Adsorption of collagen to indium oxide nanoparticles and infrared emissivity study thereon

Yu-Ming Zhou a,*, Yun Shan a, Yan-Qing Sun a, Huang-Xian Ju b

a School of Chemistry & Chemical Engineering, Southeast University, Nanjing 211189, PR China
b School of Chemistry & Chemical Engineering, Nanjing University, Nanjing 210093, PR China

Received 14 January 2007; received in revised form 17 September 2007; accepted 17 September 2007
Available online 22 September 2007

Abstract

Adsorption of collagen to indium oxide nanoparticles was carried out in water–acetone solution at volumetric ratio of 1:1 with pH value varying from 3.2 to 9.3. As indicated by TGA, maximum collagen adsorption to indium oxide nanoparticles occurred at pH of 3.2. It was proposed that noncovalent interactions such as hydrogen bonding, hydrophilic and electrostatic interactions made main contributions to collagen adsorption. The IR emissivity values (8–14 μm) of collagen-adsorbed indium oxide nanoparticles decreased significantly compared to either pure collagen or indium oxide nanoparticles possibly due to the interfacial interactions between collagen and indium oxide nanoparticles. And the lowest infrared emissivity value of 0.587 was obtained at collagen adsorption of 1.94 g/100 g In2O3. On the chance of improved compatibility with organic adhesives, the chemical activity of adsorbed collagen was further confirmed by grafting copolymerization with methyl methacrylate by formation of polymer shell outside, as evidenced by IR spectrum and transmission electron microscopy.

© 2007 Published by Elsevier Ltd.

Keywords: A. Organic compounds; A. Oxides; A. Composites; D. Optical properties

1. Introduction

Low infrared emissivity materials have been widely used to form low-e coatings, films or fibers for various applications such as window coating, thermal cover [1–5], etc. During the past decade, with an increasingly complex set of optical requirements dictated by marketplace, there has been considerable interest in unusual physicochemical and optoelectronic properties of low-e materials consisting of nanosized semiconductor particles [6], among which indium (III) oxide generally becomes the preference [7,8]. However, owing to comparatively high infrared emissivity of pure indium oxide nanoparticles, low infrared emissivity properties are generally achieved through doping or incorporation of other components [3,9]. A cursory look at the literature reveals that most studies have been devoted to inorganically doped indium oxide like tin-doped indium oxide [3], yet with few works focused on organic modification. Generally such inorganic materials can achieve low emissivity, but a fundamental and technological challenge remains to be addressed in their practical use. In most cases, these inorganic nanoparticles are required to
disperse in adhesives or polymer matrix to prepare coatings or films in changeable shapes that can be functionalized, whereas the differences in property will result in inhomogeneous mixing which may increase emissivity value of coatings or films remarkably. Hence, surface modifications of inorganic nanoparticles get quite essential to continuous efforts to optimize low-e coatings or films.

The modification technique frequently employed makes use of the interactions between the nanoparticle surface and functional groups of organic molecules, for example, silica particles and silane coupling agent [10] as well as gold particles and a functionalized thiol group [11]. There are many types of active sites such as hydroxyls, charged sites and coordinatively unsaturated metal cations on the surface of metal oxide nanoparticles, so a wide range of organic molecules could be taken into consideration for modification of such nanoparticles. One good proposal for the choice is that the organic molecule selected cannot only bring in excellent dispersion stability of these nanoparticles but also reduce emissivity values. It is easy to meet the former requirement, but as the latter one is concerned, there remain some difficulties due to the scarcity of appropriate organic molecules. In recent years, it has been found that many biomolecules have an inherent tendency to deposit on surfaces of artificial materials as tightly bound adsorbates, thus giving rise to optical, electrical, or magnetic response which is the basis of biomolecular sensors [12] and providing a new route for preparations of low-e nanoparticles ultimately.

