J Rubb. Res., **1(3)**, 154–166

Synthesis of Graft Copolymers from Highly Deproteinised Natural Rubber

YUICHI FUKUSHIMA*, SEIICHI KAWAHARA*
AND YASUYUKI TANAKA*#

Highly deproteinised natural rubber (HDPNR) latex was prepared by incubation of commercial high-ammonia latex with a proteolytic enzyme in the presence of a surfactant, followed by repeated centrifugation for two times. The nitrogen content of HDPNR was less than 0.02%, suggesting the nearly complete removal of protein from the rubber. A graft copolymer of natural rubber with styrene was made from HDPNR latex using tert-butyl hydroperoxide/tetraethylenepentamine (TBHPO-TEPA) as an organic redox initiator. The styrene content, grafting efficiency and molecular weight of grafted polystyrene were significantly increased by deproteinisation. The number of grafting sites for graft copolymer from HDPNR latex was about three times larger than that determined for graft copolymer from untreated natural rubber latex. The difference between the behaviour of HDPNR and untreated natural rubber was due to the removal of protein and of naturally occurring antioxidants present in untreated natural rubber latex.

Synthesis of graft copolymers from natural rubber has been investigated in solution, latex and solid state[1]. Oil resistance of natural rubber was reported to be improved by graft polymerisation with acrylonitrile[2]. These graft copolymers were prepared by radical polymerisation[2–6], metalation with lithium catalyst[7] and living anionic polymerisation followed by ene-reaction[8,9] for the sake of achieving a high grafting efficiency. Since natural rubber is obtained from *Hevea brasiliensis* as latex, the most desirable graft polymerisation for the practical use must be carried out in the latex state.

Several approaches for graft polymerisation in latex have been investigated for the reaction between particulate natural rubber dispersed in latex and vinyl monomer by free radical polymerisation[3,5,6,10] or γ irradiation[6]. Some important reaction conditions were suggested to accomplish a homogeneous distribution of polymerised vinyl monomer in rubber particles However, little work has been done on the molecular weight of grafted polymers, number of graft points and graft efficiency. Furthermore, thermoplastic elastomers having good physical and mechanical properties have not been prepared due to the interference of naturally occurring non-rubber constituents that are present in natural rubber, *i.e.* proteins[1,11].

In some recent studies[12–15], highly purified natural rubber was prepared by deproteinisation

* Tokyo University of Agriculture and Technology, Faculty of Technology, Division of Applied Chemistry, Koganei, Tokyo 184, Japan
# Corresponding author

with a proteolytic enzyme in the presence of surfactant(s) The residual nitrogen content of highly deproteinised natural rubber (HDPNR) was less than 0 02%, which was attributed to the di- to tri- peptide sequences, suggesting complete removal of protein The HDPNR latex was stabilised with surfactant(s) to prevent coagulation A synthetic antioxidant was usually added to HDPNR because it contained less naturally occurring antioxidants The green strength of HDPNR from commercial high-ammonia latex was almost identical to that of untreated natural rubber in spite of the absence of protein[16] Tensile strength, modulus and permanent-set were almost the same for the sulphur-cured films from HDPNR and untreated natural rubber[17] The potential of Type 1 allergy was significantly reduced for HDPNR[18] These findings imply a possible use of HDPNR for graft copolymerisation without a loss of mechanical strength to make an allergy-free thermoplastic elastomer from natural rubber

In the present paper, the preparation of a graft copolymer of HDPNR with styrene in latex by using a free radical initiator is reported The molecular weight of grafted polymer, number of graft points and graft efficiency were estimated by GPC measurements of extracted graft copolymer and its ozonolysis products The reaction conditions were investigated for graft polymerisation of HDPNR with styrene monomer without degradation of the rubber, and the results were compared with those of untreated natural rubber

EXPERIMENTAL

Materials

Natural rubber latex used was commercial high-ammonia natural rubber (HANR) latex Styrene monomer (Kishida Chemical Co) was purified by washing with 10 w/v% KOH aqueous solution followed by distillation under reduced pressure Benzoyl peroxide (BPO), tert-butyl hydroperoxide (TBHPO), cumene hydroperoxide (CHPO), di-tert-butyl peroxide (DBPO), dicumyl peroxide (DPO), 4,4'-azobis(4-cyanovaleric acid)(ACVA), 2,2'-azobis(2,4-dimethylvaleronitrile)(V-65), 2,2'-azobis-isobutyronitrile (AIBN), tetraethylenepetamine (TEPA) and dimethylaniline (DMA) were used without purification

Enzymatic deproteinisation of natural rubber was carried out by incubation of the HANR latex diluted to 30% dry rubber content (DRC) with 0 04 w/v% proteolytic enzyme (Novo Alcalase 2 0T®) and 1 w/v% sodium dodecyl sulphate (SDS) for 12 h at 38°C followed by centrifugation at 10 000 g[14,15] The cream fraction was redispersed in 1 w/v% SDS to make 30% DRC and centrifuged again to prepare HDPNR latex The HDPNR latex was diluted with distilled water to make 8% DRC and SDS was added up to 0 04 w/v% No antioxidant was added to the latex

