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Application of near infrared spectroscopy to control the protective reaction in the synthesis of emamectin benzoate

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A near infrared (NIR) method was developed for the control of the protective reaction in the synthesis of emamectin benzoate. A NIR spectrometer with a fibre-optic probe was used to analyse the residual content of avermectin and the by-product content of the shield. Partial least squares regression was used to develop the calibration models, which for the residual content were preprocessed by second derivative and for the by-product content by first derivative and straight-line subtraction preprocessing methods. After optimising the spectral pre-treatment, the coefficient of determination (R^2) of the residual content and by-product content were 0.92 and 0.99 and the root mean square errors of calibration were 0.18% and 0.46%, respectively. When the models were used to determine the residual content and by-product content, the root mean square errors of prediction were 0.20% and 0.48%, respectively. These preliminary findings suggest that the NIR method could be used to predict the residual content and by-product content simultaneously and the process could be completed within 2 min without sample destruction. The control of the protective reaction could be adjusted according to the results obtained using the NIR method. The results indicated that information contained in NIR spectra may be useful to control the protective reaction in the synthesis of the emamectin benzoate.

Keywords: avermectin, near infrared spectroscopy, residual content, by-product content

Introduction

Avermectin is well known for its insecticidal, acaricidal and antihelmintic activity and is used in world agriculture. At the same time, it is being developed as a broad-spectrum, high efficiency (low application rate) and low-residual pesticide for vegetables.[1] Emamectin benzoate is a semi-fermented and semi-synthesised insecticide of the avermectin kind. It is synthesised via oxidation, amination and deoxidation in industry. The active components of avermectin ($B_{1a,}$ B_{1b}) are shown in Figure 1. B_{1a} is the main active component of avermectin.

In order to avoid the hydroxyl reaction of the fifth carbon (C_5–OH) in the avermectin molecule, a shield is employed. A scheme for the protective reaction for avermectin is given in Figure 2. The hydroxyl of the fifth carbon in the avermectin molecule reacts with the allylchloroformate and tetramethylethylenediamine synthesises the shield of the avermectin (Reaction 1 in Figure 2), but if the allylchloroformate is present in excess in the protective reaction, the side-reaction occurs (reaction 2).

It is important to control the reaction terminus of avermectin. The residual content ($AVMB_{1a}C_5OH$) and by-product content (Figure 2) of avermectin are the key factors to ensure the synthesis of emamectin benzoate. The high residual content leads to wasted avermectin and the high by-product content causes the reaction to stop. In order to enhance the productivity and production quality, the residual content and by-product content of avermectin are controlled near zero but, industrially, it is difficult to control the protective reaction terminus of avermectin.

The traditional method of analysis of the residual content of avermectin and the by-products of the shield uses high-performance liquid chromatography (HPLC).[2] Recently, mass

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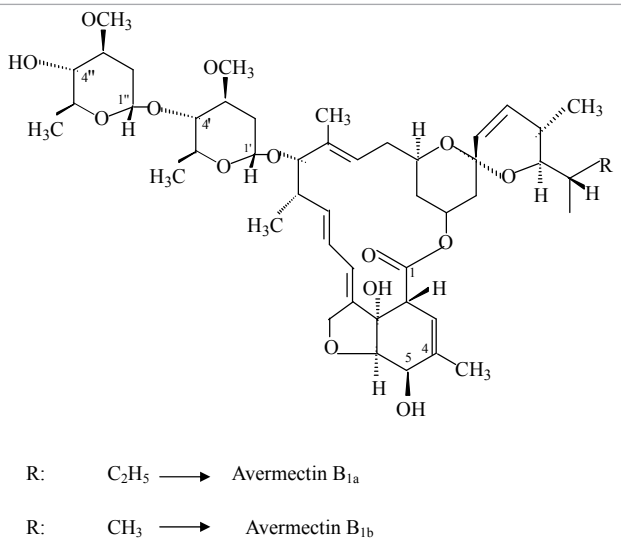

Figure 1. Chemical formulae of the active components (B_{1a} and B_{1b}) of avermectin.

