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Preparation of Low-Allergen Natural Rubber Latex by Transglutaminase Catalysis

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ABSTRACT: The technique of enzyme treatment on the water-soluble proteins and mechanical properties of natural rubber latex (NRL) films was studied. The main aim was to introduce an enzymic catalysis method to tackle the protein allergy problem in NRL product. The suitable pH value, the temperature, and the best proportion of transglutaminase to deal with NRL were found. The protein spillage of modified NRL films was greatly lower than that of unmodified NRL, and the tensile strength and the elongation at break of the modified NRL films almost had been scarcely changed. The compactness of the NRL films was improved simultaneously. These suggest that the modified NRL can be used as a kind of latex with low-allergy personal barrier products such as surgical gloves.
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INTRODUCTION

The major source of natural rubber latex (NRL) for surgical gloves is harvested from *Hevea brasiliensis* trees.[1] NRL is used superiorly in production of surgical gloves with good film-forming ability, high wet-gel strength, excellent mechanical properties, and so on.[2] NRL contains two different proteins with different molecular weights. The high molecular proteins are tightly bound to the rubber particles in the latex, and the low molecular weight water-soluble proteins (WSPs) are mainly dispersed in serum.[3,4] Most of the WSPs are removed during the concentration process and the manufacture of NRL. Even if a little of WSPs in NRL might cause allergic reaction[5,6] in somebody who are using the NRL products. The allergy reaction of NRL film articles has become an important occupational health concern, particularly among those health-care workers. The normal methods of tackling the protein allergy problems are to remove the remaining WSPs in NRL by leaching,[7] centrifugation,[8] chlorination treatment,[9] and so on. However, all these methods are not so effective for the constant decomposition of high molecular weight proteins and even lead to the environmental contamination. And, the mechanical properties of those NRL products would be reduced, when the WSP was removed from the NRL.[10,11] The other flaw of the NRL film articles is that there are a lot of natural fissures with the diameter between 500 and 7000 nm at the surface of them.[12] Some of these nature fissures are slightly larger than the effective barrier of human sperm size (3000 nm in diameter), which leads to the failure of contraception. And almost all those nature fissures are much larger than the size of HIV virus (120 nm in diameter), which even would cause the deadly damage to the surgery doctor with small wound at the surface of their hand skin.[13] Therefore, to make up for these nature fissures of NRL film articles will bring extremely great significance for medical development.

Transglutaminase (TG) is distributed intracellularly and extracellularly throughout the human body, which was used to crosslink those high molecular weight complexes chemically.[14] It has been primarily utilized to crosslink various peptides to extracellular matrix components and to prepare synthetic polymeric hydrogels. Many studies have been indicated that TG could catalyze the crosslinking of protein-bound glutamine and lysine residues with the formation of covalent *N*-ε-(γ-glutamyl) lysine amide bonds (Figure 1).[15,16] In this article, we postulate that the WSP of low molecular weight in NRL could be crosslinked as an interpenetrating network[17–19] by TG. Thereby, the dense nonporous NRL films with low allergy were prepared with satisfying mechanical properties.

