CHAPTER II
LITERATURE REVIEW

2.1 Wound Dressing

2.1.1 Wound Healing Process

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. It is a complex biological process involving haemostasis and inflammation, migration, proliferation, and maturation (Debra, 1998).

2.1.1.1 Haemostasis and Inflammation

Bleeding usually occurs when the skin is injured and serves to flush out bacteria and/or antigens from the wound. In addition, bleeding activates haemostasis which is initiated by exudate compounds such as clotting factors. Fibrinogen in the exudates elicits the clotting mechanism resulting in coagulation of the exudates (blood without cells and platelets) and, together with the formation of fibrin network, produces a clot in the wound causing bleeding to stop. The clot dries to form a scab and provides strength and support to the injured tissue. Haemostasis therefore, plays a protective role as well as contributing to successful wound healing.

The inflammatory phase occurs almost simultaneously with haemostasis, sometimes from within a few minutes of injury to 24 h and lasts for about 3 days. It involves both cellular and vascular responses. The release of protein-rich exudates into the wound causes vasodilation through release of histamine and serotonin, allows phagocytes to enter the wound and engulf dead cells (necrotic tissue). Necrotic tissue which is hard is liquefied by enzymatic action to produce a yellowish coloured mass described as sloughy. Platelets liberated from damaged blood vessels become activated as they come into contact with mature collagen and form aggregates as part of the clotting mechamism.

2.1.1.2 Migration

The migration phase involves the movement of epithelial cells and fibroblasts to the injured area to replace damaged and lost tissue, These cells

regenerate from the margins, rapidly growing over the wound under the dried scab (clot) accompanied by epithelial thickening.

2.1.1.3 Proliferation

The proliferative phase occurs almost simultaneously or just after the migration phase (Day 3 onwards) and basal cell proliferation, which lasts for between 2 and 3 days. Granulation tissue is formed by the in-growth of capillaries and lymphatic vessels into the wound and collagen is synthesized by fibroblasts giving the skin strength and form. By the fifth day, maximum formation of blood vessels and granulation tissue has occurred. Further epithelial thickening takes place until collagen bridges the wound. The fibroblast proliferation and collagen synthesis continues for up to 2 weeks by which time blood vessels decrease and oedema recedes.

2.1.1.4 Maturation

This phase (also called the "remodeling phase") involves the formation of cellular connective tissue and strengthening of the new epithelium wich determines the nature of the final scar. Cellular granular tissue is changed to an acellular mass from several months up to about 2 years.

2.1.2 Types and Properties of Wound Dressings

2.1.2.1 Ideal Wound Dressings

New occlusive dressing materials concentrate on creating the correct environment for wound healing to occur. The ideal dressing is described by Griffiths (1991) as being one that provides a moist environment; is comfortable for the patient; removes any necrotic material; promotes the production of granulation tissue; stimulates re-epithelialization; and is cost-effective. Bolton and Rijswijk (1991) state that for optimal results the wound dressing must not only meet the clinical needs of both patient and nurse, but also the wound's physiological and biochemical needs. They believe that a dressing should fulfill the following functions: conformability, particularly with uneven body surfaces, pain control, odour control, cost effectiveness, safety, aid healing, convenience, environmental acceptability, quality of life, and restores normal daily activities. Not only do these clinical needs have to be met but the specific physiological and biochemical

requirements of a wound should be addressed, such as exudate management, debridement, microbial barrier, antimicrobial, compression and adherence (Bolton and Rijswijk, 1991).

No single dressing is suitable for all types of wounds. Often a number of different types of dressings will be used during the healing process of a single wound. Briefly, dressings should perform one or more of the functions showed in Table 2.1.

Table 2.1 Properties of ideal wound dressings

Properties
- Maintain a moist environment at the wound/dressing interface
- Absorb excess exudate without leakage to the surface of the dressing
- Provide thermal insulation and mechanical protection
- Provide bacterial protection
- Allow gaseous and fluid exchange
- Absorb wound odour
- Be non-adherent to the wound and easily removed without trauma
- Provide some debridement action (remove dead tissue and/or foreign particles)
- Be non-toxic, non-allergenic and non-sensitising (to both patient and medical staff)
- Sterile

2.1.2.2 Classification of Wound Dressings

Synthetic wound dressings can be broadly categorized into the following types. (Table 2.2)

Table 2.2 Classification of wound dressings

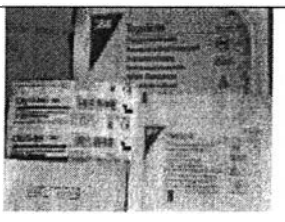
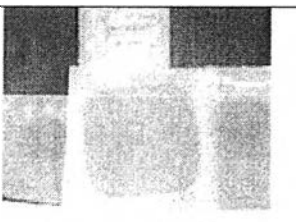
Type	Properties
Passive products	Traditional dressings that provide cover over the wound, e.g. gauze and tulle dressings
Interactive products	Polymeric films and forms which are mostly transparent, permeable to water vapour and oxygen, non-permeable to bacteria, e.g. hyaluronic acid, hydrogels, foam dressings
Bioactive products	Dressings which deliver substances active in wound healing, e.g. hydrocolloids, alginates, collagens, chitosan

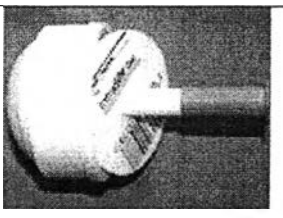
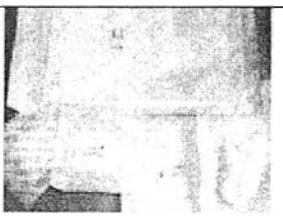
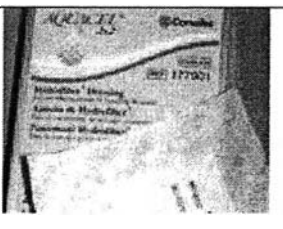
In November 1999, Food and Drug Administration of the United States of America (FDA) reclassified the dressing categories as, 1) Non-resorbable gauze/sponge dressing for external use, 2) Hydrophilic wound dressing, 3) Occlusive wound dressing, 4) Hydrogel wound and burn dressings, and 5) Interactive wound and burn dressings (Paul and Sharma, 2004).

2.1.2.3 Types of Wound Dressings and Their Main Properties

Table 2.3 describes some of the many different types of wound dressings and their main properties.

Table 2.3 Types of wound dressings and their main properties

Dressing type	Properties	Example
Gauze	- Dressings can stick to the wound surface and disrupt the wound bed when removed - Only use on minor wounds or as secondary dressings	
Tulle	- Dressing does not stick to wound surface - Suitable for flat, shallow wound - Useful in patient with sensitive skin	 Jelonet®, Paranet®
Semipermeable film	- Sterile sheet of polyurethane coated with acrylic adhesive - Transparent allowing wound checks - Suitable for shallow wound with low exudate	 OpSite®, Tegaderm®
Hydrocolloids	- Composed of carboxymethylcellulose, gelatin, pectin, elastomers and adhesives that turn into a gel when exudate is absorbed. This creates a warm, moist environment that promotes debridement and healing - Depending on the hydrocolloid dressing chosen. Can be used in wounds with light to heavy exudate, sloughing or granulating wounds - Available in many forms (adhesive or non-adhesive pad, paste, powder) but most commonly as self-adhesive pads	 DuoDERM®, Tegasorb®

