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Acrylic acid copolymer nanoparticles for drug delivery: I. Characterization of the surface properties relevant for in vivo organ distribution

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Summary

Copolymer nanoparticles of acrylic acid, acrylamide, acrylic acid butyl ester and methacrylic acid methyl ester with increasing acrylic acid content were produced by emulsion polymerization. The particles were characterized in terms of parameters relevant for in vivo organ distribution: particle size and distribution, particle charge (ζ-potential) and surface hydrophobicity. The increase in the acrylic acid content could not be correlated with the resulting particle sizes and produced no detectable effect on the ζ-potential. However, the degree of hydrophobicity of the particle surface could be distinctly reduced. The particles were polydisperse with regard to the degree of surface hydrophobicity. Subpopulations differing in the degree of hydrophobicity were detected by hydrophobic interaction chromatography (HIC). However, the reduction in surface hydrophobicity was not regarded as sufficiently large to prevent uptake by the reticuloendothelial system after intravenous injection.

Introduction

The organ distribution of intravenously administered colloidal drug carriers is determined by their physicochemical properties (Müller, 1991). In general, the particles are recognized as being foreign by the body and are rapidly taken up by liver and spleen macrophages (up to 90% within 5 min). In order to target drugs to other tissues, the properties of the carriers must be modified to avoid the liver and spleen. Many factors affect the distribution of polymeric particles, such as particle size (Davis, 1981; Illum et al., 1982), particle charge (Wilkins and Myers, 1966), complement-activating surface groups (Wegmuller et al., 1986), surface hydrophobicity (Van Oss et al., 1975, 1984), adsorption of opsonins, lipoproteins (Blunk et al., 1991) and the conformation of molecules adsorbed on the surface, as in the example of Poloxamine 908 (Müller et al., 1988). The number of factors can be reduced from sev-

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eral to two key parameters, namely, particle charge and surface hydrophobicity, since the majority of the other factors are related to these two. Charged particles are cleared more rapidly than neutral particles or those with lower charge; charged functional groups lead to complement activation. The adsorption behaviour of the blood components depends on the surface hydrophobicity of the particles.

Model carriers comprising the copolymers acrylic acid, acrylamide, acrylic acid butyl ester and methacrylic acid methyl ester were prepared in order to investigate the effect of variations in copolymer composition on the physicochemical properties of the carriers. It was considered essential to evaluate the influence of modifications of the surface properties, carried out by varying the ratio of copolymer to monomer, in order to determine whether such changes are of sufficient magnitude to result in the reduction of uptake by the spleen and liver. The particle charge and reduction in surface hydrophobicity were investigated as the major determinants of the process of recognition by the immune system. Further investigations were also performed with the objective of ascertaining whether modifying the composition of copolymers and related surface characteristics can provide a valuable tool in attempting to optimize surface modification via the adsorption of ethoxylated surfactants (Lukowski et al., 1991).

Materials and Methods

Materials

Copolymer particles consisting of acrylic acid with acrylamide, acrylic acid butyl ester and methacrylic acid methyl ester were prepared by emulsion polymerization in water (Fig. 1). Six batches of nanoparticles of different composition (Dittgen et al., 1991) were produced by increasing the acrylic acid content from 0.019 to 0.193 g per g copolymer. Simultaneously with this procedure, the amount of methacrylic acid methyl ester was reduced accordingly (Table 1). Polymerization was performed using an analogous procedure to that for copolymer consisting of acrylic acid, acrylamide, acrylic acid butyl ester and styrene (Dittgen and Zosel, 1991). The initiator was ammonium persulfate and polymerization was carried out at 80°C using a monomer concentration of 19–150 parts acrylic acid, 30 parts acrylamide, 270 parts acrylic acid butyl ester and 550–681 parts methacrylic acid methyl ester (w/w). NaCl, $CaCl_2$, Rose Bengal and ethyl-agarose (cross-linked via amine groups) were purchased from Sigma (analytical grade).

Methods

Photon correlation spectroscopy (PCS) (Cummins and Pike, 1973, 1976; Müller and Müller, 1984) was used for determination of particle sizes. A Malvern RR 100 spectrometer equipped with a 20 mW He-Ne laser (Siemens, Germany) was employed in connection with an ALV 1000 channel correlator (ALV-Laservertriebsgesellschaft, Langen, Germany) and a KWS computer system (KWS, Ettlingen, Germany). The width of the particle size distribution was evaluated as the polydispersity index (PI) (Koppel, 1972). The value of PI is zero for ideally monodisperse samples and about 0.100 for a narrow distribution, values above 0.500 representing an extremely broad distribution. The PI determined for commercially available monodisperse standard latex particles is typically in the range of 0.050.

