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Abstract

The protein allergy caused by latex products has become an acute problem to human and animals; sweat removes proteins, which then comes in contact with the skin causing the sensitization or allergic reactions. Eggshells from chickens, ducks, and birds are potential starting materials to be used in reducing the water extractable proteins in concentrated natural rubber latex (NRL). The pyrolysis of eggshells at 900°C for 2 h transforms the crystal structure of calcium carbonate into calcium oxide with a high purity, creates a small and uniform particle size, a suitable specific surface area, and an average pore diameter that is suitable for reacting with hydrochloric acid to obtain calcium chloride (CaCl₂). The additions of CaCl₂ solution and sodium dodecyl sulphate into concentrated NRL are shown and reported here to extract effectively the proteins from the NRL film.

Keywords

Concentrate rubber latex, crude eggshells, extractable proteins, modified Lowry method

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Introduction

Natural rubber latex (NRL) is one of the most important biosynthesized polymers displaying excellent chemical and physical properties that find a wide range of application in various areas.^{1–3} NRL has been used in manufacturing medical products such as medical gloves, condoms, balloons, blood transfusion tubing, catheters, injector closures, and safety bags due to its excellent elasticity, flexibility, antiviral permeation, good formability, and biodegradability.^{1,4} Rubber latex, from field collection, contains many natural substances including proteins and 33% polyisoprene. Ammonia is usually poured to the latex as a preservative to promote the alkalinity (pH) and to retard the microbial growth. The additional benefit from adding ammonia is the increase in the stability of NRL due to the increase in the negative surface charge of the rubber particles.⁵ In addition, the alkaline conditions of ammoniated latex can denature some proteins. In addition, it may even partially hydrolyze others to smaller polypeptides. The resulting NRL is further processed to concentrate latex with approximate 60% rubber content. Nonrubber material contents such as proteins are removed during this processing step. NRL proteins are organic substances that consist of carbon, hydrogen, nitrogen, oxygen, and sulfur. There are naturally occurring polymer chains that may contain hundreds of individual amino acid residues connected together by peptide bonds. Some of these proteins are considered potential allergens based on their capacity to bind to immunoglobulin E antibodies from the sera of NRL-allergic people. It is generally believed that the potentially allergic protein components are those accompanied with the aqueous soluble serum fraction of NRL. These are the proteins that are dissolved in the latex serum and tend to concentrate during product manufacturing on the film surface.⁵ Latex allergy symptoms are the result of a chain reaction that starts in the immune system. The immune system identifies what is typically harmless as an invader or allergen. The latex allergic reaction usually appears in the nose, lungs, throat, sinuses, ears, lining of the stomach, or on the skin. People with latex allergy may have symptoms such as urticaria or hives, itching or flushing, swelling, sneezing, runny nose, cough, wheeze, shortness of breath, chest tightness, nausea, dizziness, or lightheadedness, as shown in Figure 1.

There are many methods to reduce the protein content in NRL gloves: water leaching, chlorination, enzyme-treated NRL, and adding powder lubricants (i.e. silica, modified montmorillonite, starch, and ultra-fine calcium carbonate (CaCO_3)), as shown in Figure 2.

Water leaching removes hydrophilic components, reduces the formation of chemical blooming on the surface during heating and storage, reduces hydration and electrical conductivity, and reduces water-soluble components and proteins in NRL gloves; but it is not effective in leaching out larger proteins within the coagulated film. Chlorination is an effective means of reducing the extractable protein (EP) content on the glove surface, but the chlorination process is difficult to control and usually results in small fissures in the rubber film that can compromise the shelf-life and the barrier integrity of the glove.^{6–8} Protease enzyme-treated NRL is usually used in the leather manufacturing and the NRL



Figure 1. Latex glove allergy (<http://www.iacdworld.org>).

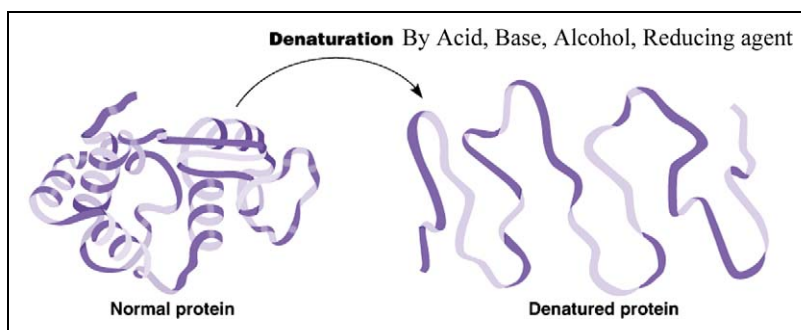


Figure 2. Protein denaturation.

device production.⁹ There are several standard methods for measuring the proteins in the concentrated NRL, such as the American Society for Testing and Materials (ASTM) Standard Method D 5712 and the International Organization for Standardization 12243 (ISO 12243).¹⁰

The objective of this work is to extract or remove the water-soluble proteins or EPs from concentrated rubber latex compounds, in order to reduce the rubber latex allergic problem, by adding calcium chloride (CaCl_2) prepared from the pyrolysis of crude eggshells, such as chicken eggshells, duck eggshells, and bird eggshells. Eggshells were pyrolyzed at 900°C for 2 h to obtain calcium oxide (CaO) and then dissolved in 2 M hydrochloric acid (HCl) along with sodium dodecyl sulphate (SDS) as the stabilizer. The method used to determine the EPs is the modified Lowry method (ISO 12243). The amount of EPs, using CaCl_2 made from eggshells, was compared with the amount of EPs using CaCl_2 made of CaO and CaCl_2 (commercial grades) of the same weight (0.25 g) by the modified Lowry method. The rubber latex compounds and latex films were characterized by Fourier transform infrared (FTIR), near infrared (NIR), and x-ray diffraction (XRD) and are reported here.

Experimental

Materials

Concentrated NRL (60% dry rubber content (DRC) and concentrated high ammonia latex) was supplied by the Rubber Research Institute of Thailand (Bangkok, Thailand).

