Interval partial least squares and moving window partial least squares in determining the enantiomeric composition of tryptophan using UV–Vis spectroscopy

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Abstract: The application of interval partial least squares (IPLS) and moving window partial least squares (MWPLS) for the enantiomeric analysis of tryptophan (Trp) was investigated. A UV–Vis spectroscopic method for determining the enantiomeric composition of Trp was developed. The calibration model was built using partial least squares (PLS), IPLS and MWPLS, respectively. Leave-one-out cross validation and external test validation were used to assess the prediction performance of the established models. The validation result demonstrated the established full-spectrum PLS model is impractical for quantifying the relationship between the spectral data and enantiomeric composition of L-Trp. On the contrary, the developed IPLS and MWPLS models are both practicable for modeling this relationship. For the IPLS model, the root mean square relative error (*RMSRE*) values of the external test validation and leave-one-out cross validation were 4.03 and 6.50, respectively. For the MWPLS model, the *RMSRE* values of the external test validation and leave-one-out cross validation were 2.93 and 4.73, respectively. Obviously, the prediction accuracy of the MWPLS model was higher than that of the IPLS model. It was demonstrated that UV–Vis spectroscopy combined with MWPLS is a commendable method for determining the enantiomeric composition of Trp. MWPLS was superior to IPLS for selecting the spectral region in the UV–Vis spectroscopy analysis.

Keywords: UV–Vis spectroscopy; interval partial least squares; moving window partial least squares; enantiomeric composition; tryptophan.

INTRODUCTION

Enantiomeric pure products are required in various fields because enantiomers may show great differences in their chemical and biological reactions.[1,2] At

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present, determining the enantiomeric composition of chiral compounds has gained much attention.

Spectroscopic methods, including UV–Vis,[3–9] NIR[10,11] and fluorescence[12–14] have been successfully used to determine the enantiomeric composition of chiral compounds. It was shown that a quantitative relationship between the enantiomeric composition and the spectra of chiral compounds exist.[3–14] Multivariable calibration methods are required to reveal this relationship. The partial least squares (PLS) method was usually used to develop the calibration model. The models reported in these studies were all full-spectrum PLS models, which were developed using the whole spectrum as the independent variable. However, a spectrum often contains spectral regions related to non-modeled information originating from unwanted constituents, background variations, random interferences and interactions. These spectral regions may degrade the calibration model and should not be used in model building.[15–17] Thus, selection of the spectral region is often required in multi-component spectroscopic analysis in order to eliminate the influence of non-modeled spectral regions and build well-fitted calibration models.[17–21]

Many studies have shown that spectral region selection can lead to significant improvement of the prediction performance over full-spectrum calibration models.[15–24] It seems spectral region selection could improve the accuracy of the calibration model when spectroscopic methods are used to determine the enantiomeric composition of chiral compounds. However, spectral region selection has not hitherto been applied to the spectroscopic determination of enantiomeric composition. Interval partial least squares (IPLS) and moving window partial least squares (MWPLS) are two local modeling methods which have been commonly used to select spectral regions in spectroscopy analysis.[16–18,22–35]

Tryptophan (Trp) is an essential amino acid for humans. The biological function of L-Trp is quite different from D-Trp. Thus, a method for the enantiomeric determination of Trp is desirable. Therefore, in the present study, IPLS and MWPLS were applied to the determination of the enantiomeric composition of Trp. UV-Vis spectroscopy was used to measure the spectra of Trp enantiomers. An IPLS model and a MWPLS model were developed and investigated.

<div align="center">EXPERIMENTAL</div>

Reagents

L-Trp and D-Trp (enantiomeric pure) were purchased from Sigma–Aldrich Co. LLC (USA). Bovine serum albumin (BSA, fraction V) was purchased from Sino-American Biotechnology Co. (Shanghai, China). Water from a Milli-Q synthesis A10 system (Merck Group, Germany) was used to prepare the samples.

Spectra

The UV–Vis spectra of the prepared samples were collected with an Agilent 8453 UV––visible spectrophotometer (Agilent, U.S.A.) over the wavelength range from 190 nm to 789 nm. Quartz cells of 1.0 cm path length were used. All the spectra were blank corrected.

Data and software

The root mean square relative error (*RMSRE*) was employed to assess the prediction performance of the obtained models. It is defined as:

$$RMSRE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(RE_i)^2} \tag{1}$$

where RE_i denotes the relative error of prediction for the ith sample and n is the number of samples.

