Synthesis and Characterization of Hybrid Chitosan/magnetite Nanocomposite Fluid

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The purpose of this work is to develop a simple, cheap and reproducible method for the synthesis of stable magnetic fluid nanocomposite chitosan/magnetite with particle size less than 100 nm and to study some of its physical and chemical properties. As a result, the method of synthesis of polyfunctional hybrid magnetic liquid nanocomposite chitosan/magnetite/sodium oleate with multilevel architecture was developed. With methods of small-angle neutron scattering, infrared Fourier spectroscopy, and transmission electron microscopy the structural characterization of the obtained magnetic nanofluids was performed. The formation of aggregates in magnetic fluids is established based on results from small-angle neutron scattering. The scattering signal from fractal particles was obtained simultaneously with the signal of small-angle neutron scattering for polydispersed spherical particles in the studied liquid systems. The mechanism of interaction of sodium oleate with chitosan in the system of chitosan/magnetite/sodium oleate is proposed. It has been shown that the binding between magnetite/sodium oleate and chitosan can be explained by the electrostatic interaction between a positively charged chitosan molecule and negatively charged coating of sodium oleate on the surface of magnetite. Strong electrostatic interaction also leads to the proton transfer from oleic acid to chitosan, thus creating a stable complex. The synthesized nanocomposite can be potentially used for targeted drug delivery and deposition of drugs in target cells and organs, for the diagnostics and therapy of diseases at the cellular level.

Keywords: Chitosan, Magnetite nanoparticles, Nanofluids, Small-angle neutron scattering, Hierarchical nanostructure.

1. INTRODUCTION

Control of the widespread diseases is an essential component of efforts currently focused on extending duration of human life. Tackling this interdisciplinary problem relies on the achievements of various fields such as medicine, biology, chemistry, physics, biomedicine, molecular biology, biophysics, biochemistry, quantum mechanics, etc. Extensive studies cover the properties of DNA structural units [1-3], the mechanisms of interaction agents with elementary cells of a living organism [4], methods of targeted drug delivery to the affected areas of the body cells [5], synthesis and properties of nanostructured materials [6, 7], new methods of numerical and experimental research of many characteristics of nanoparticles and biomolecules [8, 9].

One of the most promising trends in biomedicine during the recent years is focused on creating "nanoclinics" – the multilevel magnetosensitive nanocomposites with more than one level of organization of their chemical structure, having a functional characteristic to nanorobots, such as recognition of specific microbiological objects in biological media, targeted drug delivery to organs or cells, diagnostics and treatment of diseases at cellular and/or even molecular levels, adsorption of cells, decomposition of the drug and/or hyperthermia therapy by-products and removing them from the body using magnetic fields.

Researchers' interest in magnetosensitive biocompatible nanocomposites arises from the possibilities to control their motion in biological medium by external magnetic field. The most promising nanomaterial for magnetic drug delivery is magnetite (Fe_3O_4) nanoparticles. The characteristic features of magnetite Fe_3O_4 are low toxicity, high level of mutagenic safety, absence of adverse reactions in the organism after intravenous, intraarterial and intramuscular injections of magnetosensitive colloid. A promising application field for magnetic materials with advanced surface is preparation of their magnetosensitive nanocomposites with natural biopolymers (e.g. chitosan). In particular, combination of chitosan and magnetite properties opens the way to creation of new effective pH-controllable drug delivery and release systems with high biocompatibility.

In order to use nanoparticles for in vivo biomedical applications, they must be in the form of stable aqueous colloidal suspensions, be biocompatible, and have a size smaller than 100 nm. Particles over 100 nm are detected by the immune system and eliminated [10].

