

CHARACTERIZATION OF NANOSTRUCTURES: TOOLS AND CHALLENGES

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Abstract Apart from long-known and applied nanostructures like carbon black for tyres or pigments for coatings nanotechnology has created highly sophisticated structures used for nano/molecular electronics, diagnostics, drug delivery, UV-absorbers *etc.* Often the main question to be solved analytically is the local determination of tiny amounts of chemicals resulting in an ever increasing need for highly sensitive as well as locally resolved techniques. Applications of techniques like mass spectroscopy, transmission electron microscopy as well as ultracentrifugation to problems arising from nanotechnology in the chemical industry will be described.

Keywords: Analytical ultracentrifugation; Fuel cells; Inorganic particles; Nanostructures; Surface energy.

INTRODUCTION

Though the main hype in research as well as applications of nanostructures lasts for only the past decade as can be inferred from the number of patents using the term “nano” in the abstract (source *i.e.* European Patent Office) some large scale applications are known for much longer time. Engineered nanoparticles like titanium dioxide, amorphous silica and iron oxide are used in many consumer products like food additives, paints and cosmetics^[1]. Anthropogenic nanomaterials are created for millennia as by-products of simple combustion and food cooking and more recently by ore refining and smelting as well as combustion in vehicle and airplane engines amongst others^[2].

Some characteristics of interest for nanostructures are collected in Table 1.

Table 1. Characteristic physico-chemical features of nanoparticles and important parameters for the behavior in biological environment

Complete physical and chemical characterization	In biological environment
Shape, size, size distribution	Quantification of reactions with relevant species (proteins, lipids, enzymes,...)
Molecular structure (elemental composition)	Nanophase dispersed stability
Functionalized overlayers	Transport within cells
Extent of reaction (side reactions)	Contaminations (endotoxins)
Presence of surface contamination	Monitoring (<i>in vivo</i>) of biodistribution
Special properties (electronic, optical)	Toxicology/pharmacology standards

For the characterization of physico-chemical parameters a variety of techniques like scanning probe microscopy, light scattering, X-ray photoelectron spectroscopy, fluorescence microscopy, electron microscopy and so on are available.

The methods used for the three industrial examples (pigments, fuel cells, nanoparticles in biological environment) described in this paper are secondary ion mass spectrometry^[3], contact angle measurements^[4], transmission electron microscopy^[5] and ultracentrifugation^[6].

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EXPERIMENTAL

Mass spectra were recorded in the static SIMS mode on a TOF.SIMS 5 (ION-TOF GmbH, Münster, Germany) equipped with a Bi-cluster source.

Contact angles were measured using a DSA 10 Mk2 (Krüss GmbH, Hamburg, Germany) against water, di-jodo-methane and formamide. Surface energies were determined using Owens-Wendt theory^[7].

High resolution TEM images were obtained with a Tecnai F20 TEM (FEI Company, Eindhoven, Netherlands) equipped with a EDX detector for elemental analysis. The energy filtered images were acquired in cooperation with IFW, Dresden, Germany (Drs. Thomas and Gemming) on a Tecnai F30 TEM equipped with a Gatan Imaging Filter (Gatan Company, Warrendale, USA).

The inorganic nanoparticle size distribution was determined by analytical ultracentrifugation (AUC) of *ca.* 500 μ L of a test substance prepared by stirring *ca.* 0.1 mg/mL particle suspensions for 24 h without ultrasound treatment. At the acceleration of up to 3.0×10^5 g used in AUC, solutes and nanoparticles sediment into fractions that are separated according to their size in the range 0.5–10000 nm. Agglomerates larger than *ca.* 10 μ m diameter sediment before data acquisition starts and cannot be quantified. Simultaneous detection by synchronized optics quantifies the amount and the diameter of each fraction independently^[6]. When there is a low concentration of nanoparticles in a high-concentration protein medium, nanoparticles are easily discerned from sedimenting proteins because of their much higher density and resultant faster sedimentation of many orders of magnitude, and because of their higher refractive index and high turbidity. We used a Beckman model XL ultracentrifuge that we modified for the online recording of sedimentation with turbidity, interference, and Schlieren or ultraviolet (UV) detection^[6]. This modification allows the use of interference and turbidity optics with a ramped rpm-profile to quantify the amounts of ultrafine fraction (diameter < 100 nm), fine fraction (100 – 1000 nm), and larger-scale material (>1 μ m). The evaluation of the AUC raw data incorporates the fractal morphology of nanoparticle aggregates and applies the fractional dimension of 2.1. A single measurement represents the distribution of *ca.* 10^{12} particles and has thus high statistical relevance.

