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SYNTHESIS AND CHARACTERIZATION OF MULTI-RESPONSIVE NANO-SIZED FERROGEL BASED ON N-ISOPROPYLACRYLAMIDE AND ACRYLIC ACID

Synthesis and characterization of promising multi-responsive nano-sized hydrogel composites based on N-isopropylacrylamide, Acrylic Acid (AAc) and magnetite have been studied. Copolymer gel was used as a carrier of various drugs as well as pH and thermo-responsive component, magnetite was used as a magneto-responsive component. Composite nanoparticles were characterized by electron microscopy (TEM) and dynamic light scattering (DLS) methods. Diafiltration method was used for purification of nanogels. It was shown that the average size of nanoparticles is about 100 nm and depends on the temperature and pH of suspension. The hydrogel is characterized by clear phase transition between swollen and collapsed state upon heating above 32 °C and changing of pH value upon 4. Rapid release of incorporated drugs, used in cancer chemotherapy (photosensitizer – Methylene Blue and cytotoxic agent Doxorubicin) observed during thermo-responsive nanocomposite gels heating in the physiologically acceptable range, but still above phase transition temperature (about 40-45 °C), allows application of designed drug delivery systems in medical hyperthermia.

Keywords: *thermosensitive nanogel, ferrogels, N-isopropylacrylamid, magnetite nanoparticles, dynamic light scattering, controlled drug release, Doxorubicin*

Було синтезовано та досліджено перспективні термо- та pH-чутливі гідрогелеві композити на основі N-ізопропілакриламіду, акрилової кислоти та магнетиту. Сополімерний гель використовувався як носій різноманітних лікарських засобів, а також як термо- та pH-чутливий компонент; наночастинки магнетиту використовувались в якості магніточутливої складової композиту. Наночастинки композиту охарактеризовано за допомогою електронної мікроскопії та методу динамічного світлорозсіювання. Для очистки наногелів використано діафільтрацію. Показано, що середній розмір наночастинок становить близько 100 нм і залежить від температури та pH середовища. Гідрогелі характеризуються чітким фазовим переходом між набухлим та сколапсованим станом при нагріванні вище 32 °C та при зміні pH вище 4. Швидке вивільнення інкорпорованих ліків, які використовують в хіміотерапії раку (фотосенсибілізаттор Метиленовий Синій та цитотоксичний препарат Доксорубіцин) спостерігалося при нагріванні термочутливого гелю у фізіологічно прийнятному діапазоні, але вище температури фазового переходу (близько 40-45 °C), що дозволяє використання розробленої системи доставки ліків при медичній гіпертермії.

Ключові слова: *термочутливий наногель, ферогелі, N-ізопропілакриамід, наночастинки магнетиту, динамічне розсіювання світла, контрольоване вивільнення лікарських препаратів, доксорубіцин*

Были синтезированы и исследованы перспективные термо- и pH-чувствительные гидрогелевые композиты на основе N-изопропилакриламида, акриловой кислоты и магнетита. Сополимерный гель использовался в качестве носителя различных лекарственных средств, а также как термо- и pH-чувствительный компонент; наночастицы магнетита использовались в качестве магниточувствителый составляющей композита. Наночастицы композита охарактеризованы с помощью электронной

микроскопии и метода динамического светорассеяния. Для очистки наногелей использовалась диафильтрация. Было показано, что средний размер наночастиц составляет около 100 нм и зависит от температуры и pH среды. Гидрогели характеризуются четким фазовым переходом между набухшим и сколлапсированным состоянием при нагревании выше 32 °C и при изменении pH выше 4. Быстрое высвобождение инкорпорированных лекарств, которые используются в химиотерапии рака (фотосенсибилизатор Метиленовый Синий и цитотоксический препарат Доксорубицин) наблюдалось при нагревании термочувствительного геля в физиологически приемлемом диапазоне, но выше температуры фазового перехода (около 40-45 °C), что позволяет использовать разработанную систему доставки лекарств при медицинской гипертермии.

Ключевые слова: *термочувствительный наногель, феррогели, N-изопропилакриламид, наночастицы магнетита, динамическое рассеяние света, контролируемое высвобождение лекарственных препаратов, доксорубицин*

Over the past few decades, polymeric nanocarriers from synthetic and natural polymers such as polymeric micelles, selfassembly, and nanogels have been extensively investigated, in efforts to develop a drug delivery system with enhanced bioavailability for anti-cancer drugs, coupled with minimized side effects [1–3]. The nano-size of these carriers provides superior cancer targeting, particularly with regard to the effect of enhanced permeation and retention, which increases their potential for localization in tumor sites [3].

Temperature and pH have been utilized as candidate signals for establishing cancer targeting drug-carrying system and for controlling the release kinetics of incorporated drugs [4]. In particular, fast drug-release kinetics from the drug-carrying system at cancer sites and slow release before approaching cancer sites have been accomplished by externally heating the local cancer site [5] or by exploiting the physical properties of component polymers that can recognize the intrinsic pH differences between cancers and normal tissues [6]; the extracellular pH in most cancers is more acidic (pH 6.5–7.2) than in normal tissues. Also magnetic hyperthermia heating and external control over the drug delivery system has imbibed the interest of researchers to use magnetic nanoparticles as suitable drug carriers [7-8].

Many hydrophilic polymers, such as poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), dextran, have been used to prolong blood circulation time of drugs and drug carriers. Furthermore, some temperature-sensitive polymers had a transition between hydrophilic and hydrophobic properties at lower critical solution temperature (LCST), and could be used for enhanced passive targeting of specific tissues [9]. For example, poly(N-isopropylacrylamide) (pNIPAM), the most investigated temperature-sensitive polymer, could reduce the adsorption of serum proteins and increase the corresponding nanomedicine circulation times *in vivo* below LCST [10,11]. Recent studies [12-15] indicated that p(NIPAM-co-AAc)(PNA) nanogels were hydrophilic under physiological condition with prolonged circulation time, whereas became hydrophobic and improved cell uptake at acidic environment of tumor tissue. Due to the pH-dependent LCST, PNA nanogel can be developed as an intelligent drug vehicle. Thus, doxorubicin-loaded p(N-isopropylacrylamide-co-butyl methylacrylate) nanogels–iohexol dispersions (IBi-D) were reported for the first time for therapy of liver cancer [16].

