JST: Engineering and Technology for Sustainable Development
Volume 35, Issue 1, March 2025, 051-058

Analytical Methods for Determination of Total Nitrogen Content in the Process of Synthesizing Chemically Modified Rubber Materials

Than Van Hau, Vu Tue Minh, Dinh Duy Nham, Tran Huy Trong, Tran Quang Tung, Tran Thi Thuy*
Hanoi University of Science and Technology, Ha Noi, Vietnam
**Corresponding author email: thuy.tranthi3@hust.edu.vn*

Abstract

In the study, the total nitrogen content of natural rubber was determined to evaluate the effectiveness of deproteinization on the synthesis of chemically modified rubber materials. The remaining protein content in the obtained deproteinized natural rubber (DPNR) was analyzed as nitrogen content using the Kjeldahl method. In this method, the amine nitrogen present in the proteins was converted into ammonia, which was subsequently determined by chemical and physical methods. From the findings obtained from the examination of experimental conditions, a novel procedure was devised to determine the total nitrogen content in rubber using traditional titration, potential titration and UV-Vis spectrophotometry methods. The newly established techniques exhibited favorable results regarding the method detection limit (MDL) and limit of quantification (LOQ). Among these methods, spectrophotometry displayed exceptional sensitivity, enabling precise and accurate quantification of low nitrogen concentrations. Notably, these methods exhibit a high degree of recovery, ranging from 94.5% to 106.3%.

Keywords: Nitrogen content, deproteinized natural rubber, Kjeldahl method.

1. Introduction

In the study of synthesizing chemically modified rubber materials from natural rubber (NR), the general process includes the removal of proteins from NR, subsequent grafting polymerization with monomers, and coprecipitation of modified deproteinized natural rubber (DPNR) with appropriate fillers [1, 2].

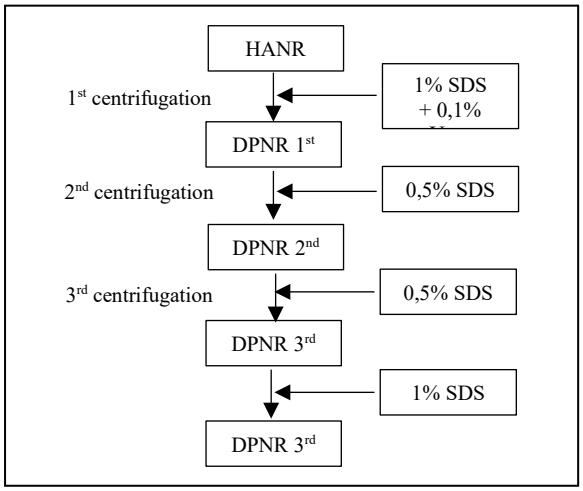

Fig. 1. Procedure of protein removal from NR

Protein removal is accomplished by removing the protein layer covering the rubber particles [3, 4]. After the rubber is deproteinized, grafting polymerization with monomers will be more effective. Examining the effectiveness of removing protein from rubber will be done by evaluating the total nitrogen content of the product after protein removal, which is called protein nitrogen content. The depletion of nitrogen content in the sample is directly correlated with the effectiveness of protein separation. As protein separation improves, the nitrogen content in the sample decreases accordingly.

The Kjeldahl nitrogen analysis method is the global standard for calculating protein content in a variety of sample materials. However, the application of this method in the synthesis of chemically modified natural rubber has not yet been scientifically and specifically evaluated with a focus on the analysis of nitrogen content. The determination of total nitrogen content through the Kjeldahl digestion process includes decomposing the sample into ammonia (NH_3). The NH_3 is subsequently distilled and then measured by photometry or titration techniques [5-9].

Total Kjeldahl nitrogen is the sum of organic nitrogen compounds and free ammonia converted into ammonium sulfate, under decomposition conditions. The rubber latex sample for total nitrogen analysis from the process of deproteinization is heated in the presence of concentrated sulfuric acid, catalyzed and evaporated until SO_3 fume is obtained and the solution becomes colorless or pale yellow [10-13]:

ISSN 2734-9381
https://doi.org/10.51316/jst.180.etsd.2025.35.1.7
Received: Aug 5, 2024; revised: Sep 30, 2024;
accepted: Oct 3, 2024

$$\text{Protein} + H_2SO_4 \rightarrow (NH_4)_2SO_4 + CO_2 + SO_3 + H_2O \quad (1)$$

During the deproteinization process, when concentrated sulfuric acid is utilized, the temperature can rise to around 330 °C. The addition of salts such as K_2SO_4 can increase the solution temperature of the decomposition mixture to 390 °C or higher, depending on the ratio of salt and acid [10-13].

