

Determination of Low Nitrogen Content of Purified Natural Rubber

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The RRIM test method used in natural rubber nitrogen content analysis was modified for the determination of low levels of nitrogen in raw deproteinised natural rubber. The modified method gives good reproducibility and accuracy as indicated by elemental analysis. FTIR analysis through the measurement of the intensity ratio of N-H stretching to =CH out-of-plane deformation band provides a rapid method for the quantification of low nitrogen content of purified natural rubber.

Natural rubber has been known to contain about 2% of naturally occurring protein. Recently, there has been a growing concern on this non-rubber component in rubber products as it can sensitise atopic persons and cause allergy^{1,2,3}. Therefore, the preparation of low protein latex and natural rubber has become increasingly important.

Protein of natural rubber is normally related to nitrogen content by the formula: Protein = $6.25 \times$ nitrogen content. Nitrogen content of commercial natural rubber can be determined by the Kjeldahl method as described in RRIM SMR Bulletin No. 7⁴. There are essentially three major steps in the method, namely; digestion, distillation and titration. The rubber sample is first digested in concentrated sulphuric acid at high temperature in the presence of a catalyst mixture. The sample is digested completely and nitrogen compounds are converted into ammonium hydrogen sulphate. The digest is then made alkaline and liberated ammonia is subsequently steam distilled into boric acid solution and titrated with standard acid. However, it is rather difficult to determine accurately low levels of nitrogen in purified natural rubber ($N < 0.03\%$) by the same method as the titer value obtained in the titration step would be much lower than that of commercial natural rubber which contains a much higher level of nitrogen. Since the

RRIM method has been commonly adopted in raw rubber analysis, the present work seeks to employ the method with some modifications for the determination of low nitrogen content of natural rubber. In addition, the presence of N-H stretching band in fourier transform infrared spectroscopy (FTIR) spectrum of purified natural rubber having nitrogen as low as 0.01% has been illustrated⁵. This offers an alternative method for the quantification of low levels of nitrogen in purified natural rubber by FTIR with suitable independent calibration.

EXPERIMENTAL

Deproteinisation of Natural Rubber

Commercial high ammonia latex was used in the present study. Deproteinisation of latex is essentially similar to that previously described⁵. Typically the latex was diluted to 6%–23% dry rubber content and stabilised with 0.2%–1% sodium dodecyl sulphate. After adjusting the pH to 9.2 with sodium dihydrogen phosphate, the latex was allowed to react with 0.04% w/v alcalase 2.0T enzyme for 24 h at 37°C. It was then either centrifuged once or twice and the rubber coagulated by acetone. The coagulated rubber was pressed and cut into small pieces, washed extensively with distilled water and dried under *vacuo*. Other

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enzymes and surfactants have also been used and details will be reported separately.

Analysis by Kjeldahl Method

The analysis of nitrogen content of natural rubber, with the exception of some modifications summarised in *Table 1*, is essentially similar to that of RRIM Test Method B7.

TABLE 1 COMPARISON OF THE CONDITIONS EMPLOYED IN NITROGEN CONTENT ANALYSIS

Method	RRIM Test Method B7	Present work (Modified method)
Sample size	0.1 g	0.3 g
Concentrated H_2SO_4	2.5 ml	4.5 ml
Catalyst ^a mixture	0.65 g	0.85 g
Ionic-exchanged distilled water for transferring digest	20 ml	40 ml
Treatment of diluted digest during addition of NaOH	—	Cooled to ice-water temperature
Distillate collection	5 min	ca 25 ml
H_2SO_4 for titration	0.01 N	0.008 N

^a Potassium sulphate-copper sulphate pentahydrate-selenium = 15 : 2 : 1 (weight ratio)

sulphuric acid used in the digestion step, 5 ml/100 mg rubber, was much higher than that of the RRIM method. The use of excessive concentrated sulphuric acid is undesirable since this would require the use of a stronger alkali particularly for the blank which may accelerate the liberation of ammonia before steam distillation.