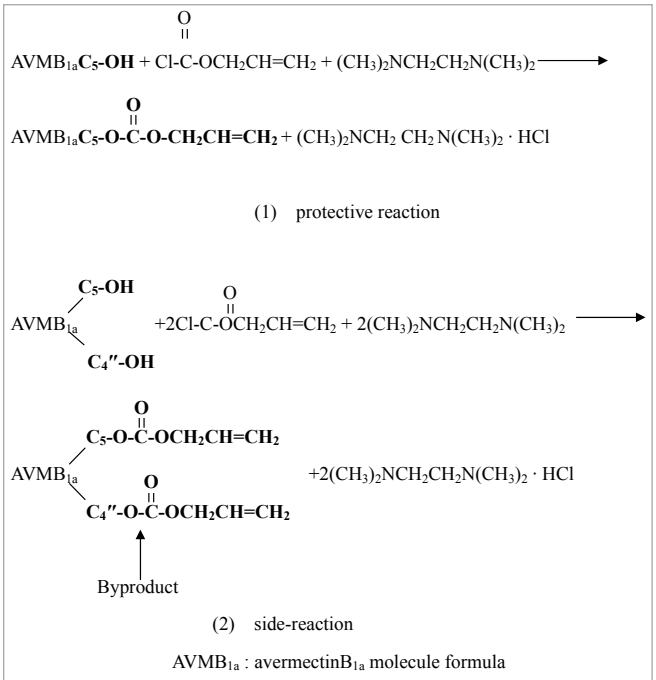

Figure 2. A reaction scheme for the protective reaction of component B_{1a} of avermectin.

spectroscopy and ultraviolet spectral analysis have also been employed.[3,4] When using these methods, it is difficult to control the reaction terminus of avermectin, because excessive time is spent on preprocessing the samples and these analytical methods require expensive chemicals. Therefore, these methods are not suitable for use in industry. A rapid method is required to monitor the protective reaction of avermectin.

Near infrared (NIR) spectroscopy is a very rapid, accurate and non-destructive method for the simultaneous measurement of different constituents in various products. Nowadays, NIR spectroscopy has been applied in many fields such as the agriculture,[5,6] environment,[7] petrochemical,[8] pharmaceutical[9–11] and food industries[12–14] and in the manufacture of certain polymers.[15,16]

The absorption of NIR energy is caused by the molecular overtone or molecular combination vibrations, typically of C–H, O–H, N–H and C–O bonds. The molecular structure of the avermectin comprises hydroxyl, methyl and phenyl groups. There are many hydrogen-containing groups in avermectin molecules, so NIR spectra can be used to study the protective reaction of avermectin. The aim of our study was to develop a control method for the protective reaction of avermectin by using diffuse reflectance NIR spectroscopy with fibre-optic probes. We used the partial least squares (PLS) method for quantification analysis of the residual content of avermectin and by-product content of the shield in the protective reaction.

Experimental

Samples

Samples of avermectin were collected from Bio-Products Co., Ltd (Da Qing, China). Samples with different residual contents of avermectin and by-products of the shield were used to develop the calibration models. The residual content and by-product content were analysed using HPLC as the reference values.

Near infrared spectroscopy

The equipment included a NIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) operating in reflectance mode with fibre-optic probes, which sourced spectra from an area with a diameter of 2–3 mm. The resolution of the spectra was 8 cm^{-1} and the average scanning times was 64 s. The spectral region covered from 12,000 cm^{-1} to 4000 cm^{-1}. Spectra of each liquid sample were collected three times and these were combined to give an average spectrum. PLS was used to develop the calibration models. Calibration models were developed using leave-one-out cross-validation. The coefficient of determination (R^2) and root mean square error of calibration (*RMSEC*) were calculated to evaluate the calibration models. Prediction accuracy was evaluated as: the root mean square errors of prediction (*RMSEP*) and relative performance determinant from the prediction (*RPD*).[17,18]

Results and discussion

Developing quantitative analysis models

A NIR spectrum of the protective reaction solution is shown in Figure 3 and peaks were interpreted as the molecular overtone or molecular combination vibrations involving hydrogen stretching and deformation modes.

