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The multivariate use of vibrational spectroscopy for chemical characterisation of chromatography media

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Abstract

Chromatography media used in purification of biomolecules are extensively tested with various methods to ensure high quality products. The spherical and porous polymer particles are derivatised to give different chromatographic properties. The variations in, for instance, ligand content and chromatographic function are generally tested with a number of conventional analytical methods (mainly wet-chemical and functional methods) and these are time consuming. However, by using vibrational spectroscopy, chemical information can be efficiently obtained from the spectral data. In combination with traditional analytical data, multivariate models can be constructed. These models can then be used as a tool for determination (prediction) of, for example, ligand content. In the present work, prediction of ionic capacity and content of allyl groups are exemplified by Raman spectroscopy of two different types of agarose-based media (Sepharose™ Fast Flow Sepharose™).

The conclusion is that vibrational spectroscopy is a simple, fast and highly informative tool for both qualitative and quantitative characterisation of adsorbents used in chromatography of biomolecules.

Keywords: Agarose; Allyl content; Ligand content; Chemometrics; Chromatography media; IR; NIR; PAS; PLS; Raman; Sepharose; Sulphopropyl; Vibrational spectroscopy

1. Introduction

Chromatography media used for purification of biomolecules are extensively tested to verify the quality of these products [1]. Some methods, such as ionic capacity, ligand content, etc. yield chemical information of the stationary phases while other methods, such as function test with various test substances (often proteins) are more related to functional properties and applications aimed for the products [2,3].

Vibrational spectroscopy techniques, such as Fourier transform (FT)-Raman, FT infrared (FT-IR) and FT near infrared (FT-NIR) have become more and more popular as tools to ensure product quality in various industrial processes [4–6]. A lot of samples can be tested in a short period of time, but this generates a large amount of spectral data to evaluate. However, by using a multivariate approach the large amount of spectral data can be efficiently evaluated. Principal component analysis (PCA) and partial least square regression (PLS) are techniques that can be used to evaluate relations and/or classification of the data and prediction of response variables, respectively [7,8]. Here the latter has successfully been used to predict different physical parameters of separation media. FT-IR, FT-NIR and FT-Raman spectroscopy [9–11] was used to characterise two different types of agarose-based media (Sepharose™ Fast Flow Sepharose™).

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The stationary phase is based on the naturally occurring polysaccharide agarose derivatised to give different chromatographic properties and prepared as porous beads with a mean particle size of 90 μm.

FT-Raman showed the best predictability judged by the highest correlation coefficient, lowest prediction error or needing the least number of PLS components in the final model when other factors, such as using the same sample preparation, were kept constant. The results are, therefore, exemplified for this technique. A summary of the predicting power obtained with the various techniques is presented.

2. Experimental

Raman spectra were collected on an NIR-FT Raman spectrometer. Before measurement, samples (chromatography media) were pre-treated by different washing steps and finally dried in vacuum. For each sample, a fine powder was obtained which could be easily packed in capillary tubes. The tubes were positioned in the spectrometer prior to measurement. The same powder samples were also directly scanned by FT-IR with a photoacoustic (PAS) detector and FT-NIR with a reflectance probe.

Details of the experimental methods are as follows: gel media (10 ml of gel slurry) was washed with 20% ethanol, 1 M NaCl and finally de-ionised water, 10 volumes of each. After dry suction the samples were dried in vacuum to <1 mbar overnight (16 h). Raman measured in back-scattering mode (Bruker RFS 100/S) were performed with 1000 scans at 8 cm^{-1} resolution, Norton–Beer medium apodisation and 600 mW laser power at 1064 nm. All spectra were normalised to 1.5 units around 1082 cm^{-1}. FT-IR/PAS of 0.1 g powdered sample with 400 scans at 8 cm^{-1} resolution at 0.2 cm/s optical path difference velocity and Norton–Beer strong apodisation (Perkin-Elmer system 2000). All spectra were normalised to 1.5 units at 1078–1086 cm^{-1}. FT-NIR were probed on top of a 6 mm height of powdered sample with 16 scans at 16 cm^{-1} resolution (Bruker Vector 22/N). All spectra were transformed with multiplicative scattering correction (MSC). Spectral calculations were made with OPUS version 3.0, Bruker and Spectrum f Windows version 1.5, Perkin-Elmer. Chemometrics were made with Unscrambler version 7.6, Camo ASA.

