Adsorption of poly(sodium(4)styrenesulfonate) on colloidal iron oxide particles

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Abstract

The adsorption of poly(sodium(4)styrenesulfonate) (PSSNa) on colloidal iron oxide has been studied with several techniques. Measurements of the adsorption isotherm as well as the electrophoretic mobility and particle size as a function of polymer concentration has been performed. Also, the electrophoretic mobility of both bare and PSSNa coated iron oxide has been studied as a function of pH. Results from the applied techniques individually demonstrate the adsorption of the polyelectrolyte to the iron oxide particle surface. In the absence of or at low polymer concentrations (and pH 4) the suspension is stabilized by a high net positive charge from the exposed iron oxide surface. Intermediate concentrations yield unstable and precipitating suspensions, probably because of a low net surface charge due to the partial coating and neutralization by adsorption of small amounts of the polyanionic polymer. At high polymer concentration the resulting suspension is again stabilized, probably due to the build up of a multi-molecular layer of polymer and a high net negative surface charge.

Keywords: Drug targeting; Iron oxide particles; Adsorption of polymer; Analytical characterisation

1. Introduction

The pharmaceutical industry has, during the recent years, been highly interested in the concept of drug targeting (Junginger, 1992). Such focused delivery of active drugs or contrast agents to selected sites or organs in the body may be achieved with a drug formulation in which the active ingredient is linked to a particulate carrier suspended in a non-active liquid (Muller, 1991). The uptake, efficacy and toxicity of such substances are known to be partly dependent upon the surface chemistry and size of the particles, and various coating materials and stabilizing agents are often important ingredients in these formulations.

An example of a targeted drug formulation is colloidal iron oxide particles which, when injected into the blood stream, are taken up by the macrophage cells of the liver and spleen. In these organs the iron oxide concentrates in healthy tissue while it is excluded from tumours, such as hepatic metastases (Bradfield, 1984), because of their decreased phagocytic activity. This in turn produces high contrast between benign and ma-

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lign tissue in Magnetic Resonance (MR) images and thus enables early detection and treatment of malignant diseases (Widder et al., 1987; Stark et al., 1988; Leander and Golman, 1989).

Characterization and analysis of such multi-phase formulations is of importance both for general understanding and for documentation and control. This is, however, a relatively new area for the pharmaceutical industry and remains a challenge for the pharmaceutical analyst. This report describes a multi-technique characterisation of the interactions between poly(sodium(4)styrene-sulfonate), PSSNa, and iron oxide particles in aqueous suspensions. The PSSNa was chosen as a model substance for polyelectrolyte stabilisation of colloidal iron oxides, and the main motive with theses studies was to investigate the quality and the consistency of the information obtained with the various techniques, applied on a pharmaceutically relevant experimental system. The adsorption of PSSNa on bare iron oxide particles has been studied by measurements of particle size and surface charge as a function of adsorbate concentration, as well as by measurements of the adsorption isotherm. Also, the effect of pH, on the surface charge of both bare as well as PSSNa coated iron oxide particles, has been studied.

2. Materials and methods

2.1. Materials

Superparamagnetic iron oxide crystals were precipitated from an aqueous solution (0.5 l) of ferric chloride-6-hydrate (34 g, Janssen Chimica, Belgium) and ferrous chloride-4-hydrate (12.5 g, Janssen) by rapid addition of ammonium hydroxide (30% solution, Janssen) while stirring vigorously at ambient temperature. The reaction mixture was kept under nitrogen except during decanting and mixing and the pH in the mixture was between 10 and 11. The reaction mixture was allowed to rest for 5 min before the particles were collected magnetically and the water decanted. The particles were rinsed with water until the pH in the wash water had stabilized at 7. To the remaining iron oxide slurry was then added water

and hydrochloric acid (1 N, Merck) to a final pH of approximately 4, and dispersed by use of an Ultrasonic W-385 sonicator for 15 min at approximately 10% output. The total iron concentration in the final suspension was 1.07 mg Fe/ml and this suspension was used throughout the study.

The poly(sodium(4)styrenesulfonate), repeating unit $[-CH_2CH(C_6H_4SO_3Na)-]_n$ was obtained from Aldrich-Chemie GmbH and Co. KG (Germany) and had a weight average molecular weight (M_w) of 70 000. An aqueous solution (7.61% w/v) of the polymer was used throughout the study.