Collagen, a biopolymer electret, consists of right-handed supercoils made from three left-handed polyproline II-type helices, with the major amino acid sequence being (Gly-Pro-Hyp)$_n$. Owing to great number of dipoles and molecule-bound charges stored in collagen molecule, collagen possesses the properties of polarization, electrostatic attraction, etc. [13]. Besides, collagen molecule abounds in chemical active sites having good affinities to metal oxide, such as amino and carboxylic acid group [14], and thus can be used to modify and stabilize metal oxide nanoparticles. Additionally, the chemical activity of adsorbed collagen is preserved, and some polymer chains can also be incorporated onto the nanoparticles to realize a good compatibility. In the following presentation, we reported adsorption of collagen to indium oxide nanoparticles in a water–acetone system and demonstrated that this binding event could cause a sharp decrease in emissivity value. Effect of pH on amount of adsorbed collagen was probed together with driving forces for adsorption. And the optimum amount of adsorbed collagen giving the lowest infrared emissivity value was determined. Finally, grafting copolymerization of methyl methacrylate onto collagen was carried out to obtain amphiphilic low-e nanoparticles.

2. Experimental

2.1. Materials

Collagen (types I and III) obtained from Taozheng Bioengineering Technology Co. Ltd. (Beijing, China) was used without further purification. Indium oxide nanoparticles were prepared using sol–gel process described in the literature [15]. One gram of indium metal ingots were thoroughly dissolved in 4 ml of concentrated nitric acid and then diluted with distilled water to total volume of 50 ml, to which required amount of citric acid was added. An indium hydroxide precipitate was synthesized by dropwise addition of 12 wt% NH$_3$H$_2$O solution to the above In(NO$_3$)$_3$ solution under vigorous stirring. The resulting precipitate was washed thoroughly with distilled water and dried at 70 °C in air, then calcined at 500 °C for 4 h to obtain indium oxide nanoparticles.

2.2. Adsorption of collagen on indium oxide nanoparticles

All adsorption experiments were conducted using suspensions including solids with total volume of 100 ml at ambient temperature. 0.5 g of collagen was dissolved in water–acetone solution at volumetric ratio of 1:1, followed by addition of indium oxide nanoparticles (0.8 g). The suspensions were sonicated for 10 min, and then the pH was adjusted to different values using 0.1 mol l$^{-1}$ HNO$_3$ or 0.1 mol l$^{-1}$ NaOH solution. The suspensions were agitated for 24 h for equilibrium to be reached. During equilibration, the suspensions were sonicated at regular intervals to break up any aggregate that might have formed. After equilibration, the samples were centrifuged for 10 min at 2500 rpm and the supernatant were carefully discarded. The resultant collagen-modified indium oxide nanoparticles were dried at 45 °C under reduced pressure in air.
2.3. Grafting copolymerization of methyl methacrylate onto collagen

Required collagen-modified indium oxide was dispersed in water/methanol solution at volumetric ratio of 1:1, into which calculated MMA monomer was added followed by calculated initiator (ammonium cerium nitrate). The reaction was carried out in N₂ atmosphere at 50 °C for 10 h. The reaction mixture was filtrated and dried, then firstly extracted with acetone to remove homopolymer, and subsequently centrifuged to discard the pure collagen-g-PMMA. The collagen-g-PMMA/indium oxide nanocomposite was dried in air at 50 °C.

2.4. Characterization

IR spectra of the samples in the form of KBr pallets were recorded on Nicolet Magna-IR 750 spectrometer (USA). The morphology of all the samples was determined by Hitachi X-650 scanning electron microscopy (SEM) and Hitachi H-600 transmission electron microscopy (TEM), respectively. Infrared emissivity value of powdery samples was measured using IRE-I Infrared emissometer of Shanghai Institute of Technological Physics, China. The amount of adsorbed collagen was evaluated by thermogravimetric analysis (TGA) carried out on TA Q600 (USA). BET specific surface area of indium oxide nanoparticles was measured by nitrogen physisorption at liquid nitrogen temperature (77 K) by taking 0.162 nm² as the area of cross-section of N₂ molecule.

