Potential modelling of acrylic acid copolymer nanoparticles by small angle X-ray scattering

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Abstract

Copolymer nanoparticles of acrylic acid, acrylic amide, acrylic butyl acrylate and methyl acrylate were characterised using a parameter relevant for in vivo organ distribution: charge distribution. Usually the zeta potential is estimated with laser Doppler anemometry as an equivalent for the particle charge. In our case, the potential distribution was investigated by small angle X-ray scattering. The nanoparticles shielded by counter-ions at high ionic strength (0.15 M NaCl solution) have short-range potentials. Different potential models were calculated: Percus–Yevick potential and Hypernetted Chain potential. The potentials estimated by small angle X-ray scattering can be divided into attractive and repulsive parts. The attractive potential, indicated by a minimum, is not significant compared with the repulsive potential and can be ignored. Consequently the tendency to form Van der Waals clusters is low. The solution shows a random order of hard spheres. Therefore, surface charges on the nanoparticles should hamper such carrier cell interaction only at short distances. In comparison with the potential estimation by laser Doppler anemometry (32.8 mV), results of the same order of magnitude were obtained using the small angle X-ray scattering method. The advantage of this method is that it provides a potential curve, which allows better appraisal of clearance by the reticuloendothelial system. By interpreting the potential curves, a more detailed prediction of steric stability of particle systems is possible. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Nanoparticles; Potential modelling; Small angle X-ray scattering; Zeta potential; Steric stabilization

1. Introduction

Among the various systems developed in drug targeting strategies, the colloidal polymeric and lipid carriers represent one of the most advanced systems for controlled drug delivery. In particular, the polylactide (PLA), polylactide/glycolide (PLA/GA), acrylate and the solid lipid nanoparticles (SLN) have been the subject of many studies (Tarcha, 1991; Müller, 1991; Löbenberg and
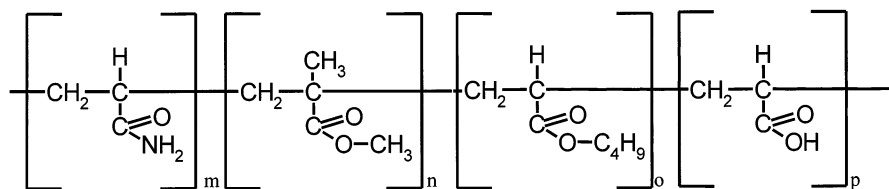
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Fig. 1. Structure of copolymer nanoparticles (m, n, o and p: number of units acrylic amide, methyl acrylate, butyl acrylate and acrylic acid (ASC), respectively).

Kreuter, 1996; Lukowski and Pflegel, 1997; Reszka et al., 1997; Omelyanenko et al., 1997). They achieve selective targeting of specialized drugs such as antineoplastics, immunosuppressants, peptide hormones or antigens.

Different parameters influence the body distribution of these nanoparticles and the recognition of the nanoparticles by the reticuloendothelial system (RES). One of these special parameters, which plays an important role for RES clearance is the particle charge. Capo et al. (1981) describe a smaller RES-mediated clearance by low-charged nanoparticles.

Laser Doppler anemometry (LDA) or electrophoresis methods are typical techniques for characterizing the zeta potential which can be treated as equivalent to the particle charge (Müller, 1996). However these methods are restricted by different assumptions (O'Brien and White, 1978) and consequently they can be taken only as an indirect measure of the surface charge of outer ionic shears. The zeta potential as a single value for a particle does not allow us to make correct predictions concerning the particle's clearance by the RES for each particle.

The second problem with zeta potential measurements is the inexact appraisal of the steric stability of colloidal carriers, as analysis of solid lipid nanoparticles shows, where nanoparticles with high zeta potentials have shown fast aggregation of the particles (zur Mühlen, 1996). New investigations are looking at the question of direct measurement of the particle potential distribution (Franosch and Singh, 1997). The aim of this study is to estimate the potential distribution around the nanoparticles and to determine the kind of potential of model acrylic acid copolymer nanoparticles by small angle X-ray scattering (SAXS). The appraisal of steric stability and the clearance should be improved.

