Polymer Bulletin 41, 601–608 (1998)

Polymer Bulletin

New evidence of the surface morphology of deproteinized natural rubber particles

P. Tangboriboonrat*, **C. Tiyapiboonchaiya**, **C. Lerthititrakul**

Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Phyathai,
Bangkok 10400, Thailand

Received: 30 July 1998/Revised version: 17 September 1998/Accepted: 14 October 1998

Summary

A new evidence of the morphology of deproteinized natural rubber (DPNR) latex particles, *i.e.*, γ-radiation vulcanized-deproteinized NR (RV-DPNR) and deproteinized-γ-radiation vulcanized NR (DP-RVNR), was obtained by transmission electron microscopy (TEM). Micrographs of the rubber particles embedding in polystyrene, prepared by using a phase transfer/ bulk polymerization process, revealed the destruction of membrane layer surrounded the DPNR particles crosslinked by γ-ray (DP-RVNR).

Introduction

It has been reported that natural rubber (NR) latex particles are stabilized by complex layer of proteins and lipids (1, 2). The presence of proteins in NR has been assumed to exert influence on some undesirable properties such as poor creep and stress relaxation, increasing storage hardening and moisture adsorption (3, 4). Recently, the extractable proteins in the latex products are found to be responsible for the allergic reactions and it is recommeded these proteins should be removed (5, 6). Tanaka and coworkers have effectively eliminated proteins from NR latex employing a proteolytic enzyme/ surfactant combination (6-8). The former breaks down the proteins linkages selectively and the latter washes out resulting oligopeptides. Results of nitrogen contents and FTIR of deproteinized natural rubber (DPNR) in the form of dried film indicated the presence of remaining proteins bound to the DPNR particles (7). However, there is no direct evidence of the morphology of the DPNR latex particle surface.

In this work, the morphology of DPNR particles is studied under transmission electron microscopy (TEM). The phase transfer/ bulk polymerization process, previously used in the study of toughening of polystyrene based on NR latex (9), is applied for embedding of DPNR particles to be further ultramicrotome sectioned. Two types of crosslinked DPNR latex particles, *i.e.*, γ-radiation vulcanized-deproteinized NR (RV-DPNR), obtained from enzymatic deproteinization of the γ-radiation vulcanized natural rubber (RVNR) latex, and deproteinized-γ-radiation vulcanized NR (DP-RVNR), prepared by irradiation of the DPNR latex with γ-ray, are titrated with an aqueous solution of cationic surfactant in the presence of styrene. At the critical transfer concentration (CTC), a hydrophobic layer around the latex particles forms and the rubber particles transfer into the styrene monomer. The styrene containing transferred rubber particles is then polymerized in bulk and the rubber particles embedding in polystyrene (PS) matrix can be sectioned for the TEM study. By using this method, the air, freeze or chemical drying step of rubber latex normally required before the embedding step for specimen preparation (10) is omitted and, hence, the disturbance of actual DPNR particle structure is minimized.

---

* Corresponding author

Experimental

Reagents

Styrene (Fluka, puriss) and *n*-butyl acrylate (Fluka, purum) monomers were purified by passing through an aluminium oxide column. Carbon tetrachloride (Merck, GR), toluene (Merck, GR), benzyldimethylhexadecylammonium chloride (Fluka, purum) and others reagents were used without further purification.

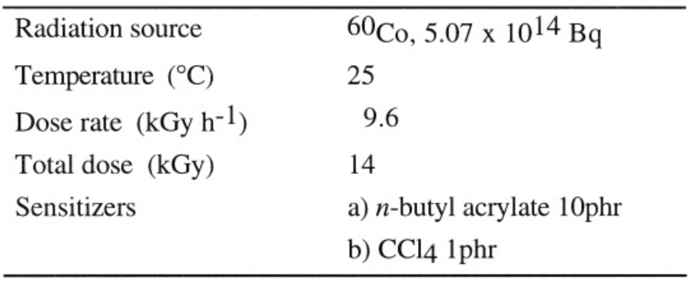
Deproteinized natural rubber (DPNR) latex

Concentrated high ammonia NR latex having *ca.* 60% dry rubber content (DRC) purchased from Rayong Bangkok Rubber Co. Ltd., was diluted with distilled water to 30%DRC. The latex was filtered through 250 mesh stainless steel sieve and stabilized with aqueous sodium dodecyl sulphate (SDS) solution (1% by weight on DRC). The stabilized latex (pH ~ 9, without any adjustment) was allowed to react with 0.04%w/v alcalase enzyme (Novo Industries, Japan) at 37°C for 24h before filtering. The mixture was centrifuged with a Supraspeed Centrifuge (Sorvall RC 28 S) at 12,000rpm for 15min after which the lower serum layer was replaced by an equal volume of aqueous solution of SDS (1%). The process was repeated twice and the DPNR latex obtained was kept at room temperature for further use.

