

Nano-Sized Hydrogel Composites Based on N-Isopropylacrylamide and Magnetite for Controlled Drug Delivery

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Synthesis and characterization studies of promising nano-sized hydrogel composites based on n-isopropylacrylamide and magnetite have been studied. N-isopropylacrylamide (NIPA) gel component was used as a carrier of various drugs, magnetite was used as a magneto-responsive component. Presence of magnetite it was proved by EPR method. Composite nanoparticles were characterized by electron microscopy (TEM) and by dynamic light scattering (DLS) method. It was shown that the average size of nanoparticles is 50 or 100 nm, depending on the method of preparation. The hydrogel is characterized by clear phase transition between swollen and collapsed state upon heating above 32 °C. Rapid release of the incorporated drug (as a model was used the photosensibilizer -Methylene Blue) observed during thermo-responsive nanocomposite gels heating in the physiologically acceptable range, but still above phase transition temperature (up to 40–50 °C), allows application of the discussed drug delivery systems in medical hyperthermia.

Keywords: hydrogels thermosensitive nanogel, N-isopropylacrylamid, controlled drug release, targeted delivery

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1. INTRODUCTION

The development of drug delivery systems that can ensure targeted drug delivery to a specific organ and their release at the previously set speed is developing intensively at the borderline of chemistry first of all polymer chemistry, nanotechnology, pharmaceuticals and bioengineering [1]. Hydrogels, sterically crosslinked hydrophilic materials of high biocompatibility that can sorb substantial water quantities and at the same time remain insoluble and retain their structural entity, seem to be most promising materials for drug transportation systems [2]. First of all, the above mentioned facts concern the so-called "smart" hydrogels that can dramatically and predictably change their characteristics, diffusional ones including, under the influence of slight changes in their environment [3]. Temperature and pH are the most widely used means to influence hydrogel transport parameters [4].

pH sensitive hydrogels mainly contain carboxyl or amine functional groups promoting binding of a drug with a polymer matrix by means of selective sorption of basic or acidic medicines, respectively [5].

Speed of the release of medical drugs incorporated into the hydrogel matrix due to ionic bonds hydrolysis is determined by the environment pH that substantially differs for different human organs, being strongly acidic for a stomach, weakly alkaline for a mouth and alkaline pH for intestines. The most promising materials, however, to prepare novel drug transportation systems are thermoresponsive hydrogels, first of all NIPA-based ones, capable of phase transition between swollen and collapsed state under heating above 32 °C

[6]. This temperature is very close to the temperature of a human organism, and during copolymerization of NIPA with monomers of various nature can purposefully shift to the region of higher (in case of hydrophilic chains incorporation, e.g. acrylamide) or lower (hydrophobic chains incorporation, for example acrylonitrile) temperatures [4]. Therefore, it is quite possible to obtain thermoresponsive hydrogel matrices with phase transition temperature in the range of 25 °C to 55 °C.

Targeted delivery of finely dispersed drug transportation systems to the specific organ can go via a bloodstream due to external low-intensive magnetic field. Recently, nano-sized systems of drug delivery based on liposomes, micelles, nanoparticles of metals and their oxides, carbon nanotubes, dendrimers, etc have been created [7, 8]. Area of the application of the above mentioned nano-sized carriers, however, is rather limited due to low stability and limited circulation time, therefore of late, the so-called nanogels, hydrogel particles of less than 200 nm appear more promising. [9]. Nanoparticles are easily accumulated in tumor cells thus allowing their application in passive targeted chemotherapy [10], and modification of their surface with biocompatible enzymes prolongs these particles circulation in bloodstream [11]. Alongside with the small size of the particles (10-200 nm), the ideal nanogel system of drug delivery should possess such properties as biocompatibility and/ or to biodegradability, substantial time of circulation via the bloodstream, good sorbability of drugs and enzymes, as well as protection of the mentioned molecules against human organism immune system response.

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Combination of the above multi-functional properties can be achieved by varying nature of the used copolymers and frequency of their crosslinking, as well as incorporated nano-fillers. In addition, incorporating of various specific vector groups (magnetic nanoparticles, for instance) that enable targeted delivery of BAP to specific organs or cells can result in better selectivity of their action in the organism.

The above mentioned facts show that development of the techniques of obtaining nano-sized thermoresponsive hydrogel drug carriers and methods of magnetic particles incorporation in their composition is of primary importance. The present article deals with this problem.

