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Recovery of Deproteinised Small Rubber Particles from Skim Natural Rubber Latex: Effect of Some Inorganic Salts

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Phase separation of cream skim natural rubber (NR) latex containing small rubber particles of about 0.1 μm was observed by the addition of inorganic salt into fresh skim latex or deproteinised skim latex. Cream skim latex was obtained by centrifugal separation of the resulting cream phase with high yield and purity by the addition of inorganic salts with protease or deproteinised skim latex. The skim rubber from deproteinised skim rubber contained less than 0.02% nitrogen content.

Key words: deproteinised skim latex; small rubber particles, inorganic salt; NR latex; protease; centrifugation

Natural rubber (NR) latex is obtained as a sap from the *Hevea brasiliensis* tree. Fresh field latex contains about 60% water, 30% rubber components and several percentages of proteins, lipids and minerals. NR latex as an industrial raw material is supplied as concentrated NR latex. It is obtained by centrifuging fresh field NR latex to get a concentration of about 60% rubber component and adding 0.2% to 0.7% of ammonia into the NR latex to prevent putrefaction. Fresh NR latex contains a wide particle size distribution ranging from 0.04 μm–3 μm[1,2]. By centrifugal separation, the larger rubber particles with an average diameter of 1 μm are separated as a cream-like rubber phase. The smaller rubber particles with an average particle size of 0.1 μm[2,3] remain in the residual serum phase with 5%~8% dry rubber content, known as skim latex. The fine rubber particles in the skim latex are known as skim rubber.

At present, the skim rubber is recovered by direct coagulation with concentrated sulphuric acid[5]. The resulting skim rubber is of little value due to the presence of many non-rubber components, as high as 20%, included in the course of coagulation[6]. These non-rubber components lower the physical properties of rubber. Furthermore, the addition of concentrated sulphuric acid can result in some side reactions to the rubber molecules, such as degradation, cyclisation, *etc*. Another problem is that if the skim latex is stored for long

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periods, the small rubber particles in the skim latex cannot be coagulated by acid coagulation[7].

Auto-coagulation of the skim latex is another method to recover the skim rubber from the skim latex[8]. This can be done by de-ammoniating the skim latex to an ammonia content lower than 0.1% and leaving it for spontaneous coagulation for at least 5 days. This method can be accelerated by adding 0.1% dioctyl sodium sulfosuccinate and 1% $CaCl_2$ to skim latex[9], which results in rapid coagulation of the skim latex within a few minutes. Morris has developed a method to recover skim rubber by the addition of a pancreatic enzyme to skim latex before coagulation with sulphuric acid[10]. It was found that the skim latex after incubating with the enzyme at 22°C–55°C for 12 h–96 h led to a simultaneous coagulation by the addition of sulphuric acid. It was reported that this method gave a low-protein skim rubber with a nitrogen content of about 0.4%[11]. This level of nitrogen content is still high and can easily cause Type I allergic reaction. Furthermore, the skim rubber obtained by the above methods contains a large amount of impurities which had been incorporated during coagulation. These would lead to poor quality of the resulting rubber.

Recently, another method was developed for recovering the small rubber particles in skim latex as concentrated skim latex by a membrane-separation procedure[4]. However, this method seems to be too expensive for the large-scale production of skim latex.

The present work is an attempt to develop a new method for the preparation of highly-purified skim rubber. A simple method of creaming is applied to the recovery of small rubber particles as concentrated skim latex by addition of an inorganic salt. The removal of contaminated proteins in the skim latex was also carried out by a proteolytic enzyme treatment, before adding the inorganic salt or using the skim latex obtained from the deproteinised NR latex. The separation of small rubber particles from water-soluble impurities such as soluble proteins can be achieved by washing the resulting skim rubber using low-speed centrifugation, to get a highly-deproteinised skim rubber.

EXPERIMENTAL

Materials

Fresh skim NR latex from centrifuged fresh NR latex, preserved with 0.3% NH_3 (TSC 8.5% and DRC 5.8%, pH 11.3), was provided by Thai Rubber Latex Company (Thailand). The fresh skim latex was kept in a refrigerator until use. Deproteinisation was carried out by incubating with 0.05 % w/v Alcalase 2.0T® at 37°C overnight[12]. Deproteinised skim latex was obtained by centrifugal separation of the deproteinised NR latex.

