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Title: A CHITOSAN SOLUTION AND METHOD OF PREPARING THE SAME

Abstract: The present invention provides a chitosan solution having a high chitosan concentration. The chitosan concentration in the chitosan solution may be >5 wt%. The chitosan concentration in the chitosan solution may be 8-20 wt%. The chitosan solution may be a chitosan dope solution. The present invention also provides a method of preparing the chitosan solution, wherein the method comprises the steps of: (i) mixing chitosan and at least one solvent to form a mixture; and evaporating the mixture to form a chitosan solution. The solvent may be an acidic solvent.
A chitosan solution and method of preparing the same

Field of the invention

The present invention relates to a chitosan solution and a method of preparing the same. In particular, the chitosan solution has a high chitosan concentration. The chitosan concentration in the chitosan solution may be \( \geq 5 \) weight \%. In particular, the chitosan concentration may be \( \geq 8 \) weight \%.

Background of the invention

Chitosan is known as a functional and biocompatible polymer and has been widely studied in many fields of application such as in water treatment, drug delivery, and for use in biomedical devices. The free amino groups in chitosan have been found to have excellent chelating capability toward the transition metal ions and adsorption property for many electrically charged substances and macromolecules such as proteins and dyes. Chitosan is also non-toxic and biocompatible and has good haemostatic and antimicrobial ability, making it suitable for many biomedical applications. Chitosan has therefore been considered for use in medical applications such as surgery suture, drug carrier, man-made skin, dialysis membranes.

The preparation of such chitosan products normally involves dissolving chitosan directly in dilute acid solutions and subsequently solidifying the chitosan solution into desired shapes with alkali solutions. However, chitosan has been difficult to be processed into products, such as hollow fibres of adequate strength. The prepared hollow fibres are usually too soft or weak for self support, attributed to the high swelling property of chitosan and, more importantly, the low concentration of chitosan dope solutions that have been used to prepare the fibres. Since chitosan solution has very high viscosity, it has been extremely difficult to prepare a homogeneous high chitosan content dope solution. Although some patents and articles have reported the preparation of chitosan
hollow fibres, these hollow fibres have poor mechanical strength or limited applications due to their physical properties.

The problem of the low mechanical strength of chitosan hollow fibre membranes is due to the low chitosan concentrations in the chitosan dope solution used to prepare the chitosan fibres or fibre membranes. Because of the high viscosity of chitosan in an acidic solution, it is very difficult to increase chitosan concentration to a greater level than 4% (by weight) using naturally occurring molecular weight chitosan (approximately 300000 Da) and to obtain a homogeneous chitosan dope solution. Usually, the final determination of the concentration of chitosan in the dope solution may be dependent on the molecular weight of chitosan. The larger the chitosan molecular weight, the more viscous the resultant chitosan solution will be. Therefore, the concentration of chitosan in the chitosan dope solution may only be higher if low molecular weight chitosan is used. Although the use of low-viscous low molecular weight chitosan may help to increase the chitosan concentration in the chitosan dope solution (for example, a maximum of 7% as reported in Modrzejewska and Eckstein, 2004), the pre-requisite of degradation of chitosan into low molecular weight is not desirable in terms of the limited supply of such products and the increase in costs incurred for the degradation process. Further, chitosan hollow fibres prepared from low molecular weight chitosan may result in fibres with reduced functionality.

Modrzejewska and Eckstein suggest that an increase in chitosan concentration in the chitosan dope solution will remarkably increase the strength of the hollow fibres fabricated from the solution. However, a high concentration of chitosan will result in a too viscous chitosan solution and hence chitosan may not be completely dissolved in the chitosan solution, i.e. some chitosan may remain in a semi-solid state, and a non-homogeneous chitosan solution will be formed making it difficult to use the solution for spinning fibres.
WO 2006/130117 described a method of preparing chitosan and cellulose acetate blend hollow fibres in which the chitosan provides the functionality and it is the cellulose acetate that provides the desired mechanical strength. However, the limitation of low chitosan concentration in the dope solution remains (chitosan content of 4% (by weight) in the dope solution). Further, the fibres require the use of an additional blend polymer.

The preparation of chitosan hollow fibres is described in US 4,464,321, Vincent and Guibal, 2000, and Modrzejewska and Eckstein, 2004. According to the literature from which Vincent et al obtained the method for the preparation of chitosan dope solution, the concentration of chitosan in the dope solution was about 4%. This concentration is recognized as not being high enough to provide the hollow fibre membranes with good mechanical strength. Even in Vincent and Guibal, the picture shows that the hollow fibre does not have self-supporting capability. In Modrzejewska and Eckstein, a low-viscous chitosan product was used to increase the chitosan concentration to 7% and a high pressure was needed to increase the shear rate of the dope solution. During the spinning process, some crystal structures were also formed in the chitosan hollow fibres. The pore structure of the prepared chitosan hollow fibres was not reported. However, it is known that to get a high enough mechanical strength, high shear rates would need to be used to gain some crystalline structure in hollow fibres which will lead to high operation pressure and increased cost. Further, to obtain low viscosity chitosan, natural polymer would need to be chemically degraded. The problem is that there is a limited supply of low molecular weight chitosan and an increase in costs incurred for the degradation process. Further, chitosan hollow fibres prepared from low molecular weight chitosan may result in fibres with reduced functionality.

Chitosan fibres have therefore been further processed to increase their mechanical strength. For example, dip coating and chemical coating of chitosan onto other base membranes (as the support) have been practiced. Examples
can be found in US 5,259,950, CN119553, JP2001038173, Edwards et al., 1999 and CN1413761. The coating method has the limitation of low chitosan content in the final hollow fibre products, and the disadvantage of possible detachment of the coated chitosan layer.

There is therefore a need in the art for a chitosan solution having a high chitosan concentration and which remains homogenous and for a chitosan construct having high mechanical strength.

Summary of the invention

The present invention seeks to address the problems above, and provides a chitosan solution and a method of preparing the same. In particular, the present invention provides a method of preparing chitosan solution with a high chitosan concentration. The chitosan solution may be a homogeneous chitosan solution.

According to a first aspect, the present invention provides a method of preparing a chitosan solution comprising the steps of:

(i) mixing chitosan and at least one solvent to form a mixture; and

(ii) evaporating the mixture to form a chitosan solution.

The chitosan solution formed in step (ii) may be a chitosan dope solution. The concentration of chitosan in the chitosan solution formed in step (ii) may be \( \geq 5 \) wt %. The concentration of chitosan in the chitosan solution formed in step (ii) may be \( \geq 8\% \). In particular, the concentration of chitosan in the chitosan solution formed in step (ii) may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 9-14 wt % or 10-12 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %. The chitosan solution formed in step (ii) may be a homogenous solution.
The concentration of chitosan used in step (i) may be ≥ 0.5 wt % based on the total weight of the mixture. For example, the concentration of the chitosan used in step (i) may be ≤ 8 wt %. In particular, any suitable concentration of chitosan may be used in step (i) to ensure complete and homogeneous dissolution of chitosan. For example, the concentration of chitosan used in step (i) may be 0.5-8 wt %, 1-5 wt % or 3-5 wt %. In particular, the concentration of chitosan may be 1-3 wt %. Even more in particular, the concentration of chitosan is 2 wt % or 3 wt %.

