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Immobilization of the proteins in the natural rubber with dialdehyde sodium alginate

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ABSTRACT

The biodegradable dialdehyde sodium alginate (DASA) was exploited to immobilize the proteins in the natural rubber latex (NRL) and the variations of the properties for the NRL films were estimated in detail. As demonstrated, the proteins were distributed more uniformly in the NRL films with DASA and the extractable protein (EP) content was effectively decreased. Particularly, the EP content was lowered to a value about 46 μg/g with 0.40% DASA, which could meet with the demands of the allergy protein threshold limit of 50 μg/g as described in ASTM D 5712 standard. Furthermore, there was some improve on the burial degradability of the NRL films modified with DASA. The mechanical properties, however, had no evident variation in the presence of DASA. In conclusion, the immobilization of the proteins with DASA should be a potential alternative to tackle the protein allergy problem for the NRL and its products.

1. Introduction

Sodium alginate (NaAlg) is a kind of chain macromolecule heteropolymer brought by copolymerization between mannuronic acid (M unit) and guluronic acid (G unit). Therefore, its chemical formula unit as $(C_6H_7NaO_6)_n$ contains free hydroxyl groups (—OH) and sodium ion substituted carboxyl group (—COONa), which results in specific swelling behavior (Maya & Havazelet, 2012) and high chemical reactivity. As we know, NaAlg dissolves slowly in water forming a viscous solution and it is extensively used as stabilizer, thickener and emulsifier. Recently, biodegradable and biocompatible NaAlg and its grafting polymers have been also manufactured into capsules, gels and "nano-in-micro" hybrid particles, which are significant in many industrial or medical applications, such as dehydration of acetic acid (Rao et al., 2006), drug delivery (Jerobin, Sureshkumar, Anjali, Mukherjee, & Chandrasekaran, 2012; Joshi, Keerthiprasad, Jayant, & Srivastava, 2012), oil-absorptive materials (Cheng, Lu, Zhang, Shi, & Cao, 2012) and flocculants (Rani, Mishra, & Sen, 2013).

As reported, *Hevea brasiliensis* tree is the most important source of natural rubber (NR), which has a significant share (more than 40%) in the elastomeric market for decades (Vaysse et al., 2012). Apart from the two major industrial applications as antivibratory parts and tires, natural rubber latex (NRL) has consistently been the most satisfactory raw material for manufacturing health care products like medical gloves. Non-rubber components in a matured commercial NRL concentrate, such as proteins, long-chain fatty acid soaps and polypeptides, are presumed to be distributed in the serum fraction as well as surrounding the rubber particle surface. This can effectively maintain the colloidal stability of NRL during the collection and transport stage, which is implemented by a core–shell structure with a mixed layer of non-rubber components covering the hydrophobic core of the polyisoprene particles (Nawamawat et al., 2011; Sansatsadeekul, Sakdapipanich, & Rojruthai, 2011; Silva et al., 2012).

As mentioned above, proteins are naturally occurring components in NRL and these proteins on the surface of NRL gloves are considered as the allergens (Jezic & Lucas, 2002; Sussman, Beezhold, & Kurup, 2002; Yagami, Sato, Nakamura, & Shono, 1995). Moreover, the extractable protein (EP) content is widely utilized to represent the protein allergens. Up to now, several methods have been developed to reduce the extractable antigenic protein content of NRL or its products. The simplest technology is adequate leaching including post-washing NRL products (Ng, Yip, & Mok, 1994; Rajammal & Thomas, 1978; Yapa & Lionel, 1979; Yapa, 1995), acid treatment (Maznah, Baharin, Hanafi, Azhar, & Hakim, 2008a) and alkali treatment (Maznah, Baharin, Hanafi, Azhar, & Hakim, 2008b). There are also some other attempts to reduce EP by the immobilization of proteins with chloride (Aziz, 1994; Elizabeth, Peethambaran, & Rajammal, 1996) or with poly(ethylene glycol) and hyaluronic acid (Yan, 2000) or with enzyme (Feng, Cheng, Sun, Tian, & Wang, 2012; Perrella & Gaspari, 2002). Although these processes are very effective in reducing EP content by removing, decomposing or cross-linking these proteins, other problems may be introduced,

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such as destabilization of NRL, variations in its coagulation properties and the pollution caused by chemicals. Furthermore, they might add the production cost of standard grades of NRL and NRL products. In a word, in order to minimize the change of the properties of NRL, it is wise to reduce EP content by immobilizing these proteins with a kind of green chemical. In this study, NaAlg was partially oxidized to obtain dialdehyde NaAlg (DASA) and then exploited to modify NRL. As confirmed, the proteins were effectively immobilized and they were distributed uniformly in the inner of NRL films with DASA. Meanwhile, the degradation of modified NRL films had some improve, while the mechanical properties had no evident variation. These results demonstrate that it is a potential technology for reducing EP content in NRL with DASA.

