Novel NIR reflection spectroscopic methods for the simultaneous determination of nanomaterial's properties

C.W. Huck

Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innrain 52a, 6020 Innsbruck, Austria. E-mail: christian.w.huck@uibk.ac.at

Introduction

In general "nano" is the collective name for investigations carried out with matter on a scale between 0.1 nm and some 100 nm, applied in physics, chemistry, pharmacy, biology, medicine, electricity, mechanics, optics, and in daily life products. Therefore, its physical, chemical and morphological characterization is an urgent and demanding challenge. Until now, particle size has been determined by electron microscopy (ELMI), light scattering or the Coulter-Counter method, surface area by nitrogen adsorption experiments (BET), and pore dimension by mercury intrusion porosimetry (MIP). For chemical characterization nuclear magnetic resonance (NMR) spectroscopy and elementary analysis are applied. Because all of these methods require expensive instrumentation and trained lab staff we propose to use near infrared reflection spectroscopy (NIRS) for the simultaneous determination of the chemical and physical properties of nanomaterials. Based on our preliminary experience in the control of fullerenes' chemical derivatization,^{1,2} and physico-chemical characterization of silica,^{3,4} the method is enhanced by enabling in addition the molecular weight of dendrimers, and the homogeneity of monolithic polymers with nano-structures, to establish a so-called "nano-intelligent" NIRS system (Figure 1).

Materials and methods

NIR spectra were either recorded with a scanning polarization interferometer Fourier-transform NIR spectrometer (FT-NIR) (Büchi, Flawil, Switzerland) or a SpectrumTM Spotlight 400 combined with a Spectrum GX IR/NIR spectrometer (Perkin Elmer, Rodgau, Germany). The FT-NIR instrument offers a resolution of 12 cm⁻¹, an absolute wavelength accuracy of ± 2 cm⁻¹ and a relative reproducibility of 0.5 cm⁻¹. The imaging system offers a signal-to-noise ratio of >12,000:1, a spectra collection speed of 160 spectra per second at 16 cm⁻¹ and a maximum number of spectra per image of >260,000. Chemometric software NIRCal 4.21 (Büchi), Unscrambler v 9.6 (CAMO, Oslo, Norway), SpectrumIMAGE-Spotlight R 1.6.0 were used for creating the principal component



Figure 1. Development of a "nano-intelligent" NIRS system for the characterization of nanomaterial's properties.

analysis (PCA) and partial least squares (PLS) regression models. For testing the models the collected spectra were divided into a learning-set (c-Set, 67%) and a test-set (v-Set, 33%) both consisting of independent samples. Measurements were carried out at 23° C from 4000 to 10,000 cm⁻¹.

Results and discussion

Dendrimers

Over the last 10 years dendrimers have attracted increasing attention in the fields of chemistry, physics, pharmacy and clinical chemistry, because of their assumed highly compact, well-defined globular shape. The branched molecular architecture, consisting of a core, an interior of shells (generations) and terminal functional groups, makes it easy to control the size, molecular weight and chemical functionality of the dendrimers. Synthesis can be performed by either the divergent or the convergent approach, meaning the stepwise construction of iterative stages around a desired core, to produce mathematically defined core-shell structures. Here we report an investigation on the adsorption and interaction of PAMAM-NH₂ dendrimers (G0-G7) onto porous silica with NIR diffuse reflection spectroscopy, in combination with multivariate data analysis

(MVA). Furthermore, GEMMA (Gas-Phase Electrophoretic Mobility Molecular Analysis) and MALDI-lin TOF-MS were drawn as reference techniques for building a multivariate calibration model, whereas capillary zone electrophoresis (CZE) was utilized to quantify the amount of adsorbed dendrimers onto the silica material. Twenty-four PAMAM-NH₂-loaded thin-layer chromatographic (TLC) plates were placed onto a horizontally-coupled integrating sphere device, and five spectra, each representing the average of 10 scans, were consecutively recorded on each of the plates, resulting in a total of 120 NIR spectra. Figure 2(a) shows the averaged, pretreated (multiplicative scatter correction (MSC)) absorption spectra, whereas Figure 2(b) depicts the second derivative (Savitzky–Golay, 9 points) spectra. The absorption intensity at specific wavenumbers exhibits a linear trend with the number of generations. In more detail, the absorption band at 4932 cm⁻¹, representing a vsym (NH) + amide II combination band, is aroused exclusively by the adsorbed dendrimers; presumably the terminal NH₂ functionalities are responsible for that. Multivariate calibration was performed with the theoretical data, with those from CE, GEMMA and MALDI-TOF/MS resulting in better results for the NIRS method in case of particle size and molecular weight, which is summarised in Table 1.⁵