After the digestion process is completed, the mixture is added with excess base (NaOH) to convert NH_4^+ into NH_3:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O + 2NH_3 \quad (2)$$

NH_3 is obtained by distillation, where it is converted into a volatile gas through the elevation of the temperature to the boiling point. The distillate vapor containing NH_3 is subsequently trapped in a solution of boric acid, sulfuric acid, or hydrochloric acid depending on the selection of different ammonium concentration measurement methods [10-13].

$$H^+ + NH_3 \rightarrow NH_4^+ \quad (3)$$

1.1. Traditional Titration Method

Protein nitrogen content is measured by titration of ammonium borate solution with standard solutions of sulfuric acid or hydrochloric acid, using appropriate indicators to determine the end point of the reaction [10-12].

$$H_3BO_3 + NH_3 \rightarrow NH_4^+ + H_2BO_3^- \quad (4)$$

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3 \quad (5)$$

The concentration of H^+ ions (mol) required to reach the titration endpoint is equivalent to the initially present nitrogen concentration, see (4), (5). The formula for determining the percentage of protein nitrogen in a rubber latex sample *m* (g) using HCl acid solution *C* (mol/l) for titration [10-13] is:

$$\%N = \frac{C.(V_s - V_b)}{1000.m} \times 14 \times 100\% \quad (6)$$

where *C* (mol/L) is the molar concentration of HCl solution, V_s and V_b (mL) are the volumes of acid solution used to titrate the sample and blank sample, respectively, *m* (g) is the mass of the decomposed sample and 14 is the equivalent weight of nitrogen. A blank sample, which contains a solution of boric acid and indicator, is often run at the same time as the analytical samples to evaluate the possible nitrogen content of the reagent used. Once the nitrogen content has been determined, it is converted to protein content using the appropriate conversion factor.

1.2. Potential Titration Method

Potentiometric titration is similar to traditional titration but does not rely on chemical indicators. In this method, the potential difference between two electrodes is used to determine the equivalence point of the titration reaction. An indicator electrode, which is immersed in the sample solution, is connected to a reference electrode by a salt bridge containing an inert electrolyte such as potassium chloride, forming an electrochemical cell. The potential of the reference electrode is constant while the potential of the indicator electrode changes depending on the ions present in the sample solution. The variation in cell potential is monitored during the addition of the titrant and is subsequently plotted as a function of the added volume. Since the potential is dependent on the concentrations of the analyte and titrant in the solution, the plot can be used to determine the equivalence point, found at the steepest section of the potentiometric titration curve [14].

1.3. Spectrophotometric Method

The concentration of NH_3 ions in the distillate can be determined by UV-Vis spectrophotometric method based on the complex chemical reaction with inorganic reagents (Nessler) or organic reagents (indo-blue) and ammonia ions in an alkaline medium. The Nessler method, historically utilized for ammonia determination, is now less commonly employed and has been substituted by other methods that offer reduced interference. In the Nessler method, K_2HgI_4 is used as the indicator, which, if not carefully controlled, can have adverse environmental implications due to the potential release of mercury [15].

In this study, we use a spectroscopic device to determine NH_3 ions according to the salicylate method. Spectrophotometric measurement at 655 nm involves the analysis of the blue compound formed by the reaction of ammonium with salicylate and hypochlorite ions. This reaction takes place in the presence of sodium nitrosopentaxyano iron (III) taxyano iron (nitroprusside). Hypochlorite ions are generated in situ by alkaline hydrolysis of N, N‘-dichloro-1,3,5-triazine-2,4,6 (1H,3H,5H)-trione, and sodium salt (sodium dichloro isocyanurate). The reaction of cioramine chloramine with sodium salicylate occurs at pH 12.6 with the participation of sodium nitroprusside. Any chloramine present in the sample is also determined. [15].