2. Experimental

2.1. Materials

High ammonia centrifuged natural rubber latex (NRL, pH 10.58) from Gold Thai Rubber Co. (Bangkok, Thailand) contained 60.2% dry rubber, 3.9% proteins and lipids, 0.5% resins and 3.0% glycosides. *Sodium alginate* (NaAlg) was purchased from Damao Chemistry Co. (Tianjin, China) with M_w about 315,400 Da and M/G ratio equal to 0.46 for M groups and a fraction of 0.54 for G groups. All other reagents were commercially available. Deionized water was used to prepare solutions.

2.2. Preparation of dialdehyde sodium alginate (DASA)

NaAlg oxidation reaction was carried out at room temperature as reported by Gomez, Rinaudo, & Villar (2007) and Kamal et al. (2001). In a dark bottle, NaAlg (10.00 g) was solubilized with deionized water (600 mL) and then an aqueous solution of *sodium periodate* (100 mL) was added under stirring to make its ratios as 2 mol%, 4 mol%, 6 mol%, 8 mol% and 10 mol% respectively. Next, the reaction mixture was diluted to a final volume of 1 L with deionized water. After 24 h, the reaction was quenched by addition of *ethylene glycol* (3.50 mL) under stirring for 0.5 h. The oxidized alginate was purified by precipitation with addition of *NaCl* (3.00 g) and *ethanol* (1 L). Finally, the precipitation was again dissolved in deionized water (200 mL), dialyzed (*Mw* = 3500) for 48 h and then lyophilized to yield a white product, which was denoted as DASA.

2.3. Characterization of DASA

2.3.1. Aldehyde group content analysis

As speculated, the hydroxyl groups of carbons 2 and 3 of the repetitive unit were oxidized by *sodium periodate* leading to the formation of two aldehyde groups in each oxidized monomeric unit of NaAlg. Therefore, the aldehyde group content in DASA could be used to represent the oxidation degree of NaAlg, which was analyzed by a Schiff base reaction with *hydroxyl groupamine hydrochloride* (Eq. (1)). Then, the aldehyde group content was calculated as Eq. (2) (Kim & Kuga, 2001; Maekawa & Koshijima, 1991).

$$\text{Alginate}\text{—}(\text{CHO})_n + n\text{H}_2\text{N—OH}\bullet\text{HCl} = \text{Alginate}\text{—}(\text{CH=N—OH})_n + n\text{H}_2\text{O} + n\text{HCl} \quad (1)$$

$$[\text{CHO}](\text{mol/g}) = \frac{(0.1 \times 10^{-3} \times V)}{w} \quad (2)$$

where [CHO] is the aldehyde group content in DASA, mol/g; V is the volume of 0.1 mol/L NaOH solution used to neutralized HCl formed during this Schiff reaction, mL; w is the weight of analyzed DASA, g.

2.3.2. IR spectra measurement

Fourier-transformed infrared spectroscopy (FTIR) of DASA was performed with AVATAR-360 FTIR spectrometer (Nicolet/Nexus 670, USA) and recorded from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1} using KBr pellets at room temperature.

2.4. Preparation of NRL films

High ammonia centrifuged natural rubber latex (50 g) was first mixed with an aqueous DASA solution in *ammonia water* and then poured into a Petri dish (diameter as 150 mm), which was horizontally placed at room temperature until the coagulation of the mixture. Next, the mixture was vulcanized at 140 °C for 15 min with 2.5 parts of *sulfur*, 5 parts of *ZnO* as activator, 0.5 part of *sulfenamide* and 0.5 part of *2-mercaptobenzothiazole* as accelerator and 1 part of *N-(cyclohexylthio)phthalimide* as inhibitor (Kumar & Nijasure, 1997) per 100 parts of rubber (phr). Finally, the vulcanization mixture was dried at 50 °C for 48 h to get a film with thickness about 2 mm. The side adjacent to the bottom of the Petri dish was denoted as the bottom side of the film, while the other side was the top side.

