

# Near infrared spectroscopy of nanostructured materials

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## Introduction

Together with the growing importance of nanotechnology, there comes a demand for a fast, easy to handle and automated analytical method to investigate new nano-materials. There are a number of conventional methods such as scanning electron microscopy (SEM), mercury intrusion porosimetry (MIP), laser diffraction, nitrogen absorption following the Brunauer–Emmett–Teller (BET) theory or size exclusion chromatography (SEC). They all have in common that they measure only one parameter and that they are invasive, time consuming, hard to automate and require experienced personnel. In contrast, near infrared (NIR) spectroscopy offers a fast, easy to handle, powerful, non-destructive, easily automated method where several parameters can be determined simultaneously. The investigated materials comprise nano-porous silica particles, dendrimers, nano-coated capillaries, lipid nano vesicles and carbon nanomaterials such as fullerenes, carbon nanotubes (CNTs) and nanocrystalline diamond (NCD). In addition, special focus is also put on particle size determination of nano-powders.

During recent years, nano materials have grown in importance for science and industry. An investigation using the CAS SciFinder software (American Chemical Society, Washington DC, USA) to find references containing the word “nano” revealed 433 references before 1989, 7108 references between 1990 and 1999 and 117,976 references for the years 2000–2011. The word “nano” has its roots in the Greek word “nannos”, which means dwarf. One nanometre equals  $10^{-9}$  m and, for comparison,

the diameter of a fibre from a spiders web measures approximately 1000 nm. When talking about nanotechnology work carried out with materials, the range from 0.1 nm to 100 nm is usually meant. With matter having sizes in the nanoscale range, special physical, chemical, electrical, medical, mechanical and optical properties can be achieved.<sup>1</sup> Together with the increasing importance of nano-materials comes the need for fast, robust and accurate analytical methods for their physical, chemical and morphological characterisation. As mentioned above, there are a number of well-established methods such as electron microscopy, laser diffraction, optical microscope image analysis or the Coulter Counter method for particle size analysis.<sup>2</sup> Specific surface area can be determined with nitrogen absorption based on the BET equation and pore size and pore volume, applying MIP or SEC. The analysis of chemical properties is not only limited to identification but also the observation of derivatisations or surface coverage. A number of methods such as nuclear magnetic resonance spectroscopy or elementary analysis are therefore applied.<sup>3,4</sup> Most of these methods are time-consuming, many are invasive, hard to automate, require experienced operators and some of them show poor reproducibility. In contrast, NIR spectroscopy offers a fast, powerful, easy to handle, non-destructive and high-throughput analysis where almost no sample preparation is needed. Having the capability to perform in- and on-line real-time monitoring of processes, NIR spectroscopy has become one of the key methods for the process analytical technology initiative of the US Food and

Drug Administration, the aim of which is to monitor and optimise a product throughout the production process and “design” the quality of a product instead of exclusively enacting quality control of a final product in a laboratory.<sup>5</sup> NIR spectroscopy is now a well-established technique with the first commercially-available, stand-alone instruments having appeared in the 1980s. In addition to the above mentioned advantages, NIR spectroscopy can, for some quantitative analyses, show higher performance compared to mid-infrared spectroscopy, mainly due to combination vibrations and overtones appearing in the wavenumber range from  $4000$ – $10,000$   $\text{cm}^{-1}$  and also offers the possibility to determine physical and chemical properties simultaneously.<sup>6</sup> A relatively new trend in the field of NIR spectroscopy is hyperspectral imaging where spectra are recorded as a function of the areal distribution. State-of-the-art instruments, in combination with microscopy, can record spectra with a lateral resolution of only a few micrometres.<sup>7</sup> Presently, NIR spectroscopy is deployed as an analytical method in agriculture, food sector, petrochemistry, pharmaceutical sector, environmental sector, clinical sector and many other fields.<sup>8</sup> This article highlights recent applications of NIR spectroscopy on nanostructured materials.