The solvent used in step (i) may be any suitable solvent. In particular, the solvent may be an acidic solvent. Even more in particular, the solvent may comprise at least one acid. For example, the solvent may be acetic acid, formic acid, hydrochloric acid, lactic acid, propionic acid, di-chloroacetic acid or mixtures thereof. According to a particular aspect, the solvent is acetic acid, formic acid, or mixtures thereof.

The step (i) of mixing may further comprise mixing water and/or at least one additive with the chitosan and at least one solvent. The addition of at least one additive in step (i) of mixing may be optional. The additive may be a viscosity-reducing additive. Any suitable additive may be added. For example, the additive may be any one of, but not limited to: NaCl, KCl, LiCl, CH₃COONa, urea, silica gel, poly(ethylene glycol) and mixtures thereof. The additives may reduce the viscosity of the mixture formed in step (i).

The evaporation step (ii) may be performed at a suitable temperature. For example, the temperature may be dependent on the solvent used in the mixing step (i) of the method. The temperature at which evaporation step (ii) is performed may be 20-120 °C. In particular, the temperature may be 40-100 °C. Even more in particular, the temperature may be 60-80 °C. For example, the evaporation step (ii) is performed at 60 °C.
The method may further comprise a step of filtering the mixture prior to the step (ii) of evaporating the mixture. The method may further comprise a step of degassing the chitosan solution formed in step (ii). Any suitable method may be used for the degassing step. For example, the degassing step may be performed by centrifugation of the chitosan solution formed in step (ii), or by placing the chitosan solution under vacuum conditions.

According to a second aspect, the present invention provides a chitosan solution prepared according to the method of any aspect of the present invention. In particular, the chitosan solution prepared is a chitosan dope solution. The concentration of chitosan in the chitosan solution may be ≥ 5 wt %. The concentration of the chitosan in the chitosan solution may be ≥ 8 wt %. In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 9-14 wt % or 10-12 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %.

The chitosan solution may be a homogeneous solution.

According to another aspect of the present invention, there is provided a chitosan solution, wherein the concentration of chitosan in the chitosan solution is ≥ 5 wt %. The concentration of the chitosan in the chitosan solution may be ≥ 8 wt %. In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 9-14 wt % or 10-12 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %. The chitosan solution may be a homogeneous solution. The chitosan solution may be a chitosan dope solution.

According to a particular aspect, the chitosan solution may be prepared according to a method comprising the steps of: (i) mixing chitosan and at least one solvent to form a mixture; and (ii) evaporating the mixture to form the chitosan solution.
Brief description of the figures

Figure 1: (a), (b) and (c) show scanning electron microscope (SEM) photographs of a chitosan hollow fibre. Figure 1(a) shows the cross-section of the fibre (x 40000), Figure 1(b) shows the inner surface of the fibre (x 40000) and Figure 1(c) shows the outer surface of the fibre (x 20000).

Figure 2: (a), (b) and (c) show a scanning electron microscope (SEM) photographs of a chitosan hollow fibre. Figure 2(a) shows the cross-section of the fibre (x 40000), Figure 2(b) shows the inner surface of the fibre (x 40000) and Figure 2(c) shows the outer surface of the fibre (x 20000).

Figure 3: Figures 3 (a), (b) and (c) show chitosan solution (concentration of chitosan in chitosan solution is 8%) prepared using the method of one embodiment of the present invention after 0, 10 and 20 minutes.

Figure 4: Figures 4 (a), (b) and (c) show chitosan solution (concentration of chitosan in chitosan solution is 5%) prepared using a conventional direct mixing method after 0, 10 and 20 minutes.

Detailed description of the invention

Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content of such bibliographic references is herein incorporated by reference.

The present invention relates to the preparation of a chitosan solution. In particular, the present invention provides a chitosan solution with a high chitosan concentration and a method of preparing the same. In particular, the present invention relates to the preparation of a homogeneous high content chitosan solution. The concentration of chitosan in the chitosan solution may be higher than 5 weight (wt) %. The chitosan solution may have various
applications. For example, the chitosan solution may be used for fabrication of various articles, such as mechanically strong chitosan hollow fibres and chitosan hollow fibre membranes. The chitosan hollow fibres may be pure chitosan fibres. The present invention also provides a chitosan construct. The chitosan construct may have a high mechanical strength making it suitable for various applications such as in bioengineering and environmental engineering. For example, the construct may be mechanically strong chitosan hollow fibres or chitosan hollow fibre membranes. The chitosan hollow fibres may be pure chitosan fibres. The mechanical strength of the hollow fibres may be 2-4 MPa in tensile stress. The present invention also provides a method of preparing the chitosan construct.

According to a first aspect, the present invention provides a method of preparing a chitosan solution, the method comprising the steps of:

(i) mixing chitosan and at least one solvent to form a mixture; and

(ii) evaporating the mixture to form a chitosan solution.

The chitosan solution formed in step (ii) may have a high chitosan concentration. The concentration of chitosan in the chitosan solution formed in step (ii) may be \( \geq 5 \) weight (wt) %. The concentration of the chitosan in the chitosan solution may be \( \geq 8 \) wt %. In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 10-15 wt %, 9-14 wt %, 10-12 wt % or 12-14 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %. The chitosan solution formed in step (ii) may be a homogenous solution.

The chitosan solution formed in step (ii) may be a chitosan dope solution. The chitosan dope solution may be used for different applications. For example, the chitosan dope solution may be used to spin fibres, prepare membranes for use in various bioengineering applications and filtration processes.
Any suitable concentration of chitosan can be used in step (i). The concentration of chitosan used in step (i) may be \( \leq 8 \) wt %. The concentration of chitosan used in step (i) may be \( \leq 5 \) wt %. For example, the concentration of chitosan used in step (i) may be \( \geq 0.5 \) wt %. In particular, any suitable concentration of chitosan may be used in step (i) to ensure complete and homogeneous dissolution of chitosan. For example, the concentration of chitosan may be \( 1-3 \) wt %. Even more in particular, the concentration of chitosan is \( 2 \) wt % or \( 3 \) wt %.

Chitosan is the deacetylated form of chitin, a naturally abundant biopolymer widely available from various resources such as seafood processing waste. Chitosan may be considered to be a cellulose-derived compound in which one of the hydroxyl groups in the cellulose ring is substituted by an amino group. The nitrogen atom accounts for up to about 7% (by weight) of the chitosan polymer.

The chitosan used in step (i) may have any suitable molecular weight. The chitosan may have a molecular weight of \( 10000 \) - \( 1200000 \) Da. In particular, naturally occurring chitosan may be used. Even more in particular, naturally occurring chitosan having molecular weight of about \( 300000 \) Da may be used. The use of naturally occurring chitosan directly avoids the need to further degrade chitosan into low molecular weight chitosan.

