Preparation and properties of natural rubber/chitosan microsphere blends

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Chitosan microspheres were prepared by ion induction and chemical cross-linking using water as the medium. Natural rubber/chitosan microsphere blends were then prepared by blending the chitosan microsphere suspension with natural rubber latex. An infrared spectrometer, particle size analyser, scanning electron microscope, surface contact angle analyser, thermogravimetric analyser, and differential scanning calorimeter were employed for structure and property characterisation. The results showed that the average particle diameter and the polydispersity index of chitosan microspheres were 323.8 nm and 0.283, respectively. The thermal stability and mechanical properties of natural rubber/chitosan blends were enhanced by introducing chitosan microspheres into natural rubber latex. The water contact angle was significantly decreased, from 94.64° to 55.47°, indicating improved hydrophilic properties for the natural rubber/chitosan film. The natural rubber/chitosan blends showed excellent antibacterial properties when the chitosan microsphere content was >4.00% w/w.

1. Introduction: Natural rubber, an important polymer with excellent physical and chemical properties, is widely used in various fields [1–3]. In particular, as a biological macromolecule, natural rubber latex is used in the production of medical products such as medical gloves, condoms, blood transfusion tubes, catheters, syringe plugs, and safety bags, because of its excellent mechanical properties, flexibility, anti-viral penetration properties, and film-forming properties [4–6]. However, natural rubber products lack antibacterial and anti-enzyme properties, and require improvement in this regard by surface modification or the introduction of antibacterial agents. Chitosan is blended with natural rubber as a reinforcing agent for its features and potential applications [7–12]. The addition of chitosan has a positive effect on the thermal stability and mechanical properties of natural rubber.

Chitosan is a natural polysaccharide obtained by the alkaline deacetylation of chitin, and is the most abundant natural polymer after cellulose [13]. Chitosan is widely used in the chemical industry, food, bio-medicines, and other fields because of its excellent safety, non-toxicity, biocompatibility, mechanical film-forming activity, and antibacterial activity [14, 15]. The form of chitosan undergoes significant changes after degradation and nanocrystallisation. Its surface area in contact with water and the activity of its ammonia groups increase [16]. Moreover, its surface and small size effects are manifested because of the high surface activity and small particle size of the nanomaterial. As the preparation of chitosan nanoparticles by ion induction relies on the mutual attraction between positive and negative charges, it is difficult to repeat centrifugation and dispersion, which limits its use in a wider range of applications. Chemical cross-linking of chitosan microspheres is generally required to obtain chitosan nanoparticles with a controlled size and stable properties. Several chemical cross-linking agents such as glutaraldehyde, formaldehyde, and glyoxal have been used [17, 18]. To avoid the disadvantages of chemical cross-linking, some physical cross-linking agents have also been used. However, physical cross-linking agents result in poor mechanical strength due to their dependence on weak electrostatic interactions between anions and chitosan [18, 19]. Therefore, a new cross-linking agent suitable for the cross-linking of chitosan microspheres is necessary.

Vanillin, extracted from tropical vanilla beans or pods [20], is often used as a flavouring agent and preservative, and is considered to be a 'generally recognised as safe' substance [21]. In addition to flavour, vanillin also has a strong antibacterial effect [22]. Furthermore, the $C{=}NH_2$ moiety of chitosan and the aldehyde group of vanillin can react to form a Schiff base, which increases the number of antibacterial activity centres, facilitating the preparation of a new natural antibacterial agent with multiple antibacterial activity centres. The vanillin cross-linked chitosan nanoparticles are circular spheres, with a smooth surface and uniform distribution [23, 24]. However, a large amount of organic solvent is used in their preparation, which is difficult to completely remove. Also, chitosan nanoparticles dispersed in water are more easily added to water-based natural rubber latex. Therefore, it is necessary to develop a new method for the preparation of chitosan nanoparticles requiring mild reaction conditions and a non-organic solvent.

The purpose of this Letter was to explore the possibility of preparing chitosan microspheres with a controlled size and stable properties in water by cross-linking vanillin with chitosan microspheres. Chitosan nanoparticles were prepared, natural rubber latex and chitosan microspheres were blended, and the physical and chemical properties of the natural rubber/chitosan blends were investigated.

