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Absorbent alginate fibres modified with hydrolysed chitosan for wound care dressings – II. Pilot scale development

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ABSTRACT

Fibres have been used extensively in wound dressing applications as they provide a high surface area for absorption, ease of fabrication and softness. It is common practice for commercial wound dressings to be produced from natural materials, such a marine polysaccharides, as they are predominantly biocompatible, non-toxic, and often display bioactive properties, such as inherent antimicrobial activity. In this study hydrolysed chitosans were utilised as a sole coagulant for the production of alginate–chitosan fibres via a one-step, direct wet-spinning extrusion process. The levels of chitosan incorporated into the fibres were analysed quantitatively via elemental analysis and qualitatively by staining using Amido Black 10B. It was estimated that the fibres contained between 4.50 and 5.10% (wt.%) chitosan. The presence of chitosan improved tensile properties such as elongation and tenacity of the base alginate fibres. The increased incorporation of chitosan into the fibres also improved the absorption of the fibres in both saline and distilled water; reaching maximum of >30 g/g and >50 g/g, respectively. This work suggests that the observed hydrolysed chitosan content within the fibre may be optimal for the preparation of a novel fibre for wound care application.

1. Introduction

A large proportion of commercially available wound dressings are produced from fibres that are woven or more extensively as nonwoven structures whereby the fibres are entangled and/or needled. Fibres are often preferentially chosen, especially when designing an absorbent wound dressing, as they have a high surface area, improved absorbency, softness and are economically viable (Knill et al., 2004; Petrulyte, 2008). Carbohydrate-based fibres, made from naturally occurring polysaccharides are desirable materials for wound dressings as they are biocompatible, non-toxic, and potentially have the ability to potentiate the healing cascade in different wounds thus reducing time to healing. Two such polysaccharides are alginate and chitosan.

Alginates are polyanionic, linear, binary copolymers of β-(1-4)-linked D-mannuronic acid (M) and its C-5 epimer α-(1-4)-linked L-guluronic acid (G) and are usually presented as the sodium salt (Draget, Oestgaard, & Smidsrod, 1990; Meng et al., 2010). Alginates are naturally occurring polysaccharides which exist widely in the cell walls and intracellular spaces of many species of brown seaweeds (*Phaeophyceae*). The source of alginate determines its monomer composition. Alginates contain three types of blocks, homopolymeric G-blocks (GGG) and M-blocks (MMM) and heteropolymeric blocks of MG (MGMG). The monomers have a tendency to stay in their most energetically favourable structure in the polymer chains so G-blocks and M-blocks have different conformations which are buckled and ribbon-like, respectively. The chemical and physical properties of the alginate are determined by the proportion and distribution of these segments, along with their relative sequencing (Batchelor et al., 2002; Davidovich-Pinhas & Davidovich-Pinhas, 2010). Alginates have the ability to gel via two mechanisms: ion dependently and ion independently. The ion dependent mechanism occurs at pH values above the pK_a of alginate, the pK_a of alginate lies between 3.38 and 3.65, and in the presence of divalent cations such as calcium ions (Ca^{2+}). Under these conditions bound sodium ions are exchanged with calcium ions, in solution, which causes the dimerisation of adjacent alginate chains, forming a three-dimensional, hydrophilic and yet insoluble network. It is believed that ion dependent gelling of alginates is decided by the average length of the poly-G-blocks as alginates containing high levels of G fractions form much stronger gels. High M alginates gel much faster and are more flexible, but lose their integrity sooner (Goh, Heng, & Chan, 2012; Grant, Morris, Rees, Smith, & Thom, 1973). Alginates also form acidic gels at pH values below the pK_a values of the uronic residues (Simsek-Ege & Bon, 2003). This sol–gel transition is due to the increase in

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intermolecular hydrogen bonds between the alginate chains as the polymer becomes uncharged due to the protonation of the carboxyl groups on the uronic residues. Both the poly-G and poly-M-blocks play a major role in the formation of the gel structure (Draget, Skjåk-Bræka, & Stokkeb, 2006).

Chitosan, the other prevalent natural polysaccharide is the partially *N*-deacetylated derivative of chitin which is found primarily in the exoskeletons of animals of the *Phylum Arthropoda* such as crustaceans, the endoskeletons of cephalopods such as squid, the radulas of molluscs, and in the cell walls of most fungi. Commercially, it is most commonly extracted from the shells of crustaceans such as crab, shrimp, lobsters and krill (Majeti & Kumar, 2000). Chitosan is a linear muco-polysaccharide, composed primarily of repeating glucosamine and N-acetyl-D-glucosamine (NAG) units. Chitosan can form gels at physiological pH if the polymer is modified to form a water-soluble derivative such as carboxymethyl chitosan. Otherwise unmodified chitosan has poor solubility (only soluble in acidic environments) and cannot gel in situ at physiological pH values (as the amino groups on the glucosamine become deprotonated and chitosan precipitates as a solid).