The distillate should be captured in 1 %V/V HCl instead of boric acid/indicator. The formula to determine the protein nitrogen percentage of rubber latex sample m (g) is as follows:

$$\%N = \frac{(C_s V_s - C_b V_b)}{10^6.m}.100\% \quad (7)$$

where V_s and V_b (mL) are the volumes of the test sample and blank sample respectively after distillation, C_s and C_b (mg/L) are the measured concentrations of the test sample and blank sample after distillation, *m* (g) is the mass of the rubber latex sample. To evaluate the potential nitrogen content of the reagent used, a blank sample containing boric acid is often analyzed concurrently with the analytical samples.

The percentage of nitrogen content of dry rubber is calculated after calculating the dry coefficient content of the rubber.

1.4. Determination of Method Detection Limit and Limit of Quantification

The method detection limit (*MDL*) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. In this study, the *MDL* was calculated based on the analysis of 10 replicates of real samples [16].

The formula to determine *MDL* is as follows:

$$MDL = t_{(n-1,\ 1-\alpha=0,99)} S_s \quad (8)$$

Where $t(n-1,\ 1-\alpha=0.99)$ is the Student's *t*-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with $n-1$ degrees of freedom and S_s is the sample standard deviation of the replicate sample analyses.

Table 1. Single-Tailed 99th Percentile *t*-Statistic

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1,\ 0,99)}$
7	6	3,143
8	7	2,998
9	8	2,896
10	9	2,821
11	10	2,764
16	15	2,602
21	20	2,528

Limit of quantification (*LOQ*) is the lowest possible concentration of the analyte that can be quantified by the method in a reliable way.

The formula for determining LOQ is as follows:

$$LOQ = 10.\ S_s \quad (9)$$

1.5. Determination of Spiked Recovery

Let *C* stand for concentration. One definition of spike recovery is

$$R\ (\%) = \frac{C_{spiked\ sample} - C_{unspiked\ sample}}{C_{added}} \times 100,\ (\%) \quad (10)$$

The acceptable recovery is specified to be in the range. This range is established in the laboratory as a primary backup on the approval method.

A spike of C_{added} was added to a replicate portion of unknown nitrogen sample ($C_{unspiked\ sample}$). Analysis of the spiked sample gave a concentration of $C_{spiked\ sample}$.

2. Materials and Methods

2.1. Materials

The experimental materials utilized in this study consisted of reagents of analytical grade and ammonium-free distilled water.

HCl 1.18 g/mL, H_2SO_4 1.84 g/mL and potassium sulfate were employed as reagents in the study.

Sodium hydroxide (NaOH) was used in the form of a 32% solution, prepared by dissolving a measured quantity of NaOH (320 g ± 20 g) in approximately 800 mL of water. The solution was then cooled to room temperature and diluted to a final volume of 100 mL using additional water. It was stored in a polyethylene bottle to maintain its integrity.

Devarda's alloy, comprising approximately 45% m/m aluminium (Al), 50% m/m copper (Cu), and 5% m/m zinc (Zn), was utilized in powdered form. Care was taken to procure an alloy with the lowest possible nitrogen content to ensure accurate results.

A boric acid (H_3BO_3) solution/indicator was prepared by dissolving bromocresol blue (0.10 g ± 0.01 g) and methyl red (0.020 g ± 0.005 g) in approximately 80 mL of ethanol. The solution was then diluted to a final volume of 100 mL using ethanol. Separately, H_3BO_3 was dissolved in warm water (20 g ± 1 g) and cooled to room temperature. To this solution, 10 mL ± 0.5 mL of the indicator solution was added, followed by dilution to a final volume of 1 liter using water. The resulting H_3BO_3 solution/indicator was employed for subsequent analysis.

A HCl standard solution with a concentration of c(HCl) = 0.10 mol/L was utilized, either prepared by mixing HCl solution and standardized through conventional analytical methods or obtained commercially.

A color reagent was formulated by dissolving sodium salicylate (130 g ± 1 g) and trisodium citrate dihydrate (130 g ± 1 g) in 1000 mL of volumetric water. The liquid volume was adjusted to approximately 950 mL by adding water. Subsequently, sodium nitrosopentaxyano iron(III) dihydrate [sodium nitroprusside, $\{Fe(CN)_5NO\}Na_2.2H2O$] weighing 0.970 g ± 0.005 g was added to the solution and dissolved completely. The resulting solution was then diluted to the final volume with water. The color reagent was stored in amber glass vials and retained stability for a minimum of two weeks.