2. Experimental

2.1. Materials: Chitosan (molecular weight 10^6 Da, deacetylation degree 90%) was supplied by Nantong Xingcheng Biological Industrial Co. Ltd. (Jiangsu, China). Natural rubber latex was purchased from China Hainan Rubber Industry Group Co. Ltd. The latex was stabilised by adding 0.70% of a 25% ammonia solution, and the dry rubber content was 60%. Vanillin (99% pure) and sodium tripolyphosphate (STPP) were purchased from Aladdin Industrial Corporation (Shanghai, China). All other chemical agents used were of analytical grade.

2.2. Preparation of chitosan microspheres [25]: Chitosan microspheres were prepared by ion induction and chemical cross-linking. First, 10.330 mg of chitosan was dissolved in 1000 ml of 1% (v/v) acetic acid solution, and the pH adjusted to 5.0–5.5 with acetic acid. Next, 1000 ml (2 mg ml^{-1}) of STPP solution was

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added dropwise to the chitosan solution, and the mixture stirred continuously for 2 h at room temperature. Then, 4.0 g of vanillin (acting as the chemical cross-linking agent) was dissolved in 10 ml of 1% (v/v) acetic acid solution, and slowly introduced dropwise into the chitosan solution, under continuous stirring for 12 h. Finally, the reaction mixture was washed three times with distilled water. Part of the product was dried in a vacuum freeze dryer for 48 h and stored in a dryer for later morphology and property analysis. The remaining chitosan microsphere suspension was directly mixed with natural rubber latex.

2.3. Preparation of natural rubber/chitosan blends: The blends were prepared by mixing the chitosan solution and natural rubber latex and stirring 30 min at room temperature, to obtain a homogeneous mixture. The loadings of the chitosan suspension (denoted 0#–8#) were 0, 5, 10, 15, 20, 30, 40, 60, and 100 g. After standing for 24 h, the mixtures were cast onto glass plates and dried at room temperature, to obtain natural rubber/chitosan composite films.

2.4. Particle size analysis: A small amount of each chitosan–vanillin suspension was diluted three times with distilled water. The chitosan particle sizes and distribution in the solution were measured by photon correlation spectroscopy and laser Doppler anemometry (Nano-ZS, Malvern, UK) after ultrasonic treatment.

2.5. Scanning electron microscopy: The morphological features of the chitosan microspheres were examined by scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). Powdered samples were sprinkled onto double-sided tape and sputter coated with a 5 nm thick gold layer. The fracture surface of the blends was obtained after the brittle failure of the bulk sample in liquid nitrogen.

2.6. Water contact angle: The prepared natural rubber/chitosan blends were fixed on an SDSL200B (KINO, USA) optical contact angle analyser, using deionised water as the test fluid at a temperature of 25°C and a humidity of 65% to measure the water contact angle on the surface of the blends. The measurement was repeated six times for each sample. The mean value θ was used as the final measurement.

2.7. Fourier transform infrared (FTIR) spectroscopy: FTIR spectra were recorded using a spectrophotometer (GX-1, Perkin Elmer Corporation, Waltham, MA, USA). Chitosan, vanillin, and chitosan microsphere samples were prepared by processing compressed potassium bromide disks and observing the range 400–4000 cm^{-1} with a resolution of 4.0 cm^{-1} in the transmittance mode. The chitosan–vanillin–natural rubber composite film composition was confirmed by using attenuated total reflection FTIR (ATR-FTIR) with a universal diamond crystal ATR accessory over the range 650–4000 cm^{-1} with a resolution of 4.0 cm^{-1} in the reflection mode.

2.8. Thermogravimetric (TG) analysis: A NETZSCH STA 449C analyser was used for the TG analysis (TGA) of the blended samples. A small amount (10 mg) of each sample was taken for the analysis and heated from 50 to 700°C at a rate of 10°C min^{-1} in a nitrogen atmosphere. The TG and derivative TG (DTG) curves were plotted for the samples.