Polyelectrolyte complexation can occur between alginate and chitosan as they are oppositely charged polysaccharides; alginate is polyanionic and chitosan is polycationic. However this interaction is dependent on the local pH. Alginate is only negatively charged above its pK_a (3.38–3.65) and chitosan is positively charged below its pK_a (~6.3) (Davis, Zivanovic, D'Souza, & Davidson, 2012). Thus, under appropriate conditions, alginate and chitosan can aggregate and coagulate to form a polyelectrolyte complex (PEC). The chitosan–alginate complex is formed by the strong electrostatic interaction between the amine and carboxyl groups of chitosan and alginate, respectively (Gierszewska-Druzynska & Ostrowska-Czubenko, 2009). The driving force behind complexation is due to the strong coulombic interactions between the opposite charges which leads to interpolymer ionic condensation. Complexation is a speedy process and is predominantly controlled by counterion diffusion (Thünemann, Müller, Dautzenberg, Joanny, & Löwen, 2004) and other factors such as the molecular weight of the polymers, the pH of the solutions, degree of deacetylation (DD) of the chitosan, charge density of the polymers, the temperature during reaction, duration of the reaction and the solvents used (Hoffman, 2002; Meng et al., 2010).

Gel-forming materials, such as alginates, can have a significant impact on the healing of wounds, when applied correctly, as they are capable of absorbing large volumes of liquid in comparison to their weight; keeping the wound site moist and allowing water vapour permeability. They also permit oxygen penetration, and can easily be removed from the site of application (Boateng, Matthews, Stevens, & Eccleston, 2008). Chitosan, although naturally non-gelling, is inherently bioactive at a cellular level which encourages wound healing. Therefore, the combination of these two polymers in the form of a fibre under an appropriate condition would create a synergistic effect; taking advantage of alginate's natural ability to gel at physiological pH and the biological attributes of chitosan, such as haemostasis, creating a fibre that could have potential in the wound care arena.

With this realisation, many research groups have produced wet-spun alginate–chitosan composite fibres. Fan et al. produced alginate–chitosan fibres by blending chemically modified water-soluble chitosans, with the sodium alginate dope before extrusion into a bath of calcium chloride (Fan et al., 2006; Fan et al., 2010). The calcium alginate–(N,O)-carboxymethyl chitosan fibres produced, although proven to be antibacterially active after the addition of silver, were not absorbent. Increasing the chitosan content within the dope improved the water solubility of the fibre; however, the fibre containing the highest chitosan concentration only absorbed 4 g/g water. Watthanaphanit et al. produced a novel spotted alginate chitosan fibre by emulsifying unmodified chitosan to blend with the sodium alginate dope before extrusion into a calcium chloride bath. Emulsifying the chitosan before blending prevented the formation of a gel between the oppositely charge polysaccharides (Watthanaphanit et al., 2009). However the distribution of chitosan along the length of these fibres was inhomogeneous, and patchy.

Core-sheath calcium alginate–chitosan fibres have been formed by extruding already formed calcium alginate fibres into a second bath containing an unmodified, high molecular weight chitosan solution (Tamura, Tsuruta, & Tokura, 2002). However, these fibres displayed only small quantities of chitosan (below 0.2 wt.%). Knill et al. (2004) demonstrated that bathing previously wet extruded calcium alginate or alginic acid fibres in hydrolysed chitosan, lower molecular weight chitosan, increased the level of chitosan incorporation into the fibre; reaching a maximum of 25 wt.%. However, since the chitosan is essentially used as a coating in this technique, the amount of chitosan on the fibre was inhomogeneous, leading to large discrepancy in chitosan contents between fibres. (1) These fibres had tenacities in the range of 2.8–10.5 cN/tex, water absorption values of between 16.3 and 35.0 g/g and saline absorption values of between 11.5 and 12.1 g/g; however, these fibres did not gel, a criteria most preferred by primary care providers. Miraftab and Smart (2008), following from Knill et al.'s research, discovered that the hydrolysed chitosan could be used as the sole coagulant for sodium alginate thus forming a hybrid fibre with inbuilt alginate and chitosan content. This new techinque allowed the user to directly extrude sodium alginate into a hydrolysed chitosan and containing bath which is a speedier process and more suitable for commerical scale-up. The aim of this study was therefore to modify this one-step, wet-extruded fibre such that gelling would become an integral part of the product.

2. Experimental

2.1. Materials

Chitosan was supplied by Kate International (Source: crab, molecular weight (Mw) ≈ 1000 kDa, degree of deacetylation (DD) 84%, viscosity (η) 50–100 cPs: 1% soln. AcOH). Sodium alginate was supplied by FMC Biopolymer, Scogin LV; M/G ratio is 45–55/45–55, viscosity 50–70 cPs, acetic acid (AcOH, CH_3COOH, 99%, Fisher Chemicals), methanol (Fisher Chemicals), calcium chloride dihydrate ($CaCl_2$, Fisher Chemicals), hydrochloric acid (HCL, 37% (w/v) analytical reagent grade, Fisher Scientific), sodium hydroxide pellets (NaOH, Fisher Scientific), sodium chloride (NaCl, Fisher Chemicals) and Amido Black 10 B dye (Sigma–Aldrich, UK).

2.2. Production of fibre

2.2.1. Dope

Sodium alginate (4.0%, w/v) was dissolved in deionised water whilst stirring for 5 h. The solution was then degassed by leaving it in the extrusion tank for 24 h.

