



Thermosensitive Hydrogels and Microgels Based on N-isopropylacrylamide and Acrylamide with Incorporated Magnetite

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Hydrogels with magnetic properties (ferrogels) are very promising for medical application, first of all, for the design of targeted delivery systems with controlled release of drugs and for magnetic hyperthermia and could be obtained using different techniques (*ex-* and *in-situ* syntheses). The present work is devoted to the study of magnetite (Fe_3O_4) formation inside the nanoreactors of (co)polymeric gels (hydrogels and microgels) based on the derivatives of acrylic acid: N-isopropylacrylamide, acrylamide, and N,N'-methylenebisacrylamide (crosslinking agent). Influence of hydrophilic-lipophilic balance of (co)polymeric templates, temperature, pH and concentration of iron cations on the magnetite formation inside polymeric matrixes were investigated along with the development of ferrogel preparation technique. Physical and chemical properties of obtained ferrogels have been studied with gravimetric analysis, thermogravimetric analysis, X-ray fluorescence spectroscopy, X-ray diffraction, optical and transmission microscopies, IR spectroscopy, etc.

Keywords: Hydrogels, Microgels, Ferrogels, Thermosensitive, Nanoreactors, Magnetite, Hyperthermia, Drug Delivery Systems.

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1. INTRODUCTION

Hydrogels (conventional or stimulus responsive) are three-dimensional high-molecular networks containing physically or chemically cross-linked (co)polymeric chains and water. Due to their high biocompatibility and the ability to incorporate drugs, the hydrogels are widely used in medicine, pharmacology, and biology to develop various biomaterials, such as implants, soft contact lenses, wound coatings, cell carriers, drug delivery systems, etc.

The most familiar representatives of the negatively thermosensitive systems are alkyl-derivatives of acrylamide, N-isopropylacrylamide (NIPAAm) and N,N-diethylacrylamide, which are studied due to their promising properties for medical application, since the systems have phase transition temperature (or lower critical solution temperature (LCST)) close to the temperature of human body. Thus LCST of polymeric systems based on NIPAAm is in the range of 32-34°C and slightly different from the temperature of human body. Depends on hydrophilic-lipophilic balance, it can be moved to any side during copolymerization of NIPAAm with hydrophilic or hydrophobic monomers [1].

Nanocomposites based on polymer hydrogels have a unique set of parameters due to the combination of polymeric matrix properties (high water content, biocompatibility, elasticity, the ability to immobilize diverse drugs, chemical and mechanical stability, functional nature, selectivity, low-cost, and low reactivity) and those of nanosized filler (high surface area to volume ratio, strength, hardness, chemical reactivity, magnetic, electric and optical properties). In addition, using "smart" (stimulus responsive) hydrogels makes it possible to develop nanocomposites responsive to many external factors. This fact essentially expands the field

of thermosensitive hydrogels application in medicine, primarily for the development of various therapeutic systems of targeted delivery and controlled release of drugs, magnetic hyperthermia of malignant tumors, etc.

Nanocomposites based on polymeric hydrogels can be obtained by various techniques, two main techniques being *ex-situ* (filler introduction before or during gelation) and *in-situ* (synthesis of nanosized filler via polymeric templates). In the first case nanosized filler in the form of a powder or stabilized dispersion is added into the reaction mixture that is later polymerized. In the second case nanocomposites are prepared using a polymer matrix as nanoreactors to synthesize the nanosized filler. The technique of using polymeric templates (different kinds of polymers and gels) allows synthesizing inorganic nanoparticles with well-defined size and morphology. Moreover, usage of hydrogel templates results in nanoparticle incorporation into hydrogel composition, as well as nanoparticle stabilization and prevention of their aggregation.

Incorporation of magnetic fillers (magnetite (Fe_3O_4), magemite ($\gamma\text{-Fe}_2\text{O}_3$), etc.) into polymeric matrixes results in the obtaining of hydrogels with magnetic properties – ferrogels, which can be remotely controlled by external magnetic field. Thus, on applying the constant magnetic field, the ferrogels can be localized closely to a target organ. This is extremely important for highly toxic drugs used, for instance, during chemotherapy of malignant tumors. Using matrixes based on "smart" polymers the ferrogels can become sensitive to various factors (e.g. temperature), which makes it possible to purposefully change their properties in a wide range.