EXAMPLES

Pigments

To assure stable dispersion of pigments in waterborne coatings special surface modification has to be undertaken. In the present case of pearl lustre pigments after longer pumping times in the coating unit agglomeration occurs with a special pigment type.

For surface characterization we applied secondary ion mass spectrometry (SIMS), which is a strictly surface sensitive technique with an information depth below 1 nm and simple contact angle measurements to the pressed pigment powder. The mass spectra given in Fig. 1 for two pigments reveal unambiguously that surface chemistry is different one pigment exhibiting a cerium oxide coating, the other one shows chromium signals which might result from uncoated pigment areas *i.e.* from the mica itself.

Following Owens/Wendt theory from the contact angle measurements the unpolar (LW; Lifshitz-van der Waals) part and the polar (AB; acid-base) part can be determined. The results given in Table 2 reveal that the pigments mainly differ in the polar part of the surface energy with a similar LW-part of about 43 mN/m.

As to be expected for the “polar solvent water” the pigment with the highest acid-base part exhibits the best dispersion stability.

Figure 2 shows a TEM image of the outer surface of single pigment particles revealing an approximately 2 nm thick amorphous layer covering the particle surface. In local EDX measurements Al and Si can be identified in this layer. The Si signal is much stronger compared to the Al signal when approaching the layer with the electron nanoprobe from the outside. This suggests that amorphous coverage consists of a Al-rich layers applied first followed by a Si-rich layer applied at the top of the pigment surface.