2. Materials

Different copolymer nanoparticles of acrylic acid (CAA) with acrylic amide, acrylic butyl acrylate and methyl acrylate (Fig. 1) were produced by emulsion polymerization in water as previously described (Lukowski et al., 1992). The CAA-nanoparticles were produced as a latex of about 50% in solid phase. The latex sample was purified by dialysis against double distilled water in open air. The polymer concentrations used for small angle experiments are 1.36, 2.26, 4.65, 8.9, 17.9 and 39.5 mg/ml.

3. Methods

3.1. Small angle X-ray scattering

The measurement of the small angle X-ray scattering intensity was done using a Kratky diffractometer (A. Paar, Graz) with electronic step scanner and data acquisition by a personal computer. The scattered Cu-K_α radiation was registered by a proportional counter after monochromatization by a 7-μm Ni filter and pulse height discrimination. The width and position of the entrance and of the receiving slit guarantees a loss-free registration of information of the scattered intensity for scattering vectors larger than $s_{min} = 0.012$ nm^{-1}. This therefore provides structural information for dimensions up to 260 nm (Damaschun and Pürschel, 1969); $s = 4\pi \sin(\Theta)/\lambda$ is the length of the scattering vector, with wavelength λ and scattering angle 2Θ.
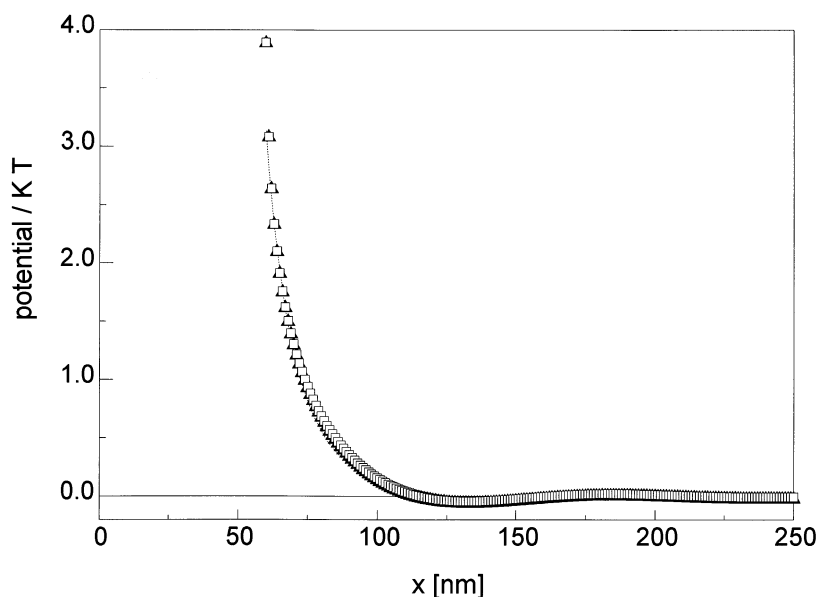
Fig. 2. Potentials of the CAA-nanoparticles dependent on the distance x in 0.15 M NaCl solution (K, Boltzmann constant; T, temperature); $\square$, Percus–Yevick potential; $\triangle$, Hypernetted Chain potential.

3.2. Zeta potential

The estimations of the zeta potential were made using a Zetaziser III (Malvern Instruments, Malvern, UK). The wavelength of the laser beam was 632.8 nm. The nanoparticles were dissolved in 0.15 M NaCl solution. The measurements were made with a small capillary.