2.5. Measurement of the proteins in the films

2.5.1. Aqueous extractable protein (EP) content analysis

The EP content in a latex film was determined according to the standard ASTM D 5712-2005. The data for three measurements were averaged to represent the EP content.

2.5.2. Protein distribution estimation

The distribution of proteins in a NRL film was evaluated by coloring with a dyestuff solution (0.1 g *coomassie brilliant blue R 250*, 450 mL *methanol*, 100 mL *acetic acid* and 450 mL deionized water) for 3 h at room temperature. Then, the unfixed dyestuff on the surface of film was washed away with a decolorizing agent solution (100 mL *methanol*, 100 mL *acetic acid* and 800 mL deionized water) for 12 h. The blue spots on the surface of the dyed specimens corresponded to the existence of proteins, and the bluer color represented more proteins.

2.6. Analysis of the mechanical properties of NRL films

Dumb-bell pieces with the narrow testing part as 25 mm × 4 mm × 2 mm were cut from a NRL film according to GB/T 528-2009 standard and then conditioned as GB/T 2941-2006 method. Next, the tensile strength (TS) and elongation at break (E) of the NRL film were determined at room temperature with a pulling speed as 100 mm/min using a Hounsfield THE 10K-S automated tensile machine (USA) according to GB/T 528 method. The data for three measurements were averaged to represent the mechanical properties of the NRL film.

2.7. Evaluation of indoor soil degradation of NRL films

Degradation of a NRL film was assessed by monitoring the change of film weight with time as described by Goheen & Wool (1991). The experiment was carried out in a series of plastic boxes (500 mm × 200 mm × 100 mm) containing common garden clay soil (Yunnan Normal University, China), which was previously treated with a 0.9 mm(diameter) mesh screening to remove lumps of earth and plant fragments and then adjusted to a relative humidity about 40% with deionized water. Rectangular samples (20 mm × 30 mm) were dried at 50 °C to get a constant initial weight (w_0). These samples were vertically buried at a depth of 20 mm from

the soil surface with the horizontal spacing as 10 mm. The relative humidity of the soil was maintained around 40% during the whole 35-day burial degradation trial.

Samples were removed from the soil every 5 days (t) and carefully cleaned with deionized water. Next, the washed specimens were dried at 50 °C to a constant weight (w_t). The weight loss (%, $w_{l,t}$) was calculated as the following Eq. (3):

$$w_{l,t}(\%) = \frac{(w_0 - w_t)}{w_0} \times 100\% \quad (3)$$

where w_0 and w_t were the dry weight of the latex film specimen before and after degradation respectively, g; $w_{l,t}$ is the weight loss for the degraded sample, %; t is the buried time, day.

3. Results and discussions

3.1. Existence of aldehyde group in DASA

The carbon(2)-carbon(3) bond in the repetitive unit of NaAlg might be broken to form two aldehyde groups (—CHO) in the presence of sodium periodate. Therefore, the existence of —CHO in oxidized NaAlg (DASA) was characterized with both FTIR spectra (Fig. 1) and the —CHO content (Table 1). As shown in Fig. 1, the peaks in FTIR (KBr pellet, cm^{-1}) for the 2 mol% oxidation of NaAlg (2% DASA) were as following: 3390, 2930, 1730 ($\nu_{C=O}$), 1600, 1410, 1310, 1040. Especially, the appearance of peak at 1730 cm^{-1} confirmed the forming of aldehyde group in the 2% DASA, which correlated well with the FTIR findings for the existence of aldehyde group in oxidized NaAlg reported by Kamal et al. (2001). What is more, similar peaks were found in the FTIR spectra for the other oxidation of NaAlg (not shown). Table 1 displayed that the —CHO content in DASA increased with the increasing dosage of sodium periodate, which was analogous to the results reported by Gomez et al. (2007).

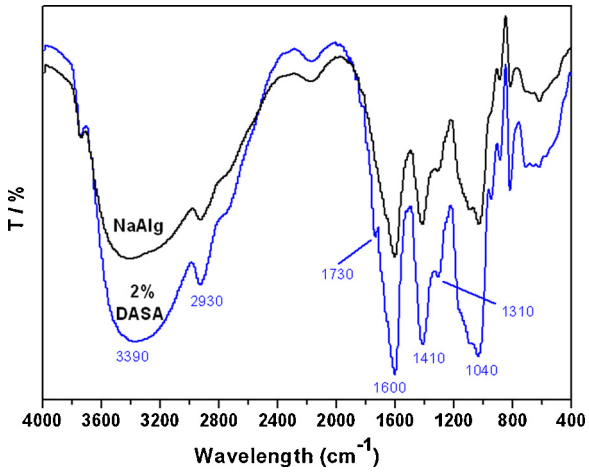


Fig. 1. FT-IR spectra of natural sodium alginate (NaAlg) and 2 mol% oxidation (2% DASA).

