CHARLES UNIVERSITY IN PRAGUE

Faculty of Science

Department of Physical and Macromolecular
Chemistry

INSTITUTE OF
MACROMOLECULAR CHEMISTRY

AS CR, v. v. i.

Department of Supramolecular Polymer Systems

Polymeric nanoparticles stabilised by surfactants

Doctoral Thesis Abstract

Jiří Pánek

Supervisor: RNDr. Petr Štěpánek, DrSc.

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INTRODUCTION

In the light of recent development, nanotechnology is the driving force of current research of new materials both in materials science and in biomedicinal applications. Nanomaterials can be constructed either by a top-down approach when the desired structure is produced in an already existing material or by a bottom-up approach when the required configuration is made by self-assembly of building blocks using various physicochemical interactions. Polymers and copolymers represent an ideal system for engineering of self-assembled nanomaterials since they offer a wide range of combinations of building materials that may serve for this purpose. The aims of my thesis follow the development and are oriented towards construction of specific polymeric nanoobjects using principles of self-assembly.

The common approach applied to all types of systems described below is based on controlling the extent of *macrophase* separation that occurs in a mixture of two compounds (solvents or polymers) that became immiscible or incompatible as a result of a change of an external variable. In the classical case the energy of the system is given by enthalpic and entropic contributions and the interaction parameter χ is given by the Flory-Huggins relation $\chi = a/T + b$ where T is absolute temperature and a and b are specific for the polymer/polymer or polymer/solvent pair. For more complex systems, a third term χ_s has to be included:

$$\chi = a/T + b + \chi_s \qquad (1)$$

In this simple representation the third term includes all additional interactions in the system, in particular the effect of different temperature expansions of the system components and that of various specific interactions in the system (hydrogen bonds, ionic interactions, etc). The formation of nanoobjects is always driven by thermodynamics (increase of χ–parameter in Eq. (1)) but controlled by specific factors of interaction with the amphiphilic molecules including kinetic and hydrodynamic effects. In this dissertation, changes in temperature and in solvent quality are used to prepare polymeric nanoparticles.

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AIMS

The simplicity of preparation methods of various polymeric nanoobjects is often preferred, since it may bring good reproducibility, effectiveness and potential of scaling up from laboratory to larger production in practise. In the presented work, physicochemical methods of preparation of polymeric nanoparticles are tested. Basically, two types of objects are involved: polymeric nanoparticles stabilised by surfactants and thermoresponsive polymeric micelles. Concerning the latter, the presence of a surfactant is replaced by the amphiphilic character of the used block copolymers. Under specific conditions, both types of objects can be prepared using the same physicochemical principle and, therefore, feature some similarities. The aims of this work are:

1. To study model surfactant-stabilised nanoparticles made of hydrophobic polymers (poly(methylmethacrylate), PMMA, or polystyrene, PS, prepared by the method of change in solvent quality. To find out the influence of various preparation conditions on nanoparticle characteristics.

2. To prepare surfactant-stabilised nanoparticles consisting of a thermosensitive polymer (poly(N-isopropylacrylamide), PNIPAM. To learn about the dependences of nanoparticle properties both on polymer and surfactant characteristics.

3. To study temperature-dependent micellar behaviour of tailor-made triblock POXZ copolymers. To find out the optimal copolymer composition considering the phase separation temperature and the size of the micelles.

4. To prepare radiolabelled polymeric micelles made of thermoresponsive copolymers described in item 3. To design their properties with respect to their possible applications.

METHODS

Scattering techniques rank among the most important instruments in polymer analysis. Exploiting the principles of interaction of radiation with matter, such methods represent a precise tool for determining both structural and dynamic characteristics of systems containing macromolecules or their assemblies. Common techniques use laser, X-ray or neutron beams since sufficiently powerful sources are available. The samples studied in this work are diluted aqueous dispersions of polymeric nanoparticles therefore dynamic light scattering (DLS), static light scattering (SLS), small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) have been used. Time resolved measurement has been used in combination with SAXS performed at high-brilliance X-ray beam generated by synchrotron.