The chitosan used in step (i) may have a suitable degree of deacetylation. For example, the degree of deacetylation may be \( \geq 55\% \). In particular, the degree of deacetylation may be \( \geq 65\% \), \( \geq 75\% \) or \( \geq 85\% \). A higher degree of deacetylation may result in a higher dissolution of chitosan being achieved in the solvent in step (i).
The solvent used in step (i) may be any suitable solvent. For example, the solvent may be any suitable solvent in which chitosan used in step (i) may dissolve. The solvent may be an acidic solvent. According to a particular aspect, the solvent may comprise at least one acid. Any suitable acid may be used. For example, the solvent may be acetic acid, formic acid, hydrochloric acid, lactic acid, propionic acid, di-chloroacetic acid or mixtures thereof. According to a particular aspect, the solvent is acetic acid, formic acid, or mixtures thereof. The concentration of the solvent used in step (i) may be any suitable concentration, depending on the type of solvent used. For example, the concentration of the solvent may vary from 0.3-100 wt%, 2-95 wt%, 5-80 wt%, 10-75 wt%, 15-60 wt%, 20-50 wt%, 25-45 wt%, or 30-40 wt%. In particular, if the solvent used in step (i) is acetic acid, the concentration of the acetic acid may be 0.3-95 wt%. If hydrochloric acid is used as the solvent in step (i), the concentration of the hydrochloric acid may be 0.5-10 wt%. If the solvent used in step (i) is formic acid, the concentration of the formic acid may be 0.5-100 wt%. For the purposes of the present invention, the concentration of the solvent is calculated from the following formula:

$\text{wt}\% = \frac{(w_{\text{weight}})_{\text{solvent}}}{(w_{\text{total\,liquid}})} \times 100$

The weight of total liquid refers to the total weight of the solvent being mixed with the chitosan.

The step (i) of mixing may further comprise mixing water and/or at least one additive with the chitosan and at least one solvent. The addition of at least one additive in step (i) of mixing is optional. The additive may be a viscosity-reducing additive. The additives may reduce the viscosity of the mixture formed in step (i). Any suitable additive may be added. For example, the additive may be any one of, but not limited to: NaCl, KCl, LiCl, CH₃COONa, urea, silica gel, poly(ethylene glycol) and mixtures thereof.
The step (i) of mixing may further comprise mixing at least one additive with the chitosan and at least one solvent. The additive may be any suitable additive which is added to vary the pore size and porosity of an article of manufacture which may be prepared from the chitosan solution according to any aspect of the present invention. The additive may be a porogen. For the purposes of the present invention, a porogen is any suitable material which enables the formation of pores in a dense structure. For example, the additive may be selected from the group consisting of, but not limited to: silica gel, glycerol, ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol, trimethylene glycol, polymers and mixtures thereof. The polymers may be undissolved polymer in small beads form, such as PVC (polyvinyl chloride), PVDF (polyvinylidene fluoride), PAN (polyacrylonitrile), PET (Polyethylene terephthalate) and mixtures thereof. The small beads of undissolved polymers may be removed using organic solvents after the preparation of the article of manufacture. The polymer may also be dissolved polymers such as cellulose acetate and nylon as supporting polymer. The additives may also be supporting polymers which are soluble in the solvent to provide additional mechanical strength to the article of manufacture which may be prepared from the chitosan solution according to any aspect of the present invention. Examples of supporting polymers include, but are not limited to, cellulose acetate, nylon and mixtures thereof.

The mixing step of step (i) may be performed with stirring to achieve higher dissolution of the chitosan in the solvent. The stirring may be continuous. The stirring may be mechanical stirring. The stirring may be achieved by using a mechanical stirrer. According to a particular aspect, the dissolution of chitosan in acetic acid may be very fast and may take less than one hour to obtain a homogeneous and clear 2 wt % chitosan mixture based on the total weight of the mixture. The mixture may be further stirred for more than one hour to ensure complete dissolution of the chitosan in the acetic acid.
The step (ii) of evaporating the mixture may be performed at a suitable temperature. For example, the temperature at which the step (ii) of evaporating the mixture is performed may be dependent on the solvent that is used in the mixing step (i). For example, the temperature at which step (ii) is performed is any suitable temperature at which the solvent may evaporate. The temperature at which step (ii) is performed may be 20-120°C. In particular, the temperature may be 40-100°C, 60-80°C or 70-75°C. For example, step (ii) is performed at 60°C.

According to a particular aspect, the mixture of step (i) may be heated to achieve the temperature at which step (ii) is performed. Any suitable method of heating the mixture may be used. For example, the mixture may be placed in a water bath until the pre-determined temperature at which step (ii) is to be performed is reached.

The evaporating step (ii) may be performed with stirring to maintain the homogeneity of the mixture. The stirring may be continuous. The stirring may be mechanical stirring. The stirring may be achieved by using a mechanical stirrer.

The step (ii) of evaporating the mixture is performed for a suitable period of time. The evaporating step (ii) may be stopped when a desired concentration of chitosan has been achieved in the chitosan solution which is formed. For example, the evaporation step (ii) may be stopped when a pre-determined weight of the total solution is achieved. The total weight of the solution may be an indication of the concentration of chitosan in the chitosan solution.

According to a particular aspect, the mixture of step (i) may be heated up to 60-80°C with mechanical stirring. When the concentration of chitosan increases to a pre-determined level (slightly lower than the final concentration needed), the stirring is stopped to decelerate the rate of evaporation of the solvent in the mixture. An optic and viscous gel may be obtained when the evaporation process is stopped. The time needed for the evaporation step may vary
depending on the final chitosan concentration that is desired. However, the method should typically be completed within a day.

The method may further comprise a step of filtering the mixture prior to the step (ii) of evaporating the mixture. A step of filtering the mixture may remove the impurities which are in the mixture and have not dissolved in the solvent. Any suitable filtration method may be used for the purposes of the present invention. For example, the mixture may be passed through a filter paper.

The method according to the present invention may further comprise a step of degassing the chitosan solution formed in step (ii). The degassing allows air bubbles entrapped in the chitosan solution to be removed from the chitosan solution. Any suitable method may be used for the degassing step. For example, the degassing step may be performed by centrifugation of the chitosan solution formed in step (ii) or under vacuum conditions.

According to a particular aspect, the chitosan dope solution is centrifuged, for example in a SIGMA 3-16-19776 centrifuge from UK, at 3000 rpm for 10 minutes to free the air bubbles entrapped in the chitosan solution. The chitosan solution may also become transparent.

According to a particular aspect, the method of the present invention comprises the following steps:

(a) preparing a clear and homogeneous 2-4 wt % chitosan solution in 1% acetic acid;

(b) filtering the mixture of step (a) to remove impurities;

(c) evaporating a certain amount of water while mechanical stirring the mixture to keep the mixture in a clear and homogeneous state until a chitosan solution is formed in which the final concentration of chitosan is about 12 wt %; and
(d) degassing the chitosan solution by centrifugation to remove any possible air bubbles entrapped in the solution.

The chitosan solution formed in step (c) may be viscous.

According to another particular aspect, the apparatus in which the chitosan solution is prepared and the stirring apparatus used in the method may be made of glassware, stainless steel or Teflon, or any suitable material which is resistant to the solvents used in the mixing step (i). For example, the apparatus may be made of material which is resistant to acids.

According to another aspect, the present invention provides a chitosan solution prepared according to the method of any aspect of the present invention. In particular, the chitosan solution prepared is a chitosan dope solution. The concentration of chitosan in the chitosan solution may be ≥ 5 wt %. The concentration of the chitosan in the chitosan solution may be ≥ 8 wt %. In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 10-15 wt %, 9-14 wt %, 10-12 wt % or 12-14 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %. The chitosan solution may be a homogeneous solution.

According to another aspect of the present invention, there is provided a chitosan solution, wherein the concentration of chitosan in the chitosan solution is ≥ 5 wt %. The concentration of the chitosan in the chitosan solution may be ≥ 8 wt %. In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 10-15 wt %, 9-14 wt %, 10-12 wt % or 12-14 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %. The chitosan solution may be a homogeneous solution. The chitosan solution may be a chitosan dope solution.