2.9. Differential scanning (DS) calorimetry: The thermal behaviour of the blends was studied using a TA Q2000 DS calorimeter. Each sample was inserted into the apparatus, and the DS calorimetry (DSC) scan was made from −100 to +100°C at a heating rate of 5°C^{-1} in a nitrogen atmosphere. The mass of each sample was about 5–6 mg. The glass transition temperature of a sample was taken at the initial change in the slope of the heat capacity of the DSC curve.

2.10. Measurement of mechanical properties: Tensile test and tear resistance experiments were conducted on an Instron Series IX 3365 Automated Materials Testing System (Instron, Acton, MA, USA). Dumbbell-shaped test pieces (for the tensile test) and sharp-angled test pieces (for the tear strength test) were cut from the chitosan–vanillin–natural rubber composite films previously conditioned for 4 h at room temperature. The tests were performed with a cross-head speed of 500 mm min^{-1} according to GB/T 528-2009/ISO 37:2005 and GB/T 529-2008/ISO 34-1:2004,28,29. The tensile moduli (M100 and M300), tensile strength, and elongation at break (EB) were obtained from the tensile tests. All the measurements were done at room temperature.

2.11. Antibacterial rate: The antibacterial efficacy was tested according to the Chinese standard QB/T 2591-2003.30 A 0.2 ml aliquot of *Escherichia coli* in DMEM was dropped onto the surface of: (a) a negative control sample, (b) a blank control sample, and (c) an antibacterial rubber sample. The samples were cultured under a daylight lamp for 24 h. The cultured *E. coli* samples were washed with culture medium three times, and the *E. coli* colonies were counted after 24 h. The antibacterial rate was calculated using

$$R = \frac{b - c}{b} \times 100 \quad (1)$$

where R is the antibacterial rate (%), b is the average number of *E. coli* colonies for the blank control sample (cfu $sample^{-1}$), and c is the average number of *E. coli* colonies for the antibacterial rubber sample (cfu $sample^{-1}$). If $R \geq 99\%$, the material has a strong antibacterial effect; if $R \geq 90\%$, the material has an antibacterial effect; and if $R < 90\%$, the material has no antibacterial effect.

3. Results and discussion

3.1. FTIR of chitosan microspheres: When comparing the FTIR spectra of chitosan microspheres and chitosan (Figs. 1 and 2 and Table 1), a broad and flat absorption band derived from chitosan (N–H overlapping O–H) and the phenolic hydroxyl group of vanillin (–OH) shifted from 3253 to 3401 cm^{-1}. This might be induced by hydrogen bonding between chitosan and vanillin. The FTIR spectrum of chitosan microspheres showed a new absorption band at 1640 cm^{-1}, which corresponds to the C=N stretching vibration, and was attributed to the formation of a new bond from the Schiff reaction between the aldehyde group of vanillin (1667 cm^{-1}) and the amino group of chitosan (1550 cm^{-1}). The characteristic absorption band of the phenolic

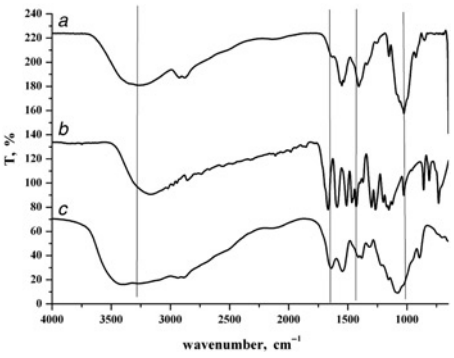


Fig. 1 *FTIR spectra of chitosan (a), vanillin (b), and chitosan microspheres cross-linked by vanillin (c)*

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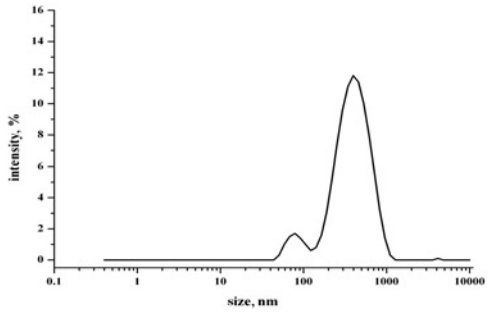


