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Phase Transfer Technique for the Study of Deproteinised Natural Rubber Latex

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Phase transfer technique, using benzyldimethylhexadecylammonium chloride as a titrant and toluene as a solvent, was employed for the characterisation of deproteinised natural rubber latex. Results from critical transfer concentration values and nitrogen contents showed that proteins bound to natural rubber particles were not completely removed by using the enzyme alcalase. Apparently, the remaining proteins might be denatured and, hence, promoted the formation of destabilised and suspended rubber at the toluene/water interphase after phase transfer.

Natural rubber (NR) latex has a sizeable impact on the overall rubber market in quantity consumed and products developed because of its distinct advantages, namely, great film clarity, high green strength and good tack. It has been reported that the non-rubber substances such as proteins and lipids, have significant effects on properties of NR[1]. Proteins and amino acids have been inferred to be responsible for the branching and crosslinking of NR and also influence storage hardening, moisture absorption and dynamic properties[1,2]. Recently, there has been a growing concern on their presence in rubber products as it can sensitise atopic persons and cause allergy. Consequently, the preparation of low protein latex and deproteinised natural rubber (DPNR) latex has become increasingly important. The development of DPNR with its low level of proteins is also aimed at engineering applications requiring low creep and stress relaxation[1-3]. Up until present, however, the study of proteins on the surface of rubber particles has not caught much attention as compared to that present in serum or in the bottom fraction due to the difficulties associated with the method of their removal from the rubber surface. Although, FTIR study of purified NR has revealed the presence of residual proteins or amino acids bonded to the NR molecules[3] their characterisation is not straight forward due to low concentration.

Phase transfer technique has been used for the surface characterisation of γ-radiation vulcanised natural rubber latex (RVNRL) and non-crosslinked NR latex[4-6]. This technique, originally developed by Heim[7] to determine the surface charge of crosslinked synthetic latex, involves titration of the anionic stabilised latex with an aqueous solution of cationic surfactant in the presence of a non-water miscible organic solvent. The surface charge of the particles is calculated from the amount of the added

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surfactant at the critical transfer concentration (CTC). For the RVNRL, the negatively charged latex particles, derived from an adsorbed layer of a protein-lipid complex, could be transferred into the organic phase by titration with cationic surfactants whose molecules bear either two long alkyl chains or one long alkyl chain and a benzyl group. The resulting CTC values were found to depend on the alkyl chain length and the optimum conditions for the phase transfer technique were obtained when the solubility parameter of solvent was close to that of natural rubber. When the technique was applied in the study of non-crosslinked NR latex by using benzyldimethyl-hexadecylammonium chloride (BHAC) as a titrant, it was found that latex (BHAC) particles were completely transferred and a direct correlation existed between the CTC and the difference between the solubility parameter of organic solvent and that of natural rubber. The transfer of rubber particles took place immediately where three phases were noted, *i.e.* the upper organic phase containing the soluble rubber, the destabilised and suspended rubber at the interphase between organic solvent and water, and the lower rubber-free serum aqueous phase. It was noted that the presence of partly coagulated and suspended rubber at the interphase between solvent and water was found only in the case of non-crosslinked NR latex. This was explained in terms of the denaturation of the proteins, linked with rubber chains, by cationic surfactant[5].

From the results mentioned above, we now wish to further apply the phase transfer technique using BHAC as a titrant for the study on deproteinised natural rubber (DPNR) latex (treatment of latex with enzyme). It was hoped that the study would lead to a wider scope of phase transfer technique, better understanding and further insight concerning the use of this technique on DPNR latex. The study involved measurements of CTC values of DPNR, control NR and adsorbed-SDS DPNR latexes. Results obtained from the determination of nitrogen contents of rubber sheets cast from DPNR latex as compared to those from whole, multicentrifuged and transferred NR latexes are also reported.