According to a particular aspect, the chitosan solution may be prepared according to a method comprising the steps of: (i) mixing chitosan and at least
one solvent to form a mixture; and (ii) evaporating the mixture to form the chitosan solution. In particular, the chitosan solution may be prepared by the method described above.

The chitosan solution according to any aspect of the present invention may have a higher chitosan concentration than that in chitosan solutions prepared by existing methods while maintaining homogeneity and/or having a suitable viscosity. In particular, the chitosan concentration of the chitosan dope solution according to any aspect of the present invention may be higher compared to the chitosan solutions having a chitosan concentration of 4% to 7% which are prepared by existing methods (US 4,464,321 and Modrzejewska and Eckstein, 2004). Accordingly, the chitosan solution according to any aspect of the present invention may have many uses. It is known that a higher chitosan concentration in the chitosan solution leads to an increase in mechanical strength of the articles manufactured from the chitosan solution (Modzejewska and Eckstein, 2004). For the purposes of the present invention, mechanical strength may be measured by tensile strength. Tensile strength is defined as the maximum tensile stress that can be sustained by an object before failure in a tensile test.

For example, chitosan may be used to make surgery sutures because of its bio-comparability, biodegradability and anti-bacterial properties. These characteristics favour and promote rapid dermal regeneration and accelerated wound healing. The chitosan solution according to any aspect of the present invention may therefore be used in bioengineering application, for example in producing stronger chitosan surgery sutures.

Chitosan is also often used as wound dressing or artificial skin because of its bio-comparability, biodegradability and anti-bacterial properties. Because of the low concentration of chitosan in the chitosan solution prepared by existing methods, the wound dressing or artificial skin that is made from these solutions are often fragile or heterogeneous (because of the drying of the solution before
solidification) or both. However, using the chitosan solution according to any aspect of the present invention which has a high chitosan concentration and is homogeneous may lead to the production of wound dressing or artificial skin which is stronger and more homogeneous.

Further, adsorption is often operated in a column configuration in which beads are filled. Chitosan beads have been studied by many researchers on their adsorption properties (WS Wan Ngah et al, 2002 and Nan Li and Renbi Bai, 2005). However, the adsorption column filled with chitosan beads have not been used in real industry because the beads made from the low concentration chitosan solution prepared by existing methods are too fragile. Accordingly, the chitosan solution according to the present invention may be used in the production of beads that made can be much stronger.

The chitosan solution according to the present invention may also be used in the preparation of chitosan constructs such as chitosan hollow fibres or chitosan hollow fibre membranes. Such constructs may have many applications such as for use in bioengineering applications, as membranes in filtration, in separation applications, in water and wastewater treatment in environmental engineering. With the high concentration of chitosan in the chitosan solution, constructs with correspondingly higher chitosan content may be prepared.

Accordingly, the present invention also provides an article of manufacture comprising chitosan solution according to any aspect of the present invention as described above. The chitosan solution may be prepared by the method according to any aspect of the present invention described above. The article of manufacture comprising the chitosan solution of the present invention may have improved mechanical strength, which may be measured by its tensile strength, and/or improved adsorption. In particular, the tensile strength of the article of manufacture may be $\geq 1$ MPa, $\geq 2$ MPa, $\geq 2.5$ MPa, $\geq 3$ MPa, $\geq 3.5$ MPa, $\geq 4$ MPa, $\geq 4.5$ MPa, $\geq 5$ MPa, $\geq 5.5$ MPa, $\geq 6$ MPa, $\geq 7$ MPa, $\geq 8$ MPa, $\geq 9$ MPa,
10 MPa, ≥ 11 MPa, ≥ 12 MPa, ≥ 13 MPa, ≥ 14 MPa, ≥ 15 MPa or ≥ 16 MPa. Even more in particular, the tensile strength may be 1-10 MPa, 2-8 MPa, 3-5 MPa. For example, the tensile strength may be 2.2 MPa or 3.5 MPa.

The article of manufacture may have any suitable shape and form. For example, the article of manufacture may be a chitosan construct. The article of manufacture may be a thread, a surgical suture, beads, membranes, flat sheet membranes, hollow fibres, wound dressing, artificial skin or beads for use as packing material in adsorption columns.

According to a particular aspect, the article of manufacture is a chitosan hollow fibre. The chitosan hollow fibre may have a tensile strength ≥ 0.1 MPa. In particular, the tensile strength of the hollow fibre may be ≥ 1 MPa, ≥ 2 MPa, ≥ 2.5 MPa, ≥ 3 MPa, ≥ 3.5 MPa, ≥ 4 MPa, ≥ 4.5 MPa, ≥ 5 MPa, ≥ 5.5 MPa, ≥ 6 MPa, ≥ 7 MPa, ≥ 8 MPa, ≥ 9 MPa, ≥ 10 MPa, ≥ 11 MPa, ≥ 12 MPa, ≥ 13 MPa, ≥ 14 MPa, ≥ 15 MPa or ≥ 16 MPa. Even more in particular, the tensile strength may be 1-10 MPa, 2-8 MPa, 3-5 MPa. For example, the tensile strength may be 2.2 MPa or 3.5 MPa.

According to another aspect, the present invention provides a chitosan construct. For the purposes of the present invention, a chitosan construct is defined as a chitosan construct which may be a 2D and/or 3D structure comprising chitosan. The chitosan construct may be of any suitable form, for example, in the form of a membrane, fibres, hollow fibres, tubular structure, and the like. According to one embodiment, the invention provides a method for preparing chitosan construct(s), wherein the construct is made from a chitosan solution having a high chitosan concentration. The construct comprising chitosan has a tensile strength of 0.1-6 MPa. In particular, the tensile strength may be 1-5 MPa, 2-5 MPa, 2.5-4.5 MPa or 3.5-4 MPa. Even more in particular, the tensile strength is 2.2 MPa or 3.5 MPa. The tensile strength of the construct
according to any aspect of the present invention may be based on the tensile strength of the wet construct.

The construct may be of any suitable form and shape. The construct may be in the form of a fibre, a membrane, beads, thread, sutures, and the like. In particular, the construct may be a fibre. The construct may be a hollow construct. According to a particular aspect, the construct may be a hollow fibre. The construct may also be a hollow fibre membrane.

The construct may have a high chitosan content. For the purposes of the present invention, chitosan content will be defined as the ratio of chitosan in the construct by weight. For example, the chitosan content in the construct may be ≥ 10%, ≥ 20%, ≥ 30%, ≥ 35%, ≥ 40%, ≥ 45%, ≥ 50%, ≥ 60%, ≥ 75%, ≥ 80%, ≥ 90% or 100%. In particular, the chitosan content in the construct may be 80-100%. Even more in particular, the chitosan content may be 100%. Accordingly, the construct may be a pure chitosan hollow fibre.

According to a particular aspect, the construct may be prepared from a suitable chitosan solution. In particular, the chitosan solution has a high chitosan concentration and is a homogeneous chitosan solution. The construct may be prepared from a chitosan solution having chitosan concentration ≥ 5 weight (wt) %. The concentration of the chitosan in the chitosan solution may be ≥ 8 wt %.

In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 10-15 wt %, 9-14 wt %, 10-12 wt % or 12-14 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %.