A laser doppler anemometry (LDA) (Cummins and Pike, 1976; Stampa et al., 1991) system was

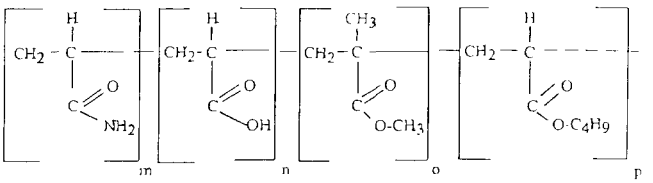

Fig. 1. Structures of the copolymer nanoparticles (*m*, *n*, *o* and *p*: number of units of acrylamide, acrylic acid, methacrylic acid methyl ester and acrylic acid butyl ester, respectively).

employed for determining the electrophoretic mobilities of particles (Zetasizer III, Malvern Instruments, U.K.). The electrophoretic mobility was converted into a ζ-potential using the Smoluchowski equation (James, 1979). The confidence intervals of the ζ-potential (ζ_{max}) are $t(P = 0.95) \cdot s_{max}$. Measurements of ζ-potential were performed in NaCl solution adjusted to a conductivity of 50 μS. pH profiles were determined in 0.1 M NaCl solution, the pH being adjusted by addition of HCl and NaOH.

Contact angles of cast polymer films were determined with a Krüss G1 goniometer (Krüss GmbH, Hamburg, Germany). The water content of nanoparticle suspensions was removed by evaporation, the dry nanoparticles dissolved in methylene chloride and a film cast on a microscope slide.

Surface hydrophobicity was evaluated by adsorption of the hydrophobic dye Rose Bengal as described previously (Müller et al., 1986). Briefly, a fixed amount of Rose Bengal (20 μg/ml) was added to suspensions of increasing nanoparticle concentration (0.1–10% w/w). Rose Bengal undergoes partitioning between the surface of the particles and the dispersion medium. At each nanoparticle concentration the partitioning quotient (PQ) was calculated according to:

$$PQ = \frac{\text{amount of Rose Bengal bound on surface}}{\text{amount of Rose Bengal in dispersion medium}}$$

From the results of the above calculations, profiles were constructed for PQ as a function of the total surface area of particles. The plots obtained were found to be linear and the corresponding slopes for all concentrations tested were taken as a measure of the degree of surface hydrophobicity.

Hydrophobic interaction chromatography (HIC) (Carstensen et al., 1991a,b) was used as an additional method of evaluating this parameter. The mini-HIC system employed has been described previously (Wallis and Müller, 1990). The system consisted of Pasteur pipettes filled with a 1 ml ethyl-agarose bed. Elution was performed using 2 ml of 0.02 M phosphate buffer pH 6.8 containing 0.3 M NaCl (= eluted peak: EP). The remaining particles were removed by washing with buffer solution containing 0.1% Triton X-100 (= wash peak: WP). Those particles that could not be eluted by treatment according to either procedure were designated as the irreversibly bound fraction. This fraction was calculated by subtracting the eluted and wash fractions from the amount of particles loaded on the column (100%). The flow rate employed was 0.02 ml/min. Particles were detected spectrophotometrically at 350 nm and registered on a chart recorder.

Results and Discussion

The size range of the nanoparticles was determined to be 102–174 nm (Table 1). No correlation could be established between nanoparticle size and the content of acrylic acid. Increasing concentrations of charged co-monomers led to a reduction in the size of copolymer particles in the

TABLE 1

Copolymer compositions, PCS diameters, PI values and ζ-potentials of the nanoparticles

Particle batch	Acrylic acid (%)	Methacrylic methyl ester (%)	PCS diameter (nm)	PI	ζ-potential (mV)
CAA 1.9%	1.9	68.6	144	0.021	−38.5
CAA 3.9%	3.9	66.6	149	0.018	−33.2
CAA 7.6%	7.6	62.7	160	0.023	−36.2
CAA 9.3%	9.3	60.7	174	0.033	−35.9
CAA 14.4%	14.4	55.3	102	0.028	−32.8
CAA 19.3%	19.3	50.4	110	0.035	−31.9