Hydrogels	- Composed mainly of water in a complex network or fibers that keep the polymer gel intact. Water is released to keep the wound moist - Used for necrotic or sloughy wound beds to rehydrate and remove dead tissue. Do not use for moderate to heavily exudating wounds	 Tegagel®, Intrasite®
Alginates	- Good for exudating wounds and helps in debridement of sloughing wounds - Do not use on low exudating wounds as this will cause dryness and scabbing - Dressing should be changed daily	 Kaltostat®, Sorbsan®
Polyurethane or silicone foams	- Designed to absorb large amounts of exudates - Maintain a moist wound environment but are not as useful as alginates or hydrocolloids for debridement - Do not use on low exudating wounds as this will cause dryness and scabbing	 Allevyn®, Lyofoam®
Hydrofibre	- Soft non-woven pad or ribbon dressing made from sodium carboxymethylcellulose fibres - Interact with wound drainage to form a soft gel - Absorb exudate and provide a moist environment in a deep wound that needs packing	
Collagens	- Dressings come in pads, gels or particles - Promote the deposit of newly formed collagen in the wound bed	

	- Absorb exudate and provide a moist environment

(Ngan, 2007)

2.2 Alginate

"Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself; in some publications the term "algin" is used instead of alginate. Alginate is present in the cell walls of brown algae such as the seaweeds *Laminaria* sp. and *Ascophyllum* sp. (Clare, 1993) as the calcium, magnesium and sodium salts of alginic acid. The calcium and magnesium salts do not dissolve in water while the sodium salt does. That is the reason why, the goal of the extraction process is to obtain dry, powdered, sodium alginate and sodium alginate is the main form of alginate in use.

2.2.1 Properties of Alginate

2.2.1.1 General Properties of Alginate

Alginate is a linear block copolymer consisting of uronic acid residues, namely β-D-mannuronic and α-L-guluronic acid, linked by (1→4)-linkages. For simplicity, alginate molecules are long chains that contain two different acidic components, abbreviated here to M and G. The way in which these M and G units are arranged in the chain and the overall ratio, M/G, of the two units in a chain can vary from one species of seaweed to another. The chemical structure of alginate/alginic acid is displayed in Figure 2.1 (Collins, 1998).

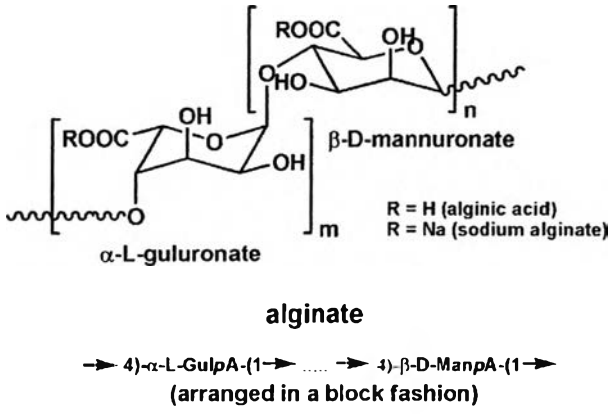


Figure 2.1 Chemical structure of alginate/alginic acid.

In other words all "alginates" are not necessarily the same. So some seaweeds may produce an alginate that gives a high viscosity when dissolved in water, others may yield a low viscosity alginate. The conditions of the extraction procedure can also affect viscosity, lowering it if conditions are too severe. All of this results in sellers normally offering a range of alginates with differing viscosities. Table 2.4 is an example of alginates with differing viscosities.

Table 2.4 An example of alginates with differing viscosities

Material	Product	Supplier	Viscosity (mPa.s, 1% soln)
Sodium alginate	ProtanalLF10/60	Pronova	20-70
Sodium alginate	Manucol®DH	ISP alginates	40-90
Sodium alginate	Manugel®GMB	ISP alginates	110-270

Information supplied by supplier. (Knill *et al.*, 2004)

2.2.1.2 Alginate Uses

The uses of alginates are based on three main properties. The first is their ability, when dissolved in water, to thicken the resulting solution (technically described as their ability to increase the viscosity of aqueous solutions). The second is their ability to form gels; in the presence of multivalent cations such as

Ca^{2+}, an aqueous solution of alginate will become a gel. Gel formation occurs due to the ionic interaction between guluronic acid residues from two or more alginate chains and cations, yielding a three-dimensional network of alginate molecules well described by the "Egg-Box Model" (see Figure 2.2) (Goth *et al.*, 2004).

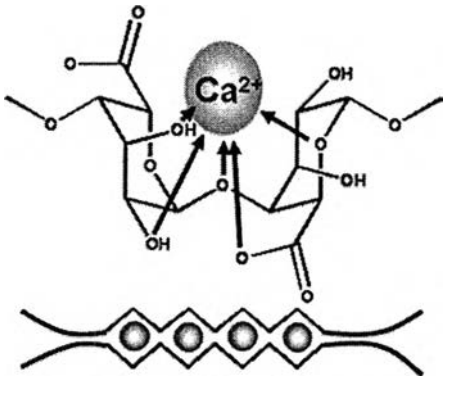


Figure 2.2 Egg-Box Model.

No heat is required and the gels do not melt when heated. This is in contrast to the agar gels where the water must be heated to about 80°C to dissolve the agar and the gel forms when cooled below about 40°C. The third is the ability to form films of sodium or calcium alginate and fibers of calcium alginates.

2.2.2 Medical Uses of Alginate

2.2.2.1 Alginate Tablet

Alginic acid powder swells when wetted with water. This has led to its use as a tablet disintegrant for some specialized applications (Khan and Rhodes, 1972). Alginic acid has also been used in some dietary foods, such as biscuits; it swells in the stomach and, if sufficient is taken, it gives a "full" feeling so the person is dissuaded from further eating. The same property of swelling has been used in products such as Gaviscon® tablets, which are taken to relieve heartburn and acid indigestion (Mandel *et al.*, 2000). The swollen alginic acid helps to keep the gastric contents in place and reduce the likelihood of reflux irritating the lining of the oesophagus.

2.2.2.2 Drug Delivery System

Because of the ability of alginate to form a gel/meshwork in the presence of divalent cations such as $CaCl_2$, this gel shrinks at acidic pH and erodes at alkaline pH. Therefore, it can be used effectively to deliver drugs to the intestine. In some applications, alginate microparticles were developed as oral sustained delivery carriers for antitubercular drugs in order to improve patient compliance (Ain *et al.*, 2003). This strategy helps to improve patient compliance in terms of reducing the dosage frequency, and may also minimize the risk of emergence of drug-resistant mutants and potential toxicity. Ain *et al.* (2003) reported that when drug-loaded alginate microparticles/free drugs were administered orally to guinea pigs, higher C_{max}, T_{max}, delayed elimination rate and higher bioavailability were observed compared with free drugs. Also, alginate has been found to have bio-adhesive properties that help in delaying the intestinal transit time of the encapsulated compound (Chickering *et al.*, 1997). Therefore, it is suggested that alginate microspheres probably adhere to the intestine mucosa for a prolonged period where they release drug in a sustained manner before being eroded off.