The chitosan solution for the preparation of the chitosan construct may be a chitosan dope solution. The chitosan solution used for the preparation of the chitosan construct may be prepared according to a method comprising the steps of: (i) mixing chitosan and at least one solvent to form a mixture; and (ii) evaporating the mixture to form the chitosan solution. In particular, the chitosan
solution may be prepared according to any aspect of the present invention, as further described below.

Any suitable solvent may be used in the preparation of the chitosan solution. In particular, the at least one solvent may be an acid. Even more in particular, the at least one solvent may be acetic acid, formic acid or a mixture thereof.

The construct may have a suitable pore structure. For example, the construct may have a porous structure. The pore structure of the construct may be such that finger-like macrovoids are absent from the construct. In particular, the construct may have a sponge-like porous structure. The porosity of the construct may be up to 80%. For example, the porosity may be about 20-80%. The construct may have interconnected pores. The interconnected pores in the structure may facilitate transport in the construct. The construct having a sponge-like porous may be suitable for use as an affinity or adsorptive membrane as it has a higher specific surface area and improved separation performance.

The construct according to any aspect of the present invention may be homogeneous, and therefore detachment or dissolution problems may be avoided which is experienced in constructs which are prepared from chitosan composites such as those described in US 5,259,950, CN1 119553, JP2001 0381 73, CN1 413761 and Edwards et al, 1999.

The construct according to any aspect of the present invention may also be modified by various ligands for a specific use. For example, the construct may be used as an affinity membrane in membrane chromatography for the pharmaceutical industry to selectively separate proteins and enzymes. The construct may be chitosan hollow fibres. Ligands such as metal ions and dyes may be immobilized onto the hollow fibres with chitosan, if desired or when necessary. For such an application, the hollow fibres may have a microporous or macroporous structure with high chitosan content.
The construct according to any aspect of the present invention may also be used in the recovery and/or removal of various valuable and/or toxic transitional metal ions from water, wastewater or industrial effluents. For example, the construct may be in the form of chitosan hollow fibres. The metal ions which may be effectively recovered/concentrated include, but are not limited to, copper, lead, gold, silver, mercury, zinc, nickel and chromium. The hollow fibres may have a microporous structure and a high chitosan content.

The construct according to any aspect of the present invention may also be used in medicine as blood or kidney dialysis membranes. The presence of chitosan in the construct may adsorb and hence remove toxic macromolecules which may be metabolised by the human body but which cannot be removed by conventional dialysis devices.

Other applications of the construct of the present invention may be in the field of seawater desalination as the presence of chitosan in the construct can enhance the rejection rate toward sodium ions and potassium ions. For example, the construct may also be suitably modified by adding N-methylglucamine groups which has affinity to boron via reaction. In this way, the construct can effectively adsorb boron. The inability of adsorb boron is the limitation of present seawater desalination systems because of its small size.

The construct according to the present invention may also be used as filler in adsorption columns. For example, the constructs may be cut into short pieces and filled into the columns. In this way, a low pressure requirement may be set up within the column, and this may lead to lower costs for running the adsorption column.

The present invention also provides a method for preparing chitosan constructs. In particular, the construct may be pure chitosan hollow fibres with high mechanical strength. The preparation of the chitosan hollow fibres may include the steps of dissolving chitosan in acidic solution to obtain a homogeneous and
viscous solution; and spinning the solution through a spinneret into a coagulation bath to form the chitosan hollow fibres. Accordingly, another aspect of the present invention is a method of preparing a construct comprising chitosan, the method comprising the steps of:

5 (i) mixing chitosan and at least one solvent to form a mixture;

(ii) evaporating the mixture to form a chitosan solution; and

(iii) preparing the construct from the chitosan solution,

wherein the construct has a tensile strength of 0.1-6 MPa.

The construct prepared by the method of the present invention may have a tensile strength of 1-5 MPa, 2-5 MPa, 2.5-4.5 or 3.5-4 MPa. In particular, the tensile strength may be 2.2 MPa or 3.5 MPa.

The chitosan solution formed in step (ii) may have a high chitosan concentration. The concentration of chitosan in the chitosan solution formed in step (ii) may be \( \geq 5 \) weight (wt) %. The concentration of the chitosan in the chitosan solution may be \( \geq 8 \) wt %. In particular, the concentration of chitosan in the chitosan solution may be 5-20 wt %, 8-20 wt %, 8-15 wt %, 10-15 wt %, 9-14 wt %, 10-12 wt % or 12-14 wt %. Even more in particular, the concentration of chitosan in the chitosan solution may be about 12 wt %. The chitosan solution formed in step (ii) may be a homogenous solution. The chitosan solution formed in step (ii) may be a chitosan dope solution.

Any suitable concentration of chitosan can be used in step (i). The concentration of chitosan used may be any suitable concentration to ensure complete and homogeneous dissolution of chitosan. The concentration of chitosan used in step (i) may be \( \leq 8 \) wt % based on the total weight of the mixture. The concentration of chitosan used in step (i) may be \( \leq 5 \) wt %. For example, the concentration of chitosan used in step (i) may be \( \geq 0.5 \) wt %. For
example, the concentration of chitosan used in step (i) may be 0.5-8 wt %, 1-5 wt %, 3-5 wt % or 4-5 wt %. In particular, the concentration of chitosan may be 1-3 wt %. Even more in particular, the concentration of chitosan is 2 wt % or 3 wt %.

The chitosan used in step (i) may have any suitable molecular weight. The chitosan may have a molecular weight of 10000 - 1200000 Da. In particular, naturally occurring chitosan or commercially available chitosan may be used. Even more in particular, naturally occurring chitosan having molecular weight of about 300000 Da may be used. The use of naturally occurring chitosan directly avoids the need to further degrade chitosan into low molecular weight chitosan.

The chitosan used in step (i) may have a suitable degree of deacetylation. For example, the degree of deacetylation may be $\geq 55\%$. In particular, the degree of deacetylation may be $\geq 65\%$, $\geq 75\%$ or $\geq 85\%$. A higher degree of deacetylation may result in a higher dissolution of chitosan being achieved in the solvent in step (i).

The solvent used in step (i) may be any suitable solvent. For example, the solvent may be any suitable solvent in which chitosan used in step (i) may dissolve. The solvent may be an acidic solvent. According to a particular aspect, the solvent may comprise at least one acid. Any suitable acid may be used. For example, the solvent may be acetic acid, formic acid, hydrochloric acid, lactic acid, propionic acid, di-chloroacetic acid or mixtures thereof. According to a particular aspect, the solvent is acetic acid, formic acid, or mixtures thereof. The concentration of the solvent used in step (i) may be any suitable concentration, depending on the type of solvent used. For example, the concentration of the solvent may vary from 0.3-1 00 wt%, 2-95 wt %, 5-80 wt%, 10-75 wt %, 15-60 wt %, 20-50 wt %, 25-45 wt %, or 30-40 wt%. In particular, if the solvent used in step (i) is acetic acid, the concentration of the acetic acid may be 0.3-95 wt %. If hydrochloric acid is used as the solvent in step (i), the concentration of the
hydrochloric acid may be 0.5-10 wt %. If the solvent used in step (i) is formic acid, the concentration of the formic acid may be 0.5-100 wt %. For the purposes of the present invention, the concentration of the solvent is calculated from the following formula:

\[
wt\% = \left( \frac{\text{weight}_{\text{solvent}}}{\text{weight}_{\text{total liquid}}} \right) \times 100\%
\]

The weight of total liquid refers to the total weight of the solvent being mixed with the chitosan.