Drug delivery may be significantly benefited by the use of magnetite nanoparticles (MNPs), because the chemotherapeutic drugs attached to these particles have the ability to target a specific site in the body, such as a tumor, by applying an external magnetic field, thereby reducing the systemic distribution of cytotoxic compounds *in vivo*, enhancing uptake at the target site and resulting in effective treatment at lower doses [17-18]. Magnetic iron oxide particles with no surface coatings or modifications have hydrophobic surfaces with a large surface area-to-volume ratio. This leads to the agglomeration of particles and formation of large clusters, resulting in increased particle size. This inherent aggregation behavior of MNPs is a crucial limiting factor that reduces the intrinsic super paramagnetic properties. Pre-coating or surface engineering of MNPs not only minimizes aggregation, stabilizes the NP suspension *in vitro*, governs their *in vivo* fates, and

minimizes remnant magnetization, but it also makes them biostable, biodegradable and nontoxic. Both synthetic and natural polymers have been employed to modify MNP surfaces [19].

The emergence of multi-stimuli responsive drug carriers has opened the door to a new generation of anti-cancer drug delivery systems that are more intelligent and more effective than conventional ones. Among multi-stimuli responsive drug vehicles, dual temperature/pH-stimuli-triggered carriers have been reported [20, 21] because many pathological processes such as inflammation, tumor, and infraction in various tissues and organs are present either a local temperature increase (by 2–5 °C) or decrease in pH (1– 2.5 pH units) [22, 23].

Nanogels are being explored as drug delivery agents for targeting cancer due to their easy tailoring properties and ability to efficiently encapsulate therapeutics of diverse nature through simple mechanisms. Nanogels are proficiently internalized by the target cells, avoid accumulating in non-target tissues thereby lower the therapeutic dosage and minimize harmful side effects [23]. Nanogels show much faster responsiveness as compared to the conventional hydrogels. Multi-stimuli responsive nanogels are more effective in targeted therapy for cancer as compared to single responsive nanogels [24].

Among multi-stimuli responsive drug vehicles, dual temperature-/pH-stimuli responsive carriers find wider application in cancer therapy because in cancer there are changes in the temperature and pH of body tissue and these two signals could be regulated easily by external triggers [25].

Qiao et al. prepared a new type of triply responsive nanogels by mini emulsion radical copolymerization of monomethyloligo(ethylene glycol) acrylate and ortho ester-containing acrylic monomer, 2-(5,5-dimethyl-1,3-dioxan-2-yloxy) ethylacrylate, with bis(2-acryloyloxyethyl) disulfide as a crosslinker [26]. The thermo/pH/redox responsive behavior of the nanogels is depending on the composition and crosslinking of the polymer.

The above mentioned facts show that development of the techniques of obtaining nano-sized thermo-responsive hydrogel drug carriers and methods of magnetic particles incorporation in their composition is of primary importance. In the present study we have prepared thermo- and pH-sensitive nano-sized hydrogel containing magnenite nanoparticles by suspension polymerization technique. The most important parameters for the prepared hydrogels were studied.

Experimental

Materials. N-isopropylacrylamide, NIPAA (Sigma-Aldrich, 97 %) was recrystallized from hexane and dried under vacuum; N,N'-methylenebisacrylamide (MBA) (Merck,98 %), acrylic acid (AAc) was purified by distillation and subsequent fractional distillation, potassium persulphate, PSP (Sigma 98 %) were used without addition purification, as well as sodium dodecylsulphate (SDS), polyethylenimine (Sigma-Aldrich MM 2000 Da) and iron salts ($FeSO_4$ and $FeCl_3$) used in magnetite synthesis. Methylene Blue was recrystallized from 50 % aqueous solution of ethanol and dried in vacuum.

Synthesis of magnetite nanoparticles

Briefly, 0,541 g of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and 0.268 g of ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) (molar ratio 2:1, respectively) were dissolved in 10 mL of deionised water and stirred vigorously under N_2 atmosphere at 70 °C. After 0,5 h, 1,6 mL of ammonium hydroxide (25 %) was rapidly injected into the mixture, stirred for another 15 min and then cooled to room temperature. The black precipitate was separated by magnet and the particles were washed five times with hot water.

After that 6.4 g of 50 % polyethylenimine in 10 ml water solution was added to magnetite nanoparticles mixture for functionalization of magnetite by amino-groups and to stabilize nanoparticles by coating low molecular weight polymer. The mixture was stirred for 1 h and then washed five times and then volume of suspension was achieved to 50 ml. The final concentration on magnetite was 4g/l. Prepared nanoparticles suspension was sonicated to get uniform dispersion.

TEM images were obtained by employing a JEOL JEM 1230 device with a 100 kV accelerating voltage. The fig. 1 shows that nanoparticles size is not exceed 20 nm.