Concentrations of acrylamide and acrylic acid butyl ester were maintained constant (3.0 and 26.6% w/w, respectively). ζ-potential measurements made in NaCl solution (conductivity 50 μS, pH 5.5).

case of styrene and styrenesulphonate (Müller, 1991). The presence of charged co-monomer increased the force of electrostatic repulsion between polymerization nuclei and stabilizes the dispersion. This effect was not observed with increasing fractions of acrylic acid. The PI values were between 0.015 and 0.040, indicating an extremely narrow distribution. The particles can thus be regarded as monodisperse. Increasing content of acrylic acid failed to influence the ζ-potential (Table 1). The ζ-potential varies as a function of particle surface charge. Increasing the amount of acrylic acid during the process of polymerization was clearly demonstrated to lack an effect on the charged surface groups and consequently the ζ-potential remained unchanged. The lack of effect on particle charge is consistent with the particles showing no evidence of size reduction with increasing content of acrylic acid. The particle charge is also affected by the concentration of initiator used (Müller, 1991). It therefore appears possible that the charge on the acrylic acid copolymer particles is little influenced by the acrylic acid content.

The pH profiles confirmed the similarity of the particles with regard to the number of surface charges. The shape of the profiles and absolute ζ-potentials were similar. A correlation to the acrylic acid content could not be established (Fig. 2). Addition of an electrolyte at low concentrations (10^{-5}–10^{-3} M) can have a distinct effect on the ζ-potential (Lucks et al., 1990, 1991). Therefore, a concentration of 0.1 M NaCl as base electrolyte was chosen to minimize the effect of added HCl or NaOH when adjusting the pH. The shape of the pH profiles is therefore mainly determined by the dissociation of the functional groups on the particle surface. At low pH, dissociation of the acrylic acid groups is depressed. Dissociation increases in extent with increasing pH, attaining a plateau above pH approx. 6. The

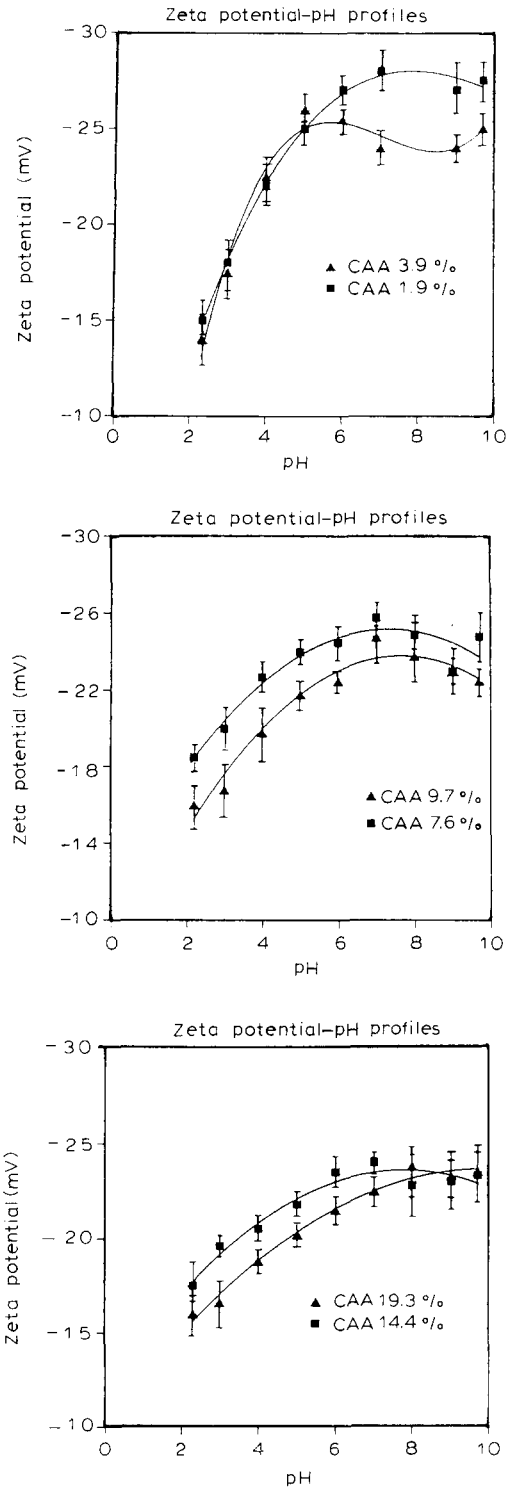

Fig. 2. ζ-potential-pH profiles of nanoparticles with increasing acrylic acid content (acrylic acid content: 1.9, 3.9, 7.6, 9.7, 14.4 and 19.3%); confidence intervals for $P = 0.95$ are indicated by vertical bars.