3.3. Theory of interparticular scattering effects

Theoretically the interaction term $a(s, c)$ can be calculated by solving the Ornstein–Zernicke equation (Eu and Gan, 1993; Henderson and Sokolowski, 1995)

$$g(r, c) = h(r, c) - \rho_0 \int g(r, c)h(r, c)\, \mathrm{d}r \qquad (1)$$

$g(r, c)$ is the particle pair correlation function calculated by

$$g(r, c) = h(r, c) + 1 \qquad (2)$$

$h(r, c)$ is the total correlation function (Zernicke and Prins, 1927) with

$$h(r, c) = \frac{10^{24}M}{2\pi^2 c N_L} \int_0^\infty s^2[a(s, c) - 1]\frac{\sin sr}{sr}\, \mathrm{d}s \qquad (3)$$

and

$$c^*(r, c) = \frac{10^{24}M}{2\pi^2 c N_L} \int_0^\infty s^2\left[\frac{a(s, c) - 1}{a(s, c)}\right]\frac{\sin sr}{sr}\, \mathrm{d}s \qquad (4)$$

is the direct correlation function taking into account only pair-interactions as discussed above (c in mg/ml, s in nm^{-1}, r in nm) and ρ_0 is the number density.

The Ornstein–Zernicke equation is an exact relation between the Fourier transforms of the distribution functions. Practically, this equation can be solved only by using different approximations for $h(r, c)$ and $g(r, c)$ (e.g. Mean Spherical Approximation, Hypernetted Chain). Each approximation is useful only if the system fulfils some preconditions for particle dimensions, counter-ions, charge and charge compensation. For a system of particles with Lenard–Jones interaction potentials, showing impenetrability and form constancy (hard spheres), the solution structure factor was derived by Fournet (1951) to be

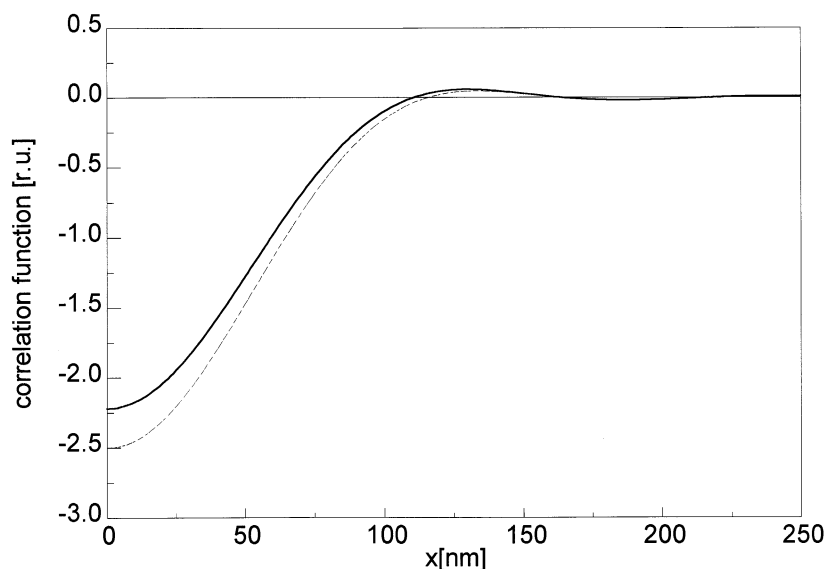$$a(s, c) = \frac{1}{1 + 8\mu \cdot \Phi(2Rs)} \qquad (5)$$

Fig. 3. Direct and total correlation function of CAA-nanoparticles with 14.4% acrylic acid in 0.15 M NaCl solution; - - -, direct correlation function; ——, total correlation function.

Table 1
Composition of the CAA-nanoparticles and its influence on the zeta potential

Nanoparticle	Methyl acrylate (%)	Acrylic acid (%)	Acrylic amide (%)	Butyl acrylate (%)	Zeta potential (mV)
CAA, 14.4%	56.2	14.4	3.1	26.3	-32.8 ± 1.8

The zeta potential measurements were made in 0.15 M NaCl solution.

where $\Phi(2Rs)$ is the scattering amplitude of spheres with a radius $2R$.

It was determined that for charged, screened spheres (Hayter and Penfold, 1981) at high volume fractions ($\mu \leq 0.2$ mg ml^{-1}) and moderate polyion–polyion interactions $0.1 \leq 2R \cdot \kappa \leq 6$

$$a(s, c) = \frac{1}{1 - 24\mu \cdot \tilde{a}(s, R, \mu, \psi_s, \kappa)} \qquad (6)$$

where $\tilde{a}$ is a function of particle radius R, the surface potential ψ_s and the Debye–Hückel inverse screening length κ.