PLS toolbox 5.5 (Eigenvector Research Inc., U.S.A.) was used to perform PLS. IPLS and MWPLS were conducted using iToolbox (programmed by Prof. Nørgaard, KVL, Denmark; shared at http://www.models.kvl.dk/iToolbox). The independent variable matrix (**X** matrix) of PLS, IPLS and MWPLS was generated by assembling the spectral data of each sample as a row vector. The dependent variable (**Y** matrix) of each model is composed by the mol fraction of L-Trp. The **X** and **Y** matrix were mean-centered in the procedure of PLS, IPLS and MWPLS.

RESULTS AND DISCUSSION

Spectra of the samples

Twenty samples were prepared. Each sample contained 0.01 mmol L^{-1} Trp and 0.02 mmol L^{-1} BSA. The enantiomeric composition of the 20 samples was varied from mol fraction 0.12 to 0.88 of L-Trp. The mol fractions of L-Trp in these samples are listed in Table I. In the prepared samples, BSA formed two different guest–host complexes with the enantiomers of Trp. The complex formed from L-Trp is diastereomeric to the complex formed from D-Trp. Hence, the two complexes were different in their UV–Vis spectra, as shown in Fig. 1. Correspondingly, the twenty prepared samples should differ in their UV–Vis spectra because they differ in their enantiomeric composition. The UV–Vis spectra of the twenty samples are shown in Fig. 2, from which the slight differences could be seen. These spectral differences are actually related to the enantiomeric composition of Trp. However, the differences are so small that a quantitative relationship between the spectra and the enantiomeric composition of Trp could not be modeled using the classical least squares method. Thus, PLS, IPLS and MWPLS were used to build calibration models between the spectral data and the enantiomeric composition of Trp.

Full-spectrum PLS model

PLS was used to build the calibration model between the UV–Vis spectra and the mol fraction of L-Trp. The spectral data of the whole wavelength range and the mol fraction of L-Trp were used as independent variable and dependent

TABLE I. Result of external test validation and leave-one-out cross validation; the samples marked by an asterisk are the samples in Group II (see text)

No.	Actual mole fraction	Predicted mole fraction			Relative error, %		
		PLS	IPLS	MWPLS	PLS	IPLS	MWPLS
1	0.12	0.249	0.128	0.128	107.50	6.67	6.67
2	0.16	0.126	0.151	0.152	−21.25	−5.63	−5.00
3*	0.20	−0.155	0.207	0.205	−177.50	3.50	2.50
4	0.24	1.100	0.217	0.240	358.33	−9.58	0.00
5	0.28	0.631	0.242	0.270	125.36	−13.57	−3.57
6	0.32	0.099	0.348	0.348	−69.06	8.75	8.75
7*	0.36	0.740	0.352	0.368	105.56	−2.22	2.22
8	0.40	0.437	0.414	0.398	9.25	3.50	−0.50
9	0.44	0.748	0.469	0.455	70.00	6.59	3.41
10	0.48	0.597	0.511	0.509	24.38	6.46	6.04
11*	0.52	0.370	0.504	0.501	−28.85	−3.08	−3.65
12	0.56	0.654	0.577	0.564	16.79	3.04	0.71
13	0.60	0.344	0.583	0.561	−42.67	−2.83	−6.50
14	0.64	−0.093	0.635	0.605	−114.53	−0.78	−5.47
15*	0.68	0.096	0.724	0.687	−85.88	6.47	1.03
16	0.72	0.394	0.744	0.733	−45.28	3.33	1.81
17	0.76	0.915	0.766	0.781	20.39	0.79	2.76
18	0.80	0.098	0.781	0.818	−87.75	−2.38	2.25
19*	0.84	0.584	0.810	0.805	−30.48	−3.57	−4.17
20	0.88	0.276	0.805	0.824	−68.64	−8.52	−6.36
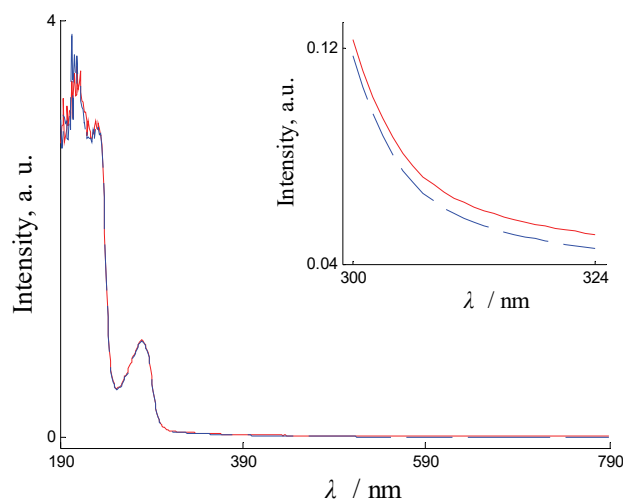
Fig. 1. UV–Vis spectra of two solutions containing 0.01 mmol L^{-1} Trp and 0.02 mmol L^{-1} BSA; solid line: D-Trp+BSA; dashed line: L-Trp+BSA. Inset: spectra from 300 to 324 nm.