CaO from eggshells (chicken, duck, and bird) was prepared from pyrolysis of eggshells. The eggshells (chicken, duck, and bird) were collected from a local cafeteria. Crude eggshells were washed with tap water and dried at room temperature. The crude eggshells were crushed with a porcelain mortar. The CaO was successfully prepared by the pyrolysis technique at 900°C for 2 h, yielding a very high purity product of 97.41 wt% (bird), 98.15 wt% (chicken), and 99.00 wt% (duck), and then kept in a desiccator.

SDS (also NaDS or $C_{12}H_{25}SO_4Na$) is an anionic surfactant with a density, melting point, and molecular weight of 1.01 g cm⁻³, 206°C, and 288.38 g mol⁻¹, respectively. The SDS compound activates in the mechanism of disrupting noncovalent bonds in the proteins, denaturing them, and causing the molecules to lose their native shape (conformation). Also, anions of SDS bind to the main peptide chains at a ratio of one SDS anion for every two amino acid residues. This effectively imparts a negative charge on the protein that is proportional to the mass of that protein (about 1.4 g SDS g⁻¹ sprotein).¹⁰ This obtained negative charge is significantly greater than the original charge of the proteins. The electrostatic repulsive interaction created by binding of SDS resulted in the proteins to unfold into a rod-like shape thereby eliminating differences in shape factor in the separation of the gel.¹¹

CaCl₂ with a higher purity of 93.0–100.0 wt% and CaO of 96 wt% purity, water-insolubility <0.5 wt%, loss on ignition 8.79 wt%, oxalate 5 wt%, and other element impurities of <0.0043 wt% was supplied by Ajax Finechem Pty. Ltd (Australia). HCl (Analytical Reagent grade, AR) was purchased from Lab-Scan Co. Ltd (Thailand).

Instruments

Laboratory Muffle Furnace. Laboratory Muffle Furnace (Linn High Thermo GmbH, LM 412.27, model DC021032 with thermocouple type K, NiCr-Ni) was used to calcine eggshell samples from 27 to 900°C to obtain CaO. The raw eggshells were put in an alumina crucible in the muffle furnace and calcined at 900°C for 2 h with a heating rate of 10°C min⁻¹.

The elemental analysis. Elemental analysis was carried out on a C/H/N analyzer (Perkin Elmer, model PE 2400 series II). The gaseous samples were freed by the pyrolysis in high-purity oxygen and were chromatographically separated by the frontal analysis with quantities detected by a thermal conductivity detector.

Fourier transform infrared spectroscopy. FTIR was used to investigate functional groups by a PerkinElmer, model Spectrum One spectrometer with a spectral resolution of 4 cm⁻¹. The samples were measured by mode of the single-crystal potassium bromide, KBr.



Figure 3. Open cup of near infrared spectroscopy.

NIR spectroscopy. NIR spectroscopy (BRAN&LUEBBE, InfraAlyzer 500) was used to study the EP spectra of the concentrated latex compounds in the region of 1100–2500 nm or the wave number $12,500\text{--}4000\text{ cm}^{-1}$. The samples were prepared within the open cup as shown in Figure 3.

The samples of the concentrated latex compounds were scanned in the reflectance mode with the NIR spectroscopy in the NIR region from 1100 to 2500 nm at 2 nm increment. The samples were packed in the standard open cup and each sample was repacked and scanned three times for the average value. The NIR absorbance spectra were stored for subsequent development of the calibration equation with the reference, that is, the EP data.

X-Ray fluorescence. XRF was used to determine the chemical compositions: Philips, model PW 2400 was used with the tube current of 1000 μA and an acquisition lifetime of 30s.

X-Ray diffraction. XRD was used to identify crystalline characteristics that were consistent with those of the International Center for Diffraction Data Standard (JCPDS) patterns. Bruker AXS analyzer (D8 Discover) was model used, which is incorporated with a Vantec-1 detector and a double-crystal wide-angle goniometry. Measurement of scanning was in the range of $5^\circ\text{--}80^\circ$ 2θ under 5° $2\theta\text{ min}^{-1}$ of scan speed in 0.05° or 0.03° 2θ increments, using CuK_α radiation ($\lambda = 0.15406\text{ nm}$).

Transmission electron microscope. TEM (JEM-2100) equipped with energy dispersive x-rays spectroscopy (EDS) for x-ray microanalysis and nano beam diffraction (NBD) was used to study microstructures. The TEM sample preparation of the crude eggshells and

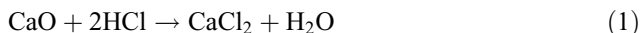
calcined eggshells was done on a grid to investigate the morphology and microstructure. An acceleration voltage of 80–200 kV with the magnifications from 50 to 1.5 million times of TEM were used.

Gas pycnometer. Gas pycnometer (Quantachrome, Ultra pycnometer 1000) was used to determine the apparent density of samples.

Autosorb-1. Autosorb-1 (Quantachrome) was utilized to investigate the porosity characteristics in terms of the specific surface area, the adsorption and/or desorption isotherms, the pore size, and the surface distribution. The quantity of gas adsorbed onto or desorbed from the solid surface at some equilibrium vapor pressures was measured in the static volumetric method. The measured absorbed or desorbed volumes of nitrogen gas were investigated in the range of 10^{-6} to slightly below 1.0 of relative pressures using krypton and the micropore options. The Brunauer–Emmet–Teller (BET) surface area (single and/or multipoint), the Langmuir surface area, the adsorption and/or desorption isotherms, the pore size and surface area distributions, the micropore volume, and the surface area were calculated via Autosorb-1 software.

Preparation of CaCl_2 solution and concentrated latex film samples

CaCl_2 solution preparation. CaCl_2 solution was made of dissolving 4.71 g of CaO powders from the calcinations of the three kinds of crude eggshells (chicken, duck, and bird) at 900°C for 2 h in 2 M of HCl as shown in equation (1). After dissolving CaO powders in 2 M of HCl, a clear solution ($\text{pH} \sim 2$) was obtained.



