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Structural Characterization of Temperature-Sensitive Hydrogels by Field Emission Scanning Electron Microscopy (FESEM)

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Summary: The submicrometer structure of the temperature-sensitive hydrogels was observed by field emission scanning electron microscopy (FESEM), using synthesized hydrogels of different outer size and shape. The hydrogel structure strongly depends on the homogeneity of the polymer chains during the crosslinking process. A porous structure of the poly(vinyl-methyl-ether) (PVME) bulkgel, synthesized by electron beam irradiation of a concentrated polymer solution, was observed in the swollen state because the phase transitions temperature is acquired through the crosslinking process. Photo-crosslinking reaction of the poly(*N*-isopropylacrylamide) (PNIPAAm) copolymer in the dry state to form PNIPAAm thin films leads to a rather homogeneous structure. In the shrunk state both gels possess structure being more compact than in the swollen state. We also synthesized PVME and PNIPAAm gels with small outer dimensions in the range of some 100 *nm*. Heating of the thermo-sensitive polymer in diluted solutions collapses the polymer chains or aggregates. The crosslinking reaction (initiated by electron beam or UV irradiation) of these phase separated structures produces thermo-sensitive microgels. These microgel particles of PVME and PNIPAAm are spherical shape having diameters in the range of 30 – 500 *nm*.

Keywords: electron microscopy; field emission scanning electron microscopy; microgels; PNIPAAM; poly(*N*-isopropylacrylamide); poly(vinyl-methyl-ether); porous structure; PVME; temperature-sensitive hydrogels

1. Introduction

Hydrogels are three-dimensional networks of crosslinked hydrophilic polymers swollen in water. Polymeric hydrogels are soft materials with properties that resemble those of materials from the human body (high water content). These materials are highly interesting for various medical

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applications (see e.g. [1-5]). Especially, sensitive hydrogels with swelling/deswelling characteristics possess a high potential of biomedical applications. Thermo-responsive gels show discontinuous volume phase transition behavior. They are in a highly swollen state at temperatures below a critical temperature (T_c) and in a shrunk state above T_c. The volume phase transition temperature is near the lower critical solution temperature (LCST) of the corresponding non-crosslinked polymers. The effect of temperature induced phase transitions of hydrogels has been intensively studied on substituted acrylamides, i.e. on poly(*N*-isopropylacrylamid) (PNIPAAm) gels (e.g. [6-8]) (LCST about 33 *°C*).

For many applications it is necessary that the swelling/deswelling kinetics of the gel is fast. These kinetics depend on the gel size. Decreasing of this dimension decreases the time, in which the polymer gel will respond to an external stimulus. One possibility of reducing the dimension consists in the preparation of thin hydrogel layers. Kuckling et al. [9] synthesized thin layers of PNIPAAm by photo-crosslinking of its copolymer with 2-(dimethyl maleinimido)-*N*-ethyl-acrylamide (DMIAAm) as chromophore. The swelling behavior of the layers was characterized by surface plasmon resonance. Another possibility in preparing small gels is the synthesis of microgel particles. Pelton [10] reported on temperature-sensitive PNIPAAm microgels (and other acrylamides) produced by emulsion polymerization of the monomer. These microgel particles are almost spherical and show fast swelling/deswelling kinetics. Vo et al. [11] synthesized PNIPAAm microgels by photo-crosslinking of the PNIPAAm-DMIAAm copolymer.

Another well-known sensitive polymer is poly(vinyl-methyl-ether) (PVME) with a LCST of about 34 *°C* [12]. A highly concentrated solution of PVME can easily be transformed into a hydrogel by high-energy radiation, e.g. electron beam or γ-ray irradiation. These so-called “clean” methods lead to an additive-free crosslinking process (no need of initiator, crosslinker etc.). Irradiation of aqueous polymer solutions fixes the structure of the polymer chains in solution. Suzuki and Hirasa [13] found a strong dependence of the gel structure on the polymer concentration and the temperature of solution during irradiation with γ-rays. With increasing temperature the polymer chains aggregate and the crosslinking reaction forms a heterogeneous gel with a sponge-like structure. The porous structure strongly affects the swelling kinetics of PVME gels [14]. High-energy irradiation of diluted PVME solutions above LCST leads to temperature-sensitive microgel particles [15]. These microgels can be used as a stabilizer of the dispersion polymerization of pyrrole [16]. At high pyrrole concentrations polypyrrole particles with a needle-like structure are formed.

