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Safety-relevant properties of Nanoparticles

Gerhard Klein

TUV SÜD Industrie Service GmbH, München, Germany

Abstract: In this paper physically measurable properties of Nanoparticles are discussed which are known or supposed to be relevant for toxicological endpoints.

First a review of well-known safety-relevant facts about Nanoparticles is given. After discussing some thermodynamic properties a list of features is derived, specifying those objective parameters of Nanoparticles which are responsible for possibly hazardous properties. These can in our opinion serve as a basis for structural descriptors for QSAR and for a more precise definition of the term “nanoparticle” which could be a contribution to forthcoming regulatory discussions.

Keywords: Nanoparticles, physicochemical properties, QSAR

1. INTRODUCTION

In the last years the discussion of detrimental effects of nanomaterials has been intensified. Lots of publications and research initiatives are exclusively devoted to nanotoxicology. On the other hand, the production of engineered nanomaterials is ongoing with increasing rates. While fundamental research is trying to identify and characterize important exposition paths, biotransformation, interaction of nanomaterials with cells and many other issues, manufacturers have to produce *today*. Since the “nano-industry” has a considerable share of SMEs, this problem of product liability is of crucial importance for all of them: How can one responsibly produce substances without being aware of all relevant safety issues?

Answering this question, the very first step should be to identify the most important physicochemical (PC) properties of nanomaterials. “Important” in this context means relevant with respect to possible toxicological effects. The review and discussion of existing proposals is the target of this paper. It has theoretical and practical implications. From the point of view of scientific reductionism, biological and especially toxicological effects can be traced back to chemical interactions which in turn are governed by physical laws. Dealing with Nanotechnology we first could think about a more phenomenological approach, which is outlined in chap. 4 where some fundamental, mainly thermodynamic properties of nanoparticles are discussed. A more fundamental approach would be possible if QSAR methods could already be applied as discussed shortly in chap. 5. Especially these methods could be quite important in the future, since the application of this approach would allow an “a-priori-evaluation” of the toxicity of nanomaterials thus accelerating the progress of nanotechnology significantly. This is the practical aspect of this discussion. But first some basic definitions in the field of “nano” shall be given.



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2. BASIC DEFINITIONS

Following essentially the conventions in [1] and [2], we give first the key definitions which are used throughout this paper:

Nanoscale - The size range from approximately 1 nm to 100 nm.

Engineered/Manufactured Nanoparticle - A particle with three dimensions at the nanoscale, engineered or manufactured by humans on the nanoscale with specific physicochemical composition and structure to exploit properties and functions associated with its dimensions. Engineered nanoparticles include particles with a homogeneous composition and structure, compositionally and structurally heterogeneous particles and multi-functional nanoparticles. In the following paragraphs we use the term “nanoparticle” for short, but exclude ultra-fine particles (UFP) or biological nanoparticles with this term. We are focusing here on Nanoparticles.

Nanomaterial - A material with one or more external dimensions or an internal or surface structure at the nanoscale, which exhibits physical, chemical and/or biological characteristics associated with its nanostructure.

Agglomerate – Collection of loosely bound particles or aggregates or mixtures of the two where the resulting external surface is similar to the sum of the surface areas of the individual components.

Aggregate – Particle comprising strongly bounded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components. The importance of understanding how the binding forces of an agglomerate or aggregate affect the dispersibility of the component particles under different conditions is noted.

3. EXISTING CLASSIFICATIONS OF RELEVANT PROPERTIES

The classical paper of Oberdörster et al. [1] concerning the elements of a screening strategy recommends that the following PC data are characterized for test materials used for toxicological screening studies (see also column 2 in Table 1):

Because of the nanometer-scale structure, *size* and structure-dependent electronic configurations are of course important. Due to these properties, particles in the nanosize range can deposit in all regions of the respiratory tract including the distal lungs, pass into cells directly through the cell membrane or penetrate between or through cells and translocate to other parts of the body. Furthermore, nanomaterials have various *shapes* and *structures* such as spheres, needles, tubes, plates, etc. and the shape of them in turn can influence the kinetics of deposition and absorption in the body (see, e. g., [3]). Also *porosity* may be important. Certainly *chemical composition* is also essential for the characterization of nanomaterials. Currently, nanomaterials are based on very different substance classes, e.g., metal/metal oxides, compounds, polymers as well as biomolecules. They can also be a combination of the above components in core-shell or other complex structures. The term chemical composition includes spatially averaged (bulk) and spatially resolved heterogeneous composition.