3. Results and discussion

3.1. IR spectra

Upon exposure to water or aqueous solutions, the surfaces of metal oxide particles or films undergo a series of chemical reactions dictated to a large extent by the chemistry of the metal ions involved. These reactions involve surface hydroxylation and hydration (dissociative and non-dissociative water chemisorption), chemisorption of solutes and charge transfer reactions [16]. Owing to large surface-to-volume ratio of nanoparticles, numbers of indium ions exist on surface of hydrophilic indium oxide nanoparticles [17,18]. These indium ions show high activity and are in favor of hydration and hydroxylation. And substitution complexation and hydrogen bonding reactions can happen between hydroxyl groups formed or water molecule adsorbed on surface of indium oxide nanoparticles and collagen macromolecule. As shown in IR spectra (Fig. 1), collagen-modified indium oxide nanoparticles show the characteristic absorption bands around 1640 cm⁻¹ and 1540 cm⁻¹ of the amide groups of collagen [19], which is an evidence of adsorption of collagen on indium oxide. And, a rise in absorption over a wide interval of wave numbers smaller than 3500 cm⁻¹ assigned to νOH due to physically adsorbed water [20] is observed, indicating the formation of hydrogen bonds between adsorbed collagen and surface water hydroxyl groups. Simultaneously, a red shift of

![Fig. 1. IR spectra of (A) collagen-modified indium oxide (B) indium oxide and (C) collagen.](image-url)
adsorption band of amide from 1650 cm\(^{-1}\) to 1640 cm\(^{-1}\) is indicative of substitution of C=O groups on collagen backbone for water molecules coordinated to unsaturated surface In\(^{3+}\) [21].

3.2. Influential factors to adsorption

3.2.1. Effect of pH

Hydroxyl groups on surface of indium oxide nanoparticles that are immersed in aqueous solution can adsorb or dissociate protons to form positive and negative surface sites, respectively [22]. In addition, collagen carries charged groups, so electrostatic interactions were expected to play a major role in pH dependence of collagen adsorption. As the isoelectric point of collagen and indium oxide is 4.5 [23] and 8.7 [24], respectively, the maximum amount of collagen adsorbed was expected to appear at pH range of 4.5–8.7, considering electrostatic attractions between negatively charged collagen and positively charged indium oxide. However, the results on pH dependence of collagen adsorption are not in good agreement with above expectations. As illustrated in Fig. 2, the maximum adsorption of collagen takes place at pH of 3.2. It should be noted that although there exist electrostatic repulsions between collagen and indium oxide at pH < 4.5 as both collagen and indium oxide carry net positive charges, more collagen is assembled onto surface of indium oxide nanoparticles than at pH of 4.5–6.4. Moreover, the amount of adsorbed collagen decreases from 2.01 g/100 g In\(_2\)O\(_3\) at pH of 4.5 to 1.87 g/100 g In\(_2\)O\(_3\) at pH of 6.4. If electrostatic interaction is the driving force over this pH range, an adsorption increase will occur owing to little charge density of collagen at pH close to isoelectric point. But it is clearly not the case for collagen/indium oxide system. These data show that electrostatic interactions do not control collagen adsorption over pH range of 3.2–6.4. The conclusion is similar to what was drawn by reports [25] on the influence of pH (range of 3–7) on the collagen adsorption onto polymer substrates.

Afore, IR spectra analysis indicates that hydrogen bonding and substitution of C=O groups for water molecules adsorbed on indium oxide nanoparticles are involved in collagen adsorption. These two interactions are related to the adsorption of water, which can provide the required number of ligands to achieve full coordination around indium ions on surface. In acidic pH range, surface hydration is the dominant interactions, compared to neutral or alkaline pH range in which surface hydroxide groups are the main form [26]. Therefore, it can be assumed that hydrogen bonding and substitution interaction of C=O groups are the dominant driving forces for collagen adsorption at pH of 3.2–6.4, considering the reductive adsorption of collagen in this pH range.

