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Short communication

Functionalized superhydrophobic biomimetic chitosan-based films

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ABSTRACT

Chitosan has been greatly applied in the fields of biomedicine, biomembranes and food/nutrition because of its nontoxic and biocompatible properties. However, water solubility of chitosan at pH < 5 strongly limits the use of chitosan-based films when pH stability and low water uptake are required in many actual applications. In this work, silyl chitosan, 3,6-*O*-di-tertbutyldimethyl silyl chitosan, a chitosan derivative, was synthesized and used to prepare extreme water-repellent films in the whole pH range of 1–14 using a phase separation method that exhibit topography with a three-level hierarchical roughness organization. The polymer also allows posterior chemical modification specifically through the amine group, permitting to control the surface chemistry and wettability. This work not only improve the stabilization of chitosan-based films but also demonstrates the possibility of manufacturing polysaccharide-based superhydrophobic surfaces with potential to be used in anti-bacterial substrates, tissue engineering, food industry and other biomedical applications.

1. Introduction

Inspired by the self-cleaning phenomenon found in nature, superhydrophobic surfaces have attracted significant attention due to their potential applications in biotechnology and water-repellent commodity materials (Blossey, 2003). Different materials and technologies have been employed in the production of superhydrophobic surfaces that typically exhibit a hierarchical roughness, at both the nano and micro scales, mimicking the topography found in the lotus leaf or other self-cleaning natural surfaces (Koch, Bhushan, & Barthlott, 2008; Sun, Feng, Gao, & Jiang, 2005). Polysaccharides are a very important class of macromolecules but there are just few reports on superhydrophobic surfaces based on such materials, being most of them derived from cellulose (Li, Zhang, & Wang, 2008). Highly water-repellent polysaccharide-based surfaces obtained by some low cost and facile processing route could have direct applications in the biomedical, biotechnological, textile or environmental field. In this work a chitosan derivative will be used to demonstrate that a simple processing procedure could be employed to obtain such kind of surfaces. Furthermore the developed surface exhibits durable extreme hydrophobic property in a wide pH range, and is chemically versatile as they process free amine groups that can allow for different chemical modifications.

Chitosan, poly-(1–4)-2-amino-2-deoxy-β-D-Glucan, as one of natural biodegradable, nontoxic and biocompatible polysaccharides has been proposed to be used in multiple fields, such as in biomedical, biotechnological, functional membranes/fibres, food/nutrition, and agricultural/environmental applications (Francis Suh & Matthew, 2000; Grini & Badot, 2008; Prabaharan & Mano, 2005; Prashanth & Tharanathan, 2007). Due to the existence of a protonable amine group, chitosan shows not only a pH responsive behaviour but also water solubility at pH < 5. This strongly limits the use of chitosan-based materials when pH stability and low water uptake are required as in many actual applications. Both amino and hydroxyl groups in the structure of chitosan permit variety of chemical modifications, which can be used to improve its stabilization and extend its applicability (Alves & Mano, 2008; Chiu et al., 2009). It is well known that the decrease of surface energy is one important way to improve stabilization (Schmidt et al., 1994; Wang et al., 2006). However, chemical modification in chitosan smooth substrates only allows controlling the wettability within the hydrophilic to the hydrophobic range (Campos, Satsangi, Rawls, & Mei, 2009; Tangpasuthadol, Pongchaisirikul, & Hoven, 2003). To our knowledge a clear relationship between topography and superhydrophobic (contact angle, CA > 150°) properties of chitosan-based systems has not been reported.

In this contribution, first a hydrophobic chitosan derivative, 3,6-*O*-di-tertbutyldimethyl silyl chitosan (SC), was synthesized using

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an improved methodology (Rúnarsson, Malainer, Holappa, Sigurdsson, & Másson, 2008). The rationale for the choice of this polymer is that: (1) the original chitosan structure may be easily recovered and (2) the hydroxyl groups are protected allowing for further chemical modifications through the amine group. Then, a straightforward method is reported to process the material into films with adequate roughness topography, so that superhydrophobic features could be displayed.