Each of the methods has specific applicability. In comparison with DLS, which is sensitive to dynamic processes such as mobility and transport, SLS and small-angle scattering techniques are sensitive to the mass and structure of molecules or particles contained within the sample. In case of SAXS and SANS, the radiation can enter the particles and not only their size and shape, but also their structure may me investigated. Synchrotron X-ray radiation offers the opportunity to use high-brilliance beams for collection of scattering patterns over short acquisition times. That way, time-resolved measurement in millisecond range may be performed. On the other hand, in SANS, the scattering amplitude can differ considerably between some chemical elements with similar atomic masses and even between isotopes of the same element. The most important pair of such isotopes is hydrogen (scattering amplitude b_i = -3.74 fm) and deuterium (b_i = +6.67 fm). By substituting one for another it is possible to make some compounds "visible" or "invisible" for neutrons, which substantially enhances the use of SANS.

RESULTS

Some of the obtained results are shown in this section. An overview of all results is given in the section Conclusion.

1. Change in solvent quality and nanoparticles stabilized by surfactants

Polymeric nanoparticles stabilized by surfactants can be prepared by acting on the parameters a and b in Eq. (1). These are system specific so that, *e.g.,* for a polymer/solvent system phase separation is induced by a change of the solvent. Self-organization of polymers with surfactants initiated by a change in the solvent quality has been successfully tested resulting in formation of stable and uniform nanoparticles. Aqueous solutions of nanoparticles made of poly(methylmethacrylate) and of polystyrene have been studied. The dispersions were prepared by a fast mixing of a solution of the polymers in an organic solvent with a solution of several ionic and non-ionic surfactants in pure water. The formation of the nanoparticles was observed DLS, SLS and SANS. The study shows how nanoparticle properties are changed by the chemical composition of surfactants, molar mass of polymers, concentrations of both components and finally, by variations in method of nanoparticles preparation [1].

Dynamic light scattering (DLS)

A systematic variation of composition was investigated. At the fixed concentration of a surfactant c_{surf} the hydrodynamic radius R_h of nanoparticles increases as the polymer concentration c_p increases. A different behaviour is observed when the surfactant concentration is increasing: R_h value decreases. Both tendencies can be arranged in a single dependence, as shown in Figure 1, by plotting R_h/f as a function of composition ratio f, where $f=c_p/c_{surf}$. All R_h/f vs. f dependences in **Figure 1** look alike. The observation shows that nanoparticles formation is controlled by the c_p and c_{surf} ratio. Besides of f, the mixing rate and temperature of the solution during preparation influence considerably the nanoparticles dimensions. Although the nature of surfactant, its hydrophobicity, charge and molar mass influence the size and density of the particles, a straightforward correlation has not been found which is probably caused by the complexity of nanoparticle process formation.
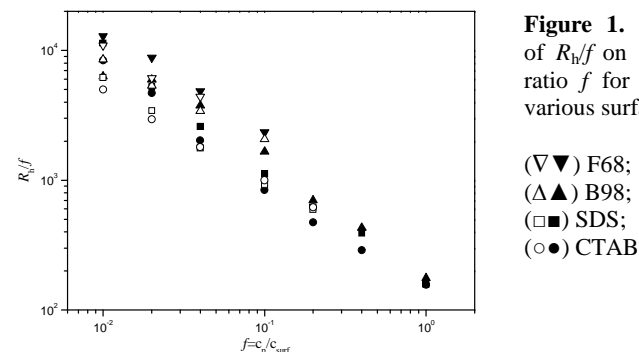

Figure 1. Dependence of R_h/f on composition ratio f for PMMA for various surfactants.