2.2.2. Coagulant

The coagulant was prepared by dissolving 60.0 g of pre-dried chitosan in aqueous acetic acid solution (1%, w/v) using deionised water. The chitosan solution was stirred until a clear, viscous solution was obtained. Hydrochloric acid (HCL, 50 ml) was then added to the solution under further stirring until homogeneity was obtained. The chitosan solution was then heated under reflux for approximately 8 h, cooled, and filtered (to remove any insoluble material). The chitosan viscosity dropped after hydrolysis from ~480 to 50 cps and the pH of the solution largely remained unchanged before and after hydrolysis (i.e. <1.00). The average

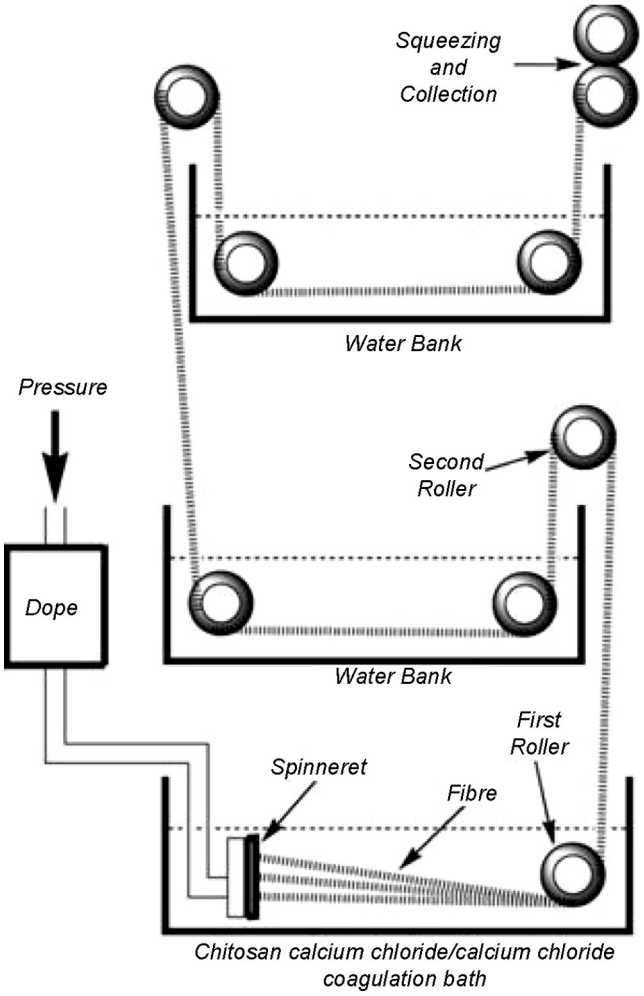

Fig. 1. An illustration of the multi-functional wet extruder (Knill et al., 2004).

molecular weights of the chitosan before hydrolysis were M_n 144.5; M_w 993.2; M_z 2666.7; M_{z+1} 4338.4 and M_v 813.6 (kDa). After hydrolysis the average molecular weights were; M_n 7.3; M_w 32.3; M_z 121.1; M_{z+1} 256.0; and M_v 25.8 (kDa). All were measured by GPC chromatographic analysis. To the hydrolysed chitosan solution was added varying concentrations of calcium chloride, 0.30%, 0.35%, and 0.40% (w/v), under constant stirring for 30 min. The pH of the coagulant was then incrementally increased using sodium hydroxide until the solution reached pH 5.0. The solution was stirred thoroughly to ensure the full dissolution of all flocculants.

2.2.3. Production of alginate–chitosan fibre on pilot scale extruder

All fibres were produced using a multi-functional laboratory wet extruder (built and designed in-house by Howden Engineering Services, UK), illustrated in Fig. 1. The degassed spinning sodium alginate dope solutions were ejected under pressure (~22 bars) at a flow rate (9 cm^3/min) through a spinneret (100 holes, 76 μm diameter) into the coagulation bath to produce the hybrid alginate–chitosan fibres. The resultant fibres were then collected by the pickup rollers and passed through a wash bath containing deionised water whilst being drawn between the first and second sets of rollers at a set draw ratio (DR) of 1.1 (DR is the speed of 2nd roller/speed of 1st roller) to improve the tenacity of the fibre by increasing the crystallinity of the fibre (Table 1). The fibres were then squeezed, between rollers, to remove excess liquid, wound up, and removed from the extruder ready for drying. Three alginate–chitosan fibres were produced: A0.30 is the alginate–chitosan fibre produced using 0.30% (w/v) $CaCl_2$ in coagulation bath, A0.35, the alginate–chitosan fibre produced using 0.35% (w/v) $CaCl_2$ and A0.40, the alginate–chitosan fibre produced using 0.40% (w/v) $CaCl_2$. All collected fibres were then dried by immersion in a series of aqueous methanol baths for 15 min. After treatment with methanol, the fibres were then shaken by hand (to remove the excess solvent) and then left overnight at room temperature to dry fully (methanol is neurotoxic by absorption through skin and by inhalation so an alternative drying method can be employed such as using acetone washes and hot air drying). Calcium alginate fibres were also produced as control fibres.

2.3. Physical and chemical testing of fibres

2.3.1. FTIR analysis

FTIR spectroscopy was adopted to analyse the composition of the alginate–chitosan and calcium alginate fibres (Nicolet Magna 750 FTIR Spectrometer coupled with a NicPlan FTIR microscope). The spectrum were acquired using a germanium ATR crystal by collecting 200–300 scans with a wavenumber range of 500–4000 cm^{-1} and 8 cm^{-1} resolution. Background spectra were acquired through the ATR element when not in contact with the sample. The fibres A0.30, A0.35, A0.40 and C were analysed by FTIR spectral analysis to see if there were any noticeable differences in the infrared spectrogram between fibres.