2.2.2.3 Wound Dressing

Among the various fibrous and hydrogel products, alginate-based products are currently the most popular ones used in wound management, since they offer many advantages over traditional cotton and viscose gauzes (Horncastle, 1995; Qin and Gilding, 1996). They are biocompatible and form a gel on absorption of wound exudate. This eliminates fiber entrapment in the wound, which is a major cause of patient trauma/discomfort during dressing removal. Such gelation prevents the wound surface from drying out, which is beneficial since a moist wound environment promotes healing and leads to a better cosmetic repair of the wound (Winter, 1962). Performance requirements for such gel led dressings (which often aim to replicate the inherent permeability/water content of natural skin) are obviously higher than mere absorbent coverings in order for the wound to remain moist during the contact period (which could be more than several days) (Thomas, 1990). Hence, it is also reported that alginate-based dressings have haemostatic properties and can enhance the rate of healing of skin wounds (Attwood, 1989; Jarvis

et al., 1987). Therefore, several alginate-based dressings are now commercially available in the market (Table 2.5).

Table 2.5 Alginate-based dressings commercially available in the market

Product	Manufacturer
AlgiDERM	Bard
AlgiSite	Smith & Nephew, Inc.
Algisteril	Johnson & Johnson
CarraSorb H	Carrington
CURASORB	Kendall
CURASORB Zinc	
Dermacea	Sherwood-Davis & Geck
FyBron	B. Braun
Gentell	Gentell
Hyperion Advanced	Hyperion Medical, Inc.
Alginate Dressing	
KALTOSTAT	Conva Tec
KALGINATE	DeRoyal
Maxorb	Medline
PolyMem	Ferris Mfg.
Restore	Hollister
SORBSAN	Dow Hickam
SeaSorb	Coloplast Sween Corp.
Tegagen HG	3M Health Care
Tegagen HI	

(Paul and Sharma, 2004)

Sayag *et al.* (1996) compared an alginate dressing with dextranomer paste in a randomised controlled trial of 92 patients with full-thickness pressure ulcers. They found a minimum 40% reduction in wound size in the alginate

group within four weeks, whereas the dextranomer group took eight weeks to achieve similar reductions in size. Sayag *et al.* (1996) concluded that, the striking healing efficacy of an alginate dressing suggests it possesses pharmacological properties which require further investigation.

Blair *et al.* (1988) found that the alginate dressing Kaltostat was significantly better at arresting haemorrhage than the control dressings (collagen, oxidised cellulose, or gauze).

In 1986, Schmidt suggested that calcium alginate might activate or stimulate the wound-healing process as it appeared to promote the growth of mouse fibroblasts. These findings were confirmed by Doyle *et al.* (1996), who suggested that calcium alginate had an effect on cell proliferation and migration that was believed to have been mediated by the release of calcium ions into the wound bed.

2.3 Chitin and Chitosan

2.3.1 Properties of Chitin and Chitosan

Chitin is a high-molecular weight linear polymer of *N*-acetyl-D-glucosamine (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose) units linked by β-D (1→4) bonds. It is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity. It may be regarded as cellulose with the hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it naturally functions as a structural polysaccharide. It is most abundant in crustaceans, insects, and fungi. Chitin is a white, hard inelastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas (Madhavan, 1992). Chitosan is the *N*-deacetylated derivative of chitin, though this *N*-deacetylation is almost never complete. A sharp nomenclature border has not been defined between chitin and chitosan based on the degree of *N*-deacetylation (Muzzarelli, 1973; Zikakis, 1984). Chemical structures of chitin and chitosan are showed in Figure 2.3.

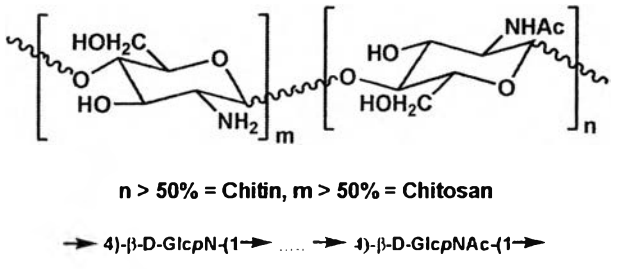


Figure 2.3 Chemical structures of chitin and chitosan.

Chitin and chitosan are of commercial interest due to their high percent nitrogen (6-89%) compared to synthetically substituted cellulose (1.25%). This makes chitin a useful chelating agent (Muzzarelli, 1973). Many reviews and articles have been published covering the applications of chitin and its derivatives in the areas of pharmaceutical and biomedical applications, paper production, textile finishes, photographic products, cements, heavy metal chelating agents, cosmetics, effluent treatment methods and in engineering applications, for example, solid state batteries (Pariser and Lombadi, 1980; Chandy and Sharma, 1990; Rathke and Hudson, 1994; Yao *et al.*, 1995; Salmon and Hudson, 1997; Ravi Kumar *et al.*, 1998).

2.3.2 Medical Uses of Chitin and Chitosan

2.3.2.1 Chitin and Chitosan Tablet

Many direct-compression diluents have been reported in the literature, but every diluent has some disadvantages (Hiromitsu *et al.*, 1981). Microcrystalline cellulose (MCC) has been widely used as a tablet diluent in Japan. Chitin and chitosan, because of their versatility, have been reported to be useful diluents in pharmaceutical preparations (Miyazaki, 1998).

Sawayanagi *et al.* (1982) reported the fluidity and compressibility of combined powders of lactose with chitin (lactose/chitin), with chitosan (lactose/chitosan) and potato starch with chitin (potato starch/chitin), and with chitosan (potato starch/chitosan). The disintegration properties of tablets made from these powders, in comparison with those of combined powders of lactose with MCC (lactose/MCC) and potato starch with MCC (potato starch/MCC) in order to

develop new direct-compression diluents, are also reported (Sawayanagi *et al.*, 1982). The fluidity of combined powders with chitin and chitosan was greater than that of the powder with crystalline cellulose. The reported hardness of the tablets follows the order: chitosan tablets>MCC>chitin. In disintegration studies, tablets containing less than 70% chitin or chitosan passed the test. Moreover, the ejection force of the tablets of lactose/chitin and lactose/chitosan was significantly less than that of lactose/crystalline cellulose tablets (Sawayanagi *et al.*, 1982). However, no reports are available on controlled drug release formulations using these tablets.

2.3.2.2 Drug Delivery System

Chitosan is non-toxic and easily bioabsorbable (Muzzarelli *et al.*, 1999) with gel-forming ability at low pH. Moreover, chitosan has antacid and antiulcer activities which prevent or weaken drug irritation in the stomach (Hou *et al.*, 1985; Miyazaki *et al.*, 1981). Also, chitosan matrix formulations appear to float and gradually swell in an acid medium. All these interesting properties of chitosan make this natural polymer an ideal candidate for controlled drug release formulations. Many excellent reviews and books deal with the properties, chemistry, biochemistry and applications of chitin, chitosan and their derivatives (Yalpani *et al.*, 1992; Pariser and Lombadi, 1980; Ravi Kumar, 1999).

2.3.2.3 Wound Dressing

Chitin and chitosan have many distinctive biomedical properties. However, chitin-based wound healing products are still at the early stages of research (Le *et al.*, 1997).

Sparkes and Murray (1986) developed a surgical dressing made of a chitosan-gelatin complex. The procedure involves dissolving the chitosan in water in the presence of a suitable acid, maintaining the pH of the solution at about 2-3, followed by adding the gelatin dissolved in water. The ratio of chitosan and gelatin is 3:1 to 1:3. To reduce the stiffness of the resulting dressing a certain amount of plasticizers such as glycerol and sorbitol could be added to the mixture. Dressing film was cast from this solution on a flat plate and dried at room temperature. It was claimed that, in contrast to conventional biological dressings, this experimental dressing displayed excellent adhesion to subcutaneous fat.