Irradiation of latex

To prepare γ-radiation vulcanized NR (RVNR) latex, the concentrated NR latex was first filtered with 250 mesh sieve and 10% aqueous potassium hydroxide solution (4 parts per hundred of rubber, phr) was added to enhance the latex stability. After 10-15min, the sensitizers, *i.e.*, purified *n*-butyl acrylate (10phr) and carbon tetrachloride (1phr) were added with stirring for 2h. The latex was kept in a 250ml bottle overnight before being subjected to irradiation with ^{60}Co γ-ray source (Gammacell 220) at a total dose of 14kGy. The vulcanized conditions are given in Table 1.

Table 1 : Irradiation conditions used for vulcanization



Radiation source	^{60}Co, 5.07 x 10^{14} Bq
Temperature (°C)	25
Dose rate (kGy h^{-1})	9.6
Total dose (kGy)	14
Sensitizers	a) *n*-butyl acrylate 10phr b) CCl_4 1phr

Two types of latices were prepared in this study; (*i*) γ-radiation vulcanized-deproteinized NR (RV-DPNR) latex which was obtained by treatment of the RVNR latex with alcalase enzyme and SDS as described above, and (*ii*) deproteinized-γ-radiation vulcanized NR (DP-RVNR) latex, prepared by irradiation of the DPNR latex with γ-ray.

Measurement of the swelling ratio

Dried latex films, prepared by spreading of the vulcanized latices on glass plates at room temperature, were cut into small pieces (1g) before immersing in toluene (150ml) to equilibrium swell. The swelling ratio of the rubber was calculated from

$$\%\ \text{Swelling ratio} = \frac{W_2 - W_1}{W_2} \times 100$$

where, W_1 : weight of dried rubber film before swelling

W_2 : weight of swollen rubber film at equilibrium

Determination of nitrogen content

Nitrogen content (%N) of the dried rubber films was determined by using Kjeldahl method as described in ASTM D 3533-90.

Phase transfer procedure

Crosslinked NR latex (RVNR, RV-DPNR or DP-RVNR) having 6%TSC (15g) was diluted with distilled water (45g) in an Erlenmeyer flask. pH of the latex was found to be ~ 9. A styrene monomer (30g) was then added and the mixture was titrated, while stirring, with 0.012 M benzyldimethylhexadecylammonium chloride (BHAC) aqueous solution, 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 lower phase and the upper phase of styrene containing swollen transferred rubber particles. The CTC values (mole of surfactant used per gram of dry latex) of the latices were calculated from the following equation

$$\text{CTC} = \frac{C \times V}{10 \times m \times \text{TSC}}$$

where, V : quantity of cationic surfactant used at the titration end point (ml)

C : surfactant concentration (mol l^{-1})

TSC : total solid content of the latex (%)

m : weight of the latex sample (g)

Bulk polymerization process

After phase transfer, the crosslinked rubber particles were allowed to swell in the styrene at room temperature for 24h. The upper styrene phase was centrifuged by using a Supraspeed Centrifuge (Sorvall RC 28 S, Du Pont) at 10,000rpm for 5min to eliminate remaining trace of water. The styrene containing transferred rubber particles was separated and then mixed with 0.2wt% of the initiator (benzoyl peroxide). The mixture was poured into a teflon coated steel mould (10.4x10.4x2.6cm) before being heated in an oven at 70°C for 24h.

Morphological study

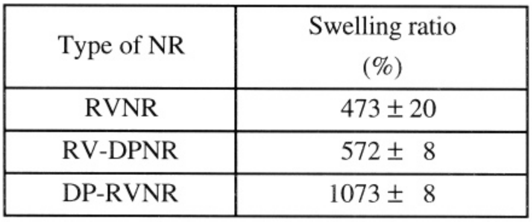
Transmission electron micrographs of rubber particles incorporated in PS matrix were obtained by using a Hitachi model H 300. Specimens were prepared according to the osmium staining principle.