2. MATERIALS AND METHODS

2.1 Materials

N-isopropylacrylamide, NIPAA (Sigma-Aldrich, 97%) was recrystallized from hexane and dried under vacuum; N,N'-methylenebisacrylamide (MBA) (Merck,98%), ammonium persulphate, PSA (Sigma 98%); N,N,N',N' tetramethylethylenediamine, TMED (Merck,99%) were used without addition purification, the same procedure was chosen for sodium dodecylsulphate (SDS) and iron salts (FeSO₄ and FeCl₃) used in magnetite synthesis. Methylene blue was recrystallized from 50% aqueous solution of ethanol and dried in vacuum.

2.2 Methods

Nanogel morphology studies in water suspensions were carried out using Transmission Electron Microscope JEOL JEM-1230. Dynamic light scattering was used to measure sizes of nanoparticles and their distribution according to sizes. The measurements were made with the help of Z-sizer of nano S-type (MALVERN). UV-spectrophotometer "SPECORD M 40 was used to study nanogel light transmission and diffusion of Methylene Blue.

3. SYNTHESIS

3.1 Nano-magnetite synthesis

Salts FeCl₃ and FeSO₄ with the ratio 1:2 mas % were dissolved in 500 ml 1N NaOH. After 30 minute stirring, 0.95 ml of concentrated HCl was added followed by another 30 minute stirring of the mixture. The prepared magnetite of black color was 5 times washed with distilled water until medium pH dropped from 12 to 8. Headers and footers are areas in the top and bottom margins of each page in a document. The authors only need to edit the headers of even and odd pages in terms of specifying the abbreviated title and abbreviated authors list. Other information will be inserted by editors.

3.2 Synthesis of thermoresponsive nanogels

NIPAA-based thermoresponsive hydrogels were synthesized by means of suspension polymerization technique. N,N'-methylenebisacrylamide was used as a crosslinking agent, while dodecylsulphonate of sodium was used as a suspension stabilizer. Initiating of the gel-formation was performed using potassium persulphate. The synthesis was carried out in three-neck flask at 65-70 C and continuous stirring (400 rotations

per minute) in argon during 6 hours. Similar method was employed in the synthesis of nanogels with incorporated magnetite. The priorly synthesized magnetite was incorporated in the reaction mixture before initiator adding. Ratio of the components used in the nanogels synthesis is given in Table 1

Table 1 – Content of the composition for nano(ferro)gel preparation.

N ₀	NIPAA, g/ml	PSA, g/ml	SDS, g/ml	H ₂ , ml	MBA, g/ml	Magnetite, g/ml
1	13,33 · 10 ⁻⁴	0,4 · 10 ⁻⁴	3,86 · 10 ⁻⁴	150	0,2 · 10 ⁻⁴	
2	13,33 · 10 ⁻⁴	0,4 · 10 ⁻⁴	15,3 · 10 ⁻⁴	150	0,2 · 10 ⁻⁴	
3	13,33 · 10 ⁻⁴	0,4 · 10 ⁻⁴	30,6 · 10 ⁻⁴	150	0,2 · 10 ⁻⁴	
4	12,0 · 10 ⁻⁴	0,4 · 10 ⁻⁴	3,86 · 10 ⁻⁴	150	0,2 · 10 ⁻⁴	1,3 · 10 ⁻⁴
5	12,0 · 10 ⁻⁴	0,4 · 10 ⁻⁴	3,86 · 10 ⁻⁴	150	0,2 · 10 ⁻⁴	2,6 · 10 ⁻⁴

All nanogels were washed by dialysis during 2 days, with 2 hour interval of changing distilled water (cut-off membranes for the dialysis - 30Da) and concentrated by four-time centrifugation at 15000 rotations per minute.

4. RESULTS AND DISCUSSION

Drug carrier size is one of the most important characteristics, since nanoparticles with the diameter less than 200 nm. can penetrate into cells and cannot be removed from the blood system by macrophages thus prolonging their presence in the organism. Dependence of the nanoparticle size on stabilizer concentration in the polymerizing composition was established using dynamic light scattering technique. (Fig.1).