Separation Method

Inorganic salt was added directly or as a concentrated aqueous solution into the fresh or deproteinised skim latex. The inorganic salts used were NaCl, Na_2CO_3, $(NH_4)_2HPO_2$, Na_2SO_4, K_2CO_3, $(NH_4)_2SO_4$, $Al_2(SO_4)_3$, $AlK(SO_4)_2$, $MgSO_4$ and $MgCl_2$. The separated skim latex was washed by low-speed centrifugation at 3000 r.p.m.

Analysis

The average particle size and zeta-potential of latex were determined by using a Malvern S4700 particle size analyser. The nitrogen content was analysed by a Yanaco CHN Corder MT-5. FTIR analysis was done by using a JASCO 460 Plus.

RESULTS AND DISCUSSION

Phase Separation of Skim Latex

Phase separation of residual rubber in the skim latex was carried out by the addition of a sufficient amount of inorganic salt into the skim latex, to recover the small rubber particles as cream phase. This is called phase separation between rubber cream fraction and serum phase. The small rubber particles of about 0.1 μm became of larger particle size with an average particle diameter of about 0.5 μm, contained in the upper phase. The serum phase was composed almost entirely of water, water-soluble components and some precipitated impurities. The impurities were not incorporated into the rubber fraction, so the purity of the rubber obtained was higher than the skim rubber obtained by the conventional method, as described above (introduction).

It was found that the suitable amount of all these inorganic salts to cause phase separation was within the range of 12% to 20 % w/v for the normal skim latex. The addition of inorganic salt resulted in the separation of a cream fraction from a clear residual bottom phase, especially in the case of sodium chloride. Clear separation was observed by standing the skim latex mixed with inorganic salt for about 12 h–24 h.

Figure 1 shows the phase separation of skim latex before and after adding 12% (w/v) sodium chloride. When the concentration of the inorganic salt was less than the above, a clear separation of the cream phase was not observed. On the other hand, the addition of excess amounts of inorganic salt gave no further separation. The cream component isolated from the skim latex could be used as latex after redispersing in water without the addition of any stabiliser. Taking advantage of good colloidal stability, it was possible to wash the recovered skim latex from salts by low-speed centrifugation, which might be optionally used to isolate or purify the cream component from the serum again.

The amount of inorganic salt added was set at four levels, *i.e.* 10 g, 12 g, 15 g and 20 g, per 100 mL of skim latex. The results are represented as (1) state of phase separation; (2) percentage of recovery of rubber and (3) average particle diameter (μm) of the recovered rubber. The results are shown in *Table 1*.

The average particle size of the original skim latex was 0.119 μm and it increased nearly to 0.5 μm–0.6 μm after phase separation. However, in some cases, such as after addition of 10% Na_2CO_3, although phase separation was not observed, the particle size increased to 0.58 μm. This indicates that phase separation is affected not only by the increase in the particle size, but also by the density of the serum phase as modified by the addition of inorganic salt.

Phase Separation of Skim Latex in the Presence of Protease

Each of the inorganic salts and 0.05%w/v of a protease were added to the fresh skim latex in various proportions. Then the mixture was allowed to stand at 37°C for 24 h. As a result, the cream component was isolated in the upper layer. The isolated rubber was subjected to the above evaluation and observation of the properties (1) to (3) with respect to the type and amount of the inorganic salt, and (4) the nitrogen content of the recovered skim rubber as determined by the CHN analyser. The results are shown in *Table 2*.

It is clear from *Table 2* that the most effective inorganic salt was sodium chloride at concentration higher than 10%w/v. It was found that the phase separation for this case

Figure 1. Phase separation of fresh skim latex before and after adding 12% NaCl.

was faster than that without the addition of protease, even though the same particle size of rubber particles was observed in both cases. Usually, the nitrogen content of skim rubber is as high as 2%–3%. However, the nitrogen content of the recovered skim rubber was dramatically reduced to less than 0.02%. This implies that the added enzyme decomposed the protein components in the skim latex effectively and the decomposed proteins dissolved in the serum phase, which was subsequently separated from the cream phase.