Monolithic poly(methylstyrene-(vinylphenyl)ethane) (MS/BVPE)

FT-NIR diffuse reflection spectroscopy was used in combination with PCA and PLS to simultaneously determine both physical and chemical parameters of a porous poly(*p*-methylstyreneco-1,2-bis(*p*-vinylphenyl)ethane) (MS/BVPE) monolithic polymer used as a stationary phase in



Figure 2. (a) NIR absorption spectra of the pure silica (dashed line) and the dendrimer loaded silica from 4000 cm⁻¹ to 7500 cm⁻¹. (b) The second derivative spectra (Savitzky–Golay, 9 points) show increasing absorbance intensity at 4932 cm⁻¹ (PAMAM-NH₂) with decreasing generation, respectively. It has to be considered that absorbance signals in (a) show negative values in (b).

Method	PLS			
Calibration wavenumbers	4000–7608 cm ⁻¹			
Data pretreatment	Multiplicative Scatter Correction (MSC)			
Calibration factors	7			
	Particle size (Å)		Molecular weight (kDa)	
	Manufacterer (Dendritech)	$\begin{array}{c} \text{GEMMA} \\ (\text{G0},\text{G1},\text{G3} = \\ \text{theor. val.}^{\text{a}} \end{array}$	Manufacterer (Dendritech)	MALDI- linTOF-MS
c-Set BIAS	4.12E–13	3.55E-13	1.10E-12	8.18E-13
v-Set BIAS	-1.44	-1.32	-5.21	-3.63
SEE	4.3	3.6	11.4	8.5
SEP	5.1	4.1	11.7	9.0
c-Set Regression coefficient	0.98	0.99	0.96	0.97
v-Set Regression coefficient	0.96	0.96	0.91	0.91
c-Set Regression intercept	1.52	1.26	2.37	1.76
v-Set Regression intercept	-0.20	-0.15	2.07	1.98
c-Set Regression slope	0.97	0.97	0.93	0.93
v-Set Regression slope	1.04	1.03	1.16	1.09

Table 1. Calibration parameters for the calculated PLS models.

^aThe theoretical values are obtained by the manufacturer (Dendritech).

separation science.⁶ Chemical variations during the synthesis of the polymer material can alter the pore volume and pore area distributions within the polymer scaffold. Furthermore NIRS chemical imaging was implemented to assess the uniformity of the samples. The relevance of the interrelation of physical and chemical parameters is highlighted whereas the amount of MS/BVPE (%, v/v) and the quantity (%) of micropores (diameter, d < 6 nm), mesopores (6 nm < d < 50 nm) and the macropores (50 nm < d < 200 nm) could be determined with one measurement. The emerged quantiles (%) and pore size distributions regulated by the amount of MS/BVPE can be gathered from Figure 3. For example, the sample which contains 35% MS/BVPE shows the greatest fraction of emerged pore volume in the meso-pore range (59.0%) whereas the quantile of micro-pore volume shows 33.7% and for the macro pore volume quantile 7.3%, respectively. The *SEP* for determining the MS/BVPE amount in the sample showed 0.35%, for pore volume quantiles 1.42–8.44% and for pore area quantiles 0.38–1.45%, respectively.⁷

Conclusions

The implication of these results is that FT-NIR spectroscopy is a suitable technique for the screening of samples with varying physico-chemical properties and to quantitatively determine the parameters simultaneously within a few seconds.



Figure 3. Resulting pore volume and pore area distributions by gradually increasing the MS/BVPE content. Note that not the entire macropore range can be assessed with nitrogen adsorption (only up to $d \sim 200$ nm).

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