As a particle decreases in size, the mass-specific *surface area* (m^2/kg in SI units) increases. More atoms/ molecules are found at the surface compared to those inside. Nanoparticles have therefore a much larger surface area per unit mass compared with larger particles. Since an increase in the surface-to-volume ratio results in the increase of the particle surface energy those particles might become also more biologically reactive.

Finally, particle *surface chemistry* (and *surface charge* as well) is important: reactive groups on a particle surface modify the biological effects. Nanoparticles are often stabilized with coatings or derivative surface to prevent *agglomeration* which would occur under ambient conditions. Surface modification can thus alter the properties and the distribution of nanoparticles in the body as well as the effects on the biological systems significantly.



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The Nano Risk Framework [4] gives an analogous base set of physical and chemical properties (see Box 2 in [4] and column 3 in Table 1). The authors propose a list starting with two formal, but important specifications: the descriptive *name* which is useful for unique designation of the material and the *common form* of the material (loose powder, dispersion, agglomerated, aerosol) which will have implications for the potential route of human or environmental exposure.

Chemical composition means here the concentrations of elemental chemicals or chemical compounds in the nanomaterial including accompanying substances (e. g. surface treatments, lattice doping, impurities which may affect toxicity and exposure). It is noted that chemical composition may change as nanomaterials are incorporated into products or break down, either during use or after disposal. Impurities in the material, and the extent of contamination, should be identified as well.

Characterization of *crystal phase* and *molecular structure* can lead to better understanding of potential structure-property relationships. *Physical Form* (crystalline or amorphous, at room temperature and pressure) and *shape* influence how the materials flow, interact with other particles (to agglomerate), how easily they disperse when entering various media or the environment, and how they interact with the biosphere. The mean *particle size*, the mean *surface area*, and the distributions around the means shall be determined as well as the mass and number-count distributions. These measures are important because an increased surface-area-to mass ratio of nanomaterials appears to be a critical feature in understanding some aspects of their toxicity, particle surface energy, and reactivity.

Particle Density shows how easily the material is dispersed in air and water and how easily it settles from air and water. This has implications for the behavior of the material in gases and liquids.

Solubility in water or other biologically relevant fluids may be important at various stages in its lifecycle as the nanomaterial interacts with other product components, materials, organisms, or the environment. *Dispersibility* is the ease with which an insoluble solid or liquid material may be dispersed uniformly in a liquid. This property influences exposure and fate throughout the product lifecycle and the partitioning of the nanomaterial should it enter an aquatic environment. *Bulk density* provides a quick indication of how much dust the nanomaterial may generate on handling while it is in its powder form. The same holds for the *agglomeration state*. Moreover, it provides information on the likely size distribution of inhalable particles as well as on their relative ease of dispersion. The *porosity* as an indication of the fraction of the particle that is *devoid* of material and pore-size distribution of the material has implications for its interaction with substances in its surroundings.

The interacting of the nanomaterial with other materials is influenced by its electric potential or the *surface charge*. In solution, the surface charge has implications for the stability and aggregation of particles. Finally, *Surface reactivity* is an indication of the likelihood and nature of a nanomaterial's interaction with other materials. Specific assays – like a Vitamin C test, a hemolysis test and a ROS assay – may here need to be tailored to specific nanomaterials.

The following table summarizes the two approaches [1] and [4] and includes also the list in [5] which details the two other ones with respect to the purposes of QSAR (see also chap. 5). As described above, [4] mentions also the parameters Name, Common Form, Molecular Structure, Particle Density, Dispersibility and Bulk Density which are not listed here explicitly.