Nara *et al.* (1987) patented a wound dressing comprising a nonwoven fabric composed of chitin fibers made by the wet spinning technique. In one of the examples, chitin powder was ground to 100 mesh and treated in 1 M HCl for 1 h at 4°C. It was then heated to 90°C where it was treated for 3 h in a 0.3% NaOH solution to remove calcium and protein in the chitin powder, and rinsed repeatedly followed by drying. The resultant chitin was dissolved in a dimethylacetamide solution containing 7 wt% lithium chloride to form a 7% dope. After filtering and allowing defoaming to occur, the dope was extruded through a nozzle of diameter 0.06 mm and 200 holes into butanol at 60°C at a rate of 2.2 g/min. The chitin was coagulated and collected at a speed of 10 m/min. The resultant strand was rinsed with water and dried to obtain a filament of 0.74 dtex with a strength of 2.8 g/den. The filaments were then cut into staple fibers. Using poly(vinyl alcohol) as a fibrous binder, nonwoven dressings were made.

Kifune *et al.* (1988) developed a new wound dressing, Beschitin W, composed of chitin nonwoven fabric which proved to be beneficial in clinical practice. Biagini *et al.* (1991) developed an *N*-carboxybutyl chitosan dressing for treating plastic surgery donor sites. A solution of *N*-carboxybutyl chitosan was dialyzed and freeze-dried to produce a 10×20×0.5 cm^3 soft and flexible pad, which was sterilized and applied to the wound. This dressing could promote ordered tissue regeneration compared to control donor sites. Better histoarchitectural order, better vascularization and the absence of inflammatory cells were observed at the dermal level, while fewer aspects of proliferation of the malpighian layer were reported at the epidermal level.

Muzzarelli (1995) introduced another chitosan derivative, 5-methylpyrrolidinone chitosan, which is believed to be very promising in medical applications. This polymer is claimed to be compatible with other polymer solutions, including gelatin, poly(vinyl alcohol), poly(vinyl pyrrolidone) and hyaluronic acid. The advantages include healing of wounded mensical tissues, and of decubitus ulcers, depression of capsule formation around prostheses, limitation of scar formation and retraction during healing. Some wound-dressing samples were prepared from an aqueous solution of this 5-methylpyrrolidone chitosan, which was dialyzed and laminated between stainless steel plates and freeze-dried to yield

fleeces. The material could be fabricated into many different forms, such as filaments, nonwoven fabrics, etc. Once applied to a wound, 5-methylpyrrolidinone chitosan becomes available in the form of oligomers produced under the action of lysozyme.

Another chitin derivative, dibutyrylchitin, was prepared by treatment of krill chitin with butyric anhydride in the presence of perchloric acid as a catalyst at 25-30°C (Szosland and East, 1995). Samples of polymers with molecular weights high enough to form fibres were obtained and dibutyryl chitin fibres were made by dry spinning a 20-22% solution into acetone. The fibres have tensile properties similar to or better than those of chitin. Moreover, it was claimed that chitin fibres with good tensile properties could be obtained by alkaline hydrolysis of dibutyryl chitin fibres without destroying the fibre structure.

As far as chitin-based commercial wound dressings are concerned, one product (Beschitin®, Unitika) is commercially available in Japan, which is a nonwoven fabric manufactured from chitin filaments.

2.4 Chitin Whiskers or Chitin Nanofibrils

Chitin has a complex, multi-level supermolecular architecture. All chitins are built from superfine fibrils having diameters in the nano scale, and each such nanofibril contains ordered nanocrystallites embedded into a low-ordered nano-domains (Muzzarelli and Muzzarelli, 2005). Figure 2.4 shows multi-level supermolecular architecture of chitin (Raabe *et al.*, 2006).

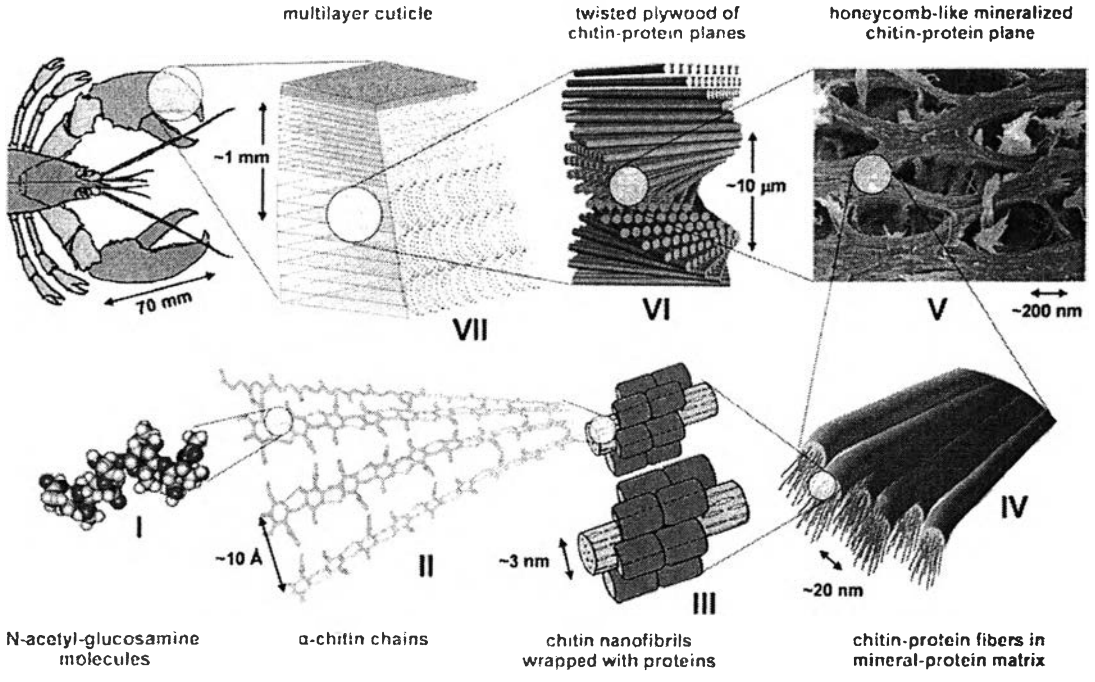


Figure 2.4 Multi-level supermolecular architecture of chitin.

Acid hydrolysis can be used to dissolve away regions of low lateral order so that the water-insoluble, highly crystalline residue can be converted into a stable suspension by subsequent vigorous mechanical shearing action. Highly crystalline chitin nanofibrils otherwise called "whiskers" therefore exhibit an enormous surface development that allows them to impart strength to several materials, such as poly(caprolactone), soy proteins, natural rubber, chitosan, and poly(vinyl alcohol). Apart from the ability to improve the mechanical integrity, chitin nanofibril may interact well with enzymes, platelets, and other cell compounds present in living tissues. Thus, the recovered peculiarity and the ability for faster adequate granulation tissue formation are accompanied and applicability of chitin nanofibrils in the medical areas is supported.

2.4.1 Application to Impart Strength

2.4.1.1 Composites with Poly(Acrylic Acid)

An extension of the above mentioned studies on the incorporation of nanofibrils in poly(acrylic acid) were those aimed at the preparation

of composites based on chitin nanofibrils. The orientation of the nanofibrils was obtained by shearing or by magnetic alignment. The X-ray diffraction data for the composites showed uni-planar orientation of the chitin crystallites, with the molecular long axes perpendicular to the direction of the magnetic field (Nge *et al.*, 2003a,b,c).

