

The rapid determination of volatile fatty acid number in para rubber latex using fourier transform-near infrared spectroscopy based on quantification and discrimination model

Sureeporn Narongwongwattana and Ronnarit Rittiron*

*Department of Food Engineering
Faculty of Engineering at Kamphaengsaen
Kasetsart University, Nakhonpathom, Thailand
fengror@ku.ac.th

Chin Hock Lim
*Thai Rubber Latex Corporation (Thailand)
Public Company Limited, Chonburi, Thailand*

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Volatile Fatty Acid number (VFA no.) is one of the parameters indicating the state of quality of Para rubber latex at that particular time. Most factories analyze this parameter using standard analytical method as in ISO 506:1992(E). Nevertheless, this procedure is complicated, chemical and time consuming, as well as skilled analyst required. Therefore, near infrared (NIR) spectroscopy which is rapid, accurate and nonchemicals method was applied to determine the VFA no. in field latex and concentrated latex based on quantification and discriminant model. The best calibration equation was obtained from standard normal variate (SNV) spectra in the region of 6109.7–5770.3, 4613.1–4242.9 cm^{-1} with $R = 0.832$, $\text{SECV} = 0.036$ and no bias. From the performance check, statistically it was found that SECV and bias were low enough for practical acceptance and the predicted VFA no. was not different significantly from actual VFA no. at 95% confidence intervals. In addition, discriminant model was developed to separate good quality latex from the deteriorated latex using VFA no. at 0.06 as standard as in ISO 2004:2010(E). The discriminant model can be used to screen the latex with overall accuracy of 91.86% in validation set.

Keywords: Hevea brasiliensis; latex quality; polyisoprene; ISO 12099:2010(E).

*Corresponding author.

1. Introduction

Para rubber tree (*Hevea brasiliensis*) is one of the important economic crops of Thailand. At least 99% of hydrocarbon of Hevea Latex consists of cis-1, 4 polyisoprene that is widely used as raw material for various industries such as condoms, gloves, latex threads, shoes, conveyor beltings and other products. The latex factories purchase latex from farmers and process into three types of concentrated latices, namely, high ammonia (HA), medium ammonia (MA) and low ammonia (LA). Finally, the concentrated latex is sold to the manufactures for processing into finished products. Throughout the whole process, the quality of field and concentrated latices is an important factor. One parameter that is used as an indicator of the quality of the latex is volatile fatty acid number (VFA no.) which is amount of volatile fatty acids originated in hydrolysis of the carbohydrates in the serum of latex. These acids consist mostly of acetic, formic and propionic acids. If the VFA no. is high, it indicates that the latex has begun to deteriorate due to the microbial infestation. High volatile fatty acids result in the loss of colloidal stability and coagulation of latex. ISO 2004:2010(E)¹ for natural rubber latex concentrate specifies that VFA no. of concentrated latex must not exceed 0.06 or as agreed between buyer and seller. In practice, VFA no. is determined by standard analytical method as in ISO 506:1992² standard for rubber latex, natural, concentrate — determination of volatile fatty acid number. Nevertheless, this procedure is destructive, complicated and chemical consuming, and also requires skilled analyst.

Near Infrared (NIR) Spectroscopy is an alternative method which is nondestructive, rapid and accurate. NIR measures absorbance of constituents which are derived from overtone vibration of molecular grouping related to the constituents at specific wavelength in range of 800–2500 nm (or wavenumber in range of 12,500–4000 cm⁻¹). For that reasons, NIR has been applied widely in agriculture and industry. For rubber, also called polyisoprene, NIR spectroscopy was used as tool to detect many parameters in rubber-producing plants such as moisture in raw rubber sheet,³ natural polyisoprene in *Eucommia ulmoides* leaves,⁴ resin content and rubber content in *Parthenium Argentatum* biomass.⁵ In Para rubber latex, Sirisomboon *et al.*⁶ studied NIR spectroscopy on evaluation of

apparent viscosity and dry rubber content (DRC).⁷ The results show that this technique is an accurate and rapid method that can be used to predict apparent viscosity and DRC with correlation coefficient of 0.974 and 0.9995, respectively. In addition, NIR were used to determine VFAs in the effluent of an anaerobic H₂-producing bioreactor with rapidity and accuracy.⁸ However, there is no publication on using NIR spectroscopy to detect VFA no. in Para rubber latex.