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Table 1: Important Safety-relevant Parameters acc. to [1], [4] and [5]

| Reference Parameter | from [1] | from [4] | from [5] |
|---|--|---|---|
| Size, Size Distribution | ✓ (only size distribution) | ✓ | ✓ |
| Shape | ✓ (including structure) | ✓ (at room temperature and pressure) | ✓ |
| Surface Area | ✓ | ✓ | ✓ (especially biologically relevant surface area involved in biological interactions) |
| Surface Structure | -- | ✓ (coating) | ✓ |
| Surface Chemistry | ✓ | ✓ (Surface Reactivity) | ✓ associated with biological reactivity |
| Chemical Composition | ✓ including spatially averaged (bulk) and spatially resolved heterogeneous composition | ✓ including surface treatment | ✓ |
| Crystal Structure | ✓ | ✓ including purity, molecular structure | ✓ called crystallinity (purity) |
| Porosity | ✓ | ✓ | ✓ |
| Surface Charge | ✓ | ✓ | Charge in biological fluid |
| Solubility | -- | ✓ in water and biologically relevant fluids | ✓ |
| Aggregation/ Agglomeration State | ✓ (only term 'agglomeration' is used) | ✓ (only agglomeration state) | ✓ |

As one can see from table 1, there is a common understanding on the most important items, but one can also assume that not all the parameters are independent and what the correlation between them could look like. To discuss this in some more detail it makes sense to have a closer look on a more fundamental description of nanomaterial, where we will focus on nanoparticles.

4. SOME THERMODYNAMIC ASPECTS

In general, the inner energy U of a system with n different species can be written as

$$U = TS + \sum_j X_j Y_j + \sum_{i=1}^n \mu_i n_i \quad (1)$$

where T is the absolute temperature, S the entropy, X_j a generalized displacement (e. g. the Volume V , Length L , Area A , Polarization \vec{P} , Magnetization \vec{M} etc.) and Y_j a corresponding generalized force (in our examples: the pressure exerted by the system ($-p$), the tension τ , the surface tension γ , the electrical field \vec{E} , the magnetic field \vec{H} etc.). μ_i is the chemical potential of species i (which can also be considered as a generalized force) and n_i is the numbers of moles in the system (some kind of a generalized displacement). A change of the inner energy can then in general be described by

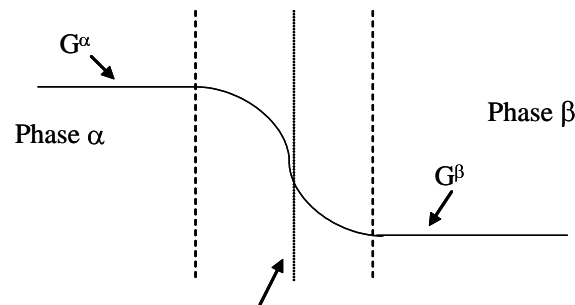
$$dU = \delta Q + \delta W + \sum_{i=1}^n \mu_i dn_i = TdS + \sum_j Y_j dX_j + \sum_{i=1}^n \mu_i dn_i \quad (2)$$

From the equations (1) and (2) also other thermodynamic potentials can be derived. Most important in the following is the Gibb's free energy:

$$G = U - TS - \sum_j X_j Y_j = \sum_{i=1}^n \mu_i n_i \quad \text{and} \quad dG = -SdT - \sum_j X_j dY_j + \sum_{i=1}^n \mu_i dn_i \quad (3)$$

Now we can consider the behavior of Gibb's free energy at a surface separating two phases α and β :