EXPERIMENTAL

Deproteinised Natural Rubber (DPNR) Latex

Commercial high ammonia-preserved NR latex concentrate having *ca.* 60% dry rubber content (DRC) (from Thai Rubber & Latex Co., Ltd., Thailand) was diluted with distilled water to 30% DRC and stabilised with aqueous sodium dodecyl sulphate (SDS) solution (1% by weight on DRC). After adjusting the pH to 9.5 with 1.66% ammonia solution the latex was allowed to react with 0.04% w/v alcalase enzyme (obtained from Novo Industries, Japan) for 24 h at 37°C. The mixture was then centrifuged with Kubota High Speed Centrifuge KR-20000T at 12 000 r.p.m. for 15 min and the lower aqueous layer was replaced by adding an equal volume of aqueous solution of SDS (1%). The process was repeated twice before preparing the NR film by casting the redispersed rubber at 50°C.

The control NR latex was prepared by employing the above procedure, omitting the alcalase treatment.

Preparation of Adsorbed-SDS DPNR Latex

To maintain the same amount of charge as that of the control NR latex, aqueous solution of sodium dodecyl sulphate (0.44% w/w of SDS on DRC of DPNR latex) was added to 100 g of DPNR latex, while stirring, and the mixture was allowed to stand overnight. The concentration of added SDS was calculated from the following equation:

$$\text{added } SDS = CTC_1 - CTC_2 \quad \text{(mol/g of dried latex)} \qquad \ldots 1$$

where,

CTC_1 = CTC of control NR latex
CTC_2 = CTC of DPNR latex.

Multicentrifuged NR Latex

Concentrated NR latex having *ca.* 60% total solid content (TSC) was diluted with distilled water to 30% TSC. The mixture was then centrifuged by using Kubota High Speed Centrifuge KR-20000T at the speed of 12 000 r.p.m. for 15 min. After first centrifugation, the lower layer was discarded and then replaced by adding equal volume of 1% w/v aqueous solution of SDS with vigorous shaking. The mixture was then centrifuged and the whole process was repeated four times. Finally, the upper layer was redispersed in distilled water and the dry rubber sheet was obtained by evaporation.

Determination of Nitrogen Content

Nitrogen contents (%N) of rubber sheets, cast from whole, enzyme deproteinised, multicentrifuged and transferred NR latexes and dried at 50°C for 24 h, were determined by using Kjeldahl method[8] as described in *ASTM D 3533-76*.

Phase Transfer Procedure

Latex sample (15 g, having 0.6% solid content) was diluted with distilled water (45 g), and the pH of the mixture was found to be ~9 without adjustment. Toluene (30 g) was added and the mixture was titrated, while stirring, with 0.0121 M aqueous solution of BHAC, using a burette. The titration end point was observed when the mixture became translucent. Stopping the agitation at this point immediately caused a phase separation with the appearance of a clear rubber-free serum aqueous phase.

The quantity of surface charge was calculated directly from the CTC by the following equation:

$$CTC = \frac{V \times C}{10 \times TSC \times m} \qquad \ldots 2$$

where,

CTC = critical transfer concentration (mole of cationic surfactant used per g of dried latex)
V = quantity of cationic surfactant used at titration end point (ml)
C = surfactant concentration (mol/l)
TSC = total solid content of latex (wt. %)
m = weight of latex sample (g).

RESULTS AND DISCUSSION

The CTC values of DPNR, control NR and adsorbed-SDS DPNR latexes determined by phase transfer technique are presented in *Table 1*.

TABLE 1 CTC VALUES (MOLE OF CATIONIC SURFACTANT PER G OF DRIED LATEX) OF DPNR, CONTROL NR AND ADSORBED-SDS DPNR LATEXES

Sample	CTC ($\times 10^{-4}$)
DPNR latex	1.49 ± 0.10
Control NR latex	1.64 ± 0.10
Adsorbed-SDS DPNR latex	1.63 ± 0.10

It is apparent from *Table 1* that the CTC values of the latexes in the present study were higher than that of the non-crosslinked NR latex

previously studied[5]. This is due to the increase in amount of negative charges on rubber particles' surface resulting from the added anionic surfactant (SDS) during the sample preparation and hence the quantity of added cationic surfactant (BHAC) needed for neutralisation to effect the transfer of the latex. The data also showed the lowest CTC value of DPNR latex relating to the remaining proteins after reacting NR with alcalase enzyme besides partly the added SDS and fatty acid. It should be observed that enzyme deproteinisation technique failed to completely remove nitrogen or proteins from NR latex (see *Table 2*).