For industrial application, International Organization for Standardization (ISO) has published ISO 12099:2010(E) which can be used as guidelines for the determination of constituents and parameters of samples by NIR spectrometer. In addition, this international standard indicates the process for validation of calibration model, statistics for performance measurement, checking instrument stability, running performance check of calibration and others.

This work aims to study the feasibility of VFA no. determination in Para rubber latex using FT-NIR spectroscopy base on quantification model development. The statistics for performance measurement is verified according to ISO 12099:2010 (E). Furthermore, discriminant model was constructed in order to separate group of latex following criterion in ISO 2004:2010(E).

2. Materials and Method

2.1. Sample preparation

The 20 field latex and 69 concentrated latex samples were collected from Research and Development department, Thai Rubber Latex Corporation (Thailand) Public Co., Ltd. in Nongyai district, Chonburi province, Thailand. In order to extend the range of VFA no., the 69 concentrated latex samples were composed of 8 low ammonia samples, 30 high ammonia latex samples and 31 samples which were sampled from latex centrifuge machine. Each sample was also kept at ambient temperature (28°C) at different time for 15–75 days to achieve such purpose.

2.2. NIR measurement

A 200 mL latex sample in beaker (250 mL) was measured by FT-NIR spectrometer (MPA model, Bruker Optics, Germany) at ambient temperature

(28°C) in diffuse reflectance mode at 32 cm⁻¹ resolution over wavenumber range of 12,500–4000 cm⁻¹. The spectrum of sample was derived from the average of 32 scans and reference spectrum was measured in every sample. The reference of FT-NIR spectrometer was internal reference which is coated with gold.

2.3. Analysis of VFA no. by the reference method

The VFA no. was analyzed using the standard analytical method as in ISO 506: 1992(E) Rubber latex, natural, concentrate — determination of volatile fatty acid number. The 50 g of latex was weighed into a stainless steel beaker. Then, 50 cm³ of the ammonium sulfate solution (30% mm⁻¹) was accurately added from the pipette while stirring the latex. The beaker was placed in the water bath which was maintained at 70°C, and continued stirring the latex until it coagulated. The beaker was covered with a watch-glass and left in water bath for a total period of 15 min. The serum was decanted and exuded through a dry filter paper. The serum was pressed out by kneading it with a pestle and filtered this serum through the same filter paper. The 25 cm³ of filtered serum was pipetted into a 50 cm³ beaker and acidify it by adding 5 cm³ of sulfuric acid solution (50% mm⁻¹) and mixed well by swirling.

Steam was passed through the steam-jacketed distillation apparatus (Markham still) for at least 15 min. The 10 cm³ acidified serum was pipetted into the tube and added with 1 drop of a silicon antifoaming. A 100 cm³ graduated cylinder was placed under the tip of the condenser to receive the distillate. The distillation was done at a rate of 3 cm³ min⁻¹ to 5 cm³ min⁻¹ until 100 cm³ of distillate has been collected.

The distillate was transferred to a 250 cm³ conical flask and any dissolved carbon dioxide was eliminated from the distillate by passing through it a stream of air free from carbon dioxide at rate of 200 cm³ min⁻¹ to 300 cm³ min⁻¹ for approximately 3 min. It was titrated with the barium hydroxide solution (0.005 mol dm⁻³) using phenolphthalein solution (0.5% mm⁻¹) as indicator. The VFA no. was calculated as,

$$\text{VFA no.} = \left[\frac{134.64cV}{m\text{TSC}} \right] \times \left[50 + \frac{m(100 - \text{DRC})}{100\rho} \right], \quad (1)$$

where

c is the actual concentration, expressed in moles per cubic decimeter, of the barium hydroxide solution;

V is the volume, in cubic centimeters, of barium hydroxide solution required to neutralize the distillate;

m is the mass, in grams, of the test portion;

DRC is the dry rubber content, expressed as a percentage by mass, of the latex concentrate;

TSC is the total solid content, expressed as a percentage by mass, of the latex concentrate;

ρ is the density, in mega grams per cubic meter, of serum; 1.02 Mg m⁻³ for centrifuged or creamed latex concentrates;

134.64 is a factor derived from relative molecular mass of potassium hydroxide, its equivalence to barium hydroxide and those parts of serum acidified and distilled.

