Progress in Silica Chemistry – Determination of Physico-Chemical Parameters *via* Near-Infrared Diffuse Reflection Spectroscopy

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Abstract: In analytical chemistry, silica gel plays a pre-dominant role in separation science. It is the most important stationary phase in chromatography and electrophoresis. Separation efficiency is directly dependent on the quality and physical properties of the chromatographic bed. Therefore, methods for the physicochemical characterisation of silica stationary phases have been developed over the past decades to fulfil the necessity of pattern control: Brunnauer Emmet Teller (BET) for determination of surface area, mercury intrusion porosimetry (MIP) and size exclusion chromatography (SEC) for pore-size measurement and light-scattering (LS) to evaluate the particle size. Beside that these methods are elaborate and time-consuming and the use of MIP is awkward due to the necessity to ply with poisonous mercury.

Therefore, we introduce near-infrared reflection spectroscopy (NIRS) in the fibre-optics mode for a fast (few seconds), easy to handle and highly reproducible new analytical technique to characterise surface area, particle size in µm- and porosity in lower nm- range. This new analytical NIRS tool is suitable for high sample throughput and therefore aims at high interests in the nano-field. Determination of particle size, porosity and surface area are achieved with a linear correlation coefficient $R^2 > 0.98$, BIAS $< 1.26 \times 10^{-14}$. Beside these advantages, our introduced NIRS approach allows physicochemical characterisation with high precision, output and performance.

Keywords: Near infrared spectroscopy, reflection, chemometrics, multivariate data analysis, silica gel.

INTRODUCTION

Research in the nano-field is demanding new analytical techniques, which enables their precise characterisation. Silica gel used in chromatographic and electrophoretic separation science possesses particle diameters in the µm- and pores in the lower nm- range. To yield highest separation efficiency in proteomics [1], metabolomics [2] and phytomics [3] of high and low molecular analytes, silica particles (porous, non-porous) and monoliths are commercialised. Electron micrographs depicted in Fig. (**1**) visualise the differences in morphology and application fields. Miniaturised separation systems such as micro liquid chromatography (µ-LC) [4], capillary electrochromatography (CEC) [5] and material-enhanced laser desorption/ionisation time of flight mass spectrometry (MELDI-TOF/MS) [6], beside sample pre-treatment techniques [7] are emerging techniques, enabling high sensitivity, selectivity for e.g. biomarker discovery. Manufacturing of well-defined particles and their morphological characterisation is a tough and exhausting job. Brunnauer Emmet Teller (BET) [8], mercury intrusion porosimetry (MIP), size-exclusion chromatography (SEC) and light scattering (LS) are the common methods applied to determine surface area, pore and particle size [9]. BET, MIP, SEC and LS require expensive laboratory equipment, well-trained staff and beside these, a measurement takes up to three hours. The inter-play of physicochemical properties and functionalisation of raw particles is essential to yield selective separation [10]. Success of derivatisation must be verified by nuclear magnetic resonance (NMR) spectroscopy. After near infrared region (780 – 2500 nm, 4000 – 12500 cm^{-1}) of sunlight had been discovered by Herschel in 1800 [11], Karl Norris from U.S. built in 1952, the first near infrared reflection spectrometer (NIRS) to quantify protein content in Manitoba wheat. From that date the number of applications in agriculture grew exponentially and became a well-established analytical method, even in phytomics and proteomics [12]. Plant ingredients used as leading compounds, e.g., 3´,4´,5´-trimethoxyflavone in *Flos Primulae veris* [13], naphtodianthrones and phloroglucines in *St. John´s Wort* [14] extracts can be quantitatively analysed, parallel to physical parameters (solvent composition) with high precision within few seconds. Qualitative cluster methods for recognition pattern control and quantitative models in coffee and wine producing industries have been described in detail and patented [15-17]. Thereby, NIRS is supported by sophisticated statistical software to perform principal component analysis (PCA) or regression (PCR) or partial least square regression (PLS) [18].

In the following work, we introduce near-infrared reflection spectroscopy (NIRS) in fibre-optics mode for a fast, easy to handle and highly reproducible new analytical technique to characterise surface area, particle size in µm- and porosity in lower nm-range based on recently carried out studies [19]. The suitability of NIRS method in nano-field is evaluated and discussed by analysing silica gel particles used in chromatographic separation science.