2. Experimental section

2.1. Synthesis of 3,6-O-di-tertbutyldimethyl silyl chitosan (SC)

The chitosan polymer HCl (G020102–1, 1 g) was stirred in methanesulfonic acid (10 mL). Dropwise addition of water (10 mL) to the heterogeneous mixture at 10 °C resulted in a clear solution. The reaction mixture was stirred for 1 h. The mesylate salt of chitosan was then precipitated with EtOH (40 mL) resulting in a gel-like precipitate. The precipitate was filtered and washed two times with EtOH (30 mL) and acetone (30 mL), allowed to air-dry for 1 h. Then this salt was re-precipitated from acetone and water and dried in a vacuum oven at 40 °C overnight to give 1.15 g as a fine white solid (90% yield).

Chitosan polymer mesylate (1.00 g, 3.97 mmol) was weighed into a flame-dried round-bottom flask that had been flushed with N_2. The polymer was dissolved completely in dry DMSO (13 mL). Imidazole (2.71 g, 39.74 mmol) and TBDMSCl (2.99 g, 19.87 mmol) were dissolved in dry DMSO (10 mL) and added dropwise into the reaction mixture at room temperature. During the addition of reagents reaction mixture turned cloudy and just 10 min after completion of addition, solid gel type material came out from solution. The reaction was continued at room temperature for 24 h. The reaction mixture was filtered through sintered funnel and washed with water 2–3 times followed by washing with acetonitrile, resulting in white fine powdered material and allowed to air-dry. The white material was dried in a vacuum oven at 40 °C overnight giving 1.46 g (96% yield).

2.2. Fabrication of the superhydrophobic SC film

SC was dissolved in dichloromethane and three concentrations 10 mg/mL, 30 mg/mL and 50 mg/mL were selected. The SC solution was dropped on the glass slide first, and then after being kept in air for 3 min, the ensemble was immersed in ethanol for 5 min. The opalescent SC film was taken out from ethanol solution, and dried under Nitrogen flow. Smooth SC films were fabricated by dropping SC solution on a glass slide and drying in air at room temperature.

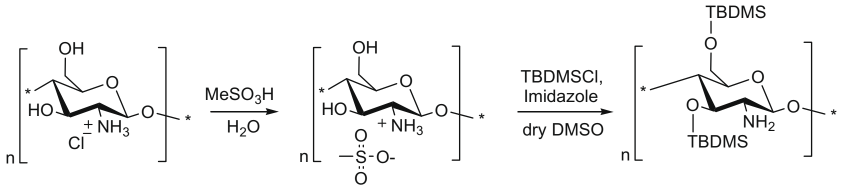
2.3. Characterization

In order to characterized SC, NMR analysis (Bruker AVANCE 400 instrument, Bruker Biospin GmbH, Karlsruhe, Germany) and FTIR-measurements (AVATAR 370 FTIR instrument, Thermo Nicolet Corporation, Madison, USA) were used in the experiment. The wettability of the surfaces was measured at room temperature by contact angle measurements OCA15+(DataPhysics, Germany). The size of drops was 3 μm. The surface morphology of the samples was analyzed using NanoSEM-FEI Nova 200 scanning electron microscope (SEM) (FEI Company, USA).

3. Results and discussion

The reaction process used for the synthesis of SC is shown in scheme 1. In the original procedure the chitosan polymer mesylate salt was reacted with 10-fold excess of tertbutyldimethyl silyl chloride (TBDMSCl) in DMSO, with imidazole as catalyst and base, to obtain full silylation of the 6-O and 3-O hydroxyl groups in 48 h reaction. Although significant excess of the reagent was used the reaction sometimes yields partially silylated material with limited solubility in organic solvents. In an effort to improve this method it was shown that better and more reproducible results were obtained when the starting mesylate salt was further purified by reprecipitation. With this starting material it was possible to get fully *O*-silylate chitosan with only 2.5-fold excess of TBDMSCl and 24 h reaction time. The resolution of the peaks in the ^{1}H NMR spectrum ($CDCl_3$) (Fig. 1A) was improved when the material was prepared by this method and the peaks could therefore be fully assigned from analysis of the COSY spectrum (Fig. 1B). Such data showed two peaks of equal intensity at 0.89 and 0.90 ppm for the tert-butyl groups and four peaks at 0.05, 0.06, 0.01, and 0.13 ppm for the methyl groups. This demonstrated that there was 100% substitution of the hydroxyl groups and this was also confirmed by the absence of the hydroxyl peak in the IR spectrum (Fig. 1C).