Duplicate determinations were carried out with a fresh 50 g test portion of latex concentrate. The average of duplicate was used to develop the calibration equation.

2.4. Quantification of VFA no. in latex sample based on partial least square regression (PLS)

The calibration equations for VFA no. determination were developed from the relations between NIR spectra of sample (absorbance) and VFA no. determined by standard analytical method (reference method). Calibration equations were developed by partial least squares regression (PLS) using OPUS 6.5 software. Due to the number of samples in this experiment is small, all 89 samples were used to develop equations by full cross validation method. Before starting the calibration equation, one is excluded from the entity of sample; this sample is used for validation. The remained samples are used to calibrate the system. The sample used for validating the system must not be part of the calibration set. The best equation was selected based on the lowest of standard error of cross validation (SECV) and high correlation coefficient (R). In addition to such parameters, ISO 12099:2010(E) for Animal feeding

stuffs, cereals and milled cereal products-Guidelines for the NIR spectrometry⁹ assigns that the performance of prediction model shall be determined by set of validation samples based on statistical parameters, bias, Standard error of prediction (SEP) and slope. The significance of the bias is checked by a *t*-test as;

$$T_b = \pm \frac{t_{(1-\frac{\alpha}{2})} \text{SEP}}{\sqrt{n}}, \quad (2)$$

where

- α is the probability of marking a type I error;
- t is the appropriate student *t*-value for two-tailed test with degrees of freedom associated with SEP and the selected probability of a type I error;
- n is the number of independent samples;

SEP is the standard error of prediction or SECV for cross validation method.

If bias value is less than T_b , the bias is not different significantly from zero.

The SEP/SECV expresses the accuracy of routine NIR results corrected for the mean difference (bias) between routine NIR and reference method. If SEP/SECV is less than the unexplained error confidence limits (T_{UE}), the SEP/SECV can be accepted whereas T_{UE} are calculated from an *F*-test as;

$$T_{UE} = \text{SEC} \sqrt{F_{(\alpha, \vartheta, M)}}, \quad (3)$$

where

- SEC is the standard error of calibration;
- α is the probability of marking a type I error;

$\vartheta = n - 1$ is the numerator degrees of freedom associated with SEP of the test set, in which n in the number of samples in validation process;

$M = n_c - p - 1$ is the denominator degrees of freedom associated with SEC; in which n_c is the number of calibration samples, p is number of terms or PLS factors of the model.

The slope, b , of the simple regression $y = a + b\hat{y}$ is often reported in NIR publication. The slope must be calculated with the reference values as the dependent variable and the predicted NIR values as

the independent variable, if the calculated slope is intended to be used for adjustment of NIR result. As for the bias, a *t*-test can be calculated to check the hypothesis that $b = 1$

$$t_{\text{obs}} = |b - 1| \sqrt{\frac{s_{\hat{y}}^2 (n - 1)}{s_{\text{res}}^2}}, \quad (4)$$

where

- b is the slope;
- n is the number of independent samples;
- $s_{\hat{y}}^2$ is the variance of the n predicted values;
- s_{res} is the residual standard deviation as defined in

$$s_{\text{res}} = \sqrt{\frac{\sum_{i=1}^n (y_i - a + b\hat{y}_i)^2}{n - 2}} \quad (5)$$

in which

- a is the intercept of equation; $a = \bar{y} - b\bar{\hat{y}}$, whereas $\bar{\hat{y}}$ is the mean of predicted value; \bar{y} is the mean of reference values; b is the slope
- y_i is the i th reference value,
- \hat{y}_i is the i th predicted value obtained when applying the multivariate NIR model.