EXPERIMENTAL

Silica Materials

ProntoSIL 60 (3 µm, 60 Å, 450 $m^2 \times g^{-1}$), ProntoSIL 120 (3 µm, 120 Å, 300 $m^2 \times g^{-1}$), ProntoSIL 300 (3 µm, 300 Å,

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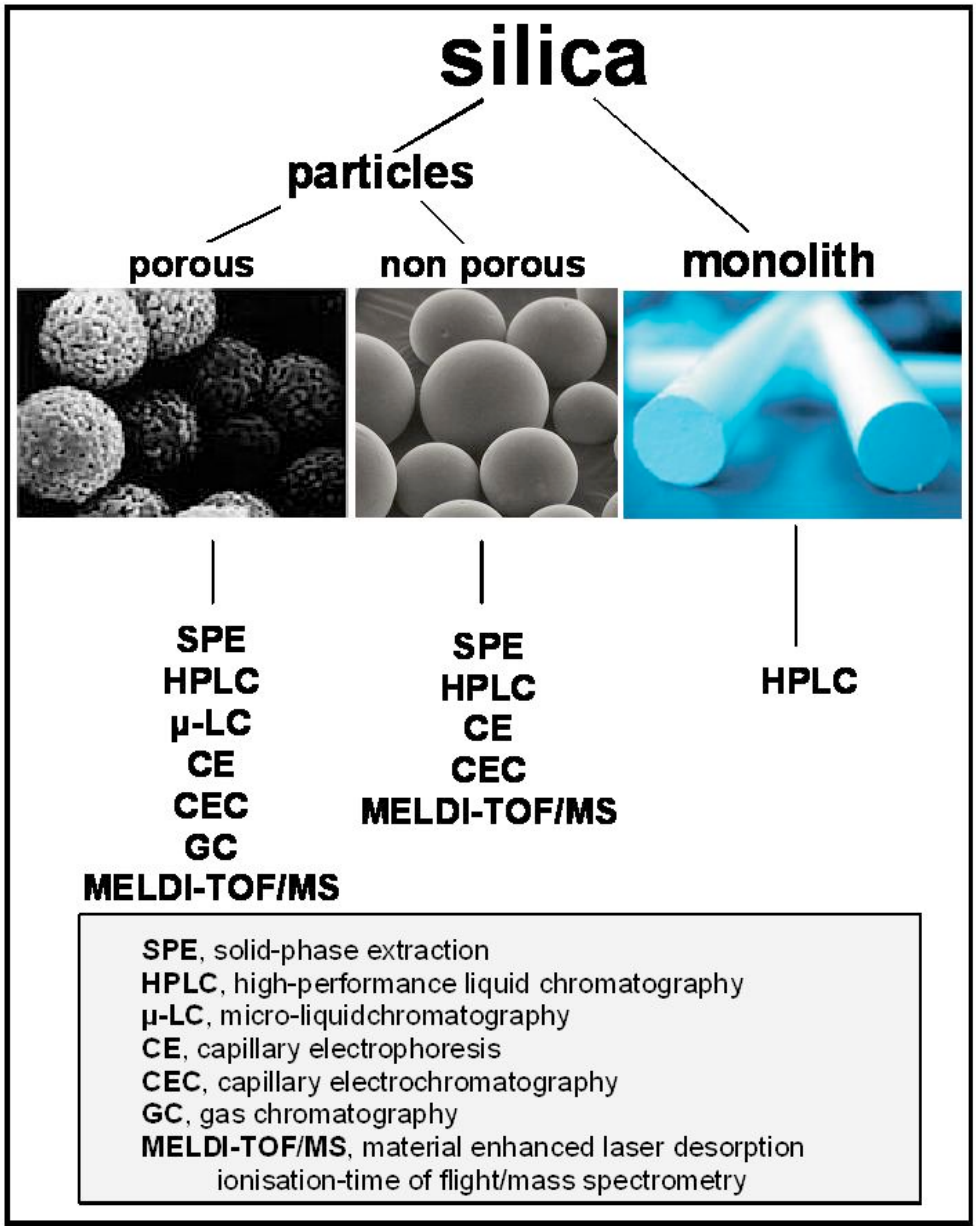

Fig. (1). Silica based stationary phases and their application in separation science.