Phase Separation of Deproteinised Skim Latex

The recovery of the small rubber particles from the deproteinised skim latex was carried out. Each of the inorganic salts was added to 100 mL of the deproteinised skim latex and the mixture was allowed to stand at 37°C for at least 1 h to 24 h. The creaming process in the case of deproteinised skim latex occurred more easily than in the case of normal skim latex with and without protease. It was observed that the phase separation was complete within 1 h in some cases, such as deproteinised skim latex with sodium chloride. This indicates that the decomposition of proteins covering the surface of rubber particles might be almost complete before the inorganic salt was added, and this assisted aggregation of the rubber particles. Thus, the creaming process was very fast in this case. The rubber obtained was characterised as shown in *Table 3*. It is clear from *Table 3* that the particle size of the small rubber particles

TABLE 1. EFFECT OF INORGANIC SALTS ON PHASE SEPARATION, PERCENTAGE OF RECOVERY AND PARTICLE SIZE OF THE RECOVERED SKIM RUBBER FROM FRESH SKIM LATEX OBTAINED FROM CENTRIFUGED NR LATEX

Type of inorganic salt	Amount (g) of inorganic salt added into 100 mL of skim latex			
	10	12	15	20
NaCl	(1) Δ	(1) ○	(1) ○	(1) ○
	(2) 60%	(2) 100%	(2)100%	(2)100%
	(3) 0.55	(3) 0.55	(3) 0.56	(3) 0.57
Na_2CO_3	(1) ×	(1) ○	(1) ○	(1) ○
	(2) –	(2) 60%	(2) 100%	(2)100%
	(3) 0.58	(3) 0.60	(3) 0.55	(3) 0.56
$(NH_4)_2HPO_4$	(1) ×	(1) Δ	(1) ○	(1) ○
	(2) –	(2) 50%	(2) 100%	(2)100%
	(3) 0.23	(3) 0.62	(3) 0.58	(3) 0.57
Na_2SO_4	(1) ×	(1) Δ	(1) ○	(1) ○
	(2) –	(2) 100%	(2) 100%	(2) 100%
	(3) 0.42	(3) 0.57	(3) 0.56	(3) 0.60
K_2CO_3	(1) Δ	(1) Δ	(1) ○	(1) ○
	(2) 60%	(2) 65%	(2) 100%	(2) 100%
	(3) 0.231	(3) 0.61	(3) 0.57	(3) 0.55
$(NH_4)_2SO_4$	(1) ×	(1) Δ	(1) ○	(1) ○
	(2) –	(2) 50%	(2) 100%	(2) 100%
	(3) 0.32	(3) 0.63	(3) 0.56	(3) 0.54

(1) Phase separation (○ : very clear; Δ : clear; × : no separation)
(2) Percentage of recovery of the skim rubber (%)
(3) Average diameter of the recovered rubber particles (μm)

increased up to 0.5 μm–0.6 μm. The nitrogen content was also dramatically reduced to the level of 0.02%, as was observed in the case of skim latex treated with protease.

It should be noted that pH of skim latex in the conditions as described above was in the range 9.8–11.4. It was interesting to check the effect of pH on phase separation of the skim latex after adding inorganic salt by using other kinds of salts. $Al_2(SO_4)_3$ and $AlK(SO_4)_2$ at a concentration of 12% caused aggregation of the skim latex and the deproteinised skim latices to occur at pH lower than 4. The skim latex was flocculated by the addition of $CaSO_4$ and $CaCO_3$. This indicates that Ca^{2+} ions are

TABLE 2. EFFECT OF INORGANIC SALTS ON PHASE SEPARATION, PERCENTAGE OF RECOVERY, PARTICLE SIZE AND NITROGEN CONTENT OF THE RECOVERED SKIM RUBBER FROM THE FRESH SKIM LATEX, IN THE PRESENCE OF 0.05% PROTEOLYTIC ENZYME