The slope, b , is considered as different from 1 when $t_{\text{obs}} \geq t_{(1-\alpha/2)}$, where

- t_{obs} is the observed *t*-value, calculated according to Eq. (4).
- $t_{(1-\alpha/2)}$ is the *t*-value obtained from table *t*-distribution for a probability of $\alpha = 0.05$.

2.5. Discrimination model

In practical application, the latex industry uses the VFA no. as one of the criteria for trading because VFA no. indicates the quality of the latex at that particular time. The latex with VFA no. lower than, or equal to 0.06 is specified as good quality latex which could be purchased while the latex with VFA no. higher than 0.06 indicates that latex started to deteriorate and would be rejected. Therefore, discrimination model was constructed for classification especially for trading of field and concentrated latices. OPUS software was used to develop partial least square discriminant analysis (PLSDA) by assigning dummy variables as 0 and 1 for good quality and deteriorated latex, respectively.

3. Results and Discussion

3.1. NIR spectra of field latex and concentrated latex samples

The NIR spectra of field latex and concentrated latex samples are shown in Fig. 1. The overall appearance of both field latex and concentrated latex spectra was not different. And all spectra revealed prominent absorption bands at 6897 cm^{-1} (1450 nm), 5154 cm^{-1} (1940 nm) which are associated with O–H overtones and combination of water,¹⁰ respectively. Furthermore, the absorbance of concentrated latex was lower than field latex due to the water content in concentrated latex less than in field latex. In addition, it also appeared absorption band at around $5900\text{--}5650\text{ cm}^{-1}$ ($1695\text{--}1769\text{ nm}$) in the spectra of both field latex and concentrated latex. This peak was assigned as C–H stretching first overtone vibration of CH_3 structure¹⁰ which was the major component of poly-isoprene. In addition, these peaks were also corresponding with absorption band of pure dried Para rubber sheet.⁷ Nevertheless, the absorption band of VFA was not noticed at the least due to the fact that the amount of VFA contained in latex is very low.

3.2. Quantification of VFA no. in latex sample with PLS

For calibration equation development, all samples were used as both of calibration set and validation

Table 1. Characteristics of VFA no. contained in field and concentrated lattices.

Parameters	VFA no.
Maximum	0.2707
Minimum	0.0188
Average	0.0639
Standard deviation	0.0588
Number of samples	86

set. The statistical parameters were shown in Table 1. The three outliers of samples were excluded based on *F*-test statistics of residuals which is difference between reference value and NIR predicted value at $\alpha = 0.05$ and ISO 12099:2010 (E) which recommend that the samples with difference exceeding ± 3 (SEP) seem to be the outliers.

The PLS regression model was developed with no-pretreatment, first derivative, multiplicative scattering correction (MSC) and standard normal variate (SNV) spectra between $4000\text{--}12,500\text{ cm}^{-1}$ (Table 2). The best equation and optimal wavelength region were selected using optimization method. The results showed that the calibration equation for VFA no. prediction derived from SNV pretreatment in the region of $6109.7\text{--}5770.3$, $4613.1\text{--}4242.9\text{ cm}^{-1}$ with six factors was the best which R and SECV were 0.832 and 0.036, respectively. From the results, the equation seems to be