Graft Copolymerisation

Graft copolymers of natural rubber with styrene were prepared from untreated HANR latex and from HDPNR latex, respectively both of which were adjusted to 8% DRC The purified styrene of 8 ml and radical initiator of $1.6 \times 10^{-5} \sim 13.0 \times 10^{-5}$ mol/g-rubber was added to 100 ml latex and the mixture was gently stirred in a reaction vessel under nitrogen atmosphere for 1 h at room temperature If necessary, 0 23~1 8 ml of 10 w/v% TEPA solution was added to the mixture just before beginning the redox reaction The reaction was carried out by stirring the latex at 400 r p m for 2 h at a definite temperature between 30°C

and 80°C. The unreacted styrene was removed by using a rotary evaporator under reduced pressure. The as-prepared graft copolymer (gross polymer) was obtained by drying the reacted latex under reduced pressure for more than a week. A control experiment was conducted without styrene.

The gross polymer was extracted with acetone/2-butanone 3:1 mixture in a Soxhlet apparatus for 24 h under nitrogen atmosphere in the dark and dried under reduced pressure for more than 3 days.

Ozonolysis

Ozonisation was carried out by blowing an equimolar amount of ozone in ozonated oxygen (1.3%) through a 0.4 w/v% methylene chloride solution of the extracted graft copolymer at –30°C. Reductive degradation of the resulting ozonide was performed by reaction with lithium aluminum hydride ($LiAlH_4$) in diethyl ether followed by decomposition of residual $LiAlH_4$ with water[19]. After reductive degradation, grafted polystyrene, thus isolated from graft copolymer, was dissolved in a small amount of chloroform. The chloroform solution was centrifuged, and the polymer was precipitated by methanol.

Measurements

Gel contents were determined by treating 0.8 g of the graft rubber with 40 ml dried toluene, which was kept without stirring for a week in the dark at ambient temperature. The gel fraction was collected as a bottom fraction obtained by centrifugation at 10 000 g for 40 min. The soluble fraction of rubber was collected as a sol fraction, which was precipitated with an excess amount of methanol. The collected gel and sol fractions were dried under reduced pressure at 40°C for a week. The gel content was estimated from the masses of the gel and sol fractions.

Nitrogen content was determined by a Yanako CHN Corder MT-5 elemental analyser, using about 10 mg rubber samples.

GPC measurements were carried out for soluble fraction of the rubbers using a JASCO 980 PU high-pressure pump, JASCO 930 RI detector and JASCO 970 UV detector. Two preparative GPC columns (7.5 mm i.d. × 500 mm and 7.5 mm i.d. × 300 mm), packed with styrene-divinylbenzene copolymer were used in series using chloroform as eluent.

^{1}H-NMR spectra were recorded with a JEOL FX-200 spectrometer using 45° pulse, with pulse repetition time of 6 s, to determine the styrene content in the graft copolymer.

RESULTS AND DISCUSSION

Radical Initiator

The radical initiators examined for the graft copolymerisation are tabulated in *Table 1*, where half-lives of the initiators are also shown. Organic free radical initiators were mainly used in the present work to attain homogeneous distribution of polymerised vinyl monomer throughout the latex particles by penetration.

Table 2 shows the gel content, number average molecular weight ($\overline{M}_n$), weight average molecular weight ($\overline{M}_w$), and polydispersity ($\overline{M}_w/\overline{M}_n$) for HDPNR coagulated from the latex stirred at 60°C or 80°C for 2 h in the absence of radical initiator. It is evident from a

TABLE 1 RADICAL INITIATORS USED IN THE PRESENT WORK

Initiator	Half-life (min) (Temperature)	Reaction Temperature (°C)
Benzoyl peroxide (BPO)	4476 (61°C)	60
Tert-butyl hydroperoxide (TBHPO)	1255 (173°C)	60
Cumene hydroperoxide (CHPO)	385 (139°C)	60
Di-tert-butyl peroxide (DBPO)	600 (126°C)	60
Dicumyl peroxide (DPO)	600 (117°C)	60
4,4'-azobis(4-cyanovaleric acid)(ACVA)	252 (70°C)	60
2,2'-azobis(2,4-dimethylvaleronitrile)(V-65)	607 (51°C)	60
2,2'-azobis-isobutyronitrile (AIBN)	678 (70°C)	60
BPO-dimethylaniline	13 (20°C)	25
TBHPO-tetraethylenepentamine (TEPA)		30
CHPO-TEPA		40