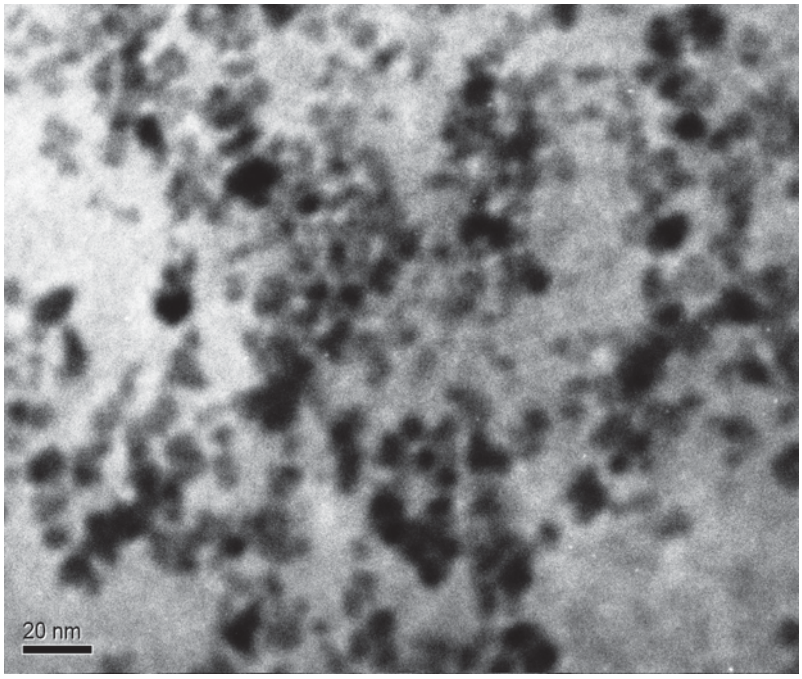

Fig. 1. TEM micrographs of magnetite nanoparticles

Syntesis of nanoferrogels

2,3 g of NIPA, 0,0393 g of MBA, 0,1124 g of SDS, 5 ml of magnetite suspension, 0,115 g of AAc and 135 g of water were placed in the beaker. Then the beaker was set on a magnetic stirrer to dissolve the reagents at room temperature. At the end of the mixing solution in the beaker was purged with argon for 2 minutes. Then it was transferred into the glass reactor equipped with a stirrer and thermometer. The reactor was placed in a water bath with thermometer connected to the relay to maintain a constant temperature. The synthesis was carried out at 68-70 °C. When the reactor temperature reaches 68-70 °C, 10 ml of 0,93 % solution of PSP in water was added. The mixing rate was 500 rotations per minute. The duration of the synthesis was additional 6 hours. During the synthesis reactor was purged with argon with a periodicity of 1-2 minutes every half hour. Reactor with nanogel solution was left to the next day and then filtered through filter paper to eliminate mechanical impurities.

Purification of nanogel suspension

Purification of nanosized hydrogels were carried out by repeated replacing of the solvent. For this purpose diafiltration method was used. Diafiltration was carry out on standard cylindrical ultrafiltration stirring cell Amicon 8050 (Millipore, USA) with a surface area of 13.4 cm^2. A cell is equipped with a magnetic stirrer, the speed of the mixer was 250 ± 10 min^{-1}. Experiments on filtration were carried out at 293 ± 2 °K and pressure of 300 kPa using compressed nitrogen. Diafiltration performed on polysulfone ultrafiltration membranes P100 with cut off 100 kDa (Microdyn Nadir). The volume flux of water throat membranes was 150-250 l/m^2h at pressure of 300 kPa. Degree of purification was evaluated by spectrophotometrically measurement of monomer concentration at 225 nm.

Morphological observations of nanogels

To observe the nanogels in a dried state, a drop of nanogel was dispersed in water and placed on a carbon-coated copper grid then dried under vacuum at 30 °C. Samples were observed after carbon coating using Transmission Electron Microscope PEM-125 K (SELMI).

The DLS measurements were performed using the Malvern Zetasizer Nano S instrument (Malvern Instruments Ltd, Malvern, United Kingdom) equipped with a He–Ne laser operated at λ = 633 nm having an operational range of 0.6 nm–6 μm. The hydrogel sample solution (5 mL) was poured into a glass cuvette. Each sample was retained inside the instrument for the period of time

required to reach a constant temperature (25, 37 or 50 °C) before the laser was passed through the sample. Data from a three measurements at different temperatures were recorded for each sample. Data on particles size were collected in three modes: intensity PSD, volume PSD, and number PSD. Were also analyzed such parameters as mean size (z-average diameter), and the width of the distribution (polydispersity index) according to the International Standard ISO13321, Methods for Determination of Particle Size Distribution.

The light transmission of nanogel suspension was investigated using UV spectrophotometer at a wavelength of 500 nm. The kinetics of phase transition was studied by determination of nanogel light transmission. For this purpose nanogel suspension was heated over temperature of phase transition (50 °C) and intensity of light transmission was measured during nanogel cooling.

The kinetics of methylene blue release was studied with Specord M40 spectrophotometer, absorption maximum was observed at 670 nm.

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. Fig. 2 shows transmission electron microscope (TEM) images of synthesized nanogels with incorporated magnetite nanoparticles. TEM results revealed the uniformity of nanoparticles in size and shape with smoothed topology and a mean diameter of approximately 100 nm.

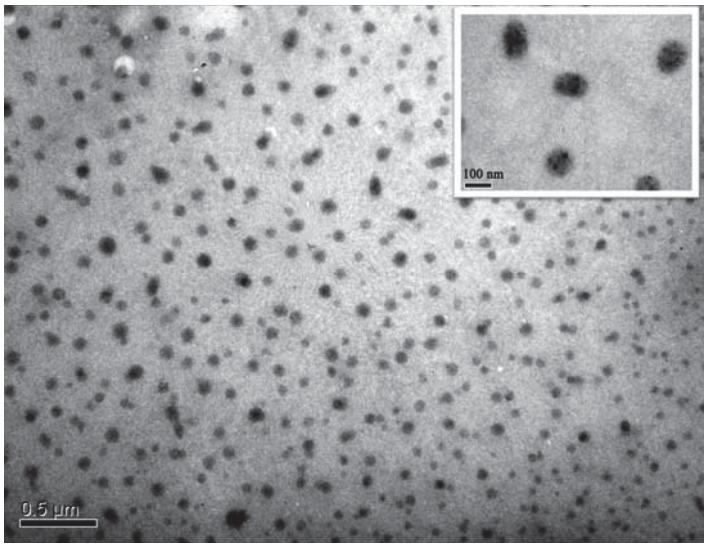

Fig. 2. TEM microphotografs of nanogel with magnetite nanoparticles.

