Amino Acids (2008) 34: 279–286
DOI 10.1007/s00726-007-0492-5
Printed in The Netherlands

Amino Acids

Nano-structured support materials, their characterisation and serum protein profiling through MALDI/TOF-MS

M. Najam-ul-Haq[1], **M. Rainer**[1], **N. Heigl**[1], **Z. Szabo**[1], **R. Vallant**[1], **C. W. Huck**[1], **H. Engelhardt**[2], **K.-D. Bischoff**[3], and **G. K. Bonn**[1]

[1] Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innsbruck, Austria
[2] Instrumental and Environmental Analytical Chemistry, University of Saarland, Saarbrücken, Germany
[3] Bischoff Analysentechnik und -geräte GmbH, Leonberg, Germany

Received October 30, 2006
Accepted December 21, 2006

Summary. In the bioanalytical era, novel nano-materials for the selective extraction, pre-concentration and purification of biomolecules prior to analysis are vital. Their application as affinity binding in this regard is needed to be authentic. We report here the comparative application of derivatised materials and surfaces on the basis of nano-crystalline diamond, carbon nanotubes and fullerenes for the analysis of marker peptides and proteins by material enhanced laser desorption ionisation mass spectrometry MELDI-MS. In this particular work, the emphasis is placed on the derivatization, termed as immobilised metal affinity chromatography (IMAC), with three different support materials, to show the effectiveness of MELDI technique. For the physicochemical characterisation of the phases, near infrared reflectance spectroscopy (NIRS) is used, which is a well-established method within the analytical chemistry, covering a wide range of applications. NIRS enables differentiation between silica materials and different fullerenes derivatives, in a 3-dimensional factor-plot, depending on their derivatizations and physical characteristics. The method offers a physicochemical quantitative description in the nano-scale level of particle size, specific surface area, pore diameter, pore porosity, pore volume and total porosity with high linearity and improved precision. The measurement takes only a few seconds while high sample throughput is guaranteed.

Keywords: Nano materials – Diamond – Nanotubes – Fullerenes – MELDI – Silica – NIRS

Introduction

Research in the nanomaterials field is much intentioned on fundamental and applied side of the particles for many categories of scientific fields like biosensors and diagnostic marker research. The size of 100–0.2 nm suits most for such applications. This size range suffices the required surface areas for better reactivity and capacity. The role of nanomaterials in the separation and bioanalysis is very well elaborated (Martin and Mitchell, 1998). Carbon nanomaterials show higher capacities due to large surface areas and additionally bind multitude of biofluid constituents through physical characteristics and the chemical functionalities. Polylysine (PL)-coated diamond nanocrystals are utilized for isolation, concentration, purification, digestion and analysis of DNA oligonucleotides from complex protein solutions and cell lysates (Kong et al., 2005). The graphite powder is reported to be packed in GELoader tips for desalting and concentration of peptide mixtures prior to desorption/ionization (Larsen et al., 2002).

Carbon nanotubes discovery (Iijima, 1991) has provided a new biocompatible material. Their diameter in the nanometers range creates great research interest in the nano field. A method with carbon nanotubes functioning as the adsorbent for small molecules in solution like quinine, N_a-benzoyl-L-arginine (B-Arg) and propranolol is developed (Pan et al., 2005). Since the first discovery of fullerenes (Osawa et al., 1987) and its mass spectrometric detection (Kroto et al., 1985), the attention of the synthetic chemist was attracted to fullerenes. The fullerene derivatives like methano fullerenes can act as binding materials in the field of life science research, because they preserve the original properties of the hydrophobic C60 core (Dietrich et al., 1994). The major advantage of using such surfaces is that more dramatic selective enrichment can be easily achieved in contrast to the standard chromatographic methods. The support materials like silica also finds its use in a wide-spread field of application e.g. as an adsorbent, drying material and an important material in chromatographic separations. The parameters like particle size, pore size, pore volume and surface area contribute towards the separation in thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

The characterization of these materials before bioanalytical applications has become an important issue over the last years. Near-infrared spectroscopy (NIRS) in combination with chemometrical software offers a powerful, rugged, non-invasive and comparatively cost-effective tool that guarantees high sample-throughput, because of short measurement time and hardly any sample preparation (Huck et al., 1999). Analysis based on near-infrared vibrational reflectance spectroscopy can be performed directly and both chemical and physical parameters are described simultaneously (Guggenbichler et al., 2006). Diffuse reflectance spectroscopy in combination with Kubelka-Munk's theory or a log (1/R)-transformation is commonly used for powder or tablet measurements with an NIR analyser, to obtain information about scattering and absorption coefficients of the samples (Cogdill and Drennen, 2005). Often a complex spectrum is achieved through NIR that is elucidated by multivariate methods like principal component analysis (PCA), partial least squares regression (PLS) and principal component regression (PCR). Calculated calibrations based on chemometrical algorithms (cluster-analysis, PCA) make it possible to distinguish and classify the materials with different particle and pore sizes.

In this particular work, the above-mentioned derivatized support materials, after thorough characterization by NIRS, are being effectively utilized for the selective enrichment and pre-fractionation of the complex biofluids like serum. The affinity-bound peptides and proteins are analyzed by matrix-assisted laser desorption/ionization time of flight mass spectrometry MALDI/TOF-MS (Karas and Hillenkamp, 1988). This material-based investigation of biofluids for marker research is termed as MELDI (Feuerstein et al., 2006; Rainer et al., 2006). The materials with the same chemical functionalities are loaded with serum constituents under developed sample preparation protocols (Huck et al., 2005). The ultimate goal of MELDI technique is to find the potential diagnostic markers for different kind of diseases like cancers, by highlighting the differences in the mass fingerprints of healthy and diseased biofluids.