TABLE 2 GEL CONTENT, $\overline{M}_n$, $\overline{M}_w$ AND $\overline{M}_w/\overline{M}_n$ FOR HDPNR AND HDPNR COAGULATED FROM THE LATEX STIRRED AT 60°C OR 80°C IN THE ABSENCE OF RADICAL INITIATOR

Rubber	Gel content	$\overline{M}_n/10^4$	$\overline{M}_w/10^5$	$\overline{M}_w/\overline{M}_n$
HDPNR	59.8	10.2	11.0	10.8
HDPNR at 60°C	66.1	9.7	10.4	10.7
HDPNR at 80°C	51.4	12.4	8.8	7.1

comparison of the stirred HDPNR with the untreated one that the values of $\overline{M}_w$ and $\overline{M}_w/\overline{M}_n$ were reduced by stirring at 80°C and the $\overline{M}_n$ value was increased. In contrast, the values of $\overline{M}_n$, $\overline{M}_w$ and $\overline{M}_w/\overline{M}_n$ did not change at 60°C. The gel content was significantly reduced by stirring at 80°C, while it was increased at 60°C. The decrease in $\overline{M}_w$ and increase in $\overline{M}_n$ at 80°C indicated that degradation occurred for mainly high molecular weight fractions of the rubber to form low molecular weight fractions This is consistent with the decrease in the gel content at 80°C. To prepare the graft copolymer at high yield, the degradation of

rubber has to be minimised Thus, it was desirable to use reaction temperatures less than 60°C for graft polymerisation with the radical initiator The reaction temperatures applied in this work are listed in *Table 1*, where the temperature was set to be low for organic redox initiators

Typical GPC curves for unreacted HDPNR and reacted HDPNR using radical initiator of 3.3×10^{-5} mol/g-rubber are shown in *Figure 1* The bimodal distribution characteristic of natural rubber was altered by the reaction with radical initiator At the reaction temperature of 60°C, BPO and AIBN changed significantly the molecular weight distribution of rubber, as shown in *Figure 1* The values of gel content, $\overline{M}_n$, $\overline{M}_w$, and $\overline{M}_w/\overline{M}_n$ are summarised for the reacted HDPNR in *Table 3* The molecular weight values were decreased to one-half or one-third by the addition of ACVA, AIBN, BPO or V65 The gel content was significantly reduced by ACVA, BPO and V65 and was about one-half by AIBN These results demonstrate that ACVA, AIBN, BPO and V65 act predominantly to degrade HDPNR On the other hand, the values of gel content, $\overline{M}_n$, $\overline{M}_w$ and $\overline{M}_w/\overline{M}_n$ for HDPNR stirred with CHPO, DBPO, DPO or TBHPO were almost identical to those of unreacted HDPNR suggesting that little reaction occurred at 60°C due to inactivity of the initiator In fact, styrene monomer was not polymerised by CHPO, DBPO, DPO and TBHPO

As shown in *Table 3*, the gel content was slightly reduced by using the organic redox initiators such as TBHPO-TEPA and BPO-DMA, but reduced significantly by CHPO-TEPA The values of $\overline{M}_n$, $\overline{M}_w$ and $\overline{M}_w/\overline{M}_n$ of the rubber were not changed by BPO-DMA, while $\overline{M}_n$ and $\overline{M}_w/\overline{M}_n$ were decreased to about one-half by TBHPO-TEPA Styrene was polymerised with TBHPO-TEPA, but not with BPO-DMA because half-life was extremely short Moreover, the ability of TBHPO-TEPA to initiate polymerisation was reported to be independent of the presence of ammonium cation[11] Consequently, TBHPO-TEPA was clarified to be the most appropriate radical initiator for the graft copolymerisation of HDPNR, this combination has been used for graft polymerisation in untreated natural rubber in previous work[3,5,6]

Graft Copolymerisation

The styrene content of the as prepared graft copolymer (gross polymer) from HDPNR grafted with polystyrene (HDPNR-PS) was compared with that of gross polymer from HANR grafted with polystyrene (HANR-PS) in *Figure 2* The styrene content of HDPNR-PS increased as the TBHPO-TEPA concentration increased, while the content of HANR-PS was almost independent of the initiator level The styrene content of HDPNR-PS was higher than that of HANR-PS by four-times at higher level of TBHPO-TEPA The difference in the styrene content may be explained due to the absence of protein and naturally occurring antioxidant in HDPNR, since the protein and naturally occurring antioxidant present in HANR react easily with free radical

The gross polymer was extracted with acetone/2-butanone 3 1 mixture In *Figure 3* the extracted soluble fraction was shown to be a mixture of polystyrene and low molecular weight rubber, the isoprene unit content of which ranged from 1 1% to 6 0% Thus, the isoprene content of the soluble fraction was

TABLE 3 GEL CONTENT, $\overline{M}_n$, $\overline{M}_w$ AND $\overline{M}_w/\overline{M}_n$ FOR HDPNR COAGULATED FROM THE LATEX STIRRED AT 60°C IN THE PRESENCE OF RADICAL INITIATOR