Any water used in the studies described below was drawn from a Milli-Q Plus (Millipore S.A., France) water purification system fed with water pre-rinsed on a Milli-RO 6 Plus (Millipore S.A., France) system.

2.2. Methods

2.2.1. Sample preparation

Samples with PSSNa at concentrations; 0.01 to 2.88 mg/ml were prepared by addition of appropriate volumes of the 7.61% (w/v) PSSNa solution to samples of the iron oxide (1.07 mg Fe/ml) suspension. Reference samples of each PSSNa concentration were prepared with water (i.e. with no iron oxide present) and served as blanks for the adsorption isotherm measurements. Immediately after addition of polymer solution the sample was whirl mixed and then allowed to equilibrate for no less than 12 h.

Samples with varying pH were prepared from the iron oxide system as well as from the PSSNa (0.05% w/v)/iron oxide system by addition of appropriate volumes of 1 N HCl (Merck) or 0.1 N NaOH (Merck) to suspension samples diluted 1:25 in water prior to pH adjustment. Before measurement of the electrophoretic mobility, the pH of each sample was measured with a Metrohm 691 pH meter (Metrohm Ltd, Switzerland).

2.2.2. Iron/sulfur content measurements

The iron content in the original suspension and the iron/sulfur content in samples from the measurements of the adsorption isotherm was determined by Inductive Coupled Plasma-Atomic

Emission Spectroscopy (ICP-AES) with a Perkin Elmer 5500B (Perkin Elmer Corp., USA). Samples were digested in nitric acid and diluted in water before measurement. The emission at wavelengths 259.940 nm (iron) and 180.731 nm (sulfur) was quantified and iron/sulfur content calculated by comparison with external standard curves.

2.2.3. Particle size measurements

The particle size of stable samples were no aggregation and sedimentation of particles were observed, was measured by photon correlation spectroscopy (PCS) on a Malvern PS/MW 4700 system (Malvern Instr. Ltd, UK) with a Multi 8 7032 correlator with 128 channels and a 75 mW Argon ion laser (Model 2113 75SLYV, Cyonics Corp., USA). Analytical conditions and settings were as follows; wavelength of incident light 488 nm, light output power 70 mW, scattering angle 90°, temperature 25°C, photomultiplier aperture 200 μm, medium viscosity 0.89 cP, medium refractive index 1.33, particle real refractive index 2.0, and particle imaginary refractive index 0.0. Sampling time and prescale values were automatically set by the software prior to each measurement. The experimental duration was set to 60 s and samples were diluted 1:80 in water immediately prior to analysis in triplicate. As response the average diameter (nm) obtained from a fit of the measured correlation function to a two parameter cumulant expression, was calculated.

The particle size of unstable samples were aggregation and sedimentation of particles was observed, was measured by the Electrical Sensing Zone (ESZ) technique on a Coulter Multisizer Mrk II (Coulter Electronics Ltd., UK) fitted with a 50 μm aperture with a nominal measuring range of 1–30 μm. Isoton II (Coulter Electronics Ltd., UK), filtered through 0.22 μm filter (Millipak 40, Millipore S.A., France) prior to use, was used as electrolyte. Samples were whirl mixed for 30 s immediately prior to sampling and each sample was measured in triplicate. As response the volume mean diameter, D(4.3), (μm) was calculated.

2.2.4. Electrophoretic mobility measurements

The electrophoretic mobility of stable samples were no aggregation and sedimentation of parti-

cles were observed was measured by Laser Doppler Velocimetry (LDV) with a Coulter Delsa 440 (Coulter Electronics Ltd., UK). Samples of various PSSNa concentrations were diluted to 1:25 in water prior to analysis. The conductivity of the diluted samples ranged from 0.06 μS/cm for the sample with no PSSNa, to 0.08 μS/cm for the sample with 2.88 mg PSSNa/ml. The conductivity of samples with variable pH ranged from 0.06 μS/cm for pH = 4.2–4.3 (i.e. unadjusted), to 0.21 μS/cm for the pH = 11.4 sample and 1.07 μS/cm for the pH = 2.0 sample.