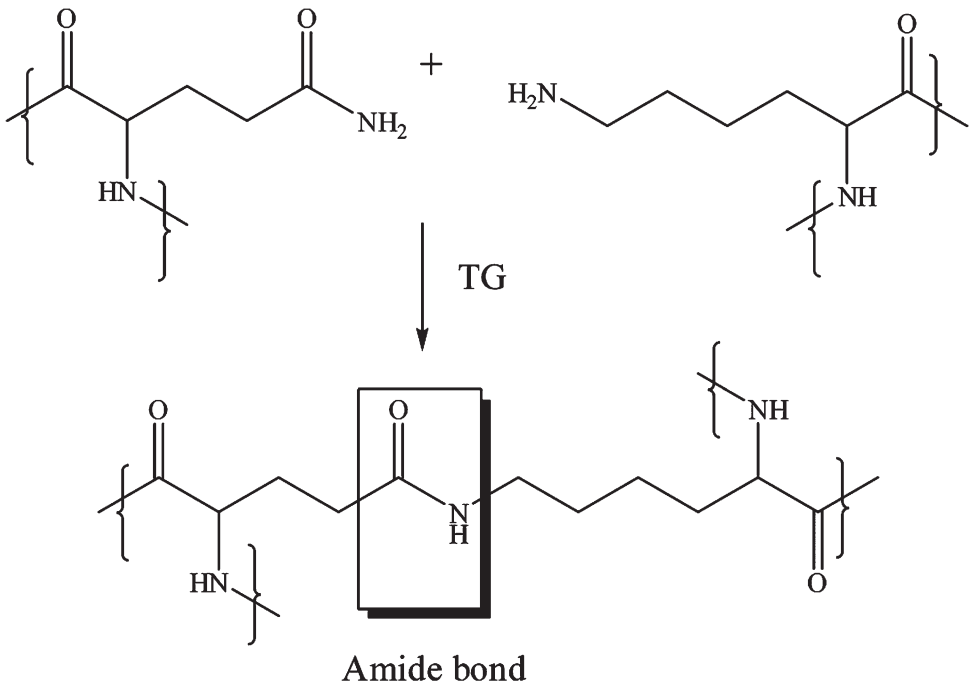

Figure 1. The schematic of the enzymic catalysis crosslinking reaction of proteins by TG.[16]

EXPERIMENTAL

Material

The NRL with a solid content of 60% (w/w) was kindly donated by Hainan American International Xianghe Industrial (Hainan China). TG was from Jiangsu Yiming Biological Products (Jiangsu China). Coomassie blue (Shanghai Yixin Biological Technology), bovine serum albumin (Shanghai Chemical Reagent Station), and folin (Sigma) were used to determine the content of protein (Shanghai China). pH buffer solution was supplied by Degussa-AJ (Shanghai China) Initiator. Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) was purchased from Shanghai LingFeng Chemical Reagent (Shanghai China).

Treatment of NRL

The acid treatment of the NRL was carried out first. NaH_2PO_4 and Na_2HPO_4 were diluted to 0.2 mol/L by the addition of distilled water. Various amounts of NaH_2PO_4 and Na_2HPO_4 were added to the latex to get NRL of various pH 7.0–10.0. The pH values of NRL were measured by using a pH meter (METTLER TOLEDO, FE20).

To research the temperature effect of TG on the NRL, 0.005 g of TG was mixed with 20.0 g of NRL at different temperatures 20.0°C (room temperature), 37.0°C (body temperature), 45.0°C (activation temperature), and 60.0°C (inactivation temperature), with the stirring velocity at 100 rpm for 1.5 h.

TG and NRL were mixed in accordance with the ratio of 1 : 2400 (the ratio of solid content, 0.417‰), before the acid treatment of the solution. The treated NRL was stirred at 45°C and 100 rpm for 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h.

After the acid treatment of the NRL solution, TG and NRL were mixed with 1 : 12,000 to 1 : 120. The NRL mixtures were stirred at 45.0°C and 100 rpm for 1.5 h.

Preparation of Latex Films

The mixtures treated like before were poured into polystyrene plate and dried at 75.0°C for 16.0 h. The thicknesses of these flat deposited films were measured by a micrometer gauge. The average thickness of the films was about 0.85 mm.

Characterizations

Fourier transform infrared (FTIR) spectroscopy was performed on a spectrometer (Magna-IR 550, Nicolet). The samples were taken at random from the flat films, and the data were collected at room temperature.