Initiator	Gel content	$\overline{M}_n/10^4$	$\overline{M}_w/10^5$	$\overline{M}_w/\overline{M}_n$
BPO	10 7	6.9	3.9	5 8
TBHPO	54.2	7.5	8 0	10 6
CHPO	61.1	14 6	11.6	8 1
DBPO	61.1	18.6	13.6	7.3
DPO	56 9	9 1	12 7	11 0
ACVA	3 5	11 0	7 0	6 4
V-65	5 5	8 2	5 3	6.6
AIBN	32.2	9 0	6 9	7.7
BPO-DMA	39.7	11 5	12.7	11 0
TBHPO-TEPA	54.1	11.3	5.8	4 6
CHPO-TEPA	19 3	7 6	4 5	6 0

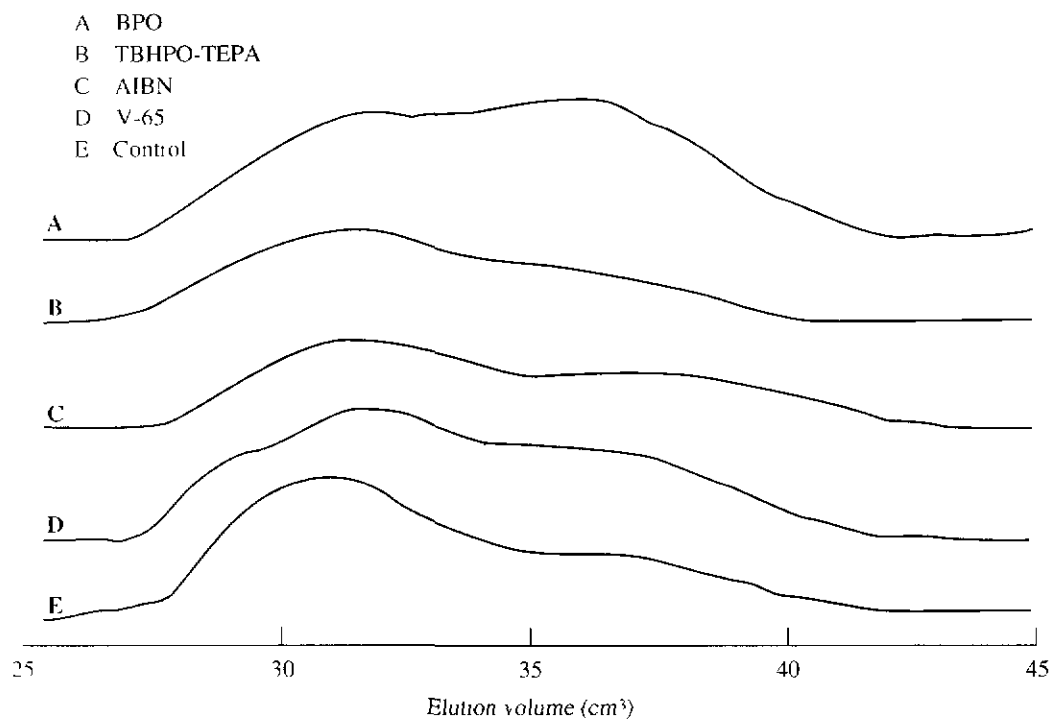

Figure 1 GPC curves for unreacted HDPNR and HDPNR reacted with radical initiators The intensity in vertical axis is represented in arbitrary unit for the comparison.