## Particle size determination

It has long been known that the particle size in a powder has a strong influence on the resulting NIR diffuse reflection spectra;<sup>9</sup> this is most obviously observed in a shift of the baseline (offset).<sup>10</sup> This

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phenomenon can be best described with the Kubelka–Munk model where reflectance ( $R_\infty$ ) is related to the scattering coefficient ( $S$ ) and the absorption coefficient ( $K$ ):<sup>11</sup>

$$f(R_\infty) = \frac{(1 - R_\infty)^2}{2R_\infty} = \frac{K}{S}$$

There are a large number of publications describing particle size determination using NIR spectroscopy. All are based on the Kubelka–Munk theory and Ciurczak's work on aspirin, ascorbic acid,  $\text{Al}_2\text{O}_3$  and  $(\text{NH}_4)_3\text{PO}_4$  for particles larger than  $85\ \mu\text{m}$ ,<sup>12,13</sup> stating that the larger the particles are, the higher the observed absorption is. Higgins and co-workers were the first to analyse nano-particles with a D90 (D90 is defined as the diameter where 90% of the particles have a smaller equivalent diameter) smaller than  $250\ \text{nm}$ .<sup>14</sup> They observed that the Kubelka–Munk theory was not applicable anymore as smaller particles showed higher absorbance than larger ones. This can be explained because the light path, after interaction with smaller particles, is longer than in powders with larger particles, as the light path increases with the number of re-reflections between the smaller particles. This explanation is illustrated in Figure 1. Bittner *et al.*<sup>15</sup> demonstrated an approach to simultaneously identify and determine the particle size of amoxicillin trihydrate particles in the sub-micrometre range (D90 =  $6.9\text{--}21.7\ \mu\text{m}$ ) observing the same effects as Higgins and proving a linearity between absorbance and reciprocal particle diameter at higher wavenumbers ( $>7000\ \text{cm}^{-1}$ ). Khanmohammadi *et*

*al.*<sup>16</sup> investigated the feasibility of NIR spectroscopy to predict the particle size of  $\text{TiO}_2$  nanoparticles. They successfully developed a model using 35 different sieved samples (five classes,  $20\ \text{nm}$  to  $>130\ \mu\text{m}$ ) applying back propagation artificial neural networks as a non-linear model.

### Nano-porous silica materials

Silica gels play the single most important role as stationary phases for chromatographic-based separation procedures. To achieve maximum separation efficiencies, it is important to know physical properties such as particle size, pore volume and specific surface area.<sup>17</sup> Silica-based stationary phases can be divided into porous-, non-porous particles and monoliths. Porous silica particles tend to be the most universally applicable ones finding use in solid phase extraction (SPE), high-performance liquid chromatography, micro liquid-chromatography, capillary electrophoresis (CE), capillary electrochromatography, gas chromatography and material enhanced laser desorption ionisation time-of-flight /mass spectrometry (MELDI-ToF/MS).<sup>18</sup> Najam-ul-Haq and co-workers<sup>19</sup> applied NIR spectroscopy, in combination with multivariate data analysis, to characterise porous silica particles. Cluster models based on the particle size and the pore size were established. Petter *et al.*<sup>17</sup> reported on quantitative partial least square regression-based models to determine particle size in the range  $3\text{--}14\ \mu\text{m}$ , pore diameters in the range  $7\text{--}400\ \text{nm}$  and specific surface in

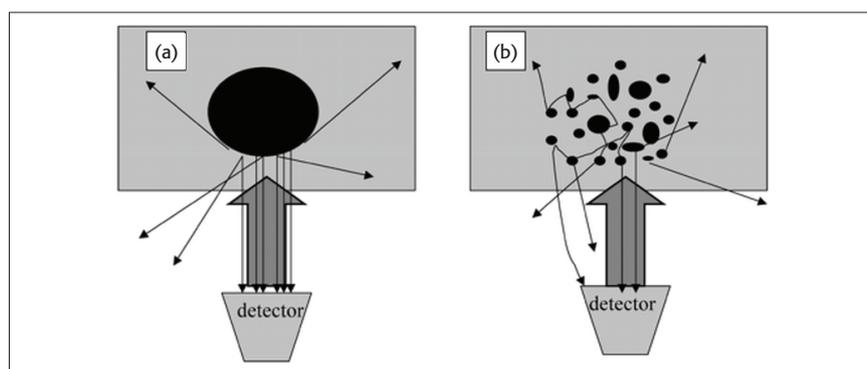
the range  $10\text{--}458\ \text{m}^2\ \text{g}^{-1}$  of porous silica particles. A principal component analysis (PCA) cluster model allowed for the classification of pure silica, amino-silica and silica-C18. This information derives from the corresponding stretching and deformation vibrations of C–H, N–H and O–H bonds in the NIR region.

### Carbon nanomaterials

Carbon nanomaterials comprise fullerenes, NCD, CNTs and graphitic-nanofibres. They show several advantages and unique properties compared to other materials. The presence of  $80\text{--}99.5\%$   $\text{sp}^2$ -hybridised carbon facilitates further derivatisation steps and the increased surface area ratio enables improved sensitivity for stationary phases, for example for SPE<sup>1</sup> or CE.<sup>20</sup> Glassy carbon electrodes modified with carbon nanomaterials can increase the performance of electrochemical applications significantly, showing higher sensitivities, lower limits of detection and faster electron transfer kinetics.<sup>21,22</sup> Figure 2 shows a range of carbon nanomaterials as well as silica particles and dendrimers that have been investigated with NIR spectroscopy.