2.3.2. Linear density

The linear density (dtex) is known as the mass per unit length of a fibre and this was calculated for all samples using a Vibromat M (Textechno, Germany) single linear density tester. During this test a single fibre is clamped vertically and tensioned by a predetermined weight (to remove any crimp) and subsequently exposed to transverse vibrations at variable frequencies (ASTM D1577). The linear density can then be calculated from the fundamental resonant frequency of transverse vibration of the fibre; measured under known conditions of length and tension. The linear density, or fibre fineness, is then calculated using the following formulae:

$$\text{Linear density (dtex)} = \frac{t}{4L^2{f_1}^2} \times 10^5$$

where t is the fibre tension (Dynes), L the effective fibre length (distance between fibre contact points) (mm) and f_1 the fundamental resonant frequency (Hz).

Table 1
The parameters and materials required for the production of the control, calcium alginate fibre (C) and alchite fibres A0.30, A0.35 and A0.40.

Fibre label	Draw ratio	Spinneret		Pump speed (cm^3/min)	Content of 1st coagulation bath	Content of 2nd bath
		No. of holes	Size of holes (μm)			
C	1.1	100	76	9.00	$CaCl_2$ (1.00%, w/v)	Deionised H_2O
A0.30					$CaCl_2$ (0.30%, w/v) + HC[a] (≈4%, w/v)	
A.035					$CaCl_2$ (0.35%, w/v) + HC[a] (≈4%, w/v)	
A0.40					$CaCl_2$ (0.40%, w/v) + HC[a] (≈4%, w/v)	

[a] HC, hydrolysed chitosan.

2.3.3. Tensile properties

The tensile properties of the fibres were measured using a Fafegraph ME (Textechno, Germany) tensile tester (EN ISO 5079). This is a semi-automatic, microprocessor controlled tensile tester, which works according to the principle of a constant rate of extension (CRE). A single fibre was clamped between too points, one moveable and one fixed, 10 mm apart, and force applied (the load cell was 10 N and set at 20 mm/min constant rate of extension). The applied force and the displacement of the moveable clamp were measured internally. To gain the mean breaking stress (tenacity, cN/tex/cN/dtex) of the fibre, the applied force was related to the pre-determined linear density (weight per unit length) of the fibre. Tenacity defines fibre strength with respect to linear density, tenacity = (B_L/D_L), where B_L is the break load (N) and D_L, the linear density (dtex), which is the fibre weight in g/10 000 m. The mean breaking strain (elongation, %) is calculated from the measured displacement related to the free length of the fibre and is expressed as a percentage of the original nominal length. The % elongation is determined from the ratio of the break length to the original length, % elongation = (L_b/L_o) × 100, where L_b is the break length and L_o, the original length (lengths must be in the same units).

2.3.4. Chitosan content of alginate–chitosan fibre

2.3.4.1. Elemental analysis. The chitosan content of the fibres was determined by analysing the nitrogen content (on a weight percentage basis, wt.%) using elemental analysis (Thermo Finnigan EA1112 Series Flash Elemental Analyzer, UK). Elemental analysis is a process that analyzes the elemental composition of a chemical compound by examining the weight percentage of each element in the compound to determine the compound's composition. The CHN elemental analyzer identifies the amount of carbon, hydrogen and nitrogen in a sample. The estimated fibre chitosan contents assume that any measured nitrogen arises solely from hydrolysed chitosan (anhydro-GlcN/GlcNAc residues) and not from any residual proteins present in either the alginate or chitosan raw materials. To convert the weight percentage of nitrogen to the chitosan content (wt.%) in the fibre sample, the following formula were used:

First, calculate the average molecular weight (Mw) chitosan (C):

$$C = (161\ \text{g/mol} \times \text{DD}) + ((1 - \text{DA}) \times 203\ \text{g/mol})$$

whereby 161 g/mol is the molecular weight of fully deacetylated chitosan and 203 g/mol, the molecular weight of fully acetylated chitosan, DD, the degree of deacetylation of chitosan raw material, and DA, the degree of acetylation (fully de-N-acetylated, i.e. degree of deacetylation = 1.00, degree of acetylation = 0.00).

Secondly,

$$\text{Chitosan}\ \ (\text{wt.\%}) = \frac{N\,(\text{wt.\%}) \times C\,(\text{g/mol})}{N\,(\text{g/mol})}$$

whereby N (wt.%) is the weight percentage of nitrogen in the fibre as determined by elemental analysis, C (wt.%) is the average molecular weight of chitosan raw material, and N (g/mol) is the molecular weight of elemental nitrogen.

2.3.4.2. Staining fibres. All fibres were dyed using an aqueous solution of 0.01% (w/v) Amido Black 10B, an amino acid staining diazo dye. The fibres were left immersed in the dye solution for approximately 8 h. After 8 h the fibres were removed and washed thoroughly using distilled water. It is expected that the dye will only stain the chitosan containing fibres (Watthanaphanit et al., 2009).

2.3.5. Absorbency

The absorbencies of the fibre samples were measured using the immersion centrifuge method (Lord, 1964) Small fibre bundles of similar weight (~0.5 g) were immersed in separate test tubes containing 5 ml of saline (0.9%, w/v sodium chloride) and distilled water for 5 min. The excess moisture from the fibre samples were removed by placing them in a centrifuge (HOWE Sigma 2-15 Centrifuge) set at 1200 revolutions per minute (RPM) for 15 min. The wetted fibres were then weighed and their wet weight recorded before placing the fibre samples into Petri dishes and into an oven held at 105 °C for 24 h. The dry fibre samples were then removed and reweighed and this was recorded as their dry weight. This process was repeated 10 times for accuracy for each fibre and each solution. The absorptions of the fibres (g/g) were calculated using the following equation:

$$\text{Absorption}\ \ (X)\ \ (\text{g/g}) = \frac{W_1 - W_0}{W_0}$$

where X, solution fibre is immersed in; W_1, wet weight of fibre; and W_o, dry weight of fibre.