Crosslinking by γ-rays needs several days (depending on the dose rate of the γ-ray source) to transfer the polymer solution into a swollen hydrogel. A very efficient method in radiation chemistry is the initiation of reactions by electron beam. Due to the high dose rate and the orders of magnitude stronger electron-matter interaction the reaction time is several minutes only to form a gel. During this short reaction time the dissipated thermal energy caused an increase of the solution temperature. We found at our experimental conditions an increase of the temperature of about 2.5 *K* per 10 *kGy* [17].

Scanning Electron Microscopy (SEM) is a very powerful tool to investigate the structure of swollen hydrogels (see e.g. [17-20]). In former studies [20] the structure of PNIPAAm hydrogels synthesized by a free radical polymerization and crosslinking was investigated. It was shown that at room temperature the formed hydrogels have a sponge-like structure. It is of particular interest to compare the results of photo-crosslinked PNIPAAm hydrogel layers with their results.

In this paper, we present the results of the structural study of temperature-sensitive hydrogels of different outer dimensions in the swollen as well as shrunk state. The investigations were performed with high-resolution field emission scanning electron microscopy (FESEM) using cryo-prepared samples of the hydrogels.

2. Materials and Methods

2.1 Synthesis of hydrogels

PVME bulkgels were prepared by irradiation of a concentrated aqueous solution with electron beam (for further details see [17]). Oxygen-free PVME aqueous solution (20 *wt.%*) was dropped onto a small piece of thoroughly cleaned aluminum and covered with mica platelets. The Al-support was irradiated with a 0.5 *MeV* electron beam (radiation dose of 50 *kGy*) with a linear accelerator ELV-2 (Budker, Novosibirsk). After irradiation the gel-coated aluminum was immersed in distilled water for 15 *min* to remove non-crosslinked PVME.

Thin layers of PNIPAAm gel were prepared by photo-crosslinking of the copolymer of *N*-isopropylacrylamide and 2-(dimethyl-maleinimido)-*N*-ethyl-acrylamide (synthesis of the copolymer, see [21]). The solution of the copolymer was spin coated on a silicon wafer dried first at air-atmosphere and then in vacuum. The films were crosslinked by irradiation with UV light (360 – 430 *nm*). Further details of preparing were described earlier [9].

2.2 Synthesis of microgels

In diluted solutions ($c_p < 0.5$ *wt.%*) PVME molecules are not monomolecularly dissolved. By light scattering measurement we found aggregates with a radius of gyration of 100 – 200 *nm*. Above the phase transition temperature these aggregates collapse to spherical particles with a radius of 90 – 100 *nm* [15]. However, these particles do not precipitate because of the low density-differences between the polymer and the solvent. This phase separated structure can be fixed by irradiation with an electron beam at 50 *°C*. The PVME microgels were used without any further purification.

Microgel particles were prepared by photo-crosslinking of the PNIPAAm copolymer (see preparation of PNIPAAm films) in diluted aqueous solution [11]. To produce PNIPAAm microgels it was necessary to heat the solution above the transition temperature (45 *°C*). The precipitation of the copolymer by heating could only be prevented by using sodium dodecyl sulfate – SDS – as stabilizer. The crosslinking reaction furthermore was initiated by UV irradiation.

2.3 Field emission scanning electron microscopy

Synthesized thermo-sensitive hydrogels in different states were investigated with an "in-lens" field emission scanning electron microscope S-5000 (Hitachi Ltd., Japan) at low acceleration voltage. The secondary electron (SE) micrographs were taken from samples in swollen states at room temperature and in the shrunk state significantly above T_c (the used temperatures depend on the polymer). The structure formed during swelling of PVME and PNIPAAm bulkgels was fixed by rapid cooling with liquid ethane (cooled to 77 *K*). Under the experimental conditions used, the ice was in an amorphous state thus the network structure of the gels was not affected. The frozen water was removed by freeze-drying at 190 *K* for 6 *h* at about $5\cdot10^{-6}$ *Torr*. After drying the samples were rotary shadowed with about 2 *nm* platinum/carbon (Pt/C) at an elevation angle of 65°. The evaporation was performed in the high vacuum chamber of a freeze-etch devise (BAF 300 with turbo molecular pump, Balzers/Liechtenstein) at room temperature. The film thickness was measured with a quartz crystal film thickness monitor (Balzers QSG 201D, Balzers/Liechtenstein).

For preparation of the crosslinked microgels (PNIPAAm and PVME) in different swelling states a small droplet of a solution at 25 *°C* and 40 *°C* was placed onto a small fragment of silicon (Si)-wafer with the corresponding temperature. The microgels were allowed for approximately 90 *sec* to adsorbed physically onto the Si-wafer, before the excess solution was removed with filter paper [15]. The following rapid cooling and freeze-drying was as described above.

