

A MATERIAL AND METHODS FOR ITS FORMATION

Technical Field

The present invention relates, in general terms, to the field of biomaterials, and more particularly, to methods for forming a material, said methods being biocompatible and suitable for use in general and biomedical manufacturing. Such materials include polysaccharides doped with a metal during vitrification.

Background

Plastics are a fundamental part of modern society. Following the creation of plastics, materials such as glass, paper and metal were rapidly displaced in their traditional applications. The development of plastics also fuelled the mass production of cheap and products with short lifespan. However, in addition to the rapid progress offered with the development of plastics, several new challenges emerged. Plastics are the first mass-produced materials that are exogeneous to Earth's ecological cycles. Every unrecovered piece of plastic that has ever been disposed of has accumulated in some form in a part of the Earth's ecosystem, from the deepest points of the ocean to the tissues of animals and humans.

Conventionally, plastics are commonly used due to their stability and persistence in water-based environments. These qualities also make plastics have little to no biodegradability. Plastic materials are also frequently made suitable for manufacturing, by increasing their crystallinity, crosslinking density, and molecular weights, simultaneously providing water stability and mechanical characteristics which are suitable for forming standalone structures. However, water plays an important role in metabolic processes which enable biodegradability, and the stability of plastics in the presence of water therefore comes with a compromise to its biodegradability. Consequently, such materials, even with biological origins, tend to be biodegradable only under specialised circumstances, making them as persistent as synthetic materials due to their limited recyclability.

There is hence a need for new ways of manufacturing biocompatible materials with similar material strengths to traditional plastics, wherein said biocompatible materials are biodegradable and sustainable. Such methods of manufacturing preferably allow for these biomaterials to be readily reassimilated into the ecological cycles of the Earth.

Summary

Disclosed herein is a composite comprising: (a) a biopolymer, and (b) a polyvalent atom or molecule, wherein the polyvalent atom or molecule is immobilised in the biopolymer in the presence of water.

Disclosed is a method for forming a material, comprising vitrifying a mixture comprising a natural polysaccharide and a metal, wherein the natural polysaccharide is doped with the metal.

Advantageously, the composite, wherein a polyvalent atom or molecule is immobilised in a biopolymer network, exhibits differential mechanical behaviour in the presence of water, and when the said water is subsequently removed.

In some embodiments, the polyvalent atom or molecule is immobilised by one of: hydrogen bonding, ionic bonding, entrapment, and cross-linking.

In some embodiments, immobilisation occurs during or after vitrification of the polyvalent atom or molecule, into the solid.

In some embodiments, the biopolymer is a polysaccharide, and the polyvalent atom or molecule is a multivalent transition metal ion, the multivalent transition metal ions being entrapped in the polysaccharide, wherein a total valency of the ions is larger than a number of sterically available hydroxyl groups in the polysaccharide.

In some embodiments, the multivalent transition metal ions are chelated to oxygen atoms of consecutive pyranose rings in the natural biopolymer.

In some embodiments, the multivalent transition metal ions are chelated to oxygen atoms of pyranose rings between one or more chains of the natural biopolymer.

In some embodiments, long-range interactions between functional groups of adjacent polymer chains of the polysaccharide are enabled by intermediate water molecules (i.e., water molecules between neighbouring polymer chains).

In some embodiments, the biopolymer is chitin or chitosan, and the multivalent transition metal ions is nickel or a nickel salt.

Disclosed herein is also a method of manufacturing the composite of any one of the above, comprising: vitrifying, via a first vitrification cycle, the natural biopolymer and during or after vitrification, doping the natural biopolymer with a metal, the metal comprising the multivalent transition metal ions, wherein vitrifying the natural biopolymer generates a waste solution consisting of a fraction of the metal comprising the multivalent transition metal ions, and wherein the waste solution is used in a second or subsequent vitrification cycle.

In some embodiments, vitrification occurs in a water-based solvent.

In some embodiments, evaporation of water from the water-based solvent forces vitrification of the material into a solid.

In some embodiments, the solution of multivalent transition metal ions is in a water-based solvent.

In some embodiments, the water-based solvent is acetic acid, with a concentration of less than 5% in water, and preferably 1% in water.