Fig. 2 *Particle size of vanillin cross-linked chitosan microspheres*

Table 1 Characteristic FTIR bands of chitosan and chitosan microspheres

Sample	FTIR, cm^{-1}
Chitosan	3253 (–OH, $–NH_2$), 1667 (C=N), 1641 (C=N), 1550 ($–NH_2$), 1026 (C–O)
Vanillin	3168 (–OH), 2736 (–CH), 1667 (C=O), 1287 (–OH)
Chitosan microspheres	3401 (–OH, $–NH_2$), 1640 (C=N), 1544 ($–NH_2$), 1265 (–OH), 1075 (C–O–C)

hydroxyl group of vanillin at 1287 cm^{-1} appeared at 1265 cm^{-1} in the FTIR spectra of chitosan microspheres. A new strong absorption band shifted from 1026 to 1075 cm^{-1} in the FTIR spectra of chitosan microspheres, which might indicate the formation of a new C–O–C structure. It arose from the C=O stretching vibration, indicating a condensation reaction between the carbonyl of chitosan and the hydroxyl of vanillin.

It can be concluded from the FTIR results that chitosan microspheres were successfully cross-linked with vanillin through the Schiff base reaction and hydrogen bonding.

3.2. Particle size analysis and morphology of chitosan microspheres: The chitosan microspheres were not uniform in size, and ranged between 160 and 1000 nm in diameter. The average particle size was 323.8 nm, and the particle dispersion index was 0.283, which indicates that the chitosan suspension has a narrow and uniform distribution. It is clear that the size of chitosan microspheres did not reach the nanometre scale, and more research is needed in this area.

SEM photographs of chitosan microspheres are shown in Fig. 3. The microspheres had a smooth surface with a few small, irregular particles, which can be attributed to the results of mechanical stress during the stirring process or the removal of moisture during the drying period.

3.3. Surface contact angle and surface free energy analysis of natural rubber/chitosan microsphere blends: The surface

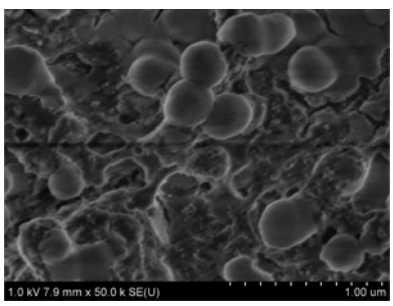


Fig. 3 *SEM photographs of chitosan microspheres*

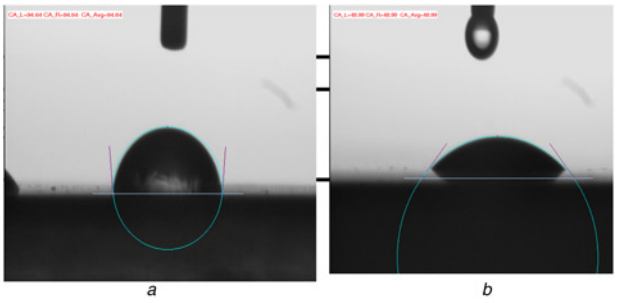


Fig. 4 *Shadow images of water droplets on (a) natural rubber and (b) a natural rubber–chitosan blend containing 6.00% chitosan microspheres*

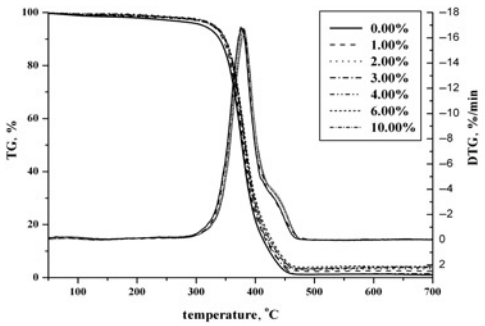


Fig. 5 *TG and DTG curves for pure natural rubber and natural rubber–chitosan blends in nitrogen*

hydrophilic and hydrophobic properties and the free energy are important factors affecting surface compatibility. A large number of studies have shown that higher surface hydrophilic properties and free energy lead, to some extent, to a higher biocompatibility. In the present experiment, the addition of chitosan nanoparticles decreased the surface water contact angle from 94.64° to 55.47° (Fig. 4), which will facilitate the surface diffusion of water molecules and improve the blend surface wettability. Moreover, the surface free energy increased from 26.32 to 50.55 mJ m^{-2}, which will promote the surface adhesion of other biological cells, thus utilising the biocompatibility of chitosan and improving the biocompatibility of natural rubber.