3. Results and Discussion

3.1 Poly(vinyl-methyl-ether) hydrogel

SE micrographs recorded at medium and high magnifications revealed that the swollen state of the PVME hydrogel at 25 °C (Fig. 1a, b) is characterized by three-dimensional sponge-like structure. The network consists of many small cavities in the range of several 10 – 100 nm. They are separated permeably from each other by thin membrane-like layers and represent the nano-reservoirs of the water. The thickness of these membranes is larger than of single network chains. It seems that some polymeric chains are aggregated forming this structure. The fine structure of the aggregated chains can be observed at high magnifications. The membrane-like layers are full of tiny holes with a typical hole-size in the order of 10 nm. This holey structure can be assigned to the structure of phase separated concentrated solution because the phase transition temperature was exceeded during the electron beam irradiation process. Above the transition temperature the polymer chains typically start to aggregate and to form polymeric bundles. These structures then were fixed by the high-energy electrons.

After heating the crosslinked PVME gel to 40 *°C*, the gel collapsed and shrunk. In this state the formed structure must be rather compact. The micrographs (Fig. 1c, d) show that the gel is still porous. The typical diameters of these cavities were in the order of up to a few 100 *nm* indicating a significant shrinkage of the mean pore size in comparison to the swollen state. Like in the swollen state these very small cavities represent the nano-reservoirs for the water. The remaining water in the gel after the collapse is the reason for the still high water content even at temperatures above LCST (degree of swelling of about 2 - 3 *g* water/*g* polymer). The membrane-like layers, which separate the cavities, appeared compact, i.e. no tiny holes in the layers were observed.

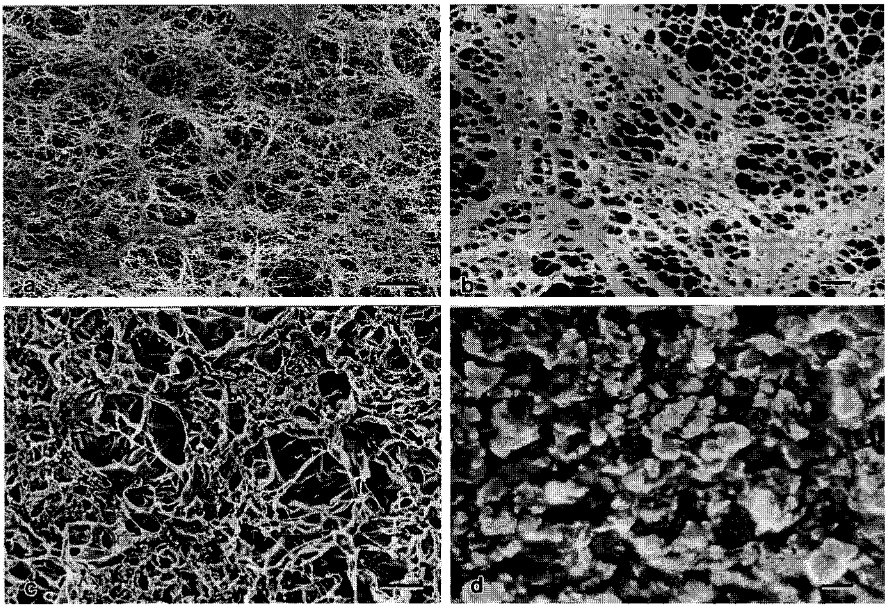

Figure 1. SE micrographs of freeze-dried PVME hydrogel synthesized by irradiation with an electron beam (a, b) in the swollen state (25 °C) and (c, d) in the shrunk state (40 °C) at different magnifications. The porous structure (due to the crosslinking of the phase separated polymer solution) of the swollen hydrogel collapsed above LCST to a more compact gel. The scale bars correspond to 1 µm (a, c) and 100 nm (b, d), respectively.

3.2 Poly(N-isopropylacrylamide) hydrogel

Fig. 2a, b display the structure of the PNIPAAm hydrogel in the swollen state at 25 *°C*. The low-magnification micrograph (Fig. 2a) displays the marginal zones of adjacent hydrogel dots synthesized by photo-patterning of the thin PNIPAAm film. The hydrogel was firmly attached to the support by an adhesion promoter [22]. A high-magnification micrograph of the PNIPAAm hydrogel (Fig. 2b) recorded in the middle region of the sample showed a gel structure with pores sizes between approximately 50 *nm* and 100 *nm*. The spatial network consisted of tiny polymeric ropes and sheet-like strands which typically have a thickness smaller than 10 *nm*.