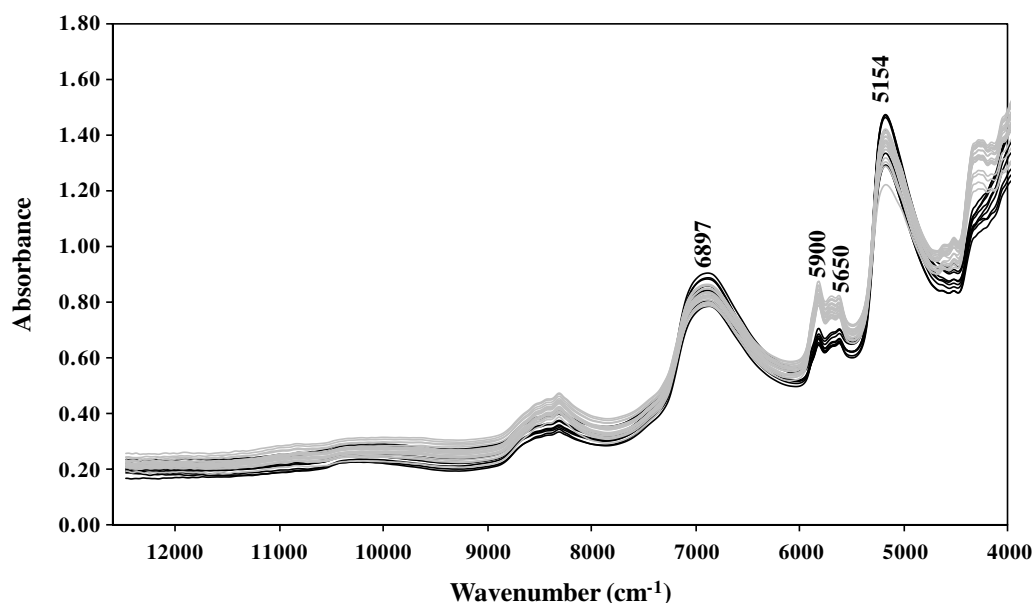


Fig. 1. NIR spectra of field latex (black line) and concentrated latex (gray line) samples measured by FT-NIR spectrometer.

Table 2. The results of PLS regression for VFA no. determination of field and concentrated lattices.

Pretreatment	Calibration		Validation				Factors	Range (cm ⁻¹)
	R	SEC	R	SECV	Bias	Slope		
No pretreatment	0.886	0.032	0.743	0.044	0.002	0.865	9	6109.7–5446.3
MSC	0.859	0.035	0.761	0.043	0.002	0.911	8	9411.4–5446.3
SNV	0.872	0.033	0.832	0.036	0.000	0.944	6	6109.7–5770.3, 4613.1–4242.9
First Derivative	0.863	0.035	0.747	0.440	0.000	0.864	9	6109.7–4242.9
Second Derivative	0.896	0.031	0.744	0.045	0.000	0.818	9	9411.4–4242.9

R: Correlation coefficient, SEC: Standard error of calibration, SECV: Standard error of cross validation, Bias: The average of residual, Factors: Number of latent variables in calibration equation, SNV: Standard normal variate, MSC: Multiplicative scattering correction.

Table 3. The statistics for performance measurement followed in ISO 12099:2010(E).

Parameters	Calculated value	Criterion	Result
Bias	0.000	$T_b = 0.008$	pass
SECV	0.036	$T_{UE} = 0.040$	pass
t_{obs} for slope test	0.283	$t_{(1-\alpha/2)} = 1.988$	pass

T_b : Bias confidence limits, T_{UE} : Unexplained error confidence limits, t_{obs} : Observe t -value, calculated from Eq. (4) $t_{(1-\alpha/2)}$: The t -value obtained from table t -distribution for a probability of $\alpha = 0.05$, SECV: Standard error of cross validation.

reasonable. Due to the fact that, after NIR spectra of samples were collected, the samples were determined VFA no. using standard analytical method taking time around 1 h. It was possible that VFA no. had been changed after the spectra measurement. Hence, VFA no. during spectra measurement was different from VFA no. obtained from standard analytical method. VFA no. could be increased

based on many factors such as quality of latex, level of bacteria population, temperature variation and time of storage.¹¹

However, from statistics for performance measurement (Table 3), there are no bias from the calibration equation (0.000). SECV was lower than T_{UE} which equals 0.040 indicating that SECV was low enough for practical acceptance. In addition, t_{obs} calculated according to equation for slope test (0.283) was lower than $t_{(1-\alpha/2)} = 1.988$ which is obtained from value of the t -distribution with a probability of $\alpha = 0.05$. It indicated that the slope was not significantly different from 1. The results showed that the NIR predicted values were not different from actual values significantly at 95% confident interval. The scatter plots of VFA no. from reference method versus VFA no. predicted from the best equation are shown in Fig. 2. It was noticed that, most of samples nearby were spreaded around target line. The regression coefficient plot of the model is shown in Fig. 3. The variable that

