VISCOELASTIC CHARACTERISATION OF CALCIUM ALGINATE GELS INTENDED FOR WOUND HEALING

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ABSTRACT

Calcium alginate gels were prepared and characterised in terms of their viscoelastic properties. For all the gel samples, the storage or elastic modulus (G') increased with increase in frequency of oscillation of the samples whilst the dynamic viscosity (h') decreased with increase in frequency of oscillation. An increase in the concentration of Ca^{2+} caused an increase in the elastic modulus of the gels. The G' of gels prepared with different grades of alginate increased in the order of increasing viscosities of the alginate grades (SOBALG PII 160 <SOBALG PII 165< SOBALG PII 167). An increase in the concentration of sodium alginate resulted in the formation of gels with reduced G' values. There was no significant difference ($p > 0.05$) between the viscoelastic properties of gels prepared using Ca^{2+} from two different sources (chloride and lactate). The G' of calcium alginate gels was significantly reduced (51 – 95%) when prepared with sodium alginate and calcium solutions autoclaved at 121 °C for 15 min. The longer the autoclaving time the greater the reduction in the G' of the gels. Ageing of calcium alginate gels resulted in a gradual increase in G' of the gels over a 24 h period. The viscoelastic properties of calcium alginate gels were dependent on the concentration of Ca^{2+} and sodium alginate, viscosity grade of sodium alginate, autoclaving and ageing of the gels formed.

Keywords: *Viscoelasticity; Calcium alginate gels; Elastic modulus; Dynamic viscosity; Wound healing.*

INTRODUCTION

Wounds cause high morbidity and mortality among several categories of people in Ghana and other developing countries. It is especially prevalent among diabetics, farmers, construction workers and the rural poor with consequent reduction in economic activities. Wound healing is, however, a much difficult and complicated process. As a result, various materials have been investigated as possible wound healing agents (Helmke, 2004).

Alginates, which are polyuronides extracted from brown seaweeds (phaeophyceae), have a

long history of use in wound healing (Passe and Blaine, 1948). Various forms of sodium alginate, especially calcium alginate fibre dressings, have been used in the management of burns, surgical wounds (Gupta *et al.*, 1991) and chronic exuding wounds such as leg and pressure ulcers (Chapius and Dollfus, 1990). Calcium alginate reacts with sodium ions in wound exudates and becomes partly converted to the sodium salt. This leads to the swelling and partial dissolution of the fibres that form a hydrophilic gel on the surface of the wound which facilitates wound healing (Thomas, 1989). Calcium alginate gels that possess the requisite viscoelastic properties could be used as vehicles for the slow delivery of drugs into wounds. The viscoelastic properties of the gels would be important determinants of their clinical performance.

The study aims at the characterisation of preformed calcium alginate gels of different physicochemical properties in terms of their viscoelastic properties for possible use in wound healing.

MATERIALS AND METHODS

Materials

Three grades of sodium alginate derived from the brown algae, *Laminaria*, namely: SOBALG PH 160, SOBALG PH 165 and SOBALG PH 167 with viscosities (in 1% solution at 20 °C) of <50 mPa.s, 450 mPa.s and 850 mPa.s, respectively, were obtained from Grindsted Products Ltd. (Denmark). Calcium chloride dihydrate and magnesium chloride hexahydrate were supplied by BDH Ltd. (Poole, UK). Calcium lactate pentahydrate was obtained from Fisher Scientific (Loughborough, UK). All other chemicals used were of analytical reagent grade.

Preparation of calcium alginate gels

Effects of calcium ion concentration

A 2% w/v sodium alginate (SOBALG PG 160) was prepared by soaking the powder in distilled water for 4 h with occasional agitation. Stock solutions (100 ml) of calcium chloride dihydrate and calcium lactate pentahydrate of concentrations 8mM, 12mM, 16mM, 20mM and 24mM were prepared in distilled water and stabilised at 20 °C for 30 min in a water bath. Each solution of calcium ion salt was added to the alginate solution (1:1) in a thin continuous stream until a final volume of 20 ml was attained. The final concentration of Ca^{2+} in the calcium alginate gels containing 1% w/v sodium alginate were: 4mM, 6mM, 8mM, 10mM and 12mM Ca^{2+}.