Materials and methods

Chemicals and reagents

Carbon nanotubes were supplied by Nanocyl S. A. Belgium. [C60]Fullerene = 99.5% were obtained from MER Corporation, (Tucson, AZ, USA). 1-Octadecanol 99%, iminodiacetic acid (IDA) 98%, *t*-butyl bromoacetate 99%, dimethyl sulphide 99%, *p*-toluenesulfonic acid 97%, thionyl chloride 99%, acetonitrile 99.9%, sinapinic acid 99.0% and malonyl dichloride 97% were purchased from Aldrich (Milwaukee, WI, USA). Diamond surfaces were provided by ρ-BeSt coating (Hartstoffbeschichtungs GmbH, Austria). Trifluoroacetic acid (TFA) 99.5% is obtained from Fluka (Buchs, Switzerland). Serum samples were provided by the Department of Urology, at Medical University of Innsbruck, Austria.

Derivatizations

The three carbon nanomaterials are derivatized as immobilized metal affinity chromatographic (IMAC) supports (Porath et al., 1975).

Diamond

Diamond surfaces were linked with glycidyl methacrylate (GMA) through ultraviolet light, derivatized with iminodiacetic acid (IDA) and loaded with copper (Cu^{+2}) ions as described elsewhere (Najam-ul-Haq et al., 2006). The final functionalities achieved are depicted in Fig. 1A.

Carbon nanotubes

Carbon nanotubes were oxidized by H_2SO_4/HNO_3 mixture, treated with thionyl chloride ($SOCl_2$), derivatized with IDA and loaded with Cu^{+2} ions as reported (Najam-ul-Haq et al., 2006b). The final product with chemical derivatizations is shown in Fig. 1B.

Fullerenes (C60)

[60]Fullereneoacetic acid was synthesized from C60 fullerenes by nucleophilic attack of a carbonyl stabilized sulfonium ylide (*t*-butyl(dimethylsulfuranylidene)acetate). The monoadduct was hydrolyzed by *p*-toluenesulfonic acid and the reaction yielded [60]fullereneoacetic acid (Ito et al., 2003). It was treated with thionyl chloride for 8 h under nitrogen to

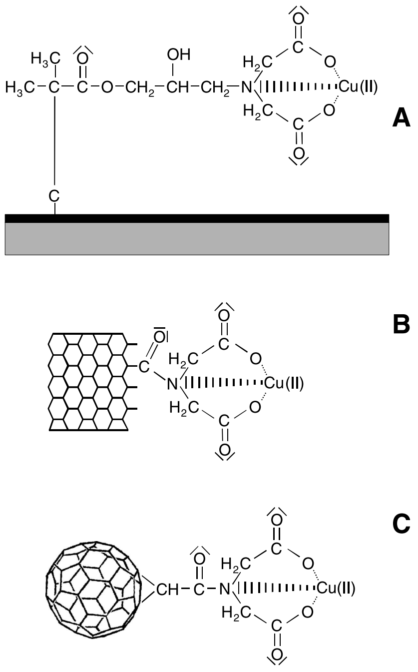

Fig. 1. The nanomaterials derivatized as NCD-IDA-Cu(II) (**A**), CNT-IDA-Cu(II) (**B**) and IDA-Cu^{+2}-[60]fullerene (**C**)

produce [60]fullerenoacetyl chloride. This was immediately used for further reaction with IDA for 8 h at 65 °C. The thoroughly purified product was loaded with copper ions to get the structure as exhibited in Fig. 1C.

Characterizations through near-infrared spectroscopy (NIRS)

A single beam polarisation NIR Fourier-Transform spectrometer (FT-NIR; Büchi, Flawil, Switzerland) equipped with a tungsten-halogen lamp, a temperated lead sulfide detector (30 °C) and a standard 2 m optic glass fibre (silica glass, Infrasil, Bes Optics Inc., Warwick, Great Britain) was used to record spectra in a wavenumber range from 4000 to 10000 cm^{-1}. Diffuse reflectance mode was employed to bulk samples. The instrument offered a resolution of 12 cm^{-1}, an absolute wavelength accuracy of $\pm 2\,cm^{-1}$ and a relative reproducibility of 0.5 cm^{-1}.

Sample temperature was kept steady in a water bath at 23 °C by a heater (Julio – model PC/4, Seelbach, Germany) with a variation limit of ±0.1 °C. The fibre-optic probe was employed to monitor the bulk samples non-invasively. To ensure sample homogeneity a rotating sample desk was used after bringing the sample to the right temperature. To enhance S/N ratio ten scans were taken for one average spectrum. For creating calibration models, i.e., selection of spectra and wavelengths, mathematical pretreatment and statistical analysis performing cluster analysis, principal component analysis (PCA) and partial least squares regression (PLSR) commercial software package NirCal 4.21 (Büchi) was used. Spectra were randomly divided into a learning-set (67%, C-set), i.e., calibration samples, and a test-set (33%, V-set), i.e., samples for testing the calibration equation. The optimum number of factors used for the individual prediction was determined by cross-validation and quality of cluster analysis was described in the Q-value. The selection of the best qualitative calibration model was based on the calculations reported elsewhere (Heigl et al., 2006).