Type of inorganic salt	Amount (g) of inorganic salt added into 100 mL of skim latex			
	10	12	15	20
NaCl	(1) Δ (2) 60% (3) 0.55 (4) 0.020%	(1) ○ (2) 100% (3) 0.55 (4) 0.018%	(1) ○ (2) 100% (3) 0.56 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.019%
Na_2CO_3	(1) × (2) – (3) 0.58 (4) –	(1) ○ (2) 100% (3) 0.58 (4) 0.018%	(1) ○ (2) 100% (3) 0.55 (4) 0.019%	(1) ○ (2) 100% (3) 0.56 (4) 0.017%
$(NH_4)_2HPO_4$	(1) Δ (2) 50% (3) 0.59 (4) 0.019%	(1) Δ (2) 100% (3) 0.62 (4) 0.018%	(1) ○ (2) 100% (3) 0.58 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.018%
Na_2SO_4	(1) × (2) – (3) 0.42 (4) –	(1) Δ (2) 70% (3) 0.59 (4) 0.021%	(1) ○ (2) 100% (3) 0.54 (4) 0.018%	(1) ○ (2) 100% (3) 0.52 (4) 0.019%
K_2CO_3	(1) Δ (2) 70% (3) 0.55 (4) 0.020%	(1) ○ (2) 100% (3) 0.54 (4) 0.018%	(1) ○ (2) 100% (3) 0.53 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.018%
$(NH_4)_2SO_4$	(1) × (2) – (3) 0.42 (4) –	(1) Δ (2) 70% (3) 0.57 (4) 0.018%	(1) ○ (2) 100% (3) 0.58 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.019%
$Al_2(SO_4)_3$	(1) × (2) – (3) 0.42 (4) –	(1) Δ (2) 50% (3) 0.62 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.019%	(1) ○ (2) 100% (3) 0.58 (4) 0.019%
$AlK(SO_4)_2$	(1) × (2) – (3) 0.45 (4) –	(1) Δ (2) 60% (3) 0.60 (4) 0.018%	(1) ○ (2) 100% (3) 0.55 (4) 0.018%	(1) ○ (2) 100% (3) 0.53 (4) 0.019%
$MgSO_4$	(1) Δ (2) 50% (3) 0.60 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.018%	(1) ○ (2) 100% (3) 0.58 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.019%

(1) Phase separation (○ : very clear; Δ : clear; × : no separation)
(2) Percentage of recovery of the skim rubber (%)
(3) Average diameter of the recovered rubber particles (μm)
(4) Nitrogen content of the recovered skim rubber (%)

TABLE 3. EFFECT OF INORGANIC SALTS ON PHASE SEPARATION, PERCENTAGE OF RECOVERY, PARTICLE SIZE AND NITROGEN CONTENT OF THE RECOVERED SKIM RUBBER FROM DEPROTEINISED SKIM LATEX

Type of inorganic salt	Amount (g) of inorganic salt added into 100 mL of skim latex			
	10	12	15	20
NaCl	(1) Δ (2) 70% (3) 0.55 (4) 0.019%	(1) ○ (2) 100% (3) 0.55 (4) 0.017%	(1) ○ (2) 100% (3) 0.56 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.018%
Na_2CO_3	(1) × (2) – (3) 0.58 (4) –	(1) ○ (2) 100% (3) 0.59 (4) 0.019%	(1) ○ (2) 100% (3) 0.55 (4) 0.019%	(1) ○ (2) 100% (3) 0.57 (4) 0.018%
$(NH_4)_2HPO_4$	(1) Δ (2) 50% (3) 0.59 (4) 0.019%	(1) ○ (2) 100% (3) 0.59 (4) 0.018%	(1) ○ (2) 100% (3) 0.55 (4) 0.017%	(1) ○ (2) 100% (3) 0.57 (4) 0.018%
Na_2SO_4	(1) × (2) – (3) 0.42 (4) –	(1) Δ (2) 70% (3) 0.57 (4) 0.018%	(1) ○ (2) 100% (3) 0.54 (4) 0.018%	(1) ○ (2) 100% (3) 0.56 (4) 0.017%
K_2CO_3	(1) Δ (2) 50% (3) 0.51 (4) 0.022%	(1) ○ (2) 100% (3) 0.54 (4) 0.018%	(1) ○ (2) 100% (3) 0.53 (4) 0.018%	(1) ○ (2) 100% (3) 0.55 (4) 0.018%
$(NH_4)_2SO_4$	(1) Δ (2) 50% (3) 0.48 (4) 0.020%	(1) Δ (2) 100% (3) 0.52 (4) 0.019%	(1) ○ (2) 100% (3) 0.58 (4) 0.018%	(1) ○ (2) 100% (3) 0.54 (4) 0.019%
$Al_2(SO_4)_3$	(1) × (2) – (3) 0.34 (4) –	(1) Δ (2) 60% (3) 0.61 (4) 0.019%	(1) ○ (2) 100% (3) 0.56 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.019%
$AlK(SO_4)_2$	(1) × (2) – (3) 0.45 (4) –	(1) Δ (2) 60% (3) 0.59 (4) 0.019%	(1) ○ (2) 100% (3) 0.55 (4) 0.018%	(1) ○ (2) 100% (3) 0.54 (4) 0.018%
$MgSO_4$	(1) Δ (2) 60% (3) 0.60 (4) 0.018%	(1) ○ (2) 100% (3) 0.57 (4) 0.020%	(1) ○ (2) 100% (3) 0.58 (4) 0.019%	(1) ○ (2) 100% (3) 0.56 (4) 0.018%