The step (i) of mixing may further comprise mixing water and/or at least one additive with the chitosan and at least one solvent. The addition of at least one additive in step (i) of mixing is optional. The additive may be a viscosity-reducing additive. Any suitable additive may be added. For example, the additive may be any one of, but not limited to: NaCl, KCl, LiCl, CHSCOONa, urea, silica gel, poly(ethylene glycol) and mixtures thereof. The additives may reduce the viscosity of the mixture formed in step (i).

The step (i) of mixing may further comprise mixing at least one additive with the chitosan and at least one solvent. The additive may be any suitable additive which is added to vary the pore size and porosity of the construct which may be prepared from the chitosan solution formed in step (ii). The additive may be a porogen. For the purposes of the present invention, a porogen is any suitable material which enables the formation of pores in a dense structure. For example, the additive may be selected from the group consisting of, but not limited to: silica gel, glycerol, ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol, trimethylene glycol, polymers and mixtures thereof. The polymers may be undissolved polymer in small beads form, such as PVC (polyvinyl chloride), PVDF (polyvinylidene fluoride), PAN (polyacrylonitrile), PET (Polyethylene terephthalate) and mixtures thereof. The small beads of
undissolved polymers may be removed using organic solvents after the preparation of the article of manufacture. The polymer may also be dissolved polymers such as cellulose acetate and nylon as supporting polymer. The additives may also be supporting polymers which are soluble in the solvent to provide additional mechanical strength to the article of manufacture which may be prepared from the chitosan solution according to any aspect of the present invention. Examples of supporting polymers include, but are not limited to, cellulose acetate, nylon and mixtures thereof.

The mixing step of step (i) may be performed with stirring to achieve higher dissolution of the chitosan in the solvent. The stirring may be continuous. The stirring may be mechanical stirring. The stirring may be achieved by using a mechanical stirrer. According to a particular aspect, the dissolution of chitosan in acetic acid may be very fast and may take less than one hour to obtain a homogeneous and clear 2 wt % chitosan mixture based on the total weight of the mixture. The mixture may be further stirred for more than one hour to ensure complete dissolution of the chitosan in the acetic acid.

The step (ii) of evaporating the mixture may be performed at a suitable temperature. For example, the temperature at which the step (ii) of evaporating the mixture is performed may be dependent on the solvent that is used in the mixing step (i). For example, the temperature at which step (ii) is performed is any suitable temperature at which the solvent may evaporate. The temperature at which step (ii) is performed may be 20-120°C. In particular, the temperature may be 40-100°C, 60-80°C or 70-75°C. For example, step (ii) is performed at 60°C.

According to a particular aspect, the mixture of step (i) may be heated to achieve the temperature at which step (ii) is performed. Any suitable method of heating the mixture may be used. For example, the mixture may be placed in a
water bath until the pre-determined temperature at which step (ii) is to be performed is reached.

The evaporating step (ii) may be performed with stirring to maintain the homogeneity of the mixture. The stirring may be continuous. The stirring may be mechanical stirring. The stirring may be achieved by using a mechanical stirrer.

The step (ii) of evaporating the mixture is performed for a suitable period of time. The evaporating step (ii) may be stopped when a desired concentration of chitosan has been achieved in the chitosan solution which is formed. For example, the evaporation step (ii) may be stopped when a pre-determined weight of the total solution is achieved. The total weight of the solution may be an indication of the concentration of chitosan in the chitosan solution.

According to a particular aspect, the mixture of step (i) may be heated up to 60-80°C with mechanical stirring. When the concentration of chitosan increases to a pre-determined level (slightly lower than the final concentration needed), the stirring is stopped to decelerate the rate of evaporation of the solvent in the mixture. An optic and viscous gel may be obtained when the evaporation process is stopped. The time required for the evaporation step may vary depending on the final chitosan concentration that is desired. However, the method should typically be completed within a day.

According to a particular aspect, the apparatus in which the chitosan solution is prepared and the stirring apparatus used in the method may be made of glassware, stainless steel or Teflon, or any suitable material which is resistant to the solvents used in the mixing step (i). For example, the apparatus may be made of material which is resistant to acids.

The method may further comprise a step of filtering the mixture prior to the step (ii) of evaporating the mixture. A step of filtering the mixture may remove the impurities which are in the mixture and have not dissolved in the solvent. Any
suitable filtration method may be used for the purposes of the present invention. For example, the mixture may be passed through a filter paper.

The method according to the present invention may further comprise a step of degassing the chitosan solution formed in step (ii). The degassing allows air bubbles entrapped in the chitosan solution to be removed from the chitosan solution. Any suitable method may be used for the degassing step. For example, the degassing step may be performed by centrifugation of the chitosan solution formed in step (ii) or under vacuum conditions.

According to a particular aspect, the chitosan dope solution is centrifuged, for example in a SIGMA 3-16-19776 centrifuge from UK, at 3000 rpm for 10 minutes to free the air bubbles entrapped in the chitosan solution. The chitosan solution may also become transparent.

According to a particular aspect, the step (iii) of preparing the construct may comprise spinning of the chitosan solution formed in step (ii). The spinning may be wet spinning. The step (iii) of preparing the construct may be as described in C Liu and R B Bai, 2005. The step (iii) of preparing the construct may result in the formation of fibres.

In particular, the step (iii) may comprise wet spinning the chitosan solution of step (ii) by means of a suitable hollow fibre spinneret, and using a coagulant bath (quench bath). The commonly available wet spinning device can be used for spinning the chitosan construct, for example in the form of chitosan hollow fibres. The tubing connecting the device and the major parts of the wet spinning device such as the dope tank, spinneret and receptacle holding the quench bath, should be made of appropriate material, such as stainless steel, which is resistant to acid.

The spinneret may comprise an annular ring and the outer and inner diameters of the annular ring are dependent on the size of the construct in the form of
hollow fibres to be prepared. The method comprises extruding the chitosan solution of step (ii) into a quenching bath while a core liquid is delivered through the lumen of the fibres. In particular, a bore liquid coagulant (or core liquid) is provided simultaneously through the inner core of the spinneret. The air gap, i.e. the distance from the outlet of the spinneret to the surface of the quenching bath can be zero or some value, depending on the characteristics of the hollow fibres to be prepared. The homogeneous chitosan dope solution is forced through the spinneret under high pressure $\text{N}_2$ gas, and protruded into a coagulation bath. The core liquid coagulant is delivered simultaneously through the inner core of the spinneret with a syringe pump. The flow rate of the chitosan solution and the core liquid can be controlled by the pressure of the compressed nitrogen gas cylinder and by a syringe pump respectively. The flow rates of the chitosan solution and the core liquid may be adjusted according to the specific requirements of the process and construct to be prepared.