variable, respectively. This model was a full-spectrum PLS model. Then, leave--one-out cross validation[36] and external test validation[37] were employed to assess

the prediction performance of this model. The prepared 20 samples were split into two groups: Group I and Group II. Group I comprised 15 samples. Group II comprised 5 samples (marked by an asterisk in Table I).
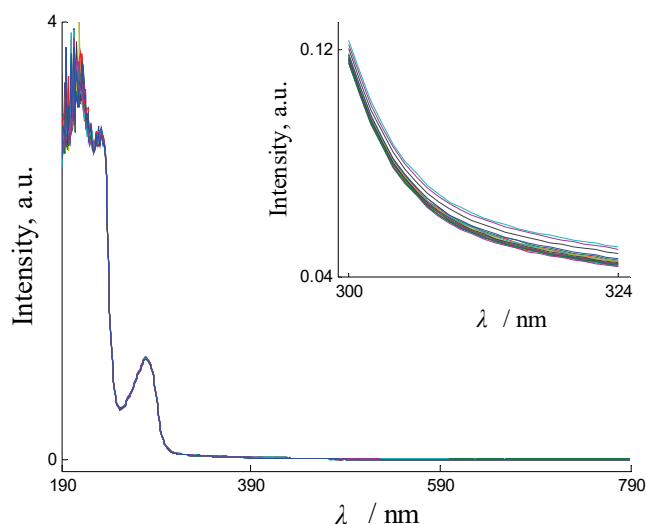

Fig. 2. UV–Vis spectra of the twenty prepared samples.
Inset: spectra from 300 to 324 nm.

First, the external test validation was performed. The PLS model was generated by using Group I as the calibration set. The obtained model contained four latent variables. Group II was used as the external test set. The mole fraction of L-Trp of the 5 samples in Group II was predicted using the obtained PLS model. The prediction result is listed in Table I. For the 5 samples, the prediction *RMSRE* was 101.77. Subsequently, the leave-one-out cross validation was performed. The mole fraction of L-Trp in Group I was predicted in turn. The used PLS model still included four latent variables. The prediction result is listed in Table I. For the 15 samples, the *RMSRE* of prediction reached 114.48. It could be seen from Table I that the predicted mole fractions were not in agreement with the actual values. In particular, the prediction results for samples 3, 4 and 14 were completely unreasonable. The results of the two validations demonstrate the full-spectrum PLS model could not model a quantitative relationship between the spectral data and the mole fraction of L-Trp. Some regions in the spectra may contain non-modeled information and should therefore, be excluded from the model. Thus, spectral region selection should be helpful in the building of a reasonable calibration model.

IPLS model

IPLS was used to perform spectral region selection and develop a calibration model. The prediction accuracy of the established IPLS model was evaluated by external test validation and leave-one-out cross validation.

In the external test validation, Group I was used as the calibration set to build the IPLS model. The full spectrum was split into 19 intervals. The wave-length range of these intervals were 190−221, 222−253, 254−285, 286−317, 318−349, 350−381, 382−413, 414−445, 446−477, 478−509, 510−541, 542−572, 573−603, 604−634, 635−665, 666−696, 697−727, 728−758 and 759−789 nm. The results of IPLS are shown in Fig. 3. According to Fig. 3, the optimal calibration model was obtained at the fourth interval (the wavelength range 286−317 nm) coupled with seven latent variables. This local model is the IPLS model was required. Then, it was used to predict the mol fraction of L-Trp of Group II. The results are presented in Table I. The *RMSRE* of the prediction was 4.03 for the five samples.

