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Nanoparticles with decreasing surface hydrophobicities: influence on plasma protein adsorption

A. Gessner [a], R. Waicz [a], A. Lieske [b], B.-R. Paulke [b], K. Mäder [a], R.H. Müller [a,*]

[a] *Department of Pharmaceutics, Biopharmaceutics and Biotechnology, The Free University of Berlin, Kelchstr. 31, D-12169 Berlin, Germany*

[b] *Fraunhofer Institute for Applied Polymer Research, Kantstr. 55, D-14513 Teltow-Seehof, Germany*

Abstract

The rapid uptake of i.v. injected nanoparticles by cells of the mononuclear phagocytic system (MPS) is a major obstacle for a long blood circulation time and a drug targeting to sites other than the MPS. The adsorption of proteins on the particles surface after i.v. administration depends on their surface characteristics and is regarded as key factor for the in vivo organ distribution. The objective of this study is to investigate changes in the plasma protein adsorption patterns in the course of surface hydrophobicity variation. Latex particles with decreasing surface hydrophobicity were synthesized as model colloidal carriers. Physicochemical characterization had been performed and considerable differences in the protein adsorption patterns on the particles could be detected by using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). Correlations between physicochemical characteristics and the protein adsorption patterns have been found and are discussed.

Keywords: Nanoparticles; Surface hydrophobicity; Protein adsorption; Two-dimensional electrophoresis; Drug targeting

Surface hydrophobicity of colloidal drug carriers is an important factor for their interaction with cells in vitro and their organ distribution in vivo (Müller, 1991). Amount and composition of adsorbed plasma proteins on the particles surface after i.v. administration is also affected by surface hydrophobicity (Brynda et al., 1984). The plasma protein adsorption pattern on the particles is regarded as a key factor for their in vivo fate (Blunk et al., 1993; Blunk, 1994). Despite the influence of surface hydrophobicity on protein adsorption is in general a well-known fact, there is a lack of more detailed studies. Thus a range of latex particles with graduated hydrophobicities were synthesized to investigate the effect on plasma protein adsorption.

Surface hydrophobicity was determined by the adsorption of Rose Bengal, a hydrophobic dye (Müller et al., 1997), and the adsorption of plasma proteins onto the surface was analyzedemploying two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). 2D-PAGE was prev-

* Corresponding author. Tel.: +49-30-77000478; fax.: +49-30-770004101.

E-mail address: mpharma@zedat.fu-berlin.de (R.H. Müller)

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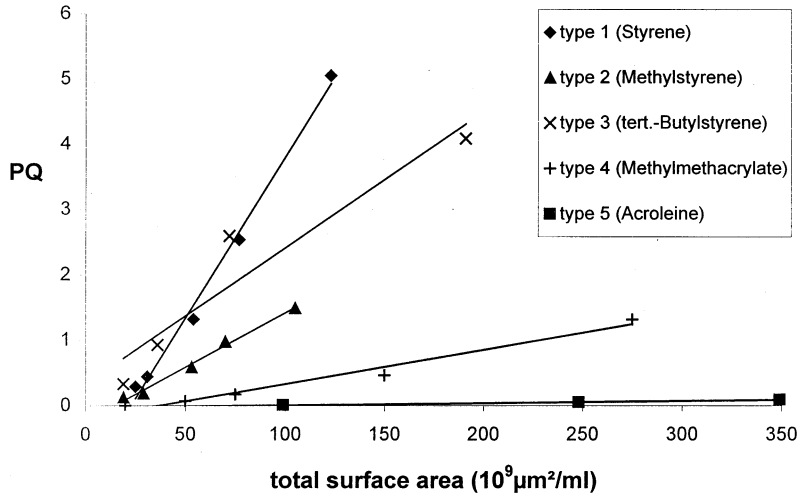

Fig. 1. Rose Bengal partitioning method. Plot of the partitioning quotient versus the total surface area of the particles. The slope of the straight lines is a measure of surface hydrophobicity.

iously established to determine plasma protein adsorption patterns on model nanoparticles for intravenous drug targeting (Blunk et al. 1993; Blunk, 1994).