2.4.1.2 Composites with Soy Protein Isolates

Soy protein isolates (SPI) of desired weight and various content of chitin were mixed and stirred to obtain homogeneous dispersions. The dispersion was freeze-dried, and 30% glycerol was added. The resulting mixture was hot-pressed at 20 MPa for 10 min at 140°C and then slowly cooled to room temperature. The SPI/chitin nanofibril composites (thickness about 0.4 mm) were thus obtained (Lu *et al.*, 2004).

Compared with a glycerol plasticized SPI sheet, the chitin filled SPI composites increase in Youngs modulus and tensile strength from 26 to 158 MPa and 3.3 to 8.4 MPa with increasing chitin content from 0 to 20 wt%. As the chitin nanofibrils increase in the SPI matrix, the composites show greater water-resistance. The improvement in all of the properties of these novel SPI/chitin nanofibril composites may be ascribed to three-dimensional networks of inter-molecular hydrogen bonding interactions between filler and filler and between filler and SPI matrix.

More than 75% of the nanofibrils have a length below 300 nm. The average length and width were estimated to be around 240 and 15 nm, respectively. The average aspect ratio (L/d, L being the length and d the diameter) of these nanofibrils is therefore around 16. These dimensions are close to those reported for chitin nanofibrils obtained from squid pen (L= 50-300 nm, d = 10 nm, L/d = 15). Sharp and well-defined diffraction rings indicated the crystalline nature (amorphous protein part and amorphous chitin domains had been removed during acid hydrolysis) of chitin nanofibrils present in the suspension. For composite materials filled with crab chitin nanofibrils, interfacial phenomena are important owing to the high specific area of the filler ca.180 $m^2.g^{-1}$). For example, for a 20 wt% chitin nanofibril filled composite, there are ca. 40 m^2 of filler surfaces in 1 cm^3 of the material.

An infrared spectrum was taken for a film of chitin nanofibrils obtained by evaporating the suspension in order to display the absence of residual proteins on the chitin fragments. In the carbonyl region, the spectrum presents three strong absorption peaks at 1658, 1622, and 1556 cm^{-1} characteristic of anhydrous chitin. The absence of the peak at 1540 cm^{-1} corresponding to the proteins proves that the successive treatments were strong enough to eliminate all the proteins and to obtain pure chitin (Lu *et al.*, 2004; Nair *et al.*, 2003a,b,c).

2.4.1.3 Composites with Natural Rubber

Reinforced natural rubber nanocomposites were developed from colloidal suspension of chitin nanofibrils and latex of unvulcanized and prevulcanized natural rubber. The chitin nanofibrils, prepared by acid hydrolysis of chitin from crab shells, consisted of slender parallelepiped rods with an average length around 240 nm and an aspect ratio close to 16. After the aqueous suspensions of chitin nanofibrils and rubber were mixed and stirred, solid composite films were obtained by casting and evaporating methods. For unvulcanized systems a freeze-drying and subsequent hot-pressing processing technique was also used. All the results lead to the conclusion that the processing technique plays a major role in the properties of final composites developed. The chitin nanofibrils form a three-dimensional rigid network only in the evaporated samples, and it is assumed to be governed by a percolation mechanism.

The preparation of the latex requires the use of poloxamer 407 BASF Lutrol F127, a surfactant, in order to obtain a stable suspension (Morin *et al.*, 2002). It is a block copolymer of ca. 70 wt% poly(ethylene oxide) and 30 wt% poly (propylene oxide) with number-average molecular weight ca. 13000 g mol^{-1}.

2.4.1.4 Composites with Poly(Caprolactone)

Poly (caprolactone) is a biodegradable, semicrystalline and thermoplastic polymer used for instance to manufacture suture threads; there is much interest in improving its mechanical properties and biochemical significance. Chitin nanofibrils were obtained from tubes secreted by Riftia, a vestimentiferan worm (much longer than those of animal origin: L = 0.5-10 m, d = 18 nm, L/d = 120). The results showed that at high temperature and above 5% nanofibrils, the chitin network

is allowed to restore thus stabilising the mechanical properties of the composite (Morin *et al.*, 2002).

2.4.1.5 Composites with Chitosan or with Poly(Vinyl Alcohol)

α-Chitin nanofibril-reinforced poly(vinyl alcohol) composite films were prepared by solution-casting technique. The as-prepared nanofibrils exhibited the length in the range of 150-800 nm and the width in the range of 5-70 nm, with the average length and width being about 417 and 33 nm, respectively. Thermal stability of the as-cast nanocomposite films was improved from those of the pure PVA film with increasing nanofibril content. The presence of the nanofibrils did not have any effect on the crystallinity of the PVA matrix. The tensile strength of α-chitin nanofibril-reinforced PVA films increased, at the expense of the percentage of elongation at break, from that of the pure PVA film with initial increase in the nanofibril content and leveled off when the nanofibril content was greater than or equal to 2.96 wt% (Sriupayo *et al.*, 2005a).

Similar preparations were made with α-chitin nanofibrils dispersed in chitosan by solution-casting, thanks to the high filmogenicity of chitosan. The length of the as-prepared nanofibrils ranged between 150 and 800 nm, while the width ranged between 5 and 70 nm, with the average values being about 417 and 33 nm, respectively. The addition of α-chitin nanofibrils did not affect much the thermal stability and the apparent degree of crystallinity of the chitosan matrix. The tensile strength of α-chitin nanofibril-reinforced chitosan films increased from that of the pure chitosan film with initial increase in the nanofibril content to reach a maximum at the nanofibril content of 2.96 wt% and decreased gradually with further increase in the nanofibril content, while the percentage of elongation at break decreased from that of the pure chitosan with initial increase in the nanofibril content and leveled off when the nanofibril content was greater than or equal to 2.96 wt%. As in the case of chitin nanofibril composites with PVA, both the addition of α-chitin nanofibrils and heat treatment helped improve water resistance, leading to decreased percentage of weight loss and percentage degree of swelling of the nanocomposite films (Sriupayo *et al.*, 2005b).

2.4.2 Application in Biomedical Field

There are several commercial hemostatic patches and gels available such as:

- Chitin-based: Clo-Sur® (Scion), Chitoseal® (Abbott), Syvek Patch® (Marine Polymer Technologies),
- Chitosan-based: Hemcon
- Collagen-based: Actifoam
- Fibrin-based: Bolheal
- Cellulose-based: Surgicel

The Syvek Patch is made of chitin microfibrils from the centric diatom *Thalassiosira fluviatilis* grown under aseptic conditions. It is seven times faster in achieving hemostasis than fibrin glue, because it agglutinates red blood cells; activates platelets whose pseudopodia make a robust contact with chitin, promotes fibrin gel formation within the patch; platelets generate force through the clot retraction process and vasoconstriction takes place very soon; and a platelet + chitin + red cells + fibrin plug is formed.

The T. *fluviatilis microfibrils* have been tested in the most demanding and crucial conditions requiring hemostasis, such as splenic hemorrage, cardiac catheterization, and bleeding esophageal varices, and found superior to all competing products. While the T. *fluviatilis microfibrils* are longer (60 x 01 micron) than crustacean nanofibrils, both chitins used in these instances have the same molecular weight ($2x10^6$ Dalton) and acetylation degree (>0.90).

It is therefore reasonable to expect that the crustacean nanofibrils will be of at least comparable efficacy, while being less expensive because their production technology is much simpler (Kulling *et al.*, 1999; Chan *et al.*, 2000; Fischer *et al.*, 2005).