2.3.6. Optical microscopy and swelling behaviour of fibres

The swelling behaviours of the fibres were determined using a Nikon-Alphaphot-2 YS2-H optical transmission microscope. Image capture was performed using a Hitachi VK-C150E video link at a magnification of 200×. Single dry fibres were placed onto microscopic glass slides and viewed under the microscope. Drops of distilled water were pipetted onto the fibres and left for 5 min to allow for maximum swelling. The fibre diameter measurements were performed using Image Pro software. Optical microscope images were taken at all stages. The swelling of the fibre samples were then calculated using the following formulae:

$$\text{Swelling}\ \ (\%) = \left[\frac{D_s - D_o}{D_o}\right] \times 100$$

where D_o is the dry fibre diameter and D_s, the swollen fibre diameter.

3. Results and discussions

It is difficult to quantify the ability of a material to gel, it can only be qualitatively assessed. A gelling material is often described as consisting of a soft, solid, or solid-like materials of two or more components, one of which is a liquid, present in substantial quantity. The process of gelation is often referred to as the linking of macromolecular chains together which initially leads to progressively larger branched yet soluble polymers depending on the structure and conformation of the starting material. As the crosslinking increases this results in more highly branched polymer with decreasing solubility (Almdal, Hvidt, & Kramer, 1993). To improve the gelling and absorption of the alginate–chitosan fibre produced by Miraftab and Smart (2008), the pH of the hydrolysed chitosan was increased to pH 5.0, a value that lies below the pK_a of chitosan (~6.3) and above the pK_a of alginate (3.38–3.65). This allows for polyelectrolyte complexation to occur between the two polymers during the wet-extrusion process and prevents the formation of an acid-based, alginic acid fibre, which does not have the ability to gel. Polyelectroyte complexation increases the physical crosslinking between the polymers which has the ability to swell. During the extrusion, the polymers self-assemble and a precipitate is formed; the removal of the precipitate from the interface of the polyelectrolyte solutions enabled the formation of a fibre as seen by Liao, Wan, Yim, and Leong (2005). Small additions of calcium chloride to the coagulant improved the precipitation of the fibre and also encouraged fibre gelling as calcium alginate is well known to gel in the presence of sodium ions. The fibre was optimised by extruding sodium alginate into three diferent coagulation baths containing hydrolysed chitosan (pH 5.0) with varying concentrations of calcium (0.30, 0.35, and 0.40%, w/v) to find the ideal concentration. Varying the calcium concentration can affect fibre

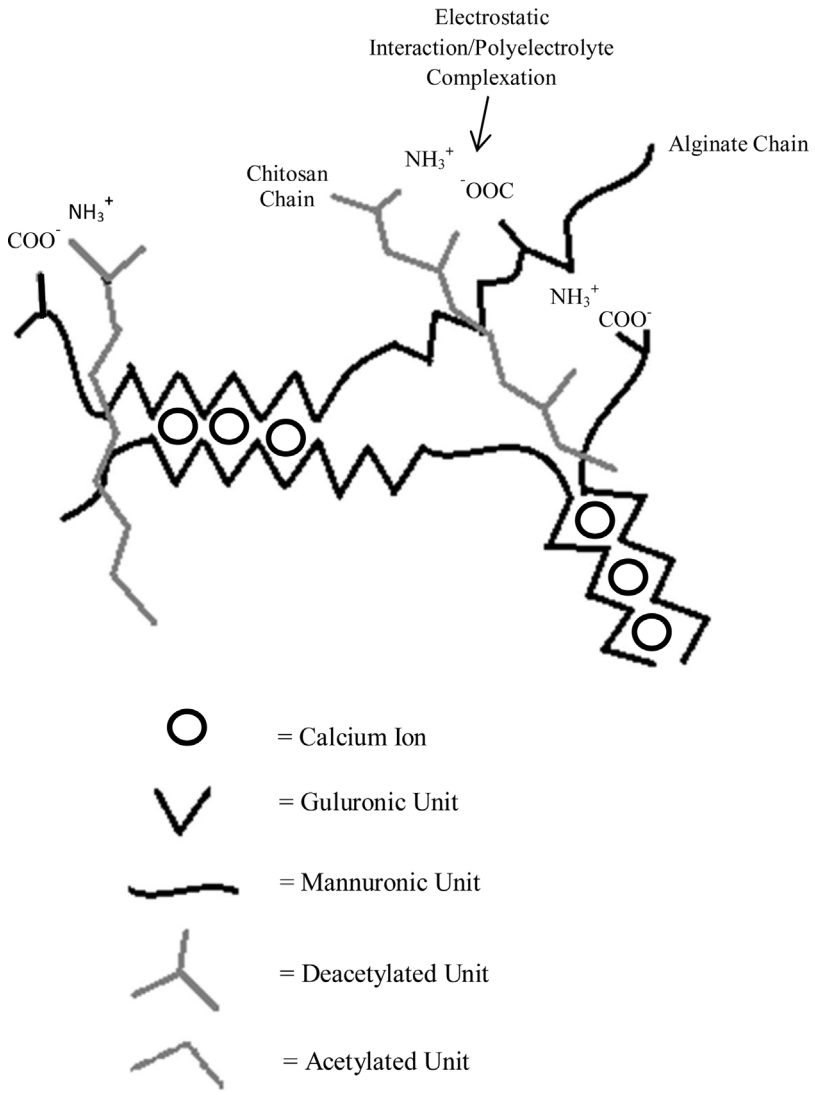

Fig. 2. The potential electrostatic interaction between the exposed, deprotonated carboxyl groups along the alginate backbone and the protonated amino groups along the hydrolysed chitosan chains and the calcium alginate "egg-box" structure caused by the dimerisation of the alginate chains by calcium ions. The positively charged hydrolysed chitosan amino groups and calcium ions compete for binding with the negatively charged carboxyl groups along the alginate backbone.

properties such as chitosan content (wt.%), absorption (g/g), and tenacity (cN/dtex). The potential interactions that occur between the materials during the extrusion process are schematically represented in Fig. 2.