3. Results and discussion

3.1. Case study 1: content of allyl groups

Normalised Raman data and content of allyl groups (variables determined by titration method) on 32 batches (objects) of Sepharose™ (an intermediate in synthesis of stationary phases based on agarose) were used to construct a data set for multivariate calibration (PLS). The variable ''content of allyl groups'' was tested against the spectral variables. The model was calculated and validated by using cross validation (Figs. 1–3). Certain regions of each spectrum were excluded due to normalisation (1075–1089 cm^{-1}) and less informative data (<800 and >1700 cm^{-1}). The loading plot in Fig. 2 reflects variations in Raman intensity due to different degree of allyl content.

Prediction of content of allyl groups with PLS showed good correlation between the spectral data and the reference data (the titration method). High correlation coefficients and a low root mean square error of prediction (RMSEP) value were found as shown in Fig. 3. The decreasing object pattern in the lower part of the plot indicates that media with low content of allyl groups are predicted to be lower in allyl content than the actual measured value obtained by the titration method. However, normal linear regression on peak maximum (allyl C=C stretch) indicates that the titration method overestimates low allyl content and that the Raman method measures correctly. Fig. 3 also shows that one medium behaves as an outlier (object 10), which is easily discovered in a ''predicted versus measured plot''.

3.2. Case study 2: ionic capacity and residual allyl groups

Normalised Raman data and wet-chemical data (ionic capacity and residual content of allyl groups (variables)) on 32 batches of the strong cation exchanger Sepharose™ Fast Flow (objects), an agarose-based stationary phase with sulphopropylated groups, were used to construct a data set for PLS. Each variable was tested separately against the spectral variables. In this study, eight batches were used as a test set for validation of the model.

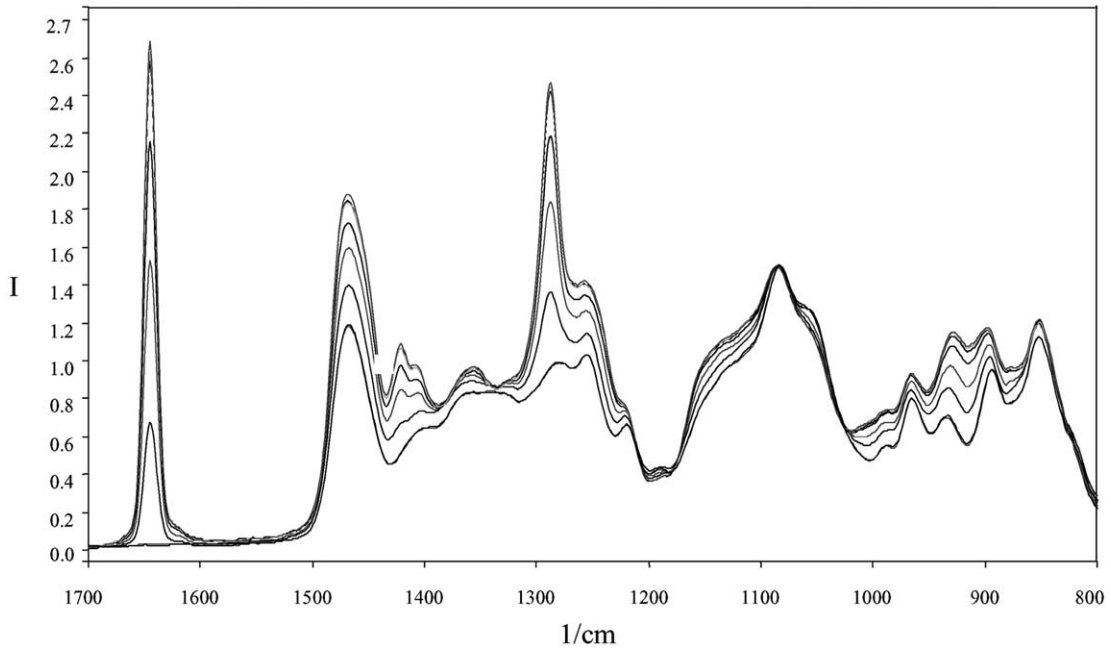

Fig. 1. Normalised Raman spectra for Sepharose™ media with different content of allyl groups.

