content in non-irradiated wafers clearly deviates from this idealized behaviour as values for c decrease from 0.82 to 0.48 for SA/MC and 0.54 to 0.49 for XG/MC. The latter range of values is in close agreement with the Casson model which states that shear stress is equal to the square root of shear rate (Casson, 1959). This model has been successfully applied to the rheology of xanthan gum (Matthews et al., 2005). Conversely, irradiation of MC modified formulations results in an increase in the degree of pseudoplasticity for all SA and XG formulations. SA containing samples become more Newtonian, or 'water-like' ($c > 0.80$) while XG gels deviate from the Casson model.

With respect to the formulation details of wafer batches (Table 1) apparent discrepancies between the calculated solids and actual weight of individual freeze-dried wafers have been attributed to residual water content (Matthews et al., 2005). Despite correcting for the water content of the polymeric materials used (Section 2.1) the measured weights exceed those calculated on the basis of a water-free sample. Thus, a mean weight of 235 (±9) mg ($n = 6$) for SA wafers is 37 mg heavier than the calculated solids content of 198 mg. This suggests a water content of 18.7% (w/w) for this batch of wafers as tested. Such levels of water in the glassy wafer structure may contribute to the overall degradation of SA and MC evident from these rheological studies. It has been reported for gamma-irradiated carageenans (Marrs, 1987) that the rate of molecular degradation in the dry state was slow compared with the gel state suggesting that the presence of water aids degradation. The extent to which the presence of significant quantities of water in lyophilised wafers

contributes to their degradation was not known but may be of interest in future studies.

Expansion ratios (Table 4) measured by consideration of the hydration and flow of disc-shaped wafers placed on gelatine and agar surfaces, reflect the relative differences in the rheological properties of the formulations tested. Originally developed as a simple model for a suppurating wound (Matthews et al., 2005) it proved to be effective at assessing the anticipated performance of the lyophilised samples in a real wound environment. The rate of production of wound exudate in vivo has been estimated to be in the region of 0.5–6.0 mL cm^{-2} in 24 h for a 'moderately' exuding wound (Thomas et al., 1996) but has not been measured for the gelatine and agar in vitro models outlined in both this and our previous study.

In these earlier studies, which used only gelatine as a model surface, there were clear and quantifiable differences in the rates of hydration, swelling and flow between the different wafer formulations. SA wafers quickly absorbed water from the polymer medium and formed viscous, pseudoplastic solutions which flowed outwards towards the perimeter of the Petri dishes. The rate of flow was proportional to the relative content of the viscosity modifying agent, MC. In contrast, XG wafers exhibited an initial swelling to a transparent gel but did not flow in the manner of the SA wafers. This was attributed to the yield stress inherent with XG gels (Whitcomb, 1978; Marcotte et al., 2001). This initially unexpected behaviour was reproduced in the current study. An expansion ratio of 1.00 at time-zero did not increase beyond a mean of $1.12(5) \pm 0.01$ (Table 4) for the XG/MC series of wafers on a 4% gelatine surface. Clearly, this was not the case on the agar surface which appeared to donate water at a faster rate to the expanding wafers than the gelatine surface (the precise difference in the rates at which both polymer media release water to the wafers is of critical importance and will be accurately quantified in future studies). For SA/MC wafers (results not included) complete coverage of the available test surface occurred in less than 24 h. On a 4% gelatine surface, unmodified SA wafers achieved an expansion ratio of 1.60 after 24 h (Matthews et al., 2005). Unmodified XG wafers, in contrast, had an expansion ratio of only 1.12 after this time in our previous study, this result being reproduced in the current study. The equivalent expansion ratio on a 1% agar surface of 1.64 at 26.50 h and 2.03 at 94.50 h (Table 4) suggests that the retarding effects on flow attributed to the persistent presence of a yield stress, did not apply in the case of contact with the agar surface. Presumably, the continued absorption of water from the agar surface by the XG wafers exceeded the critical concentration (unknown) at which the weak-gel structure of XG formulations was destroyed, resulting in continuous pseudoplastic flow. The choice of the gel medium for these measurements is therefore of critical importance, especially if such a test is to be used as a simple quality control measure. It may also be assumed that the concentration of the chosen medium will also affect the rate at which water can transfer from the gel surface to the swelling wafer. Most importantly, the performance of gamma-irradiated XG wafers was effectively unchanged following doses of 25 and 40 kGy of ionizing radiation. These results reflected the findings of the continuous shear measurements.

In conclusion, it was clear from the measurements outlined in this study that wafers containing SA and MC in particular were degraded by sterilising doses of gamma-irradiation such that their rheological properties, upon rehydration to a gel/viscous solution, were severely altered. Knowledge of the precise changes in rheology after sterilising doses of ionizing radiation is critical when pre-formulating wafers designed to remain on the surface of a range of suppurating wounds. Compensating for reductions in the viscosity of SA/MC wafers by the use of a higher molecular weight grade of SA than the one used, or increasing the MC content, may offer a solution. In contrast, XG appeared capable of being irradiated without losing its inherent rheological properties and appeared to tolerate sterilising doses of gamma-irradiation, even displaying some evidence of an increased yield stress.

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