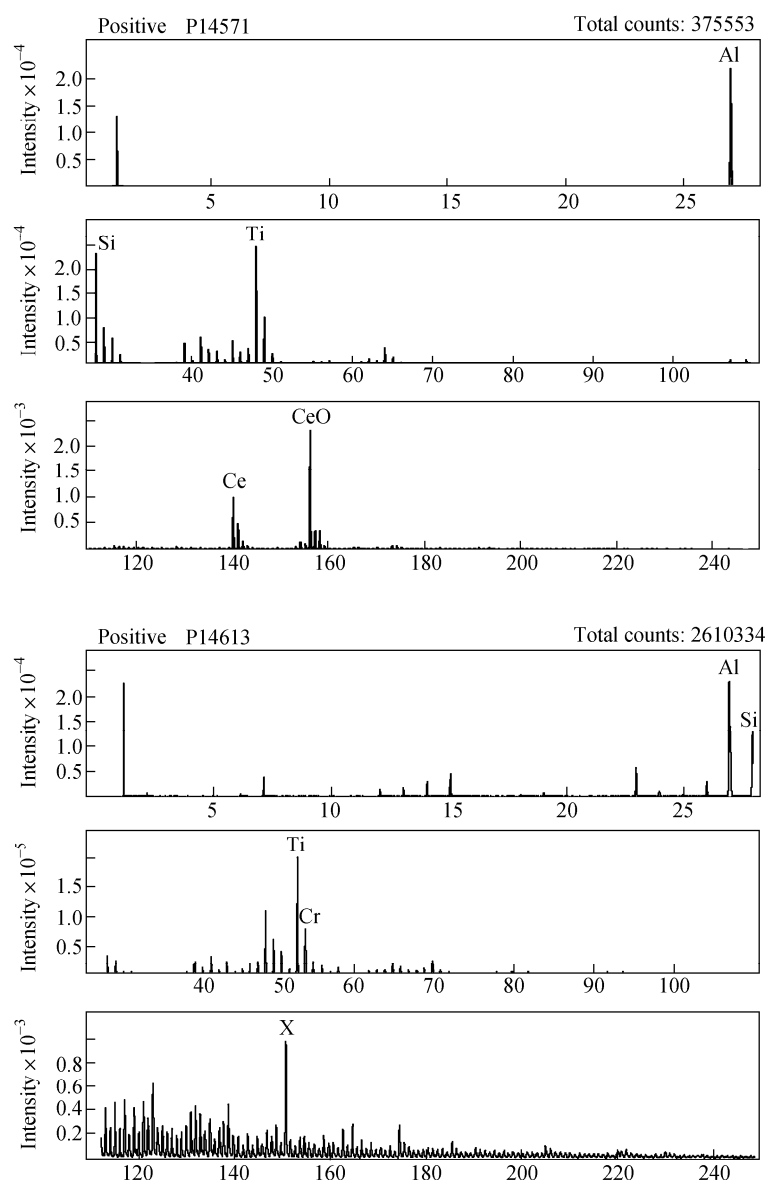


Fig. 1 SIMS spectra of two pearl luster pigments (positive ions)

Table 2. Surface energies of different pigments

Liquid name	Mica pigments		
	Sample 164185 Theta (°)	Sample 178378 Theta (°)	Sample D013301 Theta (°)
Water	99.2 ± 9.2	84.4 ± 1.7	33.3 ± 6.18
Formamide (AB)	19.4 ± 3.6	19.8 ± 5.8	26.0 ± 8.77
Alpha-bromo-naphthalene (AB)	13.3 ± 0.5	10.8 ± 1.2	15.1 ± 5.35
Calculation results (method: acid-base theory)			
LW-part	43.4 (mN/m)	43.8 (mN/m)	43.1 (mN/m)
Acidbase-part	20.6 (mN/m)	3.9 (mN/m)	8.8 (mN/m)
Total surface energy	64.0 (mN/m)	47.7 (mN/m)	51.9 (mN/m)

The example reveals that it is even possible to obtain useful information on (ensembles of) nanoparticles with methods which have a non-nm lateral resolution (SIMS about 200 nm, contact angle in the mm range).

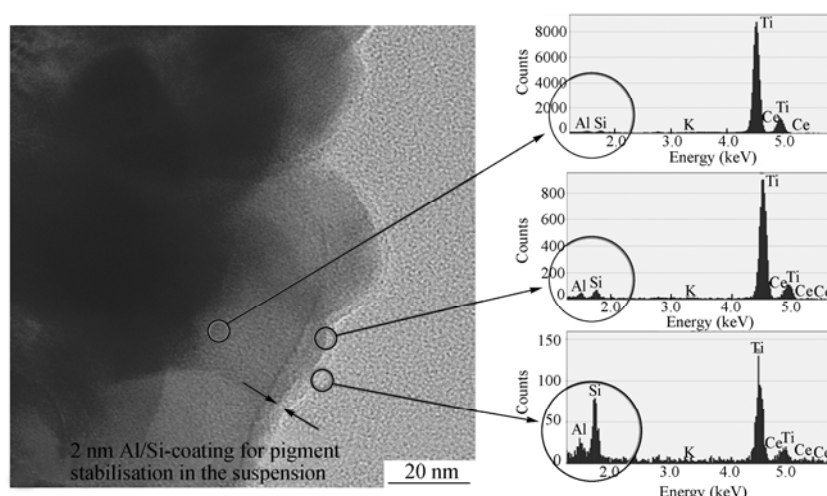


Fig. 2 TEM image with three local EDX-spectra of a pearl luster pigment taken at the encircled areas

Fuel Cells

The central element in the case of a polymer electrolyte membrane (PEM) fuel cells is the membrane electrode assembly (MEA). The MEA consists of a proton conducting membrane (the most prevalent one at the moment is NafionTM produced by DuPont) which is covered by a catalytic layer on both sides. The catalyst consists of a fine dispersion of metal particles on carbon particles (soot). When methanol is used as a fuel, the metal particles on the anode side contain Pt and Ru, whereas on the cathode side only Pt is used. Ru is needed to increase the CO tolerance of the anode catalyst. CO is generated as a by-product during the methanol oxidation and binds strongly onto Pt surface and blocks the active centers. For a good CO tolerance one needs a solid solution grade of 1:1 between Pt and Ru.