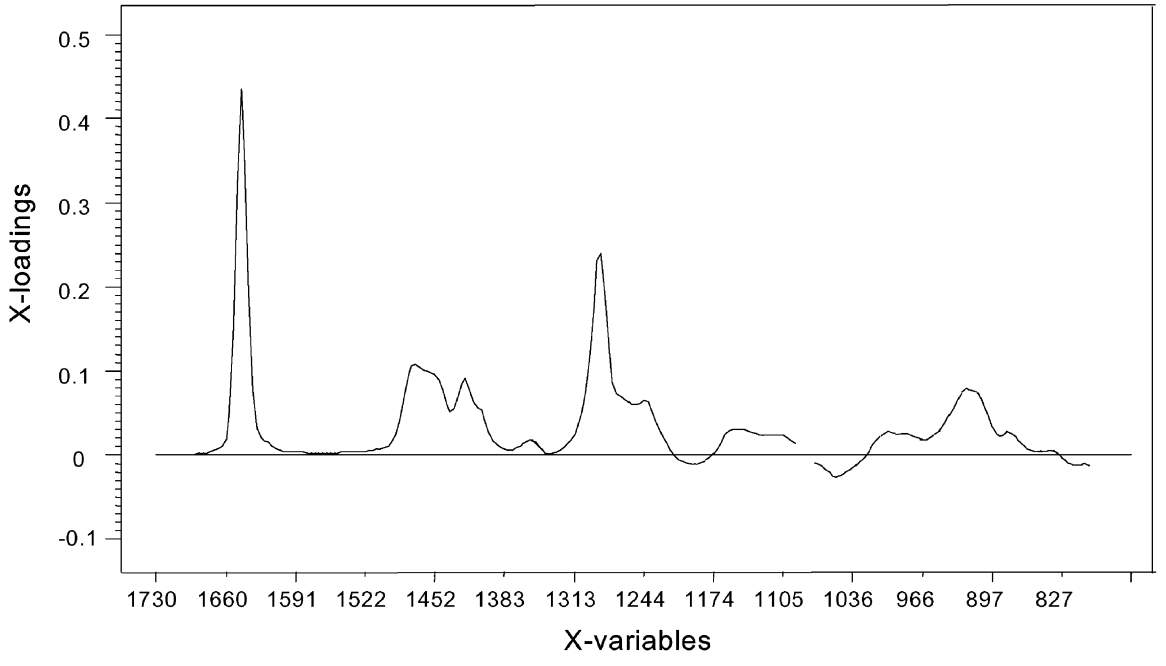

Fig. 2. *X*-loading plot for first principal component of the PLS model for Sepharose™ media with different content of allyl groups.