Effects of sodium alginate concentration

Aqueous sodium alginate (SOBALG PH 160) of concentrations 2% w/v, 3% w/v, 4% w/v and 5% w/v were prepared in distilled water. A 16mM calcium chloride solution was prepared and mixed with each alginate solution (ALG) to form calcium alginate gels of final concentrations: 8mM Ca^{2+}/1% w/v ALG, 8mM Ca^{2+}/1.5% w/v ALG, 8mM Ca^{2+}/2% w/v ALG, and 8mM Ca^{2+}/2.5% w/v ALG.

The procedure was repeated using calcium lactate as the gelling agent to obtain calcium alginate gels with the same calcium/alginate concentrations.

Effects of different sodium alginate grades

Aqueous solutions of three molecular weight grades of sodium alginate, namely: SOBALG PH 160, SOBALG PH 165, and SOBALG PH 167 each of concentration 2% w/v, were prepared in distilled water. Stock solutions of 16mM calcium chloride and calcium lactate (100 ml) were also prepared. The solutions of calcium salts and sodium alginate were stabilised and mixed to form calcium alginate gels, each of final concentration 8mM Ca^{2+}/1% w/v alginate.

Effects of autoclaving (moist heat)

Aqueous sodium alginate (SOBALG PH 160) of concentrations 2% w/v, 3% w/v, 4% w/v and 5% w/v, as well as 100 ml solutions of 12 mM, 16 mM, 20 mM and 24 mM calcium chloride was prepared. Aqueous solutions of sodium alginate (20 ml) and calcium chloride (20 ml) were

placed in 100 ml autoclave bottles, stoppered and sterilised at 121°C for 5 min, 10 min and 15 min in a small gas ring- powered autoclave. Samples were allowed to cool and stabilised at 20 °C in a water bath for 30 min. The calcium chloride/alginate solutions were mixed in a 1:1 ratio.

Effects of ageing of calcium alginate gels

A 2%w/v sodium alginate (SOBALG PH 160) and a 100 ml solution of 20mM calcium chloride dihydrate were prepared and stabilised at 20 °C for 30 min in a water bath. Aqueous calcium chloride was added in a thin continuous stream to the alginate solution in a 1:1 ratio until a volume of 100 ml was attained. The mixture was stirred gently throughout the addition period and 2 min thereafter with a small glass rod. The concentration of the Ca^{2+}/alginate in the gel formed was 10mM Ca^{2+}/1% w/v ALG. The viscoelastic properties of the gels were determined at 30 min, 3 h, 6 h and 24 h after gel manufacture.

Measurement of viscoelasticity

The viscoelastic properties of calcium alginate gels were determined by a dynamic method, namely: oscillatory testing. The viscoelastic properties of the gels were determined at 20 ± 0.1°C with a Controlled Stress Rheometer (Carrimed CSL 150 Rheometer, UK) fitted with a parallel plate measuring system with a gap of 400 mm and system inertia of1.440 micro $N.m.s^{-2}$. Gel samples were placed onto the rheometer plate and subjected to forced sinusoidal strains at frequencies of 1 Hz to 10 Hz. Measurements were started 1 min after the sample was placed onto the rheometer plate. The viscoelastic properties of each sample, namely: storage or elastic modulus (G′), loss or viscous modulus (G^2) and the complex or dynamic viscosity (h′) were determined by computer software developed for the rheometer. Greatest emphasis, for data representation purposes, was however, placed on the reporting of storage modulus or elastic modulus (G′) of the gel samples. All experimental determinations of viscoelastic parameters were carried out in the linear viscoelastic region. Five replicate viscoelastic measurements were performed on each gel sample.