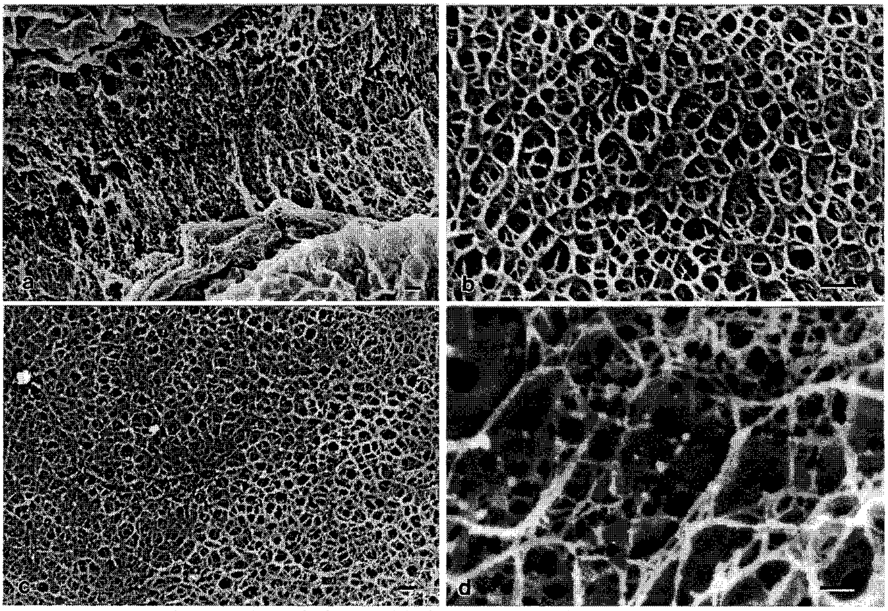

Figure 2. SE micrographs of freeze-dried PNIPAAm hydrogel synthesized by photo-crosslinking (a, b) in the swollen state (25 *°C*) and (c, d) in the shrunk state (35 *°C*) at different magnifications. The swollen PNIPAAm gel shows a homogeneously structure and generate polymer bundles due to the phase separation. The scale bars correspond to 100 *nm*.

In the shrunk state at 35 *°C* (Fig. 2c) the hydrogel surface appeared rather flat, i.e. grooves like in the swollen state were not found. A fine porous structure is visible. At high magnification a spatial network (Fig. 2d) of polymeric bundles with pores having diameters in the range of typically 10 *nm* could be observed.

3.3 Poly(vinyl-methyl-ether) microgel

Fig. 3 displays the structure of thermo-sensitive microgel particles synthesized by irradiation with a dose of 80 *kGy* at temperatures below (Fig. 3a) and above (Fig. 3b) the LCST.

In the swollen state at 25 *°C* the microgel particles are almost spherical with a creased surface. The particles seemed to have also a sponge-like internal structure. Small holes in the range of 10 *nm* in the polymeric material were observed sporadically at the particles surface. The outer diameter of these particles typically was in the range of 250 – 450 *nm*. Beside the particles we found at some locations of the sample an irregular polymeric net that can be assigned to the non-crosslinked polymers in the solution.

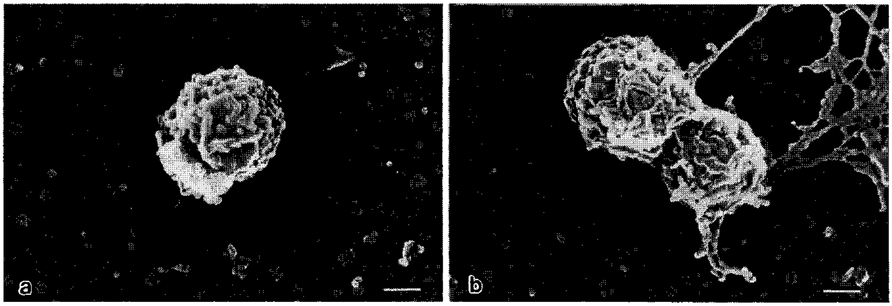

Figure 3. SE micrographs of freeze-dried PVME microgels synthesized by crosslinking the phase separated structure of diluted polymer solutions with electron beam (a) at 25 *°C* and (b) at 40 *°C*. Isolated particles in the swollen state have typically a diameter of approximately 300 *nm* and are porous. In the shrunk state the particles are compact. The scale bars correspond to 100 *nm*.