Sample preparation

Three milligrams of copper loaded nanomaterials were filled into micro centrifuge tubes and equilibrated twice with sodium acetate buffer (pH = 4.0) and Phosphate-buffered saline (PBS; pH = 7.4, created by 0.01 M Na_2HPO_4/NaH_2PO_4, 0.15 M NaCl). 40 µl of the prepared blood serum sample (10 µl of raw serum diluted in 90 µl of PBS, pH = 7.4) was incubated with equilibrated materials. The unspecifically bound serum components were washed away by three times washing with PBS and once with deionized water. One microliter of the resulting slurry was placed on a stainless steel target (Bruker Daltonics GmbH, Bremen, Germany), followed by adding 1 µl of saturated sinapinic acid in 50% (v/v) acetonitrile, 0.1% trifluoroacetic acid. The sample preparation on diamond surfaces was carried out according to the method reported earlier (Najam-ul-Haq et al., 2006a).

MALDI/TOF-MS analysis

The prepared samples were directly analysed by MALDI/TOF-MS (Ultraflex MALDI-TOF-TOF, Bruker Daltonics, Bremen, Germany), in linear mode with the detector energy of 1623 V. The comparative data was collected by averaging 300 laser shots in the mass range of 2–10 kDa. The validation of all data, including external calibration using Protein Standard I (Bruker Daltonics, Bremen, Germany) and all further data processing, was carried out by Flex analysis 2.4 post analysis software and for data acquisition Flex Control 2.4 was utilized.

Results and discussion

Near-infrared reflectance spectroscopy (NIRS) is a non-invasive spectroscopic tool applied in a wide field of analytical chemistry. In the following chapters its advantages for the characterisation of nano-structured separation materials are shown and discussed.

Characterizations of silica with NIRS

Five silica materials with particle sizes in the µm- and pore sizes in the nanometer-range, namely ProntoSIL 120 (3 µm, 120 Å), ProntoSIL 120 (5 µm, 120 Å), ProntoSIL 120 (10 µm, 120 Å), ProntoSIL 120 (15 µm, 120 Å) and ProntoSIL 120 (20 µm, 120 Å) have been investigated by FT-NIR spectroscopy to distinguish and further classify the samples with different particle sizes. Figure 2 shows the spectra where intense absorptions appear especially in wavenumber ranges between 4000–4600 cm^{-1}, 5000–5400 cm^{-1} and 7000–7400 cm^{-1} that can be attributed to combinations and overtones of fundamental vibrations. Especially at 5268 cm^{-1} silica shows a clear absorption signal that can be assigned to a stretching first overtone vibration aroused by the Si=O bonding. An absorption band at 7104 cm^{-1} can also be associated with a first overtone of Si=O stretching vibrations. The growth in absorbance from 3 up to 20 µm is caused by the higher number of Si=O stretching vibrations present in particles with increased size. The spectra depicted in Fig. 3 show the absorptions of the silica material with different pore size but same particle size. Additionally to the absorptions mentioned above, one intense absorption also occurs in the lower wavenumber range at 4524 cm^{-1}. A stretching vibration mode is responsible for that. According to Babinet's theory pores are recognised as columns where diffusion following Raleigh's equation takes place. The fact that all spectra overlap at wavenumbers rich of physicochemical information requires the use of chemometrics to extract unique spectral information. Therefore the Büchi NIRCal 4.21 chemometric software was used to calculate 2 qualitative models to distinguish and classify, on one hand the silica material with different particle sizes but same pore size, and on the other hand with different pore size but same particle size. The 3-dimensional factor plot depicted in Fig. 4 shows the correlation between recorded pretreated spectra based on their loading values. Four primary factors and 1–4 secondary factors were chosen with the help of calculated PRESS (Predicted Residual Error Sum Square) function and the loading values of the absorption spectra over the primary factors. Proper wavelength for the calibration (4440–9000 cm^{-1}) were chosen by means of Property Wavelength Regression that shows the regression coefficient of the spectra over the whole wavenumber range from 4000 to 10000 cm^{-1}. First derivative BCAP and normalization by closure were