In some embodiments, a concentration of multivalent transition metal ions during doping is in the range of 0.3 M to 2 M, preferably in the range of 0.5 M to 1.6 M, more preferably in the range of 0.6 M to 1.2 M, and even more preferably in the range of 0.8 M to 1.0 M.

In some embodiments, the water used is environmental water.

In some embodiments, the method further comprises washing the material to form a final material.

Disclosed herein is a method for manufacturing a material, comprising doping a natural biopolymer with a solution comprising multivalent transition metal ions, wherein doping the polysaccharide with the metal comprises:

- doping the polysaccharide with a first metal salt solution of a first concentration;
- washing the material, thereby producing a final material, a water byproduct and a second metal salt solution of a second concentration that is lower than the first concentration;
- adding metal salt to the second metal salt solution to bring the second concentration up to the first concentration; and
- forming a further material by vitrifying a natural polysaccharide in the water byproduct and, during vitrification, doping the natural polysaccharide with the second metal salt solution.

Brief description of figures

Embodiments of the present invention will now be described with reference to the following figures, in which:

Figure 1 shows an outline of the manufacturing cycle of a material, in accordance with a preferred embodiment of the present invention.

Figure 2 illustrates the production and characterisation of Nickel-doped chitosan, according to an embodiment of the invention. Figure 2(a) shows a local production of chitinous polymer in the natural environment. Figure 2(b) illustrates a qualitative comparison of vitrified chitinous films. Figure 2(c) provides a schematic of the chemical structure of chitosan. Figures 2(d) and 2(e) provide a Fourier-transform Infrared (FTIR) spectra of Nickel-doped chitosan films. Figure 2(f) shows the crystal conformation of chitinous films. Figure 2(g) illustrate representative tensile stress-strain curves of the Nickel-doped films. Figure 2(h) shows a plot of tensile strength for samples of varying Nickel concentrations before and after immersion in water.

Figure 3 illustrates the optical absorbance of Cs and CsNi composite films, obtained via UV-vis absorption spectroscopy.

Figure 4(a) illustrates an Ashby plot, showing material properties of tensile strength and density. Figure 4(b) shows representative stress-strain curves of Nickel-doped chitosan. Figure 4(c) shows a series of scanning electron microscopic (SEM) images of the film's surfaces. Figure 4(d) shows the X-ray photoelectron spectroscopy (XPS) C1s spectra of a chitosan film, before doping with Nickel, immediately after doping with Nickel, and after a first immersion in water. Figure 4(e) shows a molecular diagram of the role of Nickel and water in vitrified chitinous films. Figure 4(f) shows the thermogravimetric analysis plots of the chitinous films in the temperature range for water evaporation. Figure 4(g) shows the colorimetric analysis plots of the release of inconsequential Nickel from the chitinous film to its surroundings during a first contact with water.

Figure 5(a) illustrates such a production cycle, according to a preferred embodiment of the present invention. Figure 5(b) shows a replica of a drinking cup manufactured using the production cycle of Figure 5(a). Figure 5(c) shows the Nickel-doped chitosan cup filled with water, over a period of one week. Figure 5(d) shows a photo of the clinostat used to replicate negative molds for the Nickel-doped chitosan. Figure 5(e) shows a comparison between objects produced using positive molds and negative molds. Figure 5(f) shows an example of a three square-metre film made of a Nickel-doped chitosan.

Figure 6 shows a series of X-ray diffraction (XRD) data was collected for wet, dry and fresh films.

Detailed description

Disclosed herein are composites, comprising a biopolymer and a polyvalent atom or molecule, wherein the polyvalent atom or molecule is immobilised in the biopolymer in the presence of water (immobilisation may occur after vitrification, during washing, or during vitrification). Advantageously, the composite, wherein a polyvalent atom or molecule is immobilised in a biopolymer network, exhibits differential mechanical

behaviour in the presence of water, and when the said water is subsequently removed.

As used herein, the term "immobilised" may refer to one of: hydrogen bonding, ionic bonding, entrapment, and cross-linking.

In some embodiments, immobilising the polyvalent atom or molecule within the biopolymer occurs during a vitrification cycle, wherein the immobilisation occurs during or after vitrification.

In some embodiments, the biopolymer is a polysaccharide, and the polyvalent atom or molecule is a multivalent transition metal ion, and the multivalent transition metal ion is entrapped in the polysaccharide. Preferably, the total valency of the ions is larger than the number of hydroxyl groups in the polysaccharide.