Figure 1: Free Enthalpy between two phases



Surface S (Phase σ) with Area A

Somewhere in between the phases we have a the interface region (surface) separating α and β . For each component i the number of moles is given by $n_i = n_i^\alpha + n_i^\beta + n_i^\sigma$, where $n_i^\alpha = c_i^\alpha V^\alpha$, c_i^α being the concentration [mol/m³] of i in phase α and V^α the total volume of phase α and analogous for β . If we take now only surface-surface tension-work into account, we find the following expression for an infinitesimal change of Gibb's free surface energy at constant pressure:

$$dG^\sigma = -S^\sigma dT + \gamma dA + \sum_{i=1}^n \mu_i dn_i^\sigma \quad (4)$$

where μ is the chemical potential of the species considered.¹ At const. T , p and in the case of just one component we find

$$dG^\sigma = \gamma dA + \mu dn^\sigma \quad \text{or} \quad \mu^\sigma := \left(\frac{\partial G^\sigma}{\partial n^\sigma} \right)_{p,T} = \gamma \left(\frac{dA}{dn^\sigma} \right) + \mu \quad (5)$$

¹ Here, $\gamma > 0$ is chosen which means that force is exerted *on* the system and "directed out of the particle"



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μ^σ is the chemical potential of the component at the surface. Assuming now that we have spherical particles with radius r in equilibrium with the pure solid phase (infinitely large, so $r = \infty$ for it), we find the Kelvin-equation

$$\mu^\sigma - \mu = \gamma \left(\frac{dA}{dn^\sigma} \right) = \frac{2\gamma V_m}{r} \quad (6)$$

(V_m : molar volume of the component). The largest area we can create is just the case of Phase σ being the gas phase itself; the “particle”-diameter is then the diameter of the molecule (or ion-pairs or atoms etc.) of the component. We then have

$$\mu^\sigma \cong \mu_{Gas} = \mu_{Gas}^0 + RT \ln p_{gas} \quad \text{and} \quad \mu = \mu_{Bulk} = \mu_{Bulk}^0 + RT \ln p_{Bulk} \quad (7)$$

The upper index “0” means standard conditions ($T = 298$ K, $p = 1$ atm). So we get

$$\mu_{Gas}^0 - \mu_{Bulk}^0 = \frac{2\gamma V_m}{r} \quad (8)$$

for standard conditions. With (8) one can calculate the value of γ for different compounds; if r is taken as the “radius” of the compound or the atom one finds results which are considerably higher than those calculated than by profound methods like DFT, e. g. But this is reasonable, since we include here also the breaking of all bonds within the solid. If we now assume that our phase σ is the nanoparticle-phase, we find from (7) and (8)

$$\mu^{nano} \cong \mu_{Bulk}^0 + \frac{2\gamma V_m}{r} + RT \ln p_{nano} \quad (9)$$

This holds for the gas phase. The generalization of (9) to arbitrary states and phases means substituting pressures by activities, and as is known the activity (denoted by “a”) is the relevant parameter for the chemical equilibrium. So for our nanoparticle we have the following form of the chemical potential:

$$\mu = \mu_{Bulk}^* + RT \ln a + \frac{2\gamma V_m}{r} \quad \text{or} \quad \mu = \mu_{Bulk}^* + RT \ln \left(a e^{\frac{2\gamma V_m}{RT r}} \right) \quad (10)$$

Two things should be noted in (10): First, there is an exponential dependence of the equilibrium constant for a given chemical reaction on the radius of the particle². This is in our simple model the cause for increasing “reactivity”. And we use μ^* instead of μ^0 , since the definition of the standard state depends now on “the environment” of the particle which can be any “biologically relevant fluid” (see [4] and [5]). As it stands, (10) is not complete. What is missing is the fact that particles are very often charged, depending on the electrolytes and the pH in the medium of the particle. In a mere thermodynamic discussion, the concept of activity is generalized, including now also activity coefficients. To discuss this important item in more detail, a microscopic model must be used. Here, reference is made to the Stern model for colloids as it is shown in fig. 2 below.