The WSP of the NRL films was measured according to the Modified Lowry Protein Test Method, ASTM D 5712 (1999) with optional correction.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) can make sure of molecular weight of the protein in NRL. Before doing SDS-PAGE, the NRL needs to deal with high-speed centrifugation, which could help us to obtain the whey in which WSP mainly dissolved. The liquid supernatants and TG were separately mixed with an equal amount of SDS-PAGE sample buffer (0.5*M* Tris–HCl of pH 6.8, 10% SDS, 12% 2-mercaptoethanol, and 50% glycerol). SDS-PAGE[20,21] was performed according to the method of Laemmli using a ready-made 5–10% gradient gel. After electrophoresis, the gel was stained with 0.25% CBB R-250. Molecular weights of protein bands were estimated by using Precision Protein Standards (Beijing Zoman Biotechnology, Beijing, CA).

The particle size distribution of NRL and the optimum treated latex were measured by a light-scattering spectrophotometer (Nano ZS, ZEN3600, Malvern Instruments, UK).

The micromorphology of the pure NRL particles and NRL particles treated by TG were investigated by transmission electron

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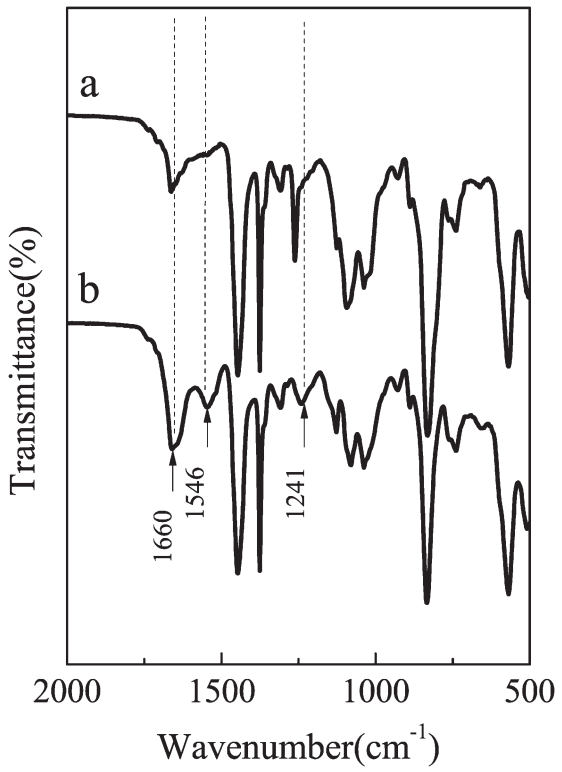

Figure 2. FTIR spectra of the films of pure NRL (a) and NRL treated by TG (b).

microscopy (TEM, JOEL JEM-1200EX), with an acceleration voltage of 200 kV, by drying a drop of the washed colloidal dispersion onto a copper grid covered with a conductive polymer.

The surface morphology of the pure NRL and the TG-treated optimal latex films were studied by scanning electron microscope (SEM, JSM-6360LV), and all of these film surfaces were sprayed gold.

Tensile property tests have been done according to GB 7543-2006. Five dumb bell test pieces with 50.0-mm long and 4.0-mm wide rectangular working sections were cut from each sample which thicknesses at the middle part have been measured. The elongation at break and tensile strength of the samples were measured by universal test tension machine (CMT2202) and pilled at a rate of 400.0 mm/min.