Concentrated rubber latex films preparation. The formulae of concentrated rubber latex films are shown in Table 1. CaCl_2 of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 g was used to prepare solutions according to CaCl_2 Solution Preparation section according to equation (1), then mixed with 10 wt% SDS as a stabilizer, which was added into the concentrated latex. The concentrated latex compounds were stirred at 1300 r min^{-1} for 30 min. The concentrated NRL compounds were cast onto the plastic Petri dishes, dried at room temperature for 24 h, and kept in a dessicator. The other parts of concentrated latex compounds were poured into the sample cells of FTIR and NIR spectroscopy to determine the chemical functional groups including peptide and amino acid spectra of proteins.

Chemical analysis. The EPs of the concentrated latex compounds in the film samples were determined by the modified Lowry method (International Organization for Standardization 12243 (ISO 12243)). In general, the modified Lowry method was used for the determination of water EP in the medical gloves made from NRL. The measurement was repeated four times for each sample and the measured values were averaged. The precipitated protein was redissolved and quantified colorimetrically by the modified Lowry method using a protein standard.

Table 1. Formulation of concentrated natural rubber latex compounds for films preparation.

Compositions	Dry weight (g)	Wet weight (g)
Concentrated latex, 60% HAL	100	167
10% SDS ^a	20	200
4.71 g CaO in 2 M HCl	0.25	2.41
	0.50	4.83
	0.75	7.24
	1.00	9.66
	1.25	12.07
	1.50	14.49
	1.75	16.90
	2.00	19.32

^aSDS is sodium dodecyl sulphate, an anionic surfactant.

NRL film samples preparation for the EP testing by the modified Lowry method. The cast film was cut into smaller and rectangular pieces and the mass of each sample (M_p) was recorded with an accuracy of approximately 1.5 ± 0.001 g.

Determination of the EPs by the modified Lowry method

Preparation of the pure protein concentration precipitate for the modified Lowry method. According to ISO 12243 (medical gloves made from NRL—determination of water EP using the modified Lowry method), the reagent A (alkaline copper citrate), the reagent B (dilute Folin reagent prepared by diluting 72 cm³ of 2 N Folin reagent with 28 cm³ of water), the reagent C (a solution of 6 g of sodium carbonate in 100 cm³ of water), the reagent D (a solution containing 1.5 g of copper sulfate and 3 g of sodium citrate in 100 cm³ of water), the sodium deoxycholate solution, and the trichloroacetic acid solution were prepared. The rubber latex films were cut in small pieces in a rectangular shape, and the mass (M_p) of each sample was determined. The rubber latex film sample was extracted at $25 \pm 5^\circ\text{C}$ for 120 min, followed by shaking for 15s. The extract was decanted and removed of any particulate matters by centrifuging for 15 min. The extract was stored for 48 h at a temperature below 7°C , and the molecular weight (M_w) was measured. The protein concentration precipitate was divided and put into four sample tubes for testing by the ultraviolet (UV) spectrophotometer, which calculated and reported the average EP values.

According to the modified Lowry method, the cast film pieces were put into a conical flask, the distilled water of 20 ml was added, then the sample flasks in a water bath were shaken at 37°C for 5 min. The shaking water bath was set at the speed of 200 r min^{-1} for 1 h to extract the water soluble proteins. Water soluble proteins were poured out of the conical flask into a 50-ml plastic tube and the weight of water in the plastic tube (M_w) was measured. The plastic tube was put in a centrifuge with the high speed of 3000 r min^{-1} for 15 min. The protein concentration precipitate was divided into four

Table 2. Chemical composition analysis of crude eggshell.

Compounds	Raw chicken eggshells (wt%)	Raw duck eggshells (wt%)	Raw bird eggshells (wt%)
Na ₂ O	—	<0.1	0.23
MgO	—	0.10	1.12
SiO ₂	—	0.01	0.04
P ₂ O ₅	0.80	0.46	1.19
SO ₃	1.79	0.96	0.98
Cl	—	—	0.06
ZrO ₂	—	0.02	—
K ₂ O	0.21	0.20	0.11
SrO	779 ppm	0.02	0.02
CaCO ₃	96.46	96.35	96.23

—: compounds that cannot be measured.

sample tubes for testing by a UV spectrophotometer that calculated and reported the average EP values.

Calculation of the extractable protein contents. The EP contents were determined from equation (2) as follows

$$\frac{C \times M_w}{M_p \times V} \quad (2)$$

where C is the absorbance from a UV spectrophotometer or the protein concentration in the redissolved protein solution, in micrograms per cubic centimeter ($\mu\text{g cm}^{-3}$), M_w is the mass in grams of the distilled water, M_p is the mass in grams of a rectangular piece of the cast film, and V is the volume of the extractant used, in cubic centimeters (cm^3).

Results and discussion

Physical and chemical characteristics of CaO made from chicken, duck, and bird eggshells

The chemical compositions of crude chicken, duck, and bird eggshells by XRF are tabulated in Table 2. Most of the crude eggshells contain approximately 96 wt% of CaCO₃ and the rest belonging to other oxide compounds: Na₂O, MgO, SiO₂, K₂O, SrO, and ZrO₂. Table 3 shows the organic compositions of the crude eggshells from chicken, duck, and bird as measured by the elemental analyzer. The data of specific surface area, the average pore diameter, the true density, and the average particle size of CaO are tabulated in Table 4. The specific surface area and average pore diameter values of CaO made from the chicken eggshells are $7.79 \text{ m}^2 \text{ g}^{-1}$ and $17 \text{ \AA}$, respectively, which are close to the values of the duck eggshells of $7.70 \text{ m}^2 \text{ g}^{-1}$ and $17.69 \text{ \AA}$, respectively. The specific surface area and average pore diameter of CaO from the bird eggshells are $3.47 \text{ m}^2 \text{ g}^{-1}$

Table 3. Organic composition of crude eggshells as measured by elemental analyzer (Perkin Elmer PE2400 SeriesII).