For strong, charge-coupled interactions of the macroions (small ionic-strength of the counterions) and low polymer concentrations ($\mu < 10^{-3}$ mg ml^{-1}), the main part of the repulsive Coulomb potential has been replaced by hard sphere impenetrability in the calculation procedure for $a(s, c)$, leading to a rescaled structure factor in the

Mean Spherical Approximation (Hayter and Penfold, 1981; Hansen and Hayter, 1982).

On the other hand, the interaction term can be estimated directly from the experimental X-ray curves. A comparison with the theoretical $a(s, c)$ can be used to confirm the type of the potential and to determine its free parameters or if the theoretical parts exist, the function can be experimentally estimated and compared for different particle systems. Therefore, the approximation Percus–Yevick and Hypernetted Chain can be easily calculated.

4. Results and discussion

For investigation of the solution structure and for characterization of the nanoparticles by their potentials we tried to solve the Ornstein–Zernicke
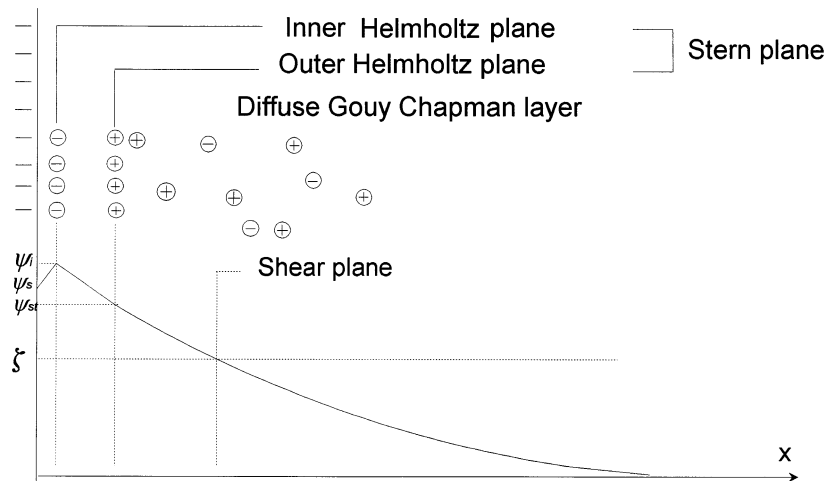
Fig. 4. Schematic drawing of the potential curve at the phase border with a negative surface potential at distance x of the solid surface: ψ_s, surface potential; ψ_i, potential of the inner Helmholtz plane; ψ_{st}, Stern potential and ζ, zeta potential (Müller, 1991).

equation analytically as an exact relation between the Fourier transforms of the particle distribution functions by using approximations for $h(r, c)$ and $g(r, c)$ with the Mean Spherical Approximation. However the preconditions for particle dimensions, counter-ions, charge and charge compensation were not fulfilled. So it was impossible to solve the equation for the nanoparticles with such formalism. That is why the potential curves were estimated by numerical methods directly from the X-ray data.

The potentials, Percus–Yevick and Hypernetted Chain, of the CAA-nanoparticles dissolved in 0.15 M NaCl solution were calculated using the experimentally available pair correlation function $g(r, c)$ and the direct correlation function $c*(r, c)$ (see Section 3.3). The Percus–Yevick potential (Percus and Yevick, 1958; Franosch and Singh, 1997) was given by Eq. (7)

$$\phi_{PY} = KT \log(1 - c*(r, c)/g(r, c)) \qquad (7)$$

The second pair potential was estimated by Hypernetted Chain theory (Salgi et al., 1992; Eu and Gan, 1993).

$$\phi_{HC} = KT[g(r, c) - \log g(r, c) - 1 - c*(r, c)] \qquad (8)$$

Both types are short range potentials (Fig. 2). This is confirmed by the experimentally determined direct and total correlation functions (Fig.