TABLE 2

Decrease in contact angles of nanoparticles with increasing acrylic acid content

Acrylic acid content of particles (%)	Contact angle (°)
1.9	83
3.9	75
7.6	68
9.3	62
14.4	57
19.3	48

ζ-potential increased only by 10 mV when shifting the pH from 2 to 9. This indicates that the dissociation of acrylic acid groups has a limited effect on the absolute surface charge. In the case of strongly dissociating groups, distinct increases can be found in the ζ-potential vs. pH profiles, e.g., in the styrene copolymers (Dittgen et al., 1988).

The contact angles decreased with increasing acrylic acid content (Table 2). This reduction in surface hydrophobicity is attributed to the increase in number of hydrated COOH groups on the surface. The presence of such hydrated groups has been reported to lower the surface hydrophobicity of polystyrene particles (Table 3, lower; after Müller, 1991). The CAA 1.9% particles were relatively hydrophobic as shown by the con-

TABLE 3

Slopes of the plots shown in Fig. 3 for the six batches of CAA and those determined on surface-modified polystyrene particles

Particle batch	Surface area per unit mass CAA (nm^2/ng)	Slope from partitioning experiment ($ml\ m^{-2}$) (Fig. 3)
CAA 1.9%	41.7	6.87
CAA 3.9%	40.4	4.63
CAA 7.6%	37.5	4.85
CAA 9.3%	34.5	2.94
CAA 14.4%	58.9	2.88
CAA 19.3%	54.5	2.36
PS-(140 nm)	33.6	63.9
PS-(900 nm)	63	60.2
PS-AR-NH_2 (190 nm)	32.1	52.9
PS-OH-(250 nm)	22.9	1.42
PS-COOH (190 nm)	30.1	0.51

PS, polystyrene particles; PS-COOH, PS-AR-NH_2 and PS-OH, PS particles surface-modified by introduction of COOH, aromatic amino and hydroxyl groups, respectively (lower part after Müller, 1991).

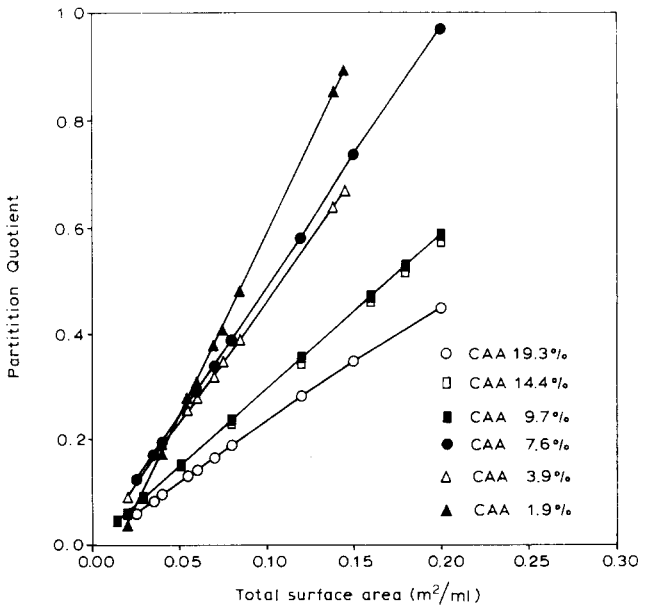

Fig. 3. Plot of Rose Bengal partitioning coefficient PQ vs increasing surface area (corresponding to increasing particle concentration) for the six batches of CAA nanoparticles.