The obtained images directly correlated with the data provided by dynamic light scattering. Representative DLS spectra of individual replicates are expressed as percentages of total intensity or volume or number. Fig. 3 shows that one or three size populations were observed in the intensity-based spectra (a, d), two or one in the volume-based view (b, f), and one in the number-based examinations (c, i). Up to 1-μm-sized particle, was observed in the intensity-based and the volume-based particles size distributions (PSDs) and nanosized in the number-based PSDs. The supramicrometer-sized population observed is noted to occur near the maximum of the analytical range (0.6 nm–6 μm) of the instrument. Because the intensity-based data are biased toward larger particles due to their ability to scatter light thousands of times more than smaller particles, the volume- and number-based PSDs provide a more realistic representation of the importance of each peak [28]. Thus fig. 3c demonstrates that nanoparticles of homogeneous distribution near 100 nm are formed.

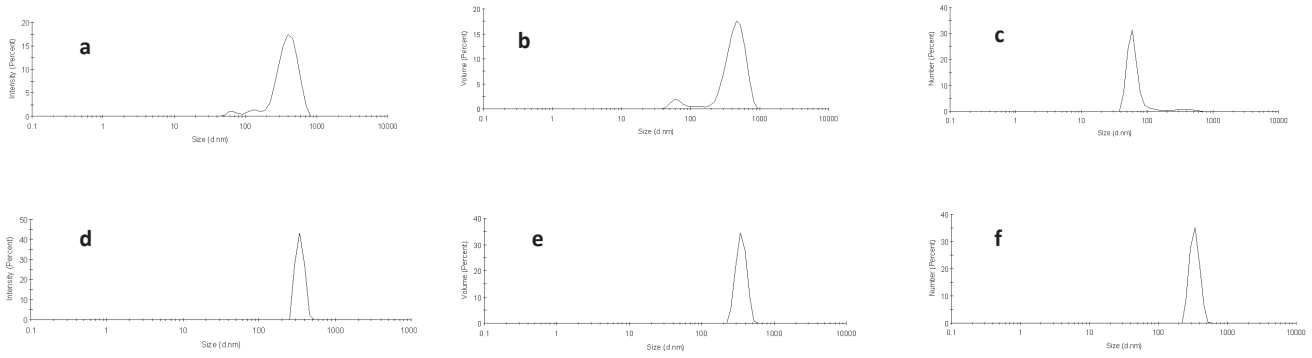

Fig. 3. Intensity-, volume-, and number-based particles size distributions (PSD) spectra unpurified nanogel samples of (a–c) and purified by diafiltration (d–f)

Taking into account medical scope of application of synthesized nanogels extremely important problem is washing them from unreacted monomers. Gelling reaction is never 100 % by output, and practically all acrylic monomers are toxic, unlike the corresponding polymers. In the case of medical macrogels, their cleaning is performed by prolonged extraction in an appropriate solvent (preferably water), with its multiple replacement. The process of the complete washing takes several days and requires the consumption of large amounts of solvent (hundreds of times the mass of hydrogel medical devices). In this work for nanogel purification and separation from unreacted monomers diafiltration have been successfully applied. Diafiltration is an ultrafiltratiom membrane technique based on replacement of solvent in constant volume. So the initial concentration of nanogel is constant that predict nanoparticles conglomerations. For this process were selectively used permeable (porous) membrane filters with cut off 100 kDa. That means membrane separate the components of suspensions with MW more than 100 kDa. Smaller molecules such as monomer, solvents, and water pass freely through the ultrafiltration membrane in permeate. The Fig.4 demonstrated the NiPA concentration in permeate vs number of solvent replacement. It was found that the initial concentration of unreacted NIPA immediately after synthesis significantly exceeded the maximum allowable level. After a five-fold solvent replacements concentration of monomer decreased 50 times, and after 7-fold - more than 500 times.

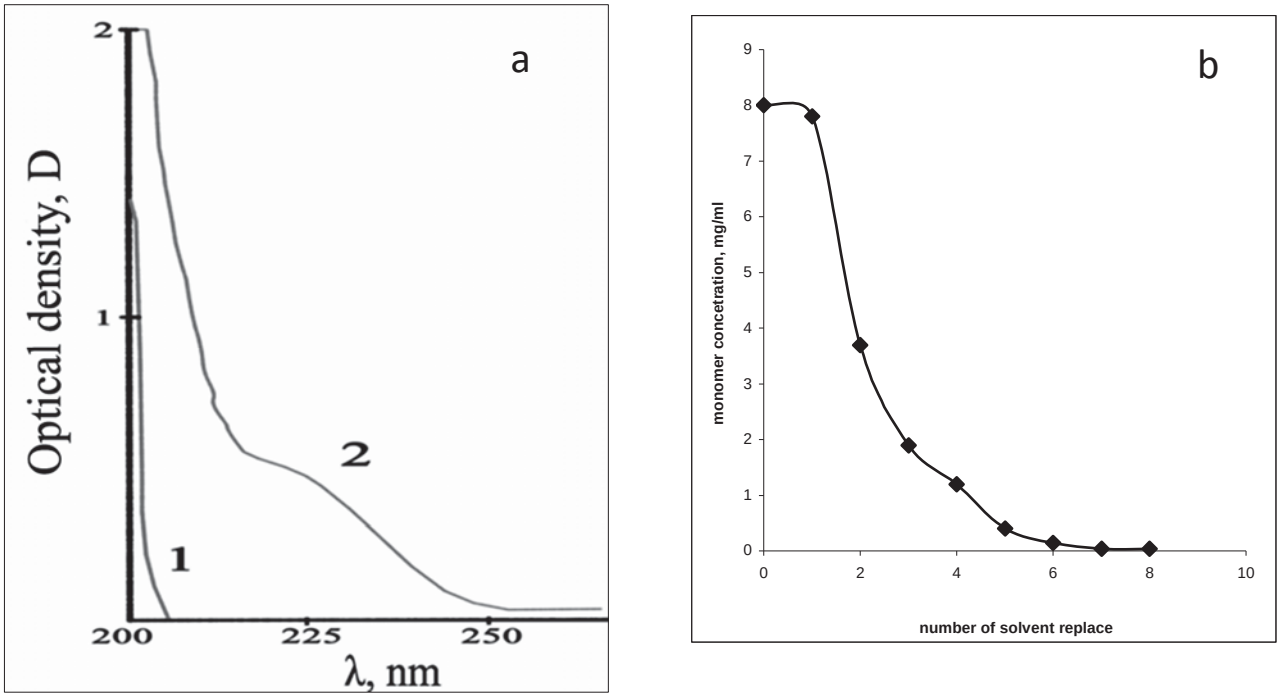

Fig. 4. Concentration change of NIPA monomer during nanogel purification by diafiltration: a (1) – purified, a (2) – unpurified; b – reduction of monomer concentration.