The liquid in the quench bath and the core liquid are also referred to as the external and internal coagulants, respectively. The external coagulant and the internal coagulant may be composed of non-solvents which are mixable with the solvent in which the chitosan is dissolved in step (i) but in which the chitosan will precipitate and form solid. Any suitable non-solvent may be used. The non-solvent may be an alkali solution. For example, the non-solvents which may be used include, but are not limited to, KOH, NaOH solution, $\text{Na}_3\text{PO}_4$, sodium acetate (NaAc) solution, ammonia solution, tripolyphosphate solution, alginate solution and mixtures thereof. The choice of external and internal coagulants depends on the amount of chitosan to be fixed and the structure of the hollow fibres to be prepared. A higher concentration of the quenching bath will lead to a denser construct being formed. The concentration of the quenching bath should be high enough to ensure adequate hardening rate of the chitosan solution to get the desired hollow fibre configuration.
Once the chitosan hollow fibres are drawn out of the external coagulant bath, the fibres are collected by a PVC drum and at the same time are rinsed with clean water. The collection rate of these hollow fibres is accurately controlled by a motor so that a pre-determined dragging force is imposed on the hollow fibres. Alternatively, the collection rate may be adjusted such that there is no dragging force imposed on the hollow fibres. After removing the fibres from the drum, the chitosan hollow fibres are rinsed again and immersed in DI water to clean the excessive coagulant.

According to a particular aspect, the hollow fibres may be treated, before drying, with a plasticizer which acts as a softening agent. Examples of plasticizers include, but are not limited to, glycerol, ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol and trimethylene glycol. In particular, the plasticizer is glycerol. The treatment of hollow fibres with plasticizers may be carried out by immersing the hollow fibres in a 10% (by weight) of glycerol aqueous solution for a number of days, for example three days.

To obtain dry hollow fibres, the plasticized hollow fibres may be dried directly in air under tension. For getting a small amount of dry sample for analyses, a multi-step solvent exchange method that is rapid may be used. Such methods involve immersion of the hollow fibres in 1-propanol or ethanol for 2 hours and subsequently in 1-heptane or 1-hexane for another two hours. The replacement of water in the hollow fibres with organic solvents can prevent the pore collapse of the hollow fibres.

According to a particular aspect, the construct prepared from the method of the present invention is a fibre. Even more in particular, the construct prepared is a hollow fibre.

According to a particular aspect, the method of preparing a construct of chitosan comprises the steps of:
(a) preparing a clear and homogeneous 2-4 wt % chitosan solution in 1% acetic acid;

(b) optionally filtering the mixture of step (a) to remove impurities;

(c) evaporating a certain amount of water while mechanical stirring the mixture to keep the mixture in a clear and homogeneous state until a chitosan solution is formed in which the final concentration of chitosan is up to about 12 or 18 wt %;

(d) degassing the chitosan solution by centrifugation to remove any possible air bubbles entrapped in the solution; and

(e) extruding the degassed chitosan solution through a spinneret into a quenching or coagulant solution/bath in which the solvent is soluble but the chitosan is insoluble to form the chitosan construct.

The chitosan solution formed in step (c) may be viscous. The construct prepared from the method of the present invention may have a high mechanical strength and high adsorption capacity and/or reactivity. The construct may be in the form of chitosan hollow fibres.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

The chitosan used in the representative examples below is supplied by Sigma-Aldrich (practical grade from crab shells, Brookfield viscosity > 200 cps having a molecular weight of about 319,000 Da. Glacial acetic acid (100% acetic acid) was obtain from Merck (Germany). NaOH was provided by Sino Chemical Co., Singapore.
Example 1

A mixture of chitosan in acetic acid was prepared using 8 g of chitosan, 5 ml acetic acid and 400 ml DI water with mechanical stirring at room temperature of about 23°C to form a fully dissolved homogeneous mixture. The mixture was then filtered by a Whatman Grade No. 1 filter paper to remove any impurities which had not dissolved in the acetic acid. The mixture was then heated to and maintained at a temperature of 60°C by a heating water bath. Mechanical stirring was still performed on the mixture during the heating. The solvent gradually evaporated. The weight of the solution was monitored frequently. The heating was continued until the total weight of the solution decreased to about 100 g. The mechanical stirring was then stopped a chitosan solution was formed. The chitosan solution was degassed by centrifugation at 3000 rpm for 10 minutes using a SIGMA 3-16-19776 centrifuge from UK. The concentration of the chitosan in the chitosan solution was 8 wt %.

The chitosan solution was then used as a dope solution for preparing chitosan hollow fibres. In particular, the chitosan hollow fibres were prepared through wet spinning the chitosan dope solution into a coagulant by the method described in C Liu and R B Bai, 2005. The homogeneous chitosan dope solution was forced through a stainless steel spinneret comprising an annular ring (with outer diameter and inner diameter of 1.72 mm and 0.72 mm respectively) under high pressure N₂ gas adjusted to 50.0 psi, and was protruded into a coagulation bath. A bore liquid coagulant was delivered simultaneously through the inner core of the spinneret with a syringe pump and the flow rate of the bore fluid flow rate was set at 0.12 ml/min. NaOH (10% m/v) was used as both the external coagulant (in the coagulation bath) and the internal coagulant (the bore flow).

The chitosan hollow fibres which were prepared from the method above showed high mechanical strength. In particular, the tensile stress, elongation ratio and Young's modulus at break of the wet hollow fibres were measured and shown to
be 2.2 MPa, 84% and 3.6 MPa, respectively. The mechanical properties of the wet hollow fibres were evaluated through the measurement of tensile strength, elongation ratio, and Young’s modulus at break. Tests were conducted with an Instron-5542 (UK) Materials Testing Machine equipped with Bluehill (Instron) Ver. 2.5 software under the conditions of room temperature (about 23 °C) and a relative humidity of about 60%. The initial gauge length was set to 100 mm and the drawing speed at 2 mm/min. The sample hollow fibre was cut into a 140 mm length, and attached onto the two clamps of the machine. The test data were recorded and the values of the tensile stress, elongation ratio at break and Young’s modulus of the hollow fibres were calculated by the software. The tensile strength value of the resultant hollow fibres appeared to be greater than that of many other hollow fibres known in the art and the Young’s modulus was generally comparable with those of other hollow fibres known in the art.

The morphologies and structures of the resultant hollow fibres are shown in Figure 1. The hollow fibres show a spongy-like and porous structure on cross-section and inner surface (Figure 1(a) and Figure 1(b), respectively), and a dense structure on outer surface (Figure 1(c)). The outer and inner diameters of the hollow fibres were 1.335 mm and 0.576 mm, respectively.

**Example 2**

A mixture of chitosan in acetic acid was prepared from 12 g chitosan, 5 ml acetic acid and 400 ml DI water with mechanical stirring. The remaining processing of the mixture to form the chitosan dope solution was the same as previously done in Example 1. The concentration of the chitosan in the chitosan dope solution was 12 wt%.

The chitosan solution was then used as a dope solution for preparing chitosan hollow fibres in the manner described in Example 1 above. However, the nitrogen gas pressure was adjusted to 60.0 psi instead of 50.0 psi.
The chitosan hollow fibres which were formed from the spinning process showed high mechanical strength. The tensile strength, elongation ratio and Young's modulus at break of the wet hollow fibres were measured as in Example 1 and found to be 3.5 MPa, 125% and 4.7 MPa, respectively. The tensile strength of the resultant hollow fibres was greater than that of many other hollow fibres known in the art. The elongation ratio and Young's modulus were generally comparable with or better than those of other hollow fibres known in the art. Further, it was observed that when the chitosan concentration in the chitosan dope solution increased from 8% (chitosan dope solution prepared in Example 1) to 12% (chitosan dope solution prepared in Example 2), the tensile strength of the chitosan fibres prepared from the dope solution increased from 2.2 MPa to 3.5 MPa. Therefore, it can be concluded that an increase in chitosan concentration leads to an increase in the mechanical strength of the fibres.