RESULTS AND DISCUSSION

The FTIR Characterization of Pure NRL and NRL Treated by TG

The structural feature of the pure NRL and the NRL treated by TG could be characterized by FTIR spectroscopy. Figure 2 shows the FTIR spectrum of pure NRL and the NRL treated by TG. For the modified NRL, the characteristic peaks of amide bonds at 1660.0, 1546.0, and 1241.0 cm^{-1} correspond to the vibrations of C═O, C—N, and N—H. But for the pure NRL, the amide bands of characteristic absorption peak is emerged only at 1660.0 cm^{-1}, which is mainly caused by the C═O stretching vibration of Amide I. The characteristic lines at 1546.0 and 1241.0 cm^{-1} are attributed to Amide II (C—N stretching vibration and N—H bending vibration) and Amide II (C—N stretching vibration and N—H plane bending vibration).[22] During the crosslinking process, certain lysine and hydroxylysine residues are deaminated by the action of the located lysyl oxidase, resulting in the formation of highly reactive aldehyde groups that spontaneously form covalent bonds with each other or with other lysine or hydroxylysine residues.[23] Obviously, the FTIR spectrum of covalent *N*-ε-(γ-glutamyl) lysine amide bonds confirms that the residual protein-bound glutamine and lysine can be crosslinked with the catalysis of TG. The results of FTIR spectrum verified the proteins at the surfaces of latex particles that had been crosslinked in the TG-treated NRL.

The Contribution of the Enzyme Addition, the Temperature, pH, and the Reaction Time to the Content of WSP in NRL Treated by TG

The effect of the different enzyme addition on the WSP contents is shown in Figure 3(a). It can be seen that the WSP of the NRL decreases rapidly with the increasing of the enzyme addition until 0.417‰, and then, it will increase with the enzyme addition. The reduction of the WSP with the enzyme addition is due to the crosslinking of the proteins with TG catalysis. Those low molecular weight WSPs are crosslinked as water-insoluble high molecular protein interpenetrating network. At the same time, they prevent the residual low molecule proteins traversing the films effectively. But the more enzyme addition makes the excess TG traverse the NRL film surface, which leads to the increasing of the WSP. Figure 3(b) gives the optimum crosslinking catalytic reaction temperature of TG. When the WSP reaches the minimum, the optimum reaction temperature is found at 45.0°C, which coincides with the activation temperature of TG. The optimal pH value of the catalytic crosslinking reaction is pH 9.0, as shown in Figure 3(c). Figure 3(d) demonstrates the existence of the optimum reaction time in this reversible reaction. When the reaction time attached at 1.5 h, the crosslinking reaction reaches equilibrium. Figure 3 indicates that the optimum reaction conditions are at 45.0°C, pH 9.0, and 1.5 h, with the enzyme addition of 0.417‰.

The Molecular Weight of the Protein in Pure NRL and NRL Treated by TG

The SDS-PAGE method is mainly used to describe different molecular weights of the proteins. Using this method, the catalytic action of TG in the crosslinking process can be discussed. The different whey samples of the TG-treated NRL, TG, and pure NRL were examined by SDS-PAGE. The proteins in the pure NRL whey migrate as a fuzzy ranging in relative size from 40 to 65 kDa on a Coomassie blue-stained gel (Figure 4; lane d), which correspond to the molecular weights of WSPs. TG shows two clear bands of about 55 and 65 kDa, three faint bands of around 40 kDa (Figure 4; lane c). As shown in Figure 4 lane b, the whey of NRL treated by TG emerges a new band of 160 kDa except for the TG bands of 55 and 65 kDa. The bands between 40 and 65 kDa that exist in the pure NRL almost disappeared. When the pure NRL was incubated with TG, a majority of protein constituents in NRL were effectively crosslinked or polymerized by TG to form high molecular weight biopolymers.[24] It confirms that the proteins of 40–65 kDa in pure NRL have been crosslinked to form big proteins of 160 kDa. These