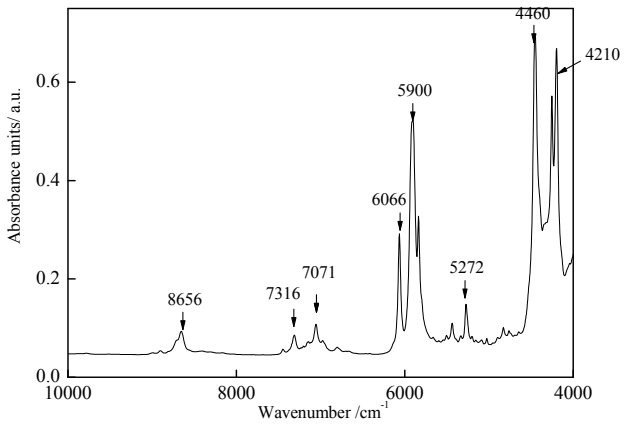

Figure 3. NIR spectrum of the protective reaction solution.

The samples included in the calibration set should encompass the maximum possible variability and span the whole range of values of the property to be measured. Table 1 shows the number (*N*), range, mean and standard deviation (*SD*) of the reference data for the residual content of avermectin and the by-product content of the shield.

Various calibration models, based on diverse spectral pretreatments, the number of PLS factors and spanning variable wavenumber ranges, were tested. Second derivative preprocessing was selected for the residual content of the avermectin; the R^2 was 0.92 and the *RMSEC* was 0.18%. The spectral ranges which gave the best model were 8800–7500 cm^{-1}, 6100–5400 $cm^{-1}$and 4600–4200 cm^{-1}. The model for by-product content of the shield is also shown in Table 2. The optimum number of PLS factors was identified as the point where the mean square error of cross-validation decreased to a minimum and started to plateau while R^2 increased towards a maximum. Correlation PLS factor numbers for R^2 and *RMSEC* for the residual content of avermectin and the by-product content are shown in Figures 4(a) and (b). The plots of the actual values against NIR predicted values for

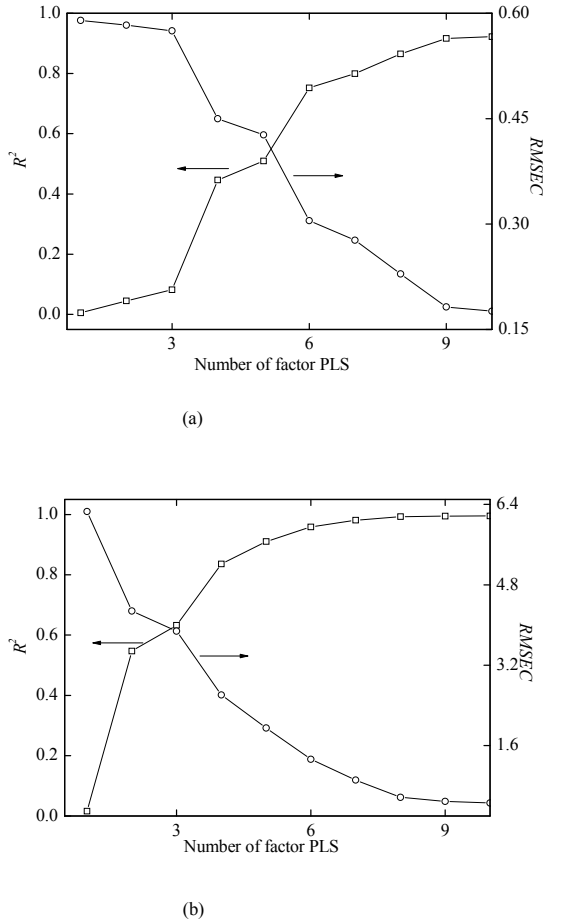

Figure 4. Changes in R^2 and *RMSEC* with increasing correlation PLS factor number: (a) residual content, and (b) by-product content.