As can see from Fig. 3 (d-f) the average size of nanoparticles gels after their diafiltration increases significantly. Increasing of nanogels size associated with separation of surfactant (sodium dodecyl sulfate) from nanogel dispersions which leads to a reduction of their stabilizing influence. However, even after purification the size of nanogels do not exceed 300 – 500 nm.

Temperature of hydrogel phase transition for macrogels can be found gravimetrically, however this is practically unattainable in case of nanogels. Nevertheless, analogous clear dependence was established by measuring temperature dependence of the hydrogel nanodispersions light transmission. As is seen in Fig. 5, 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 hydrophilic 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 nanogel containing magnetite constitutes about 35 °C.

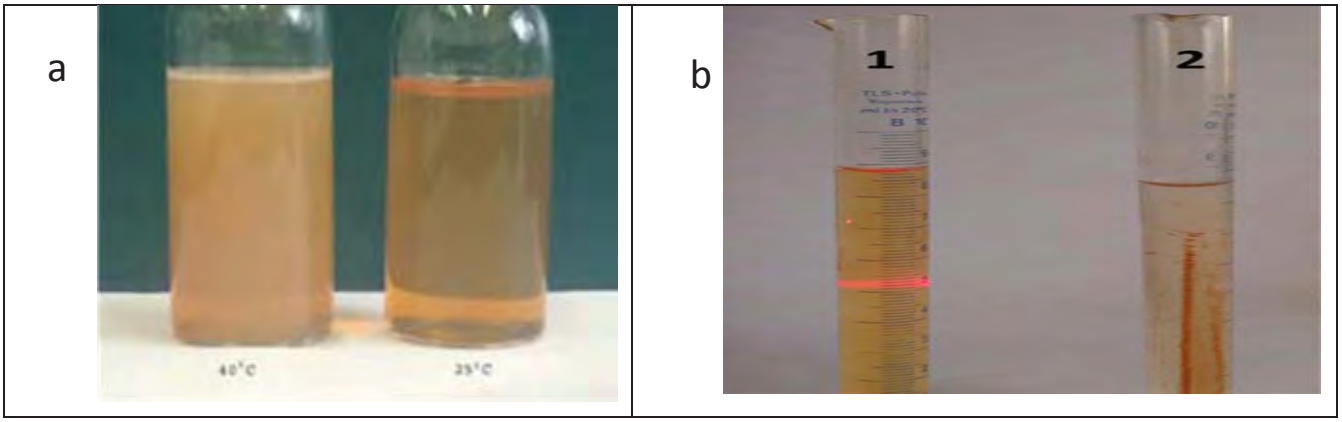

Fig. 5. a – nanogel with MNPs looks at 25 and 40 °C. b – concentrating of nanoferogel under the influence of a constant magnetic field: 1- nanoferogel without imposing magnetic field (with demonstration of Tyndall cone), 2 - nanoferohel at imposing a magnetic field with induction 1 T.

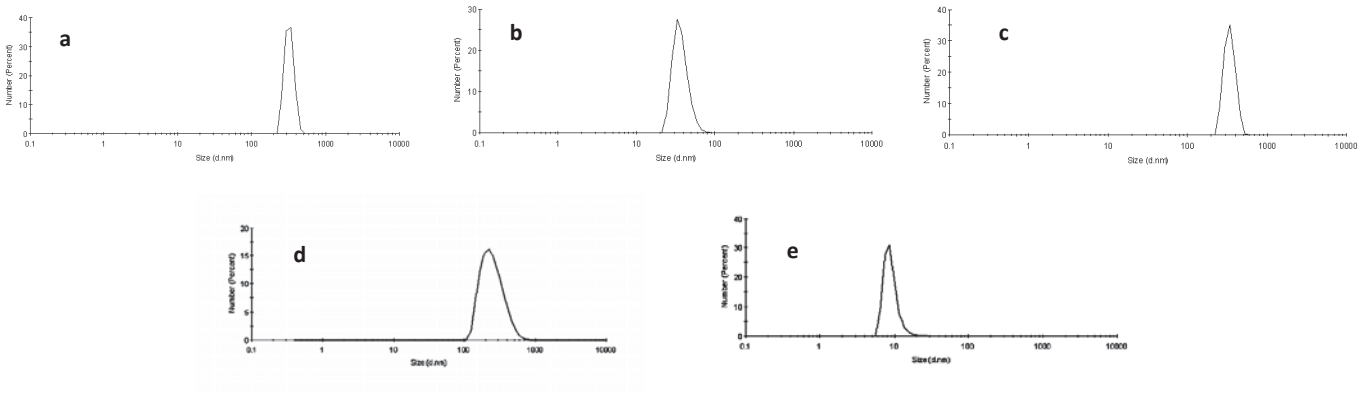

Fig. 6. Number-based PSD spectra of nanogels samples with MNPs at different temperature: a – 25 °C, b – 37 °C and c – 25 °C and different pH: d - pH 10, e - pH 2.

Fig. 6 demonstrates that pH and temperature has significant influence on nanogels size. Thus in acidic pH nanogels have size about 10 nm (fig. 6e). In the base conditions size of nanoparticles rather increased more than 10 times. At the same time, the increase in temperature from 25 to 50 °C leads to a significant (5-6 times) reduce their size. Note that the specified process is reversible. Further cooled of nanogels to the starting temperature returns their size to baseline values (fig. 6 a-c).