Table 2 illustrates the results of five nitrogen content analyses effected on the same purified natural rubber. Analysis performed with the modified method shows good reproducibility with a variation of 1.5%. Digestion of the same sample using less amount of

TABLE 2 REPRODUCIBILITY OF NITROGEN CONTENT ANALYSIS OF PURIFIED NATURAL RUBBER

Run No	Catalyst mixture/g	Nitrogen content/ $\times 10^2\%$ w/w
1	0.85	2.33
2	0.85	2.31
3	0.85	2.30
4	0.85	2.23
5	0.85	2.27
Average		2.29 \pm 0.03
6	0.65	2.09
7	0.65	2.08
Average		2.09 \pm 0.01

Conditions employed: Modified method described in *Table 1* except for the catalyst mixture

FTIR Analysis

Approximately 0.05 g of sample was dissolved in 4 ml chloroform and cast on potassium bromide disk, dried under a stream of nitrogen gas. FTIR absorbance measurements were made with a JASCO 5300 FTIR spectrometer with auto gain in the presence of desiccant such that moisture absorption by KBr disk or sample can be minimised. The spectra are obtained from an average of 300 scans.

RESULTS AND DISCUSSION

Modified RRIM Method

Tristram⁶ used the Kjeldahl method in the determination of rubber nitrogen content at the level of 0.01%. However, the amount of

TABLE 3 ANALYSIS OF NITROGEN CONTENT OF NATURAL RUBBER

Sample	Nitrogen content/ %w/w	
	Kjeldahl method ^a	Elemental analysis ^b
DPNR	0.015	0.015
Pale crepe	0.38	0.38
Phenacetin ^c		7.82

^a Average of triplicate

^b Analysed by Yanaco Analytical Industries, Japan using Yanaco CHN Corder MT-5 Elemental Analyser

^c Calculated value: %N = 7.82

catalyst mixture (0.65 g) gives a slightly lower nitrogen content value. This probably is an indication that complete digestion of the samples has not been achieved within the same period of time (approximately 1 h) and thus longer digestion is probably required. However, it should also be noted that excessive heating after digestion is complete and may result in oxidation of ammonium ion and consequently liberation of nitrogen gas⁷.

The accuracy of the modified method in nitrogen content determination was checked by elemental analysis of two samples contain-

ing different levels of nitrogen. Analysis of a standard sample, phenacetin (%N = 7.82) by the elemental analyser shows good accuracy when compared with the calculated value. Table 3 shows that analysis results obtained from the modified method agree well with that of elemental analysis for both commercial pale crepe with normal level and deproteinised natural rubber with a low level of nitrogen. This is not totally unexpected since both methods analyse nitrogen content of the sample after complete destruction. Therefore, the modified method is also applicable to commercial natural rubber.