In the leave-one-out cross validation, the used IPLS model was again developed using the spectral data for 286−317 nm and it included seven latent variables. The prediction results are presented in Table I. The *RMSRE* of this prediction was 6.50 for the 15 samples. As shown in Table I, the predicted mole fractions were almost in agreement with the actual values, which showed that IPLS was practicable for modeling the quantitative relationship between the
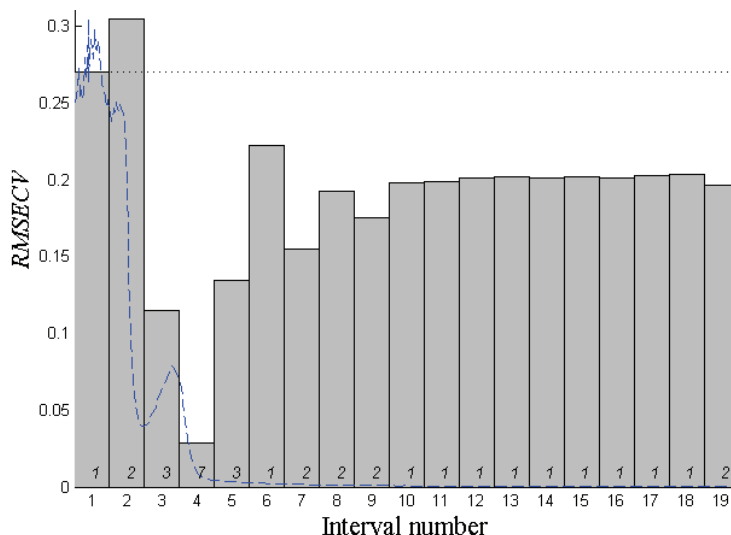

Fig. 3. Root mean square error of cross validation (*RMSECV*) plot obtained from IPLS. Vertical bar is the *RMSECV* for each local model. The figure in each bar is the optimal number of latent variables for each local model. The dashed line gives the mean spectrum of the 20 samples. The dotted line is the *RMSECV* of the full-spectrum PLS model.

spectral data and the mol fraction of L-Trp. Obviously, selection of the spectral region is a crucial step to build a feasible calibration model. IPLS was superior to PLS for building the calibration model, because it involves the procedure for the selection of the spectral region.

Although the obtained IPLS model was acceptable, it was still worthwhile to search for a better calibration model because the prediction error of samples 5 was somewhat large. IPLS can only divide the spectrum into a series of equidistant intervals at certain positions. The spectral intervals built by IPLS may still contain non-modeled information and so was not the best region for building the model. If a more informative spectral region could be found to build the model, a better calibration model should be obtained.

MWPLS model

MWPLS finds the optimal spectral region by moving the window through the total spectral range. It can inspect more potential spectral regions than IPLS. Thus, a MWPLS model was developed in order to improve the obtained calibration model.

The prediction performance of the obtained MWPLS model was still assessed by external test validation and leave-one-out cross validation. In the external test validation, Group I was used as the calibration set to build the model. The window size was set to 31 nm. The results of MWPLS are illustrated in Fig. 4. In terms of the *RMSECV*, the optimal calibration model was the local model built at the position 306 nm, *i.e.*, the established MWPLS model was actually the local PLS model developed from the spectral region 291−321 nm. This model includes five latent variables. The mol fraction of L-Trp of Group II was then predicted using this model. The results are listed in Table I. The *RMSRE* of the prediction was 2.93 for the five samples. In the leave-one-out cross validation, the used MWPLS model was also developed from the spectral region of 291−321 nm and it included five latent variables. The prediction results are presented in Table I. For the 15 samples, the *RMSRE* of the prediction was 4.73. As shown in Table I, the predicted mol fractions were in good agreement with the actual values.

The results presented above demonstrate MWPLS to be a practicable method for modeling the quantitative relationship between the spectral data and the mol fraction of L-Trp. As expected, the prediction accuracy of the obtained MWPLS model was higher than that of the IPLS model. It was shown that using MWPLS was better than using IPLS for the determination of the mol fraction of L-Trp. MWPLS is a more promising method for analyzing UV–Vis spectrum. Obviously, using the MWPLS selected spectral region instead of the IPLS selected spectral region as the independent variables substantially improved the prediction accuracy of the calibration model. It was demonstrated that the selection of the