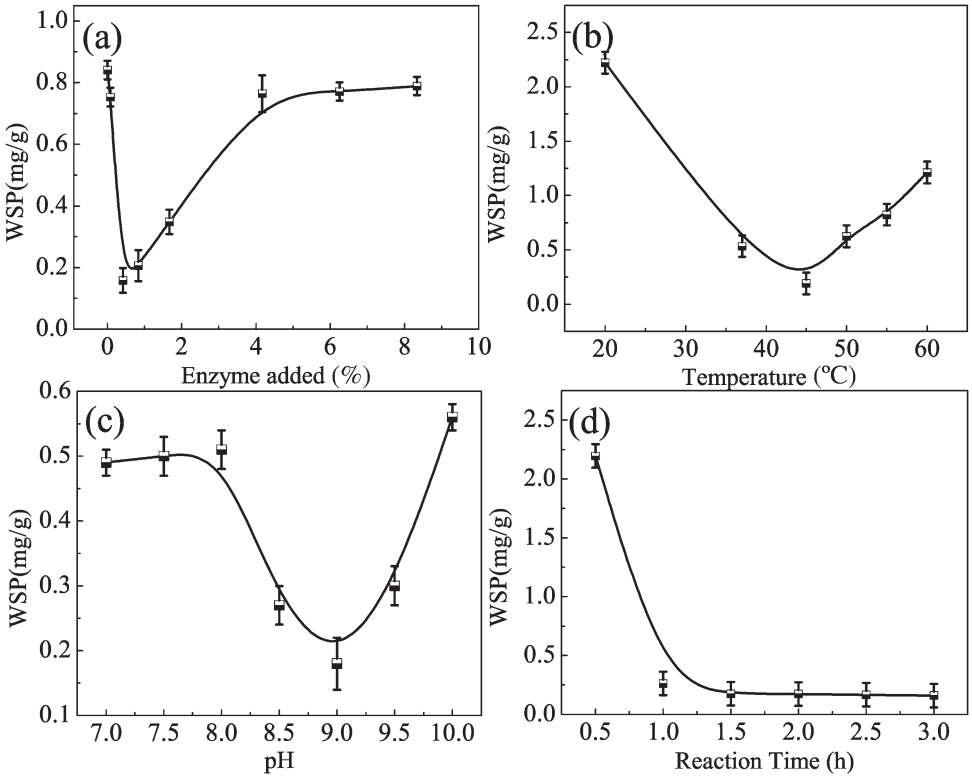

Figure 3. WSP contents depend on the enzyme addition (a), temperature (b), pH (c), and reaction time (d).

results demonstrate that the proteins in NRL could be crosslinked by TG catalysis.

The Enzyme Addition Dependence of Average Particle Size

By crosslinking the proteins on the surface of the latex particles, the particle size of the latex particles will be increased. So, the average particle size can indirectly reflect the crosslinking status of the proteins in NRL. Figure 5 displays the average particle size and the particle size distribution curves of the NRL with different additive amounts of enzyme. The particle size distribution of pure NRL owns the lowest average particle size at 420.0 nm. As the increase of enzyme addition, the average particle size increases rapidly, until the additive amount of enzyme at 0.417‰, the average particle size reaches the maximum. With the additive amount of enzyme increasing, the more proteins that always exist at the surface of the latex particles were crosslinked by TG catalysis. By the proteins crosslinking process, a lot of small latex particles had been crosslinked to form as big ones which cause the average particles size increased. When the average particles size reached the maximum at 987.0 nm, almost all the small molecule proteins had been crosslinked in the latex. If the amount of enzyme increased continually, the average particles size in latex would present downward trend. It is due to the hydrolysis of the —carboxyamide group of Gln residues in the absence of amine substrate by TG biphasic catalysis.[25] Small additive amount of TG would promote the crosslinking. Nevertheless, the excess TG addition would lead to the decomposition of proteins. The decomposition velocity of proteins is slower than that of the crosslinking. When the enzyme addition attached 5.0‰, the system would achieve a new dynamic equilibrium. After that the more enzyme addition did not work in the latex. Obviously, the optimal additive amount of enzyme is about 0.417‰, when the average particle size reached the maximum.