3.2. Proteins in latex films

Fig. 2 suggested that the extractable protein (EP) content was decreased with both the rise of DASA content in NRL films (Fig. 2(a)) and the increase of the CHO content in DASA (Fig. 2(b)). Moreover, it fell to a value lower than 50 μg/g with 0.40% DASA ([CHO] content as 0.85 ± 0.07 mmol/g) in Fig. 2(a) or with 0.09% DASA ([CHO] content as 3.84 ± 0.10 mmol/g) in Fig. 2(b), which could meet with the demands of the protein allergy threshold limit as described in ASTM D 5712-2005. Furthermore, the EP content dropped to zero in the presence of 1.20% DASA (seen in Fig. 2(a)) indicating that the proteins in the NRL were almost immobilized with DASA. In a word, the level of EP content was expected to be correlated to the total aldehyde group content in the NRL films and the lower EP content was detected with the higher aldehyde group content.

Fig. 3 displayed the surface of the rubber films after coloring. As for the natural rubber latex film ('NRL film'), the blue color was only found on the top side. An explanation was that the proteins were prone to migrate to the top side of lower surface tension during the formation of film as compared with the other five sides, which was similar to the findings provided by Frank & Gaspari (2002). The proteins, however, were distributed on all the six faces (top, bottom and four lateral sides) of the films containing DASA as confirmed by the existence of the blue color. That is, the migration of proteins

Table 1
The aldehyde group content in the oxidized NaAlg (DASA).

	Mole ratio of sodium periodate to NaAlg (mol %)				
DASA	2	4	6	8	10
[CHO] content (mmol/g)	0.85 ± 0.07	1.78 ± 0.10	2.77 ± 0.12	3.84 ± 0.10	4.79 ± 0. 12

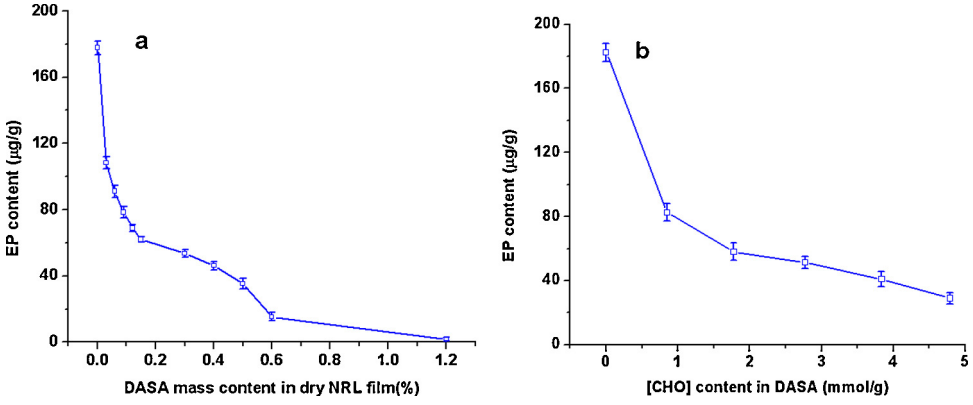


Fig. 2. The extractable protein (EP) content in the natural rubber latex (NRL) film modified with dialdehyde sodium alginate (DASA). (*a*, DASA mass content was as 0, 0.03%, 0.06%, 0.09%, 0.12%, 0.15%, 0.30%, 0.40%, 0.50%, 0.60% or 1.20% and its [CHO] content was 0.85 ± 0.07 mmol/g; *b*, DASA mass content was 0.09% and its [CHO] content was 0.85 ± 0.07 mmol/g, 1.78 ± 0.10 mmol/g, 2.77 ± 0.12 mmol/g, 3.84 ± 0.10 mmol/g or 4.79 ± 0.12 mmol/g respectively).