spectral region significantly influences the calibration results of PLS in the analysis of UV–Vis spectroscopic results. Inspecting more regions of the spectra is helpful in obtaining better calibration results.
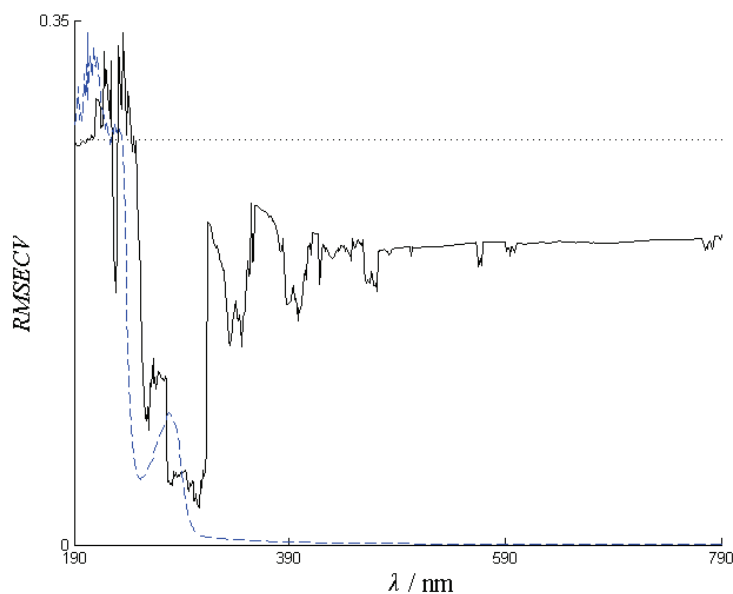

Fig. 4. *RMSECV* plots obtained from MWPLS. The solid line gives the *RMSECV* for all the local models. The dotted line represents the *RMSECV* for the full-spectrum PLS model. The dashed shows the mean spectrum of the 20 samples.

CONCLUSIONS

A UV–Vis spectroscopic method for the determination of the enantiomeric composition of Trp was developed. The result of external test validation and leave-one-out cross validation demonstrated that both IPLS and MWPLS are practicable for modeling the quantitative relationship between the spectral data and the mol fraction of L-Trp. On the contrary, full-spectrum PLS is unable to model this relationship. It was shown that the selection of the spectral region is an indispensable step to determine the enatiomeric composition of Trp by UV––Vis spectroscopy.

The MWPLS model showed higher prediction accuracy compared with the IPLS model. It was demonstrated that a more suitable spectral region for building the calibration model was found by MWPLS, leading to the establishment of a better calibration model. Obviously, MWPLS was better than IPLS for determin-ing the enatiomeric composition of Trp. Since the results of PLS, IPLS and MWPLS were quite different, it was shown that the selection of the spectral region has a significant influence on the calibration result in UV–Vis spectro-

scopic analysis. MWPLS is superior to IPLS for selecting the spectral region in UV–Vis spectroscopy analysis.

The combination of UV–Vis spectroscopy and MWPLS is an easy-to-use and commendable method for determining the enantiomeric composition of Trp at low concentration levels. The enantiomeric composition of Trp can be accurately determined provided there is 0.01 mmol L^{-1} Trp in the sample. Although this method was only applied to Trp in the present study, it should be a promising method for other chiral compounds.

И З В О Д
МЕТОДЕ ПАРЦИЈАЛНИХ НАЈМАЊИХ КВАДРАТА УЗ ФИКСНУ И МОБИЛНУ СЕЛЕКЦИЈУ ИНТЕРВАЛА У ОДРЕЂИВАЊУ ЕНАНТИОМЕРНОГ САСТАВА ТРИПТОФАНА ПРИМЕНОМ UV–VIS СПЕКТРОСКОПИЈЕ

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Испитивана је примена методе парцијалних најмањих квадрата (PLS) уз фиксну и мобилну селекцију спектралних интервала (IPLS и MWPLS, редом) у анализи енантиомера триптофана (Trp). Развијена је UV–Vis спектроскопска метода за одређивање енентиомерног састава Trp. Добијени су калибрациони модели применом PLS, IPLS и MWPLS приступа. Методом унакрсне валидације појединачних изостављања (leave-one--out) и валидацијом на екстерном сету објеката процењена је поузданост предвиђања добијених модела. Резултати валидације показују непрактичност примене PLS приступа у моделовању целог спектралног опсега, у циљу одређивања енантиомерног састава L-Trp на основу спектралних података. Насупрот томе, оба новоразвијена приступа, IPLS и MWPLS, су погодна за моделовање. У IPLS моделу, релативна грешка изражена као корен средње вредности квадрата одступања измерених од предвиђених вредности (*RMSRE*) у случају екстерне калибрације и унакрсне валидације појединачних изостављања износе 4,03 и 6,50, редом. За MWPLS модел, *RMSRE* теста екстерне калибрације и унакрсне валидације износе 2,93 и 4,73, редом. Очигледно је да је предвиђена тачност MWPLS модела виша него код IPLS модела. Показано је да је UV–Vis спектроскопија комбинована са MWPLS погодна за одређивање енантиомерног састава Trp. MWPLS модел је супериорнији у односу на IPLS за селекцију спектралне области и UV–Vis спектроскопску анализу.

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