2.2.5. Adsorption isotherm measurements

The adsorption isotherm of PSSNa on the iron oxide surface was obtained by measurements of the equilibrium polymer concentration in the aqueous matrix after removal of particles. The removal of particles was achieved either by ultracentrifugation or by filtration of the samples. Filtrates were prepared by using 0.025 μm single use syringe filters (ANOTOP™, Anotec Separation Ltd., UK). Supernatants were prepared by ultra-centrifugation with a Beckman TL-100 ultracentrifuge. Sample aliquots of 3.5 ml in polycarbonate tubes were centrifuged at 4°C and 100 000 rev./min for 30 min before 2.5 ml of supernatant was carefully withdrawn. The polymer concentration was determined by measurements of the sulphur content in the matrix samples by ICP-AES. As each ionized monomer unit contains 17.5% (w/w) sulphur, calculated from the repeating unit, the polymer concentration (P) was calculated as 5.72 times the measured sulphur content. To control the actual amount of added adsorbate, corrected for possible adsorption of polymer to system surfaces (tube walls, filters etc.), reference samples containing PSSNa, but no iron oxide was measured. At the lowest PSSNa concentrations, typically 5% of the added polymer was lost to system surfaces. The measured matrix concentrations in the reference samples were used as actually added adsorbate, and the adsorbed concentration was calculated in as $P_{reference} - P_{sample}$.

As control of the completeness of the separation, the Fe content of all samples was measured by ICP-AES.

3. Results and discussion

The Fe content remaining in the water matrix after separation was found to be in the range 0.025–0.035 mg/ml. This amounts to some 3% of the initial content and demonstrates the near completeness of the separation techniques. It is likely that the remaining amount is ionic iron, not completely removed during the washing step in the preparation procedure. The amount of residual iron was independent of polymer concentration, thus no interaction between this and the polymer was indicated.

Fig. 1 shows the adsorption isotherm of PSSNa on the iron oxide particles and, as seen from this figure, adsorption is detected independent of separation technique. The amount of adsorbed polymer increases with increasing adsorbate concentration up to some 1 mg/ml, reaching a maximum adsorbed amount of ca. 0.25 mg/ml.

The uncoated iron oxide particle in water was found to have a hydrodynamic average diameter of 130 ± 5 nm. Using a particle density of 5.2 g/ml (Weast, 1983) and assuming a spherical particle, one may estimate the surface area of the particles in the suspension as

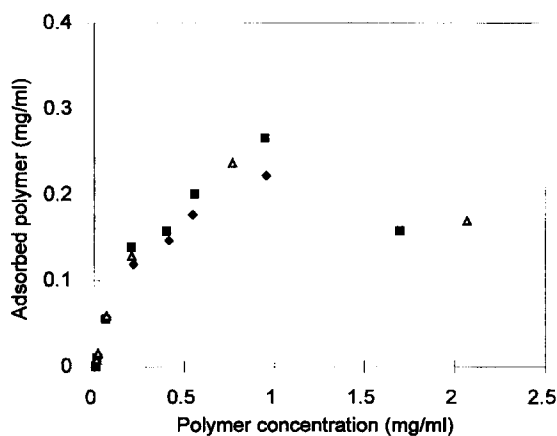Surface area $(m^2/ml) = m_p \cdot A_p/\rho_p \cdot V_p$

Fig. 1. The adsorption of PSSNa on colloidal iron oxide. Adsorbed amount (mg/ml) versus concentration of PSSNa (mg/ml). ■ and ◆ is fractionation by centrifugation, ▲ is fractionation by filtration.

where m is the mass of particles per ml, ρ is the density of the particles and A and V is the area and volume of a single particle respectively. Thus estimated, the particle surface area in the suspension was found to some $9.5 \cdot 10^{-3}$ m^2/ml and the maximum adsorbed amount consequently represents as much as 26 mg/m^2.