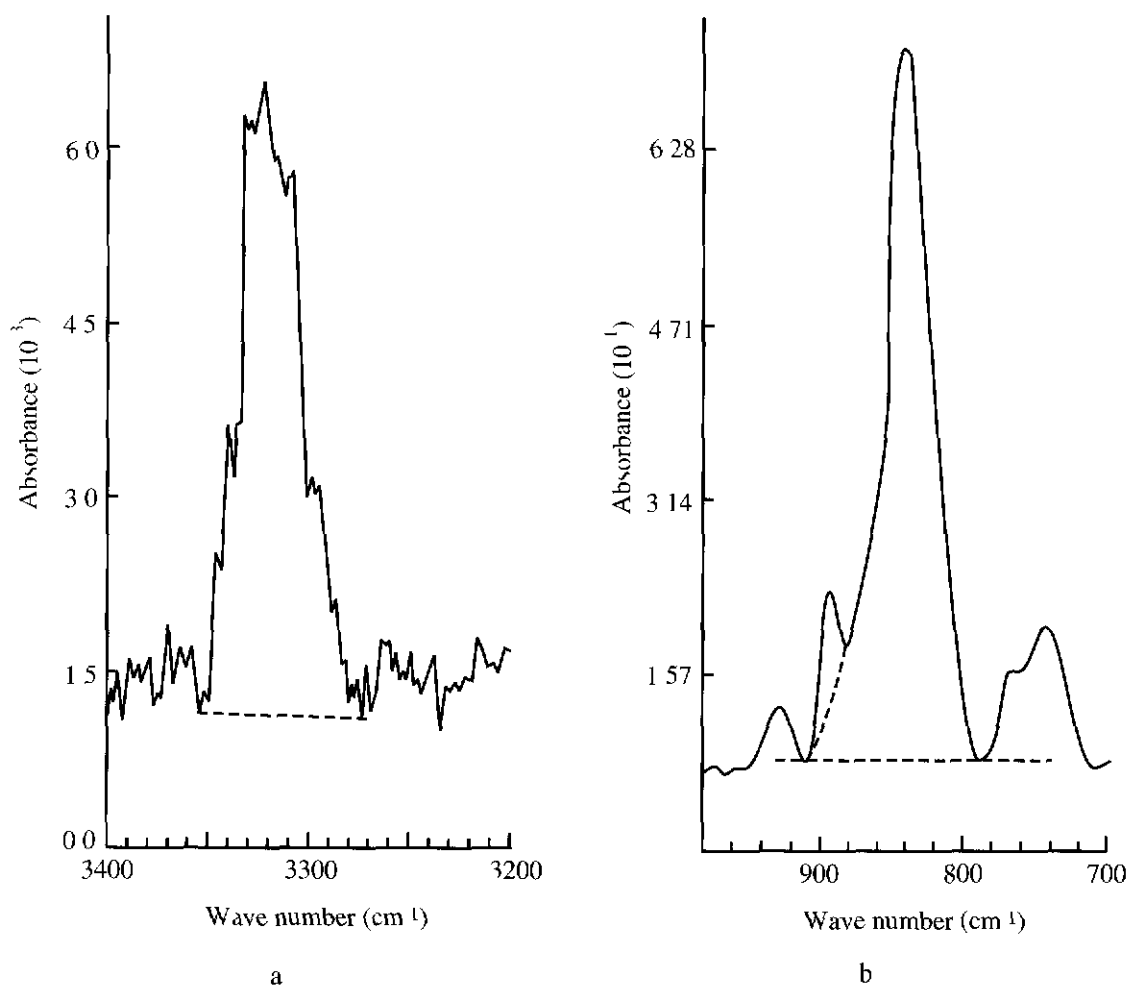


Figure 1 FTIR spectra of deproteinised natural rubber a) N-H stretching, b) =CH out-of-plane deformation band

FTIR Analysis

It has been disclosed⁵ that non-purified raw natural rubber shows N-H stretching band at 3280 cm^{-1} , which is a characteristic of natural rubber protein. Deproteinised rubber, on the other hand, has the vibrational frequency centred at about $3316\text{--}3320\text{ cm}^{-1}$ due to residual peptide or amino acid. Since almost all the nitrogenous materials in the raw natural rubber are peptides, the measurements of the intensity of N-H stretching band together with suitable vibrational band arising from rubber polyisoprene as reference, can be used for the determination of nitrogen content of deproteinised rubber if an independent calibration is made.

Figure 1 a) and b) show the N-H stretching and $=\text{CH}$ out-of-plane deformation bands of purified natural rubber, respectively. The integrals of these bands were extended from 3347 cm^{-1} to 3277 cm^{-1} and 907 cm^{-1} to 780 cm^{-1} , respectively. Figure 2 illustrates the relationship between natural rubber nitrogen content analysed by the modified method and area ratio of N-H stretching to $=\text{CH}$ out-of-plane deformation band. Band area ratio was used such that the N-H stretching band intensity can be measured with better accuracy. A linear relationship (2.22% w/w nitrogen/unit ratio) was obtained for samples with low levels of nitrogen ($\leq 0.02\%$). For samples with higher nitrogen content, deviation from this linear relationship was found. Apparently, this is due