There is a number of articles reporting on synthesis and properties of magnetite/chitosan nanomaterials. For instance, a hydrogel, chitosan crosslinked carboxymethyl-β-cyclodextrin (CM-β-CD) polymer covering Fe_3O_4 magnetic nanoparticles was synthesized in [11]. However, the average diameter of the composite particles in [11] was estimated to be 80-200 nm. In [6], magnetic nanocapsules, based on N-palmitoyl chitosan and magnetite and loaded with anticancer drug doxorubicin, were prepared using a double emulsion method. SEM micrographs showed that magnetic nanocapsules prepared in this way had a narrow size distribution in

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the range of 100-150 nm and dynamic light scattering measurements evidenced an average hydrodynamic size of magnetic nanocapsules to be 215 ± 23.33 nm [6]. Authors of [12] reported synthesis of doxorubicin-conjugated Fe_3O_4 nanoparticles and chitosan-coated nanocarriers. The final product (Fe_3O_4-doxorubicin-chitosan) showed an average diameter of 136 nm with a polydispersity index of 0.493 [12]. As an another example [13], magnetic nanoparticles double coated with different concentrations of dextran sulfate or reduced dextran and chitosan solutions were formed by layer-by-layer deposition. The second layer of chitosan gave the nanoparticles the hydrodynamic diameters ranging from 230 nm to 250 nm. The resulting colloidal systems were stable over the time period of 60 days [13]. Similarly, PLGA/magnetite composite particles obtained by the method [14] had a size of around 200 nm.

At the same time, colloidal stability of the above-mentioned systems is not clear from the cited papers. Indeed, most of the SEM and/or TEM images presented in [6, 11-13] demonstrate substantial presence of aggregates, which may cause the formation of blood clots and blocking of blood vessels thus preventing the use of these nanoparticles in biomedical applications. In addition, the reported size of the particles is greater than 100 nm, which is not desirable for in vivo biomedical applications. Thus, the problem of creation of stable magneto-sensitive biocompatible colloids remains relevant.

The main goal of this paper is to develop a simple, cheap, and reproducible synthesis procedure for creation of stable magnetite/chitosan nanocomposite magnetic liquids with particles size smaller than 100 nm and to investigate some physicochemical properties of the obtained nanofluid.

2. EXPERIMENTAL METHODS

All the chemicals were of analytical grade and were used as purchased. Double distilled water was used as a solvent whereas sodium oleate ($C_8H_{17}CH = CH(CH_2)_7CO - O^-Na^+$) was used as the surfactant. Chitosan (low molecular weight, 50-190 kDa) was purchased from Sigma. Chitosan solution was prepared by dissolution of chitosan in 1 % acetic acid.

Preparation of magnetite. 24 g of ferrous chloride ($FeCl_2$) and 48 g of Ferric chloride solution ($FeCl_3$) were dissolved in 1 l of deionized water. This solution was added dropwise to 250 ml of ammonia solution (NH_4OH, 25 % in water). Black precipitate was collected and washed several times by distilled water to pH = 7.

The synthesis of magnetite was carried out by the co-precipitation of iron salts according to the reaction:

$$Fe^{+2} + 2Fe^{+3} + 8NH_4OH \rightarrow Fe_3O_4 + 4H_2O + 8NH_4^+$$
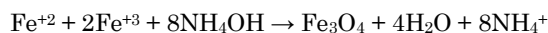
Preparation of magnetic fluid (magnetite/sodium oleate nanofluid). To obtained magnetite, 15 g of sodium oleate was added and the suspension was kept on water bath (80°C) for 1 hour with stirring. Obtained magnetic fluid with magnetite concentration of 50 mg/ml was used as a concentrate for further experiments.

Preparation of chitosan/magnetite magnetic fluid (chitosan/magnetite/sodium oleate nanofluid). Magnetite was coated with chitosan by physical sorption process in acidic media (chitosan/magnetite nanocompo-

site). To 25 ml of 1 % chitosan solution 6 ml of magnetic fluid concentrate was added and solution was sonicated in ultrasound bath over 15 min. Obtained composite was collected by permanent magnet and washed with water 20 times. Chitosan/magnetite nanocomposite was additionally stabilized by agitation with of 0.1 g of sodium oleate and 15 ml of water with heating to 75 °C during 30 min (sodium oleate as a surfactant provides additional solubility of chitosan and whole stability of the colloidal system). Aggregates were separated by permanent magnet. Nanoparticles were obtained by centrifugation of colloidal solution at 15000 rpm during 15 min.