The surface potential in this model is caused by the surface charge density (assumed as positive in fig. 2 below). In an electrolyte solution with positive Co-ions and negative counterions, the negative ions will displace the water molecules attached to the surface and create a stable and fixed “Stern-layer”, such decreasing the potential to the stern potential. Non-fixed hydrated negative counterions and positive Co-ions form then a more or less diffuse Gouy-Chapman-layer which is characterized by the Debye-Hueckel-Parameter κ

$$\kappa^2 = \frac{2e^2}{\epsilon_0 \epsilon kT} I \quad , \quad I = \frac{1}{2} \sum_i n_i z_i^2 \quad (11)$$

where I is the ionic strength, n_i the density of particles i [$1/m^3$] and z_i the valences of the ions (with sign).

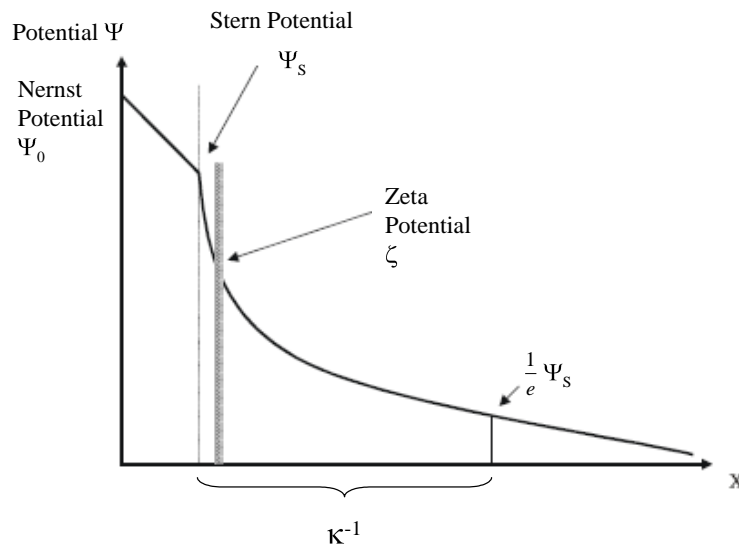
² Note that for $r = \infty$ (plane surface) we find the usual formula $\mu = \mu_{Bulk}^* + RT \ln a$

In DLVO-theory [6] in its simplest form, one can give quantitative formula for the repulsive potential E_{rep} (r : radius of the particle, x : distance d of two particles, normalized to their diameter,³ Z_p : number of net charges on the particle's surface, Ψ_S : Stern potential, see fig. 2) for two limiting cases ($\kappa r \ll 1$ and $\kappa r \gg 1$):

$$(i) \quad \kappa r \ll 1: \quad E_{rep}(x) = \Psi_S^2 4\pi \varepsilon_0 \varepsilon r \frac{\exp(-2\kappa x)}{2(1+x)}; \quad \Psi_S = \frac{Z_p e}{4\pi r \varepsilon_0 \varepsilon (1+\kappa r)} \quad (12)$$

$$(ii) \quad \kappa r \gg 1: \quad E_{rep}(x) = \frac{\Psi_S^2 4\pi \varepsilon_0 \varepsilon r}{2} \ln(1+\exp(-2\kappa x)); \quad \Psi_S = \frac{2kT}{\varepsilon_0 \varepsilon} \sinh^{-1} \left(\frac{Z_p e^2}{8\pi r^2 \kappa \varepsilon_0 \varepsilon kT} \right)^4$$

Figure 2: Potential of a charged Nanoparticle



The attractive London - van-der-Waals-Potential E_{attr} is given by

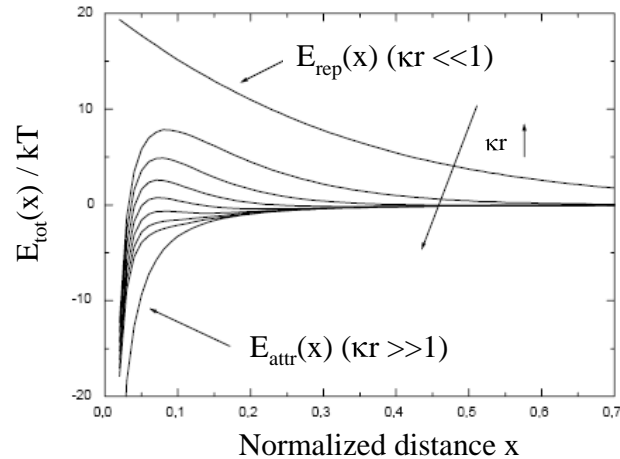
$$E_{attr}(x) = \frac{A_H}{12} \left[\frac{1}{(x^2 + 2x)^6} + \frac{1}{(x+1)^6} + 2 \ln \left(\frac{x^2 + 2x}{(x+1)^2} \right) \right] \quad (13)$$