3.1. FTIR analysis

Four fibres were examined using FTIR analysis: A0.30, A0.35, A0.40 and C (Fig. 3). The calcium alginate spectra (*C*) showed a peak at 3327 cm^{-1} indicative of O—H stretching, another at 1600 cm^{-1} indicative of asymmetric carboxylate stretching and a peak at 1409 cm^{-1} due to symmetric carboxylate stretching.

Spectra A and B were produced using decreasing amounts of calcium chloride in the coagulation bath, respectively. Spectrum A, the A fibre, has the highest chitosan content (wt.%) from elemental analysis (Table 2), and it appears that the intensities of the 1600 cm^{-1} and 1409 cm^{-1} peaks, associated with asymmetric carboxylate and symmetric carboxylate stretching, respectively, decrease with increasing chitosan content within the fibre. A peak appears to form at 1750 cm^{-1} which is indicative of amide bond stretching. These spectra do indicate that the fibres produced with a lower concentration of calcium ions within the coagulant contain more chitosan (wt.%). However, further analyses were required to quantitatively confirm the presence of chitosan.

3.2. Linear density and tensile properties

The linear densities of the alginate–chitosan fibres decreased with increasing calcium content within the fibre from A0.30 through to A0.35, as did the value for standard deviation, which indicates that the fibres A0.35 and 0.40 are more homogenous in structure (Table 2). Fibre elongation (%) decreases with increasing calcium concentration, implying that the fibres become less flexible with increasing calcium content (wt.%). All alginate–chitosan fibres have higher values of elongation than the control calcium alginate fibre which again may well be due to the presence of chitosan within the alginate–chitosan fibres.

3.3. Chitosan content of alginate–chitosan fibre

3.3.1. Elemental analysis

The chitosan contents (wt.%) of fibres C, A1, A2 and A3 were obtained using elemental analysis, via the detection of nitrogen, and these results are adduced in Table 2. It is clearly evident that the

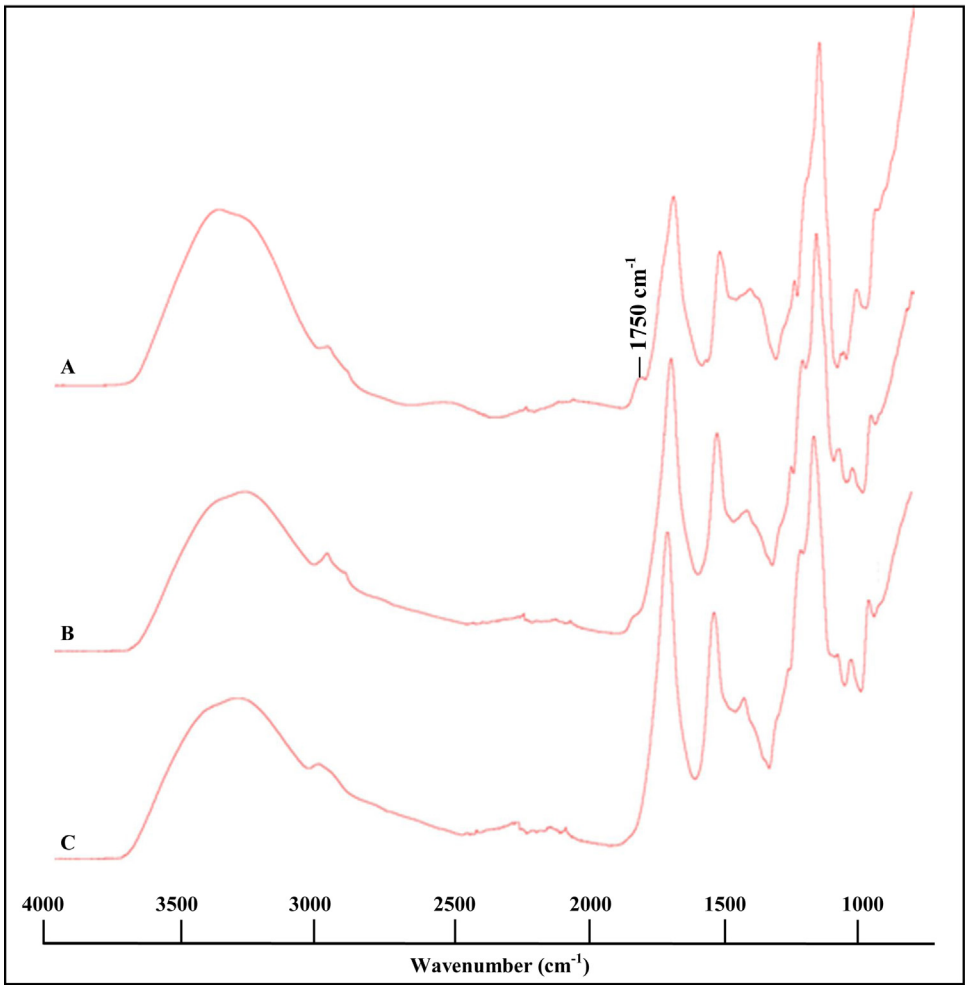

Fig. 3. FTIR absorbance spectra of (A) the fibre A0.30, (B) the fibre A0.40, and (C) the fibre C.