Although publications on nanocomposites are numerous, the information about synthesis of nanoparticles, especially magnetite ones, via hydrogel nanoreac-

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tors is very scarce, and sometimes contradictory. Despite this, investigation of the influence of diverse factors on nanoparticles formation was hardly investigated.

Therefore, the present work will be devoted to the study of magnetite formation inside hydrogel nanoreactors based on NIPAAm and acrylamide with the development of ferrogel preparation techniques. The influence of hydrophilic-lipophilic balance of polymeric templates and concentration of iron cations on the magnetite formation will be investigated. Additionally, physicochemical and biomedical properties of thermosensitive hydrogels and ferrogels on their base will be described in details.

2. MATERIALS AND METHODS

2.1 Materials

N-isopropylacrylamide (NIPAAm) (Sigma-Aldrich, 97%) was recrystallized from n-hexane and dried under vacuum. Acrylamide (AAM) (Merck, 99.9%), N,N'-methylenebisacrylamide (MBA) (Merck, 98%), ammonium persulfate (PSA) (Sigma, 98%), N,N,N',N'-tetramethylethylenediamine (TMED) (Merck, 99%), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and NaOH were used as received without preliminary purification.

2.2 Synthesis of hydrogels and microgels

(Co)polymeric hydrogels and microgels based on NIPAAm and AAM were synthesized by means of radical polymerization in water medium. Covalent cross-linking of copolymeric chains was accomplished using bifunctional monomer MBA. Initiation of the polymerization was performed with the help of the redox system composed of ammonium persulfate (PSA) and N,N,N',N'-tetramethylethylenediamine TMED [2].

(Co)polymeric gel composition was determined by the composition of the reaction mixture and was found to be as follows: weight fraction of monomers constituted 20% (ratio of monomers was changed through all concentration range), MBA – 0.20%, concentration of the equimolar initiating mixture was 0.04 mol/L and 0.12 mol/L respectively for hydro- and microgels.

2.3 Synthesis of ferrogels

Magnetic hydrogels or ferrogels with incorporated magnetite were synthesized based on the (co)polymeric matrixes described above. The formation of magnetite was carried out in the nanoreactors through the precipitation reaction below:



At the first stage of the synthesis, the polymeric matrixes were saturated with iron cations. In order to perform the saturation, dried polymeric matrixes were covered with an excess of water solution of di- and trivalent iron salts with the ratio 1:2 for 12h. Then, the polymeric matrixes saturated with iron cations, were treated for 3h in case of hydrogels and for 30min in case of microgels with 10% solution of NaOH for the formation of magnetite. Then, the resulting ferrogels was washed extensively in distilled water at room temperature to remove residues.

3. RESULTS AND DISCUSSIONS

Phase transitions of (co)polymeric hydrogels were studied by a gravimetric analysis to measure equilibrium swelling degree depending on temperature (see Fig. 1). It shows that copolymerization of NIPAAm with hydrophilic AAM leads not only to the shift of LCST to higher temperatures as it was intended, but also to the changes in the nature of the dependence. LCST of NIPAAm-based homopolymeric matrix is about 34 °C, but during NIPAAm chains substitution by AAM ones, it grows and becomes 37 °C (2.5% AAM), 42°C (5% AAM) and 47 °C (10% AAM) respectively. Thus, by copolymerization of NIPAAm and AAM phase transition temperature can be controlled and varied over a wide range. It is very important for the usage of these copolymeric hydrogels for hyperthermia and controlled release of drugs.

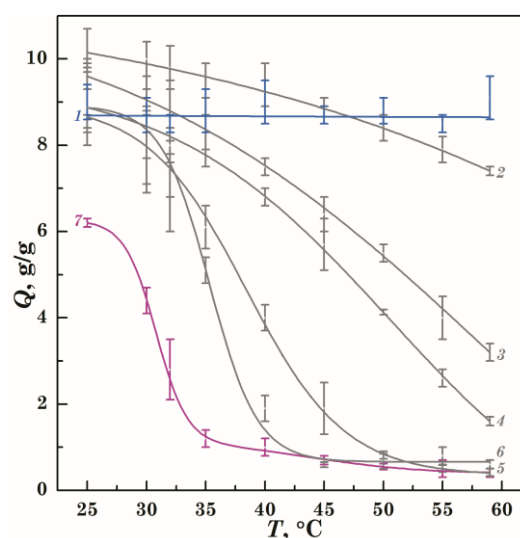


Fig. 1 – Dependence of equilibrium swelling degree of (co)polymeric hydrogels based on NIPAAm and AAM in distilled water on temperature: 1 – 0% NIPAAm; 2 – 50% NIPAAm; 3 – 75% NIPAAm; 4 – 80% NIPAAm; 5 – 90% NIPAAm; 6 – 95% NIPAAm; and 7 – 100% NIPAAm.