### Carbon nanotubes (CNTs)

CNTs have unique features, being able to display metallic, semiconducting as well as superconducting electron transport. CNTs possess a hollow core, allowing guest molecules to be stored and show the largest elastic modulus of any known material.<sup>21</sup> Generally, CNTs can be divided into two groups: single wall carbon nanotubes (SWCNT) having a cylindrical nanostructure, which is formed by a single, rolled-up graphite sheet, showing high aspect ratio, and multi-walled carbon nanotubes (MWCNT), which are assemblies of several layers of graphene cylinders with varying diameter encircling one another with an interlayer space of  $0.34\ \text{nm}$ . While SWCNTs typically show diameters of  $0.4\text{--}3\ \text{nm}$ , MWCNTs have diameters of  $1.4\text{--}100\ \text{nm}$ .<sup>23</sup> NIR spectroscopy has been well established as a powerful tool for the characterisation of the electronic band structure of CNTs. One of the first reports of NIR spectroscopy as a tool for the investigation of



**Figure 1.** Schematic of light scattering by (a) large particles and (b) smaller particles. Reproduced with permission from Reference 14.

CNTs was published by Chen *et al.*,<sup>24</sup> followed up by others such as Itkis *et al.*<sup>25,26</sup> and Liu *et al.*<sup>27</sup>

## C<sub>60</sub> fullerenes

Since the discovery of the buckminsterfullerene (C<sub>60</sub>) in 1985 research on it has subsequently grown. Various fullerenes with diameters in the range of 20–100 nm are of high interest in the field of peptide and protein identification for diagnostic marker research.<sup>28</sup> To shorten the turnaround time in the lab, NIR spectroscopy, in combination with multivariate data analysis, was introduced to monitor and verify derivatisation steps. A robust PCA model to identify C<sub>60</sub>, C<sub>60</sub>-aminosilica, C<sub>60</sub>-epoxysilica, dioctadecylmethano-[60]-fullerene and IDA-[60]-fullerene was established.<sup>17</sup>

## Nanocrystalline diamond (NCD)

NCD can be deposited forming a thin layer on several substrates such as nickel, graphite and titanium to selectively bind several molecules from biological fluids. Using the MELDI screening approach, NCD showed the highest sensitivity of all carbon nanomaterials with a lower limit of detection of 600 attomol. Due to the combined characteristics of chemisorption and physisorption NCD offers excellent regeneration capabilities.<sup>29</sup> The surface can be either –O or –H terminated showing hydrophilic or hydrophobic properties, respectively. For a successful further derivatisation it is crucial to have information about the termination type. X-ray photoelectron spectroscopy can be carried out to distinguish between –H and –O termination. This requires trained personnel and enormous expenditure on instrumentation. Heigl *et al.*<sup>1</sup> developed a NIR spectroscopic method to determine the termination mode with high precision applying a fibre-optic probe under nitrogen atmosphere.

## Nano-coated capillaries

Inner wall coatings of fused silica capillaries for CE help to prevent irreversible protein absorption during the electrophoretic separation process.<sup>20</sup> Near infrared hyperspectral imaging can be used as a tool for quality control of the derivatisation of the capillaries. We developed a PCA-based cluster model to identify uncoated fused silica, latexdiol-coated- and fullerenol-coated fused silica capillaries. Having an inner diameter of <75 µm and a coating thickness of only 100 nm, a special experimental set-up was created whereby the end of the capillary was sanded down using sandpaper P1000 at a 45° angle prior to being measured in NIR reflection mode with a lateral resolution of 6.25 µm (Figure 3).

## Guest–host interactions among dendrimers

Dendrimers feature a well-defined globular shape, having a branched structure consisting of an interior core of shells (generations) and terminal functional groups. Size molecular weight and chemical functionality are easily controllable. There is a wide variety of applications in the fields of chemistry, physics, pharmaceuticals and clinical chemistry. Heigl *et al.*<sup>30</sup> used NIR spectroscopy to guest–host interactions between G0–G7 amine-terminated poly(amidoamine) as “guests” and porous silica materials as