tact angle being close to the values described for polystyrene (90°). They were distinctly greater than the angles determined with the polymethylmethacrylic nanoparticles (72.7°) (Kreuter, 1983). Taking the hydrophobicity of CAA 1.9% into account, it can be predicted that the particles will be heavily opsonized after i.v. injection and rapidly cleared by the liver and spleen. Increasing the acrylic acid content reduced the hydrophobicity markedly, resulting in a contact angle of 48°. However, this reduction is not sufficient to reduce opsonization in the blood. Above a certain degree of hydrophobicity, no difference is found in the extent of adsorption among several hydrophobic materials (Brynda et al., 1984). For example, the adsorption of human fibrinogen on carbon (contact angle 60°) is identical to that on polyethylene (contact angle 95°) (0.77 and 0.73 $\mu g/cm^2$, respectively). Therefore, the reduction in contact angle of the CAA 19.3% nanoparticles does not prevent opsonization or uptake by liver/spleen macrophages in vivo.

The Rose Bengal partitioning experiments yielded straight lines on plotting PQ vs the surface area per ml of nanoparticle suspension (Fig. 3). The slope decreased with increasing acrylic acid content from CAA 1.9% to CAA 19.3% nanoparticles (Fig. 3 and Table 3). This confirmed the decrease in surface hydrophobicity observed during the contact angle measurements. The slope evaluated for the CAA 1.9% nanoparticles is distinctly below that measured for un-

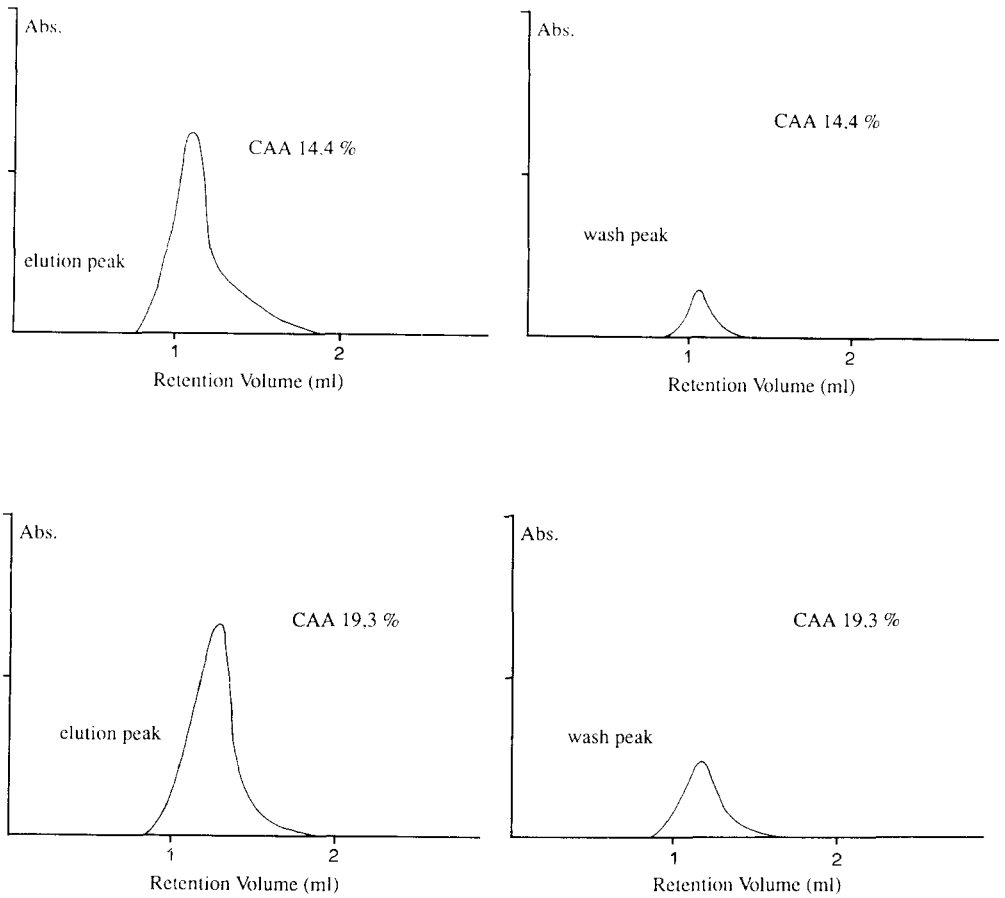

Fig. 4. Chromatograms of CAA 14.4% (upper) and CAA 19.3% (lower) obtained on ethyl-agarose (left: elution peaks with buffer; right: wash peaks with buffered Triton X-100 solution).