In some embodiments, the multivalent transition metal ions are chelated to oxygen atoms of consecutive pyranose rings in the natural biopolymer, and in other embodiments, the multivalent transition metal ions are chelated to oxygen atoms of pyranose rings between one or more chains of the natural biopolymer.

Disclosed herein are also methods for manufacturing the composite, comprising vitrifying, via a first vitrification cycle, a mixture comprising a natural polysaccharide doped with a metal. Advantageously, the material formed is biodegradable under standard conditions, is flexible, and may be moulded into objects with complex shapes. Further advantageously, methods of production of such a material produce negligible to no waste – i.e. any "waste solution" generated during the first vitrification cycle, such as any additives or by-products, consist a fraction of the metal comprising the multivalent transition metal ions, which may be used in a second or subsequent vitrification cycle.

Figure 1 shows an outline of the manufacturing cycle of the material. In step 102, a natural polysaccharide is dispersed in a water-based solvent, forming a dispersion. This can be performed using any known technique such as grinding, or otherwise particulating, the polysaccharide. Per step 104, a metal is then added to the dispersion. The metal may be in the form of a metal salt solution of a first concentration. The metal may also be a transition metal or inner transition metal,

such as Nickel. Water is then allowed to evaporate in step 106, to cast a desired object. Evaporation forces vitrification of the natural polysaccharide, resulting in a solid material in the form of the object. Thus, evaporation step 106 can involve pouring or depositing the mixture (polysaccharide and metal salt solution) in a mould, and then evaporating water from the mould. In some embodiments, the mould is moved during evaporation to ensure even distribution, or a desired distribution, of the mixture in the mould. This results in a formed object, per step 108. Since the water has been evaporated, the object formed at step 108 may not have optimum strength. Thus, per step 110, the solid material may then be washed in water 112 to produce a "washed object". Waste solution 114 is separated from the washed object, resulting in a "final object" 116. The waste solution is a mixture of the metal salt solution in a second concentration (120), and water (i.e., a water byproduct 118), the second concentration being lower than the first concentration. Additional metal salts are added to the waste solution per step 122, resulting in a metal salt solution of the desired (first) concentration, and this may then be added to a dispersion in a second or subsequent manufacturing cycle of the material.

Advantageously, the method of production outlined here does not result in any waste, as the by-product of a first manufacturing cycle may be reused for a second and subsequent manufacturing cycles.

The natural polysaccharide used may be a material comprising chitosan. Chitosan may be extracted from discarded shrimp (*Penaeus monodon*) shells, fungi and other sources. The natural polysaccharide may also be chitin and other derivatives of chitin, starch, hemicellulose, cellulose, pectin, and alginic acid.

The water-based solvent may be a low concentration of acetic acid in water. The low concentration of acetic acid may be as low as about 0.5% acetic acid in water (in comparison, table vinegar is a solution of 5-8% of acetic acid in water). The concentration of acetic acid may be 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or, more preferably, 0.5% as set out above.

The metal salt solution may be Nickel chloride dissolved in water. The metal salt solution may also be other transition metal salts, including zinc and copper. The concentrations of Nickel chloride, or other transition metal salt, in water may be about 0.3 M (mol/L) to about 2.0 M, more preferably have a lower concentration bound of

about 0.35 M, 0.4 M, 0.45 M, 0.5 M, 0.55 M, 0.6M, 0.65 M, 0.7 M or 0.75 M, and an upper bound of about 1.95 M, 1.9 M, 1.85 M, 1.8 M, 1.75 M, 1.7 M, 1.65 M, 1.6 M, 1.55 M, 1.5 M, 1.45 M, 1.4 M, 1.35 M or 1.3 M, and most preferably be between 0.6 M and 1.4 M.

Figure 2 illustrates the production and characterisation of Nickel-doped chitosan, according to a preferred embodiment of the invention. Figure 2(a) shows a local production of chitinous polymers as part of regionalised circular economies. Chitin and chitosan are typically by-products of the shrimp and crab processing industry and play a crucial role as structural components in most heterotrophs used in the bioconversion of organic waste and the local production of nutrients. Conventionally, chitin may be incorporated into production cycles through waste management or natural ecological cycles.