Results and Discussion

Viscoelastic materials are those that over normal time scales exhibit both viscous and elastic properties. The elastic modulus (G′) and dynamic viscosity (h′) describe the elastic and viscous properties of viscoelastic materials, respectively. Calcium alginate promotes wound healing (Morgan, 1997) and may also be used for controlled drug delivery (Shiraishi *et al.*, 1993) into wounds. For calcium alginate gels to be useful as vehicles for the slow delivery of drugs into wounds, it must possess the requisite viscous and elastic properties. The viscous property will ensure the smooth flow of the gels from containers and also on the wound surface, while the elastic property will enhance the mechanical strength of the gels and its ability to retain the drug particles within the calcium alginate gel matrices so as to ensure continuous release of drugs into wounds. Calcium alginate gels can stimulate wound healing as it has been shown to promote the growth of mouse fibroblasts (Schmidt, 1986). Doyle *et al* (1996) have also shown that calcium alginate have an effect on cell proliferation and migration which was mediated by the release of Ca^{2+} into wounds.

Sodium alginate readily formed translucent gels with calcium chloride or calcium lactate solutions, demonstrating a high affinity of Ca^{2+} for sodium alginate. An increase in concentration of the gelling agents resulted in the rapid formation of firmer, translucent gels, with a corresponding increase in the G′ of the gels (Figures 1 and 2). Both G′ and h′ were dependent on the frequency of oscillation of the samples, showing the gels to be viscoelastic. The G′ increased with increase in frequency of oscillation of the samples whilst h′ decreased with increase in frequency of oscillation.

Table 1: The elastic modulus (G′) and dynamic viscosity (η′) of calcium alginate gels containing 1% w/v sodium alginate (SOBALG PH 160) produced from different calcium salts

Concentration of calcium salt (mM)	Calcium lactate		Calcium chloride	
	G′ (Pa)	η′ (Pa.s)	G′ (Pa)	η′ (Pa.s)
6	21 ± 2.2	0.5 ± 0.001	19 ± 2.1	0.5 ± 0.001
8	90 ± 6.7	1.7 ± 0.01	93 ± 6.9	2.0 ± 0.01
10	145 ± 12.4	4.7 ± 0.3	145 ± 11.9	4.5 ± 0.3
12	309 ± 22.7	13.9 ± 1.1	312 ± 24.0	13.9 ± 1.2

Viscoelastic properties were determined at 1 Hz, 20 ± 0.1°C
Data reported as the mean ± SD of 5 gel samples

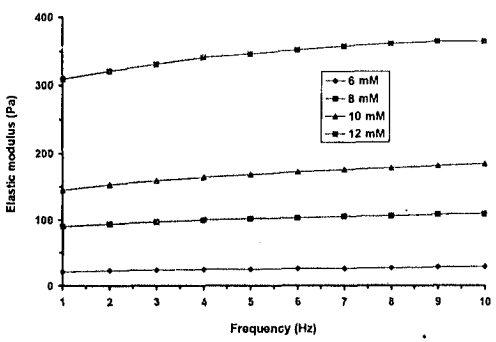

Fig. 1: Effects of calcium chloride concentration on the elastic modulus of calcium alginate gets containing 1% w/v sodium alginate (SOBALG PH 160)

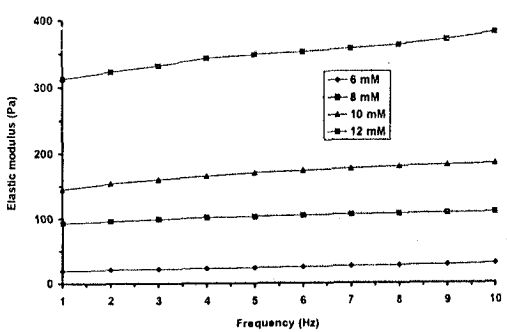

Fig. 2: Effects of calcium lactate concentration on the elastic modulus of calcium alginate gets containing 1% w/v sodium alginate (SOBALG PH 160)