TABLE 2 NITROGEN CONTENTS (%N) IN THE NR SAMPLES DETERMINED BY USING KJELDAHL METHOD

Code	Sample sheet prepared from	%N
A	Whole NR latex	0.53 ± 0.01
B	Deproteinised NR (DPNR) latex	0.06 ± 0.01
C	Multicentrifuged NR latex	0.27 ± 0.01
D	Sample transferred from whole NR latex (A)	0.49 ± 0.04

The difference of CTC values between DPNR and control NR latexes of 1.5×10^{-4} correlated to the charges of proteins removed by the enzyme because the same amount of SDS was added in both latexes. Consequently, a calculated volume of SDS was added to DPNR latex to replace the removed proteins and the phase transfer of the adsorbed SDS-DPNR latex was then studied. It can be concluded from the result shown in *Table 1*, from which only small difference of CTC values between control NR and adsorbed-SDS DPNR latexes was observed, that the added SDS could replace the indigenous proteins of NR latex, removed by enzyme, and produce the same amount of negative charges on the surface of rubber particles in DPNR latex.

Similar to that observed in the case of non-crosslinked NR[5], the transfer of rubber particles in all latex samples in the present study took place immediately where three phases were noted, *i.e.* the upper organic phase containing soluble rubber, the destabilised and suspended rubber at the organic solvent/water interphase and the lower rubber-free serum aqueous phase. The partly coagulated and suspended rubber at the interphase could also be explained in terms of the neutralised polyisoprene chains linked with proteins denatured by added cationic surfactants[9,10]. However, in the case of synthetic latex, the unstable polymer at the interphase was not observed. This was explained by the fact that the particles of synthetic latex emulsified by low molecular weight surfactant were stabilised in the organic phase after phase transfer process due to polymer-solvent interaction[7]. We, therefore, expected that the DPNR latex having low protein content would be transferred and promoted the stable rubber solution in organic phase when compared to the control NR. Also the adsorbed-SDS DPNR latexes should provide the same result as that observed in DPNR latex itself.

Unexpected results were obtained in all latexes studied after phase transfer. At their CTC, similar observations were noted, *i.e.* all latexes were transferred where three phases were noted. This behaviour of coagulated rubber at the toluene/water interphase might be attributed to the remaining proteins in DPNR latex, bonded to rubber chain, which could not be removed by the enzyme and the proteins were consequently denatured by added cationic surfactants. The amount of denatured proteins might be enough to cause the coagulated and suspended rubber at the interphase. It was reported[11] that the proteins tightly bounded to the rubber particles remained in the leached latex products directly influenced their properties. In the case of adsorbed-SDS DPNR

latex, the low molecular weight surfactant (SDS) added could be pulled off when it was neutralised with cationic surfactant and the rubber particles behaved as DPNR latex. Therefore, it is not unreasonable to conclude from our results that denatured proteins linked with rubber chains are responsible for the presence of unstable rubber at the interphase.

Nitrogen contents (referred to proteins) of NR sheets prepared from whole NR (A), DPNR (B) multicentrifuged latexes (C) and rubber transferred from whole NR latex (D) were determined by using Kjeldahl method. Results are given in *Table 2*.

It is apparent from *Table 2* that enzyme deproteinisation and multicentrifugation methods reduced the nitrogen content of proteins in the NR samples. According to Hasma[11], proteins of high ammoniated NR latex concentrate distributed in two main fractions: the rubber and serum fractions. The rubber fraction consisted primarily of the 14 kDa and, the minor, 24 kDa proteins, both of which were extractable by ammonia, SDS or chloroform-methanol solution while the serum fraction comprised of the 14, 24, 29, 36 and 45 kDa proteins and one with molecular weight greater than 100 kDa. Since reasonably low value of nitrogen content in NR sheet from DPNR latex (B) was obtained (*Table 2*) the efficiency of deproteinisation, a chemical process in which the enzyme reacts directly to proteins both in rubber and serum fractions under certain conditions, can be assumed. Also proteins associated with the rubber particles and presumed to be bound to the rubber membrane were expected to be eliminated by enzymes. However, it was found that the proteins in the studied NR latex were not completely removed by using the alcalase enzyme.