Above the phase transition temperature (40 *°C*) the microgels collapse to more compact particles having still a creased surface like in the swollen state. The shrinkage of particles above T_c is difficult to demonstrate with only a few imaged individual particles in single micrographs because of (i) one and the same particle cannot be imaged and compared in both states, and (ii) different particles usually differ significantly in diameter caused by the wide diameter-range in the swollen state.

3.4 Poly(N-isopropylacrylamide) microgel

Fig. 4 shows typical FESEM micrographs of PNIPAAm microgels in the swollen (Fig. 3a) and in the shrunk state (Fig. 3b). The outer dimension of the almost spherically formed PNIPAAm microgels is smaller than the one of the PVME microgels. In Fig. 4a "aggregates" consisting of several microgels having different diameters were found. The particles were also synthesized above the critical temperature. However, the surface of these microgels in the swollen state was rather smooth. There are two possible reasons for this effect: Firstly, for the stabilization of the suspensions SDS was used. The surfactant may be still at the surface of the particles smoothing their possibly creased surface. Secondly, a rather smooth surface could be caused by a higher crosslinking density. In the shrunk state (Fig. 4b) the microgel particles are collapsed and possess a creased surface which is similar to the one of the collapsed PVME microgels.

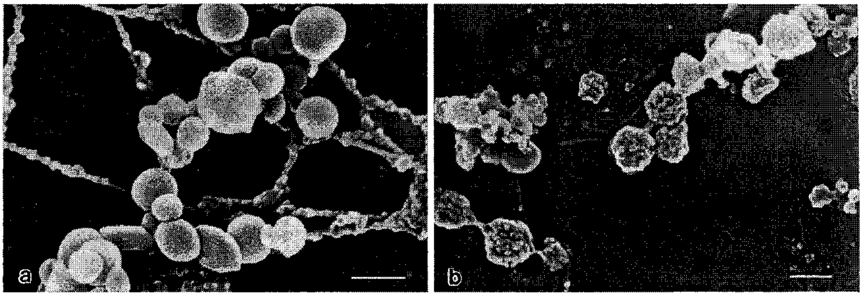

Figure 4. SE micrographs of PNIPAAm microgels synthesized by photo-crosslinking of the diluted polymer solution above LCST (a) in the swollen state (25 °*C*) and (b) in the shrunk state (40 °*C*). The swollen microgels have a smooth surface. In the shrunk state a compact rough surface is visible. The scale bars correspond to 100 *nm*.

4. Conclusions

The field emission scanning electron microscopy at low acceleration voltage proved to be a very valuable tool to observe the spatial structure of freeze-dried temperature-sensitive hydrogels in the swollen and in the shrunk state down to the dimensions of several nanometers. The cryo-preparation and subsequent Pt/C coating of these fragile structures with high water content (similar to biological specimens like soft issues) ensured the preservation of their structure also under high vacuum conditions required for electron microscopy. It is obvious that the observed structures would hardly be accessible by other high-resolution imaging methods like scanning force microscopy or conventional transmission electron microscopy thus FESEM represents an unique imaging method for hydrogels. The structure of thermo-sensitive hydrogels strongly depends on the synthesis conditions (Fig. 5). The network formation at lower temperatures or in the air-dried state leads to homogeneous structures (Fig. 5a). Crosslinking at temperatures above the phase transition leads to porous structures (Fig. 5b). In the case of diluted aqueous solution of thermo-sensitive polymers we use the phase separated structure (globular particles) to synthesize temperature-sensitive microgels. These particles are cross-linkable by UV irradiation (in case photo-sensitive PNIPAAm copolymers) or by irradiation with high-energy electrons (in case PVME). The structure of these microgel both in the swollen and in the shrunk state particles was almost globular with diameters ranging typically from about 100 *nm* to 500 *nm*.

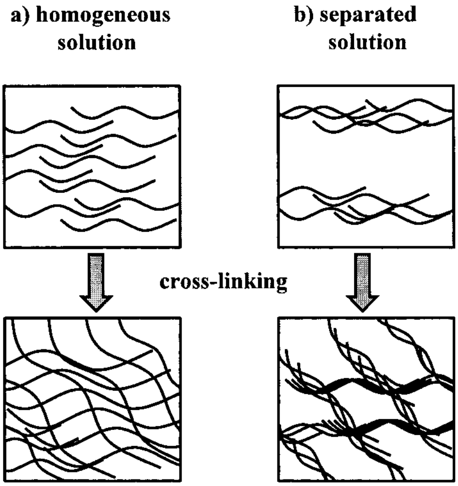

Figure 5. Schemes of crosslinking reaction of (a) homogeneous solution and (b) phase separated polymer solutions.

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