(1) Phase separation (○ : very clear; Δ : clear; × : no separation)
(2) Percentage of recovery of the skim rubber (%)
(3) Average diameter of the recovered rubber particles (μm)
(4) Nitrogen content of the recovered skim rubber (%)

not suitable for effective phase separation of the small rubber particles from the skim latex.

The occurrence of phase separation of skim rubber in the above three types of system can be explained by considering the colloidal stability of latex as indicated by the zeta-potential of each system. Sodium chloride was used as the inorganic salt in all systems. As can be seen in *Figure 2*, the zeta-potentials were about –59, –61 and –56 mV for the fresh skim latex, skim latex with proteolytic enzyme and the deproteinised skim latex, respectively, in the absence of added sodium chloride.

Increasing the amount of sodium chloride to 10%, the zeta-potential value showed insignificant change in the case of fresh skim latex. Then, the phase separation was not observed in the first system if sodium chloride was less than 10%. However, a decrease in the absolute magnitude of 61 mV to 56 mV and from 56 mV to 52 mV was observed in the case of the fresh skim latex with proteolytic enzyme and the previously deproteinised skim latex, respectively. This implies that sodium chloride is not the only factor affecting the colloidal stability of the small rubber particles in the fresh skim latex, but the reduction of surface charge due to the loss of proteins is also an important factor. As noted previously, the proteolytic enzyme could decompose protein components, resulting in a decrease of the colloidal stability of the small rubber particles. Thus, the clear separation of cream phase was very fast, especially, in the case of deproteinised skim latex.

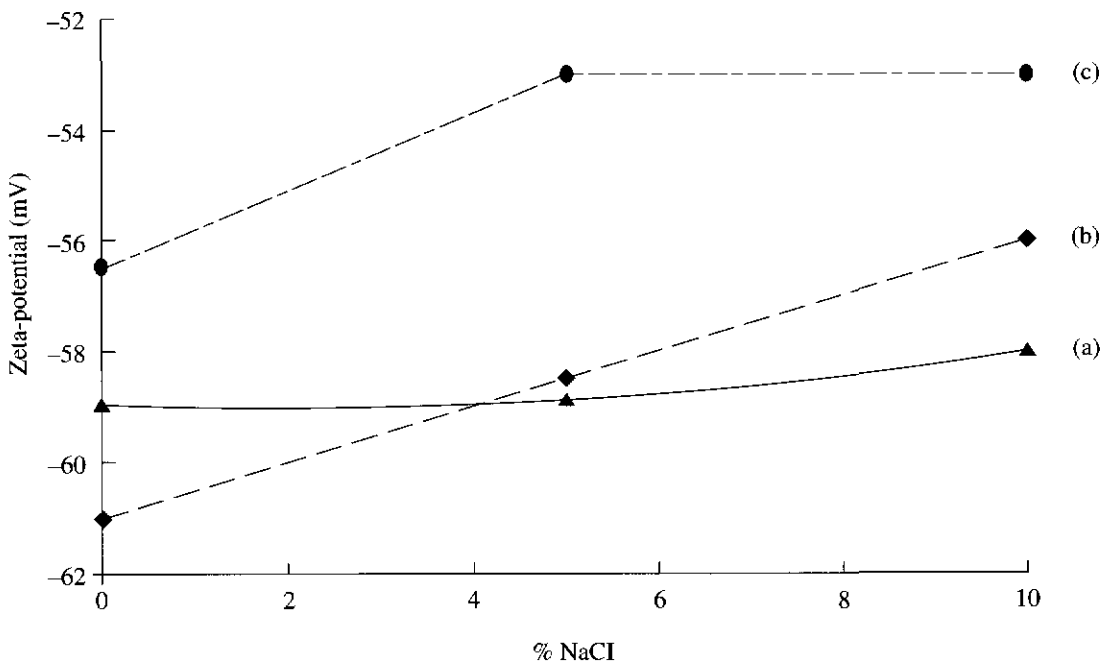

Figure 2. Changes in Zeta-potential of latex from three systems: (a) fresh skim latex; (b) fresh skim latex + 0.05% proteolytic enzyme + NaCl; and (c) deprotenised skim latex + NaCl.