2.4.2.1 Wound Dressings for Scarless Healing

Early demonstrations of the efficacy of chitin/chitosan in wound healing by Malette *et al.* (1986) were based on irregularly shaped, high mesh powders. Later freeze-dried layers were adopted that permitted scarless restoration of vascularized tissue, and complete healing even in aged patients. Microspheres are

under study. Chitin nanofibrils are expected to conform to the wound geometry, to have immediate contact with cells in all the usual presentations. Moreover they can be suspended in gels, including chitosan gels, prepared to solidify upon application as a consequence of photocrosslinking reactions, enzymatic reactions, and spontaneous rapid drying.

2.4.2.2 Immuno-Stimulating Products

The intravenous administration of chitin particles has been found to promote macrophage priming in mice. The particles become bound to macrophage plasma membrane mannose/fucose receptors that mediate internalization. They are then degraded by lysozyme. Within 3 days macrophages give a large oxidative burst when elicited with phorbol myristate acetate. The mechanism involves the production of endogenous interferon-gamma by natural killer cells NK1.1 due to macrophage/NK1.1 interaction. These responses are similar to those generated by microbial particulate components. It is expected that chitin nanofibrils will be more effective in view of their larger surface, and easier to administer (Shibata *et al.*, 1997).

2.4.2.3 Activation of Macrophages

Chitin is phagocytosed and is a potent macrophage stimulator. Oral administration of chitin micro-/nano-particles is effective in down-regulating serum IgE and lung eosinophilia in a mouse model of ragweed allergy. The intranasal application of microgram doses of chitin microparticles is an effective treatment for reducing serum IgE and peripheral blood eosinophilia, airway hyperresponsiveness and lung inflammation in allergy models. It results in elevation of cytokines, IL-12, interferon-gamma and TNF-alpha and reduction of IL-4 production during allergen challenge (Han *et al.*, 2005).

2.4.2.4 Cosmetic Fillers for Aesthetic Medicine

Work in progress indicates that chitin nanofibrils suspended in saline can be injected under the wrinkled skin to restore its normal look, with the aid of a G30 needle. The nanofibrils last longer than hyaluronic acid, and do not give rise to any adverse effect. Facial masks can also be manufactured with dibutyryl chitin incorporating chitin nanofibrils (Morganti *et al.*, 2006).

The chitin nanofibrils may be incorporated in a number of biological agents capable of different combined functions. The ability of nanofibrils to travel through the intercellular spaces of stratum corneumis is probably due to their diffusion along the polar head groups of the intercellular lipids. The intercellular lipid pathway provides, in fact, the primary barrier to the passive diffusion of water-soluble and lipid-soluble molecules across the stratum corneum, whose porous/polar organization may favour their permeation. Advantages are expected from this emerging technology, based on chitin nanofibrils, useful for the development of advanced functional products needed to improve the quality of life.

2.4.2.5 Drug Delivery

Chitin nanofibrils may be used as drug carriers thanks to their ability to deliver active compounds across the skin. They may be useful as injectable systems in plastic surgery to restore the mechanical stability of the skin, or for the regeneration of any other tissue. The injectable systems have great potential for applications in interactive tissue engineering approaches as they can be designed with a wide range of properties and configurations.

2.5 Wet Spinning

Wet spinning is one of the most complex spinning techniques. Some wet spinning process requires the polymer solution to be kept above or below ambient temperature and thus a heat exchanger is used. The spinnerets are immersed in tanks containing the coagulation into which the filaments are extruded. The extrusion direction is variable from vertically upwards to horizontal. The spinnerets used in wet spinning can have up to 2,000 holes for commercial applications. However, conventional wet spinning is the slowest of the principal processes having wind-up speeds to approximately 150 m/min. The schematic drawing of the wet spinning apparatus is shown in Figure 2.5.

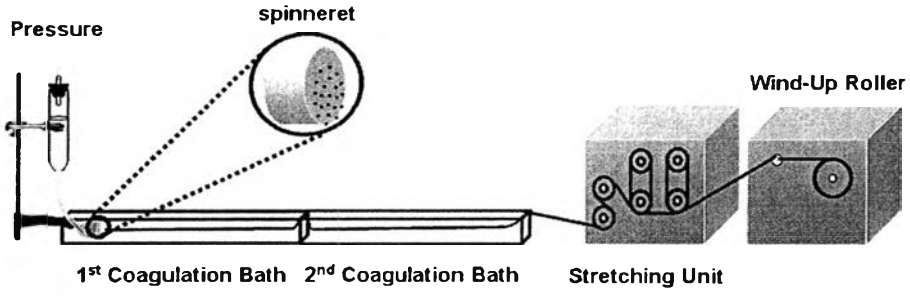


Figure 2.5 Schematic drawing of the wet spinning apparatus.

This process is based on precipitation only, without chemical regeneration. The solvent diffuses out of the extrudate into the bath, and a non-solvent diffuses from the bath into the extrudate. The polymer precipitates as a gel initially at the extrudate-coagulant interface but progressively throughout the extrudate. The coagulation rate has a large influence on the gel structure and the final fiber properties. The processing variables are concentration and temperature of the spinning solution, composition, concentration and temperature of the coagulation bath, and the stretch applied during spinning. These conditions lower the coagulation bath temperature and lower stretch during spinning. These conditions lead to greater homogeneity and higher orientability in a subsequent stretching or drawing process and hence to better tensile properties. The coagulation bath and spinning solutions may include small amounts of modifying agents that tend to improve homogeneity of the spun yarn. The coagulated filaments pass over a guide to driven rollers. The steps succeeding coagulation vary according to the product but typically include washing, stretching, finish application, drying, crimping, controlled relaxation for a tow, plus cutting for a staple fiber product (Kroschwitz, 1986). Table 2.6 shows typical wet spinning solvents and coagulants.

Table 2.6 Typical wet spinning solvents and coagulants

ISO Fiber Class	FTC Fiber Class	Chemical Structure of Fiber	Solvent	Coagulant
viscose	rayon	cellulose	aqueous solution of sodium salt and xanthate ester	dilute sulfuric acid + sodium sulfate + zinc sulfate water
modal	rayon		aqueous solution of sodium salt and xanthate ester	dilute sulfuric acid + sodium sulfate + zinc sulfate
cupro	cupra	cellulose	aqueous cuprammonium hydroxide	water
alginate		calcium salt of alginic acid	aqueous solution of sodium salt of alginic acid	slightly acidic aqueous calcium hydroxide calcium chloride aqueous solution
protein	azlon	arachin, zein, or casein	dilute sodium hydroxide	dilute sulfuric acid + sodium sulfate
acrylic	acrylic	copolymer of acrylonitrile (<85 wt %) with other monomers	dimethylformamide dimethylacetamide (DMA) dimethyl sulfoxide	50 % aqueous dimethylformamide 50% aqueous dimethylacetamide 50% aqueous dimethylsulfoxide

			50% sodium thiocyanate 60% zinc chloride 70% nitric acid 90% ethylene carbonate	10% sodium thiocyanate 30% zinc chloride 30% nitric acid 30% ethylene carbonate
modacrylic	modacrylic	copolymer of acrylonitrile, 35-85 wt%, with other monomers	acetone, up to 50% acrylonitrile dimethylformamide (DMF) >50% acrylonitrile	aqueous acetone aqueous dimethylformamide
vinylal	vinal	poly(vinyl alcohol), posttreated with formaldehyde	water	aqueous sodium sulfate
aramid	aramid	poly(p-phenylene-terephthalamide)	100% sulfuric acid	water or dilute sulfuric acid
elastine	spandex	segmented polyurethane	DMF DMA	30% aqueous DMF 30% aqueous DMA

(Kroschwitz, 1986)

2.6 Wet Spinning of Alginate and Alginate-Based Fibers for Wound Dressing Applications

In the wet-spinning process in which alginate is converted from powder into fiber form, alginate is first dissolved in water to form a homogenous solution. Sodium alginate is typically used as the raw material, since it is easily soluble in water. In preparing a spinning solution, it should be pointed out that the properties of the alginate fibers depend on a number of factors, such as the molecular structure and molecular weight of the alginate, the coagulation bath composition, the processing

temperatures and speeds, etc. As the first step in the production process, the spinning solution has an important effect on both the production efficiency and the product performances. Some of the factors involved in the preparation of an alginate spinning solution are discussed below.