Furthermore, amount of adsorbed collagen decreases more sharply over pH range of 7.6–9.3 than in pH < 6.4, as the curve of pH dependence shows. If hydrogen bonding and substitution interaction of C=O groups still solely govern collagen adsorption at pH of 7.6–9.3, a similar curve slope will be obtained. It is known that electrostatic repulsion occurs between collagen and indium oxide at pH > 8.7 owing to net negative charges of both of them and electrostatic interactions are weak at pH value close to isoelectric point, which will result in the reduction of adsorption if collagen adsorption relies on electrostatic interaction. This indicates that electrostatic interaction has a bigger influence on collagen adsorption over pH range of 7.6–9.3 than at pH less than 6.4. However, the contribution of hydrogen bonding and substitution interactions to this result cannot be ruled out. van Oss et al. [27] have found that protein, an analogue.
to collagen, is incorporated onto SiO$_2$, talc, SnO$_2$ particles at neutral pH through both electrostatic interaction and other driving forces, such as charge transfer interaction between protein and cations imbedded in surface of particles. So, it may be hypothesized that this result originates from cooperation of electrostatic interactions and driving forces mentioned earlier. To further clarify the electrostatic interactions, we have characterized the $\text{d(amount of adsorption)}/\text{dpH}$ as a function of pH. These data (inset in Fig. 2) show that two inflexion points appear at pH about 4.5 and 8.7 that correspond to isoelectric points of collagen and indium oxide separately, which better supports the discussion above and indicates that electrostatic interactions cannot be dropped despite not dominating the adsorption of collagen in acidic pH range.

### 3.2.2. Hydrophilic interaction

Adsorption of a biopolymer on a substrate is a complex system in which intermolecular interactions between biopolymer also need consideration besides interactions between biopolymer and substrate. Solvent-induced hydrophobic or hydrophilic interaction that depends on the nature of solvent and biopolymer is intermolecular interaction of considerable importance. Collagen is a macromolecule having hydrophilic segments, and is insoluble in acetone. In this collagen/water–acetone system, it is found that hydrophilic interaction is also contributing force for collagen adsorption. Here, the term “hydrophilic interaction” is used as a convenient short hand for the complex thermodynamic process in which hydrophilic regions of molecules tend to be forced together by their repulsion from a hydrophobic liquid phase [28,29]. Thermogravimetric analysis shows that the amount of collagen adsorbed goes up with increase of ratio of acetone versus water. The amount adsorbed at different ratio of 1:10, 1:1 (acetone:water) is found to be 1.75 and 1.87 g/100 g In$_2$O$_3$, respectively. Simultaneously, this conclusion is further confirmed by SEM of collagen-modified indium oxide prepared at ratio of 1:10 and 1:1 (Fig. 3). As shown in Fig. 3(a), collagen-modified indium oxide nanoparticles assemble into close aggregates the outside of which is wrapped by several layers of collagen when the ratio of acetone versus water is 1:1. Whereas collagen-modified indium oxide nanoparticles only aggregate loosely with no clear layer of collagen to be seen as the ratio decreases to 1:10 (Fig. 3(b)). It is clear that hydrophilic segments of collagen conglomerate together at high ratio of acetone versus water due to repulsion from acetone.

### 3.3. TEM images

TEM images of indium oxide nanoparticles prior to and after collagen adsorption are shown in Fig. 4. It is clearly seen that several layers of collagen surrounds indium oxide nanoparticles that are spherical solid of 30–50 nm in...
diameter (Fig. 4(a)) prior to collagen adsorption, as illustrated in Fig. 4(b). Collagen (molecular mass of about 300,000 Da) is about 300 nm long and 1.5 nm in diameter. The amounts adsorbed for a close-packed monolayer of molecules lying flat (“side-on” position) or standing perpendicular (“end-on” position) to the surface would be $0.1 \mu g \text{cm}^{-2}$ and $25 \mu g \text{cm}^{-2}$, respectively [25,30]. Based on specific surface area of 18.3 m$^2$/g for indium oxide nanoparticles, the maximum value of collagen adsorbed is 0.114 $\mu g \text{cm}^{-2}$, which is higher than expected for a “side-on” position. This datum indicates that while portions of the molecule are lying flat on the surface, other portions are forming multi-layers at the surface through intermolecular interactions. In addition, multi-layers of collagen can be found in TEM images of all the other samples, even for least amount that is 0.062 $\mu g \text{cm}^{-2}$, which shows that first layer of collagen does not cover the surface of indium oxide nanoparticles thoroughly. The result may be attributed to semi-flexible chain of collagen molecule and incomplete coverage of surface reactive sites.