($\nabla\blacktriangledown$) F68;
($\triangle\blacktriangle$) B98;
($\square\blacksquare$) SDS;
($\circ\bullet$) CTAB

Small angle neutron scattering (SANS)

SANS experiments were performed at CEA-Saclay on the spectrometer PAXY of the Laboratoire Leon-Brillouin. In SANS, contrast variation can be used to investigate various structural details of the particles. Through a variation of scattering by selective substitution of protons for deuterium, detailed structure can be visualized as was demonstrated in the past for copolymers, polymer-coated particles, micelles and other self-assembled structures. For this reason, deuterated sodium dodecyl-d_{25} sulfate (d-SDS) and deuterated polystyrene (d-PS) were used in combination with protonated compounds (h-SDS, h-PS) as components of the nanoparticles. Three types of systems were tested:

(*i*) deuterated polymer + protonated surfactant in D_2O where the coherent scattering, which is very small, originates only from the surfactant. To increase the scattering intensity the concentration of solution was increased by evaporation.

(*ii*) protonated polymer + protonated surfactant in D_2O where the scattering comes both from the polymer and the surfactant, thus the whole nanoparticle is "visible."

(*iii*) protonated polymer + deuterated surfactant in D_2O. The scattering length density of the deuterated surfactant is almost matched by D_2O. In this case most of the scattering is produced by the polymer.

Typical scattering curve for the d-PS + h-SDS system, where the surfactant is visible, is plotted in **Figure 2**. The contrast variation experiment shows that the nanoparticles are entities with uniform density. Polymeric and surfactant molecules are evenly distributed within the nanoparticles. Such structure distinguishes them from other architectures like micelles, vesicles or complexes. One possible distribution of surfactant and polymer inside of a nanoparticle is in a simplified way shown in **Figure 3.**
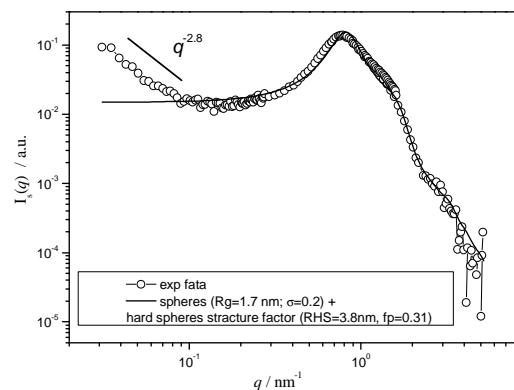

Figure 2.
Scattered intensity I_s as a function of the scattering vector q for various systems. (○) d-PS/ h-SDS; (▲) h-PS/h-SDS; (□) h-PS/d-SDS. Solid line is fitting curve.
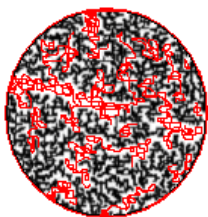

Figure 3. Possible distribution of a surfactant (red color) and polymer (black color) inside of a nanoparticle by SANS data.

Time-Resolved Small-Angle X-ray Scattering (SAXS)

The time development measurement of formation of the abovementioned polymeric nanoparticles was studied. A series of SAXS experiments were performed on the high brilliance beam line ID02 at the ESRF (Grenoble, France). Time-dependent experiments were performed using a home-made apparatus. Equal volumes of the aqueous solution of a surfactant and organic solution of a polymer in separate syringes were injected simultaneously the measurement cell placed directly in the beam. The mixing was followed by scattering data acquisition.

The results suggest that the kinetics of the formation of nanoparticles comprising ionic surfactants, such as sodium dodecyl sulfate (SDS), is much faster (in the range of milliseconds – all scattering curves are almost identical up to 2 minutes after mixing, **Figure 4**) than that for nanoparticles comprising non-ionic surfactants, such as Pluronic F68 (on the time scales of several seconds – the scattering curves are changing during 2 minutes, **Figure 5**). The transformation of particle surface from transient structure to hard sphere one has been observed in real time. The results also suggest that the formation of the nanoparticles is a two stage process: in the beginning a nucleation stage occurs which is followed by a growth regime. [2].
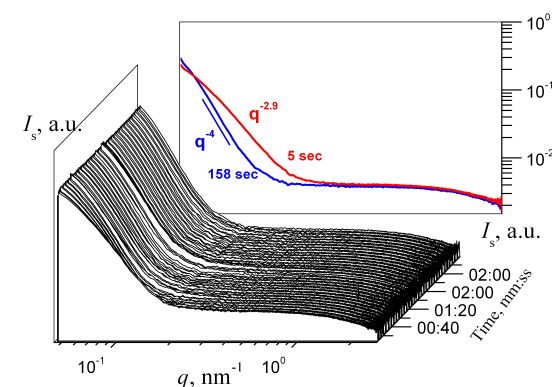