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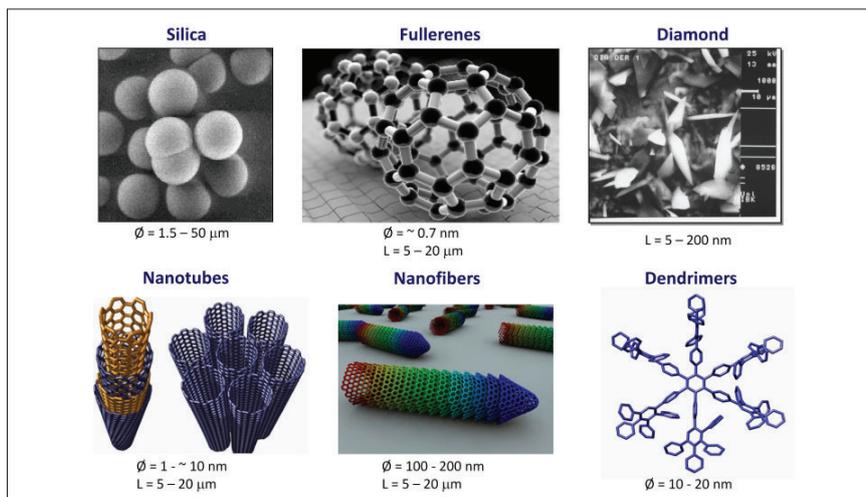
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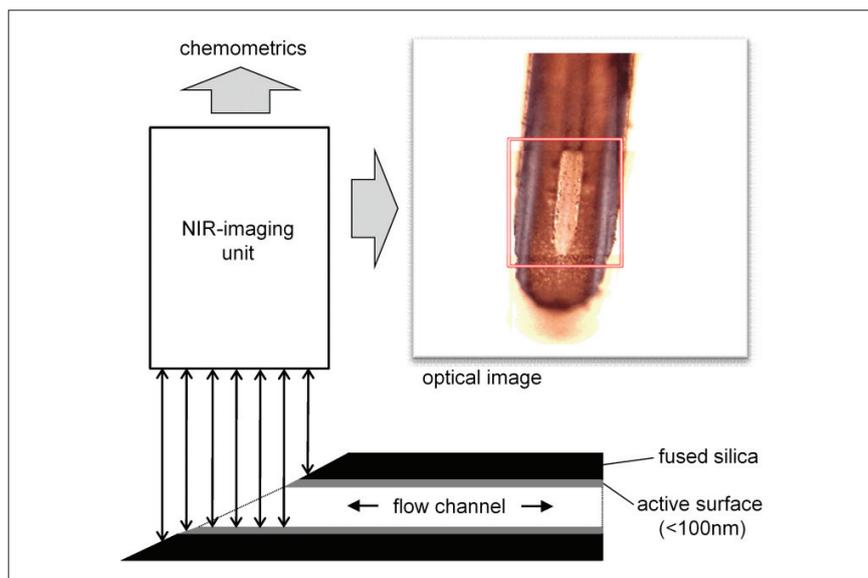
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**Figure 2.** Nanomaterials investigated with NIR spectroscopy. Reproduced with permission from Reference 1.



**Figure 3.** Schematic NIR imaging measurement setup of nanocoatings in fused silica CE-capillaries.

“hosts” to predict molecular weights ranging from 0.52 kDa to 116.49 kDa and particle diameters ranging from 1.5 nm to 8.1 nm. Porous silica particles have a constant pore size, with dendrimers showing smaller particle size than the pore size, having access to the interior of the silica particles, while larger particles can only interact with the silica surface.

### Lipid nano vesicles

Lipid nano vesicles are applied in drug delivery and as membrane substances due to their unique osmotic activ-

ity, membrane permeability, chemical composition and surface characteristics. Such liposomes are spherical, self-closed nanostructures consisting of lipid bilayers that enclose parts of the surrounding solvent into their interior. Bista *et al.*<sup>31</sup> applied NIR spectroscopy to investigate synthetic polyethyleneglycolated lipids that spontaneously form liposomes upon hydration. NIR absorption spectroscopy is deployed for the band component analysis of the samples in liquid form to get a broader understanding of the molecular structure and conformation.

### Conclusion

The suitability of NIR spectroscopy as a tool to replace numerous conventional methods for the investigation of nanostructured materials such as nanoporous silica particles, dendrimers, nano-coated capillaries, lipid nano vesicles and carbon nanomaterials was illustrated and summarised. Due to all the advantages listed below, this method has high potential to become well-established and widely used in the field of nanotechnology, particularly with regard to PAT applications:

- non-invasive
- simultaneous determination of physical and chemical properties
- short analysis time
- high throughput analysis
- easy measurement
- no/almost no sample preparation necessary.

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