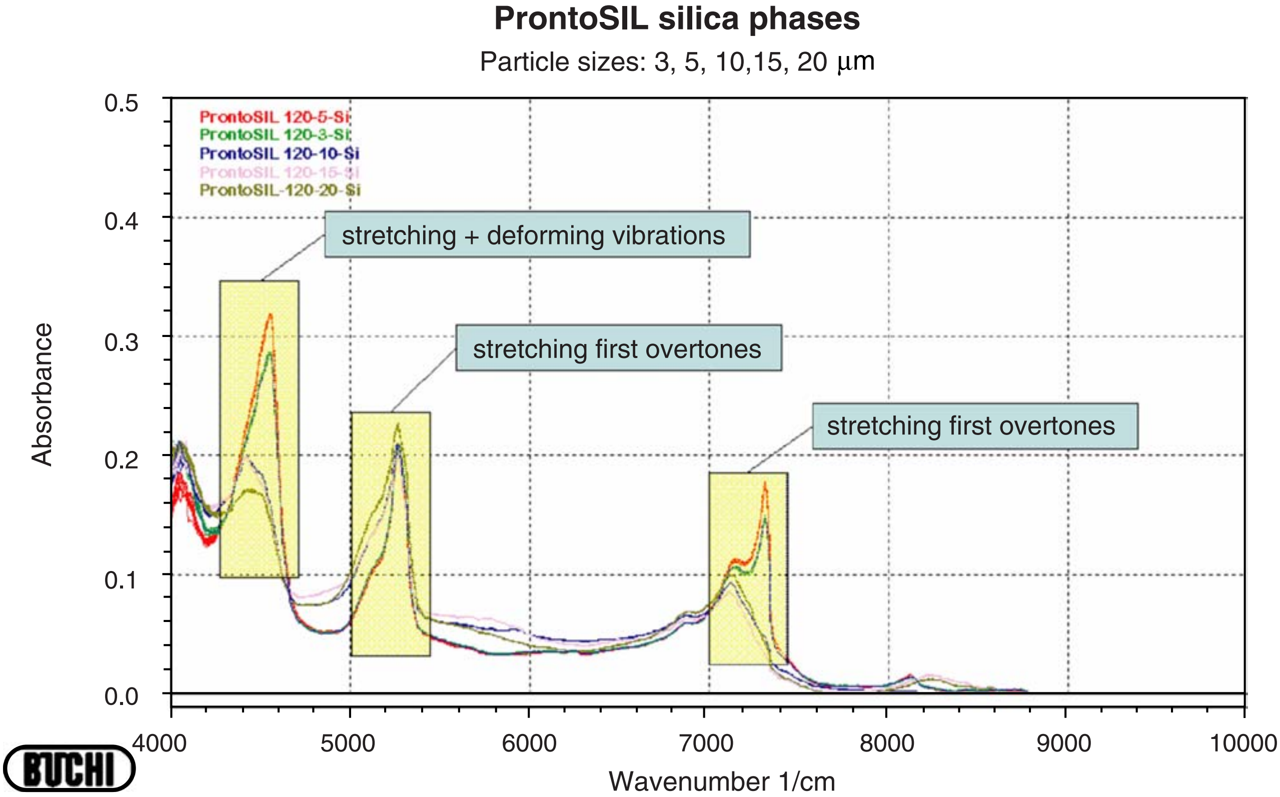

Fig. 2. Recorded spectra of ProntoSIL 120-3, ProntoSIL 120-5, ProntoSIL 120-10, ProntoSIL 120-15, ProntoSIL 120-20 marked with characteristic vibrational absorbances in the near-infrared wavenumber range from 4000 to 10000 cm^{-1} (2500–1000 nm) (colour reproduction of this figure available in the online version of this paper under www.springerlink.com)

applied to the spectra with regard to correct offset variations. The given spectra residuals and spectra radii considered all within the pre-set values calculated by the NirCal 4.21 Software. The estimated values give promise to a robust and reliable calibration that confirms a Q-value, quality of calibration, of 0.981321. To classify the four silica materials with different pore sizes three primary factors and 1–3 secondary factors showed the best results as given in Fig. 5. Wavenumber range was chosen from 4596 to 9996 cm^{-1}. The three-dimensional plot shows that every single sample could be classified and assigned to a single cluster. The given spectra residuals and spectra radii considered all within the pre-set values and a Q-value of 0.990448 describe the high sensitivity and specificity of this calibration. The two cluster models show clearly that every single sample could be classified and assigned to a single cluster, what makes NIR a very powerful and promising tool in the characterization of separation materials. Compared to other more conventional techniques including Coulter Counter or light scattering, this NIR method allows not only a much faster (only a few seconds) but also more precise analysis offering higher reproducibility.

Characterizations of fullerenes with NIRS

Fullerenes, a special form of carbon clusters, possess high symmetry and form closed molecular systems with sp^2 hybridised carbon as a common building block, are of high interest in the field of peptide and protein identifications for diagnostic marker research (Vallant et al., 2006). Six samples consisting of C60, dioctadecylpropandioate, dioctadecylmethano[60]fullerenes, [60]fullereneacetic acid, iminodiacetic acid (IDA) and Cu^{+2}-IDA-[60]fullerenes have been investigated to distinguish and further classify the derivatizations for marker research (Fig. 6). Intense absorptions appear in the low wavenumber range between 4000 and 5000 cm^{-1} that can be attributed to combinations of fundamental vibrations. Dioctadecylpropandioate, and dioctadecylmethano[60]fullerene shows clear absorption signals at 4320 cm^{-1} that can be assigned to combinations involving C–H stretching + C–H deforming modes. Absorption bands at 5796 cm^{-1} are associated with the first overtones of C–H stretching vibrations, while an absorption band consisting of 2 × C–H stretching + C–H deforming vibration modes can be observed at 7176 cm^{-1}. A C–H stretching second overtone is placed