Table 2
Values of absorption of fibres C, A0.30, A0.35, and A0.40 in deionised water and saline and their respective tensile properties and chitosan contents.

Fibre label	Deionised water absorption (g/g)[a]	Saline absorption (g/g)[a]	Elongation (%)[a]	Linear density (dtex)[a]	Tenacity (cN/dtex)[a]	Chitosan content fibre (wt.%)[a]
C	2.34 ± 0.43	18.30 ± 1.13	6.97 ± 0.87	5.20 ± 0.74	8.32 ± 0.96	0.00
A0.30	56.76 ± 1.57	32.30 ± 2.24	13.60 ± 1.80	11.50 ± 3.31	11.42 ± 2.76	5.11 ± 0.18
A0.35	52.83 ± 1.46	29.21 ± 1.71	11.69 ± 1.49	6.14 ± 0.93	4.52 ± 1.21	4.92 ± ±0.20
A0.40	43.26 ± 1.34	25.24 ± 1.68	9.67 ± 1.39	5.15 ± 0.63	7.88 ± 0.78	4.51 ± 0.07

[a] Results are represented as a mean ± S.D.

chitosan contents within the fibres increased with the utilisation of a decreased concentration of calcium within the coagulation bath, e.g. the A0.30 fibre contained a maximum chitosan content of 5.11 (wt.%) and this reduced to a maximum of 4.51 (wt.%) within the A0.40 fibre.

3.3.2. Staining fibres

Amido Black 10 B was used to stain all the hybrid alginate–chitosan fibres produced and the pure calcium alginate fibres as a control. Amido Black 10 B is an anionic dye and can easily be absorbed by the cationic amino groups present along the chitosan backbone ($-NH^{3+}$) (Bingchao, Ruihua, & Qian, 2012). It was observed that the fibres that contained a higher chitosan content (wt.%) ultimately absorbed more dye and hence were darker in colour (Fig. 4). This trend has also been observed by Watthanaphanit et al. (2009). The calcium alginate fibres absorbed a small amount of the dye but the colour difference between the chitosan-containing fibres was clearly apparent. This qualitative data confirm the data observed from the quantitative analysis and thus implies that the positively charged calcium ions and positively charged amino groups on the chitosan glucosamine monomers compete for the negatively charged carboxyl groups along the sodium alginate backbone. Thus a decrease in the concentration of calcium ions within the bath will allow more of the hydrolysed chitosan chains to electrostatically bind and coagulate the sodium alginate dope which ultimately increases the chitosan content (wt.%) within the fibre.

3.3.3. Absorbency and gelling

Overall, a noticeable difference between the absorption capacities of the calcium alginate fibres (C) and the hybrid alginate–chitosan fibres were observed. Additionally a marked variation in absorption between the different alginate–chitosan fibres using ascending calcium chloride concentrations (0.3–0.4%,

Fig. 4. Photographic images of fibres A0.30, A0.35, A0.40, and C, left-to-right, after staining with 0.01% (w/v) Amido Black 10B aqueous solution for 12 h.

w/v), was also observed. The water and saline absorption of the alginate–chitosan fibres decreased with increasing calcium concentration within the coagulation bath from a maximum of 56.76 ± 1.57 to 43.26 ± 1.34 (g/g) and 32.30 ± 2.24 to 25.24 ± 1.68 (g/g), respectively. A reduction in the saline absorption, with increasing calcium concentration in the bath, could be explained by the reduction of sodium within the fibre as more and more calcium ions exchange with the sodium ions within the fibre. The higher the calcium concentration within the bath, the faster the rate of diffusion of calcium ions into the fibre, until a saturation point is reached whereby all of the guluronic groups are fully occupied in the "egg-box" formation as observed by Cuadros, Skurtys, and Aquilera (2012). For all Alginate–chitosan fibres saline absorption was approximately 20 (g/g) lower than water absorption. These results indicate that when 0.3% (w/v) calcium chloride is used during coagulation, a large portion of the sodium ions within the fibre do not exchange for the divalent calcium ions in the coagulant and thus, the resultant fibres, have a greater propensity to absorb liquid due to the soluble nature of sodium alginate. As the concentration of calcium increases, the rate of ion exchange between the fibre and coagulant increases, resulting in a fibre that is more calcium-based and hence less absorbent, as calcium alginate fibres are water insoluble.

The control calcium alginate fibre absorbed approximately 15 (g/g) more saline than water which is indicative of calcium alginate fibres. These results also indicate that the presence of hydrolysed chitosan in the alginate–chitosan fibres also plays a role in enhancing the absorption capacities of the fibres as the fibres produced using lower calcium concentrations (w/v %) have a higher chitosan content (wt.%) and are more absorptive in both water and saline (Table 2). The presence of hydrolysed chitosan within the coagulant aids the formation of a solid alginate–chitosan fibre by forming a polyelectrolyte complex with the alginate. This allows for a lower concentration of calcium to be used within the bath which improves the gelling of the fibre as the fibres become more water soluble. Often calcium alginate dressings have sodium ions reintroduced by treating the fibres with a sodium sulphate solution as this improves the gelling and absorbency of the fibres (Qin, 2005). All the alginate chitosan fibres gelled well, for this reason; however, the fibres did start to gel less as more calcium was added to the bath. If a fibre gels well then immediately it starts to lose its fibrous structure and form an homogenous mass that is highly absorptive. Fibres that are absorbent, but not gelling, swell, but do not lose their fibrous structure; these fibres have a greater wet tensile strength.