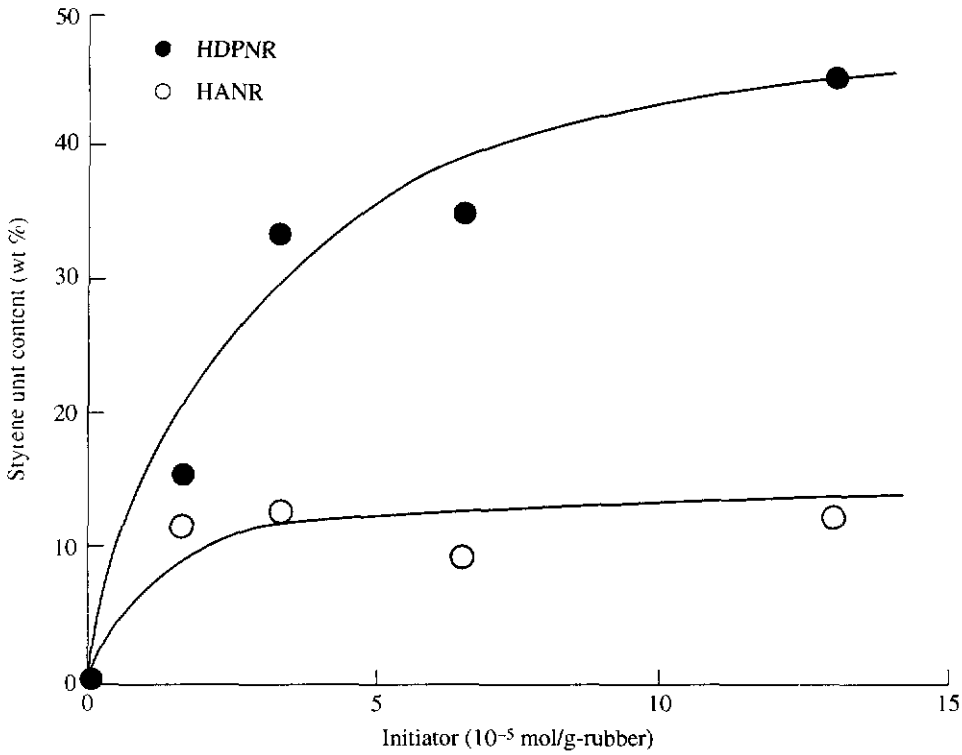

Figure 2. Styrene content of as-prepared graft copolymer (gross polymer) prepared from HDPNR and from HANR.

negligibly low and the graft copolymer was assumed to be free from PS.

The grafting efficiency (γ) was estimated as follows:

$$\gamma = \frac{\text{Mass of grafted monomer (g)}}{\text{Mass of polymerised monomer (g)}} \times 100 \quad \ldots 1$$

where the mass of polymerised monomer is the styrene content (by mass) of the gross polymer and the mass of grafted monomer is the styrene content of the acetone/2-butanone insoluble fraction. In *Figure 4*, the dependence of grafting efficiency on the initiator concentration is shown for HDPNR and HANR. The value of grafting efficiency for HDPNR was distinguishably higher than that of HANR. This demonstrates that the ability to form graft copolymer for HDPNR is significantly enhanced by removing protein, a part of which covered the surface of the HANR particles. The plot of grafting efficiency *versus* initiator concentration showed a smooth maximum, from which the largest grafting efficiency provided the best initiator concentration, being 3.3×10^{-5} mol/g-rubber. The decrease in grafting efficiency with further increasing initiator concentration may be a consequence of side reactions such as homopolymerisation of styrene monomer occurring at higher initiator concentrations.

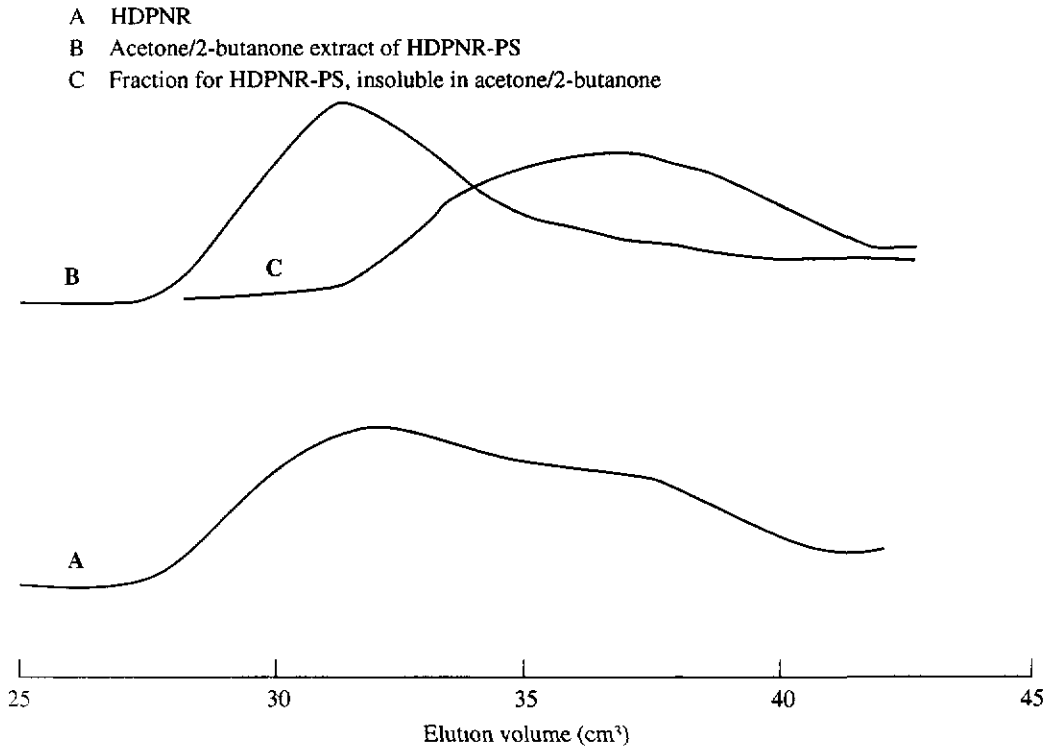

Figure 3. GPC curves for HDPNR, acetone/2-butanone extract of HDPNR-PS and fraction for HDPNR-PS, insoluble in acetone/2-butanone.