The Morphology of Latexes and Films

The micromorphologies of the diluents of pure NRL and NRL treated by TG (with the additive amount of enzyme at 0.417‰) are observed by TEM. Figure 6 shows the TEM images for the pure NRL particles [Figure 6(a)] and the NRL particles treated by TG [Figure 6(b)]. The pure NRL particles are spherical with smooth surfaces. These NRL particles distribute in the latex independently, but the NRL particles treated by TG proteins were aggregated to form micelles by the TG crosslinking catalysis. These phenomena are consistent with the size distribution test results that we have just discussed earlier. The interpenetrating network that was formed by the proteins crosslinking could also prevent the small molecule proteins traversing, which made the NRL treated by TG to form low-allergen membrane products.

SEM is used to study the surface morphology of membranes. The SEM images of the pure NRL film and the NRL film treated by TG are shown in Figure 7. There are many small holes with the diameter from 500 to 5000 nm at the pure NRL film surface, which were leaded by the interspaces of large and small rubber particles. The modified films exhibit much smoother. The gap at it is even less than 50 nm, which is much smaller than the human sperm size 3000 nm and the HIV virus size 120 nm. The cause of the compactness films formation is that the crosslinking proteins at the surface of the particles

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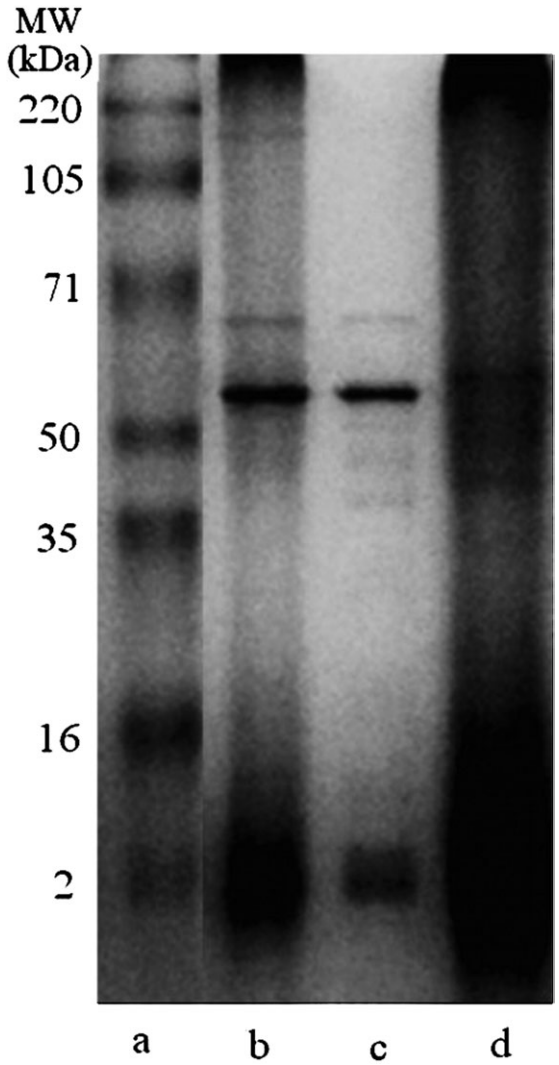


Figure 4. SDS-PAGE patterns of the whey of NRL treated by TG (lane b), TG (lane c), and pure NRL (lane d).