Figure 2(b) shows a qualitative comparison of vitrified chitinous films, with an increasing concentration of Nickel used in the product cycle as the comparison progresses to the right of the image. Undoped chitosan takes on a pale-yellow colour. When doped with Nickel, the chitinous films may turn green, which corresponds to the colour of a Nickel (II) salt in an aqueous solution. Figure 2(b) illustrates an increase in intensity of the colour as the concentration of the Nickel salt solution increases.

Figure 3 shows the optical absorbance of Cs and CsNi composite films, obtained via UV-vis absorption spectroscopy. The interaction of Ni with chitosan may be seen from the visible peak originating around 400 nm and 700 nm, illustrating an interaction between the Nickel ion and its nearby molecules, particularly the hydroxyl groups. This may also suggest that Ni, when present in the Cs composite, modifies the structure of chitosan by breaking and reforming new bonds. The chelation of Cs with Ni may change the crystalline nature by deforming the crystal structure, as observed in the X-ray diffraction data (Figure 2(f), discussed below).

Figure 2(c) provides a schematic of the chemical structure of chitosan. The most likely locations of Nickel, when Nickel is used to dope chitosan, have been marked (i.e. Position I and Position II). Considering only Nickel (II) ions on chitosan, the most stable location for Nickel ions in chitosan polymer chains may be the space between the primary and secondary hydroxyl groups of consecutive pyranose rings. This has

been marked as Position I in Figure 2(c). At Position I, Nickel weakly interacts with the fully coordinated oxygen atom in the ring. However, in the presence of other elements and compounds, such as water, Nickel ions may be stably formed at Position II, between the primary amino and secondary hydroxyl groups. At Position II, Nickel ions may coordinate with water molecules and the sterically available groups of adjacent chitinous chains. In some embodiments, ions (e.g., of Nickel) dynamically change their bonding sites using water to mediate the bonding. Nickel ions may also reside between the primary amino and the primary hydroxyl groups of consecutive rings.

Figure 2(d) shows the Fourier-transform Infrared (FTIR) spectra of the Nickel-doped chitosan films. A blue shift of the amide II band may be observed, from 1542 cm^{-1} to 1561 cm^{-1} . This is likely due to the presence of Nickel in the chitinous structure. This is consistent with the interaction with the primary amines (marked by Position II in Figure 2(c)). A second blue shift may be apparent in the band located at 1325 cm^{-1} , corresponding to the bending of the CH_2 group, likely consistent with Nickel in the inter-ring position (Position I in Figure 2(c)).

The presence of Nickel on the chitosan chain may be further confirmed by the moderate modifications of the spectra in the $500\text{-}700\text{ cm}^{-1}$ region, where the vibrations of the bond between the Nickel ions and the hydroxyl groups appear. The impact of Nickel on the FTIR spectra may arise primarily from the changes introduced by new water molecules associated with the Nickel ions.

Nickel forms weak hydrogen bonds with water molecules — up to six when in solution — and while the new interactions with the chitinous chains replace some of those molecules, the Nickel-doped films incorporate several times as many water molecules as the number of Nickel ions introduced. The effect of this additional water is observable by the peak in the band at 1628 cm^{-1} , which likely corresponds to the bending vibration of the hydroxyl groups in water molecules. While this vibration is overshadowed by the amide I band (1636 cm^{-1}) in pristine chitosan films, it appears to dominate the fingerprint region in Nickel-doped films with even the smallest amount of Nickel and rapidly grows as the concentration increases. A similar effect may also be clearly seen in the intensity of the broad band at 3250 cm^{-1} (as illustrated in Figure 2(e)), where the intensity of the vibrations corresponding to the stretching of the O–H bond rapidly increases with the amount of Nickel in the system.

Figure 2(f) shows the crystal conformation of chitinous films. Regular chitosan films typically have a hydrated crystal structure, with two distinct peaks at 9.5° and 20° regions, corresponding to the hydrated crystal conformation, and a broad amorphous region in the $15\text{--}30^{\circ}$. As the amount of Nickel and water increases, the amorphous regions appear to dominate the structure (i.e. the two distinct peaks are diminished). It can be demonstrated that strain stiffening, which reorganises chitosan films into a more crystalline structure using external forces, can also result in closer packing by reducing the free volume and expelling water molecules from the material as new chain-chain direct interactions replace their binding sites. A similar but opposite effect can be observed in the Nickel-doped films, where the additional Nickel and its associated water may result in lower crystallinity.