To prepare the sodium dichloroisocyanurate solution, NaOH (32.0 g ± 0.1 g) was dissolved in 500 mL ± 50 mL of water and allowed to cool

to room temperature. Subsequently, sodium dichloroisocyanurate (2.00 g ± 0.02 g) was added to the solution and dissolved completely. The entire solution was transferred to a 1000 mL volumetric flask and diluted to the mark with water. The sodium dichloroisocyanurate solution was stored in amber glass vials, maintaining stability for at least two weeks.

2.2. Methods

2.2.1. Sampling samples

Samples were transferred to polyethylene bottles and either immediately processed for analysis or stored at temperatures between 2 °C and 5 °C until analysis.

The digestion process was performed using the DK6 digestion equipment, which includes a Kjeldahl digester with 800 mL digestion vessels. For the blank test, 50 mL of water was taken instead of the sample. To prepare the sample for analysis, 5 ÷ 10 g of the sample was placed in a digestion tube. Distilled water was added to reach a total volume of 50 mL. Next, 4.0 mL ± 0.1 mL of H_2SO_4, 0.20 g ± 0.01 g of Devarda's alloy, and 2.00 g ± 0.05 g of K_2SO_4 were added to the digestion tube. After a minimum of 60 minutes, a few grains of pumice were added, and the contents of the flask were boiled under a fume hood. The volume gradually decreased due to water evaporation. When white smoke became visible, the neck of the Kjeldahl flask was covered with a small funnel to reduce evaporation. Care was taken not to boil the contents until completely dry. The temperature during this period should not exceed 370 °C. Once the fuming ceased, the decomposed liquid sample turned colorless or slightly green. The boiling process was continued for an additional 60 min ± 5 min to ensure complete digestion [17-18]. After decomposition, the sample was allowed to cool to room temperature before proceeding with the analysis using the specified methods.

In the distillation step, the UDK142 automatic steam distillation unit was used. For the traditional titration method, a distillation vessel was filled with 50 mL ± 2 mL of boric acid/indicator solution. For the potential titration method, only the boric acid solution was used. It was ensured that the condenser tube's tip was fully immersed in the indicator solution. Subsequently, a neutralized sample flask received a careful addition of 10 mL ± 1 mL of water, followed by the introduction of 25 mL of 32% NaOH solution. The flask was immediately connected to the distiller. The flask was heated to maintain a distillation rate of approximately 5 mL/min, and the distillation process was stopped upon collecting about 30 mL of distillate. If needed, the system could be cleaned using a rinsing solution after storage.

2.2.2. Determination of nitrogen content by traditional titration method

In the traditional titration method, the distillate obtained previously was titrated with HCl (0.02 mol/L) using the red indicator present in the receiving flask. The volume of HCl consumed during titration was recorded. The %N can be calculated using (7).

2.2.3. Determination of nitrogen content by potential titration method

The potential titration method was implemented as depicted in Fig. 2. A HCl standard solution (0.04 mol/L) was prepared and placed in a burette for titration. An electrode specifically prepared for this purpose was inserted into the analytical solution, and the change in mV electrode potential (pH) was monitored during the titration process. The mV (pH) electrode potential value at the end of the titration is recorded. By utilizing this information, the V_{eq} value can be determined, allowing for the calculation of the analyte's concentration in the analytical solution.

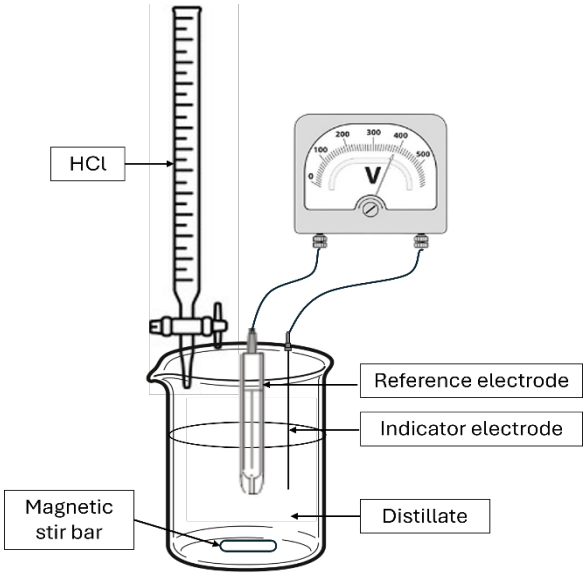

Fig. 2. Schematic diagram for potential titration method

2.2.4. Determination of nitrogen content by spectrophotometric method

To perform the analysis, 50 ml of the digested sample was taken and mixed with 5.00 ml ± 0.05 ml of a color reagent. The resulting mixture was thoroughly shaken. Subsequently, 5.00 ml ± 0.05 ml of a sodium dichlororoisocyanurate solution was added, and the mixture was shaken thoroughly once again. The flask containing the solution was then placed in an incubator, maintaining a temperature of 25 °C ± 10 °C. After a minimum of 60 minutes, the flask was removed from the incubator, and the absorbance of the solution was measured at a wavelength of 655 nm. The samples were measured and analyzed using the Agilent Cary 60 spectrophotometer.