There was no significant difference ($p > 0.05$) between the viscoelastic properties of gels prepared using Ca^{2+} from two different sources (chloride and lactate) (Table 1). The problem of syneresis was however, less pronounced in gels produced with calcium lactate than with calcium chloride. Calcium alginate gels are formed by cation exchange between Na^{+} in sodium alginate and Ca^{2+} in calcium chloride or calcium lactate (Haug and Smidsrod, 1965). The gel formation and cross-linking processes are due to the stacking of the guluronic acid blocks (G-blocks) of sodium alginate with the formation of an egg-box junction (Grant *et al.*, 1973; Bryce *et al.*, 1974). The elastic modulus (G′) gives an indication of the mechanical strength of calcium alginate gels. As higher Ca^{2+} concentrations resulted in the formation of firmer gels with higher G′ values, it is presumed that an increase in Ca^{2+} leads to the strengthening of the forces by which the ions bind sodium alginate with subsequent enhancement of the mechanical strength of the calcium alginate gels. The increased strength of the cross-linked gel matrices would help in reducing the rate of erosion of the calcium alginate gel matrices in

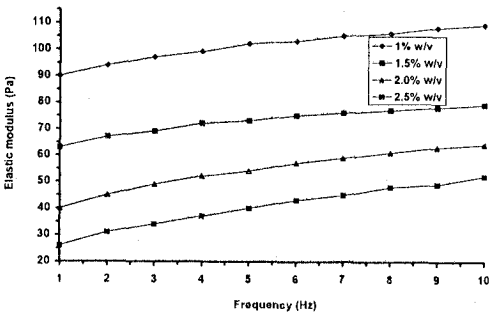

Fig. 3: Effects of sodium alginate (SOBALG PH 160) concentration on the elastic modulus of calcium alginate gels containing 8 mM calcium chloride

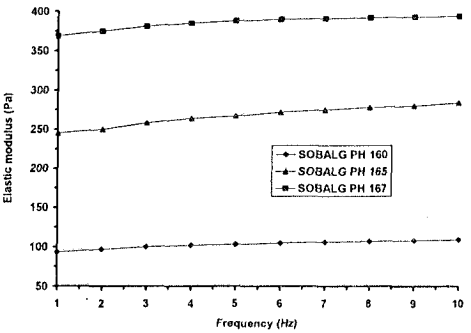

Fig. 4: Effects of different sodium alginate grades on the elastic modulus of calcium alginate gels (8 mM calcium lactate/1% w/v sodium alginate)

aqueous fluids thereby preventing the rapid release of incorporated medicaments when utilised as a slow-release drug delivery system into wounds.

At a constant Ca^{2+} concentration of 8mM, an increase in sodium alginate concentration (SOBALG PH 160) from 1% w/v to 2.5% w/v resulted in a decrease in the G′ of the gels (Figure 3), and the formation of loosely formed calcium alginate gels. This occurred when both calcium chloride and calcium lactate were used as gelling agents. It is presumed that at a concentration of 1% w/v, the Ca^{2+} concentration used in forming the gels was enough for complete reaction with the alginate. Thus, as the sodium alginate concentration was raised to 1.5% w/v and above, the Ca^{2+} became inadequate for complete reaction with the alginate leading to the formation of poorly formed gels with loose gel matrices.

Figure 4 shows the effect of different alginate grades on the G′ of calcium alginate gels. At a particular frequency of oscillation, the G′ of a gel containing 1% w/v sodium alginate and 8mM calcium chloride or calcium lactate increased in the order of alginate grade used as follows: SOBALG PH 160 <SOBALG PH 165 <SOBALG PH 167. The molecular weights and viscosities of the different grades of alginate used as well as the firmness of the gels formed also follow this order. Alginates are block-copolymers containing the homopolymeric blocks, MM and GG, together with blocks with an alternating sequence (Smidsrod, 1974). The GG-blocks have better selective binding with Ca^{2+} in solution than the MM and MG-blocks. The affinity of alginates for Ca^{2+} and the stability of the gels formed, increases with increasing content of L-guluronic acid (G) residues in the alginate. The proportion of guluronic acid (G) residues in the different alginate grades used also followed the order outlined above.