100 $m^2 \times g^{-1}$), ProntoSIL with 120 Å and different particle diameters 3, 5, 10, 15, 20 µm, ProntoSIL with 5 µm particle and 60, 120, 200 and 300 Å pore diameter were purchased from Bischoff (Leonberg, Germany), Nucleosil C8 (5 µm, 300 Å, 100 $m^2 \times g^{-1}$), Nucleosil C18 (5 µm 300 Å, 100 $m^2 \times g^{-1}$), Nucleosil 1000 C18 (7 µm, 1000 Å, 25 $m^2 \times g^{-1}$) and Nucleosil 4000 C18 (7 µm, 4000 Å, 10 $m^2 \times g^{-1}$) from Agilent (Waldbronn, Germany), Hypersil C18 BDS (3.5 µm, 130 Å, 170 $m^2 \times g^{-1}$), Spherisorb C18 (3, 5, 10 µm, 80 Å, 220 $m^2 \times g^{-1}$), Spherisorb C8 (3, 5, 10 µm, 80 Å, 220 $m^2 \times g^{-1}$) were from Waters (Milford, USA). For the determination of particle size non-porous silica Micra (1.5 µm), purchased from Micra Scientific (Northbrook, IL, USA), was included in the model. All other silica phases, Amino 4 µ (4 µm, 126 Å, 317 $m^2 \times g^{-1}$), Amino A21 (10 µm, 114 Å, 317 $m^2 \times g^{-1}$), Amino A23 (6 µm, 117 Å, 310 $m^2 \times g^{-1}$), Silica 622 (14 µm, 91 Å, 431 $m^2 \times g^{-1}$), Silica 191-1 (6 µm, 74 Å, 458 $m^2 \times g^{-1}$), Silica 191-2 (6 µm, 74 Å, 458 $m^2 \times g^{-1}$), Silica 191-3 (6 µm, 74 Å, 458 $m^2 \times g^{-1}$), ODS 2 (6 µm, 74 Å, 308 $m^2 \times g^{-1}$, non endcapped), ODS 3 (6 µm, 78 Å, 267 $m^2 \times g^{-1}$, endcapped) were homemade.

Scanning Electron Microscopy (SEM)

The silica particles with different porosities and particle sizes were examined by the Zeiss scanning electron microscope (SEM) with the combination of higher magnification, larger depth of focus and greater resolution. The particles were mounted on the stub by using an adhesive prior to the microscopic inspection. SEM's were operated under high vacuum conditions (10^{-3} Pa) to ensure good resolution and high signal to noise levels.

NIR-Spectroscopy

NIR spectra were recorded with a scanning polarization interferometer NIR-spectrometer (Büchi, Uzwil, Switzerland), crosswise over a wavelength range from 4500 to 10000 cm^{-1} using an optic glass fibre (silica glass, Infrasil, Bes Optics Inc., Warwick, Great Britain, length 2 m) in re-

flection mode. There was no distance between the top of fibre and sample; measurements were carried out by applying slight pressure onto the sample.

10 scans were used for one average spectrum to equilibrate inhomogeneities. Chemometric software NirCal 3.0 and 4.0 (Büchi) was used for creating a model. Samples were thermostated in a water bath to 23°C (model PC/4, Julabo, Seelbach, Germany), randomly divided into a so called learning-set (75%), and a test set (25%). Measurements were conducted at 23 °C in a wavenumber range from 4000 – 10000 cm^{-1}.

Optimum number of factors used for the individual prediction was determined by cross-validation. Quality of cluster analysis was described in the Q-value calculated by NirCal 4.0 software. Selection of the best quantitative regression model is based on the following calculated values:

1) BIAS, i.e., the average deviation between predicted values (y_n) and actual values (x_n), in the calibration-set; should be close to zero.

$$Bias = \frac{1}{N}\sum\left(x_n - y_n\right)$$

2) PRESS, Predicted Residual Error Sum Square is the sum of square of deviation between predicted and reference values. The PRESS value of validation set should be as small as possible and similar to that of the calibration set.

$$PRESS = \sum\left(x_n - y_n\right)^2$$

3) Standard error of estimation (SEE), i.e., the standard deviation of differences between reference values and NIRS results in the calibration set.

$$SEE = \sqrt{\frac{1}{N}\sum\left(x_n - y_n - Bias\right)^2}$$

4) Standard error of prediction (SEP), i.e., the counterpart for the test-set samples. SEE and SEP should be as small as possible.

$$SEP = \sqrt{\frac{1}{N}\sum\left(x_n - y_n - Bias\right)^2}$$

5) The correlation coefficient (R^2) should approach 1.

Preparation of the Homemade Starting Silica Gels

Starting silica has been sized in our laboratory with an average particle size, pore diameter, specific surface area and pore volume, provided above. Further preparation of the phases was carried out as described earlier [8-10].

Reference Measurements

Particle size, pore diameter, specific surface area, total porosity, pore porosity and pore volume were determined and calculated following the size-exclusion chromatographic methods published [9]. In case of commercially available materials the information provided by the producer was used.