Figure 3 shows a high resolution TEM image of some Pt/Ru particles on carbon carrier particles, which are partially graphitized through the production procedure. The metal particles are about 5 nm large and single crystalline indicating that indeed a solid solution is formed. However local EDX analyses show a strong variation of the Pt/Ru concentration ratio from particle to particle. This is an important information, since excess Ru not bound into crystal lattice can migrate through the membrane onto the cathode side leading to a performance loss of the fuel cell.

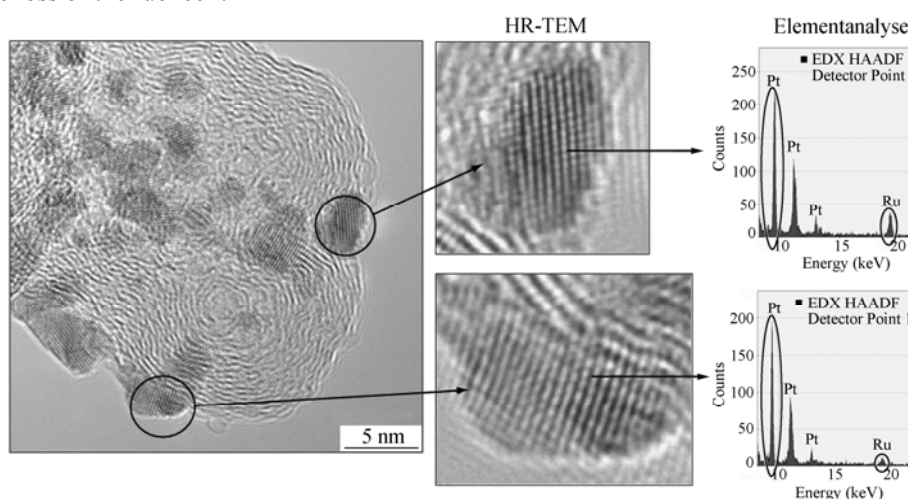


Fig. 3 High resolution (HR) TEM picture of catalyst nanoparticles on soot including elemental analysis

Besides a good electron conduction to the outer circuit one also needs good proton conduction from the anode through the membrane to the cathode side. This is achieved by adding a F containing ionomer like NafionTM to the anode, where the protons can move *via* a hopping mechanism along the polymer chain. Ideally the ionomer should form a filament like network between the metal and the carbon particles. In Fig. 4 one can see these filaments connecting different carbon particles separated by a pore.

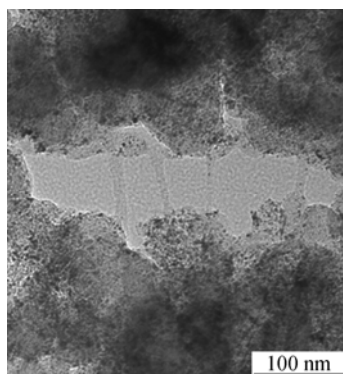


Fig. 4 TEM picture of the catalyst ionomer interface
Ionomer should form a filament like network between the metal and soot particles assuring a good proton conductivity to the membrane.

However the ionomer distribution between the carbon and the metal particles cannot be imaged by conventional bright field TEM, because the contrast difference between the two is too low.

Since the Ionomer contains F as an inorganic element, the ionomer distribution can be imaged using energy filtered (EF) TEM at the F absorption k-edge^[5]. Challenges for these measurements are the low F concentration, the radiation sensitivity of the ionomer and the fact that samples have to be very thin for EFTEM.

Figure 5 shows the zero loss TEM image, the F distribution image and a superposition of both images, but taken at a different sample area. The images demonstrate that the ionomer filaments indeed form contact between the Pt/Ru particles and the carbon carrier thus ensuring a good proton conductivity from the active metal site over the carbon carrier to the membrane.