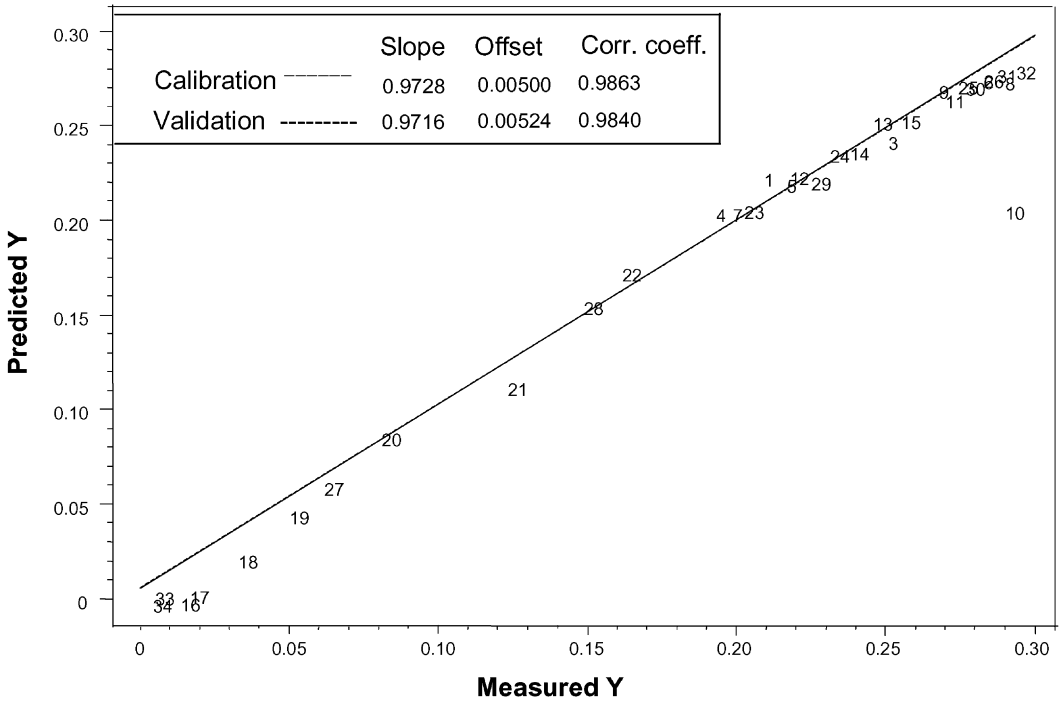

Fig. 3. Regression data (predicted vs. measured plot) for content of allyl groups (range 0.015–0.30 mol/l medium) with two PLS components, RMSEP = 0.0083 mol/l (outlier excluded).

Certain regions of each spectrum were excluded in the calculations due to normalisation (1078–1089 cm^{-1}) and less informative data (<700 and >1800 cm^{-1}).

Prediction of ionic capacity and content of residual allyl groups showed good correlation between the spectral data and the wet-chemical data (titration methods) (Tables 1 and 2). High correlation coefficients and a low RMSEP value were found as shown in Figs. 4 and 5. An outlier was found during evaluation of ionic capacity data. The wet-chemical value (0.206 mol/l medium) was predicted as 0.218 mol/l. However, after re-measurement by titration the wet-chemical value was corrected to 0.220 mol/l.

Table 1
PLS models built on vibrational spectra of Sepharose™ Fast Flow for prediction of ionic capacity (mol/l)

Method	Slope	Offset (mean)	Correlation coefficient	RMSEP (mean)	PLS components
FT-Raman	0.946	0.011	0.973	0.0025	5
FT-IR/PAS	0.998	0.001	0.998	0.0097	8
FT-NIR	0.866	0.028	0.931	0.0070	5

Table 2
PLS models built on vibrational spectra of Sepharose™ Fast Flow for prediction of allyl groups (mol/l)

Method	Slope	Offset (mean)	Correlation coefficient	RMSEP (mean)	PLS components
FT-Raman	0.867	0.008	0.931	0.005	1
FT-IR/PAS	0.985	0.001	0.992	0.005	5
FT-NIR	0.931	0.005	0.965	0.006	2