The scattering curves on Fig. 7a show that the phase transition for the NiPA–based nanogels is observed at temperature about 32 °C. Adding magnetite nanoparticles does not change phase transition temperature of polyisopropylacrylamide. But after purification of nanogel suspension some changes were observed. The phase transition temperature shifts several degrees higher and reach 34-35 °C. In addition, the phase transition kinetics study showed that after purification the rate of the suspension phase transition is also reduced. So purification reduces the speed of the phase transition from 35 to 11 min^{-1}. Obviously this behavior of the phase transition after purification is connected with increase of average size of particle in suspension.

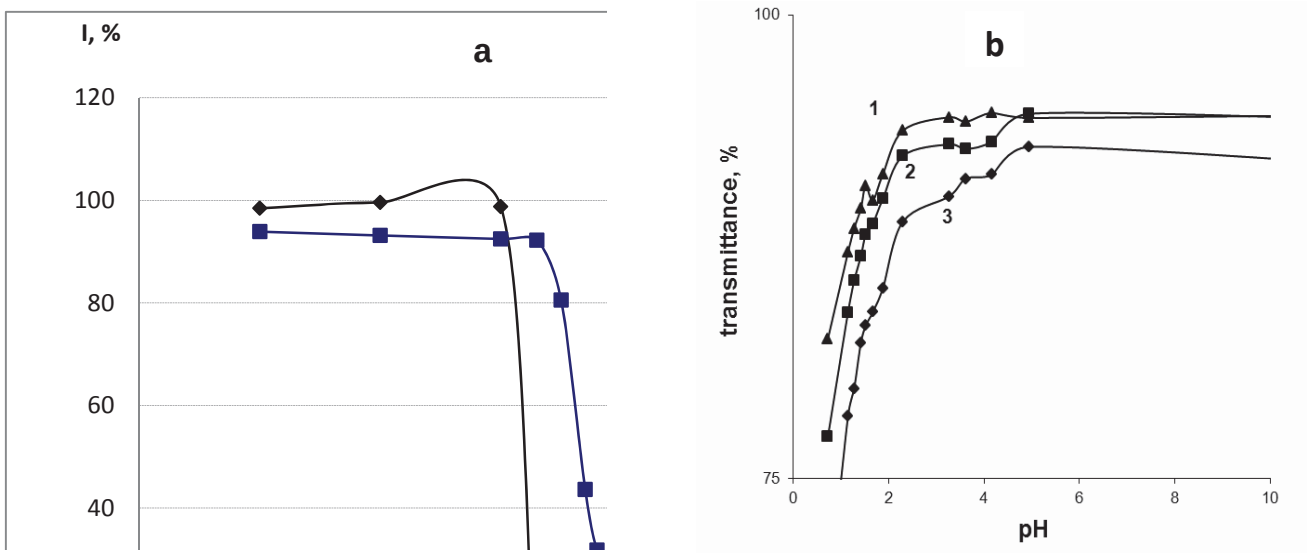

Fig. 7. Nanogel (1.05 % NIPA, 0,26 % AAc) light transmission change versus temperature (a) and pH (b) at a constant T (17 °C) with different wave lengths: 1 – 550 nm, 2 – 500 nm, 3 – 440 nm.

On the other hand, with increasing pH (over 4) due to the electrostatic repulsion between similarly-charged dissociated ionic carboxyl groups of the acrylic acid component, macromolecule alters its conformation, from the collapsed compact – into swollen unfolded, which is accompanied by the sharp change of the light transmission (Fig. 7b).

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-vis spectroscopy. In addition, this compound is used in medicine as an efficient antibacterial agent as well as a photosensitizer in photodynamic cancer therapy.

Fig. 8a demonstrates Methylene Blue release from obtained nanogels at temperature before and after phase transition. As can be seen different kinetic curves was observed. At the temperature before phase transition (20 °C) equilibrium concentration of 7 mg/l achieved within one hour. Over the same period of time at a temperature temperature 50 °C when nanogels transformed into a compact collapsed state threefold released of drug is observed.

Fig. 8b shows that after the photosensitizer prior washing out at 20 °C, its diffusion stops in 1 hour at the mentioned temperature, while on heating above phase transition temperature, intensive drug release is observed and rate of release increases several times.

The thermo-initiated diffusion from nanoferrogels were also investigated applied to incorporated Doxorubicin – medication, commonly used in cancer chemotherapy (Fig. 9). When ferrogels heated above the LCST the diffusion rate increases threefold.