3.4. TG and DSC analysis of natural rubber/chitosan microsphere blends: The thermal stability of natural rubber/chitosan blends can be assessed from the investigation of thermal decomposition. Fig. 5 is the TG and DTG curves of the pure natural rubber and natural rubber/chitosan blends in nitrogen. There is only one

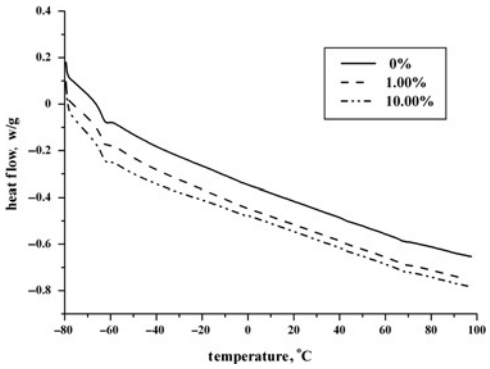


Fig. 6 *DSC curves for pure natural rubber and natural rubber–chitosan blends*

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Table 2 Effect of CS loadings on mechanical properties of CS/NR blends

Property	Chitosan microsphere loading, wt%								
	0	0.50	1.00	1.50	2.00	3.00	4.00	6.00	10.00
Tensile strength, MPa	3.20	3.24	3.37	3.68	4.02	4.18	4.39	5.87	5.61
100% elongation, MPa	0.41	0.41	0.41	0.41	0.42	0.43	0.45	0.49	0.46
300% elongation, MPa	0.49	0.51	0.51	0.51	0.52	0.52	0.53	0.57	0.56
Tear strength, kN m^{-1}	10.85	7.56	8.04	9.35	9.82	10.83	11.06	11.75	12.28

obvious thermal decomposition step in the DTG curves, primarily initiated by thermal scission of C–C chain bonds.

The thermal stability of the prepared blends is dominated by the distribution of chitosan microspheres. Since the chitosan microspheres are homogeneously distributed in the natural rubber matrix as nanoclusters when the chitosan content is <6.00 wt%, the chitosan and natural rubber molecular chains interact through various effects such as branching, size, and surface effects. Therefore, the decomposition behaviour of natural rubber latex changes with the addition of chitosan. When the chitosan microsphere loading is <6.00 wt%, the maximum initial temperature (T_0) of the blend is 354.9°C, which is 5.3°C higher than that of pure natural rubber. This indicates that the thermal stability of the blends has been improved.

The glass transition temperatures (T_g) of natural rubber/chitosan blends containing 1.00 and 10.00% of chitosan microspheres were −65.74 and −65.96°C, respectively, which were slightly lower than that of pure natural rubber (T_g = −64.87°C) (Fig. 6). A glass transition was not detected for the chitosan microspheres within the experimental temperature range of −80 to +100°C. The T_g of the blends decreased slightly with increasing chitosan microsphere content. The decrease of T_g is related to the transition of the resulting blend from non-crystalline to an amorphous form, i.e. to a more disordered internal molecular chain arrangement.

The polymer blend is miscible if its components form a single homogeneous phase at the molecular scale. Therefore, the compatibility of a blend system can be determined by measuring the T_g. Test results confirmed that the natural rubber/chitosan composite had a single phase and was thermodynamically compatible.