The morphologies and structures of the resultant hollow fibres are shown in Figure 2. The hollow fibres made in this example had similar surface morphology and membrane structure to that of the hollow fibres made in Example 1. The hollow fibres showed a spongy-like and porous structure on cross-section and inner surface (Figures 2(a) and 2(b), respectively), and a dense structure on outer surface (Figure 2(c)). The outer and inner diameters of the hollow fibres were 1.369 mm and 0.604 mm, respectively. Further, the pore sizes of the fibres prepared in Example 2 were smaller than the pore sizes of the fibres prepared in Example 1.

**Example 3**

Two chitosan dope solutions were prepared - one using a method whereby a high concentration of chitosan was mixed directly in the solvent to obtain the chitosan solution and the other using the method of the present invention. For
example, the direct mixing method is used in US 4,464,321 to prepare the chitosan dope solution.

5% chitosan solution (in 2% glacial acetic acid (100% acetic acid)) was prepared by the direct mixing method and a chitosan solution in which the concentration of the chitosan was 8% was prepared by the method of the present invention in the manner described in Example 2 above. The resultant chitosan solution prepared using the direct mixing method was not homogeneous for being able to be used to fabricate hollow fibres by the spinning method described in Examples 1 and 2 above, whereas the chitosan solution prepared using the method of the present invention which contained a higher chitosan concentration was a homogeneous chitosan solution. This can be seen by comparing Figures 3 (a), (b) and (c) with Figures 4 (a), (b) and (c). The chitosan solution prepared from the direct mixing method was highly viscous and gel-like, and therefore unsuitable for spinning into fibres.

The chitosan solution prepared by the direct mixing method, even after 7 days of stirring, the air bubbles in the solution were still severely entangled and not released. The solution still lacked homogeneity. On the other hand, for the 8% chitosan solution prepared using the novel method in this invention, the air bubbles were easily released and the homogeneity of the chitosan solution was maintained.

Therefore, it can be concluded that it is difficult to prepare a suitable homogeneous chitosan solution with a high chitosan concentration using the direct mixing method. However, a chitosan dope solution with a high chitosan concentration which is suitable for use in the spinning of fibres can be prepared using the method of the present invention.
References

7. CN1119553
8. CN1413761
9. JP2001038173
10. WO 2006/1 301 17
11. US4464321
12. US5259950
Claims

1. A method of preparing a chitosan solution, the method comprising the steps of:
   
   (i) mixing chitosan and at least one solvent to form a mixture; and

2. The method according to claim 1, wherein the concentration of the chitosan mixed in step (i) is \( \leq 8 \) weight (wt) %.

3. The method according to claim 1 or claim 2, wherein the concentration of chitosan in the chitosan solution of step (ii) is \( \geq 5 \) wt %.

4. The method according to any one of the preceding claims, wherein the concentration of chitosan in the chitosan solution of step (ii) is \( \geq 8 \) wt %.

5. The method according to claim 4, wherein the concentration of chitosan in the chitosan solution of step (ii) is 8-20 wt %.

6. The method according to any one of the preceding claims, wherein the at least one solvent comprises at least one acid.

7. The method according to claim 6, wherein the at least one solvent is selected from the group consisting of: acetic acid, formic acid, hydrochloric acid, lactic acid, propionic acid, di-chloroacetic acid and mixtures thereof.

8. The method according to claim 7, wherein the solvent is acetic acid, formic acid, or mixtures thereof.
9. The method according to any one of the preceding claims, wherein the step (ii) of evaporating the mixture is performed at a temperature of 20-120 °C.

10. The method according to any one of the preceding claims, wherein the step (ii) of evaporating the mixture is performed at a temperature of about 60°C.

11. The method according to any one of the preceding claims, wherein the step (i) of mixing further comprises mixing water and/or at least one viscosity-reducing additive with the chitosan and at least one solvent.

12. The method according to claim 11, wherein the at least one viscosity-reducing additive is selected from the group consisting of: NaCl, KCl, LiCl, CH₃COONa, urea, silica gel, poly(ethylene glycol) and mixtures thereof.

13. The method according to any one of the preceding claims, further comprising a step of filtering the mixture prior to the step (ii) of evaporating the mixture.

14. The method according to any one of the preceding claims, further comprising a step of degassing the chitosan solution of step (ii).

15. The method according to claim 14, wherein the step of degassing is performed by centrifugation of the chitosan solution.

16. The method according to any one of the preceding claims, wherein the chitosan solution of step (ii) is a chitosan dope solution.

17. A chitosan solution prepared according to the method according to any one of claims 1 to 16.
18. A chitosan solution comprising chitosan, wherein the concentration of chitosan in the chitosan solution is ≥ 5 wt %.

19. The chitosan solution according to claim 18, wherein the concentration of chitosan in the chitosan solution is ≥ 8 wt %.

20. The chitosan solution according to claim 19, wherein the concentration of chitosan in the chitosan solution is 8-20 wt %.

21. The chitosan solution according to any one of claims 18 to 20, wherein the chitosan solution is a chitosan dope solution.

22. The chitosan solution according to any one of claims 18 to 21, wherein the chitosan solution is prepared according to a method comprising the steps of:

(i) mixing chitosan and at least one solvent to form a mixture; and

(ii) evaporating the mixture to form the chitosan solution.
Figure 1

(a)

(b)

(c)
Figure 2

(a)

(b)

(c)
### INTEGRATIONAL SEARCH REPORT

**International application No.**
PCT/SG2008/000338

#### A. CLASSIFICATION OF SUBJECT MATTER

<table>
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<th>Int. Cl.</th>
<th>C08B 37/08 (2006.01)</th>
<th>C08L 5/08 (2006.01)</th>
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</table>

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. MINIMUM DOCUMENTATION SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

#### B. DOCUMENTS SEARCHED

**Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched**

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

WPI, EPDOC, USPTO & Keywords: chitosan, solution, concentrate, evaporate and the like

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<tbody>
<tr>
<td>X</td>
<td>WPI/Thompson Accession No. 1981-74529 D[41] JP 56106901 (MITSUBISHI RAYON CO-LTD) 25 August 1981 See Abstract</td>
<td>1-2, 6-11, 14, 16-17, 12, 15</td>
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<tr>
<td>Y</td>
<td>WPI/Thompson Accession No. 2005-284693 [29] WO 2005/25520 (CNRS CENTNAT RECH SCI) 24 March 2005 See Abstract</td>
<td>1-2, 6-9, 11, 14, 16-17, 12, 15</td>
</tr>
<tr>
<td>X</td>
<td>US 4983304 A (TSUGITA et al) 08 January 1991 See Examples 1-14; see also column 5, lines 40-60</td>
<td>1-9, 11, 13-14, 16-22, 12, 15</td>
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<tr>
<td>Y</td>
<td>US 4464321 A (PITIALIS et al) 07 August 1984 See whole document</td>
<td>1-3, 6-9, 11, 14, 16-18, 21-22, 12, 15</td>
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<td>Further documents are listed in the continuation of Box C</td>
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* Special categories of cited documents:

  - **A** document defining the general state of the art which is not considered to be of particular relevance
  - **E** earlier application or patent but published on or after the international filing date
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  - **O** document referring to an oral disclosure, use, exhibition or other means
  - **P** document published prior to the international filing date but later than the priority date claimed
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  - **X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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**Date of the actual completion of the international search**

03 October 2008

**Date of mailing of the international search report**

17 OCT 2008

**Name and mailing address of the ISA/AU**

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