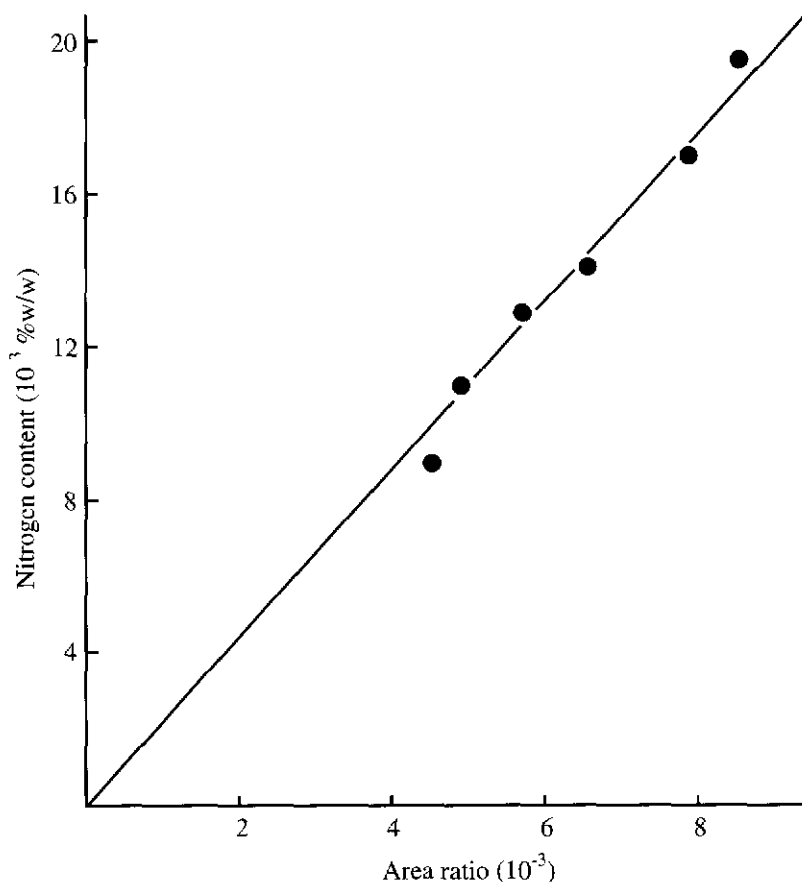


Figure 2. Relationship between nitrogen content and intensity ratio of N-H stretching to $=\text{CH}$ out-of-plane deformation band.

to the broadening of N-H band arising from the formation of more hydrogen bonding as shown in *Table 4*.

TABLE 4 BAND BROADENING OF N-H STRETCHING

Nitrogen content/%w/w	N-H stretching ^a band's width/cm ⁻¹
0.009 – 0.020	70
0.039	107
0.049	118
0.067	130

^aLength of base-line as indicated in *Figure 1a*

Although FTIR offers rapid determination of nitrogen content of deproteinised natural rubber, however, care must be taken to exclude moisture, hydroxyl containing additive or nitrogenous materials without N-H group from the sample or the validity of the calibration would be nullified.

A comparative study on the analysis of a low nitrogen natural rubber sample using Kjeldahl, elemental analyser and FTIR is shown in *Table 5*. The results obtained from Kjeldahl and FTIR methods show fairly good agreement with that from the elemental analyser. However, it must be noted that the use of elemental analyser requires special care in trace analysis of nitrogen because the quantity of sample used is very much lower than that of Kjeldahl method and therefore it is very susceptible to the presence of small amounts of contaminant.

TABLE 5 A COMPARATIVE ANALYSIS OF NITROGEN CONTENT OF DEPROTEINISED NATURAL RUBBER

Nitrogen content/%w/w	Kjeldahl ^a	Elemental analyser ^{a,b}	FTIR ^a
DPNR	0.009 ± 0.001	0.010 ± 0.002	0.009 ± 0.001

^aAverage of triplicate analysis

^bYanaco CHN Corder MT-5 Elemental Analyser

CONCLUSION

The modified RRIM test method has been shown to be a more sensitive method than the original method for the determination of low levels of nitrogen in highly purified natural rubber. The accuracy of this method has been shown to be satisfactory by elemental analysis. FTIR analysis provides a rapid method for the quantification of low levels of nitrogen provided that no significant amount of moisture, hydroxyl containing additives or nitrogenous materials without N-H group are present.

ACKNOWLEDGEMENT

The authors thank Eno Science Foundation, Japan for providing financial support to Eng Aik-Hwee to carry out part of this work.

Date of receipt: November 1992

Date of acceptance: April 1993

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