RESULTS AND DISCUSSION

Qualitative Analysis

For the identification of silica particles with different morphology and derivatisation, a qualitative model based on principal component analysis (PCA) was established, which allows clustering with differences in particle size, surface area, pore structure and derivatisation. Differences in pore structure beside particle size can be visualised by scanning electron microscopy (SEM). Fig. (**2**) depicts micrograph pictures of non-porous (1.5 μm), 300 Å (5 μm) and 1000 Å (7 μm) particles. For measurement, all samples were thermostated to 23 °C to avoid baseline shifts in a wavenumber range from 4500 to 9996 cm^{-1}, following the Kubelka-Munk equation in diffuse reflection mode: $f(R) = (1-R)^2/2R$; $R = I/I_0$. The applied analytical strategy is shown in NIR workflow depicted in Fig. (**3**). Sample is measured in diffuse reflection; Si-O, O-H, N-H and C-H functional groups are excited to stretching and bending vibrations and absorbed energy is recorded. The intensity of light reflected is sent towards a PbS-detector and spectrum is generated by Fourier transformation (FT). As differences in spectra resulting from different morphology are in some cases hard to interpret, chemometric algorithms including principal component analysis (PCA), principal component regression (PCR) and partial least squares regression (PLS) are applied. By this qualitative and quantitative analysis can be achieved. Fig. (**4**) shows the spectral differences between raw, octadecylated and amino derivatised silica. For spectral interpretation absorption spectra of these three materials are recorded. The most intense band belongs to the stretching vibration of O-H

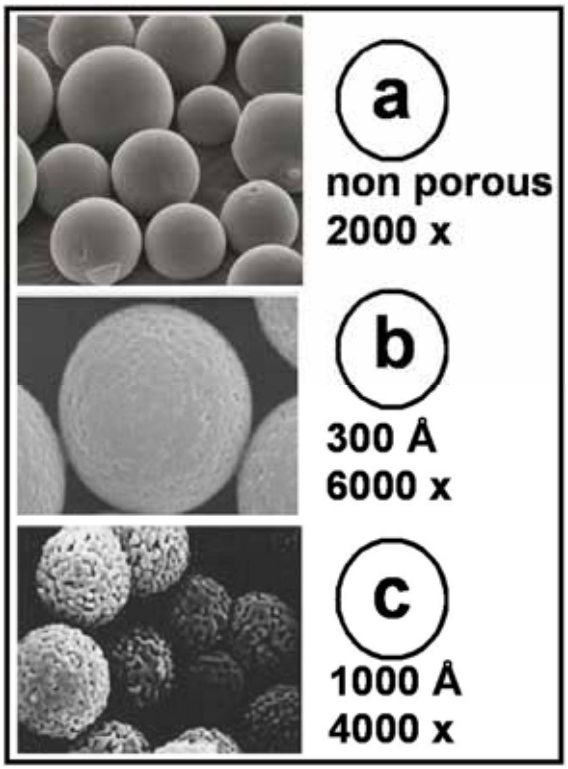

Fig. (2). Scanning electron micrograph (SEM) pictures of (**a**) non-porous (1.5 μm), (**b**) 300 Å (5 μm) and (**c**) 1000 Å (7 μm) particles.