(A_H is the Hamaker constant which is 10^{-19} to 10^{-21} J at room temperature). Summing (12) and (13), one finds the following qualitative behaviour of the potential as shown in fig. 3.

³ Exact definition: $x := (d - 2r) / 2r$

⁴ \sinh^{-1} is the inverse function of \sinh ; we're neglecting here Born's repulsion term.

Figure 3: Qualitative DLVO-Interaction-Potential of charged Nanoparticle



Several facts can be learned from the discussion in this chapter:

- (1) Measurements of the Zeta-Potential are in fact approximate measurements of the Stern-Potential. The Nernst Potential, which would allow us to infer on surface charge, can only be measured at low electrolyte concentrations ($< 10^{-6}$ molar) which are not found under physiological conditions. Surface charge (of the particle itself and/or of reactive groups on its surface) is of course the physical cause of all the potentials. But it is not a very practical parameter from the viewpoint of measurement in contrast to the Zeta-Potential. However, the conditions of measurement (biological relevant conditions) must be specified carefully.
- (2) The total energy of interaction of charged nanoparticles depends also critically on the size of the particle, see (12) and (13).
- (3) “Agglomeration” and “aggregation” are valuable qualitative indicators, but not fundamental ones. Aggregation can occur if the energy barrier shown in fig. 3 can be overcome, agglomeration might be attributed to possible secondary minima (not shown in fig. 3). Agglomeration and aggregation in turn determine dispersibility.
- (4) “Surface chemistry” should be term referring to surface treatment (reactive groups, coatings). Insofar it also influences Surface structure, charge, “reactivity” and agglomeration/aggregation.
- (5) “Reactivity” itself is also not well defined. What is defined is the concept of chemical potential which influences the equilibrium of any chemical reaction. The increase of the chemical potential with decreasing particle size makes size the obviously important parameter when talking about “nanos”.
- (6) “Surface area” is determined by size and shape of the particle. It can be interesting to identify possible parts of the surface which might be important for special biological interactions. Since surface can become manifest as ‘internal’ or ‘external’ surface (the first one being porosity), the term should be reserved for external surface area.
- (7) “Chemical composition” and “crystal structure” are not completely independent: One should remember the distinction between (non-)stoichiometric *compositions* and modification or phase/homogeneity/purity/defects in the *crystal*. Some defects are again due to thermodynamics (e. g. $Zn_{1+x}O$ and its semiconducting behaviour or the different phases of Ti_nO_{2n-1} with probably delocated electrons in Ti’s d-Orbitals)

In the last two chapters reference shall be made to QSAR and the correlation of the different quantities in this chapter shall be discussed.



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5. QSAR

Quantitative Structure-Activity Relationships or QSARs are theoretical models that can be used to predict the physicochemical and biological properties of molecules⁵. A Structure-Activity Relationship (SAR) rests upon the proven hypothesis that chemicals with similar molecular structures have similar effects in physical and biological systems. Therefore they allow predicting the potential of a chemical containing the substructure to exhibit a certain biological effect if the chemical substructure is known. To describe the variations of the extent of an effect with variations in molecular structure, a quantitative method is required. QSAR as a mathematical model relates a quantitative measure of a structure (e.g. a physicochemical property) to a biological effect (e.g. a toxicological endpoint). In principle, (Q)SARs can be used to supplement experimental data - it helps supporting priority setting of chemicals, guiding experimental design (e.g. selection of tests /doses) and providing mechanistic information - or to replace testing by grouping chemicals into chemical categories and filling in data gaps for classification and labeling as well as for risk assessment. Thus this method allows an understanding of the correlation between PC-data and corresponding biological effects as well as an economic prediction of those effects. Therefore it entered also the European Chemical legislation (REACH). To extend the traditional (Q)SAR paradigm to nanoparticles, one needs among others structural descriptors for physicochemical properties and calculated structural descriptors; QSAR modelling should in principle be possible then, provided that relevant descriptors can be identified and sufficient experimental data can be obtained ([5], [7]). The present paper supports this approach.