Gel Content and Molecular Weight of Graft Copolymer

Table 4 shows the gel content and estimated values of $\overline{M}_n$, $\overline{M}_w$ and $\overline{M}_w/\overline{M}_n$ for sol fractions of HDPNR-PS and HANR-PS. The values of $\overline{M}_n$, $\overline{M}_w$, and $\overline{M}_w/\overline{M}_n$ for the sol fraction of HDPNR-PS were quite similar to those for HANR-PS, and they decreased as initiator concentration increased. On the other hand, the gel contents of HDPNR-PS, which appeared to be increased to some extent by graft copolymerisation of HDPNR, was smaller than that of HANR-PS. The difference in gel content between HANR-PS and HDPNR-PS must be due to the influence of non-rubber components. The decrease in molecular weights of the sol fraction and increase in the gel content with increasing initiator level demonstrate that not only graft copolymerisation but also degradation and gelation occurred as the initiator concentration was increased.

The styrene content and grafting efficiency for the graft copolymers prepared from HDPNR were significantly larger than those for the graft copolymers from HANR despite the fact that the molecular weights of the sol fractions were similar for HDPNR-PS and HANR-PS. To explain the relationship among styrene content, grafting efficiency and molecular

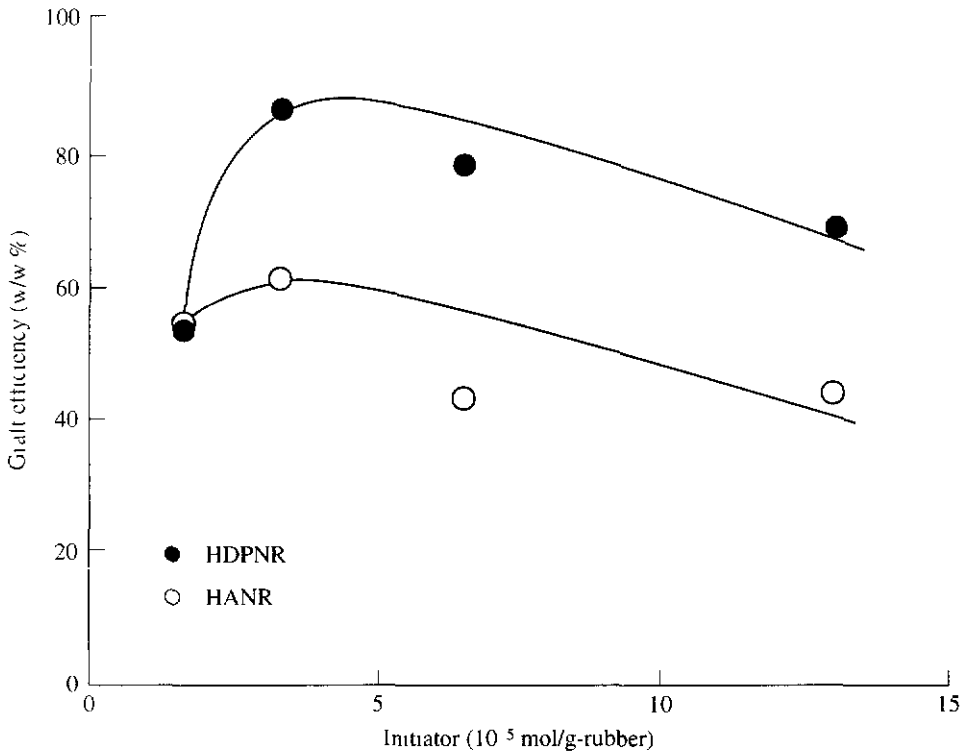

Figure 4 Grafting efficiency versus *initiator, TBHPO-TEPA, concentration, for HDPNR and HANR*

weight of graft copolymer, it was important to estimate the molecular weight of the grafted PS for the sol and gel fractions of both rubbers The grafted PS was isolated from the gross polymer by extraction with acetone/ 2-butanone 3.1 mixture followed by ozonolysis of the insoluble fraction at low temperature The estimated values of $\overline{M}_n$, $\overline{M}_w$, $\overline{M}_w/\overline{M}_n$ for the grafted PS are listed in *Table 5* The values for the grafted PS from both HDPNR-PS and HANR-PS did not vary greatly with the initiator concentration. The values for grafted PS from HDPNR were higher than those for grafted PS from HANR

An average number of grafted sites per chain ($\bar{n}$) for each sol fraction from HDPNR and HANR was estimated from the molecular weights of both grafted polystyrene and graft copolymer as follows

$$\bar{n} = \frac{(\overline{M}_n \text{ for graft copolymer}) \times (\text{mole fraction of styrene units})}{(\overline{M}_n \text{ for grafted polystyrene})} \qquad 2$$