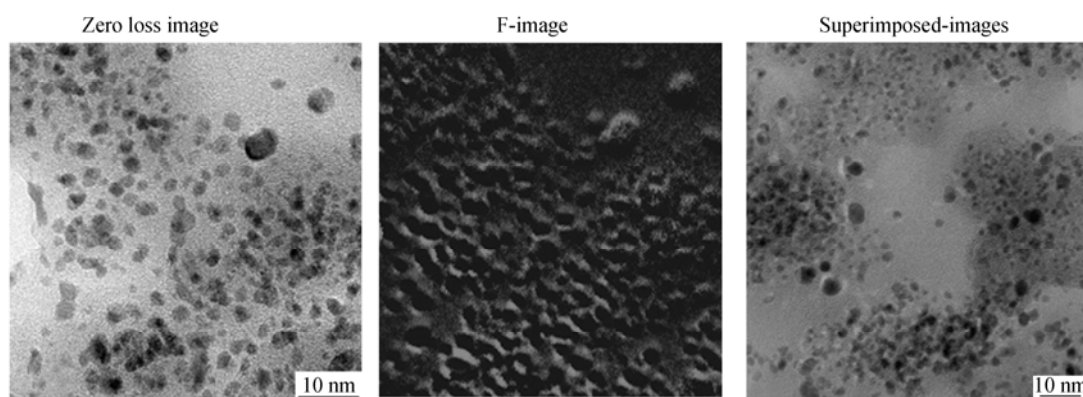


Fig. 5 EFTEM of the F distribution and superposition with the zero loss image

This example demonstrates that electron microscopy can be a very powerful tool for the characterization of structures on the nm-scale.

Inorganic Particle Suspensions

As can be seen from the literature (for example [2]) toxicity of nanoparticles is an even growing topic of nanoscience with high complexity and without simple answers^[8]. It turns out that for toxicity questions the information needed is mostly different from the ones described above. A collection of challenges for the characterization needed (taken from ref. [9]) is given in Table 3.

Table 3. Examples for the information needed to estimate toxicity of nanostructures

Known limitations of translating <i>in vitro</i> techniques to <i>in vivo</i> behaviour
New techniques to provide information on phys./chem. state of nanostructures in complex physiological milieu
Limit degradation and changes in the analysis
New techniques and protocols to analyze individual nanostructures and produce ensemble averages and statistics
Control of the ubiquitous surface contamination potential:
Adventitious adlayers, mixed oxides, surface active microbial endotoxins, corrosion products, Ostwald ripening, contaminations (PDMS; PAHs)

A variety of recent publications demonstrates that subtle details in the experimental testing conditions and differing nanoparticle physicochemical properties play a role. Especially the questions of particle size distribution and of surface contamination — *i.e.* the question, what is the nanostructure intended and what is the structure my test system encounters — seemed to play an important role. Directly interrelated is the question if and how the nanostructures are altered through contact to physiological surroundings like body fluids and so on. So in contrast to the measurements described above the characterization of ensembles of nanostructures in physiological media comes into play. A method well suited to these conditions is the analytical ultracentrifuge (AUC). One of the incontestable strength of the AUC is determination of particle size and particle size distribution from the nm to the μm scale (other methods like light scattering in general only deliver mean values) in the solutions of interest including cell culture suspensions containing fetal calf serum (FCS) and Dulbeccos modified eagle medium (DMEM). As test particles we choose the oxides of titanium, zirconium and cerium. Figure 6 displays the result of an AUC run with TiO_2 of 21 nm size (irregular but globular). The first peak corresponds to the serum proteins, mainly BSA as can be inferred from the inset. Further analysis of the AUC results tells us that there is no protein aggregation through the nanoparticles. The AUC method is thus the only method that can identify in a single experiment both proteins and primary particles and agglomerates with correct concentrations (absolute measurement through the interference detector) and correct molar masses (through the characteristic sedimentation speed). The curve obtained *via* laser diffraction tells us that this method overestimates the bigger particles, and by principle the diffraction will not detect 99% of the colloidal system, *i.e.* the proteins and the comparably small nanoparticles!