3.4. Infrared emissivity

Infrared emissivity values at wavelength of 8–14 $\mu$m of all the samples prepared at different pH are listed in Table 1. Pure indium oxide nanoparticles (30–50 nm) and collagen have high infrared emissivity values (0.903 and 0.851, respectively). In contrast, all modified indium oxide nanoparticles are of quite lower infrared emissivity values from 0.679 to 0.587 than collagen and pure indium oxide nanoparticles. In order to find explanations for these results, we prepared collagen-modified analogues using indium oxide nanoparticles of 80–100 nm in diameter. It is found that no significant decrease in emissivity value is observed, even for similar amount of collagen adsorption. This fact gives evidence that the decrease results originate from interfacial interactions between collagen and indium oxide nanoparticles. SEM and TEM analysis shows that collagen-modified indium oxide nanoparticles are of core–shell structure with the collagen as shell and indium oxide nanoparticles as core. It is clear that there is a special interfacial layer between core and shell, in which vibration modes of molecules, atoms, and pendant groups are different from

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Amount of collagen</th>
<th>Solution pH</th>
<th>Infrared emissivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.09</td>
<td>3.2</td>
<td>0.603</td>
</tr>
<tr>
<td>2</td>
<td>2.06</td>
<td>4.0</td>
<td>0.595</td>
</tr>
<tr>
<td>3</td>
<td>2.01</td>
<td>4.5</td>
<td>0.590</td>
</tr>
<tr>
<td>4</td>
<td>1.94</td>
<td>5.3</td>
<td>0.587</td>
</tr>
<tr>
<td>5</td>
<td>1.87</td>
<td>6.4</td>
<td>0.601</td>
</tr>
<tr>
<td>6</td>
<td>1.79</td>
<td>7.0</td>
<td>0.610</td>
</tr>
<tr>
<td>7</td>
<td>1.71</td>
<td>7.6</td>
<td>0.625</td>
</tr>
<tr>
<td>8</td>
<td>1.53</td>
<td>8.2</td>
<td>0.641</td>
</tr>
<tr>
<td>9</td>
<td>1.35</td>
<td>8.7</td>
<td>0.661</td>
</tr>
<tr>
<td>10</td>
<td>1.14</td>
<td>9.3</td>
<td>0.679</td>
</tr>
</tbody>
</table>
that in body of core and shell due to interfacial interactions [31]. The vibration mode alteration results in a change in infrared emissivity value [32] so that interfacial layer has dissimilar infrared emissivity value to pure indium oxide nanoparticles and collagen. Furthermore, large ratio of surface-to-volume of nanoparticles brings in big ratio of interfacial layer. As a result, collagen-modified indium oxide nanoparticles show quite different infrared emissivity values, too. An analogue could be found in literature [4] by Kittle that interfacial interactions between metal oxide and adhesive agents leaded a big change in infrared emissivity values.

Moreover, all collagen-modified indium oxide nanoparticles have different infrared emissivity values. When the amount of collagen adsorbed is 1.94 g/100 g In$_2$O$_3$, the lowest infrared emissivity value of 0.587 is obtained. It is hypothesized that an optimum amount of collagen exists for infrared emissivity, at which maximum collagen molecules belong to interfacial layer. When the amount excels optimum value, excess collagen would increase infrared emissivity, and inversely, it is also the case owing to insufficient coverage of collagen molecules to form interfacial layer. Here, it is clearly seen that the optimum amount for sake of infrared emissivity is 1.94 g/100 g In$_2$O$_3$.