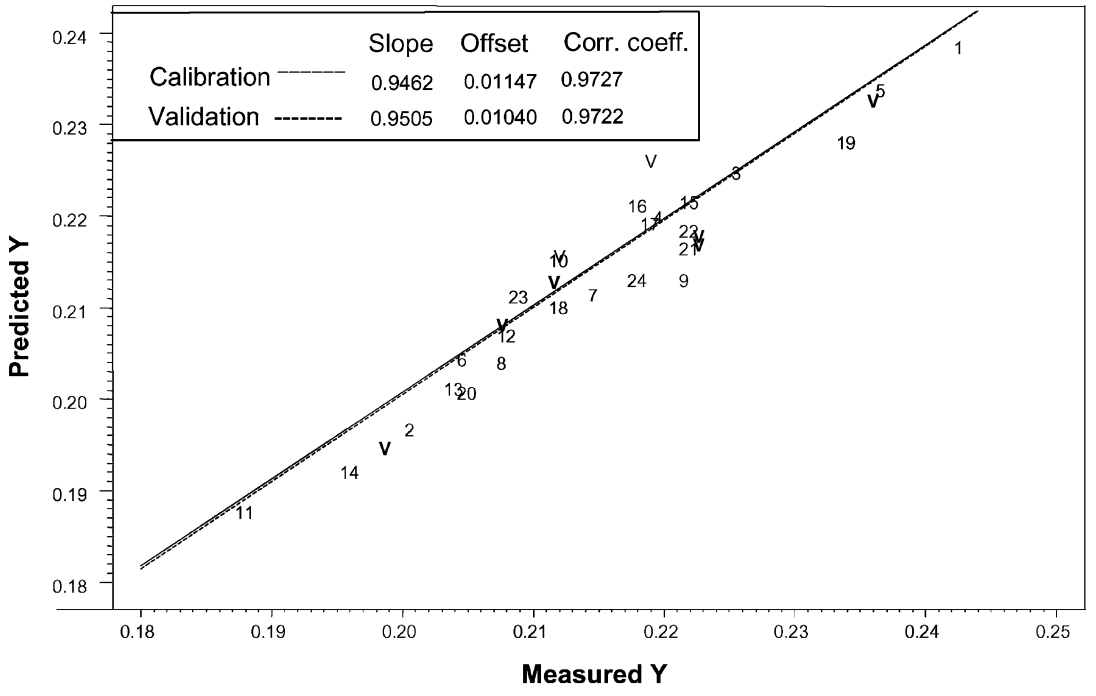

Fig. 4. Regression data (predicted vs. measured plot) for ionic capacity (range 0.186–0.241 mol/l medium) for Sepharose™ Fast Flow with five PLS components; calibration (numbers), validation (V), RMSEP = 0.0025 mol/l.

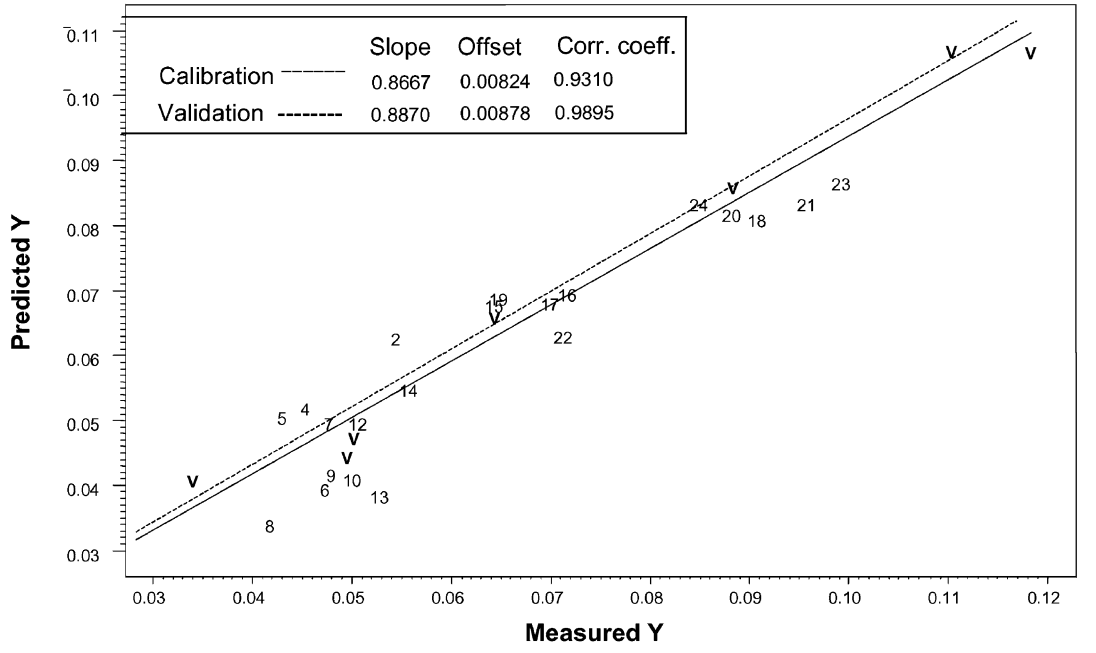

Fig. 5. Regression data (predicted vs. measured plot) for content of residual allyl groups (range 0.034–0.118 mol/l medium) for Sepharose™ Fast Flow with one PLS component; calibration (numbers), validation (V), RMSEP = 0.005 mol/l.

3.3. Comparison of vibrational methods for predicting ability

The FT-Raman method showed the best predicting ability judged by the lowest predicting error in combination with the least number of PLS components. In practical interpretative spectroscopy, both Raman and IR are a first choice as interpretation of chemical functionality are more straightforward. The FT-NIR method seems to be attractive for multivariate calibration, considering the scan time needed is around 10 s compared with 10–15 min for the other two methods. In this study, the sampling requirements for all methods are the same as measurements are made on the same powdered sample with no need of dilution. Rather the techniques are complementary and should be used in combination.

4. Conclusions

Raman and IR spectroscopy are suitable for first-order qualitative and quantitative characterisation of stationary phases used in chromatography of biomolecules. Chemical information can be predicted by combining vibrational spectroscopy (IR, Raman and NIR) with chemometrics.

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