Fourier transform infrared spectroscopy (FTIR) of the samples was performed by a «Perkin Elmer» (model 1720H) spectrometer. 20 µl of magnetic fluid was dried on KBr transparent glass, and transmittance spectra were collected in the wavenumber range of 500-4000 cm^{-1}.

Small-angle neutron scattering (SANS) experiment was carried out on the small-angle neutron time-of-flight YuMO instrument at the pulsed IBR-2 reactor at the Joint Institute for Nuclear Research. A two-detector set-up with ring wire detectors were used. The neutron scattering vector module range was 0.05-0.80 nm^{-1}. The measured scattering curves were corrected for background scattering from buffer solutions. For absolute calibration of the scattered intensity during the measurements, a vanadium standard was used. For the measurements, the solutions were put in 1 mm thick quartz plain cells (Helma) and kept at room temperature.

Transmission electron microscopy (TEM) was carried out using Transmission Electron Microscope JEOL 2010.

3. RESULTS AND DISCUSSION

Obtained nanosized magnetite had a zero-residual magnetization and was in superparamagnetic state so that each particle can be considered as being a single-domain but interparticle magnetic interaction can still be neglected. Such a state is important for biomedical applications due to its convenience for magnetic field-driven transportation applications. Such magnetite nanoparticles (Fig. 1) were used for the following modifications.
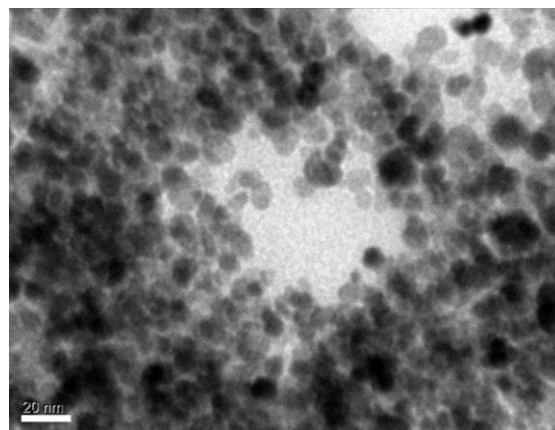

Fig. 1 – TEM-image of initial magnetic fluid concentrate

Chitosan is soluble in water only in acidic media (pH = 2-6). At pH = 2-6, chitosan swells and its chains undergo deploying due to electrostatic repulsion of positively charged $-NH_3^+$ groups (Fig. 2). Magnetite nano-

particles are positively charged in aqueous media from pH = 2 to pH = 8, this is why negatively charged oleate residue has good adsorption of magnetite surface. SANS experiment was done to perform structural characterization of the synthesized magnetic composite at the nanoscale. From the SANS data we can see scattering from polydisperse nanoparticles with radius of gyration $R_g \approx 7.5$ nm as well as SANS signal from quite big nanoparticles with radius of the gyration $R_g \approx 7.5$ nm as well as SANS signal from quite big aggregates (size > 100 nm) (Fig. 3).
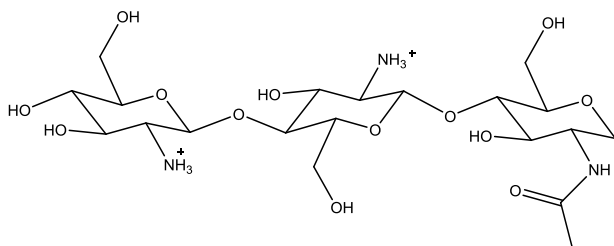