Figure 2(g) shows representative tensile stress-strain curves of the Nickel-doped films. All samples studied, at varying concentrations of Nickel, have tensile strengths in the range of 30 MPa to 40 MPa. This does not appear to depend on the concentration of Nickel nor the crystallinity of the film. Such strength is similar to commodity plastics. Concentrations of Nickel below 0.8 M have limited impact on the material's tensile strength, despite the additional water and lowered crystallinity. Above 0.8 M, the material typically gains elasticity without sacrificing strength, simultaneously achieving strength and toughness, which is characteristic of the functional versatility of structural biomaterials. The strength of the material is preserved while its Young's modulus (which is a measure of stiffness) falls significantly, marking the material's increased ability to be stretched (i.e. elasticity), and therefore, its ability to absorb more energy without breaking (i.e. increased toughness).

Advantageously, the introduction of Nickel into chitosan increases both the material's flexibility as a plasticiser and its strength as a crosslinker (i.e. the material is both tough and strong). These are conventionally seen as incompatible material properties but have been shown to exist simultaneously in materials and processes presented herein.

Figure 2(h) shows a plot of tensile strength for samples of varying Nickel concentrations before (in the empty bars) and after (in the solid bars) immersion in water. The pristine chitosan films without Nickel are represented in black, while the

other colours represent the different concentrations of Nickel in the solution, for concentrations between 0.6 M to 1.4 M. The dry samples are represented in unfilled bars.

Advantageously, the material is not only tough and strong, it gets stronger when immersed in water (Figure 2(h)). The strengths of all Nickel-doped chitosan films, for concentration of Nickel of above 0.6 M, appear to increase in strength when immersed in water. For the film doped with a 0.8 M Nickel solution, an increase of almost 50% was observed. This suggests that there is an optimal balance in the roles of the Nickel and water, in simultaneously enhancing the intermolecular bonds through new interactions and disrupting them by preventing direct chain-chain interactions.

Figure 4(a) illustrates an Ashby plot, showing material properties of tensile strength and density, for a variety of natural and synthetic materials. The properties of tensile strength and density for Nickel-doped chitosan, in a water environment (marked as "CsNi wet") and after removing additional water at 60 °C for 24 hours (marked as "CsNi dry") are represented in the plot for comparison. These Nickel-doped films have a tensile strength of about 36.12 ± 2.21 MPa when dry, and an increased tensile strength of about 53.01 ± 1.68 MPa when immersed in water. The tensile strength of dry films is comparable to commodity plastics, such as propylene, polystyrene and polylactic acid, and the tensile strength of wet films are comparable to those of engineering plastics, such as polycarbonate, polyethylene terephthalate glycol and polyoxymethylene.

Using a 0.8 M concentration for fabricating the Nickel-doped films appears optimal for achieving this mechanical improvement in water. Excess, or inconsequential, nickel is introduced for the purpose of ensuring all possible links are formed, and any unconsumed nickel may be removed. Doping may comprise doping at a concentration that results in excess polyvalent atoms or molecules (e.g., Nickel). While this excess results in polyvalent atoms or molecules being left over (for reuse in a subsequent vitrification cycle, for example), it has been found to assist with fabrication. Figure 4(b) shows representative stress-strain curves of Nickel-doped chitosan. The curves suggest that the changes to Nickel-doped chitosan are permanent after the release of inconsequential Nickel ions (as indicated by "washed"), and reversible if intermolecular water is extracted by oven-drying (as indicated by "dry") and replenishing it by wetting the films (as indicated by "wet"). The macroscopic change

after the first immersion may also be observable as an accumulation of Nickel on the surface of a freshly made film, which later disappears.

Figure 4(c) shows a series of scanning electron microscopic (SEM) images of the film's surfaces. The top image is that of a pure (i.e. undoped) chitosan film, the centre image is that of a Nickel-doped chitosan (CsNi) film ("fresh"), immediately after fabrication, and the bottom image is that of a washed film ("washed"). White precipitates were observed in the centre image, and this may indicate Nickel accumulation on the surface. Said excess Nickel appears to be washed off after the first immersion.