TABLE 4

HIC data: percentages eluted with buffer, washed off with Triton X-100 and irreversibly bound

Particle batch	Eluted by buffer (%)	Washed off with Triton X-100 (%)	Irreversibly bound (%)
CAA 1.9%	–	39	61
CAA 3.9%	–	40	60
CAA 7.6%	19	30	51
CAA 9.3%	13	31	56
CAA 14.4%	44	15	41
CAA 19.3%	55	29	16

modified polystyrene latex particles (Table 3, lower). Attachment of hydrophilic groups to the polystyrene nanoparticles (OH) reduced the surface hydrophobicity as indicated by a slope of 1.42. The hydrophobicity of the CAA nanoparticles could be lowered to a similar extent by using 19.3% acrylic acid. However, both the hydrophilized CAA and polystyrene particles are still too hydrophobic to prevent clearance by the RES.

The HIC results provide further evidence in support of the decrease in surface hydrophobicity. The percentage eluted with buffer increased with increasing acrylic acid content. Consequently, the percentage washed off with Triton-X-100 and the fraction irreversibly bound both decreased (Table 4). The CAA 1.9% particles proved to be the most hydrophobic, since elution was impossible with buffer. The CAA 19.3% are the least hydrophobic as indicated by the high percentage eluted and the minor proportion irreversibly bound. Typical chromatograms are shown in Fig. 4. Methods such as the measurement of contact angles and Rose Bengal partitioning entail a few major disadvantages. Particles must be removed from their dispersion medium or be dissolved for casting a film in order to measure contact angles. This could result in modification of the surface properties. Neither method can distinguish subpopulations differing in surface hydrophobicity, since only an averaged value is yielded for the surface hydrophobicity. Chromatographic techniques allow the polydispersity in surface properties to be resolved. This could be demonstrated for polystyrene particles which had been surface-modified during a second stage of the preparation procedure by the introduction of COOH groups. The particles adopt a bimodal distribution, one subpopulation consisting of unmodified particles and the other comprising successfully carboxylated particles (Müller, 1991). The CAA nanoparticles also exhibited polydispersity in their surface properties as indicated by tailing of the elution peak (Fig. 4, upper). The tail of the peak corresponds to the more hydrophobic particles. Even more strongly hydrophobic particles are retained on the column and must be washed off with Triton X-100. The most hydrophobic particles are irreversibly bound. One can therefore distinguish between at least four subpopulations of increasing degree of surface hydrophobicity: e.g., for CAA 14.4%, (fraction 1) particles which pass the column without undergoing interaction and appear after the void volume (fraction at 0.7–1.2 ml); (fraction 2) retarded fraction which is eluted with buffer at 1.2–2.0 ml; (fraction 3) particles which can only be eluted with surfactant solution; and (fraction 4) particles irreversibly bound by strong hydrophobic interaction with the column.

The polydispersity of nanoparticles has important implications for their in vivo distribution. The measured organ distribution is a superposition of the distribution of subpopulations. A reduction in uptake by the liver observed with surface-modified particles (e.g., from 90 to 60%) can be explained in terms of surface polydispersity. The most strongly hydrophilic of the particle subpopulations (30%) circulates in the blood whereas those that are more hydrophobic are captured by macrophages in the liver. Similar polydispersity effects have been described for particles coated with Antarox CO 990 (Müller and Heinemann, 1989).

Conclusions

Increasing the amount of acrylic acid in the copolymer with acrylamide, acrylic acid butyl es-

ter and methacrylic acid methyl ester did not affect the particle size in the polymerization process. Measurements of ζ-potentials could not detect an effect exerted by the increase in acrylic acid content on the number of charges on the particle surface. This is favourable because an increase in particle charge would promote uptake by the liver and spleen. However, acrylic acid reduced the surface hydrophobicity as could be shown by the evaluation of contact angles, Rose Bengal partitioning and HIC. The reduction in surface hydrophobicity is attributed to the presence of hydrated COOH groups. HIC demonstrated that the particles are polydisperse with regard to surface properties (hydrophobicity). This implies that the organ distributions observed with such particles result from the superposition of the distributions of each of the subpopulations. The extent of the reduction in surface hydrophobicity achieved by chemical means (variation of acrylic acid content) is not regarded as sufficient to bring about a distinct reduction in uptake by the RES.

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