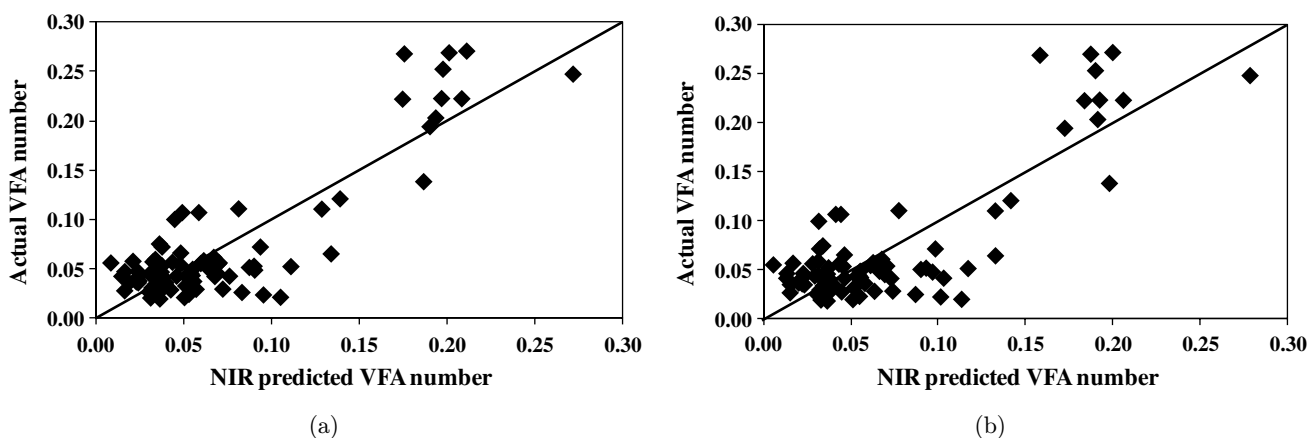


Fig. 2. Scatter plots of predicted VFA no. and VFA no. obtained from standard analytical method in calibration set (a) and cross validation set (b).

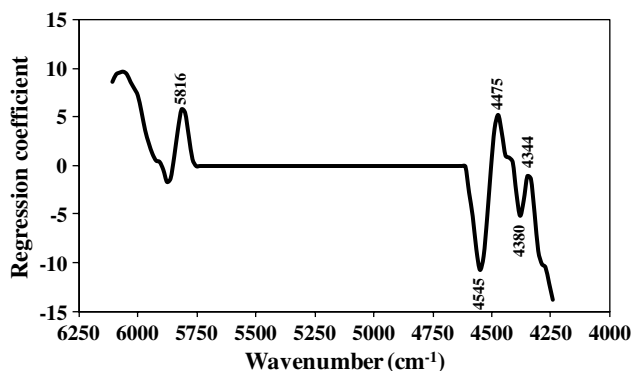


Fig. 3. Regression coefficient plot of calibration equation based on PLS for VFA no. determination in both field and concentrated lattices.

influenced on the model appeared at 4545 cm^{-1} (2200 nm) which is the absorption band of C–H stretching and C=O stretching combination vibrations¹⁰ of acetic acid, formic acid and propionic acid. Furthermore, the peaks at 5816 cm^{-1} (1719 nm) and 4475 cm^{-1} (2234 nm) were also the absorption band of formic acid (Fig. 4). In addition, regression coefficient plot also appeared absorption band of acetic acid at 5816 cm^{-1} (1719 nm) and 4380 cm^{-1} (2283 nm) as shown in Fig. 5. All mentioned-acids is the main acids contained in volatile fatty acid.

3.3. Discriminant model development

The latex industry uses the VFA no. as the criteria to indicate the quality of the latex for commercial trading. Generally, the latex with VFA no. lower than or equal to 0.06 is purchased whereas latex with VFA no. higher than 0.06 is rejected.