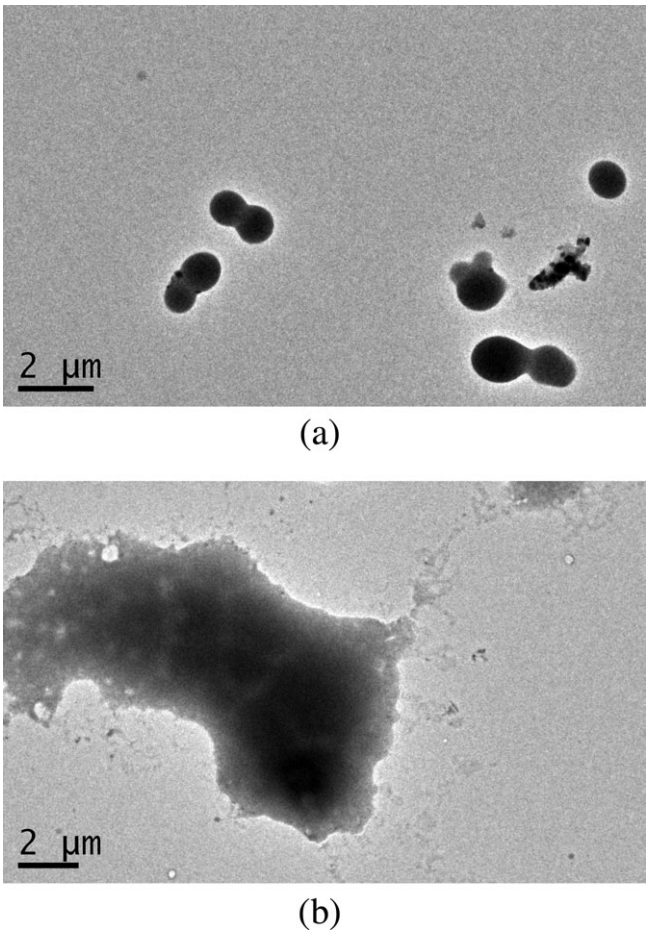


Figure 6. TEM photograph of the pure NRL particles (a) and the NRL particles treated by TG (b).

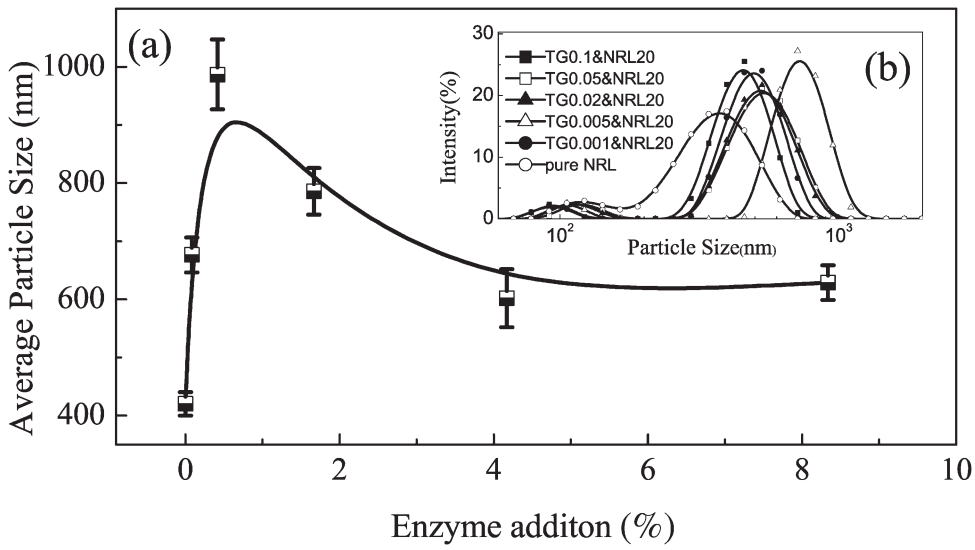
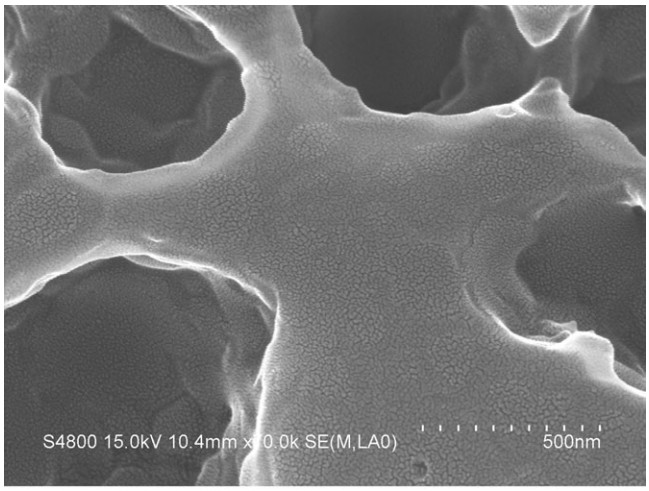
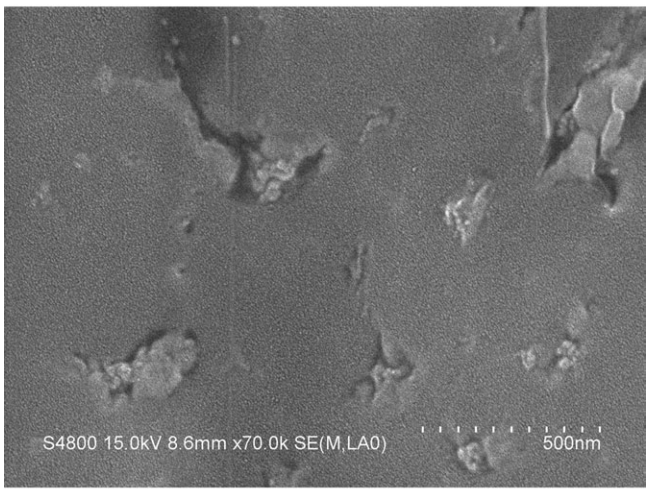