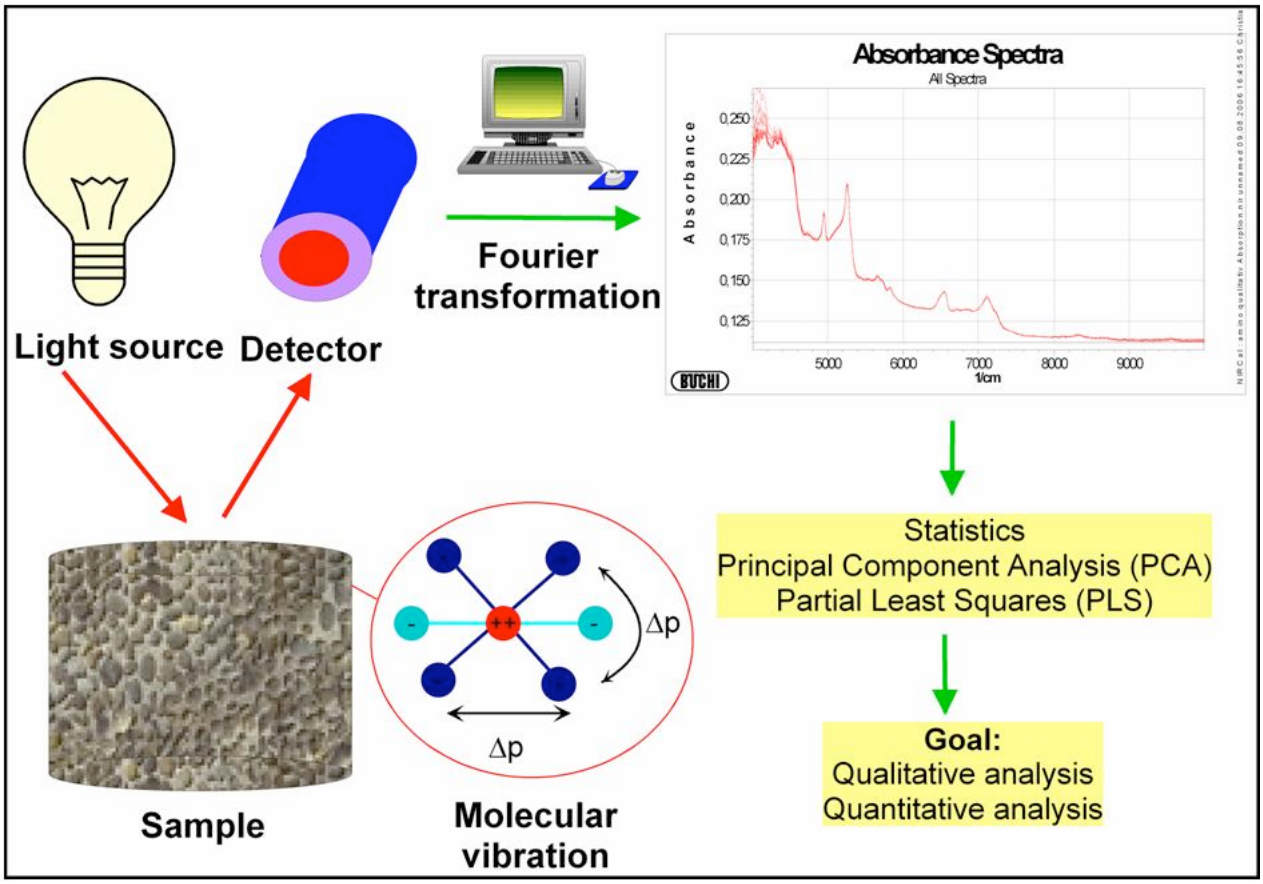

Fig. (3). Analytical workflow in near infrared reflection spectroscopy (NIRS).

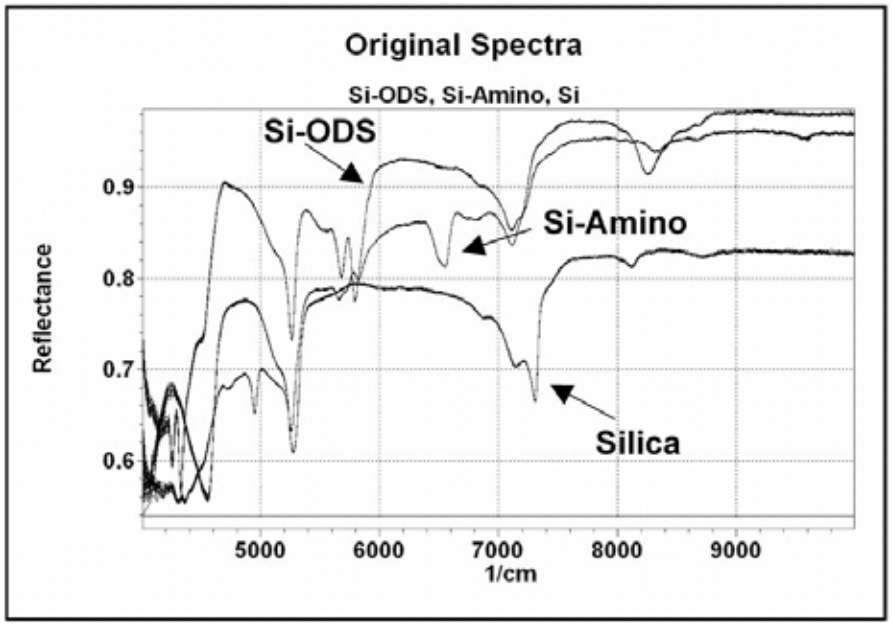

Fig. (4). Original NIR spectra of raw, octadecylated and amino derivatised silica. Conditions: Wavenumber range, 4500 - 9996 cm^{-1}; scans, ten; temperature, 23 °C.

near 5200 cm^{-1}. In case of raw silica the first overtone of O-H stretching vibration is present at 7200 cm^{-1}. The absorption spectrum of octadecylated silica additionally shows C-H stretching overtones between 5600 - 5800 cm^{-1} and at 7300 cm^{-1}. Amino derivatisation shows a distinct N-H stretching first overtone vibration at 6500 cm^{-1}. The characteristic vibrations responsible for spectral differences are marked with broken lines. Normalisation by closure improved signal-to-noise ratio and allowed to distinguish between raw, octadecylated and amino-derivatised silica in a 2- and 3-

dimensional factor-plot (Fig. **5**). Three principal components were required for separation into different clusters with a Q-value of 0.989. 75% of 30 recorded spectra were used for calibration, 25% for validation. As the investigated particles possess different porosity and particle size beside different derivatisation, it is obvious that the established qualitative NIRS model is suitable to distinguish between different morphology and derivatisation.