Element	Crude chicken eggshells (%wt)	Crude duck eggshells (%wt)	Crude bird eggshells (%wt)
C	14.001 $\pm$ 0.345	13.640 $\pm$ 0.230	14.270 $\pm$ 0.110
H	0.097 $\pm$ 0.025	0.490 $\pm$ 0.050	0.850 $\pm$ 0.077
N	0.145 $\pm$ 0.031	0.630 $\pm$ 0.100	1.170 $\pm$ 0.023

Table 4. Physical characterization of calcium oxide made from eggshells calcined at 900°C for 2 h.

Raw materials	Specific surface area (m ² g ⁻¹)	Average pore diameter (Å)	True density (g cm ⁻³)	Average particle size (micron)
CaO powder from chicken	7.79	17.00	2.16	2.00
CaO powder from duck	7.70	17.69	2.84	12.12
CaO powder from bird	3.47	82.99	2.88	12.44

and 82.99 Å, respectively. CaO here possesses the pore diameter in the range of mesopore: 20 Å–500 Å (2–50 nm). True density values of CaO of the chicken, duck, and bird eggshells are 2.16, 2.84, and 2.88 g cm⁻³, respectively. The average particle size of CaO of the chicken, duck, and bird eggshells are 2.00, 12.12, and 12.44 µm, respectively. The particle size of CaO is very small, which is suitable for using as filler or dispersed phase in concentrated NRL.

TEM micrographs of CaO from chicken (Figure 4(a) and (a1)), duck (Figure 4(b) and (b1)), and bird (Figure 4(c) and (c1)) eggshells are shown in Figure 4 with the magnifications of 25,000 $\times$ and 12,000 $\times$. The TEM micrographs show uniformly particle size distribution and an agglomeration due to fine particles, consistent with the results obtained from the particle size measurement.

FTIR spectra of CaO made from the crude chicken, duck, and bird eggshells show the same peak positions at 3643 cm⁻¹ strong ν (O–H), 3435 cm⁻¹ ν (O–H), 1630 cm⁻¹ strong ν (C=O), and 500–580 cm⁻¹ ν (Ca–O), as shown in Figure 5.

Properties of concentrated rubber latex

The properties of concentrated rubber latex are tabulated in Table 5, where the data were obtained following the ISO2004-1997. The properties of the concentrated latex compounds measured are the total solid content (ISO124); the DRC (ISO126); the nonrubber solids per rubber; the alkalinity content (ISO125); the volatile fatty acid number (ISO 506); the KOH number (ISO127); the pH values (ISO976); the specific gravity (ISO705); the mechanical stability (ISO35); and the apparent viscosity by the Brookfield test (ISO1652), according to the International Organization for Standardization.

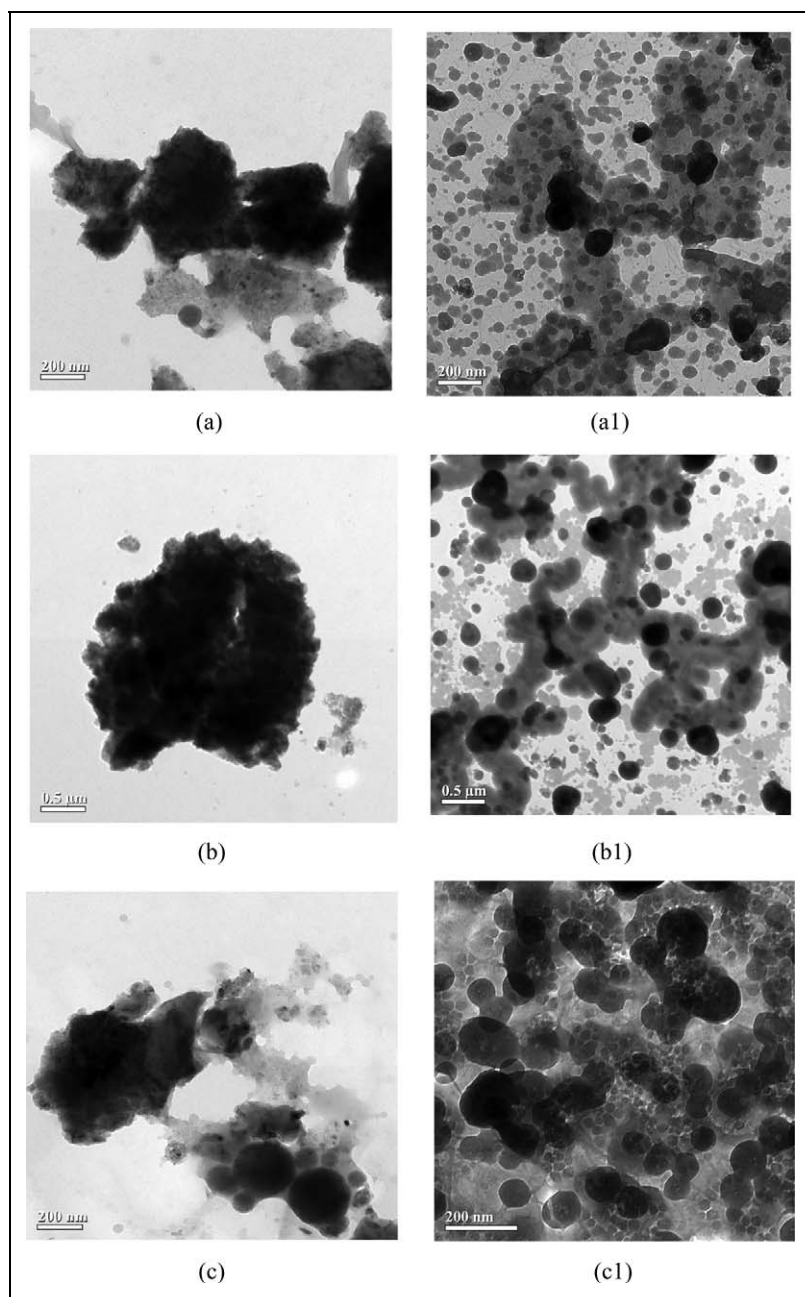


Figure 4. Transmission electron micrographs of eggshells with the magnifications of $\times 25,000$ and $\times 12,000$. (a) Crude chicken eggshells; (a1) chicken eggshells calcined at 900°C for 2 h; (b) crude duck eggshells; (b1) duck eggshells calcined at 900°C for 2 h; (c) crude bird eggshells; and (c1) bird eggshells calcined at 900°C for 2 h.