3. Results and Discussion

3.1. Method Detection Limit and Limit of Quantification Survey

The results of graphing the standard curve at 5 standard points using the photometric method are presented in Table 2.

Table 2. Results of building the standard curve

C (mg/L NH_4-N)	0.05	0.1	0.2	0.3	0.4
Absorbance	0.156	0.285	0.560	0.825	1.029
R^2			0.9985		

The results of building standard curves show that the standard curves are linear and relatively stable with good correlation coefficients.

Given the minimal standard deviations observed across all three methods, it can be affirmed that the repeatability of these approaches is acceptable. This characteristic not only underscores the methods' reliability but also indicates a high level of accuracy, rendering them well-suited for precise determination of nitrogen content within the sampled specimens.

The average nitrogen content values exhibit minimal variation among the different methods, which are presented in Table 3. Notably, the *MDL* and *LOQ* values, as well as the standard deviation, of the spectrophotometric method were lower than those of the corresponding titration methods. Collectively, these results denote the elevated sensitivity and precision characteristic of the spectrophotometric approach.

Table 3. Results from experiments involving the repetition of spiked samples tẹn times to determine *MDL* and *LOQ* values through traditional titration, potential titration, and spectrophotometric methods

Method	Acid-base titration	Potential titration	Spectrophotometric method
Result (% N)	0.042	0.043	0.041
	0.046	0.041	0.040
	0.045	0.043	0.041
	0.048	0.044	0.039
	0.043	0.044	0.039
	0.043	0.044	0.041
	0.044	0.045	0.040
	0.042	0.044	0.042
	0.043	0.040	0.040
	0.045	0.040	0.041
Average (% N)	0.0440	0.0429	0.0405
SD	**0.0018**	**0.0017**	**0.0009**
MDL (% N)	**0.0051**	**0.0049**	**0.0024**
LOQ (% N)	**0.0180**	**0.0174**	**0.0087**

3.2. Sample Measurement Results

The first, second, and third protein removal samples were collected and analyzed using the acid-base titration method to determine the protein nitrogen content, thereby evaluating the effectiveness of protein removal from natural rubber. The analytical findings presented in Table 4 indicate that the initial sample, prior to protein separation, exhibited a nitrogen concentration of 0.257%. Subsequent separations resulted in a significant reduction in protein content in the sample, with the third separation yielding a nearly negligible protein nitrogen content, falling below the detection limit of the employed method. The degree of recovery ranged from 94.5% to 106.3%.

Plotting of pH and potential difference values against the volume of added titrant showed an equivalent point at V_{eq} = 1.40 mL (Fig. 3 and Fig. 4).

Table 4. Results of analysis of real samples by traditional titration method

Sample	Original	1st protein removal	2nd protein removal	3rd protein removal
Nitrogen content (%N)	0.257	0.044	0.020	0.012
Degree of Recovery (%)	95.6	98.4	106.3	94.5

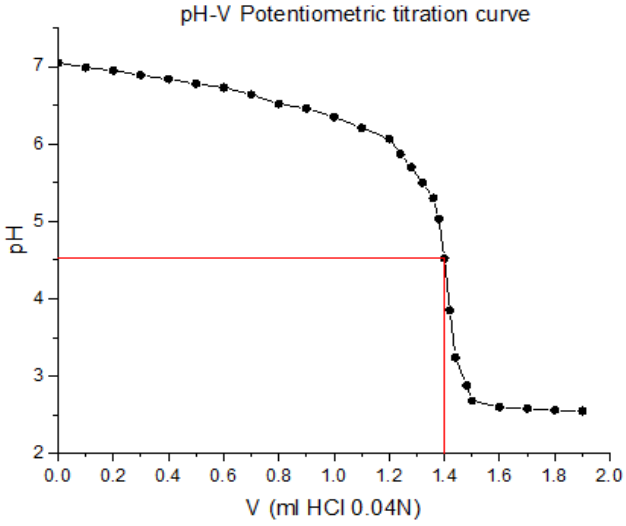

Fig. 3. pH-V potentiometric titration curve chart for nitrogen content analysis in latex samples