Additionally, when AAM content increases, there is a change from the abrupt dependence (at high NIPAAm concentrations) to smooth one (concentration of NIPAAm being 80–25%) followed by the loss thermosensitivity by polymeric matrix at NIPAAm concentration less than 25%.

Thus, copolymeric hydrogels containing 95% of NIPAAm and 5% of AAM are the most suitable to develop systems for magnetic hyperthermia and chemotherapy due to their properties (the presence of thermosensitivity, abrupt change of swelling degree on temperature, and the value of LCST).

Magnetic sensitivity of the obtained ferrogels is determined by the quantity and size of incorporated magnetite nanoparticles. Therefore, the study of swelling of hydrogels having different chemical composition versus concentration of iron cationic solutions permits optimization of magnetic nanoparticle synthesis in polymeric templates. This is especially important for hydrogels based on NIPAAm, which are very sensitive to the ionic strength of the solution, and at its certain

values the thermosensitive hydrogel can collapse.

It has been determined that the equilibrium swelling degree of AAm-based homopolymeric hydrogel increases with the increase of iron cation concentration followed by slight decreases at the concentrations higher than 6.0% (see Fig. 2.1). The hydrogels containing NIPAAm chains behave differently. NIPAAm-based homopolymeric hydrogel transfers to the collapsed state at mass concentration of iron cations 2.2% (see Fig. 2.3). For copolymeric hydrogel (95% NIPAAm) critical concentration, at which phase transition occurs, grows and becomes 4.5% (see Fig. 2.2).

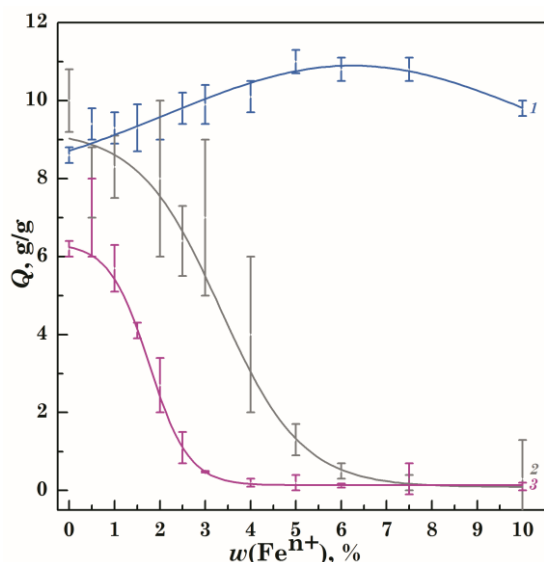


Fig. 2 – Equilibrium swelling degree of (co)polymeric matrixes based on NIPAAm and AAm in distilled water and water solutions of iron cations ($\text{Fe}^{2+}/2\text{Fe}^{3+}$) with different concentrations at 25 °C: 1 – 0% NIPAAm, 2 – 95% NIPAAm, and 3 – 100% NIPAAm.

In addition, investigation of hydrogel swelling degree dependence in iron cations solutions with different concentrations prepared based on 0.1M H_2SO_4 and at different temperatures has been carried out (see Fig. 3). The data comparison allows drawing conclusions that swelling degree of copolymeric matrix with 95% NIPAAm content increases (in 1.5 times) with decreasing temperature from 25 °C to 5 °C. Moreover critical concentration of iron cations increases from 4.5% to 10% respectively. The difference in swelling degree of hydrogels in iron cations solutions based on water and 0.1M H_2SO_4 is not significant.

Knowledge of swelling dependence of (co)polymeric matrixes on iron cation concentration and temperature allows picking up conditions of magnetite synthesis in the hydrogel nanoreactors. Therefore thermosensitive ferrogels have been synthesized at 5 °C in iron cations solutions with concentrations up to 10% based on 0.1M H_2SO_4 . These conditions allow increasing the magnetite content in polymeric matrix, and reduce the degree of hydrolysis of iron cations during synthesis.

As can be seen from electron micrograph (see Fig. 4) synthesized magnetic particles are nanosized with the average particle size of about 5-10nm.