6. CONCLUSIONS

As discussed in chap. 3 and 4, there are different potentially relevant PC-parameters to characterize nanoparticles. They are not mutually independent as fig. 4 shows and if we wonder which parameters should be measured we should emphasize that we have (at least) four parties with different interests: the theorist, the QSAR-expert, the toxicologist and the practitioner. Theoretically, the specification of the chemical potential is the phenomenological decisive quantity; looking into the microscopic world, it is determined by parameters like *size*, *surface chemistry* ("non-electric" part), *porosity*, *chemical composition* and/or *structure*, respectively. These parameters are measurable and we suggest that they must be specified. *Surface charge* is interesting in theory and for QSAR; for measurements and specifications, *zeta potential* is the better quantity. For interactions in the biosphere, QSAR people and toxicologists must know the *solubility*, *shape* and the *aggregation/agglomeration state* as well. Prescriptions should be developed in which biologically relevant environment they are to be investigated. From a practical point of view it cannot be the task for a nanoparticle-producer to characterize his/her product in all (possibly) relevant toxicological aspects. All that the producers can do is characterizing *qualitatively* shape and state of agglomeration/aggregation and solubility for (not yet) specified reference conditions under which the product is usually handled.

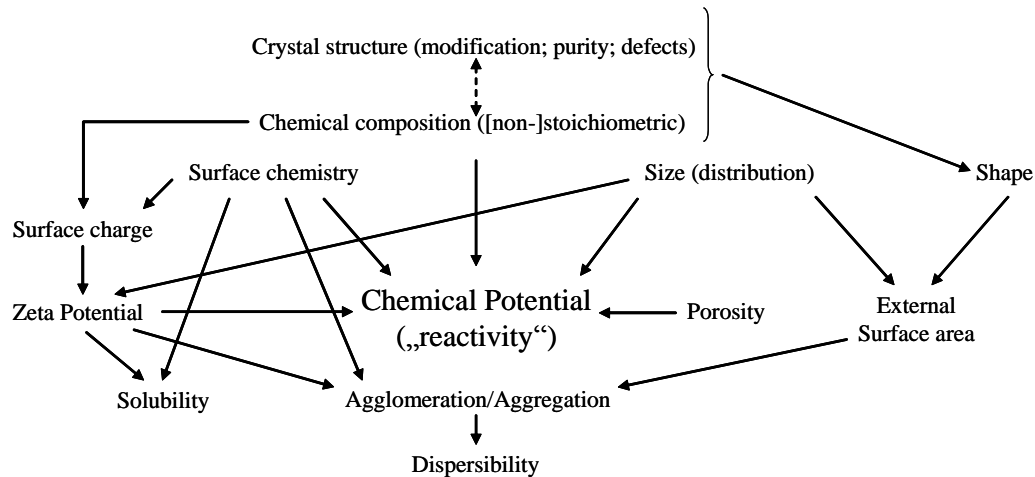
The discussion of this paper is certainly not the last word: In addition to the previous discussion one should also remember that it's not only thermodynamics which determines whether a chemical reaction will occur or not and which is the focus of this paper. *Chemical kinetics* finally determines whether a reaction will take place or not and will also play a crucial role in biological systems. Further biological effects will result from the solid state physics of nanomaterials which should include further potentially biologically important effects. Examples could be the surface electronic configuration or the formation of polarons and excitons whose discussion is beyond the scope of this paper.

⁵ The presentation here follows [7]; for an overview about QSAR see [8], e. g.



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Figure 4: Important parameters for characterizing nanoparticles



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