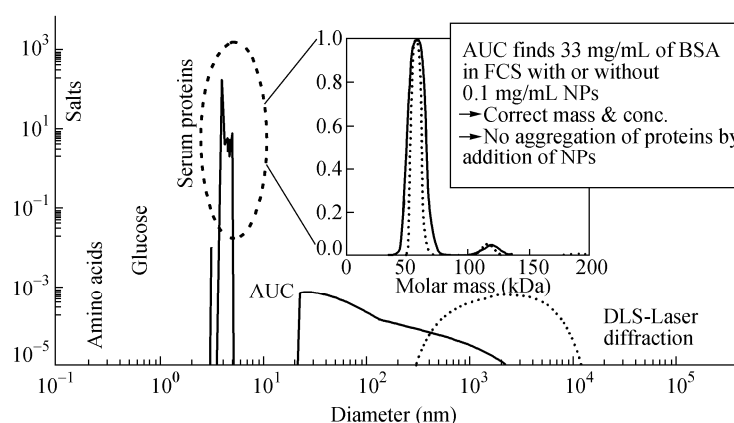


Fig. 6 Particle size distribution of TiO_2 in FCS by AUC (solid line) and by DLS (dotted)
Inset: molar mass resolution of the protein signal without nanoparticles (solid curve) and with TiO_2 nanoparticles added (dotted), demonstrating that AUC determines correctly all colloidal components from proteins to nanoparticles

This point is further clarified in Fig. 7 which includes two different investigations: the influence of protein concentration on particle size and the comparison of various techniques for particle size determination. The original particle size for the different oxides was around 20 nm. Figure 7 reveals that proteins can disperse nanoparticles — at 100% FCS the original size appears whereas at lower concentration strong aggregation is observed. A comparison of different techniques (dynamic light scattering (DLS), laser diffraction, X-ray AUC slow, Cryo TEM, DLS + prefilter, X-ray AUC fast and turbidity AUC) at the highest FCS concentration demonstrates that apart from turbidity AUC all other methods deliver too high particle sizes for the oxides.

Further details can be found in ref. [10].

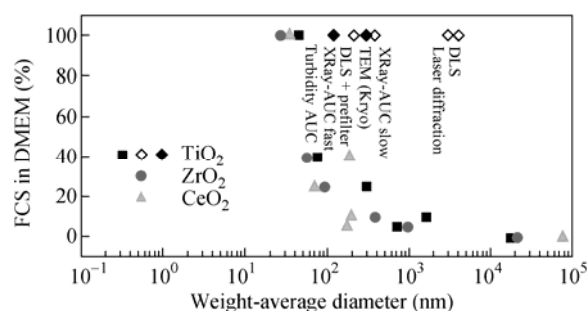


Fig. 7 Influence of protein content (FCS) on particle size and comparison of different techniques for determination of particle size

DISCUSSION

In this paper we have shown that for selected technical nanosystems characterization down to the nanoscale is possible especially if the system and the problem under investigation allows the application of vacuum conditions (with exception of scanning force microscopy and the contact angle measurements in particular).

But these methods in general do not allow a characterization of an ensemble of nanoparticles in their natural, physiological environment. A sufficient complete picture of nanostructures as demonstrated by the last examples can only be achieved through a combination of various methods as well as a proper choice of methods necessitating techniques with even better lateral resolution and higher chemical sensitivity on highly structured materials.

REFERENCES

- 1 Borm, P., *Nanotoday*, 2008, 3(1-2): 56
- 2 Buzea, C., Blandino, I. and Robbie, K., *Biointerphase*, 2007, 2(4): MR17
- 3 Benninghoven, A., Hagenhoff, B. and Niehuus, E., *Anal. Chem.*, 1993, 65: 630A
- 4 van Oss, C.J., Good, R.J. and Chaudhury, M.K., *J. Colloid Interf. Sci.*, 1986, 111: 376
- 5 Williams, D.B. and Carter, C.B., “*Transmission Electron Microscopy*”, Plenum Press, New York, London, 1996
- 6 Mächtle, W. and Börger, L., “*Analytical Ultracentrifugation of Polymers and Nanoparticles*”, Springer-Verlag, Berlin Heidelberg, 2006
- 7 Owens, D.K. and Wendt, R.C., *J. Appl. Polym. Sci.*, 1969, 13: 1741
- 8 Lewinski, N., Colvin, V. and Drezek, R., *Small*, 2008, 4(1): 26
- 9 Grainger, D.W. and Castner, D.C., *Adv. Mater.*, 2008, 20: 867
- 10 Schulze, C., Kroll, A., Lehr, C.-M., Schäfer, U.F., Becker, K., Schnekenburger, J., Schulze-Isfort, C., Landsiedel, R. and Wohlleben, W., *Nanotoxicology*, 2008, 2: 51