Figure 5. (a) Enzyme addition dependence of average particle size and (b) distribution of particle size.



(a)



(b)

Figure 7. SEM photograph of the pure NRL film (a) and the NRL film treated by TG (b).

make the adjacent rubber particles to range more regular and eliminate the holes between them.

Mechanical Properties

The data of elongation at break and tensile strength with different enzyme addition of NRL films are shown in Figure 8. The elongation at break of the pure NRL films is about 1300.0%, which is just the same as that of the modified films [Figure 8(a)]. The tensile strength of both the pure NRL films and the modified films are almost consistent at 11.0 MPa [Figure 8(b)]. The additive amount of TG in NRL makes actually no difference to the result of the elongation at break and the tensile strength at all. These suggest that the mechanism properties of the modified films are as good as that of the pure NRL films.

CONCLUSIONS

To conclude, we have demonstrated an effective and relatively inexpensive method of reducing the allergenicity of NRL films and promoting the density of it, which can be carried out by

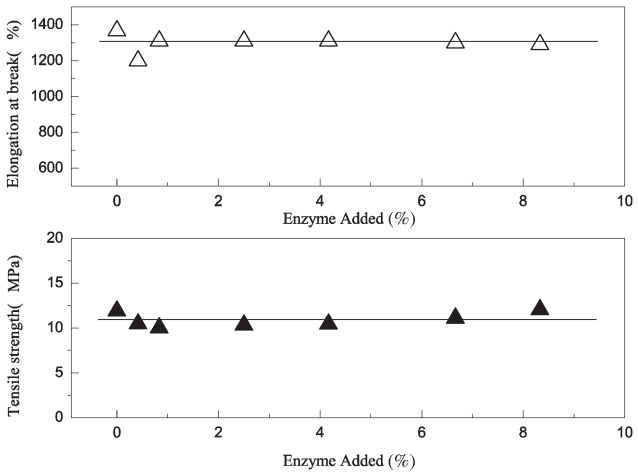


Figure 8. Elongation at break (a) and tensile strength (b) for different enzyme additions of NRL films.

the proteins crosslinking with TG catalysis. In this method, the interpenetrating network can be formed by the crosslinking of the small molecular proteins in NRL, which prevent the WSP traversing from the NRL film effectively. The modified films showed tensile strength and elongation at break values comparable to those unmodified NRL films. This method would help people to obtain low-allergy medical NRL membrane articles with smoothness, comfortableness, and densification.

ACKNOWLEDGMENTS

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