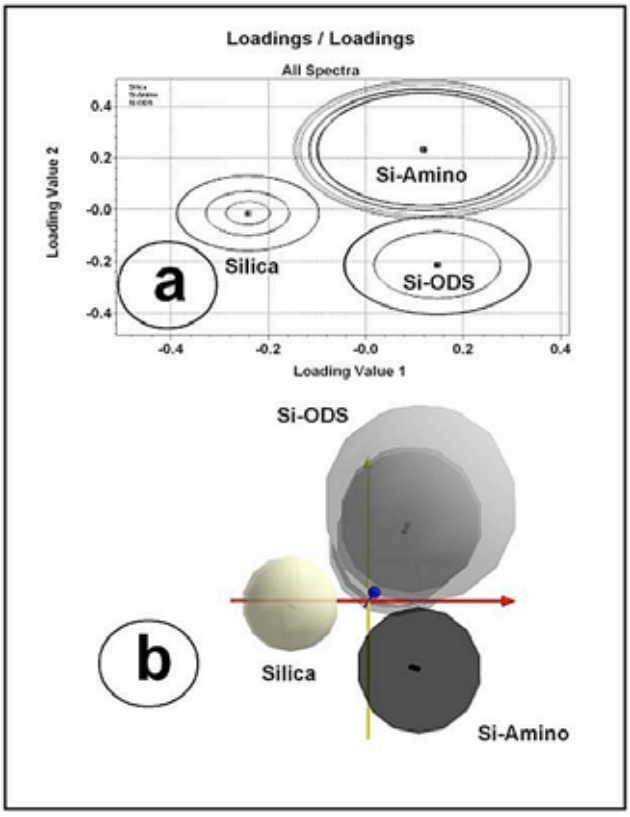

Fig. (5). 2-dimensional (**a**) and 3-dimensional factor plot (**b**) of raw, octadecylated and amino derivatised silica. Conditions: Wavenumber range, 4500 - 9996 cm^{-1}; scans, ten; temperature, 23 °C; 30 recorded spectra; data pre treatment, normalization by closure.

Quantitative Analysis

Particle Size

Particle size is routinely determined by electron microscopy, light scattering or Coulter-Counter (Halasz, 1978). For the establishment of a NIR-model, particle diameters provided by individual manufacturers were taken for calibration. 330 spectra of 33 different silica phases (see Experimental) were recorded in a wavenumber range between 4440 and 9000 cm^{-1} in diffuse reflection mode. Mathematical pre-treatment and statistical analyses were carried out using partial least square regression (PLS), normalisation by closure and following calculation of first derivative. Fig. (**6a**) shows the correlation between pre-treated spectra and true values. 10 primary factors were chosen with the help of calculated PRESS (Predicted Residual Sum Square, Fig. **6b**). The estimated values give promise to a robust and reliable calibration that confirms a R^2, SEP, SEE and BIAS of 0.985, 0.45 µm, 0.46 µm and 5.85 $\times 10^{-15}$ (Table **1**). Even the prediction of particle size in case of ProntoSIL phases with same porosity showed high linearity. Compared to the Coulter-Counter method, NIR model allowed the determination of particle size with a relative standard deviation in percent (RSD %), not more than 8.6% compared to +15/-12% by CC (Table **2**).

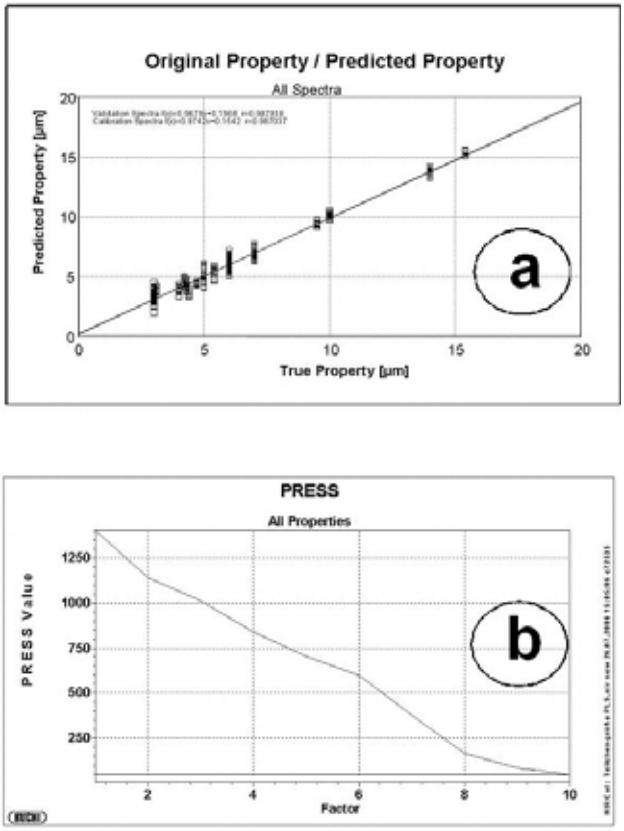

Fig. (6). (**a**) Predicted (NIRS) versus reference property for the determination of particle diameter; (**b**) PRESS (Predicted Residual Sum Square. Conditions: Data pre treatment, normalization by closure, first derivative BCAP (4440 - 9000 cm^{-1}); scans, 10; reflectance; temperature, 23°C.