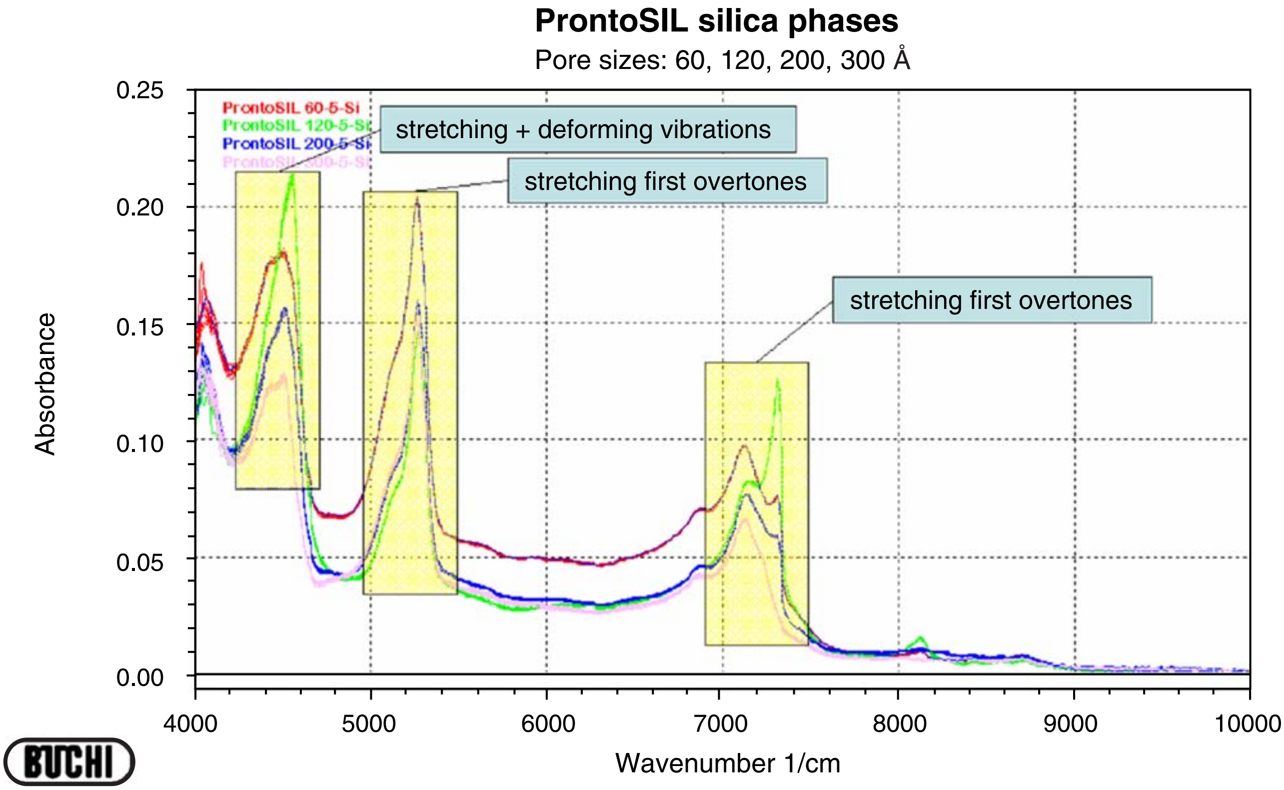

Fig. 3. Recorded spectra of ProntoSIL 60-5, ProntoSIL 120-5, ProntoSIL 200-5, ProntoSIL 300-5 marked with characteristic vibrational absorbances in the near-infrared wavenumber range from 4000 to 10000 cm^{-1} (2500–1000 nm) (colour reproduction of this figure available in the online version of this paper under www.springerlink.com)

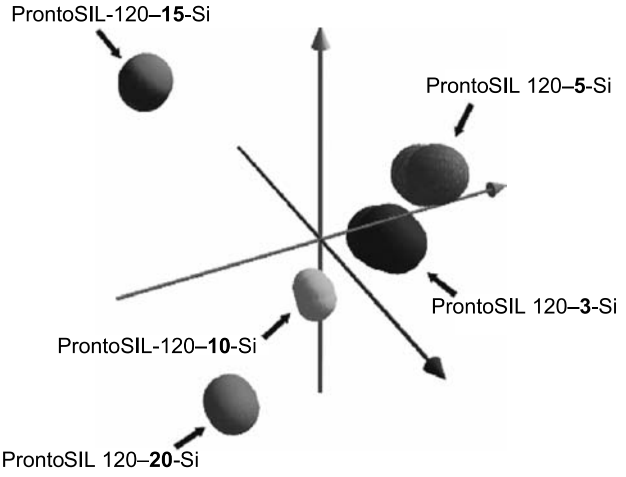

Fig. 4. 3-dimensional factor plot of five silica phases separated on the basis of their different particle sizes (3, 5, 10, 15, 20 µm) – calculated by principal component analysis (PCA) algorithm. Conditions: wavenumber range, 4400–9000 cm^{-1}; spectral pretreatments, First Derivative BCAP, Normalization by Closure; measurements, 10-scans, 10 for each sample; temperature, 23 °C

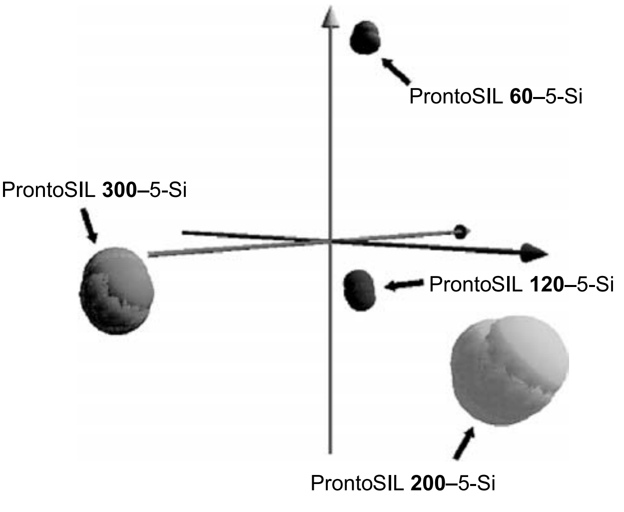

Fig. 5. 3-dimensional factor plot of four silica phases separated on the basis of their different pore sizes (60, 120, 200, 300 Å) – calculated by principal component analysis (PCA) algorithm. Conditions: wavenumber range, 4596–9996 cm^{-1}; spectra pretreatments, First derivative BCAP, Normalization by Closure; measurements, 10-scans, 10 for each sample; temperature, 23 °C

at 8220 cm^{-1} for both samples mentioned above. C60 fullerenes show several absorption peaks between 4000 and 4600 cm^{-1} that are related to C–H + C–C stretching combinations at 4068 cm^{-1} and C–H stretching + C–H deforming combinations at 4320 cm^{-1}. Cu^{2+}-IDA-[60]fullerenes show two intense absorptions; first at 4404 cm^{-1}