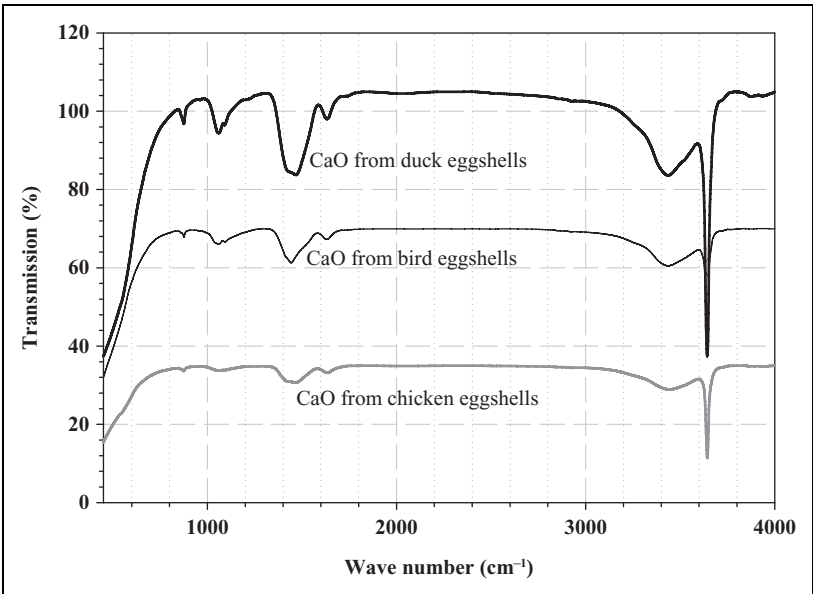


Figure 5. Fourier transformation infrared spectra of calcium oxide obtained from chicken, duck, and bird eggshells calcined at 900°C for 2 h.

Table 5. Properties of concentrated natural rubber latex compounds.

Properties	Concentrated latex	ISO 2004-1997	References
TSC per rubber weight (%)	61.86 ± 0.11	>61.50	ISO 124
DRC per rubber weight (wt%)	59.97 ± 0.10	>60.00	ISO 126
Non rubber solids per rubber weight (wt%)	1.89 ± 0.01	<2.00	–
NH ₃ per rubber weight (wt%)	0.53 ± 0.08	>0.60	ISO 125
NH ₃ per water weight (wt%)	1.38 ± 0.22	–	–
VFA number	0.050 ± 0.009	<0.20	ISO 506
KOH number	0.78	<1.00	ISO 127
pH	10.05 ± 0.09	–	ISO 976
Specific gravity	0.948 ± 0.001	–	ISO 705
MST (s)	852 ± 39	>650	ISO 35
Viscosity spindle no. I		–	ISO 1652
Speed 6 r min ⁻¹ (cps)	186 ± 62		
Speed 30 r min ⁻¹ (cps)	111 ± 37		
Speed 60 r min ⁻¹ (cps)	90 ± 27		

TSC: total solid content; DRC: dry rubber content; VFA: volatile fatty acids; MST: mechanical stability time.

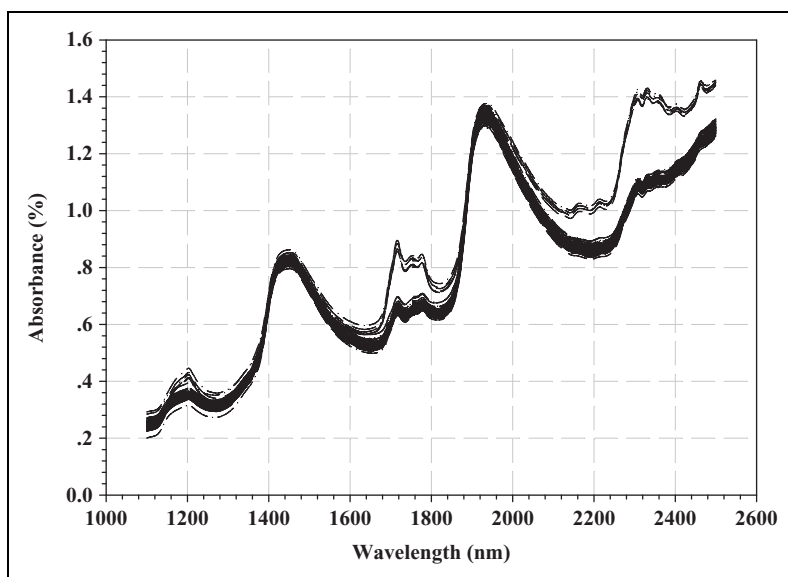


Figure 6. Near infrared spectroscopy spectra of natural rubber latex compounds added calcium chloride and sodium dodecyl sulphate.

Quality and quantity of EP removed from concentrated NRL surface films with the addition of CaCl_2 and SDS

NIR spectra of the concentrated latex compounds added with CaCl_2 solution and the stabilizer (SDS) in liquid state were measured in the region of 1100–2500 nm ($12,500\text{--}4000\text{ cm}^{-1}$), as shown in Figure 6. The characteristic peaks indicate the percentages of absorbances at the wavelengths of 1520 and 1980 nm, which are related to the EP contents in terms of peptide or amino acid of the first overtone N–H ($\nu_1\text{N-H}$) and the asymmetric combination N–H, respectively, as previously reported by Osborne and Fearn¹² and Murray and William.¹³ Furthermore, the absorbance peaks at the wavelengths of 1450 and 1920 nm indicate the first overtone of O–H ($\nu_1\text{O-H}$) of the concentrated NRL compounds.