3). The difference between the direct and total correlation functions of the nanoparticles in 0.15 M NaCl solution is small because the negative charges are shielded at the particle surface by sodium counter-ions. The Debye–Hückel radius, defined by

$$\mu = \frac{4\pi}{3} R^3 (\text{nm}^3) \cdot \frac{N_L}{M} \cdot c(\text{mg/ml}) \cdot 10^{-24} \qquad (9)$$

(N_L, Loschmidt's number, M, molecular mass), is about 0.8 nm for the ionic condition used. That means the nanoparticles should interact almost as independent hard spheres.

The solution shows a random order of hard spheres, which means there is no significant correlation between the position of the nanoparticles. The number of the next nanoparticles is 1.2 in a shell between 110 and 160 nm. That corresponds to a value calculated from a volume concentration $\mu = 0.0367$ mg ml^{-1} for a statistically random distribution of nanoparticles.

It was found that the Percus–Yevick potential and the Hypernetted Chain potential are very similar, although these potentials take into account interaction terms. This result can be explained by the interaction of the nanoparticles. The Percus–Yevick potential only describes the interaction of two particles while the Hypernetted Chain potential considers interactions between

three particles. It can be concluded that the shielding of the potentials by sodium counter-ions is very strong and that multiple interactions of the nanoparticles has no significant influence on the course of the potentials.

Furthermore, it was found that the potentials are mainly repulsive. The repulsive potential decreases relatively quickly in relation to the distance r. The estimated minimum at a distance of 120 nm is smaller than kT. It is insignificant and cannot indicate a weak attractive term. From this it can be concluded that a supply of kinetic energy, for instance by shaking, will not result in particles forming a lot of clusters, which means the dispersion is sterically stable. This is in agreement with the other observation (Müller et al., 1994) that only 0.5% of the nanoparticles form clusters. By calculating the potentials, 15–40 mV was determined at distances of 60–90 nm. These values agree well with the zeta potential where 32.8 ± 2.1 mV was estimated under the same ionic conditions (Table 1). Nevertheless, it is difficult to compare the zeta potential exactly with the potential functions derived by the SAXS-method. The main problem is the correct estimation of the real position of the shear plane defining the zeta potential. The zeta potential only describes the potential of the outer shears corresponding to the GCGS-model (Gouy, 1910; Chapman, 1913; Stern, 1924; Grahame, 1947) which means it is the difference between the potentials on the shear plane and the neutral part of the diffuse layer (Fig. 4). It is calculated using the mobility V_E (relation of velocity v and field strength E) of the particles (Eq. (10))

$$\zeta = \frac{4\pi\eta V_E}{\epsilon} \text{ (mV)} \tag{10}$$

with η the viscosity [Pa·s] and ϵ the dielectric constant (Müller, 1996).

The Percus–Yevick potential and the Hypernetted Chain potential calculated from SAXS characterize spherical symmetric potential curves starting from the surface of the particle to infinity. In contrast to the zeta potential, it is possible to discuss the curves in more detail and to detect attractive and repulsive terms, that is only one absolute value. This might be also relevant to recent stabilization problems of other colloidal particles (Weyhers, 1995; zur Mühlen, 1996).

References

Capo, C., Garrouste, F., Benoliel, A.M., Bongrand, P., Depieds, R., 1981. Non-specific binding by macrophages, evaluation of the influence of medium-range electrostatic repulsion and short range hydrophobic interaction. Immunol. Commun. 10 (1), 35–43.

Chapman, D.L., 1913. A contribution to the theory of electrocapillarity. Phil. Mag. 25, 475–481.

Damaschun, G., Pürschel, H.V., 1969. Über die Meßstrategie bei der Untersuchung der Röntgenkleinwinkelstreuung von verdünnten Lösungen von Biopolymeren. Monatshefte Chemie 100, 274–280.

Eu, B.C., Gan, H.H., 1993. Integral equations of the correlation functions for polymeric liquids. J. Chem. Phys. 99 (5), 4084–4102.

Fournet, G., 1951. Diffusion des rayons X par les fluides. Acta Cryst. 4, 293–301.

Franosch, T., Singh, A.P., 1997. Solution of the Percus–Yevick equation for the molecular pair correlation function of a linear solute molecule in a simple liquid. J. Chem. Phys. 107 (14), 5524–5530.