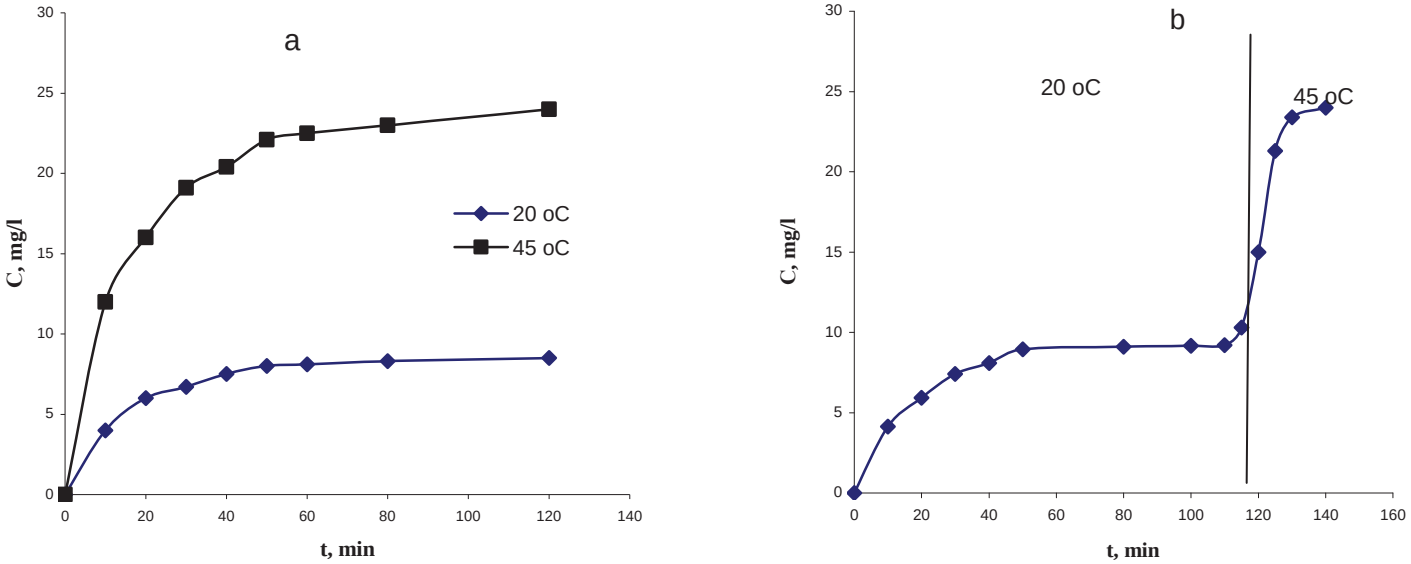

Fig. 8. Methylene blue release kinetics from nanogels at 20 °C and 45 °C (a) and changes of methylene blue release rate from nanogels vs. temperature changes from 20 °C to 45 °C (b)

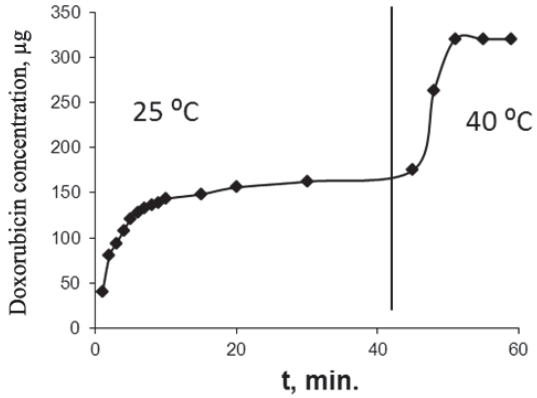

Fig. 9. Doxorubicin release from nanogels at 20 °C and 40 °C

Conclusion

Thus the present studies enabled the development of synthetic techniques for NiPAA-based nano-sized hydrogels with an average particle size about 100-200 nm. Hydrogels demonstrate clear phase transition between swollen and collapsed states on heating above 32 °C and upon acidification to a pH value below 5. It is shown that imparting of magnetoresponsive 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 near the target organ. Synthesized nanoscale ferrogels have been studied using electron microscopy (TEM) and by dynamic light scattering (DLS) methods. Obtained nanogels were purified by diafiltration method. The particles size distribution spectra show that nanogels size increased after diafiltration which is explained by reducing of concentration of stabilizing agent. Also the nanogels size depends on temperature and pH. In collapsed state after temperature of phase transition the nanogels size reduced to 20 – 50 nm and upon acidification – to10-20 nm. Rapid release of the incorporated drugs, used in cancer chemotherapy (photosensitizer – Methylene Blue and cytotoxic agent Doxorubicin) observed during thermo-responsive nanocomposite gels heating in the physiologically acceptable range, but still above phase transition temperature (up to 40–45 °C), allows application of the discussed drug delivery systems in medical hyperthermia.

REFERENCES

1. ***W.Park, S.Park, K. Na.*** Potential of self-organizing nanogel with acetylated chondroitin sulfate as ananti-cancer drug carrier. // Colloids and Surfaces B: Biointerfaces. – 2010. – V. 79. – P. 501-508.
2. ***K.S. Soppimath, T.M. Aminabhavi, A.R. Kulkarni, W.E. Rudzinski.*** Biodegradable polymeric nanoparticles as drug delivery devices. // Journal of Controlled Release. – 2001. – V. 70. – P. 1–20.
3. ***S. Mitra, U. Gaur, P. Ghosh, A. Maitra.*** Tumour targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. // Journal of Controlled Release. – 2001. – V. 74. – P. 317–323.
4. ***Nam Muk Oh, Kyung Taek Oh, Hye Jung Baik, Bo Reum Lee, A. Hyeong Lee, Yu Seok Youn, Eun Seong Lee.*** A self-organized 3-diethylaminopropyl-bearing glycol chitosan nanogel for tumor acidic pH targeting: In vitro evaluation. // Colloids and Surfaces B: Biointerfaces. – 2010. – V. 78. – P. 120–126.
5. ***A.M. Ponce, Z. Vujaskovic, F. Yuan, D. Needham, M.W. Dewhirst.*** Hyperthermia mediated liposomal drug delivery // Int. J. Hyperthermia. – 2006. – V. 22. – P. 205–213.
6. ***Y. Bae, N. Nishiyama, S. Fukushima, H. Koyama, M. Yasuhiro, K. Kataoka.*** Preparation and Biological Characterization of Polymeric Micelle Drug Carriers with Intracellular pH-Triggered Drug Release Property: Tumor Permeability, Controlled Subcellular Drug Distribution, and Enhanced in Vivo Antitumor Efficacy // Bioconjug. Chem. – 2005. – V. 16. – P.122–130.
7. ***E. Alison Deatsch, A. Benjamin Evans.*** Heating efficiency in magnetic nanoparticle hyperthermia. // J. Magn. Magn. Mater. – 2014. – V. 354. – P. 163–172.
8. ***TejabhiramYadavalli, Shivaraman Ramasamy, Gopalakrishnan Chandrasekaran, Isaac Michael, Helen Annal Therese, Ramasamy Chennakesavulu.*** Dual responsive PNIPAM–chitosan targeted magnetic nanopolymers for targeted drug delivery. // Journal of Magnetism and Magnetic Materials. – 2015. – V. 380. – P. 315–320.
9. ***Lingyu Jiang, Qing Zhou, Ketao Mu, Hui Xie, Yanhong Zhu, Wenzhen Zhu,Yanbing Zhao, Huibi Xu, Xiangliang Yang.*** pH/temperature sensitive magnetic nanogels conjugated with Cy5.5-labled lactoferrin for MR and fluorescence imaging ofglioma in rats. // Biomaterials. – 2013. – V. 34. – P. 7418-7428.
10. ***Yallapu M.M., Jaggi M., Chauhan S.C.*** Design and engineering of nanogels forcancer treatment. // Drug Discov Today. – 2011. – V. 16. – P. 457-63.
11. ***Saunders B.R., Laajam N., Daly E., Teow S., Hu X., Stepto R.*** Microgels: from responsive polymer colloids to biomaterials. // Adv Colloid Interface Sci. – 2009. – V. 147-48. – P. 251-62.
12. ***Chen H., Zhu H., Hu J., Zhao Y., Wang Q., Wan J., et al.*** Highly Compressed assembly of deformable nanogels into nanoscale suprastructures and their application in nanomedicine. // ACS Nano. – 2011. – V. 5. – P. 2671-80.
13. ***Xiong W., Gao X., Zhao Y., Xu H., Yang X.*** The dual temperature/pH-sensitive multiphase behavior of poly(N-isopropylacrylamide-co-acrylic acid) microgels for potential application in in situ gelling system. // Colloids Surf. B Biointerfaces. – 2011. – V. 84. – P. 103-10.
14. ***Xiong W., Wang W., Wang Y., Zhao Y., Chen H., Xu H., et al.*** Dual temperature/pH-sensitive drug delivery of poly(N-isopropylacrylamide-co-acrylic acid)nanogels conjugated with doxorubicin for potential application in tumorhyperthermia therapy. // Colloids Surf. B Biointerfaces. – 2011. – V. 84. – P. 447-53.
15. ***Zhao Y., Zheng C., Wang Q., Fang J., Zhou G., Zhao H., et al.*** Permanent andperipheral embolization: temperature-sensitive p(N-isopropylacrylamideco-butyl methylacrylate) nanogel as a novel blood-vessel-embolic material in the interventional therapy of liver tumors. // Adv. Funct. Mater. – 2011. – V. 21. – P. 2035-42.
16. ***Kun Qian, Yingying Ma, JiangshanWan, ShinanGeng, Han Li, Qianwen Fu, XiaolePeng, Xuefeng Kan, Guofeng Zhou,Wei Liu, Bin Xiong, Yanbing Zhao, Chuansheng Zheng, Xiangliang Yang, Hui Bi Xu.*** The studies about doxorubicin-loaded p(N-isopropyl-acrylamide-