Concentrated NRL films were added with the CaCl_2 solution as obtained from the pyrolysis of the chicken, duck, and bird eggshells dissolved in HCl and SDS. The FTIR peaks in the range of $400\text{--}4000\text{ cm}^{-1}$ are shown in Figure 7. The FTIR spectra are encoded as: Latex film_chicken_05; Latex film_duck_05, and Latex film_bird_05; they show the same peak positions identifying the chemical functional groups at 2956 cm^{-1} $\nu(\text{C-H of CH}_3)$, 2919 cm^{-1} $\nu(\text{C-H of CH}_2)$, 2875 cm^{-1} $\nu(\text{C=O})$ and $\delta(\alpha\text{-CH}_3)$, 1468 cm^{-1} $\delta(\text{CH}_2)$, 1248 cm^{-1} $\nu(\text{S-O of SO}_4)$, 1221 cm^{-1} $\nu(\text{C-O, C-O-C, and C-C-O})$, 1071 cm^{-1} $\nu(\text{S-O of SO}_4)$, 830 cm^{-1} $\nu(\text{S-O-C})$, 722 cm^{-1} $-(\text{CH}_2)_n\text{-(n>3)}$, and 633 and 587 cm^{-1} $\delta(\text{SO}_4)$. Furthermore, the FTIR spectra of concentrated rubber latex films added with CaCl_2 and SDS are compared with the FTIR spectra of CaO powder made from the calcined crude eggshells and the pure concentrated latex film. The FTIR spectrum of CaO

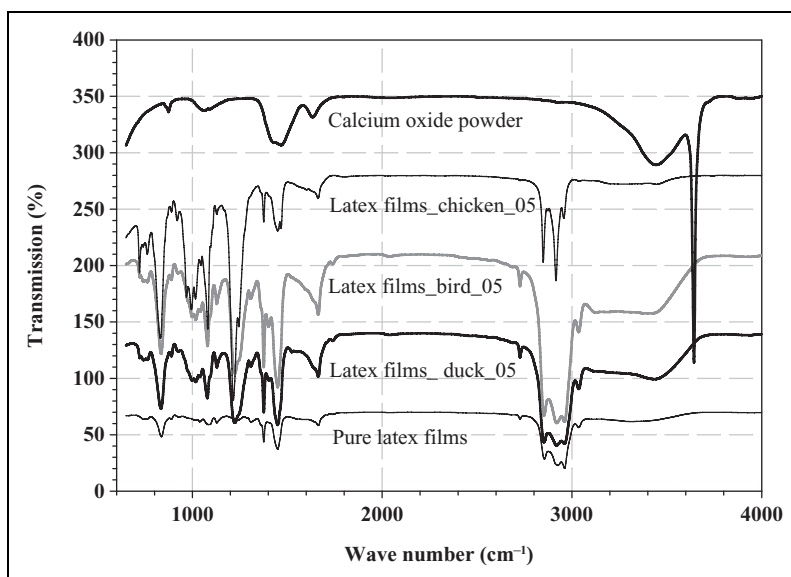


Figure 7. Comparison of Fourier transformation infrared spectra of natural rubber latex films added calcium chloride and sodium dodecyl sulphate.

shows peaks at 3600 cm^{-1} strong $\nu(\text{O-H})$, 3450 cm^{-1} $\nu(\text{O-H})$, $2800\text{--}2970\text{ cm}^{-1}$ $\delta(\text{C-H})$, 1750 cm^{-1} $\nu(\text{C=O})$, 1650 cm^{-1} strong $\nu(\text{C=O})$, and $500\text{--}580\text{ cm}^{-1}$ $\nu(\text{Ca-O})$. The characteristic peaks of the pure concentrated latex are at $3000\text{--}2900\text{ cm}^{-1}$ $\nu(\text{C-H})$ and $(=\text{CH})$, $2900\text{--}2800\text{ cm}^{-1}$ $\nu(\text{CH}_2)$, $1800\text{--}1600\text{ cm}^{-1}$ $\nu(\text{C=C})$, $1500\text{--}1300\text{ cm}^{-1}$ (CH_3 asymmetric deformation), $1300\text{--}1100\text{ cm}^{-1}$ $\nu(\text{C-O-C})$, and $1100\text{--}740\text{ cm}^{-1}$ ($=\text{CH}$ bending). All FTIR spectra encoded with Latex film_chicken, Latex film_bird, and Latex film_duck show the same peak positions, corresponding to the peak positions of pure latex films and the CaO powder, except for the peak positions at 3600 cm^{-1} strong $\nu(\text{O-H})$ and 3450 cm^{-1} $\nu(\text{O-H})$, which are due to dried latex films. These FTIR spectra assignments encoded Latex film_chicken, Latex film_bird, and Latex film_duck are of the same functional groups of the rubber latex as matrix phase and CaO as dispersed phase.

The XRD peak patterns of pure NRL films, latex films added with CaCl_2 and SDS, and CaO powder from eggshells are shown in Figure 8. The XRD peak pattern of pure NRL film shows an amorphous phase, whereas the XRD peak pattern of CaO powder indicates a crystalline phase of the lime face-centered cubic phase formation (Fm3m) which corresponding to CaO and consistent with JCPDS No.82-1690. Moreover, the XRD peak patterns of latex film added with CaCl_2 solution and SDS for all the three kinds of eggshells (chicken, duck, and bird) show a semicrystalline phase due to the amorphous phase and a small amount of crystalline phase. The crystalline phase found in the semicrystalline phase formation comes from adding SDS as the stabilizer and the CaCl_2 solution that contains ionic charge contents (Na^+ , Ca^{2+} , O^{2-} , H^+ , and Cl^-). The quantities of EPs, removed from concentrated NRL surface films added with CaCl_2