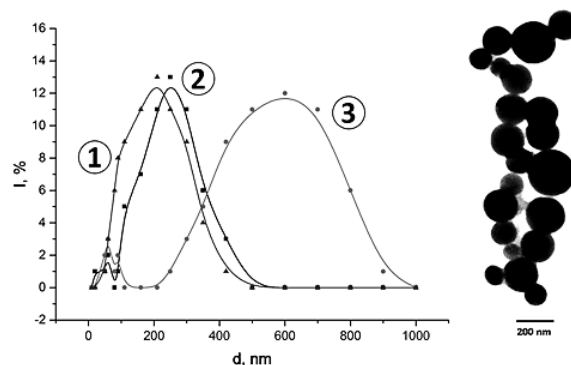


Fig. 1 – Curves of distribution of synthesized nanogels versus SDS concentration according to dynamic light scattering (1-0.0386%; 2-0.153%; 3-0.306%). Inset – Nanogel microstructures (TEM) (C_{SDS} = 3.86mg/ml).

The performed investigations showed that the size of the prepared nanoparticles diminishes with the increase of the stabilizer concentration in the range of 0.027 to 0.324 mas.%. When the stabilizer concentration equals 0.027%, the size of the obtained hydrogels is mainly 600nm. If the stabilizer concentration is 0.054%, the size constitutes 260nm, and the concentration 0.0108 gives the size of 200nm. Time of the composition clouding that signals the gel formation initiation increases and constitutes 2.4 and 5.5 hours respectively.

Further growth of emulgator concentration causes even bigger decrease of hydrogel nanoparticle size, as well as polymerization inhibition that requires varying initiator concentration and conditions of the polymerization reaction.

The obtained micropictures directly correlating with the data provided by dynamic light scattering demonstrate that at the emulgator concentration near 3.86 $\mu\text{g/ml}$, nanoparticles of homogeneous distribution from 100 to 200 nm are formed.

Optical properties of the synthesized nanogels are characterized by clear Tindale cones at laser irradiation transmission of 650 nm wave length. These characteristics show colloid size of hydrogel particles in the range of 30 to 1000 nm. The synthesized nanohydrogels are also characterized by intensive opalescence accompanied by dichroism- they are blue in the reflected light, while in the transmitted light the nanohydrogels become yellow. Such intensive opalescence is known to be observed when the particle size does not exceed 1/10 of the wave length, that is from 38 to 75 nm for visible light. This phenomenon testifies to the presence of significant number of nanoparticles of the size considerably smaller than 200 nm. This reveals itself at the initial phase of the distribution curves obtained by means of dynamic light scattering [Fig.1].

The synthesized nanogels are characterized by distinct phase transition between swollen and collapsed state observed at about 32 C that fully coincides with the relevant parameter of the hydrogels of similar chemical composition synthesized before in macro state [4].

Temperature of macrogel phase transition for such macrogels was found gravimetrically, however this is practically unattainable in case of nanogels. Nevertheless, analogous clear dependence was established by measuring temperature dependence of the hydrogel nanodispersion light transmission. As is seen in Fig.2, at the temperature lower than 30 C, the hydrogels have expanded conformation, while on heating above 35 C phase transition to a compact collapsed state is observed due to breaking of H-bonds between water molecules and hydrophil amide groups of N-isopropylacrylamide under the influence of Brownian motion and intensification of hydrophobic interactions of polymer isopropyl groups. Light transmission of water dispersions in this case drops dramatically. Temperature of phase transition for the nanoferrgel containing magnetite constitutes about 35 C, and its transparency is slightly smaller than that for non-filled nanogel.

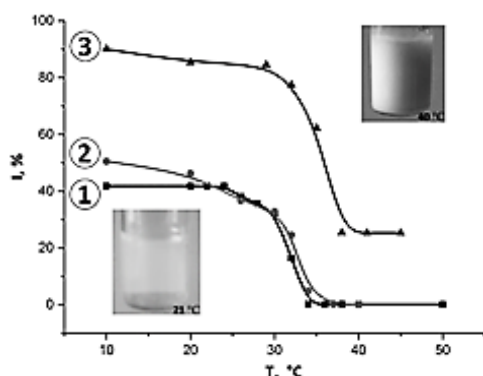


Fig. 2 – Nanogel light transmission change versus temperature (1-15.3 $\mu\text{g/ml}$ SDS; 2-30 $\mu\text{g/ml}$ SDS; 3-15.3 $\mu\text{g/ml}$ SDS + 1.3 mg/ml of magnetite.) Insertion – Nanogel (1) look at 21 and 40 C.