Smooth SC film was obtained by solvent casting and exhibited water contact angle (CA) of 103.6 ± 2.7°. In order to prepare SC films exhibiting the desired hierarchical roughness, a phase separation based method was employed in this work adapted from a previously reported procedure (Song, Veiga, Custodio, & Mano, 2009; Zhao et al., 2006). Briefly, rough opalescent SC film was observed after the polymer solution film cast on a glass slide was immersed in ethanol and dried in a nitrogen flow. The contact angles of the rough SC films processed using solutions with concentrations of 10 mg/mL, 30 mg/mL and 50 mg/mL were 137.9 ± 1.6°, 153 ± 2.3° and 151.5 ± 1.9°, respectively. Therefore superhydrophobicity was achieved on the SC film with concentration of both 30 mg/mL and 50 mg/mL. In order to explain why the film showed such unique water-repellency property, scanning electronic microscopy (SEM) and atomic force microscopy (AFM) were used to characterize the surfaces. Fig. 2A and B display representative SEM images of smooth and rough chitosan films, respectively. The rough surface, as seen at a lower magnification, is composed by irregular elements with a length scale of tens of microns. The magnified SEM image (Fig. 2C) shows that such larger structures are built with smaller spherical objects with two different length scales: micro-spheres of around 1–2 μm in diameter and nano-sized spheres with diameters from 10 to 200 nm. The further magnified image (Fig. 2D) shows the surface of the micro-spheres is also not smooth, exhibiting also some nanoscale roughness. The AFM image in Fig. 2E also strengths the existence of nanoparticles distributed between the micro-spheres, which is consistent with the SEM images. Such hierarchical structure, developed along three differ-



Scheme 1. Chemical reaction scheme used for the synthesis of di-tertbutyldimethylsilyl- chitosan.