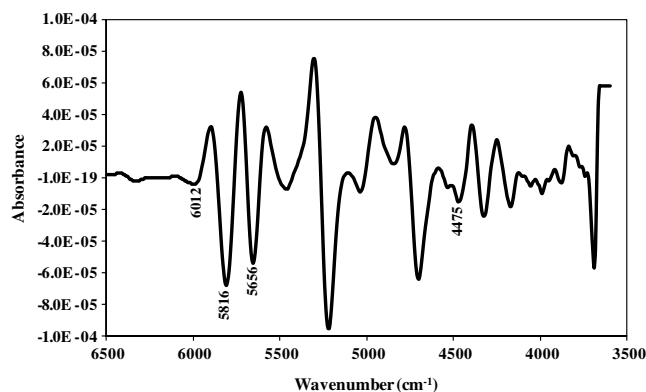


Fig. 4. The second derivative spectra of formic acid measured by FT-NIR spectrometer in the region of $6500\text{--}3500\text{ cm}^{-1}$.

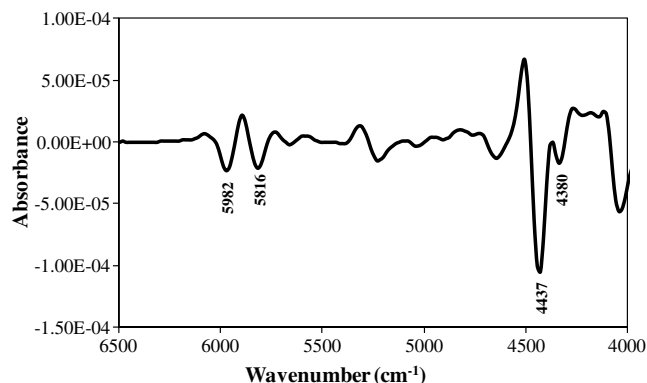


Fig. 5. The second derivative spectra of acetic acid measured by FT-NIR spectrometer in the region of $8000\text{--}4000\text{ cm}^{-1}$.

Therefore, discrimination model was constructed to separate group of samples by using VFA no. at 0.06 as criterion. Sample with VFA no. lower than or equal to 0.06 is represented by a dummy variable equal to 0, which means the latex quality was good enough for industry acceptance. Sample with VFA no. higher than 0.06 is represented by a dummy variable equal to 1. The PLS discrimination model were developed by the relations between NIR spectra of sample (X-variable) and dummy variable as Y-variable. The best model was selected from the percentage of correct classification. The predicted value of sample which was considered as correct classification must be in range of -0.50 to 0.50 for samples in group 0 and more than 0.51 for samples in group 1. Apart from that, it was assigned as the error of prediction. Similarly to quantification model, discriminant models were developed with pretreatment spectra such as first derivative, second derivative, MSC and SNV. The correction of discriminant models were shown in Table 4.

From Table 4, the discrimination model was obtained from second derivative pretreatment spectra over the wavenumber region of $7513.7\text{--}5446.3\text{ cm}^{-1}$. The developed model could correctly classify quality of latex with 92.06% for good latex and 91.30% for deteriorated latex, or equal to 91.86% for overall classification in validation set. Moreover, the number of factors using in this model was only three factors indicating that developed model was more robust than others. The score plots are shown in Fig. 6. From the regression coefficient plot of discriminant model (Fig. 7), the main variables were 6012 cm^{-1} and 5656 cm^{-1} corresponding with absorption of formic acid (Fig. 4) which is one of the main acids containing in VFA no.

Table 4. The percentage of correct classification of models for VFA no. prediction.