Incorporation of the previously synthesized nanosized magnetite (Fig.3) in the nanogel composition imparts magnetically controlled properties to relevant nanoferrgels, that is ensures an addressed localization

under the influence of constant magnetic field. The nanoferrgels containing 8 and 13 mass% of magnetite in the entire monomer were synthesized. It should be noted that prior investigations, where macrogels of similar chemical composition were used, proved the absence of cytotoxicity towards two lines of cells of revaccinated testicles of piglets and larynx cancer cells Hep-2 for magnetite concentration from 0 to 20.5 and 33%, respectively [7].

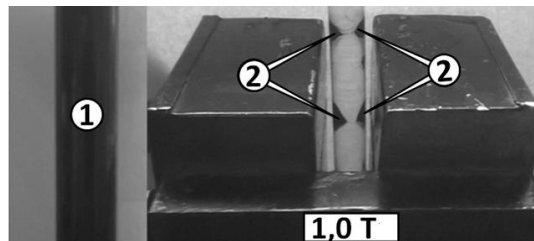


Fig. 3 – Nanomagnete concentration under constant magnetic field with induction 1 T. 1-nanomagnete without magnetic field application; 2-nanomagnete under magnetic field.

According to the data obtained by means of dynamic light scattering, the distribution curve peak constitutes about 15 nm of the particle size in the absence of the nanomagnete stabilizer. The nanogel size of ex situ incorporated magnetite is about 200 nm and actually is identical to the corresponding parameter of non-filled hydrogel matrix.

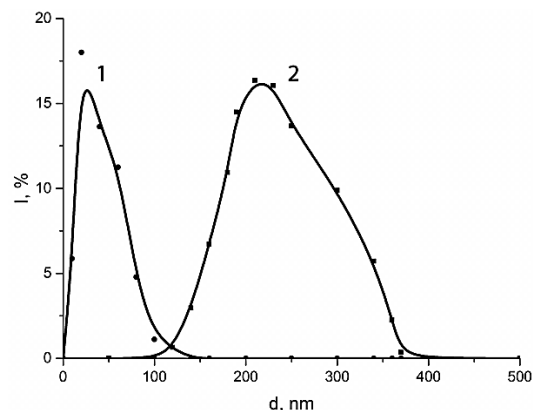


Fig. 4 – Distribution curves of synthesized nanomagnete (1) and magnetite-filled hydrogel (2) according to dynamic light scattering.

Methylene blue was used as a model compound to study heat-initiated diffusion of incorporated drugs from thermoresponsive hydrogel matrices. The compound demonstrates intensive absorption bands at 290 and 760 nm thus allowing control of its diffusion by UV-spectroscopy. In addition, this compound is used in medicine as an efficient antibacterial means and a photosensibilizer in photodynamic therapy [12].

NiPAA-based thermoresponsive nanogels behave as water reservoirs, and on heating spontaneously release an incorporated drug passing to the collapsed state. If at phase transition temperature, 4 mg/g of a photosensibilizer is released during 10 minutes, and 6 mg/g -during 30 minutes, then at 45 C the corresponding parameters are 3- time increased and constitute 12 and 19 mg/g, respectively.

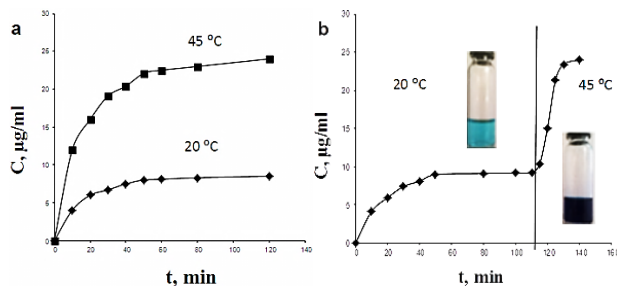


Fig. 5 – Methylene blue release kinetics from nanoferragels at 20 C and 45 C -(a) and changes of methylene blue release rate from nanoferragels vs. temperature changes from 20 C to 45 C (b). Insertion – Nanodispersion colour changes on heating.

After the photosensibilizer prior washing out at 20 C, its diffusion stops in 1 hour at the mentioned tempera-

ture, while on heating above phase transition temperature, intensive drug release is observed (Fig.5) and its concentration increases several times.

5. CONCLUSION

Thus the present studies enabled the development of synthetic techniques for NiPAA-based nano-sized hydrogels with an average particle size of approximately 200 nm. The hydrogel shows distinct phase transition between swollen and collapsed states on heating above 32 °C. It is shown that imparting of magneto-responsive properties to hydrogel can be achieved via ex situ incorporation of magnetite nanoparticles with an average size of about 15 nm, thus creating conditions for their addressed localization at a specific organ.

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