TABLE 4 GEL CONTENT, $\overline{M}_n$, $\overline{M}_w$ AND $\overline{M}_w/\overline{M}_n$ FOR HDPNR-PS AND HANR-PS COAGULATED FROM THE LATEX GRAFTED AT 30°C TBHPO-TEPA AS INITIATOR

Initiator concentration (10^{-5} mol/g-rubber)	Gel content	$\overline{M}_n/10^4$	$\overline{M}_w/10^5$	$\overline{M}_w/\overline{M}_n$
HANR-PS				
1 6	70 7	7 2	7 3	10 1
3 3	64 4	8 6	5 0	5 8
6 5	76.8	6 0	3 9	6.5
13 0	72.2	5 8	2.1	3 6
HDPNR-PS				
1 6	42.0	7.2	7.0	9.7
3 3	64.0	5.8	4.3	7.4
6 5	60.0	5 8	3.9	6 7
13 0	61.3	4 8	3.0	6.3

where the value of $\overline{M}_n$ for grafted PS is assumed to be similar for the sol and gel fractions. As shown in *Table 5*, the value of $\bar{n}$ for HDPNR-PS was larger than that of HANR-PS by three times, except for the graft copolymer prepared with the initiator concentration of $1\,6 \times 10^{-5}$ mol/g-rubber. The value of $\bar{n}$ was the largest at the initiator concentration of 3.3×10^{-5} mol/g-rubber for the both rubbers, respectively. This implies that the initiator is consumed mainly for graft copolymerisation at 3.3×10^{-5} mol/g-rubber, but less for degradation and homopolymerisation.

In a previous work[5], the grafting efficiency and average number of grafted sites of graft copolymer, prepared from untreated natural rubber latex by radical copolymerisation, was reported to be such that the reaction product was unsuitable for commercial use as thermoplastic elastomer In the present work, the grafting efficiency and average number of grafted sites were significantly increased by deproteinisation. This demonstrates that the reaction of the rubber molecule with radical initiator was made more effective by removing both protein and naturally occurring antioxidant. Furthermore, it is clearly shown that, at the initiator concentration of 3.3×10^{-5} mol/g-rubber, the graft copolymerisation of HDPNR occurred preferentially over degradation and homopolymerisation.

CONCLUSION

Highly deproteinised natural rubber (HDPNR) latex was prepared by incubation of commercial high-ammonia latex with a proteolytic enzyme in the presence of a surfactant, followed by centrifugation twice.

TABLE 5 $\overline{M}_n$, $\overline{M}_w$, $\overline{M}_w/\overline{M}_n$ AND AVERAGE NUMBER OF GRAFTED SITES PER CHAIN, $\bar{n}$, OF GRAFTED COPOLYMER EXTRACTED FROM HDPNR-PS AND HANR-PS COAGULATED FROM THE LATEX GRAFTED AT 30°C USING TBHPO-TEPA AS INITIATOR

Initiator concentration (10^{-5} mol/g-rubber)	$\overline{M}_n/10^4$	$\overline{M}_w/10^5$	$\overline{M}_w/\overline{M}_n$	$\bar{n}$
HANR-PS				
1 6	6.4	1.6	2.6	0.50
3 3	5.5	0.9	1.6	0 95
6.5	5 8	1.2	2.0	0.30
13 0	5.1	0.9	1.6	0.45
HDPNR-PS				
1 6	10 4	3 3	3 2	0.41
3 3	4.3	1.2	2 9	3 13
6.5	9 3	5.1	5 5	1 36
13.0	9.0	4.1	4 5	1.48

The molecular weight of HDPNR was not changed when the latex containing cumene hydroperoxide (CHPO), di-tert-butyl peroxide (DBPO), dicumyl peroxide (DPO) or tert-butyl hydroperoxide (TBHPO) was stirred. The gel content of HDPNR increased by stirring with TBHPO-tetraethylenepentamine (TEPA) at 30°C, whereas the values of $\overline{M}_n$, $\overline{M}_w$ and $\overline{M}_w/\overline{M}_n$ were reduced. Use of the initiator TBHPO-TEPA at 30°C and a concentration of 3.3×10^{-5} mol/g-rubber produced graft copolymer of high styrene content with good grafting efficiency, as well as suppressing both degradation of the rubber and homo-polymerisation of styrene. The grafting efficiency and styrene content of HDPNR-PS copolymers were higher than for HANR-PS copolymers.

The graft copolymer was isolated from the gross polymer by extraction with acetone/ 2-butanone 3:1 mixture. The molecular weight of the grafted polystyrene, which was determined by GPC measurement of ozonolysis products of the extracted graft copolymer, was significantly increased by the removal of protein from HANR. The number of grafted sites for HDPNR-PS copolymers was larger than that for HANR-PS copolymers. The differences in styrene content, grafting efficiency, molecular weight, gel content and number of grafted sites between HDPNR and untreated natural rubber were explained on the basis of the removal of protein and naturally occurring antioxidant from untreated natural rubber, since they react easily with free radical.