From values for the Mark-Houwink constants the intrinsic viscosity of PSSNa in water may be calculated, and from this parameter the radius of gyration and the corresponding cross-sectional area of a single polymer coil may be estimated (Branderup and Immergut, 1989). The size of the coil is strongly dependent on the ionic strength of the medium which, due to the surface charge and adsorbed counter ions, is rather difficult to estimate close to the particle surface. Assuming a relatively high ionic strength close to the surface, and consequently assuming Θ-solvent conditions, calculations yield a radius of gyration of approximately 5 nm for the PSSNa molecules. If, then, each adsorbed molecule occupies a surface area equivalent to the cross-sectional area of the coil, one may estimate the mass/area for a monolayer of molecules to approximately 1.5 mg/m^2. As the polymer is a polyelectrolyte it may, however, not adsorb as a coil sitting on the surface, but be flattened by multiple attachments. In this case a different approach may be used to estimate the mass/area ratio for a monolayer. Simple molecule modelation estimates the spatial extension of a monomer unit to approximately 0.5 by 0.7 by 1.2 nm. Assuming that the polymer adsorbs so that the monomers are closely packed on the surface occupying an area equal to the monomer extension in two dimensions, one may estimate the mass/area ratio of a monolayer to approximately 0.4 to 1.0 mg/m^2, dependent on which plane of the monomer that adsorbs. Although both these estimates are partly based on uncertain assumptions, they do indicate that the measured 26 mg/m^2 represents the build up of multiple layers of adsorbed polymer or trains/loops of polymer extending from the surface.

Fig. 2 and Fig. 3 shows the electrophoretic mobility (E) of the particles and their hydrodynamic diameter as a function of polymer concentration, respectively. The electrophoretic mobility
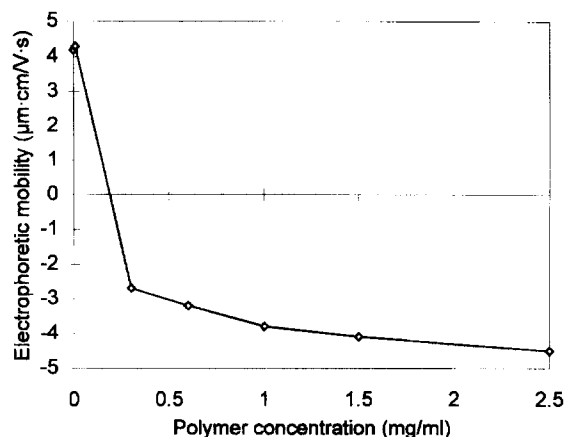
Fig. 2. The electrophoretic mobility (μm · cm/V · s) of colloidal iron oxide versus concentration of PSSNa (mg/ml).

Fig. 4. The electrophoretic mobility (μm · cm/V · s) of pure colloidal iron oxide (■) and PSSNa coated colloidal iron oxide (○) versus pH.

was found to be 4.2 ± 0.6 μm · cm/V · s for the uncoated iron oxide particle at pH 4, and decreased rapidly with increasing polymer concentration. At the highest polymer concentration pH lowered only some 0.5 units from its initial value, thus changes in this parameter does not account for the observed changes in the mobility. The isoelectric point is located between 0.01 and 0.3 mg/ml. Concentrations intermediate to these (0.03 and 0.1 mg/ml) yields destabilised samples with observable sedimentation within a few hours. At these intermediate concentrations, the net charge of the particle is probably close to zero as the
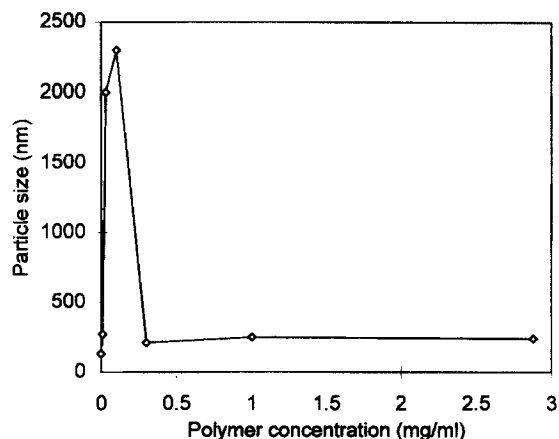