Figure 4(d) shows the X-ray photoelectron spectroscopy (XPS) C1s spectra of a chitosan film, before doping with Nickel, immediately after doping with Nickel, and after a first immersion in water. The XPS C1s spectra of a pristine (i.e. undoped) chitosan film shows three peaks, namely the C-(C,H) aliphatic peak, the C-(N,O) peak, and the C=O/O-C-C peak. For a Nickel-doped film, these three peaks have higher binding energies than the same peaks in the pristine film. There also appears to be a notable reduction of the area under the deconvoluted C-(N,O) peak (i.e. at 286.37 eV), suggesting that the main interaction between Nickel and chitosan occurs through the functional groups of chitosan. However, after the first immersion of a Nickel-doped film in water, these differences disappear, resulting in a surface analysis that resembles that of pure chitosan films. The same effect is observed in the O1s and N1s spectra, supporting a hypothesis that the permanent change in the material is likely due to a loosely bound Nickel and associated water is removed upon first immersion. This hypothesis is also substantiated by an observed weight loss of the Nickel-doped films immediately after immersion, in contrast to a weight gain normally expected of a typically hydrophilic material (i.e. chitosan) due to a water uptake.

Figure 4(e) shows a molecular diagram of the role of Nickel and water in vitrified chitinous films. The solid lines indicate direct interchain interactions, and the dashed line indicate interchain interactions across water molecules. The inclusion of Nickel, which can simultaneously link several chains and water molecules, adds a new level of possible weak and easily reconfigurable bonds, including the complete or partial coordination of Nickel ions with water, without significant bonds to the polymeric chains, and their release to the environment.

Chitinous materials are typically organised as hydrated crystals in which the relatively long-range interactions between the functional groups of adjacent polymer chains are enabled by intermediate water molecules. The inclusion of Nickel during the formation of the chitinous structure may cause a formation of weak hydrogen bonds with multiple water molecules, both dissociated and not, and with the oxygen and nitrogen in the functional groups of the chitosan chains. While some water is bound within the polymeric chains, large amounts are also introduced from the environment alongside Nickel. This may result in a structure of stiff polymeric chains which are bound together by a combination of direct interchain bonds and weak and rapidly configurable bonds, mediated by highly motile particles (i.e. Nickel and water) trapped within the structure.

Figure 4(f) shows the thermogravimetric analysis plots of the chitinous films in the temperature range for water evaporation. The percentage of water in a film comprising freshly-Nickel-doped chitosan is about 43% (marked by "Cs (fresh)"). This value drops to about 20% when the films are immersed in water for the first time, and remains stable after drying for 24 hours at 60 ° (marked by "Cs (washed)" and "Cs (washed + dry)" respectively). Notably, the percentage of water in these films is higher than the about 17% of water present in undoped chitosan films (marked by "Cs"). The thermogravimetric analysis plots may suggest that Nickel particles and associated water molecules do not contribute to the structure and are released during the first immersion.

Figure 4(g) shows the colorimetric analysis plots of the release of inconsequential Nickel from the chitinous film to its surroundings during a first contact with water. While the films retain their strength under water, about 90% of entrapped Nickel may be weakly bound to the chitosan and is released during the first immersion. Advantageously, this suggests that despite the amount of Nickel introduced to the dispersion, only one Nickel ion may be required for every 7.91 pyran rings, for achieving the water-strengthening effect in chitosan. To illustrate this, it may be said that the Nickel content of one discarded AAA battery (about 2.2 g of Nickel) may be sufficient to dope and manufacture more than a dozen typical plastic cups (about 4.7 g each), using Nickel-doped chitosan.

The above evidence suggests that most inconsequential Nickel may be released during a first immersion in water, and may provide evidence of an optimisation of

the material, in which the arbitrary entrapment of Nickel needed to saturate the system is followed by a process that removes all but the Nickel that contributes to the material's structure. This process is done in water, the environment in which the films are formed. Any resulting by-products from the optimisation process is therefore itself an ingredient for producing the films.

Advantageously, this production cycle, in which water used to remove the inconsequential Nickel, may be used as an input for fabricating films (i.e. zero waste is generated).