Latex particles were synthesized at the Fraunhofer Institute for Applied Polymer Research, Teltow-Seehof. Emulsion polymerization technique as well as the characterization methods for particle sizing and determination of surface charge are described elsewhere (Paulke et al., 1992; Paulke and Möglich, 1995). Five latex types were investigated.

Surface hydrophobicity was evaluated by adsorption of the hydrophobic dye Rose Bengal as described previously (Müller, 1991; Müller et al., 1997). Briefly, a fixed amount of dye was added to nanoparticle suspensions of increasing concentration. Rose Bengal undergoes partitioning between the particles surface and the dispersion medium. The partitioning quotient (PQ) was calculated according to PQ = amount Rose Bengal bound on surface/amount Rose Bengal in dispersion medium (for each particle concentration). PQ was plotted as a function of the total surface area resulting in straight lines (Fig. 1). The slopes were taken as a measure of the degree of surface hydrophobicity; the steeper the slope, the more hydrophobic is the surface. Table 1 lists the characteristics of the particles being investigated in this study.

To analyze the plasma protein adsorption on the particles, suspensions of the particles contain-

Table 1
Particle characteristics[a]

Latex type	Polymer	Particle diameter (nm)	Surface charge (μC/cm²)	Degree of hydrophobicity (ml/m²)
1	Styrene	91	−3.2	49
2	Methylstyrene	89	−2.97	17
3	Tert-butylstyrene	87	−1.95	21
4	Methylmethacrylate	120	−2.07	5
5	Acroleine	340	−8.34	0.3

[a] The degree of hydophobicity is the slope of the lines from Fig. 1.

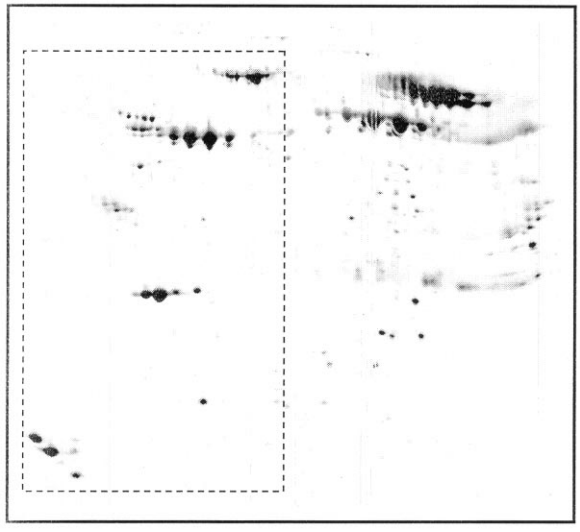

Fig. 2. Gel of plasma proteins adsorbed on latex type 1 (Polystyrene). The area of the close-ups (Figs. 3 and 4) is indicated.

ing constant surface areas were incubated in citrate-stabilized human plasma for 5 min at 37°C. The particles were separated from the plasma by centrifugation and washed extensively with bidistilled water (Blunk, 1994). The adsorbed proteins were desorbed by protein solubilizing solutions (Blunk, 1994; Lück et al., 1998), and the sample was applied to the 2D-PAGE and processed like described previously (Bjellqvist et al., 1993; Harnisch and Müller, 1998). After 2D-PAGE the gels were silver stained and scanned with a laser densitometer. The gel images were analyzed by employing an automated computer analysis system (Blunk, 1994; Harnisch and Müller, 1998).