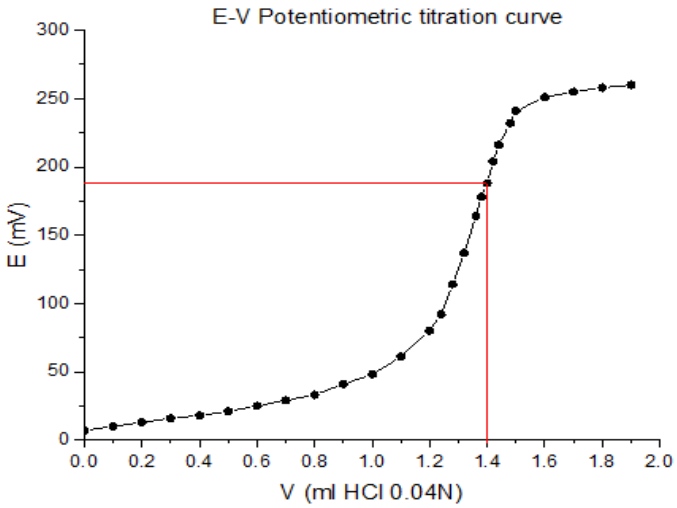

Fig. 4. E-V potentiometric titration curve chart for nitrogen content analysis in latex samples

A similar analysis was applied to the potential titration methods, with the outcomes detailed in Table 5. Notably, the degree of recovery fell within a more constrained range of 96.5% to 104.8%, indicating a narrower variability in the recovery rates.

Table 5. Results of analysis of real samples by potential titration method

Sample	Original	1st protein removal	2nd protein removal	3rd protein removal
Nitrogen content (%N)	0.269	0.043	0.022	0.010
Degree of Recovery (%)	96.5	97.4	104.8	97.2

Table 6. Results of real sample analysis by spectrophotometric method

Sample	Original	1st protein removal	2n protein removal	3rd protein removal
Nitrogen content (%N)	0.283	0.043	0.024	0.008
Degree of Recovery (%)	101.2	102.7	96.0	96.1

In the case of the spectrophotometric method, the value for nitrogen content of the original sample was higher, while values of the protein removals were lower than those of the two titration methods, respectively. When evaluating the degree of recovery, the initial two samples subjected to this method demonstrated notable precision and dependability. Conversely, the outcomes from the subsequent protein extraction steps may have been influenced by contaminants present during the analysis.

4. Conclusion

The removal of all proteins from natural rubber was proved by evaluating the total nitrogen content of the product after protein removal using the Kjeldahl method. This study applied three analytical methods to determine protein nitrogen content in rubber latex. Comparative analysis revealed negligible disparities between the methodologies, affirming the effectiveness of protein separation. After 3 times of protein nitrogen separation, the nitrogen content of the DPNR decreased to 0.012 wt%.

The utilized method demonstrated exceptional repeatability, conforming to ISO 17025 standards. The *MDL* and *LOQ* were 0.0051 (%N) and 0.0180 (%N) for the acid-base titration method, 0.0049 (%N) and 0.0174 (%N) for the potential titration method, and 0.0024 (%N) and 0.0087 (%N) for the spectrophotometric method, respectively. The spectrophotometric method exhibited lower *MDL* and *LOQ* compared to titration methods.

The titration method is effective for samples rich in nitrogen, but the accuracy and recovery can depend on the skill of the operator, with minimal equipment requirements. Spectrophotometric methods are characterized by their quickness and sensitivity to low levels of proteins, requiring clear, diluted solutions free from contaminants that could interfere with protein analysis.

Acknowledgments

This work was supported by Ministry of Science and Technology of Vietnam under the Grant NĐT/JP/23/03.

References

[1] H. L. Fisher, Vulcanization of rubber, Industrial & Engineering Chemistry, vol. 31, iss. 11, pp. 1381-1389, 1939. https://doi.org/10.1021/ie50359a015

[2] L. Bateman, Chemistry and Physics of Rubber-Like Substances, Wiley, New York, 1963, 784 pp.