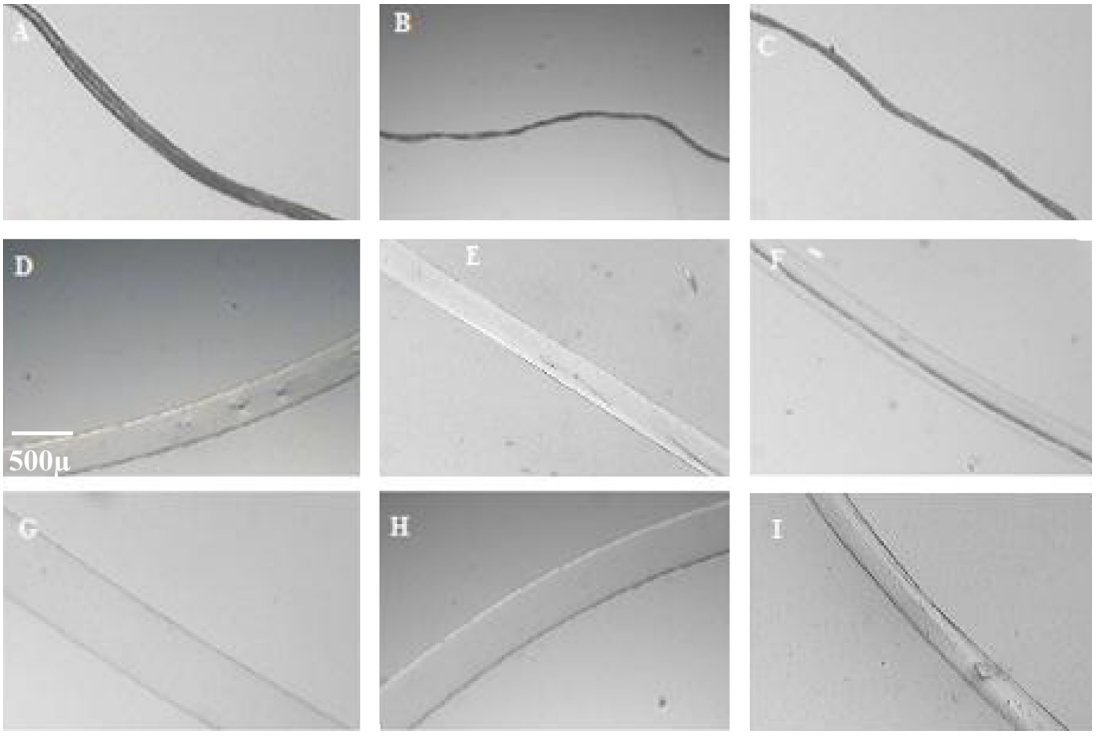

Fig. 5. Optical micrograph images of fibres dry: A (A0.30), B (A0.35), and C (A0.40), after immersion in saline: D (A0.30), E (A0.35), and F (A0.40), and after immersion in deionised water: G (A0.30), H (A0.35), and I (A0.40) at magnification 40×.

3.3.4. Optical microscopy and swelling behaviour of fibres

Fibres A0.30, A0.35 and A0.40 were examined by optical microscopy and the resultant images analysed using Image Pro software in order to compare and contrast their swelling abilities (Fig. 3). The dry fibres were all flat and ribbon-like despite the spinneret holes being circular but after immersion became swollen and cylinderical in shape (Fig. 5). The average dry diameters of the A0.30 and A0.35 fibres both increased by >5 times after immersion in deionised water and increased >3.5 times after immersion in saline. The average dry diameter of the A0.40 fibre increased by >4.5 times after immersion in water and >3 times after immersion in saline. In general fibre swelling decreased with increasing calcium content within the alginate–chitosan fibres, substantiating the fact that there are fewer sodium ions present at higher coagulant calcium concentrations.

4. Conclusion

The preparation of a one-step wet-extruded, highly absorbent, gelling alginate–chitosan fibre was achieved by carefully adjusting the pH of the acid-soluble hydrolysed chitosan to prevent the formation of alginic acid during coagulation. The hydrolysed chitosan forms a polyelectrolyte complex with the alginate to form a physically crosslinked network which allowed for a lower concentration of calcium to be used within the bath which improved the gelling of the fibre as the fibres became more water soluble. The addition of calcium also aided gelling as calcium alginate is widely acknowledged to gel in the presence of sodium ions; prevalent in wounds. The fibre selected for further optimisation was the hybrid alginate–chitosan produced using 0.35% (w/v) calcium chloride as this fibre provided a balance between absorption and tensile properties. Fibre A0.35 absorbed >50.0 g/g deionised water and >28.0 g/g saline, had an elongation of >11.0%, linear density >7.00 dtex, and a maximum chitosan content >5.00 wt.%. This fibre has the potential to be produced on a larger scale for the production of a nonwoven dressing for the treatment of exuding wounds where absorbent biomaterials are required. The next step will be to produce a nonwoven prototype and assess its efficacy in terms of its wet tensile strength and absorption. This absorption may increase, due to absorption between the interlocked fibres. Finally we will compare this novel dressing against commercially available gelling dressings, such as Aquacel® and Kaltostat® (Convatec Ltd.), which target exuding wounds.

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