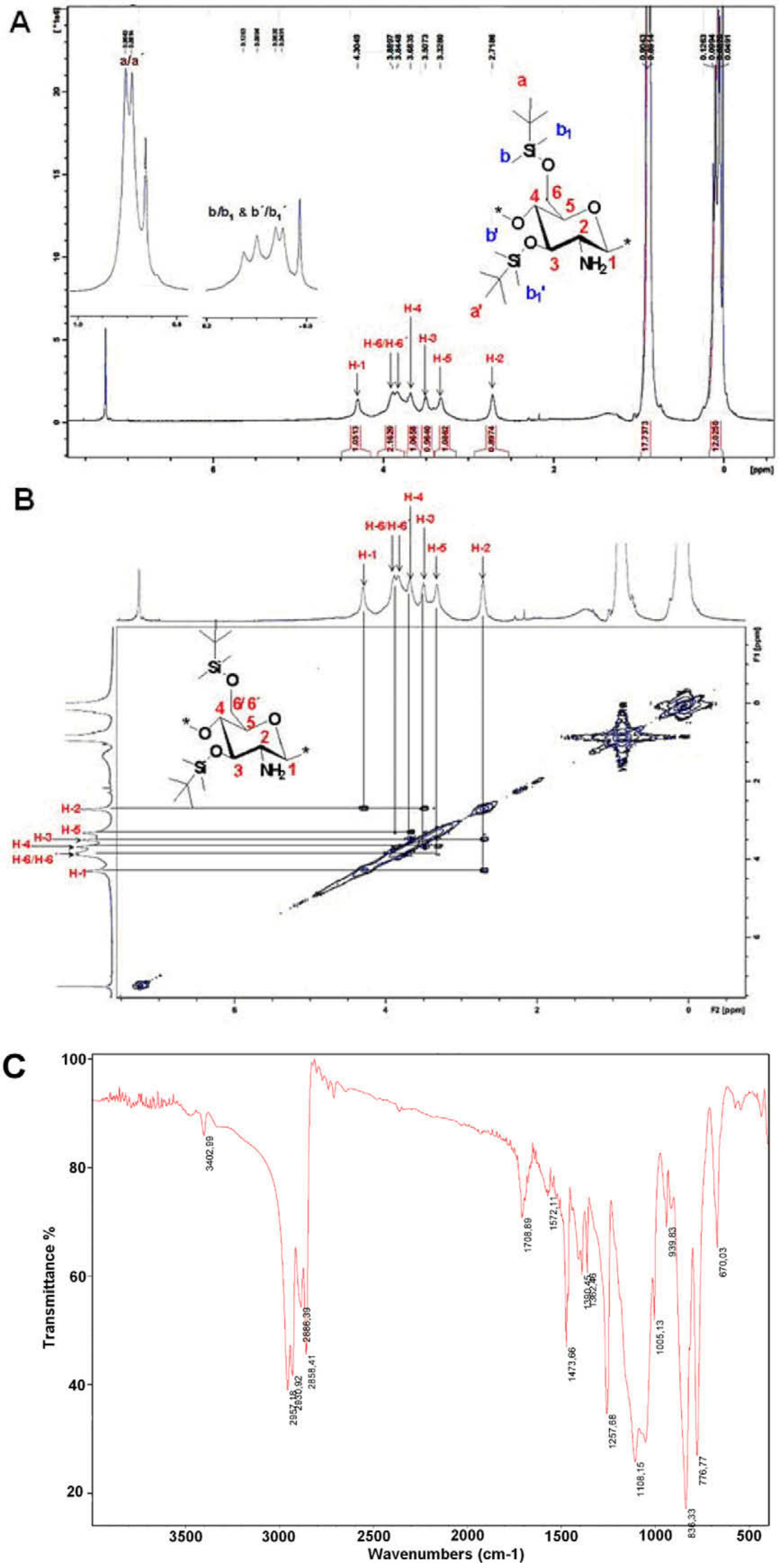


Fig. 1. A. ^{1}H NMR spectrum ($CDCl_3$) and structure of di-tertbutyldimethyl chitosan, was improved when the material was prepared by this method and the peaks could therefore be fully assigned from analysis of the COSY spectrum. B. COSY spectrum of SC with peak assignment; C. FTIR spectrum of SC.

ent size levels, play a crucial in the superhydrophobic features of the prepared film.

A possible principle for the formation of this unique hierarchical rough structure may be the following: when the SC-