Fig. 2 – Scheme 1. Structure of protonated chitosan molecule monomer unit
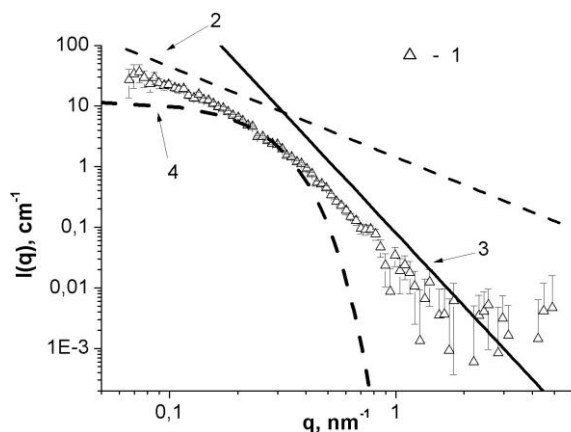

Fig. 3 – SANS scattering spectrum for liquid system of magnetite nanoparticles covered with chitosan. 1 – primary particles of magnetic fluids chitosan; 2 (aggregates) and 3 correspond to power laws $I \sim q^{-1.5}$ and $I \sim q^{-4}$ respectively. Dashed line 4 corresponds to Guinier approximation for aggregates with radius of gyration $R_g = 7.5$ nm

The nature of the latter can be deduced from the behavior of the scattering curve in the range of small scattering vector module q. This behavior can be described by the power law with the exponent 1.5, so that according to the theory and interpretation of small-angle scattering [15] it can be inferred that the aggregates correspond to branched rod-like mass fractal. Due to their quasi one-dimensional particles with smoothed surface exist in the system as well. It should be mentioned that SANS structure these 'aggregates' can be thought to be rather fragile in contrast to the bulk ('three-dimensional') aggregates observed in [10-13]. In large q-values we can see power law behavior with the power exponent 4 which means that individual polydisperse spherical results are in good correlation with previous results for similar kind magnetic fluids and its component [16-18]. Also obtained SANS and TEM data on the particle sizes agree between each other rather well.

Obtained nanofluids were characterized by FTIR spectroscopy (Fig. 4). In the FTIR spectrum of chitosan/magnetite and magnetite/sodium oleate magnetic fluids (Fig. 4), the broad adsorption band from 3600 to 3100 cm^{-1} corresponds to the stretching vibrations O–H of hydroxyl groups. Adsorption bands at 2920 and 2851 cm^{-1} were attributed to the asymmetric and symmetric CH$_2$ stretch vibrations, respectively.

The peaks at 1637 and 1564 cm^{-1} were derived from the existence of the symmetric (vs) and asymmetric (vas) stretching vibrations of –COO$^-$ group, respectively. Strong adsorption band at 1564 cm^{-1} indicates non-coordinated –COO$^-$ group due to the excess of sodium oleate in solution. Adsorption band at 1417 cm^{-1} can be attributed to –OH bending vibrations (Fig. 4, line 1).

According to the literature data, binding of –COO$^-$ group to the magnetite surface can be characterized by four types of complexation: monodentate, bridging (bidentate), chelating (bidentate), and ionic interaction. Type of carboxylate interaction with magnetite surface can be distinguished by measuring wavenumber separation (Δ) between v_{as} and v_s. If Δ is smaller than 110 cm^{-1}, binding is bidentate. According to that, we can conclude from the obtained FTIR spectrum that sodium oleate interacts with magnetite by two oxygen atoms [19, 20] of carboxylic group (Fig. 5).

Reduction of adsorption band (v_{as}) absorption intensity in chitosan/magnetite spectra as compared to the magnetite/sodium oleate one can be attributed to the amide absorption band and can be explained by electrostatic interaction between the nitrogen atom of the amino group of the chitosan and carboxyl group of oleic acid (Fig. 6).