Figure 4. The temporal evolution of the SAXS intensity for a PMMA-SDS system.
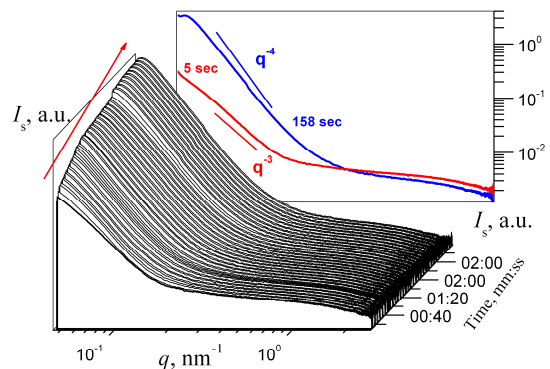
Figure 5. The temporal evolution of the SAXS intensity for a PMMA-F68 system.

2. Thermoresponsive polymeric nanoparticles stabilized by surfactants

The second part of results is devoted to the case where the phase separation is achieved by a change in temperature T in Eq.(1). In aqueous environment some of the polymers undergo fast and reversible changes in microstructure, induced by a change in temperature, from a hydrophilic to a hydrophobic state and are called "thermoresponsive". One of the most interesting is poly(*N*-isopropylacrylamide), PNIPAM, which is a water-soluble polymer with many attractive properties. For example, it undergoes a coil-to-globule transition on heating at 32 °C (lower critical solution temperature, LCST); this property is the result of rather complex polarity of this molecule.

Solutions of PNIPAM, with ionic and non-ionic surfactants were investigated by light scattering methods in temperature range 15 – 45 °C. In contrast to previous studies of the other authors, when surfactants were used in excess, low concentrations of surfactant ($c_{surf} \leq 5 \times 10^{-4}$ g · mL^{-1}) are used in this investigation. Under such conditions, the surfactants are not able to prevent intermolecular aggregation and the formation of well defined nanoparticles of PNIPAM is observed in aqueous solution on heating above the LCST. The effect of PNIPAM or surfactant

concentrations and molecular weight of PNIPAM on nanoparticle parameters and on the phase transition temperature of PNIPAM solutions was investigated. An example is shown in **Figure 6** where the temperature dependence of the hydrodynamic radius, R_h, was measured [3].
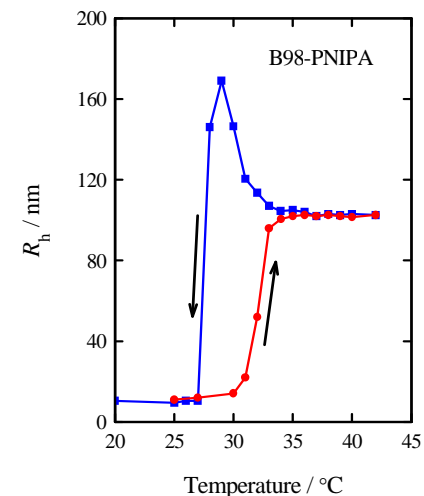

Figure 6. Red curve shows slow stepwise heating and blue curve corresponds to slow stepwise cooling after fast heating of solution to 40° C.
Polymer concentration: $c_{PNIPA} = 5 \times 10^{-4}$ g · mL^{-1}.
B98 concentration: $c_{B98} = 5 \times 10^{-5}$ g · mL^{-1}. M_η of the PNIPAM: 122 000.

The structure of particles is supposed to be similar to block copolymer micelles. Hydrophobic PNIPAM molecules form the insoluble core of particles and their hydrophilic shell consists of hydrophilic parts of surfactants. An intermediate shell at the core-shell interface contains both the hydrophobic parts of surfactants and PNIPAM chains. In the case when a Pluronic [poly(ethyleneoxide) – *block* – poly(propyleneoxide) – *block* – poly(ethyleneoxide)] surfactant is used, the nanoparticles are covered with biocompatible poly(ethyleneoxide) shells and such particles may be good candidates for prospective applications.