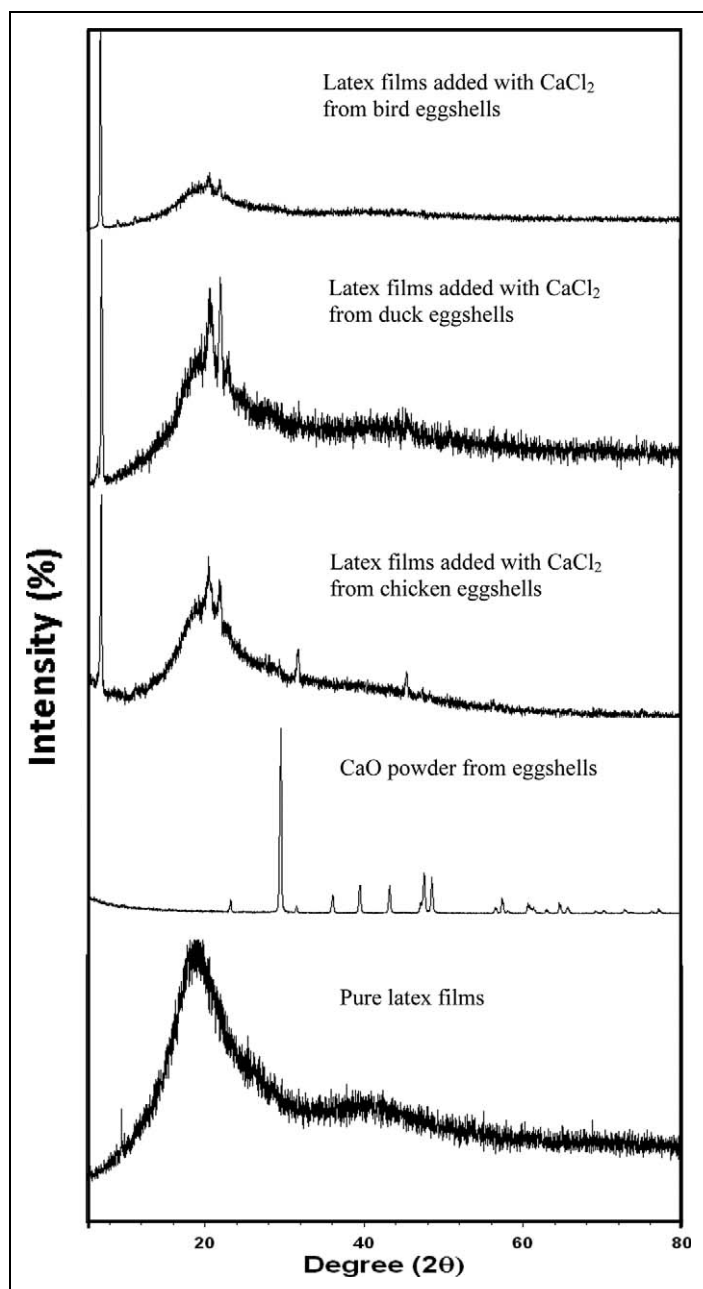


Figure 8. Comparison of x-ray diffraction patterns of pure concentrated natural rubber latex film, calcium oxide, rubber latex films added calcium chloride solution made from calcium oxide of eggshells dissolved in 2 M HCl.

solution and SDS, according to the formulation of Table 1, were determined four times for each sample. The water EP contents, as measured by the modified Lowry method, are shown in Table 6. The small amount of the EP of the pure NRL films is equal to $38.84 \mu\text{g g}^{-1}$. The amount of EPs of Latex_01, Latex_02, and Latex_03 films added with only 2 M HCl (no CaO) are equal to 4515, 4360, and $6109 \mu\text{g g}^{-1}$, respectively. Adding only HCl into the concentrated rubber latex can reduce the EP because HCl is a strong acid containing ionic charges of H^+ and Cl^- , which can form bonds with proteins, thus denaturing proteins and reducing the crosslink densities of concentrated rubber latex films. However, the comparison of the amount of EP values added with 0.5 and 1.0 g of 2 M HCl and encoded Latex_01, Latex_02, and Latex_03 are slightly lower than those of EPs with CaCl_2 as prepared from the CaO (chicken, bird, and duck eggshells) mixed with 2 M HCl to form a CaCl_2 solution. Latex films added with the CaCl_2 solution made from CaO of chicken, duck, and bird eggshells dissolved in 2 M HCl and mixed with SDS are encoded as Latex_chicken_01-07, Latex_duck_01-07, and Latex_bird_01-07, respectively. Latex_chicken_05, Latex_duck_05, and Latex_bird_03 possess the highest values of the water EP contents: 7338, 5803, and $7008 \mu\text{g g}^{-1}$, respectively. Especially, adding 0.75 g of CaCl_2 solution made from dissolving CaO bird eggshells in 2 M HCl is the most suitable for the extraction of proteins at the film surfaces. The higher amounts of CaCl_2 in the solutions, such as 1.75 and 2.00 g, yield lower water EPs contents than those of CaCl_2 in the range of 0.75–1.50 g CaCl_2 . The amounts of water EPs from Latex_01 and Latex_02 are lower than the latex added with CaCl_2 dissolved in 2 M HCl. It can be concluded that the CaCl_2 solution made from the eggshells is of a high purity, a high specific surface area, and containing more ionic specie contents (Ca^{2+} , H^+ , Cl^- , and O^{2-}), suitable to denature proteins because CaO is a porous-solid material functioning as a heterogeneous catalyst. The different values of the obtained EPs are due the differing specific surface areas and pore diameters of CaO. The specific surface areas and the average pore diameters of CaO made from chicken and duck eggshells are nearly the same. They differ distinctly from the specific surface area and the pore diameter of CaO made from bird eggshells. The main advantage of a large average pore diameter of CaO from bird eggshells is the ability to trap the large and small molecular proteins from NRL more easily.

Conclusion

CaCl_2 made from CaO was produced by the pyrolysis of crude eggshells (chicken, duck, and bird) calcined at 900°C for 2 h and dissolved in 2 M HCl. The CaO made from eggshells are of high purity ($\sim 99 \text{ wt}\%$), of small and uniform sizes ($2\text{--}12 \mu\text{m}$), of low true density, with high specific surface areas and average pore diameters, especially the bird eggshell with $3.47 \text{ m}^2 \text{ g}^{-1}$ and $82.99 \text{ \AA}$, and possess more ionic specie contents (Ca^{2+} , O^{2-} , H^+ , and Cl^-). CaO is a porous-solid material that can be used as a heterogeneous catalyst to absorb and denature proteins. The eggshells can be used as a starting material or the dispersed phase to extract the proteins from NRL. The proteins are known to cause latex-allergic problems to human skins. The effective way to prevent the allergic sensitization is to remove the EPs. Controlling the level of proteins on the finished products such as medical gloves, condoms, balloons, and medical/dental parts to a lower level is expected to reduce the sensitization of NRL product users.