For the gels to be used in wound healing, it is important that an appropriate method of sterilization be found to prevent possible cross-infection of wounds. Gels produced with autoclaved sodium alginate and calcium chloride solutions had a remarkably lower G′ as compared to those produced with un-autoclaved sodium alginate and calcium chloride solutions. Also, the longer the time of autoclaving the lower the G′ values (Figure 5). The viscosities of sodium alginate solutions reduced after autoclaving at 121 °C for 5, 10 and 15 min. The reduction in viscosity increased with an increase in autoclaving time. No change in the flow properties of calcium chloride solutions was observed after autoclaving. The high temperature (121°C) that the sodium alginate solutions were subjected

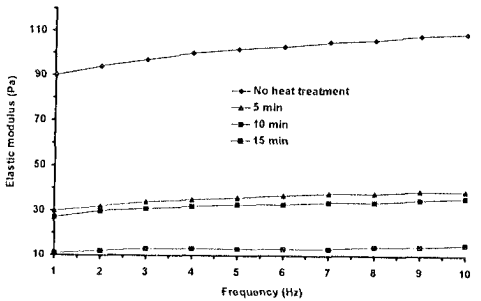

Fig. 5: Effects of autoclaved sodium alginate and calcium Chloride solutions on the elastic modulus of calcium alginate gels (8 mM calcium chloride/1% w/v alginate (SOBALG PH 160)

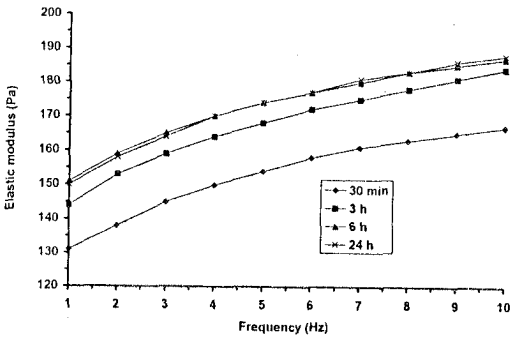

Fig. 6: Effects of ageing on the elastic modulus of calcium Alginate gels (10 mM calcium chloride/1% w/v sodium alginate (SOBALG PH 160)

to would cause the depolymerisation of the alginate structure, resulting in loss of viscosity and subsequent reduction in the G ′ of the gels. Autoclaving different concentrations of sodium alginate at 121 °C for 15 min resulted in a reduction of 51-95% of the G ′ of the resultant calcium alginate gels. The results of the gel sterilization appear to be similar to those reported in the literature. Vandenbossche and Remon (1993) have reported that autoclaving caused a 64% decrease in viscosity while heating during ethylene oxide sterilization resulted in reduced viscosity and breakdown of alginate dispersions. Leo *et al* (1990) observed that the gel strength of calcium alginate beads decreased with increasing sterilization temperature due to an increase in depolymerisation of the alginate molecules and that ethylene oxide and gamma-irradiation modes of sterilization caused similar effects. Thus, a more appropriate method of sterilization that causes no loss of viscosity and no reduction in gel strength should be considered for the sterilization of calcium alginate gels intended for wound healing.

The G′ of the calcium alginate gels increased with ageing of the gels (Figure 6). When the gel sample was oscillated at a frequency of 1 Hz, the G′ increased by 15% after 24 h storage at room temperature. The increase in G′ appeared to peak 24 h after gel manufacture. This appears to be in agreement with observations made by Mitchell and Blanchard (1976) that the strength of calcium alginate gels increases for a period up to 24 h after preparation, though Bolister (1989) has shown by atomic absorption that all calcium binding by alginates occurs within 30 min of exposure to calcium ions. It is believed that the strength of the inter-chain binding between alginates and Ca^{2+} increases with ageing of the gels up to a point. It thus seems advisable to age calcium alginate gels for 24 h in order to obtain gels with much strengthened cross-linked matrices.

CONCLUSION

The study has shown that it is possible to confer different structural and viscoelastic properties upon alginate gels by varying the type and concentration of Ca^{2+} used as the gelling agent and the concentration and viscosity of sodium alginate used. Autoclaving of sodium alginate solutions causes a significant reduction in the viscosities and G′ values of the resultant calcium alginate gels hence sterilization of the gels by moist heat is inappropriate. Ageing of calcium alginate gels increases the mechanical strength of the gels making them more appropriate as vehicles for drug delivery.

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