3. Thermoresponsive polymeric micelles for radionuclide delivery

Polymer micelles rank among most often studied drug delivery systems. The use of drug delivery vehicles in nuclear medicine brings some extra benefits. The most remarkable one is that the radionuclide does not need to leave the complex with the carrier to maintain its function of radiodiagnostics or radiotherapeuticals. The bearer of the demanded effect is the emitted ionizing radiation controlled by nuclear decay, which is not significantly influenced by chemical environment. Moreover, if the effective range and biological effect of ionizing particle is sufficient, the drug delivery vehicles need not even enter the cell.

Thermoresponsive polymer micelles with a thermoresponsive core and hydrophilic corona can be easily produced by simple heating of aqueous solution of the block or graft copolymer from the room to body temperature. If the copolymer is properly designed, the size of micelles can be suitable, *e.g.*, for spontaneous accumulation of the micelles in the target tissue.

New tailor-made copolymers based on poly(2-alkyl-2-oxazolines) have been tested. Poly(2-alkyl-2-oxazolines) attract increasing attention in biomedicinal research due to their peptide-related structure and adjustable physicochemical and biological properties. Dr. Hrubý from our institute synthesized ABA triblock copolymers poly[2-methyl-2-oxazoline – *block* – (2-isopropyl-2-oxazoline – *co* – 2-butyl-2-oxazoline) – *block* – 2-methyl-2-oxazoline]. The polymers have two hydrophilic A blocks and one central thermoresponsive B block with different monomer units ratios. These polymers are soluble in aqueous milieu, molecularly dissolved below the cloud point temperature (CPT) of the thermoresponsive block and form micelles at higher temperatures.

The temperature-dependent micellar behaviour has been tested in aqueous solutions both by dynamic (DLS) and static (SLS) light scattering. Micelles are formed within a narrow temperature range. The CPT value is a function of the copolymer composition, the CPT increases as the content of hydrophilic 2-methyl-2-oxazoline groups increases. The optimized triblock copolymer contains 15 mol-% of hydrophobic 2-butyl-2-oxazoline monomeric unit in the central thermoresponsive block with 1:1 thermoresponsive to hydrophilic block weight ratio. For that

copolymer, the formation of micelles with R_h of 80 - 100 nm above its CPT (28 °C) is observed [4]. An overview of the temperature-dependent micellar behaviour of the studied systems is shown in **Figure 7.**
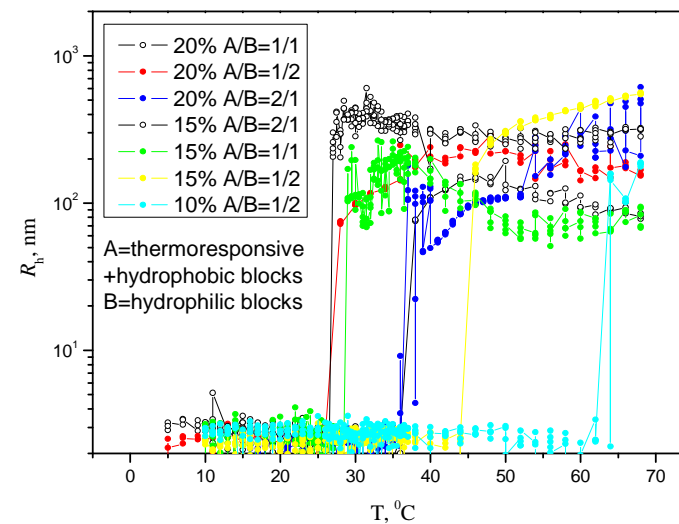

Figure 7. Temperature dependence of volume-weighted R_h of nanoparticles, cooling ($c_P = 0.5 \text{ g} \cdot \text{L}^{-1}$).