3.5. Effect of chitosan microspheres on the mechanical properties of natural rubber/chitosan blends: The introduction of chitosan microspheres improved the mechanical properties of natural rubber to some extent. Table 2 shows the mechanical properties of natural rubber/chitosan blends with different chitosan microsphere contents. Owing to effective reinforcement by chitosan, the tensile strength at different elongations was improved to a degree. As filler–polymer interactions play an important role at low elongations, the tensile strength was measured at elongations of 100 and 300% only. The tensile strength of the blends increased when the chitosan microsphere content was <6.00 wt%. Compared with pure natural rubber, the mechanical properties of the blends with 6.00 wt% chitosan increased from 3.20 to 5.87 MPa, representing an increase of 82.50%. However, the tensile strength decreased with further addition of chitosan due to the aggregation of chitosan microspheres. Insignificant effects of chitosan on EB and the stretching strength of natural rubber were observed.

Poor tear resistance is one of the main problems of natural rubber latex products. However, the tear resistance of the natural rubber/chitosan blends did not increase with an increase in the chitosan content. The tear resistance of the blend decreased significantly when 0.50 wt% of chitosan microspheres was added. The tear resistance of the blend (10.83 kN m^{-1}) was nearly the same as that of pure natural rubber (10.85 kN m^{-1}) when 3.00 wt% of chitosan microspheres was added. The tear resistance of the blend increased with the content of chitosan microspheres when more than 3.00 wt% of chitosan microspheres was added.

Similarly, the improvement in the thermal stability and mechanical properties of the blends was also strongly dependent on the degree of chitosan dispersion. The maximum increase in the mechanical properties of the blends appeared at a chitosan microsphere content of 6.00 wt%: large particles began to aggregate after this point.

3.6. Effect of chitosan microspheres on the antibacterial properties of natural rubber/chitosan blends: Fig. 7 shows the colony morphology of natural rubber/chitosan blends after 24 h of antibacterial testing. The number of *E. coli* colonies is listed in Table 3. According to the antibacterial plastic standard (QB/T 2591-2003), materials with an antibacterial rate of >90% are considered to have an antibacterial function. Pure natural rubber essentially did not have antibacterial properties for its largest number of *E. coli* colonies of 309 cfu $sample^{-1}$. By contrast, the blend containing 3.68% of chitosan microspheres had excellent antibacterial properties. In addition, the antibacterial rate of the blends increased with an increase in the content of chitosan microspheres. Therefore, natural rubber/chitosan blends have excellent antibacterial properties when the chitosan microsphere content exceeds 4.00%.

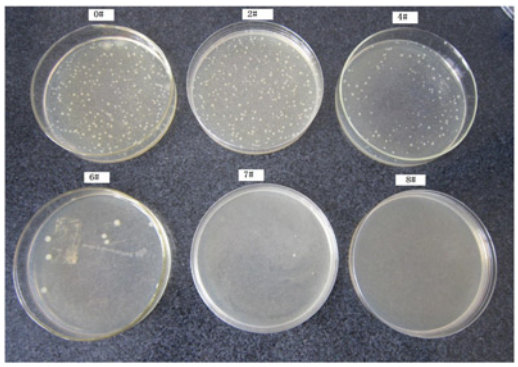


Fig. 7 *Colony morphology of pure natural rubber (0#) and natural rubber–chitosan blends (2#–8#)*

Table 3 Antibacterial properties of chitosan/natural rubber films with different chitosan microsphere loadings

Chitosan microsphere loading (wt%)	Escherichia coli Viable count (cfu ml^{-1})	Inhibitory rate (%)
0	309	—
1.00	296	4.21
2.00	186	39.81
4.00	8	97.41
6.00	3	99.03
10.00	0	100.00

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4. Conclusions: In this Letter, a chitosan microsphere suspension with stable properties was successfully prepared by ion induction and chemical cross-linking. Compared with previous methods of preparing chitosan microspheres, this method has several advantages including mild reaction conditions and the use of water instead of an organic solvent as the medium. The antibacterial activity of chitosan microspheres was enhanced by cross-linking chitosan nanoparticles with vanillin, which has a strong antibacterial activity. The microsphere suspension prepared using this new method can be directly blended with natural rubber latex to obtain natural rubber/chitosan blends, whose thermal stability, mechanical, hydrophilic, and antimicrobial properties are substantially improved compared with natural rubber.

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