Results and Discussion

Swelling ratio and nitrogen content of latices

The values of swelling ratio, inversely proportional to the degree of crosslinking, of dried films casted from RVNR, RV-DPNR and DP-RVNR latices are presented in Table 2.

Table 2 : Swelling ratio of films casted from RVNR, RV-DPNR and DP-RVNR latices

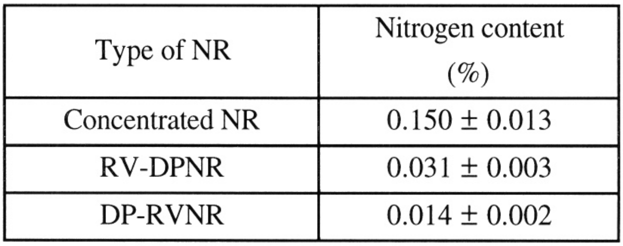


Type of NR	Swelling ratio (%)
RVNR	473 ± 20
RV-DPNR	572 ± 8
DP-RVNR	1073 ± 8

As shown in Table 2, the swelling ratio of the films casted from the RV-DPNR latex, prepared by deproteinization of RVNR latex, was about 570%, indicating moderately crosslinked rubber. This value was not greatly altered as compared to that of RVNR which implied that the enzymatic deproteinization did not significantly affect the crosslink density of the vulcanized NR. On the contrary, a high swelling ratio (~1070%) was obtained in the case of DP-RVNR, in which proteins were removed from the concentrated NR latex before irradiation, indicating the lightly crosslinking. It can be, therefore, assumed that the low amount of remaining proteins in DP-RVNR latex caused a decrease in efficiency of vulcanization of NR latex with γ-ray.

The amount of proteins in NR latex can be generally presented by nitrogen content. %Nitrogen of dried films casted from the concentrated and deproteinized NR latices, *i.e.*, RV-DPNR and DP-RVNR, were determined and shown in Table 3.

Table 3 : Nitrogen content of dried films casted from concentrated and deproteinized NR (RV-DPNR and DP-RVNR) latices



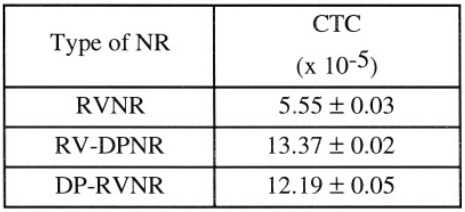
Type of NR	Nitrogen content (%)
Concentrated NR	0.150 ± 0.013
RV-DPNR	0.031 ± 0.003
DP-RVNR	0.014 ± 0.002

As expected, the data revealed that the nitrogen content of both deproteinized NR latices (RV-DPNR and DP-RVNR) was lower than that of concentrated NR latex because some of proteins in the former latices were eliminated by using the enzyme alcalase/ SDS system. This deproteinization technique was very effective in removal of more than half of the original proteins. It was reported that the nitrogen content of rubber was reduced from 0.3% to 0.01% by using this technique (8, 11, 12). However, the figures also indicated that proteins bound to NR particles were not completely removed. It is of interest to note that the nitrogen content of the NR latex which was vulcanized before deproteinization; RV-DPNR, were greater than those of DP-RVNR. It is believed that the enzyme deproteinization was less efficient when the rubber particles had been previously irradiated. This might be due to the change of proteins structure when subjected to γ-ray which resulted in the decrease of enzyme activity.

Study of phase transfer technique

Phase transfer technique has been used to characterize the surface of non-crosslinked, γ-radiation vulcanized and deproteinized NR latices (13-15). This technique was applied for the study of the two types of crosslinked DPNR latices (RV-DPNR and DP-RVNR) in the present work. The CTC values obtained are presented in Table 4.