Fig. 3. The particle size (nm) of colloidal iron oxide versus concentration of PSSNa (mg/ml).
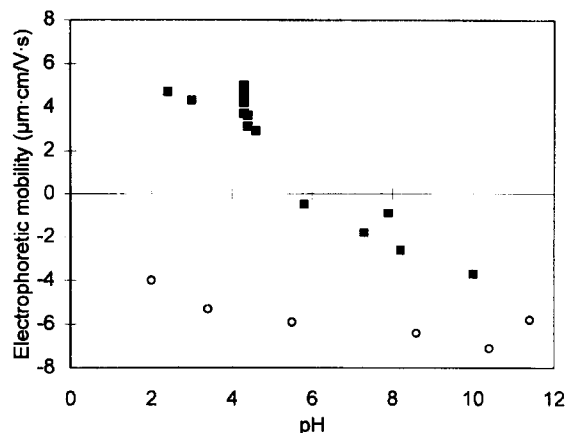
original positive surface is neutralized by the adsorption of negative polymer. This again leads to a lessened electrostatic stabilisation and rapid agregation of the particles in the intermediate concentration regime. This phenomenon is also clearly shown in Fig. 3, with the sharp increase in particle size, due to aggregation, at intermediate polymer contents. At high polymer concentration the electrophoretic mobility levels out around − 4 μm · cm/V · s and the particle size seems to stabilize at 230 ± 20 nm. The re-stabilisation of the system at high polymer concentrations is probably caused by the now highly negative surface with a sufficient electrostatic stabilisation, arising from the multiple layer of adsorbed polymer. Is also probable that steric stabilisation contribute to the stability of the system at these polymer concentrations, although the observed electrophoretic mobility should represent a surface potential which is sufficient to stabilize the system on its own. The increase in size, from the original uncoated particle, indicate the build up of an approximately 50 nm layer of polymer. With an estimated radius of gyration of approximately 5 nm, the observed thickness seems quite consistent with a multilayer of adsorbed polymer, as indicated from the measured adsorption isotherm.

The results from the measurements of the electrophoretic mobility of uncoated as well as PSSNa coated iron oxide as a function of the

Table 1
Effect of various PSSNa concentration on colloidal iron oxide suspension

Polymer concentration	Observations	Physicochemical explanation
$\leqslant 0.01$ mg/ml	High positive mobility, stable suspension, 0–0.5 mg polymer/m^2	Bare or only slightly covered surface, stabilized by electrostatic repulsion between positively charged iron oxide surfaces
0.03–0.10 mg/ml	Mobility close to zero, unstable suspension, 1–5 mg polymer/m^2	Surface partially covered or covered with thin layer of polymer leading to low net surface charge and insufficient electrostatic stabilisation
> 0.3 mg/ml	High negative mobility, stable suspension, 10–20 mg polymer/m^2	Surface covered with thick layer of negatively charged polymer leading to sufficient steric/electrostatic stabilisation

suspension pH is shown in Fig. 4. The bare iron oxide surface display a typically amphoteric nature going from the high positive electrophoretic mobilities at low pH to increasingly negative values with higher pH. The isoelectric point in this system is located around between pH 6 and 7. When coated with PSSNa the characteristics of the particle surface is completely changed to display a persistently acidic nature. In this system the surface is negative even at the lowest pH, changing slightly to a somewhat more negative at higher pH. Whereas the variation in conductivity (i.e. ionic strength) between the samples of different pH may be responsible of some of the variation within in each series, the functional difference observed between the two different surfaces should not influenced by this variation.

The results in Figs. 1–4 are thus internally consistent and the observed behaviour may be summarized and explained schematically as indicated in Table 1.

4. Conclusion

Results from all the employed techniques individually demonstrate the adsorption of the polyelectrolyte, PSSNa, to the iron oxide surface. In the absence of polymer or at low polymer concentrations the suspension is stabilized by a net positive charges from the exposed iron oxide surface. Intermediate concentrations yield unstable and precipitating suspensions, probably because of a low net surface charge due to the

partial coating and neutralization by adsorption of small amounts of the anionic polymer. At high polymer concentration the resulting suspension is stabilized, probably due to the build up of a multimolecular layer of polymer and the resulting stabilization by a now high net negative surface charge.

The combined results of the applied analytical techniques yield information which contributes to the fundamental understanding of the investigated system. The internal consistency between results also demonstrates the qualitative accuracy and usefulness of these methods as tools for analysis and characterisation of pharmaceutical formulations of colloidal/disperse nature.

Acknowledgements

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