From the FTIR data, we can also conclude that the sodium oleate interacts with chitosan by electrostatic mechanism. This process can affect the charge distribution of chitosan molecule, which can result in shrinking of chitosan and lead to the formation of weak aggregates. This conclusion is in good agreement with SANS data.

Summarizing SANS and FTIR data, the following mechanism responsible for structural reorganization at chitosan addition to aqueous ferrofluids can be proposed (Fig. 7).

In the proposed model, the bonding of magnetite/sodium oleate with chitosan can be attributed to the electrostatic interaction between the positively charged chitosan molecule and negatively charged sodium oleate coating on magnetite surface. Strong electrostatic interaction also leads to the proton transfer from oleic acid to chitosan thus creating a stable complex.

4. CONCLUSIONS

Treatment of the most threatening diseases requires the development of effective methods for directed transport of drugs, due to which healthy tissues will not be exposed to the molecules of potentially highly toxic drugs. One of the methods for achieving the necessary concentration of drug molecules in a given region of a living organism is the use of nanoparticles as a carrier of the drug and control of their movement within the body with the help of a magnetic field. Nanoparticles of magnetite are the most promising nanomaterial for magnetoconveiled drug delivery.
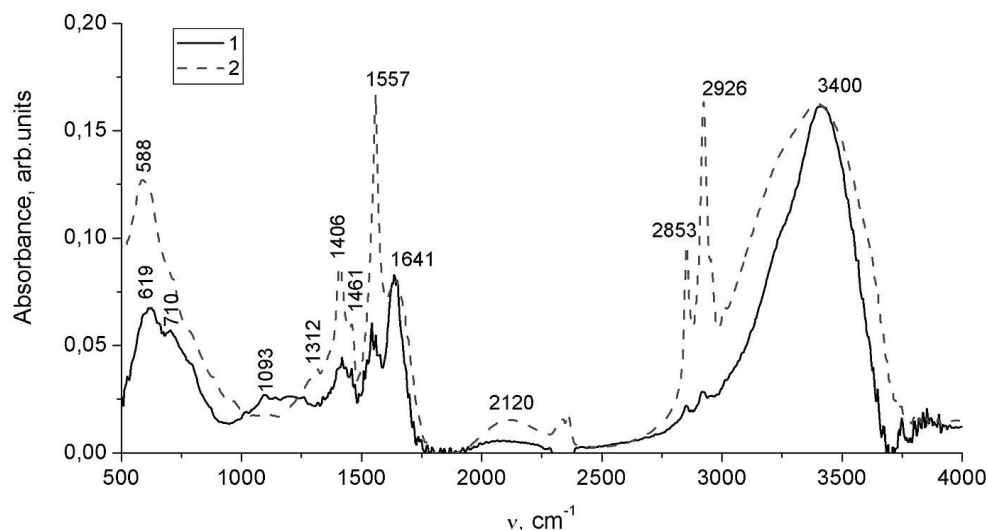
Fig. 4 – FTIR spectra of magnetite/sodium oleate (line 1) and chitosan/magnetite/sodium oleate (line 2) magnetic fluid
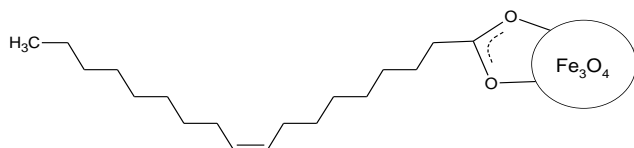

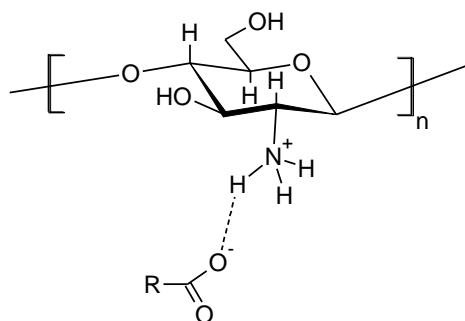**Fig. 5** – Scheme 2. Sodium oleate interaction with magnetite surface