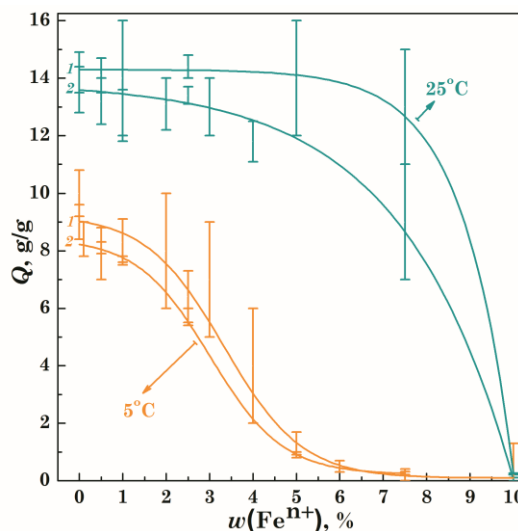


Fig. 3 – Equilibrium swelling degree of (co)polymeric matrixes based on NIPAAm (95%) and AAm (5%) in iron cations solutions ($\text{Fe}^{2+}/2\text{Fe}^{3+}$) with different concentrations at 5 °C and 25 °C prepared based on: 1 – water, 2 – 0.1M H_2SO_4 .

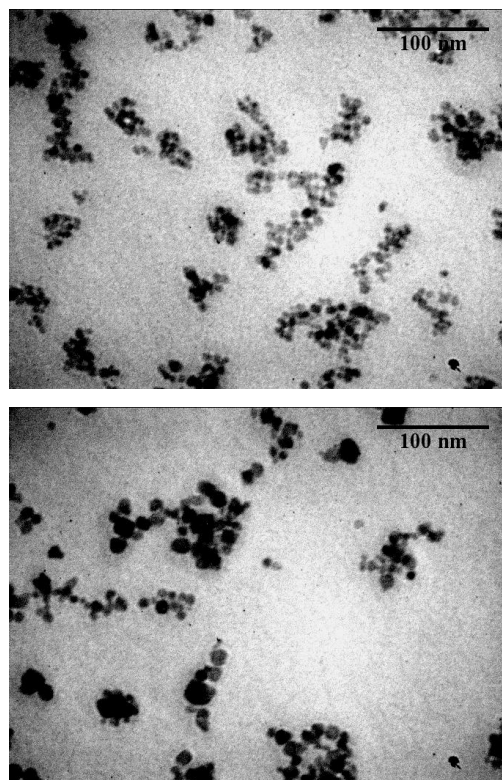


Fig. 4 – Micrographs (TEM) of cross section of copolymeric ferrogels based on NIPAAm (95%) and AAm (5% AAm) obtained in solutions based on 0.1M H_2SO_4 with iron cations concentration: 1 – 0.50% Fe^{n+} , 2 – 2.50% Fe^{n+} .

The phase fractions in ferrogels have been determined by X-ray diffraction. XRD patterns of magnetite (Fe_3O_4) and magemite ($\gamma\text{-Fe}_2\text{O}_3$) are similar, but they can be identified by comparing the relative peak intensities. Thus, identification of magnetic filler inside ferrogels was carried out according to the ASTM card index on the basis of 5 reflexes and its relative peak intensities with Miller indexes (220), (311), (400), (511),

and (440), which correspond to inter-plane distances 0.2966 (28%), 0.2530 (100%), 0.2096 (20%), 0.1712 (25%), and 0.1614 (34%) nm [3]. These diffraction lines provide clear evidence of the formation of pure Fe_3O_4 nanoparticles. No foreign phases were detected, ensuring the phase purity of the samples.

TGA-DTG thermogravimetric analysis of thermosensitive hydrogels is shown in Figure 5. The TGA-DTG thermograms of homopolyisopropylacrylamide hydrogel (see Fig. 5.1) show the first step weight loss, which can be attributed to the removal of absorbed water at temperature maxima of 75 °C and the second step weight loss of bounded water, which appears as a shoulder on the DTG curve. The weight loss of homopolyacrylamide hydrogel (see Fig. 5.3) occurs with two well-defined steps weight loss at 90 °C and 133 °C. For copolymeric hydrogel (see Fig. 5.2) containing 95% NIPAAm and 5% AAm the first and the second step weight loss occur at temperature maxima of 62 °C and 112 °C. Also it should be noted that the ratio between the two steps weight loss varies: with increasing of hydrogel hydrophilicity the second step weight loss attributed to the removal of bounded water becomes more intense and shifts toward higher temperatures.