Preferential adsorption of plasma proteins on nanoparticles was shown previously in several studies (Blunk, 1994; Harnisch and Müller, 1998; Lück et al., 1998), i.e. some proteins were enriched on the particle surface, whereas others were

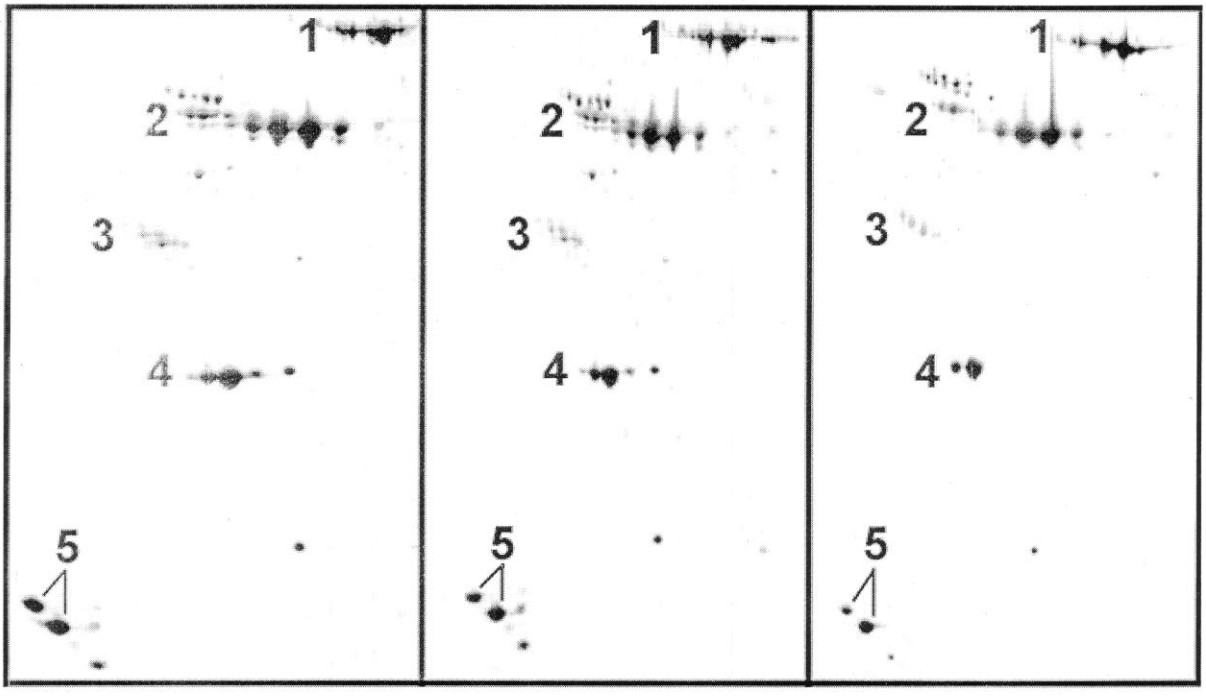

Fig. 3. Plasma protein adsorption pattern on latex particles: left, type 1; middle, type 2; right gel, type 3. Close-ups of the left part of the gels ranging from PI 4,5 (left) to 6 (right) and MW 70 kD (upper) to 6 kD (lower). (1) Albumin; (2) fibrinogen γ-chain; (3) ApoJ; (4) ApoA-I; (5) ApoC-III.