Fig. 6 – Interaction of sodium oleate and chitosan
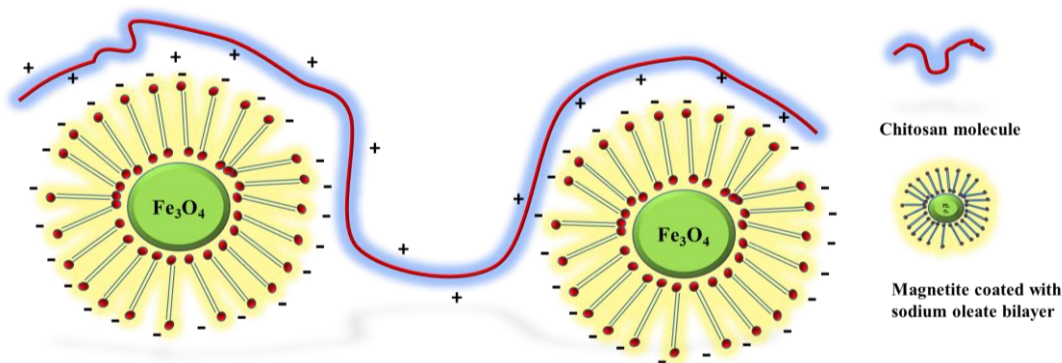

Fig. 7 – A possible mechanism responsible for structural organization at chitosan/magnetite/sodium oleate magnetic fluid

In this paper, the method for the preparation of multifunctional chitosan/magnetite nanocomposites with multilevel hierarchical architecture is developed. Created nanocomposite structure has been characterized by transmission electron microscopy, FTIR spectroscopy and small-angle neutron scattering technique. The results obtained by different methods are in good agreement and confirm that the proposed synthesis procedure achieves its objectives. The synthesized nanocomposite can be used for the targeted drug delivery and deposition of drugs in target cells and organs, for the diagnostics and therapy of diseases at the cellular level. The obtained nanostructures are also promising for preparation of novel medicinal drug forms with high efficiency and complex (cytotoxic, immunotherapeutic and hyperthermic) activity.