[3] K. Berthelot, S. Lecomte, Y. Estevez, and F. Peruch, *Hevea brasiliensis* REF (Hev b 1) and SRPP (Hev b 3): an overview on rubber particle proteins, Biochimie, vol. 106, pp. 1-9, Nov. 2014. https://doi.org/10.1016/j.biochi.2014.07.002

[4] T. A. Dung *et al*., Modification of Vietnam natural rubber via graft copolymerization with styrene, Journal of the Brazilian Chemical Society, vol. 28, no. 4, pp. 669-675, 2017. https://doi.org/10.21577/0103-5053.20160217

[5] N. T. Ha, K. Kaneda, Y. Naitoh, L. Fukuhara, K. Kosugi, and S. Kawahara, Preparation and graft-copolymerization of hydrogenated natural rubber in latex stage, Journal of Applied Polymer Science, vol. 132, iss. 34, May. 2015. https://doi.org/10.1002/app.42435

[6] H. Nguyen Duy *et al*., Improvement of thermal properties of Vietnam deproteinized natural rubber via graft copolymerization with styrene/acrylonitrile and diimide transfer hydrogenation, Polymers for Advanced Technologies, vol. 32, iss. 2, pp. 736-747, Oct. 2020. https://doi.org/10.1002/pat.5126

[7] N. Rimdusit *et al*., Radiation graft-copolymerization of ultrafine fully vulcanized powdered natural rubber:

effects of styrene and acrylonitrile contents on thermal stability, Polymers, vol. 13, iss. 19, Oct. 2021. https://doi.org/10.3390/polym13193447

[8] P. Yu, H. He, and A. Dufresne, A novel interpenetrating polymer network of natural rubber/regenerated cellulose made by simple co-precipitation, Materials Letters, vol. 205, pp. 202-205, Oct. 2017. https://doi.org/10.1016/j.matlet.2017.06.088

[9] R. Blanchard, E. O. Ogunsona, S. Hojabr, R. Berry, and T. H. Mekonnen, Synergistic cross-linking and reinforcing enhancement of rubber latex with cellulose nanocrystals for glove applications, ACS Applied Polymer Materials, vol. 2, iss. 2, pp. 887-898, Jan. 2020. https://doi.org/10.1021/acsapm.9b01117

[10] P. Sáez-Plaza, M. J. Navas, S. Wybraniec, T. Michałowski, and A. G. Asuero, An overview of the Kjeldahl method of nitrogen determination, Part II. Sample preparation, working scale, instrumental finish, and quality control, Critical Reviews in Analytical Chemistry, vol. 43, iss. 4, pp. 224-272, Jul. 2013. https://doi.org/10.1080/10408347.2012.751787

[11] P. Y. Lim, Determination of chemical properties of meat: protein determination by Kjeldahl method, Laboratory Manual on Analytical Methods and Procedures for Fish and Fish Products, Marine Fisheries Research Department, Southeast Asian Fisheries Development Center, 2nd ed., 1992, pp. B-1.1- B-1.3.

[12] N. L. Qi, P. W. Li, X. H. Zeng, H. H. Huang, Z. M. Yang, and X. Gong, Comparison of Kjeldahl and the elemental analysis methods for determination of nitrogen content in raw natural rubber, Advanced Materials Research, vol. 815, pp. 722-726, Oct. 2013. https://doi.org/10.4028/www.scientific.net/AMR.815.722

[13] ISO 6778:1984, Water quality - Determination of ammonium - Potentiometric method, 1984.

[14] A. Hulanicki, M. Maj-Zurawska, and S. Głąb, Titrimetry | Potentiometry, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Encyclopedia of Anlytical Science, 3rd edi., pp. 121-128, 2013. https://doi.org/10.1016/B978-0-12-409547-2.00546-1

[15] M. D. Krom, Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate, Analyst, vol. 105, iss. 1249, pp. 305-316, 1980. https://doi.org/10.1039/an9800500305

[16] U. S. E. P. Agency, Definition and Procedure for the Determination of the Method Detection Limit, EPA 821-R-16-006, Revision 2, 2016.

[17] C. F. Liao, Devarda's alloy method for total nitrogen determination, Soil Science Society of America Journal, vol. 45, iss. 5, pp. 852-855, Sep-Otc. 1981. https://doi.org/10.2136/sssaj1981.03615995004500050005x

[18] R. R. I. Malaysia, R.R.I.M test methods for Standard Malaysian Rubbers, SMR Bulletin, no.7, 1973.