2.6.1 Factors Involved in the Preparation of an Alginate Spinning Solution

2.6.1.1 Molecular Weight of the Alginate Powder

In theory, a higher molecular weight would result in a higher level of inter-chain bonding and a greater fiber strength. During the preparation for spinning dope, it should be noted that as the molecular weight increases, the solution viscosity increases sharply, making it difficult to dissolve a high concentration of alginate in a given amount of water. Although alginate is a natural polymer, manufacturers can control the molecular weight (or degree of polymerization, DP) by varying the severity of the extraction conditions to produce products with viscosities in a 1% solution ranging from 10 to 1000 mPa.s, with a DP range of 100-1000 units. In order to extrude the alginate solutions through spinneret holes to form filaments, the spinning solution typically has a viscosity in the range 10000-20000 mPa.s, which can be obtained either from a high concentration solution, made of alginate with a low molecular weight, or a low concentration with a high molecular weight. For the production of alginate fibers, in order to balance the production efficiency with product performance, the raw material typically has a viscosity in 1% solution in the range 40-100 mPa.s (Qin *et al.*, 1996).

2.6.1.2 Concentration of the Spinning Solution

The viscosity of the alginate solution increases sharply as the concentration increases. McDowell (1960, 1977) found a useful empirical equation which can be applied to a wide variety of alginates:

$$\log_{10}(\text{viscosity}) = a\sqrt{(\text{concentration})} - b$$

Where a is a constant related to the DP of the alginate and b is a constant for a particular type of alginate. It should be noted that higher

concentrations are normally preferred in the wet-spinning process for a high production efficiency and generally good fiber properties, since as-made fibers from a spinning solution with a high solid content have a higher wet strength and are relatively easy to carry out the stretching and washing processes. However, high solution viscosities make it difficult to remove bubbles in the solution brought in during the mixing process. For practical reasons, wet-spinning processes typically use aqueous solutions containing 5-6% sodium alginate as the spinning solution.

2.6.1.3 Temperature of the Solution

The viscosity of alginate solutions decreases as temperature increases, at a rate of about 2.5% per degree Celsius. Since viscosity drops sharply on heating, it is useful to heat a solution during the dissolution process. However, if alginate solutions are kept above 50°C for several hours, depolymerization may occur, giving a permanent loss of viscosity and molecular weight. During the preparation of alginate spinning solutions, it is usual to use high-shear mixers and the solution temperature is raised by the heat generated from high levels of shearing. This is beneficial since the reduced viscosity helps the bubbles to rise from the spinning solution. However, a prolonged storage of the alginate solution at high temperatures will have a detrimental effect on its molecular weight.

2.6.1.4 pH of the Solution

The viscosity of alginate solutions is unaffected over the range pH = 5-11. Below pH = 5, the free ions in the chain start to become protonated, -COO to -COOH, and as the electrostatic repulsion between chains is reduced, they are able to come closer and form hydrogen bonds, producing higher viscosities (King, 1983). When the pH is further reduced, a gel will form, usually between pH = 3 and 4. Above pH = 11, slow depolymerization occurs on storage of alginate solutions, giving a fall in viscosity. For the wet-spinning of alginate, the spinning dope is normally prepared by dissolving sodium alginate in deionized water, with pH at around 7.

2.6.2 Production of Calcium Alginate Fibers

In terms of the manufacturing process, calcium alginate fiber can be made via one of the most basic spinning processes. The spinning solution can be

made by dissolving sodium alginate powder in water and, after degassing to remove the bubbles in the solution, a concentrated sodium alginate solution can be extruded through fine spinneret holes into a calcium chloride bath, whereby sodium alginate is precipitated out in filament form as a calcium alginate fiber (the later being insoluble in water). The as-made fibers can then be stretched, washed and dried to produce calcium alginate fibers. It should be pointed out that solutions of sodium alginate can react with many di- and tri- valent cations to form gels; hence it is possible to use a variety of metal ions to precipitate sodium alginate solution during the wet-spinning process. In the production of alginate fibers, calcium has found greatest popularity as the divalent ion for gel formation, mainly because its salts are cheap, readily available and non-toxic. Zinc chloride has also been used for the production of zinc alginate fibers (Qin *et al.*, 1996).

During the extrusion process where sodium alginate is extruded into a calcium chloride bath, the buckled chain of guluronic acid units acts as a two-dimensional analogue of a corrugated egg-box (see Figure 2.2) with interstices in which the calcium ions may pack and be coordinated, and while calcium ions help to hold the alginate molecules together, their polymeric nature and their aggregation bind the calcium ion more firmly, resulting in a firm gel structure.

During the production of calcium alginate fibers, calcium ions from the coagulation bath diffuse into the fiber to form a swollen fibrous gel. Thomas *et al.* (1995) studied the diffusion of calcium ions during the wet-spinning process. Their results showed that the gelling time of calcium alginate fiber varied linearly with alginate concentration, increased markedly with fiber radius and decreased with increasing calcium concentration. It is interesting that the gelling time is independent of the guluronic acid and mannuronic acid contents.

2.6.3 Production of Silver-Containing Alginate Fibers

Silver has a long history as an antimicrobial agent, especially in the treatment of burns (Klasen, 2000; Klasen 2000). While metallic silver is relatively inactive, silver ions are effective against a wide range of bacteria. When low concentrations of silver ions accumulate inside cells, they can bind to negatively charged components in proteins and nucleicacids, thereby effecting structural

changes in bacterial cell walls, membranes and nucleic acids that affect viability. Although silver is a highly effective antimicrobial agent, it has a limited toxicity to mammalian cells (Lansdown, 2002). In recent years, silver has been gaining importance in the wound management industry, and a number of silver-containing wound dressings have been developed. These function by the sustained release of low concentrations of silver ions over time, and generally appear to stimulate healing, as well as inhibiting microorganisms. A number of laboratory studies have shown the excellent antimicrobial performances of silver-containing wound dressings (Furr *et al.*, 1994; Lansdown *et al.*, 2003; Thomas and McCubbin, 2003; Thomas and McCubbin, 2003).

Since alginate wound dressings are highly absorbent, they are mainly used on highly exuding wounds where microbial infection is common. By incorporating silver ions into alginate fibers, it is possible to obtain highly absorbent wound dressings with good antimicrobial properties.

Since alginate is a polymeric acid, it can form salt with silver ions. However, unlike calcium alginate, which is highly insoluble in water, silver is a monovalent ion and when sodium alginate solution is extruded into a silver nitrate solution, it is difficult to form silver alginate fiber. A mixed solution of calcium chloride and silver nitrate can be used to produce fibers that are a mixture of calcium alginate and silver alginate.