co-butyl methylacrylate) temperature-sensitive nanogel dispersions on the application in TACE therapies for rabbit VX2 liver tumor. // Journal of Controlled Release. – 2015. – V. 212. – P. 41–49.
17. ***L. Agiotis, I. Theodorakos, S. Samothrakitis, S. Papazoglou, I. Zergioti, Y.S.*** Raptis Magnetic manipulation of superparamagnetic nanoparticles in a microfluidic system for drug delivery applications. // Journal of Magnetism and Magnetic Materials. – 2016. – V. 401. – P. 956-964.
18. ***Thayyath.S. Anirudhan, Peethambaran.L. Divya, Jayachandran Nima*** Synthesis and characterization of silane coated magnetic nanoparticles/glycidylmethacrylate-grafted-maleated cyclodextrin composite hydrogel as a drug carrier for the controlled delivery of 5-fluorouracil. // Materials Science and Engineering: C on ScienceDirect, 1 October 2015. – 2015. – V. 55. – P. 471-481.
19. ***Dilnawaz F., Singh A., Mohanty C., Sahoo S.K.*** Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. // Biomaterials. – 2010. – V. 31. – P. 3694–3706.
20. ***Guo S., Qiao Y., Wang W., He H., Deng L., Xing J., Xu J., Liang X.-J., Dong A.*** Poly(e-aprolactone)-graft-poly(2-(N,N-dimethylamino) ethyl methacrylate) nanoparticles: pH dependent thermo-sensitive multifunctional carriers for gene and drug delivery. // J. Mater. Chem. – 2010. – V. 20. – P. 6935–6941.
21. ***Chang B., Sha X., Guo J., Jiao Y., Wang C., Yang W.*** Thermo and pH dual responsive, polymer shell coated, magnetic mesoporous silica nanoparticles for controlled drug release. // J. Mater. Chem. – 2011. – V. 21. – P. 9239–9247.
22. ***Liu C., Guo J., Yang W., Hu J., Wang C., Fu S.*** Magnetic mesoporous silica microspheres with thermo-sensitive polymer shell for controlled drug release. // J. Mater. Chem. – 2009. – V. 19. – P. 4764–4770.
23. ***Zhang L., Guo R., Yang M., Jiang X., Liu B.*** Thermo and pH dual responsive nanoparticles for anticancer drug delivery. // Adv. Mater. – 2007. – V. 19. – P. 2988–2992.
24. ***Govind Soni, Khushwant S. Yadav.*** Nanogels as potential nanomedicine carrier for treatment of cancer: A mini review of the state of the art. // Saudi Pharmaceutical Journal. – 2014;
25. ***Morinloto N., Qiu X.P., Winnik F.M., Akiyoshi K.*** Dual stimuli-responsive nanogels by self-assembly of polysaccharides lightly grafted with thiol-terminated poly(Nisopropylacrylamide) chains. // Macromolecules. – 2008. – V. 41. – P. 5985–5987.
26. ***Qiao Z.Y., Zhang R., Du F.S., Liang D.H., Li Z.C.*** Multiresponsive nanogels containing motifs of ortho ester, oligo(ethyleneglycol) and disulfide linkage as carriers of hydrophobic anti-cancer drugs. // J. Control. Release. – 2011. – V. 152. – P. 57–66.
27. ***C. A. Staples, S. R. Murphy, J. E. McLaughlin, H. W. Leung, T. C. Cascieri and C. H. Farr.*** Determination of selected fate and aquatic toxicity characteristics of acrylic acid and a series of acrylic esters // Chemosphere. – 2000. – V. 40.1. – P. 29-38.
28. ***Milad Rabbani Esfahani, Holly A. Stretz, Martha J.M.*** Wells. Abiotic reversible self-assembly of fulvic and humic acid aggregates in low electrolytic conductivity solutions by dynamic light scattering and zeta potential investigation. // Science of the Total Environment. – 2015. – V. 537. – P. 81–92.

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