Figure 5(a) illustrates such a production cycle. Due to a "zero-waste" process, the production cycle may be applied to several objects which may be manufactured with an approach similar to the use of chitosan in product manufacturing. This allows for the production of common plasticware, such as containers and cups.

Figure 5(b) shows a replica of a drinking cup manufactured using the production cycle of Figure 5(a), i.e. vitrifying it on a positive mould. The photo on the left shows the replica right after production (i.e. 100% Ni). After inconsequential Nickel has been removed by immersion in water, there appears to be no apparent impact on the geometry of the cup is observed (Figure 5(b), right).

Figure 6 further supports this observation, where a series of X-ray diffraction (XRD) data was collected for wet, dry and fresh films. The data suggests that Nickel and its associated water in the chitosan structure may significantly hamper the crystallinity. At an optimal Nickel concentration, the additional water-mediated bonds compensate for this effect, resulting in a more robust material with enhanced underwater performance. For a freshly-prepared Nickel-doped chitosan post-preparation, a substantial amount of inconsequential Nickel may remain trapped within the structure. It is postulated that two things occur when these samples are immersed in water. First, Nickel, which lacks bonds to the chitosan chains, may be released. Second, additional water may infiltrate the system, filling intermolecular spaces and facilitating new water-mediated bonds. The "wet" sample represents a sample which has been immersed in water for 24 hours. When the samples are subsequently dried and water is expelled (i.e. the "dry" sample), the spectrum closely resembles that of a "fresh" film. This suggests that crystallinity of the chitosan chains remain unaffected throughout the process, and that the varying mechanical properties of the films may

be attributed to the water-mediated bonds between them rather than any reorganisation of the chains.

Figure 5(c) shows a Nickel-doped chitosan cup filled with water, over a period of one week. The images demonstrate an impermeability of the Nickel-doped chitosan, i.e. the manufactured drinking cup is water resistant.

Figure 5(d) shows a photo of a clinostat used to replicate negative moulds for the Nickel-doped chitosan. While there are several challenges associated with negative moulding, such as a necessity for the material to maintain constant contact with the mould while shrinking during the vitrification process, it was found that negative moulding may result in a qualitative improvement in the aesthetics of the objects, as compared to those produced with positive moulds (see Figure 5(e), for an example). Replication of the negative moulds may be achieved by a random positioning machine, i.e. a two-axis clinostat, with two perpendicular frames and an attached negative mould. A continuous repositioning of the mould forces the polymeric solution to maintain contact during the vitrification process, enabling moulding of closed geometries.

Advantageously, this method of manufacturing may be scaled to production of large-area sheets. Figure 5(f) shows an example of a three square-metre film made of a Nickel-doped chitosan. Such a film was found to be capable of holding its weight after 24 hours of water immersion. This may suggest an absence of scalability restrictions and highlights a potential for rapid scaling of the results and principles to ecologically relevant scales.

Materials and Methods

Chitosan used here was sourced from the by-products of shrimp processing factories in India, courtesy of iChess Pvt. Ltd., Mumbai, India. Industrial-grade acetic acid and Nickel chloride hexahydrate (98%, Sigma Aldrich) were obtained from local vendors and utilised as received.

3 wt. % chitosan solution was prepared by dissolving chitosan flakes in 1% acetic acid with continuous stirring for 48 hours at room temperature. Nickel chloride solutions with concentrations ranging from 0.6 M to 1.4 M were prepared separately

in 10 mL distilled water. Both solutions (i.e., chitosan and Nickel) were mixed through continuous string for 6 hours to ensure uniform distribution of Nickel ions in the chitosan solution. The solution was poured into a Petri dish to prepare the standard samples and dried in an air oven at 40 °C for 24 hours. Similar results have been obtained with other molds and drying conditions (e.g., 3 m films dried at room temperature). The samples are coded as Cs, 0.6 M, 0.8 M, 1.0 M, 1.2 M, 1.4 M, relative to the molar concentration of Ni in the original solution. Depending on the state of the film, they are labelled as:

- "fresh" for chitosan and chitosan-Nickel films or constructs after they are dried/vitrified from the original solution;
- "washed" for Nickel-doped constructs (e.g., films) that have been immersed in water for more than 24 hours at least once, and with inconsequential Ni removed;
- "wet" for constructs currently or recently immersed in water and still soaked, retaining large amounts of excess water; and
- "dry" for constructs that have been oven-dried and their excess water removed.