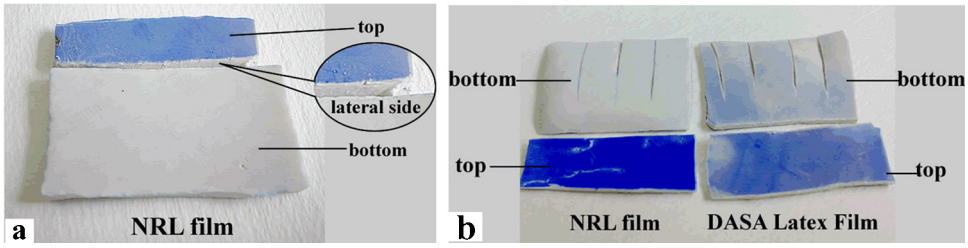


Fig. 3. NRL films colored with a *coomassie brilliant blue 250* solution. ('NRL film' and 'DASA Latex Film' represented the natural rubber latex film and the film containing 0.40% DASA with its aldehyde group content as 0.85 ± 0.07 mmol/g).

Table 2
The weight loss of NRL films with different DASA contents during the burial trial.

DASA content (%)	Weight loss (%)						
	0 day	5 days	10 days	15 days	20 days	25 days	35 days
0	0	0.21 ± 0.08	2.10 ± 0.25	2.88 ± 0.26	4.14 ± 0.25	4.83 ± 0.32	4.95 ± 0.35
0.09	0	0.45 ± 0.09	2.48 ± 0.23	3.18 ± 0.28	4.38 ± 0.28	5.20 ± 0.36	5.87 ± 0.36
0.40	0	0.55 ± 0.17	2.64 ± 0.29	3.43 ± 0.32	4.62 ± 0.32	5.69 ± 0.29	8.72 ± 0.38
1.20	0	0.62 ± 0.22	2.90 ± 0.40	3.66 ± 0.32	5.16 ± 0.43	6.80 ± 0.34	10.23 ± 0.26

to the top side was effectively inhibited resulting in more uniform distribution in 'DASA Latex film'. These phenomenon displayed that the proteins were evidently immobilized with DASA, which was also proved by the decrease of EP content (seen in Fig. 2).

3.3. Mechanical characteristics

The tensile strength and the elongation at break for the NRL films with DASA were shown in Fig. 4(a) and (b) The mechanical properties had some change with the variation of the total aldehyde group content in the films brought by DASA, but the increment was lower than 3%. Moreover, the tensile strength and the elongation at break for all the films containing DASA were higher than 25 MPa and 750% respectively, which could meet with the demands of rubber gloves as required by GB 7543-2006 standard and GB 24787-2009 standard. As a result, there is little impact of DASA on the mechanical properties of the latex films.

3.4. Burial degradation of latex films

The weight loss results for the NRL films during the burial trial were indicated in Table 2. The weight loss increased with both the content of DASA and the prolonged buried time. Furthermore, the weight loss reached to a value as high as 10.3% for the film containing 1.20% DASA after 35-day bury, which was much higher than that for the NRL film without DASA. These data suggested that the degradation of NRL film was enhanced with DASA.

During the bury degradation trial, molds were observed on the surface when the film was pull out from the soil and the district corresponding to the growing region of the molds would get black after drying. As displayed in Fig. 5(a), more molds were propagated on the surface of the film with higher DASA content leading to larger holes. Similarly, Fig. 5(b) suggested that the more molds and holes were found with the prolonged buried time. That is, the degradation degree was enhanced with the increasing DASA content and the prolonged buried time, which was consistent to the variation of the weight loss of films shown in Table 2.

3.5. Discussions for the immobilization of proteins

As demonstrated by FTIR analysis, aldehyde groups were formed by the rupture of C(2)–C(3) bond in the repetitive unit of NaAlg in the presence of sodium periodate. As displayed in Fig. 6, these reactive aldehyde groups may be reacted to the amino group (in the form of $-NH_2$) of the proteins in the latex (pH 10.58) by a beta-elimination reaction. As pointed out, NaAlg used to prepare DASA is a polysaccharide with *Mw* about 315,400 Da. Thus, the molecule weight would have a sharp rise for the cross-linked proteins, leading to evident decrease of water solubility and migration ability. That is, the proteins were effectively stabilized with DASA, which was further confirmed with both the decrease of EP content and the more uniform distribution of the proteins in the NRL films. Specially, EP in the film with few DASA (i.e. with 0.40% DASA containing