Grahame, D.C., 1947. The electrical double layer and the theory of electrocapillarity. Chem. Rev. 41, 441–501.

Gouy, M., 1910. Sur la constitution de la charge électrique a la surface d'un électrolyte. J. Phys. 9, 457–468.

Hansen, J.P., Hayter, J.B., 1982. A rescaled MSA structure factor for dilute charged colloidal dispersions. Mol. Phys. 46 (3), 651–656.

Hayter, J.B., Penfold, J., 1981. An analytic structure factor for macroion solutions. Mol. Phys. 42 (1), 109–118.

Henderson, D., Sokolowski, S., 1995. Hard-sphere bridge function calculated from a second order Percus–Yevick approximation. J. Chem. Phys. 103 (17), 7541–7551.

Löbenberg, R., Kreuter, J., 1996. Macrophage targeting of azidothymidine, a promising strategy for AIDS therapy. AIDS Res. Hum. Retroviruses 12 (18), 1709–1715.

Lukowski, G., Pflegel, P., 1997. Electron diffraction of solid lipid nanoparticles loaded with aciclovir. Pharmazie 52 (8), 642–643.

Lukowski, G., Müller, R.H., Müller, B.W., Dittgen, M., 1992. Acrylic acid copolymer nanoparticles for drug delivery I: characterization of surface properties relevant for the in vivo organ distribution. Int. J. Pharm. 84 (1), 23–31.

Müller, J.J., Lukowski, G., Kröber, R., Damaschun, G., Dittgen, M., 1994. Acrylic acid copolymer nanoparticles for drug delivery, structural characterization of nanoparticles by small angle X-ray scattering. Colloid Polym. Sci. 272, 755–769.

Müller, R.H., 1991. Colloidal Carriers for Controlled Drug Delivery and Targeting—Modification, Characterization and In Vivo Distribution. Wissenschaftliche Verlagsgesellschaft CRC Press, Boca Raton, FL.

Müller, R.H., 1996. Zetapotential und Partikelladung in der Laborpraxis. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

O'Brien, R.W., White, L.R., 1978. Electrophoretic mobility of a spherical colloidal particle. J. Chem. Soc. Faraday Trans. II 74, 1607–1626.

Omelyanenko, V., Kopeckova, P., Prakash, R., Clemens, C., Ebert, C., Kopecek, J., 1997. Biorecognition of HPMA copolymers mediated by synthetic receptor binding epitopes. Proc. Int. Symp. CRS 24, 51–52.

Percus, J.K., Yevick, G.J., 1958. Analysis of classical statistical mechanics by means of collective coordinates. J. Phys. Rev. 110, 1–13.

Reszka, R., Beck, P., Fichtner, I., Hentschel, M., Richter, J., Kreuter, J., 1997. Body distribution of free, liposomal and nanoparticle-associated mitoxantrone in B16-melanoma-bearing mice. J. Pharm. Exp. Ther. 280 (1), 232–237.

Salgi, P., Guerin, J.F., Rajagoplan, R., 1992. Structure of colloids and macroionic solutions with soft long range interactions. Colloid. Polym. Sci. 270, 785–793.

Stern, O., 1924. Zur Theorie der elektrischen Doppelschicht. Z. Elektrochem. 30, 508–516.

Tarcha, P.J., 1991. Polymers for Controlled Drug Delivery. CRC Press, Boca Raton, FL.

Weyhers, H., 1995. Feste Lipid Nanopartikel für die gewebsspezifische Arzneistoffapplikation. Thesis, FU Berlin.

Zernicke, F., Prins, J.A., 1927. Die Beugung von Röntgenstrahlen in Flüssigkeiten als Effekt der Molekülanordnung. Z. Phys. 41, 184–195.

zur Mühlen, A., 1996. Feste Lipid Nanopartikel mit prolongierter Wirkstofffliberation, Herstellung, Langzeitstabilität, Charakterisierung, Freisetzungsverhalten und mechanismen. Thesis, FU Berlin.