REFERENCES

1. O.O. Brovarets, D.M. Hovorun, *J. Biomol. Struct. Dynam.* **37** No 7, 1880 (2019).
2. T.Yu. Nikolaienko, L.A. Bulavin, D.M. Hovorun, *Biopolym. Cell.* **27** No 1, 74 (2011) [in Ukrainian].
3. T.Yu. Nikolaienko, L.A. Bulavin, D.M. Hovorun, *Biopolym. Cell.* **27** No 4, 291 (2011) [in Ukrainian].
4. S. Cai, S. Thati, T.R. Bagby, H.-M. Diab, N.M. Davies, M.S. Cohenc, M.L. Forrest, *J. Control. Release.* **146**, 212 (2010).
5. Jose L. Arias, L. Harivardhan Reddy, Patrick Couvreur, *J. Mater. Chem.* **22**, 7622 (2012).
6. V. Balan, G. Dodi, N. Tudorachi, O. Ponta, V. Simon, M. Butnaru, L. Verestiuc, *Chem. En. J.* **279**, 188 (2015).
7. L.F. Sukhodub, K. Dyadyura, *J. Nano-Electron. Phys.* **10** No 6, 06003 (2018).
8. V. Sureshkumar, S.C.G. Kiruba Daniel, K. Ruckmani, M. Sivakumar, *Appl. Nanosci.* **6**, 277 (2016).
9. Ie.V. Pylypchuk, D. Kołodyńska, M. Kozioł, P.P. Gorbyk, *Nanoscale Res. Let.* **11** No 1, 168 (2016).
10. B.S. Zolnik, Á. González-Fernández, N. Sadrieh, M.A. Dobrovolskaia, *Endocrinology* **151** No 2, 458 (2010).
11. Y. Ding, S.Z. Shen, H. Sun, K. Sun, F. Liu, Y. Qi, J. Yan, *Mater. Sci. Eng.: C.* **48**, 487 (2015).
12. S. Sadighian, K. Rostamizadeh, H. Hosseini-Monfared, M. Hamidi, *Colloid. Surf. B: Biointerface.* **117**, 406 (2014).
13. L. Barbosa-Barros, S. García-Jimeno, J. Estelrich, *Colloid. Surf. A: Physicochem. Eng. Asp.* **450**, 121 (2014).
14. A. Pérez, C. Herrero, C. Mijangos, R. Hernández, *Colloid. Surf. A: Physicochem. Eng. Asp.* **456**, 108 (2014).
15. M.V. Avdeev, V.L. Aksenov, *Phys. Usp.* **53** No 10, 971 (2010).
16. M.V. Avdeev, A.V. Feoktystov, P. Kopcansky, G. Lancz, V.M. Garamus, R. Willumeit, M. Timko, M. Koneracka, V. Zavisova, N. Tomasovicova, A. Jurikova, K. Csach, L.A. Bulavin, *J. Appl. Cryst.* **43**, 959 (2010).
17. V. Závišová, M. Koneracká, M. Múčková, J. Lazová, A. Juríková, G. Lancz, N. Tomašovičová, M. Timko, J. Kováč, I. Vávra, M. Fabián, A.V. Feoktystov. V.M. Garamus, M.V. Avdeev. P. Kopčanský, *J. Magn. Magn. Mater.* **323** No 10, 1408 (2011).
18. V.I. Petrenko, M.V. Avdeev, V. M. Garamus, L.A. Bulavin, P. Kopčanský, *Colloid. Surf. A: Physicochem. Eng. Asp.* **480** No 5, 191 (2015).
19. Y. Ren, K. Iimura, T. Kato, *Langmuir* **17**, 2688 (2001).
20. L. Zhang, R. He, H.-C. Gu, *Appl. Surf. Sci.* **253** No 5, 2611 (2006).

Синтез та характеризація гібридної нанокомпозитної рідини хітозан/магнетит

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Метою даної роботи є розробка простого, дешевого і відтворюваного методу синтезу стабільних нанокомпозитних магнітних рідин хітозан/магнетит з розміром частинок менше 100 нм і дослідження деяких їхніх фізико-хімічних властивостей. В результаті проведених досліджень розроблено метод синтезу поліфункціональних гібридних нанокомпозитних магнітних рідин складу хітозан/магнетит/ олеат натрію з багаторівневою архітектурою. Для структурної характеризації отриманих магнітних нанорідин були використані методи малокутового розсіяння нейтронів, інфрачервоної Фур'є-спектро-скопії та просвічуючої електронної мікроскопії. Методом малокутового розсіяння нейтронів встановлено формування агрегатів в магнітних рідинах. Сигнал розсіювання від фрактальних частинок був отриманий одночасно з сигналом малокутового розсіяння нейтронів для полідисперсних сферичних частинок в вивчаємих рідких системах. Запропоновано механізм взаємодії олеату натрію з хітозаном в системі хітозан/магнетит/олеат натрію. Показано, що зв'язок магнетиту/олеату натрію з хітозаном можна пояснити електростатичною взаємодією між позитивно зарядженою молекулою хітозану і негативно зарядженим покриттям з олеату натрію на поверхні магнетиту. Сильна електростатична взаємодія також призводить до переносу протона з олеїнової кислоти в хітозан, створюючи таким чином стабільний комплекс. Синтезований нанокомпозит може бути використаний для адресної доставки ліків і осадження ліків в клітинах і органах-мішенях, для діагностики і терапії захворювань на клітинному рівні.

Ключові слова: Хітозан, Наночастинки магнетиту, Нанорідини, Малокутове розсіяння нейтронів, Ієрархічні наноструктури.