Part of the 2-butyl-2-oxazoline monomeric units was replaced by equimolar number of 2-(3-butenyl)-2-oxazoline monomeric units during the synthesis and a phenolic moiety was subsequently introduced into the optimized triblock copolymer. Such polymer was then radiolabelled with ^{125}I in good yield with sufficient in vitro stability under model conditions [4].

<u>**CONCLUSION**</u>

1.

Polymeric nanoparticles prepared by a change in solvent quality and stabilised by surfactants have been systematically investigated by DLS/SLS techniques. Model hydrophobic polymers (PMMA, PS) and different ionic and non-ionic surfactants were used in the study and resulting particles with hydrodynamic radii in range 50-250 nm were stable in aqueous environment. The important parameter for nanoparticles dimensions is the composition ratio $f=c_p/c_{surf}$ of the incoming compounds. The size of nanoparticles increases as f increases. Besides of f, the mixing rate and temperature of the solution during preparation influence considerably the nanoparticles dimensions. Although the nature of surfactant, its hydrophobicity, charge and molar mass influence the size and density of the particles, a straightforward correlation has not been found which is probably caused by the complexity of nanoparticle process formation.

The results demonstrate that nanoparticles have a spherical shape and sharp boundaries. The architecture of the nanoparticles has been investigated by the contrast variation SANS experiment showing that the nanoparticles are entities with uniform density where the polymer and surfactant molecules are evenly distributed within the nanoparticle volume. Such structure distinguishes them from other architectures like micelles, vesicles or complexes. The kinetics of formation of those nanoparticles has been observed *in situ* by time-resolved-SAXS measurement and the results suggest that the formation of the nanoparticles features two stages. At the beginning, a nucleation stage occurs which is subsequently followed by a growth regime. The charge of the surfactant plays an important role in the formation process. The kinetics of growth of the nanoparticles comprising non-ionic surfactants is much slower in comparison with those containing charged surfactants.

2.

It has been shown by DLS/SLS measurement that well defined thermoresponsive nanoparticles, with hydrodynamic radii in range 21-136 nm, can be easily prepared by heating PNIPAM solutions with low addition of surfactants above the LCST. A variety of surfactants, both ionic and non-ionic ones, were tested. Changes in the chemical composition of surfactants, concentration of surfactants or PNIPAM polymers and the molecular weight of PNIPAM modify nanoparticle parameters in a wide range of M_w and R_h values. In the first approximation, the structure of the particles is supposed to be similar to block copolymer micelles. Hydrophobic PNIPAM molecules form the insoluble core of particles, their hydrophilic shell consists of hydrophilic parts of surfactants and an intermediate shell at the core-shell interface contains both the hydrophobic parts of surfactants and PNIPAM chains.

3.

The temperature dependent micellar behaviour of new ABA triblock copolymers, synthesised by Dr. Hrubý, has been determined. The copolymers of oxazoline type consist of two hydrophilic A-blocks and one central thermoresponsive B-block, which is a statistical copolymer of 2-isopropyl-2-oxazoline and 2-butyl-2-oxazoline monomeric units. The CPT value is a function of the polymer composition. Increase in the hydrophobic monomer content in the thermoresponsive B-block results in the CPT reduction. The comparison of the copolymers with different thermoresponsive to hydrophilic block weight ratio shows that the increase in the hydrophilic block content considerably increases the CPT of the whole copolymer. The overall hydrophilicity/hydrophobicity of the triblock copolymer is at least of the same importance as the ratio of monomeric units of the thermoresponsive B-block itself. The A to B block weight ratio influences also the size of the micelles. Optimisation of the composition leads to the weight ratio of thermoresponsive to hydrophilic block of 1:1 and the hydrophobic monomer content in the B-block of 15 mol-%. That copolymer forms micelles with hydrodynamic radii of 80-100 nm above 28 °C, *i.e.* close to the physiological temperature.

4.

A phenolic moiety was introduced into the optimised polyoxazoline copolymer during its synthesis to allow radionuclide labelling with iodine radioisotopes. The polymer was radiolabelled with ^{125}I radionuclide in good yield and with sufficient stability *in vitro*. Subsequently, radioactive polymeric micelles, potentially applicable in nuclear medicine, can be prepared.