Table 4 : CTC values of RVNR, RV-DPNR and DP-RVNR latices

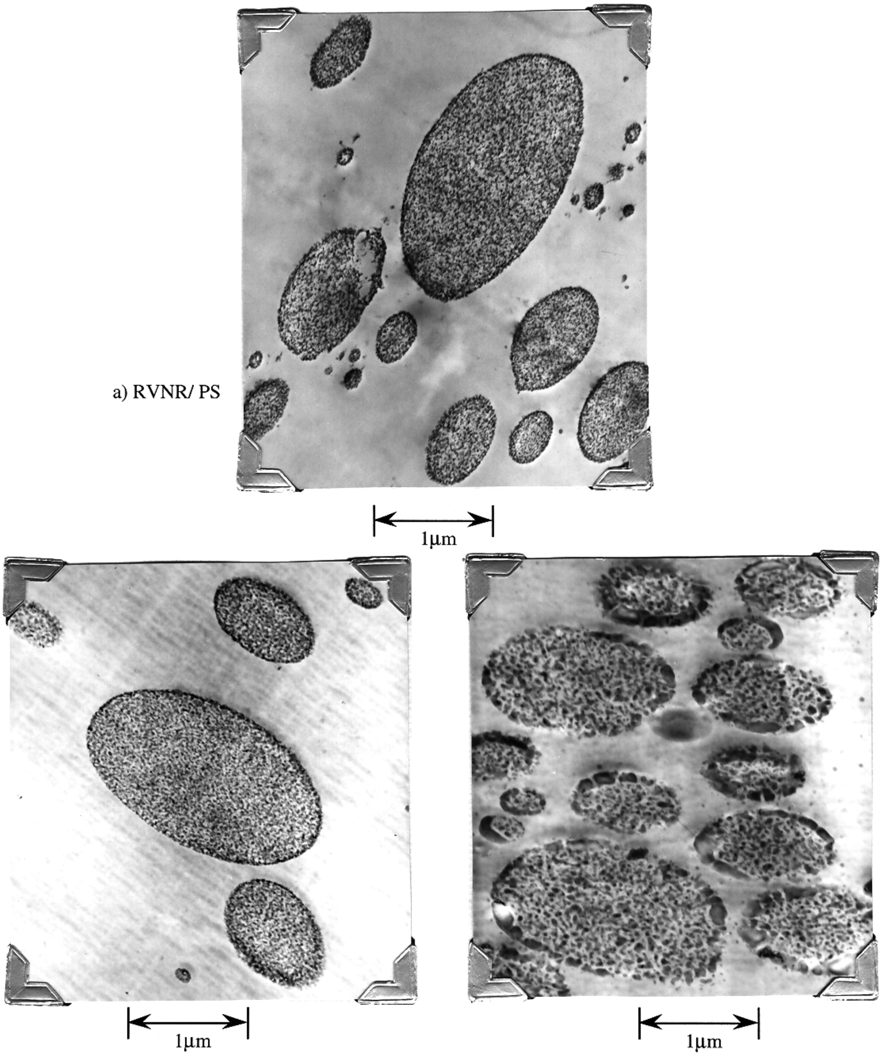


Type of NR	CTC (x 10^{-5})
RVNR	5.55 ± 0.03
RV-DPNR	13.37 ± 0.02
DP-RVNR	12.19 ± 0.05

From Table 4, it was found that the CTC values of the deproteinized latices, *i.e.*, RV-DPNR and DP-RVNR were approximately twice the value of RVNR latex. This could be due to an increase in the amount of negative charges on the surface of the DPNR particles resulted from the added anionic surfactant (SDS) during the deproteinization process. Consequently, the high quantity of the added cationic surfactant (BHAC) for neutralization of the rubber particles was required. Although, the CTC values of both deproteinized latices were similar, it was observed that the CTC of RV-DPNR latex was slightly higher than that of DP-RVNR. The results obtained indicated the greater amount of surface charge on the particles of the former latex which agreed well with the higher remaining proteins as shown by their higher nitrogen content in Table 3. Therefore, it was reasonable to assume that the phase transfer technique could be employed to indicate the presence of negative charges, partly derived from residual proteins, on the surface of DPNR latices (13).

Morphology of DPNR latex particles

Morphology of the crosslinked rubber particles (3%) incorporated in PS matrix, prepared by using the phase transfer/ bulk polymerization process, was studied under TEM. The micrographs of RVNR, RV-DPNR and DP-RVNR latex particles in PS are respectively illustrated in Figures 1a, b and c.