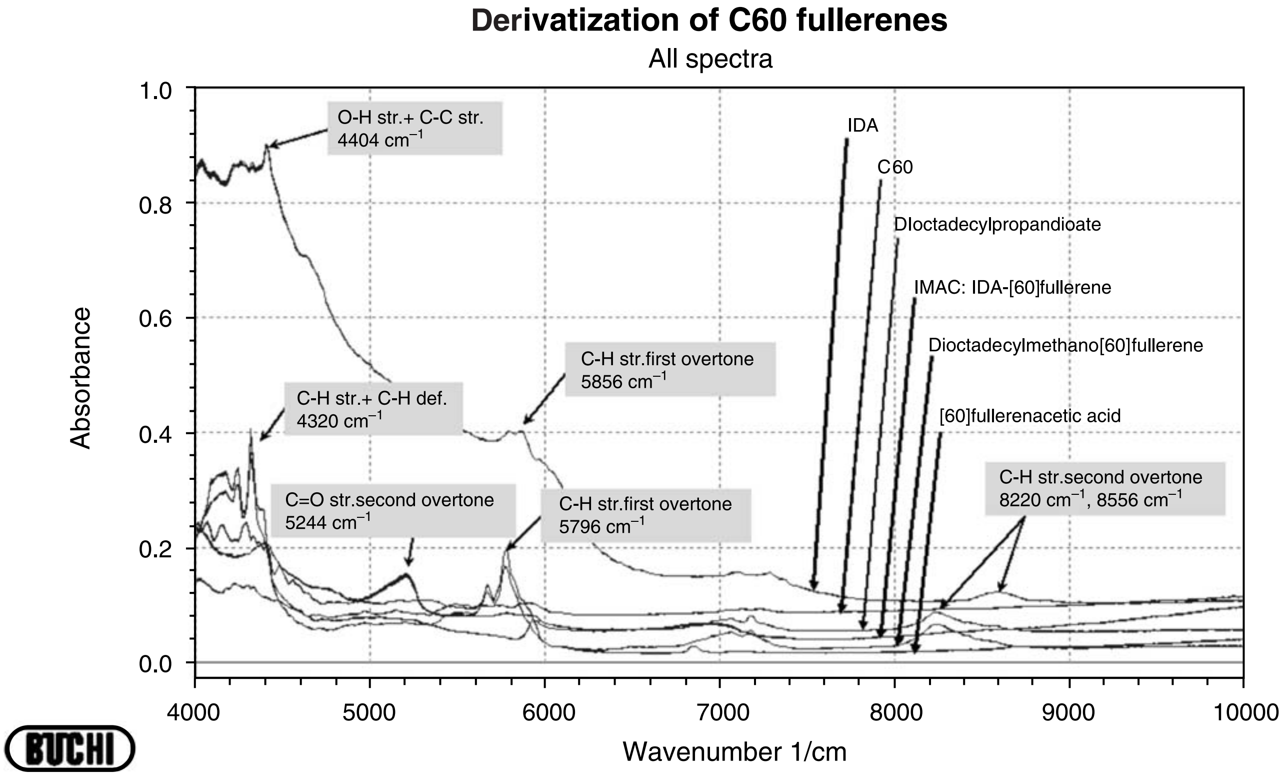

Fig. 6. Recorded spectra of C60, dioctadecylpropandioate, dioctadecylmethano[60]fullerene, [60]fullerenacetic acid, IDA (iminodiacetic acid) and Cu^{+2}-IDA-[60]fullerenes, marked with characteristic vibrational absorbances in NIR wavenumber range from 4000 to 10000 cm^{-1}

i.e. O–H stretching + C–C stretching, and the second at 5244 cm^{-1}; C=O stretching second overtone. The recorded [60]fullereneacetic acid spectra shows a series of weak absorptions from 4000 to 4350 cm^{-1} responsible for C–H stretching and C–H deforming vibrations. The iminodiacetic acid (IDA) absorption spectra consist of a very broad band that loses intensity at higher wavenumbers. Absorptions arise at 4404 cm^{-1}, O–H stretching + C–C stretching, at 5856 cm^{-1} a stretching first overtone can be observed, and at 8556 cm^{-1} a C–H stretching second overtone appears. The fact that all six spectra overlap quite strong at wavenumbers rich of physicochemical information that requires chemometrics to extract the spectral information. Büchi NIRCal 4.21 chemometric software was used to calculate a qualitative model to distinguish and classify the different fullerenes based samples. The 3-dimensional factor plots depicted in Fig. 7 shows correlation between the recorded pre-treated spectra based on the loading values. Five primary factors and 1–5 secondary factors were chosen from calculated PRESS (Predicted Residual Error Sum Square) function and the loading values of the absorption spectra over the primary factors. Proper wavelengths for calibration (4440–9000 cm^{-1}) were chosen by Property Wavelength Regression that shows the regression coefficient of the spectra over the whole wavenumber range from 4000 to 10000 cm^{-1}. The given spectral residuals and radii considered all within the pre-set values calculated by the NirCal 4.21 Software. The estimated values give promise