3.5. Collagen-g-PMMA copolymer/In$_2$O$_3$ core–shell nanocomposites

In practical applications, most adhesives or polymers in which the low-e nanoparticles dispersed are hydrophobic, therefore, it is of great importance to prepare amphiphilic nanoparticles. Here, hydrophobic PMMA chains are incorporated onto adsorbed collagen by grafting copolymerization [33]. The IR spectrum for the collagen-g-PMMA copolymer/In$_2$O$_3$ nanocomposites shows that except the characteristic adsorption bands of collagen and indium oxide a new band at 1730 cm$^{-1}$ appears, which is assigned to $\nu_{C=O}$ in ester, and adsorption bands at 1243 cm$^{-1}$ and 1150 cm$^{-1}$ correspond to $\nu_{C-O}$ in ester [19]. In addition, there are also characteristic adsorption bands of PMMA at 1064 cm$^{-1}$, 840 cm$^{-1}$ and 750 cm$^{-1}$. These are evidences for the existence of PMMA chains. In order to confirm that the PMMA chains are grafted onto collagen, we put the nanocomposites in the strong nitric acid aiming to remove the indium oxide, the solids left were dissolved with acetone. It was found that no dissolving occurred. Hence, we can conclude that the nanocomposites are composed of indium oxide and collagen-g-PMMA copolymer.

Unlike the collagen-modified indium oxide, these particles are regular core–shell spheres of 40–60 nm in diameter (Fig. 5). This suggests that the hydrophobic PMMA chains and hydrophilic collagen macromolecules assemble into compact shell after grafting copolymerization. When water, acetone and chloroform are used as dispersing solvents, respectively we obtain similar TEM micrographs, indicating that these core–shell particles well dispersed in both aqueous and organic media are amphiphilic.

Moreover, collagen-g-PMMA copolymer/In$_2$O$_3$ nanocomposites are found to have a 5–8% increase in emissivity value compared to collagen-modified indium oxide. The lowest and highest values are 0.62 and 0.72, respectively. We note that infrared emissivity value of the nanocomposites prepared by collagen-modified indium oxide at maximum adsorption only rises by 5% which is the minimum increment, and that some loosely bound collagen macromolecules may disassemble from the indium oxide during grafting copolymerization. This result shows that disassembly of some excess collagen adsorbed may counteract the addition of PMMA chains partly.

Fig. 5. TEM of collagen-g-PMMA copolymer/In$_2$O$_3$ nanocomposites.
4. Conclusions

In the present work, adsorption of collagen on indium oxide nanoparticles was performed over pH range of 3.2–9.3 in water–acetone solution. Maximum amount of collagen is found to assemble onto surface of indium oxide nanoparticles at pH of 3.2. Hydrogen bonding and substitution interaction of C=O groups are assumed to be the dominant driving forces for collagen adsorption at pH of 3.2–6.4. However, in the higher pH range of 7.6–9.3, electrostatic interactions as well as other driving forces such as hydrogen bonding, play an important role in collagen adsorption. Additionally, hydrophilic interactions are involved in adsorption over the whole pH range of 3.2–9.3. Resulted collagen-modified indium oxide nanoparticles have significantly lower infrared emissivity values, with the lowest value down to 0.587. It is assumed that interfacial interactions account for the decrease in infrared emissivity value. And organically compatible low-e materials are finally obtained as formation of core–shell nanocomposites by grafting copolymerization on collagen-modified indium oxide with methyl methacrylate.

Acknowledgements

This work was financially supported by “The Six Top Talents” of Jiangsu Province of China (06-A-033), Program for New Century Excellent Talents in University of China (NCET-04-0482), the National Natural Science Foundation of China (no. 50377005), innovation plan to postgraduates in University of Jiangsu Province and Excellent Doctoral Thesis Foundation of Southeast University.

References