Table 1. Calibration Parameters of Quantitative Particle Size Determination

Wavelengths calibration set	4596 – 9996 cm^{-1}
Data pre-treatment sequence	Normalisation by closure, 1st derivative
Number of primary factors	10
C-Set BIAS [µm]	-5.85165×10^{-15}
V-Set BIAS [µm]	0.00677028
C-Set SEP [µm]	0.469259
Consistency	96.8421
C-Set regression coefficient	0.987037
V-Set regression coefficient	0.987818
Q-Value	0.843123

Pore Size

Pore size is routinely determined by mercury intrusion porosimetry (MIP) or size exclusion chromatography (SEC). For NIR investigation the same 330 spectra described in the prior chapter were used for establishing the model. Applying principal component regression (PCR) calculated of first derivative and normalised spectra resulted in the calibration equation depicted in Fig. (**7a**). The values for statistical

Table 2. NIR Results on Particle Size (Chosen Examples)

Material	Particle Size [µm] ± 15 % SD[1] Reference	Particle Size [µm] ± SD [%] NIR
Amino, 4 µ	4.00	4.00 ± 6.5.00
Amino, A21	10.00	10.00 ± 2.60
Amino A23	6.00	6.00 ± 4.30
Silica 622	14.00	14 ± 1.70
Silica 191-1	6.00	6.00± 4.30
Silica 191-2	6.00	6.00 ± 4.30
Silica 191-3	6.00	6.00 ± 4.30
ODS 2 (non endc.)	6.00	6.00 ± 4.30
ODS 3 (endcap.)	6.00	6.00 ± 4.30
Nucleosil 1000	7.00	7.00 ± 4.30
S80 Resin	5.40	5.4 ± 4.80
Prontosil 200	3.00	3.00 ± 8.60
Prontosil 120	3.00	3.00 ± 8.60
Prontosil 60	3.00	3.00 ± 8.60
Prontosil 300	3.00	3.00 ± 8.60
Nucleosil C8	5.00	5.00 ±5.20
Nucleosil C18	5.00	5.00 ± 5.20

[1]According to method, literature reference [8,9].

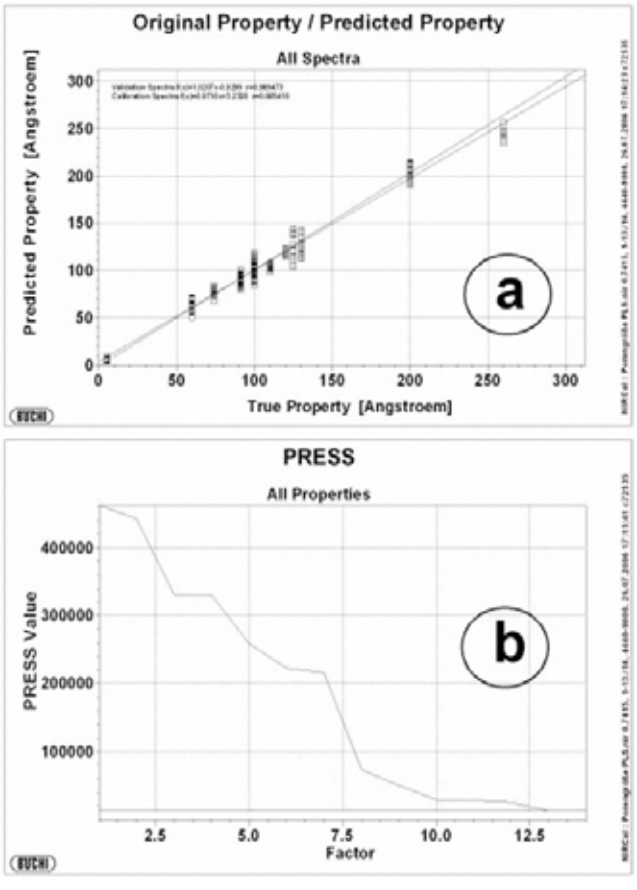

Fig. (7). (**a**) Predicted (NIRS) versus reference property for the determination of pore diameter; (**b**) PRESS (Predicted Residual Sum Square. Conditions: Data pre treatment, first derivative, normalisation by closure (4440-9000 cm^{-1}); scans, 10; reflectance; temperature, 23°C.

parameters R^2, SEP, SEE and BIAS were 0.985, 8.3 Å, 7.3 Å, 1.26 $\times 10^{-14}$ (Table **3**). 14 primary factors were applied following the PRESS function (Fig. **7b**). According to Babinet´s theorem pores are recognised as small columns where diffusion following Raleigh´s law takes place. The maximum relative standard deviation in percent (RSD %) for the NIRS method was 8.6% compared to 6% by size exclusion chromatography (SEC) (Table **4**). ProntoSIL phases with the same particle size but different pore sizes could be analysed with high linearity.