ACKNOWLEDGEMENT

This work was supported partly by a Grant-in-Aid (No. 07555594) from the Ministry of Education, Science, Sports and Culture of Japan.

Date of Receipt: March 1998
Date of Acceptance: August 1998

REFERENCES

1 CAMPBELL, D S (1988) *Natural Rubber Science and Technology* (Roberts, A.D. ed.), p 679. Oxford: Oxford University Press.

2. ALLEN, P.W. (1963) *The Chemistry and Physics of Rubber-like Substances* (Bateman, L. ed.), Chap. 5. London: Maclaren.

3. BLOOMFIELD, G.F. AND SWIFT, P.M. (1955) The Polymerization of Vinyl Monomers in Natural Rubber Latex *J. Appl. Chem.*, **5**, 609–615

4 LUNDSTEND, O.W. AND BEVILACQUA, E.M. (1957) Grafting in Natural Rubber. *J. Polym. Sci.*, **24**, 292–297

5. ALLEN, P.W AND MERRETT, F.W. (1956) Polymerization of Methyl Methacrylate in Polyisoprene Solutions *J. Polym. Sci.*, **22**, 193–201

6 COCKBAIN, E G., PENDLE, T.D. AND TURNER, D.T. (1959) Formation of Graft Polymers by γ Irradiation of Natural Rubber Latex and Methyl Methacrylate. *J. Polym. Sci.*, **39**, 419–426.

7. TATE, D.P., HALASA, A.F, WEBB, F.J., KOCH, R.W. AND OBERSTER, A.E. (1971) Metallation of Unsaturated Polymers and Formation of Graft Copolymers *J. Polym. Sci.*, Part A-1, **9**, 139–145.

8. CAMPBELL, D S, LEOBER, D.E. AND TINKER, A J. (1978) Preparation of Polydiene-g-polystyrene Graft Copolymers. *Polymer*, **19**, 1106–1107.

9. CAMPBELL, D.S AND SEOW, P.K (1990) Graft Copolymers of *cis*-1,4-Polyisoprenes with Poly(methyl methacrylate) *J. nat. Rubb. Res.*, **5(2)**, 135–143.

10 ERBIL, H.Y. (1986) Graft Copolymerization of Some Hydrophilic Vinyl Monomers in Natural Rubber. *J. nat. Rubb. Res.*, **1(4)**, 234–239.

11. HO, C.C., KONDO, T., MURAMATSU, N. AND OHSHIMA, H. (1996) Surface Structure of Natural Rubber Latex Particles from Electrophoretic Mobility Data. *J. Coll. Interface Sci.*, **178**, 442–445

12. ENG, A.H., TANAKA, Y. AND GAN, S.N (1992) FTIR Studies on Amino Groups in *Hevea* Rubber *J. nat. Rubb. Res.*, **7(2)**, 152–155

13. ENG, A.H, KAWAHARA, S AND TANAKA, Y. (1993) Determination of Low Nitrogen Content of Purified Natural Rubber *J. nat. Rubb. Res.*, **8(2)**, 109–113.

14. ENG, A.H., EJIRI, S, KAWAHARA, S AND TANAKA, Y. (1994) Structural Characteristics of Natural Rubber – Role of Ester Groups *J. Appl. Polym. Sci. Polym. Symp.*, **53**, 5–14.

15. TANAKA, Y, KAWAHARA, S. AND TANGPAKDEE, J. (1997) Structural Characterization of Natural Rubber. *Kautsch. Gummi Kunstst.*, **50**, 6–11.

16. SAKAKI, T., HIOKI, Y., KOJIMA, M., KUGA, A AND TANAKA, Y. (1996) Highly Purified Natural Rubber I Preparation of Anionic Latex and its Physical Properties *Nippon Gomu Kyokaishi*, **69**, 553–556

17 NAKADE, S , KUGA, A , HAYASHI, M. AND TANAKA. Y (1997) Highly Purified Natural Rubber IV Preparation and Characterisation of Gloves and Condoms. *J. nat. Rubb. Res.*, **12(1)**, 33–42.

18. HAYASHI, M.. NAKADE, S., SUNGAGAWA, M., KANO, T., TSUKAMOTO, K. AND TANKA, Y. (1996) Highly Purified Natural Rubber III Biological Properties *Nippon Gomu Kyokaishi*, **69**, 562–565

19 TANAKA, Y., KAWAHARA, S , IKEDA, T AND TANAMAI. H (1993) Analysis of the Sequence Distribution of 1,2 Units in Polybutadiene by an Ozonolysis-GPC Method *Macromolecules*, **26**, 5253–5255.