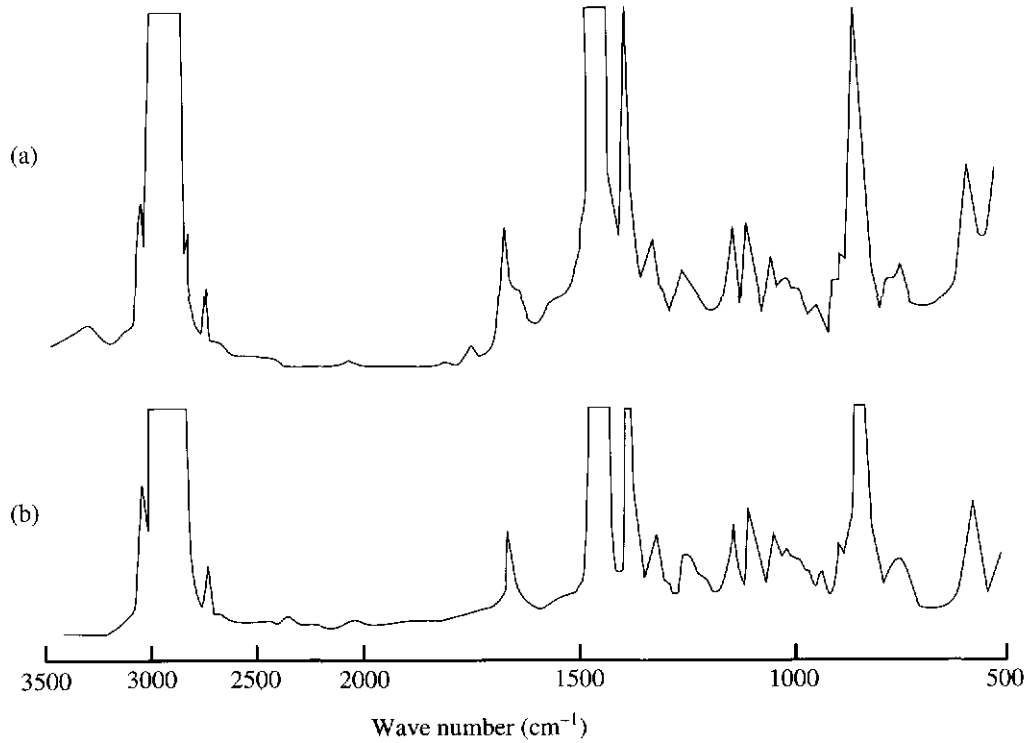

Figure 3. FTIR spectra of rubber from (a) fresh skim rubber and (b) deproteinised skim latex + 10% NaCl.

The purity of the recovered skim rubber by the addition of sodium chloride into the deproteinised skim latex was compared with that of the original fresh skim rubber, by FTIR. As shown in *Figure 3*, there are clear absorbances at 1738 cm^{-1} and 3280 cm^{-1} due to fatty ester and protein, respectively. However, the FTIR spectrum of the recovered skim rubber from the deproteinised skim latex showed no absorbance in these regions. This indicates that the skim rubber obtained by this method is a highly purified skim rubber.

CONCLUSIONS

The recovery of the residual rubber in skim latex and deproteinised skim latex was carried out by the addition of inorganic salts. Sufficient inorganic salt such as sodium chloride increases the particle size from 0.1 μm to 0.5μm–0.6 μm. The addition of 0.05%w/v protease into the fresh skim latex together with an inorganic salt accelerated the phase separation of skim latex. Deproteinised skim latex showed good phase separation, which was complete within 1 h in the case of sodium chloride. The nitrogen content of the recovered skim rubber from the fresh skim latex with protease and the deproteinised skim latex was dramatically reduced from 2%–3% to less than 0.02%.

The occurrence of phase separation of skim rubber was presumed to be due to the increase in density of the serum phase and the loss of

colloidal stability of latex by a decrease in the absolute magnitude of the zeta-potential of the rubber particles. The absence of absorbances at 1738 cm^{-1} and 3280 cm^{-1} due to fatty ester and protein, respectively, implies that highly purified skim rubber can be recovered by this method.

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