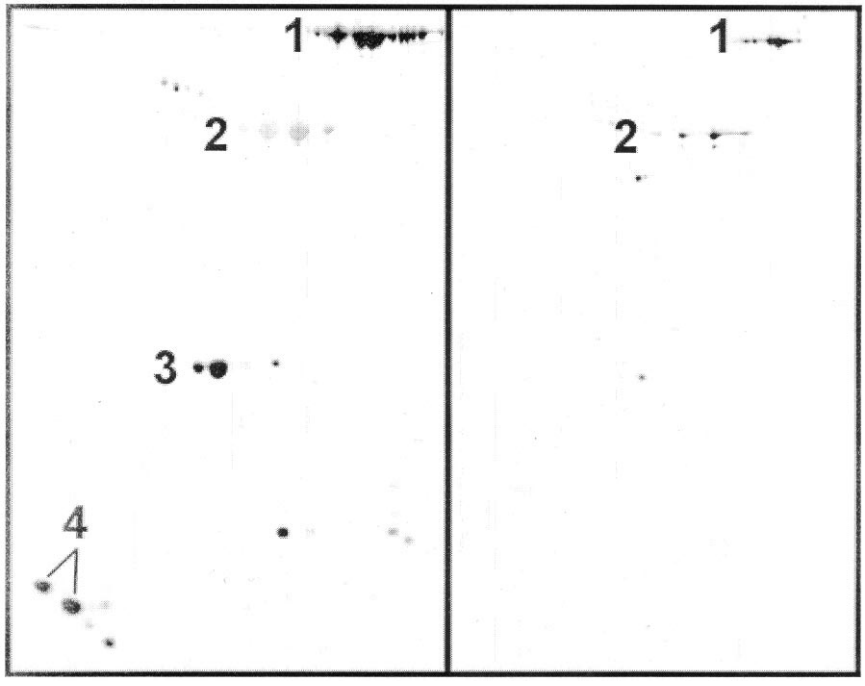

Fig. 4. Plasma protein adsorption pattern on latex particles: left, type 4; right gel, type 5. Close-ups of the left part of the gels ranging from PI 4 (left) to 5.5 (right) and MW 55 kD (upper) to 6 kD (lower). (1) Albumin; (2) fibrinogen γ-chain; (3) ApoA-I; (4) ApoC-III.

diminished compared to bulk plasma. The same applies to the particles presented here (Figs. 2–4).

The amount of adsorbed proteins on the different particle types is expressed in arbitrary units, selected proteins are listed in Table 2.

Acroleine particles (type 5) showed by far the lowest hydrophobicity (Table 1) and protein adsorption (Fig. 4). The value of the total counts detected on the gel of the acroleine particles was six times lower as compared to the particles synthesized of styrene (type 1). The major proteins adsorbed on the particles of each type were Fibrinogen (7.7 up to 35% of the overall pattern) and IgG (13 up to 35.7% of the overall pattern). The particle surface of type 1 was the most hydrophobic and showed the highest amount of adsorbed proteins. In contrast to that, a reduction of adsorption could be observed on the gel of particle type 2 (methylstyrene) possessing an about three times lower hydrophobicity. The total protein amount decreased by 30% as compared to the particles of type 1. Especially the adsorption of Fibrinogen and the Apolipoproteins was affected by diminished surface hydrophobicity.

Though hydrophobicity of methylstyrene (type 2) and tert-butylstyrene (type 3) particles is in the same order of magnitude, protein adsorption onto tert-butylstyrene particles is strongly decreased. The total protein amount was more than three

Table 2
Proteins adsorbed on the different particle types[a]

Latex type	1	2	3	4	5
Albumin	6.6	6.1	2.0	0.9	0.6
Fibrinogen	19.4	13.2	5.2	1.6	5.0
IgG	4.6	7.0	1.1	2.9	2.3
Ig light chain	10.7	6.1	1.0	3.0	1.7
Apo A-I	6.4	3.6	1.8	3.3	0.1
Apo A-IV	1.5	0.5	0.01	1.3	0.1
Apo C-III	3.8	3.0	1.7	2.4	0.1
Apo J	4.2	1.5	0.2	1.9	–
Total counts	86.0	57.2	16.6	20.7	14.0

[a] Total adsorbed protein mass expressed in arbitrary units. The values are the mean of three experiments.

times lower as compared to the particles of type 2. The adsorption of the large proteins Albumin, Fibrinogen and IgG decreased, but also the Apolipoproteins A-IV and J (MW 45 and 52 kD) were strongly diminished. This probably could be attributed to the fact, that protein adsorption is hindered sterically by the tert-butyl groups on the particles surface of type 3. Further decrease in the particles hydrophobicity (methylmethacrylate/type 4 and acroleine/type 5) is followed by diminished protein adsorption.

In conclusion, 2-D PAGE and a well defined range of latex particles could prove the influence of surface hydrophobicity on protein adsorption. Decreasing surface hydrophobicity leads quantitatively to decreasing amounts of adsorbed proteins and qualitatively to changes in the obtained protein adsorption patterns. However, the study also clearly demonstrated that not only surface hydrophobicity, but also the functional groups present affect the protein adsorption (shown by comparison of particle type 2 and 3). Future studies have to show in which way different functional groups present on the particles surface influence the change in the particles adsorption patterns.

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