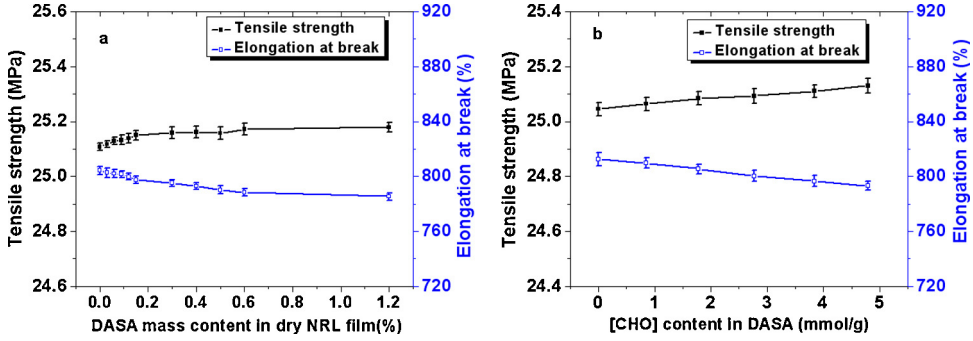


Fig. 4. The mechanical properties of the NRL films with DASA. (*a*, DASA mass content was as 0, 0.03%, 0.06%, 0.09%, 0.12%, 0.15%, 0.30%, 0.40%, 0.50%, 0.60% or 1.20% and its [CHO] content was 0.85 ± 0.07 mmol/g; *b*, DASA mass content was 0.09% and its [CHO] content was as 0.85 ± 0.07 mmol/g, 1.78 ± 0.10 mmol/g, 2.77 ± 0.12 mmol/g, 3.84 ± 0.10 mmol/g or 4.79 ± 0.12 mmol/g respectively).

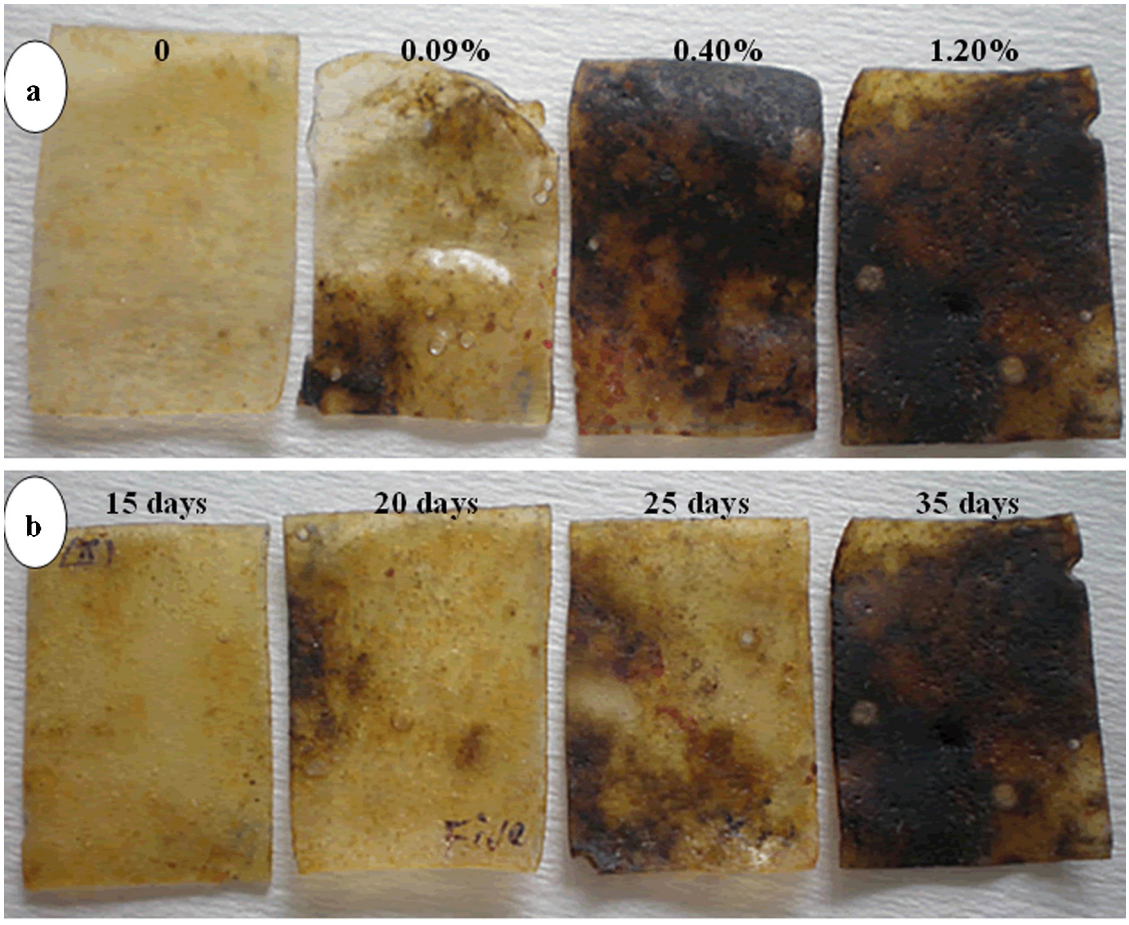
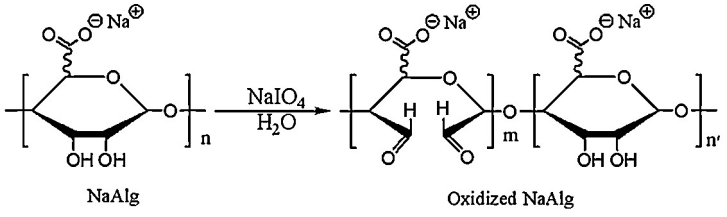