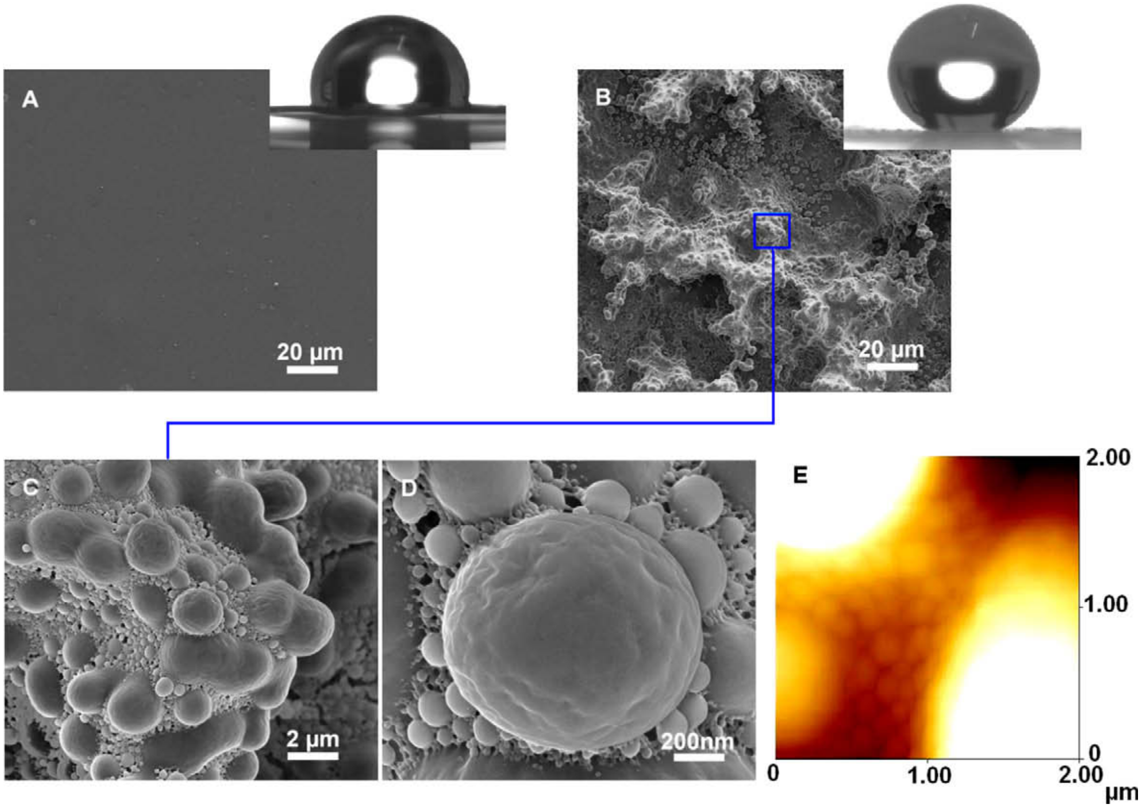


Fig. 2. A and B: representative SEM images of smooth and rough chitosan film (obtained using a 50 mg/mL SC solution); on the top right corner of these micrographs, the profiles of the water drops deposited onto the films are shown, with CAs of 103.6 ± 2.7° and 151.5 ± 1.9°, respectively. C and D: magnified images of the superhydrophobic surface, which displayed abundant number of micro and nano particles. E – AFM image on the superhydrophobic surface.

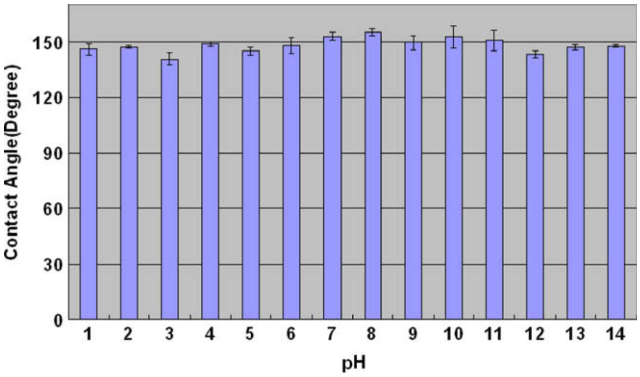


Fig. 3. pH dependence on the contact angle on the rough SC film.

dichloromethane film was immersed in ethanol, the non-solvent (ethanol) tended to diffuse in the polymer solution as both liquids are miscible. The mass transfer of the non-solvent and solvent across the interfaces causes the homogeneous solution to become thermodynamically unstable (Coombes & Heckman, 1992; Wang et al., 2005; Witte, Dijkstra, Van den Berg, & Feijen, 1996). Polymer precipitation takes place on the interface between the SC solution and the non-solvent which resulted of solid–liquid demixing accompanying the liquid–liquid demixing (Witte, Dijkstra, Van den Berg & Feijen, 1996). This will induce the creation of particular structures onto the surface exhibiting peculiar hierarchical roughness architectures. At this point polymer nuclei are then formed, growing into micro-spheres. The nanospheres may be the result of the second-stage precipitation of reminiscent polymer that was not involved in the development of the bigger structures.

The stability of the superhydrophobic characteristics of the developed films was assessed by CA measurements in the pH range from 1 to 14, using hydrochloric acid and sodium hydroxide aqueous solutions. Fig. 3 shows that SC film shows extreme hydrophobic property in the whole pH range, where the CAs are always higher than 140°, and often higher than 150°. These results show that even at acidic conditions, where the amine groups are protonated, the developed films maintained their extreme water-repellency. Moreover, after being kept in air for one month, the SC films still showed stable superhydrophobic properties at neutral conditions.

4. Conclusions

In conclusion, the synthesis method of 3,6-*O*-di-tertbutyldimethyl silyl chitosan (SC) was improved and this polymer was used to prepare durable superhydrophobic films using a simple and low-cost phase separation method. The roughness of such films exhibited three levels of hierarchical organization and their extreme hydrophobic properties could be detected in the whole pH range. The polymer presents free amine groups and protected hydroxyl groups, indicating that its properties could be changed by simple chemical modification. We foresee that such methodology could be extended to other polysaccharides and that these materials could find applications in anti-bacterial or impermeable textiles, tissue engineering and other biomedical applications, or membrane technology.

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