Table 1. Statistical parameters of the samples used in the calibration models.

Quality indexes	Range (%)	Mean (%)	*SD* (%)	*N*
Residual contents	0.50– 3.05	1.56	0.59	74
By-product contents	0.47–24.20	6.31	6.27	72

Table 2. Statistical results of calibration models for residual content and by-product content.

Quality indexes	Pre-processing routine	Spectral range (cm^{-1})	PLS factors	R^2	*RMSEC* (%)	Slope	Intercept (%)
Residual content	Second derivative	8800–7500 6100–5400 4600–4200	9	0.92	0.18	0.92 ± 0.03	0.13 ± 0.05
By-product content	First derivative and straight line subtraction	7500–4500	10	0.99	0.46	0.99 ± 0.01	0.04 ± 0.07

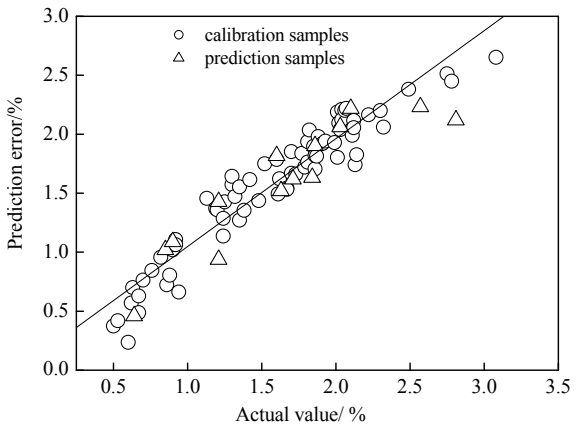

Figure 5. Correlation between predicted and actual values for the calibration (○) and the prediction (△) set samples: (a) residual content; (b) by-product content in the calibration and prediction sample set.

the calibration set samples of the residual content and the by-product content are shown in Figures 5(a) and (b).

Prediction

Both the residual content and the by-product content were predicted in 14 prediction set samples. The ability of the models to determine the residual content and the by-product content is presented in Figures 5 (a) and (b). The *RMSEP* values were 0.20% and 0.48%, and the *RPD* values were 3.17 and 14.60, respectively.

Analysis using the reference method (HPLC) to determine the residual content and the by-product content takes about 120 min including preparation time, but analysis using the NIR method could be completed within 2 min, without destructing the sample. In order to enhance productivity, economise on consumable materials and ensure the synthesis of the emamectin benzoate, the residual content and by-product content could be controlled within 2.00% and 2.50% using HPLC analysis. The above results suggest that, by using the NIR method, the residual content and by-product content could be controlled near zero, but the maximal prediction error is about 1.00%, because the *RMSEP* for the residual content and the by-product content were 0.20% and 0.48%, respectively. The features of the NIR method and the reference method are compared in Table 3. Adoption of the NIR method would lead to economic use of material and decreased cost to control the protective reaction.

Conclusion

It may be deduced that the NIR method is a good alternative for the analysis of the residual content and by-product content. Control of the protective reaction was performed based on NIR spectra combined with PLS methods. The results of this study showed that NIR spectra were a reasonable and rapid method. Further studies will be directed to analyse the feasibility of monitoring the oxidation, amination and deoxidation reactions of avermectin using information in the same NIR spectra.

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Table 3. Advantages of the NIR method compared to the HPLC method.

	Reference method	NIR method
Analysis of samples	Destructive	Non-destructive
Solvent	Acetonitrile, water, standard solution	No
Instrument	HPLC	NIR spectroscopy
Analytical process	Preprocess	On-line analysis gives an immediate result
Time	120 min sample^{-1}	2 min sample^{-1}

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