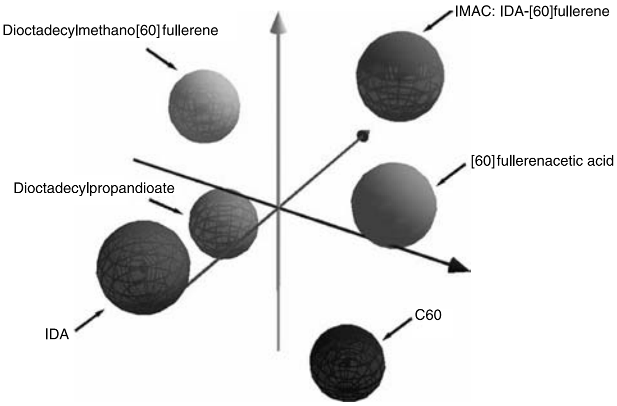

Fig. 7. 3-Dimensional factor plot of six separated samples calculated by principal component analysis (PCA) algorithm. Conditions: wavenumber range, 4440–9000 cm^{-1}; measurements, 10-scans, 10 for each sample; temperature, 21 °C

to a robust and reliable calibration what confirms a Q-value, quality of calibration, of 0.98712. The cluster model shows unambiguously that every single sample could be classified and assigned to a single cluster.

MELDI application

Material-enhanced laser desorption/ionisation (MELDI) is a material based approach, which utilizes different derivatized carrier materials for the selective binding and enrichment of biomolecules and their further analysis with MALDI-TOF mass spectrometry. Biomolecules bind to these modified nano-materials through hydrophobic, electrostatic, coordinate covalent bond or Lewis-acid/base interactions. The technique enables study of peptides and proteins from complex biological systems after reducing the complexity by built-in desalting and pre-fractionation. The MELDI workflow includes the sample preparation, laser desorption/ionisation to acquire mass spectra, and at later stage, the bioinformatics for recording the differences among spectra from control and diseased fluids. Compared to the existing surface-assisted techniques, MELDI does not only focus on the carrier's affinity, but moreover includes the material's morphology, which plays a crucial role in terms of analyte capacity and selectivity. By placing the material into the centre of the investigations, it could be clearly shown that parameters such as pore size, particle diameter, surface area or natural characteristics like hydrophobicity or hydrophilicity can influence the peak-pattern significantly. Such differences in protein-profiles from derivatized diamond, carbon nanotubes and fullerenes are demonstrated in Fig. 8. Although all carriers possess the same IDA-Cu^{+2} affinity, they are leading to different mass spectra and especially in the small mass range (<5000), the remarkable differences can be observed. Furthermore NCD-IDA-Cu(II) is showing a characteristic pattern around 8000 Daltons (Da) which cannot be found in the other two spectra (A) and (B). Nevertheless some peaks which are specific for IDA-Cu(II) affinity are present in all of the three carbon materials and can be clearly seen between 6200 and 7000 or 8400 and 9600 Da. It explains how the inherited physical characteristics of nanomaterials can make a difference in the capturing nature, in spite of the similar functionalities. However, one can rule out the fact that some differences in the spectra can arise due to the distinct surface concentrations of IDA-Cu^{2+} on the materials' surfaces and in addition, the differences can be attributed to the extent of non-specific interactions. This underlines the importance of characterisation of the support materials, via near-infrared reflectance spectroscopy before further usage. Thus, the small but very demonstrative differences depicted in comparative spectra emphasize the influence of material characteristics in surface-assisted mass fingerprinting and underline the advantage of MELDI in this regard.

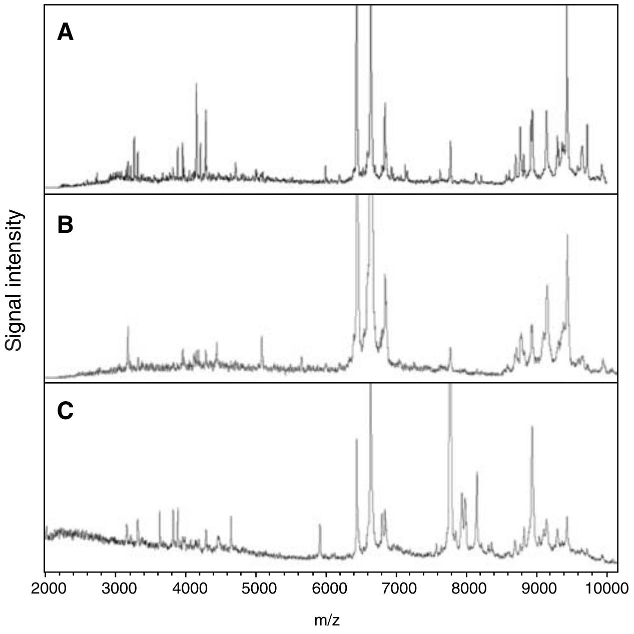

Fig. 8. MELDI mass-fingerprints recorded from 2000 to 10000 Da performed on [60]fullerene-IDA-Cu(II) (**A**), CNT-IDA-Cu(II) (**B**) and NCD-IDA-Cu(II) (**C**) by adding 300 laser shots. For analysis, the standard serum sample was used and sinapinic acid in 50% ACN/0,1% TFA was taken as a matrix

Acknowledgements

This work was supported by the Austrian Genome Program (GEN-AU), Vienna, Austria and by the special research Program "Cell Proliferation and Cell Death in Tumors" (SFB021, Austrian Science Fund), FWF, Vienna, Austria and MVIT-WINN (West Austrian Initiative for Nano Network), Austria.