Pretreatment	Range	Factors	% Correct classification in calibration set			% Correct classification in validation set		
			Good latex	Deteriorated latex	Overall	Good latex	Deteriorated latex	Overall
No pretreatment	6109.7–5446.3	6	92.06	91.30	91.86	92.06	86.96	90.70
MSC	7513.7–5446.3	5	92.06	91.30	91.86	92.06	78.26	88.37
SNV	7513.7–5446.3	5	92.06	86.96	90.70	90.48	73.91	86.05
First derivative	9411.4–6094.3	8	92.06	91.30	91.86	92.06	82.61	89.53
Second derivative	7513.7–5446.3	3	92.06	91.30	91.86	92.06	91.30	91.86

Good latex: Samples which VFA no. were lower than or equal to 0.06, deteriorated latex: Samples which VFA no. were higher than 0.06, Factors: number of latent variables in discriminant model, SNV: Standard normal variate, MSC: Multiplicative scattering correction.

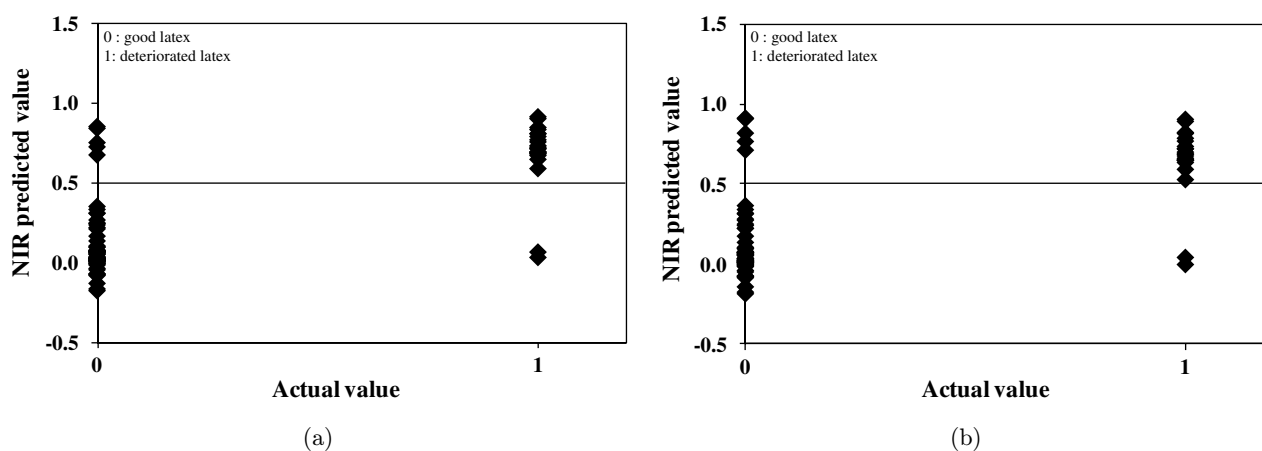


Fig. 6. Score plots of predicted VFA no. versus dummy variable in calibration set (a) and cross validation set (b).

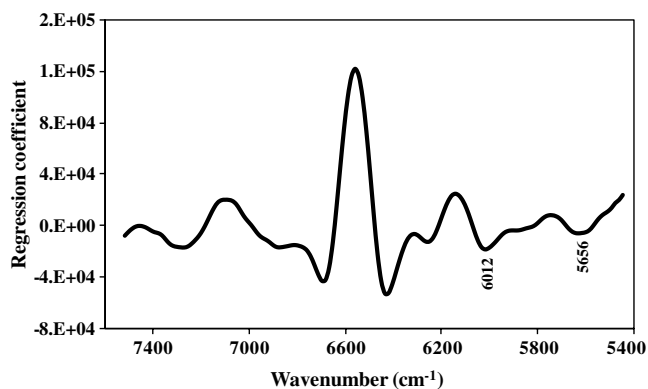


Fig. 7. Regression coefficient plot of discriminant model based on PLSDA for VFA no. classification in both field and concentrated lattices.

4. Conclusion

NIR spectroscopy is rapid and accurate method for determination of VFA no. in field and concentrated lattices without chemical. The developed calibration

equation could predict VFA no. accurately with $R = 0.832$, $SECV 0.036$ and no bias. Moreover, these statistics also overcome the statistics test for performance measurement as followed ISO 12099:2010(E). All the same, the discriminant model also can be used to sort the good quality latex out from deteriorated latex with 91.86% of correct classification.

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