The pristine chitosan films used to compare the underwater properties of pure chitosan with Nickel-doped chitosan were neutralised using 1 M NaOH to avoid their dissolution when immersed. Freshly prepared films were washed five times in a beaker containing NaOH solution, followed by washing in double distilled water in another container.

In all other cases, pure chitosan and Nickel-doped chitosan films were prepared without neutralisation.

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications and variations that fall within the spirit and the scope of the appended claims.

Throughout this disclosure, and unless the context requires otherwise, the word "comprise" and variations such as "comprises" and "comprising" will be understood

to imply the inclusion of a stated integer or step or group of integers or steps, but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this disclosure is not, and should not be taken as, an acknowledgement or any form of suggestion that the prior art forms part of the common general knowledge.

CLAIMS

1. A composite comprising: (a) a biopolymer, and (b) a polyvalent atom or molecule, wherein the polyvalent atom or molecule is immobilised in the biopolymer in the presence of water.
2. The composite according to claim 1, wherein the polyvalent atom or molecule is immobilised by one of: hydrogen bonding, ionic bonding, entrapment, and cross-linking.
3. The composite of claim 1, wherein immobilisation occurs during or after vitrification of the polyvalent atom or molecule, into the solid.
4. The composite according to 1 to 3, wherein the biopolymer is a polysaccharide, and the polyvalent atom or molecule is a multivalent transition metal ion, the multivalent transition metal ions being entrapped in the polysaccharide, wherein a total valency of the ions is larger than a number of sterically available hydroxyl groups in the polysaccharide.
5. The composite of claim 4, wherein the multivalent transition metal ions are chelated to oxygen atoms of consecutive pyranose rings in the natural biopolymer.
6. The composite of claim 4, wherein the multivalent transition metal ions are chelated to oxygen atoms of pyranose rings between one or more chains of the natural biopolymer.
7. The composite of claim 4, wherein long-range interactions between functional groups of adjacent polymer chains of the polysaccharide are enabled by intermediate water molecules.
8. The composite of any one of claims 4 to 7, wherein the biopolymer is chitin or chitosan, and wherein the multivalent transition metal ions is nickel or a nickel salt.

9. A method of manufacturing the composite of any one of claims 4 to 8, comprising: vitrifying, via a first vitrification cycle, the natural biopolymer and during or after vitrification, doping the natural biopolymer with a metal, the metal comprising the multivalent transition metal ions, wherein vitrifying the natural biopolymer generates a waste solution consisting of a fraction of the metal comprising the multivalent transition metal ions, and wherein the waste solution is used in a second or subsequent vitrification cycle.
10. The method of claim 9, wherein vitrification occurs in a water-based solvent.
11. The method of claim 9 or claim 10, wherein evaporation of water from the water-based solvent forces vitrification of the material into a solid.
12. The method of any one of claims 8 to 11, wherein the solution of multivalent transition metal ions is in a water-based solvent.
13. The method of claim 12, wherein the water-based solvent is acetic acid, with a concentration of less than 5% in water, and preferably 1% in water.
14. The method of any one of claims 8 to 13, wherein a concentration of multivalent transition metal ions during doping is in the range of 0.3 M to 2 M, preferably in the range of 0.5 M to 1.6 M, more preferably in the range of 0.6 M to 1.2 M, and even more preferably in the range of 0.8 M to 1.0 M.
15. The method of any one of claims 8 to 14, wherein the water used is environmental water.
16. The method of any one of claims 8 to 15, further comprising washing the material to form a final material.
17. A method for manufacturing a material, comprising doping a natural biopolymer with a solution comprising multivalent transition metal ions, wherein doping the polysaccharide with the metal comprises:
 - doping the polysaccharide with a first metal salt solution of a first concentration;

washing the material, thereby producing a final material, a water byproduct and a second metal salt solution of a second concentration that is lower than the first concentration;
adding metal salt to the second metal salt solution to bring the second concentration up to the first concentration; and
forming a further material by vitrifying a natural polysaccharide in the water byproduct and, during vitrification, doping the natural polysaccharide with the second metal salt solution.

18. A material formed using the method of claim 17.

19. A film comprising the material of any one of claims 1 to 8.