a) RVNR/ PS

b) RV-DPNR/ PS

c) DP-RVNR/ PS

Figure 1 : TEM of crosslinked rubber particles incorporated in PS matrix

All micrographs revealed two-phase morphology of the rubber particles (dark) dispersed in PS matrix (light). The size distribution of crosslinked NR particles was rather broad and their shape elliptical. In the rubber particles, the presence of internal PS was found and the size controlled by the crosslink rubber network as normally occurred in interpenetrating polymer networks (IPNs) (16). Moreover, it was important to observe, in Figures la and 1b, that the RVNR and RV-DPNR latex particles were surrounded by a membrane layer, possibly derived from protein-lipid complex (1, 17). This membrane was clearly broken out in the case of the DP-RVNR particles (Figure 1c). From the nitrogen content in Table 3 and the morphology in Figure 1, it is evident that the membrane of RV-DPNR particle was not significantly disturbed due to the remaining proteins on the vulcanized particles. On the contrary, deproteinization of the latex before irradiation (DP-RVNR) removed a large proportion of the proteins and therefore the membrane might be partly destroyed during deproteinization. These micrographs can be regarded as the direct evidence to confirm the presence of proteins in the membrane layer around the NR particles and, moreover, the elimination of major proteins in DP-RVNR. Our results are in agreement with the previous observation which reported that the deproteinization by proteolytic enzyme selectively cleavages peptide linkages (11). It should be mentioned that in the previous studies of the DPNR latex structure, the latex was firstly dried before analysis (3, 7, 8, 11) and the data were then used to interprete the DPNR structure. The phase transfer/ bulk polymerization process used in our work therefore successfully provided a direct information of the morphology of DPNR particles.

Conclusions

The degree of crosslinking of γ-radiation vulcanized NR latex was found to decrease when the proteins had been previously removed by using proteolytic enzyme. Inversely, this deproteinization method was less efficient, indicated by the lower nitrogen content, in the case of the crosslinked rubber particles. In addition, the CTC values, determined by phase transfer technique, of the DPNR latices indicated the presence of negative charges, partly derived from residual proteins, on the surface of rubber particles. By bulk polymerization of the styrene monomer containing the transferred deproteinized γ-radiation vulcanized NR (DP-RVNR) latex particles, the direct evidence of the remaining membrane layer around the particles was clearly obtained under TEM.

Acknowledgements

The authors gratefully acknowledge the ENO Science Foundation (Japan) and the Thailand Research Fund (TRF) for financial support. We would like to thank Prof. Y. Tanaka, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184 (Japan) and Dr. K. Suchiva, Mahidol University, Bangkok (Thailand) for stimulating discussions and very helpful suggestions. Special thanks go to the Office of Atomic Energy for Peace (Thailand) for the support of using ^{60}Co γ-ray source.

References

1. Gazeley KF, Gorton ADT, Pendle TD (1988) Latex concentrates: properties and composition. In: Roberts AD (ed) Natural Rubber Science and Technology. Oxford Science Publications, Oxford (Ch. 3)
2. Ho CC, Ng WL (1977) Colloid Polym Sci 257: 406
3. Othman AB, Hasma H (1988) Proceedings IRC. Penang, Malaysia

4. Sakaki T, Hioki Y, Kojima M, Kuga A, Tanaka Y (1995) Proceedings IRC. Kobe, Japan
5. Russel-Fell R (1993) Internat. Conference. “Latex protein allergy: the present position” Vrije Universiteit, Amsterdam, The Netherlands
6. Hayashi M, Nakade S, Sunagawa M, Kano T, Tsukamoto K, Tanaka, Y (1995) Proceedings IRC. Kobe, Japan
7. Eng AH, Kawahara S, Tanaka Y (1993) J nat Rubb Res 8: 109
8. Eng AH, Tanaka Y, Gan SN (1992) J nat Rubb Res 7: 152
9. Tangboriboonrat P, Tiyapiboonchaiya C accepted for publication in J Appl Polym Sci (July 18, 1998)
10. Sawyer LC, Grubb DT (1996) Polymer microscopy (2nd edition). Chapman&Hall, London (ch. 4)
11. Tangpakdee J (1998) Ph.D. Thesis, Tokyo university of agriculture and technology, Tokyo, Japan
12. Tanaka Y, Kawahara S, Tangpakdee J (1997) Kautschuk Gummi Kunststoffe 50: 6
13. Tangboriboonrat P, Suchiva K, Kuhakarn S, Tuchida P (1996) J nat Rubb Res 11: 26
14. Tangboriboonrat P, Suchiva K, Kuhakarn S (1994) Polymer 35: 5144
15. Tangboriboonrat P, Suchiva K, Riess G (1995) Polymer 36: 781
16. Sperling LH (1985) Pure and applied research on interpenetrating polymer networks and related materials. In: Walsh DJ, Higgins JS, Maconnachie A (ed) Polymer Blends and Mixtures. Martinus Nijhoff Publishers, Dordrecht (pp 267-287)
17. Gomez JB, Subramaniam A (1985) Proceedings IRC. Kuala Lumpur, Malaysia