Table 3. Calibration Parameters of Quantitative Particle Size Determination

Wavelengths calibration set	4440 - 9000 cm^{-1}
Data pre-treatment sequence	1st derivative, normalisation by closure
Number of primary factors	14
C-Set BIAS [Å]	1.26726 $\times 10^{-14}$
V-Set BIAS [Å]	-0.918256
C-Set SEP [Å]	8.33681
Consistency	114.17
C-Set regression coefficient	0.985416
V-Set regression coefficient	0.989473
Q-Value	0.635189

Table 4. NIR Results on Pore Diameter (Chosen Examples)

Material	Pore Diameter [Å] ± 28 % SD[1] Reference	Pore Diameter [Å] ± SD [%] NIR
Amino, 4 µ	126.00	126.00 ± 6.50
Amino, A21	114.00	114.00 ± 7.20
Amino A 23	117.00	117.00 ± 7.00
Silica 622	91.00	91.00 ± 9.00
Silica 191-1	91.00	91.00 ± 9.00
Silica 191-2	74.00	74.00 ± 11.10
Silica 191-3	74.00	74.00 ± 11.10
ODS 2 (non endc.)	74.00	74.00 ± 11.10
ODS 3 (endcap.)	78.00	78.00 ± 10.90
Nucleosil 1000	1000.00	1000.00 ± 0.80
S80 Resin	100.00	100.00 ± 8.20
Prontosil 200	200.00	200.00 ± 4.10
Prontosil 120	120.00	120.00 ± 6.80
Prontosil 60	60.00	60.00 ± 13.70
Prontosil 300	300.00	300.00 ± 2.70
Nucleosil C8	120.00	120.00 ± 6.80
Nucleosil C18	300.00	300.00 ± 2.70

[1]According to method, literature reference [8,9].

Specific Surface Area

Specific surface area is usually determined by the BET-method with a maximum RSD% of ± 7.8%. Establishment of a partial least square regression (PLS) model, calculation of first derivative using eight primary factors provided values for R^2, SEP and SEE of 0.99, 13.48 $m^2 \times g^{-1}$ and 13.55 $m^2 \times g^{-1}$ (Table **5**). Found RSD % was < 6.8 compared to 7.8% by BET (Table **6**).

Table 5. Calibration Parameters of Specific Surface Area Determination

Wavelengths calibration set	**4596 – 9996 cm^{-1}**
Data pre-treatment sequence	**Normalisation by closure, 1st derivative**
Number of primary factors	**8**
C-Set BIAS [m^2]	$4{,}65196 \times 10^{-14}$
V-Set BIAS [m^2]	13.48 wrong value? should be much <<
C-Set SEP [m^2]	13.55
Consistency	98.9421
C-Set regression coefficient	0.997654
V-Set regression coefficient	0.989965
Q-Value	0.867543

Table 6. NIR Results on Specific Surface Area (Chosen Examples)

Material	**Specific surface area [$m^2 \times g^{-1}$] ± 7.8 % SD[1] Reference**	**Specific surface area [$m^2 \times g^{-1}$] ± SD [%] NIR**
Amino, 4 µ	317.00	317.00 ± 4.20
Amino, A21	317.00	317.00 ± 4.20
Amino A 23	310.00	310.00 ± 4.30
Silica 622	431.00	431.00 ± 3.10
Silica 191-1	458.00	458.00 ± 2.90
Silica 191-2	458.00	458.00 ± 2.90
Silica 191-3	458.00	458.00 ± 2.90
ODS 2 (non endc.)	308.00	308.00 ± 4.30
ODS 3 (endcap.)	267.00	267.00 ± 5.00
Nucleosil 1000	25.00	25.00 ± 3.60
S80 Resin	458.00	458.00 ± 2.90
Prontosil 200	200.00	200.00 ± 6.70
Prontosil 120	300.00	300.00 ± 4.50
Prontosil 60	450.00	450.00 ±- 0.70
Prontosil 300	100.00	100.00 ± 6.80
Nucleosil C8	350.00	350.00 ± 3.80
Nucleosil C18	350.00	350.00 ± 3.80

[1]According to method, literature reference [8,9].

CONCLUSIONS

The shown results point out the high suitability of NIRS to be used in the field of nano-technology for qualitative and quantitative investigations in material science. Each model established is characterised by high linearity, precision, reproducibility and speed of analysis. As measurement is non-invasive and extremely fast, this NIRS method can be used as a supplementary tool to support commonly used techniques like BET, MIP, LS and SEC to reach higher performance and output, as no expensive technical equipment is required.

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