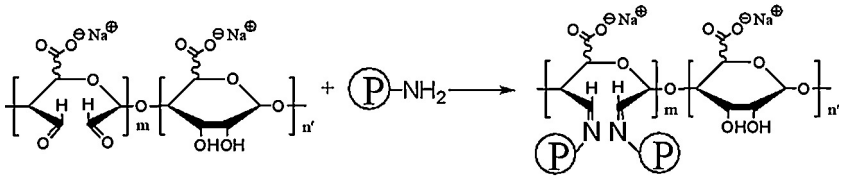
Fig. 5. The photographs for the NRL films with DASA containing 0.85 ± 0.07 mmol/g aldehyde group during the burial degradation trial. (*a*, the mass content for DASA was as 0, 0.09%, 0.40% and 1.20% in the NRL film and the buried time was as 35 days; *b*, the mass content for DASA was as 1.20% and the buried time was as 15 days, 20 days, 25 days and 35 days).

0.85 ± 0.07 mmol/g aldehyde group) was reduced to a value lower than 50 μg/g, which would meet with the demands of the protein allergy threshold limit as required by ASTM D 5712-2005. In conclusion, considering the preparation and usage for DASA and its dosage as described in Section 2, the reducing in the allergic proteins with DASA would not evidently add costs to the production of NRL products.

As reported by Kamal et al. (2001), NaAlg is a kind of polysaccharide easy to be broken down by the microorganisms. DASA, as an oxidized alginate, is also helpful for the propagation of molds. As displayed, more holes were formed for the films containing DASA accompanying with higher weight loss, which may be due to the more uniform distribution of proteins and the degradation of both the proteins and the cross-linked DASA. This can further enlarge the



(a) oxidation of NaAlg forming aldehyde groups on the main chain



(b) reaction between oxidized NaAlg (DASA) and proteins

Fig. 6. Schematic diagram of the reaction between DASA and proteins in the NRL film.

effective contact between the molds propagated in the burial process and the rubber solid component, resulting in the enhancement of the degradation of the rubber component. Furthermore, it should be pointed out that the DASA modified natural rubber maintained excellent mechanical properties required for medical gloves. In a word, the reduction in the allergic proteins with DASA would not result in pollution and evident variation of the properties of NRL, and it is a promising alternative for NRL and its products.

4. Conclusions

In this study, a novel solution to immobilize the proteins in latex films was proposed by adding dialdehyde sodium alginate (DASA). The proteins were distributed more uniformly and the aqueous extractable protein (EP) content was lower in the films with DASA as compared with the natural rubber latex (NRL) films. Moreover, the EP content in the film containing 0.40% DASA (aldehyde group content about 0.85 mmol/g) was reduced to a value lower than 50 μg/g, which could meet with the demands of the protein allergy threshold limit as required by ASTM D 5712-2005. Furthermore, there was some improvement in the burial degradability, but little impact on the tensile strength and the elongation at break with the addition of DASA. In a word, the protein immobilization with DASA should be a potential and green option to solve the protein allergy problem for NRL and its products.

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