In order to attach silver ions onto the alginate fibers, calcium alginate fibers can be treated with aqueous solutions of silver nitrate. The silver ions in the solution exchange with calcium ions in the fiber, resulting in the formation of calcium alginate fiber containing silver ions. These fibers are highly antimicrobial. However, due to the oxidative power of the silver ions, they are sensitive to light exposure and can be come dark-to-black in appearance (Le *et al.*, 1997).

Adding particles of water-insoluble silver compounds into the alginate fiber is one way to avoid oxidation and maintain the white physical appearance that is highly desirable for a biomedical material. Le *et al.* (1997) developed a method to incorporate silver sulfadiazine (SSD) into alginate fibers by mixing water-soluble sodium sulfadiazine with sodium alginate to form a spinning solution, which was then extruded into a 2% calcium chloride solution containing silver nitrate. During

the fiber-forming process, sodium alginate reacts with calcium ions to form the filament, whilst sodium sulfadiazine reacts with silver ions to form SSD, which is deposited inside the fiber structure. Alternatively, after sodium sulfadiazine and sodium alginate are dissolved to form a spinning solution, silver nitrate is added into this solution before extrusion. In this process, the SSD particles formed through the reaction between sodium sulfadiazine and silver nitrate is dispersed in the spinning solution. When extruded to form fiber, the SSD particles are embedded in the fibers.

As mentioned before, although silver is a highly effective broad-spectrum antimicrobial agent, it is also highly oxidative to organic materials. Skin discoloration and irritation associated with the use of silver nitrate is well known. In order to protect the host material from oxidation and discoloration, some novel silver-containing compounds have been developed in recent years and these have been made into fine particles that can be blended with fiber-forming polymers during extrusion.

AlphaSan RC5000 is a silver sodium hydrogen zirconium phosphate. This microbiologically active ingredient is a synthetic inorganic polymer. Under a scanning electron microscope, it resembles cube-shaped crystals, with an average particle size of about a micrometer. It consists of a three-dimensional, repeating framework of sodium hydrogen zirconium phosphate, with many equally spaced cavities containing silver. Silver (at 3.8% by weight) provides the main anti microbial properties, while the framework matrix acts to distribute silver evenly (without clumping or pooling) through out the individual fibers where the AlphaSan particles are added.

When AlphaSan RC5000 is mixed with sodium alginate solution, the fine particles can be evenly distributed in the spinning solution under a high rate of shearing. Because the particles are very fine, they can be suspended uniformly while the solution is extruded to form fibers. Since the sodium hydrogen zirconium phosphate framework prevents the silver ions from oxidizing the alginate, this type of silver-containing alginate fiber remains white even after sterilization through irradiation (Qin and Groocock, 2002).

When alginate fibers containing AlphaSan RC5000 particles are in contact with wound exudates, the silver ions can be released into the wound exudates

by three mechanisms. First, there is an ion exchange between the silver ions in the fiber and the sodium and calcium ions in the wound fluid. Second, silver ions can be chelated by protein molecules in the wound fluid. Third, AlphaSan particles attached on the surface of the fibers can also be detached from the fibers and get into the wound exudate.

2.6.4 Production of Chitin-, Chitosan- Containing Alginate Fibers

Combination of alginate and chitosan can compliment each other in the properties of the final products. One additional factor enabling the development of tailored biomaterials based on the use of both alginate and chitosan is through their potential to form a polyelectrolyte complex via ionic interactions. It is assumed that the carboxylate moieties on alginate molecules will ionically interact with the protonated amino groups on chitosan counterparts to form a three-dimensional matrix, known as a physically cross-linked hydrogel. Because of these opposite charges, a direct mixing of alginate and chitosan solutions would readily coagulate or form gels, leading to unfeasibility in the production of the respective alginate/chitosan fibers from mixed dope solutions of the polymers. Problems in the direct production of alginate/chitosan fibers can be overcome by several means. Some examples are the blending of separately fabricated alginate and chitosan fibers; the utilization of chitosan cationic core for the production of an alginate fiber (Cole and Nelson, 1993); and the coating of chitosan on calcium alginate filament (Tamura *et al.*, 2002).

Tamura *et al.* (2002) prepared chitosan-coated alginate filament. The results showed that the chitosan of high molecular weight tended to precipitate in the presence of calcium ion, therefore, a minimum amount of chitosan should be applied to the coagulation bath. For chitosan of a molecular weight 40,000 Da, significant increase of wet/dry ratio of knot strength was observed. In general, filament in dry condition is stronger than in wet condition. However, the present chitosan-coated alginate filament showed higher strength in wet condition. For chitosan of a molecular weight 160,000 Da, the tensile strength in dry state and wet state were improved compared with the filaments coated by the chitosan of lower molecular

weight. The optimum concentration of chitosan used in the first coagulation bath was suggested to be 0.014% w/v.

Knill *et al.* (2004) reported the results of alginate fibers modified with unhydrolysed and hydrolysed chitosans. Modification of fibers with unhydrolysed chitosan generally resulted in a significant reduction in tenacity, and elongation if a water washing stage was not used, implying that the unhydrolysed chitosan is more like a coating rather than penetrating/reinforcing the alginate fiber. Reduction of chitosan molecular weight had a positive effect on its ability to penetrate the alginate fibers. Not only increase chitosan content in the fiber but also reinforce fiber structure and thus enhance tensile property (compared with unhydrolysed alginate/chitosan fibers). Hydrolysed alginate/chitosan fibers demonstrated antimicrobial effect (in terms of bacterial reduction) with an initial used and had the ability to provide a slow release of antibacterially active components. Notwithstanding, the majority of these methods have been based on the principle of coating via ionic interactions.

2.6.5 Production of Other Novel Alginate Fibers

From a wet-spinning point of view, the production process for alginate fibers is one of the cleanest processes used for manmade fibers. The dissolution of sodium alginate takes place in pure water and at neutral pH, whilst coagulation can take place in a dilute $CaCl_2$ aqueous solution, again at a neutral pH and at room temperature. This makes it easy for alginate to be used as a carrier for biologically active compounds that can be added into the fibers and still maintain their bioactivities in the finished fibers.

Fan *et al.* (2005) prepared fibers from alginate and gelatin blends by spinning their solution through a viscose-type spinneret into a coagulation bath containing aqueous $CaCl_2$ and ethanol. The highest tensile strength was obtained when the gelatin content was 30 wt% of the overall solid content. The water retention values of the blend fibers increase with increasing gelatin contents. There was strong interaction and good miscibility between alginate and gelatin molecules, as a result of intermolecular hydrogen bonds.

Wang *et al.* (2006) prepared fibers from blends of alginate and soy protein by spinning their solution through a viscose-type spinneret into a novel coagulating bath, containing aqueous $CaCl_2$, HCl and ethanol. Fibers with 10% soy protein isolate had a tensile strength of 14.1 $cN.tex^{-1}$ in the dry state and 3.46 $cN.tex^{-1}$ in the wet state, with elongation at break of 20.71 and 56.7%, respectively.

Kobayashi *et al.* (1987) used alginate fiber for enzyme immobilization. They spun an aqueous mixture of sodium alginate and enzymes into divalent metallic ion solution as a coagulating bath to produce enzyme-containing fibers. The entrapment yields of enzymes, such as glucoamylase, cyclodextrin glucanotransferase, endo-polygalacturonase and protease, were higher in the calcium alginate fibers than those found in calcium alginate beads made under similar conditions. It was found that the yields increased with increasing extrusion rate through the spinning nozzle because at a higher extrusion rate the polymeric molecules are more highly oriented along the fiber axis, which can help prevent leakage of the entrapped enzymes.