It was reported that the nitrogen content of NR was affected by many factors such as speed, number of centrifugations and temperature, *e.g.* percents of nitrogen (0.56%) were found to be 0.25%, 0.13%, 0.09% after first, second and third centrifugation, respectively[12]. Therefore it can be assumed that some of the proteins were lost in the process of producing multicentrifuged NR latex (C). Centrifugal force should effect principally on proteins having high molecular weight in the serum fraction and cause precipitation to the bottom phase which was discarded. After several centrifugations, all proteins in the serum and those physically linked with rubber particles were removed. The data obtained in *Table 2* indicated that nitrogen content of NR sheet prepared from latex C was indeed lower than that of whole NR (*Sample A*) by about 50%.

It can be noticed that the nitrogen content of sample prepared from phase transferred latex (D) and whole NR (A) remained unchanged, therefore it was concluded that the phase transfer method could not be used to remove proteins from NR. Proteins in both serum and rubber fractions might be neutralised by added cationic surfactant and then transferred with rubber particles from the aqueous phase into organic phase or at the interphase.

CONCLUSIONS

A study of phase transfer technique in DPNR latex (treatment of latex with alcalase enzyme) indicated the presence of negative charges partly derived from residual proteins. The remaining proteins in DPNR latex could be denatured by added cationic surfactant, hence, the coagulated and suspended rubber at the toluene/water interphase after phase transfer was promoted. When a calculated amount of SDS was added to DPNR latex to replace the removed proteins the rubber could be

transferred and the CTC values of control NR and adsorbed-SDS DPNR latexes were similar. The nitrogen contents of DPNR, multicentrifuged and phase transfer of whole NR latexes indicated that enzyme deproteinisation method removed major part of proteins from NR while those physically lined with rubber were removed by multicentrifugation. However, the phase transfer method failed to eliminate proteins from NR.

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REFERENCES

1. NAIR, S (1988) Characterisation of Natural Rubber for Greater Consistency. *Rubber World*, **198(4)**, 27

2 ENG, A H, KAWAHARA, S AND TANAKA, Y (1993) Determination of Low Nitrogen Content of Purified Natural Rubber. *J nat Rubb Res*, **8(2)**, 109

3 ENG, A H, TANAKA, Y AND GAN, S N (1992) FTIR Studies on Amino Groups in Purified *Hevea* Rubber *J nat Rubb Res*, **7(2)**, 152

4. TANGBORIBOONRAT, P (1991) Contribution a l'etude du Latex de Caoutchouc Naturel par Transfert de Phase. Ph.D. Thesis. Universite de Haute Alsace, Mulhouse, France.

5 TANGBORIBOONRAT, P., SUCHIVA, K. AND KUHAKARN, S (1994) Characteristation of Non-crosslinked Natural Rubber Latex by Phase Transfrer Technique *Polymer*, **35(23)**, 5144.

6. TANGBORIBOONRAT, P., SUCHIVA, K AND RIESS, G (1995) Surface Characterisation of a γ-radiation Crosslinked Natural Rubber Latex by a Phase Transfer Technique with Cationic Surfactants *Polymer*, **36(4)**, 781.

7. HEIM, Ph (1987) Contribution a l'etude de la Polymerisation en Suspension de PMMA Choc. Mise au point de Procedes de Transfert de Phase de Latex Acryliques Ph.D. Thesis, Universite de Haute Alsace, Mulhouse, France.

8 ANNUL BOOK OF ASTM STANDARDS (1979) *ASTM D3533-76*, part 37, 769.

9. BREUER, M M. AND ROBB, I D. (1972) Interactions between Macromolecules and Detergents. *Chem Ind.*, **1**, 530.

10 HIRAMATZU, K, UEDA, C, IWATA, K, ARIKAWA, K AND AOKI, K (1977) The Interaction of Bovine Plasma Albumin with Cationic Detergent, Studies by Binding Isotherm, Optical Rotation and Difference Spectrum. *Bull Chem Soc Japan*, **50(2)**, 368.

11. HASMA, H (1992) Proteins of Natural Rubber Latex Concentrate *J nat Rubb Res*, **7(2)**, 102.

12. GREGORY, M J AND TAN, A S (1975) Some Observations on Storage Hardening of Natural Rubber *Proc Int Rubb Conf, Kuala Lumpur*, **4**, 28