Table 6. Water EP contents from the concentrated natural rubber latex films as measured by the modified Lowry method.^a

Codes	Concentrated latex (g)	10 wt% SDS (g)	CaCl ₂ ^b solution or HCl ^c (g)	Water EP (μg g ⁻¹)
Pure latex films	100	—	—	38.84
Latex_01^c	100	20	0.50 g of 2 M HCl	4515
Latex_02^c	100	20	1.00 g of 2 M HCl	4360
Latex_03^c	100	20	2.00 g of 2 M HCl	6109
Latex_chicken_01	100	20	0.25 g of CaCl₂^b	3449
Latex_chicken_02	100	20	0.50 g of CaCl ₂ ^b	4210
Latex_chicken_03	100	20	0.75 g of CaCl ₂ ^b	4212
Latex_chicken_04	100	20	1.00 g of CaCl ₂ ^b	4957
Latex_chicken_05	100	20	1.50 g of CaCl₂^b	7338
Latex_chicken_06	100	20	1.75 g of CaCl ₂ ^b	6508
Latex_chicken_07	100	20	2.00 g of CaCl ₂ ^b	6517
Latex_bird_01	100	20	0.25 g of CaCl₂^b	6237
Latex_bird_02	100	20	0.50 g of CaCl ₂ ^b	5588
Latex_bird_03	100	20	0.75 g of CaCl₂^b	7008
Latex_bird_04	100	20	1.00 g of CaCl ₂ ^b	5512
Latex_bird_05	100	20	1.50 g of CaCl₂^b	5174
Latex_bird_06	100	20	1.75 g of CaCl ₂ ^b	4169
Latex_bird_07	100	20	2.00 g of CaCl ₂ ^b	5129
Latex_duck_01	100	20	0.25 g of CaCl₂^b	3219
Latex_duck_02	100	20	0.50 g of CaCl ₂ ^b	4834
Latex_duck_03	100	20	0.75 g of CaCl ₂ ^b	4731
Latex_duck_04	100	20	1.00 g of CaCl ₂ ^b	5639
Latex_duck_05	100	20	1.50 g of CaCl₂^b	5803
Latex_duck_06	100	20	1.75 g of CaCl ₂ ^b	5706
Latex_duck_07	100	20	2.00 g of CaCl ₂ ^b	3965
Latex_CaCl₂_01	100	20	0.25 g of CaCl ₂ ^d	2830
Latex_CaO_01	100	20	0.25 g of CaCl ₂ ^e	1989

EP: extractable protein; SDS: sodium dodecylsulphate.

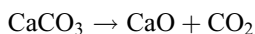
^aAll data are the average values from experimental testing three times per one sample, and data in the same evaluation.

^bThe weight of CaCl₂ solution obtained from CaO of chicken, bird, and duck eggshells dissolved in 2 M HCl as given in the below equations.

^cAdding only 2 M HCl solution (not contain CaO) of 0.50 g (latex_01), 1.00 g (latex_02), and 2.00 g (latex_03) into the latex compound samples.

^dThe weight of CaCl₂ solution produced by dissolving CaCl₂ (commercial grade) with water.

^eThe weight of CaCl₂ solution produced by dissolving CaO (commercial grade) in 2 M HCl as given in the below equations



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References

1. Peng Z, Kong LX, Li SD, Chen Y, and Huang MF. Self-assembled natural rubber/silica nanocomposites: its preparation and characterization. *Compos Sci Technol* 2007; 67: 3130–3139.
2. Angellier H, Molina-Boisseau S, and Dufresne A. Mechanical properties of waxy maize starch nanocrystal reinforced natural rubber. *Macromolecules* 2005; 38: 9161–9170.
3. Sato S, Honda Y, Kuwahara M, Kishimoto H, Yagi N, and Muraoka K. Microbial scission of sulfide linkages in vulcanized natural rubber by a white rot basidiomycete, *Ceriporiopsis subvermispora*. *Biomacromolecules* 2004; 5: 511–515.
4. Rogero SO, Lugaõ AB, Yoshii F, and Makuuchi K. Extractable proteins from irradiated field natural rubber latex. *Radiat Phys Chem* 2003; 67: 501–503.
5. Perrella FW, and Gaspari AA. Natural rubber latex protein reduction with an emphasis on enzyme treatment. *Methods* 2007; 27: 77–86.
6. Truscott W. Sensitivity to latex in medical devices. In: *International latex conference, US Food and Drug Administration, CDRH*, Baltimore, MD, 5-7 Nov 1992.
7. Lai PF, and Ng KP. Production of high quality latex products: Malaysian efforts in meeting future requirements. In: *Latex: education symposium No. 36, Rubber Division, American Chemical Society Educational Symposium on Natural Rubber*, Philadelphia, 2 May 1995.
8. Aziz NAA. Chlorination of Gloves. In: *Latex Protein Workshop of the International Rubber Technology Conference (Rubber Research Institute of Malaysia)*, Kuala Lumpur, 14-16 June 1993.
9. Truscott W. The industry perspective on latex. *Immunology and Allergy Clinics of North America* 1995; 15: 89-115.
10. Marrakchi S, and Maibach HI. Sodium lauryl sulfate-induced irritation in the human face: regional and age-related differences. *Skin Pharmacol Physiol* 2006; 19(3): 177–180.
11. Mukerjee P, and Mysels KJ. *Critical micelle concentration of aqueous surfactant systems NSRDS-NBS 36*. Washington, DC: US Government Printing Office, 1971.
12. Osborne BG, and Fearn T. Theory of near infrared spectrophotometry. In: *Near infrared spectroscopy in food analysis*. England: Longman Scientific and Technical, Longman Group UK Limited, 1986, pp. 20–42.
13. Murray I, and Williams PC. Chemical principles of near infrared technology. In: Williams PC and Norris KH (eds) *Near infrared technology in agricultural and food industries*. St Paul, MI: American Association of Cereal Chemists Inc, 1990, pp. 17–31.