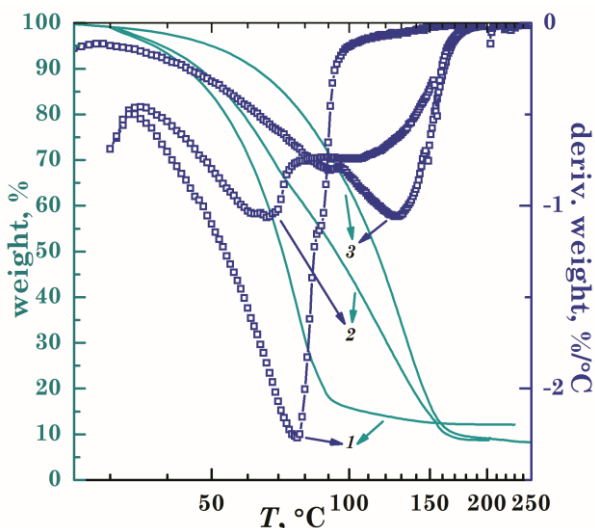


Fig. 5 – Thermogravimetric (TGA) and differential thermogravimetric (DTG) analysis of (co)polymeric hydrogels based on NIPAAm and AAm: 1 – 100% NIPAAm, 2 – 95% NIPAAm, 3 – 0% NIPAAm.

TGA-DTG thermogravimetric analysis has been also carried out for ferrogels based on copolymeric matrix containing 95% NIPAAm and 5% AAm (see Fig. 6). Incorporation of Fe_3O_4 nanoparticles into the hydrogel composition causes changes in the shape of the TGA-DTG curves.

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The first weight loss of the DTA curve at 90 °C (see Fig. 6.1) and 88 °C (see Fig. 6.2) is corresponding to the absorbed water. These temperatures are higher than the temperature of pure copolymeric hydrogel. This result indicates that the inorganic particles of Fe_3O_4 enhanced the thermal stability of thermosensitive hydrogels, the organic moieties.

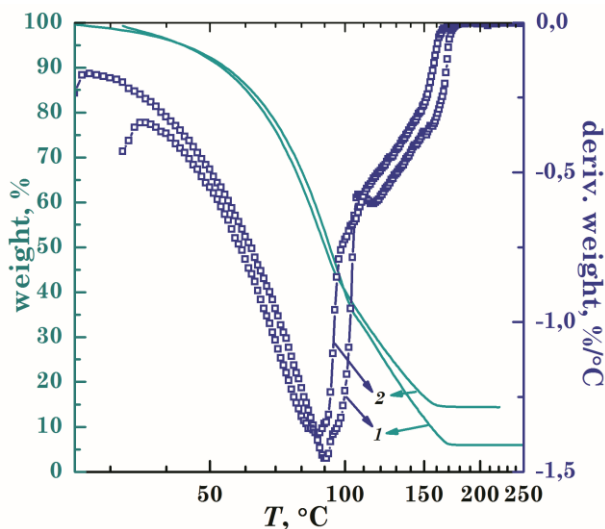


Fig. 6 – Thermogravimetric (TGA) and differential thermogravimetric (DTG) analyses of copolymeric ferrogels (95% NIPAAm and 5% AAm) obtained in solutions with iron cation concentration: 1 – 1.00% Fe^{3+} , 2 – 5.00% Fe^{3+} .

4. CONCLUSIONS

Ferrogels sensitive to both physiologically acceptable temperature and external magnetic field have been prepared based on covalently cross-linked NIPAAm and AAm-based (co)polymeric hydrogels and microgels via *in-situ* technique. Obtained results help to choose suitable conditions (range of iron cation concentration and temperature) for ferrogel synthesis.

The technique developed for the synthesis of magnetite particles in nanoreactors of thermosensitive copolymeric hydrogels permits their stabilization, and prevention of nanoparticle aggregation, and allows for obtaining magnetic particles with an average size of about 5-10nm. Copolymer ferrogels having 95% of NIPAAm and 5% of AAm with 0.2% MBA content have been shown to possess the best properties for biomedical application. Ferrogels with such composition are characterized by the abrupt phase transition at about 42°C (most efficient release of incorporated medicine) and by high magneto-sensitivity that grows with the increase of magnetite concentration.

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