References

Cogdill RP, Drennen JK (2005) Near infrared spectroscopy in the pharmaceutical industry. NIR News 16: 23–26

Dietrich F, Isaacs L, Philip D (1994) Syntheses, structures, and properties of methanofullerenes. Chem Soc Rev 23: 243–255

Feuerstein I, Najam-ul-Haq M, Rainer M, Trojer L, Bakry R, Aprilita NH, Stecher G, Huck CW, Klocker H, Bartsch G, Guttman A, Bonn GK (2006) Material enhanced laser desorption/ionization (MELDI) – a new protein profiling tool utilizing specific carrier materials for TOF-MS analysis. J Am Chem Soc Mass Spectrom 17: 1203–1208

Guggenbichler W, Huck CW, Kobler A, Bonn GK (2006) Near infrared reflectance spectroscopy as a tool for quality control in wine production. J Food Agric Environm 4: 98–106

Heigl N, Huck CW, Rainer M, Najam-ul-Haq M, Bonn GK (2006) Near infrared spectroscopy, cluster and multivariate analysis hyphenated to thin layer chromatography for the analysis of amino acids. Amino Acids 31: 45–53

Huck CW, Bakry R, Bonn GK (2005) Sample preparation techniques for mass spectrometry in proteomics using recently developed highly selective stationary phases. Curr Proteomics 2: 269–285

Huck CW, Maurer R, Popp M, Basener N, Bonn GK (1999) Quantitative fourier transform near infrared reflectance spectroscopy (NIRS) compared to high performance liquid chromatography (HPLC) of a flavone in flos primulae veris extractives. Pharm Pharmacol Lett 9: 26–29

Iijima S (1991) Helical microtubules of graphitic carbon. Nature 354: 56–58

Ito H, Tada T, Sudo M, Ishida Y, Hino T, Saigo K (2003) [60]Fullerenoacetyl chloride as a versatile precursor for fullerene derivatives: efficient ester formation with various alcohols. Org Lett 5: 2643–2645

Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 60: 2299–2301

Kong X, Huang LCL, Liau SCV, Han CC, Chang HC (2005) Polylysine-coated diamond nanocrystals for MALDI-TOF mass analysis of DNA oligonucleotides. Anal Chem 77: 4273–4277

Kroto HW, Heath JR, O'Brian SC, Curl RF, Smalley R (1985) C60: Buckminsterfullerene. Nature 318: 162–163

Larsen MR, Cordwell SJ, Roepstorff P (2002) Graphite powder as an alternative or supplement to reversed-phase material for desalting and concentration of peptide mixtures prior to matrix-assisted laser desorption/ionization-mass spectrometry. Proteomics 2: 1277–1287

Martin CR, Mitchell DT (1998) Nanomaterials in analytical chemistry. Anal Chem 70: 322A–327A

Najam-ul-Haq M, Rainer M, Huck CW, Stecher G, Feuerstein I, Steinmüller D, Bonn GK (2006a) Chemically modified nano crystalline diamond layer as material enhanced laser desorption ionisation (MELDI) surface in protein profiling. Curr Nanosci 2: 1–7

Najam-ul-Haq M, Rainer M, Schwarzenauer T, Huck CW, Bonn GK (2006b) Chemically modified carbon nanotubes as material enhanced laser desorption ionisation (MELDI) material in protein profiling. Anal Chim Acta 561: 32–39

Osawa E (1987) Soccer ball molecule: C60. Kagaku 42: 60–61

Pan C, Xu S, Zou H, Guo Z, Zhang Y, Guo B (2005) Carbon nanotubes as adsorbent of solid-phase extraction and matrix for laser desorption/ionization mass spectrometry. J Am Soc Mass Spectrom 16: 263–270

Porath J, Carlsson J, Olsson I, Belfrage G (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature 258: 598–599

Rainer M, Najam-ul-Haq M, Huck CW, Feuerstein I, Bakry R, Huber LA, Gjerde D, Zou X, Qian H, Du X, Wie-Gang F, Ke Y, Bonn GK (2006) Ultra fast mass fingerprinting by high-affinity capture of peptides and proteins on derivatized poly(glycidyl methacrylate/divinylbenzene) for the analysis of serum and cell lysates. Rapid Commun Mass Spectrom 20: 2954–2960

Vallant RM, Szabo Z, Trojer L, Najam-ul-Haq M, Rainer M, Huck CW, Bakry R, Bonn GK (2006) A new analytical material-enhanced laser description ionization (MELDI) based approach for the determination of low-man serum constituents using fullerene derivatives for selective enrichment. J Proteome Res 6: 44–53

Authors' address: A. Univ. Prof. Mag. Dr. Christian W. Huck, Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innrain 52a, A-6020 Innsbruck, Austria,
Fax: +43-512-507-2965, E-mail: christian.w.huck@uibk.ac.at