Diagnostic and analysis of aggregation stability of magnetic fluids for biomedical applications by small-angle neutron scattering

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Abstract. Diagnostics of aggregation and determination of the aggregation regimes and their control in biocompatible magnetic fluids are necessary for their development in biomedical applications. Small-angle neutron scattering (SANS) method was applied in the structure analysis of various types of magnetic fluids for biomedical applications. Additionally the interaction characteristics between surfactant/polymer molecules used in stabilization of magnetic fluids were investigated, which is very important for understanding the synthesis procedure of highly stable magnetic fluids with controllable properties.

1. Introduction
Cancer remains one of the most widespread diseases and leading cause of death worldwide, despite a certain progress in diagnosis and treatment methods in recent years. During last decade researchers have been investigating applications of colloidal particles (or nanoparticles) that could act as delivery systems for targeted cancer drugs [1,2]. The nanoparticles with radii between 1 – 10 nm provides large specific surface for functionalization (e.g. chemically active substances interacting with cancer cells). Along with it, if the magnetic nanoparticles are used, then, their transport and concentration in a given place can be additionally controlled by an external magnetic field, thus increasing the efficiency of the treatment, and, avoiding the spread of the nanoparticles in healthy tissues in the organism. Moreover, new possibilities for employing magnetic properties of the particles in medical diagnostics (magnetic resonance tomography) and therapy (magnetic hyperthermia) of cancer tumors appear [1,2].

Biomedical applications require that magnetic nanoparticles are to be placed in liquid conditions. The corresponding systems are known as magnetic fluids (or ferrofluids) [3].
stabilization of magnetic fluids, the particles are coated with special shells of surfactants or polymers. Despite the fact that magnetic fluids are actively used in technical applications for rather long time, still it is not an easy task to place magnetic nanoparticles in highly polar water without aggregation [4]. The main problem is a strong interaction of surfactants with water, which is competitive with their adsorption on magnetic nanoparticles. At the moment, there are several ways to stabilize water-based ferrofluids, but what concerns the biological media it is not possible to prevent aggregation completely. In biomedical context the formation of aggregates has side effects relating to the difficult elimination of nanoparticles from organisms, the possible appearance of blood clots, as well as the reduce in the therapeutic efficiency. Thus, knowledge of aggregation regimes in magnetic fluids is a key point for their development in biomedical applications. In this connection, important goal is a reliable diagnostics of aggregation and determination of the aggregation regimes and their control in biocompatible magnetic fluids.

For this purpose we develop the method of small-angle neutron scattering (SANS), which is quite sensitive to the aggregation processes in nanosystems. SANS is actively applied in structure research of magnetic fluids [5]. Wide possibilities of the contrast variation (hydrogen/deuterium isotopic substitution) in neutron experiments allow us to ‘look’ inside the aggregates. Also, the additional magnetic scattering of neutrons can be used for studying magnetic correlations in nanosystems with magnetic inclusions. Taking into account the complexity of magnetic fluids (which are mostly polydisperse), reasonable tasks for SANS in this case are to find out the type of aggregates formed in them under different conditions, conclude about their inner nuclear, as well as magnetic structures, and obtain information about interaction between their different components. The present work shows how the SANS method is applied in the structure analysis of various types of magnetic fluids for biomedical applications. Additionally the interaction characteristics between surfactant/polymer molecules used in stabilization of magnetic fluids are investigated, which is very important for understanding the synthesis procedure of highly stable magnetic fluids with controllable properties.

2. Experiments
SANS experiments were performed at the Yellow Submarine small-angle instrument at the steady-state reactor of the Budapest Neutron Centre, Hungary and the SANS-1 instrument located at the Neutron Facility at the Helmholtz Zentrum Geesthacht, Germany. The differential cross-section per sample volume (hereafter referred to as scattered intensity) was obtained as a function of the scattering vector module, \( q = (4\pi/\lambda)\sin(\theta/2) \), where \( \lambda \) is the incident neutron wavelength and \( \theta \) is the scattering angle. At the Yellow Submarine instrument the fixed wavelength of \( \lambda = 0.386 \) nm and \( 1.2 \) nm (monochromatization \( \Delta\lambda/\lambda = 20\% \)) and sample-detector distances of \( 1.3 \) and \( 5.6 \) m (detector size \( 64 \times 64 \) cm\(^2\); resolution \( 1 \times 1 \) cm\(^2\)) were used to cover the \( q \)-interval of \( 0.15-4.5 \text{ nm}^{-1} \). In the measurements performed on the SANS-1 instrument, we used the wavelength \( \lambda = 0.81 \) nm (monochromaticity \( \Delta\lambda/\lambda = 10\% \)) and the sample–detector distances ranging from \( 0.7 \) to \( 9.0 \) m (detector size \( 55 \times 55 \) cm\(^2\); resolution \( 0.7 \times 0.7 \) cm\(^2\)) and, therefore, the measurements were performed in the range of scattering vectors from \( 0.04 \) to \( 2.00 \text{ nm}^{-1} \). All SANS measurements were performed at \( 25 \) °C. The calibration on 1-mm water sample was made after the background, buffer and empty cuvette corrections in a standard way [6].

3. Results and discussion
Different aggregate classes depending on the stabilization mechanism (steric, electrostatic and steric/electrostatic stabilization) of magnetic particles (magnetite) in physiological conditions are studied. In figure 1 example of the SANS contrast variation application [7,8] is given for water-based magnetic fluids used as a source of magnetic nanoparticles in the therapy of the brain cancer glioblastoma. These fluids are characterized by record achievable concentrations of magnetic material (up to 10 vol. %) with keeping high stability [9]. The nanomagnetite was stabilized by double layer of fatty mono-carboxylic acids with short alkene chains, namely lauric (C12) and myristic (C14) acids. Despite high concentration and long time stability, SANS shows the presence of aggregates in the
systems. The found effective match points (see insets to figure 1) are shifted and show a difference in the composition of the aggregates. Schematic views of the fluid structures in figure 1 illustrate the main important conclusion of the analysis: there is a different rate in the surfactant coating of the particles in the aggregates, which is consistent with the longer chain of myristic acid. Among the studied systems in this way are water-based magnetic fluids with substitution of sodium oleate as surfactant by biocompatible polymer polyethyleneglycol at the magnetite surface, which aims at the increase in the life time of magnetic nanoparticles in living organisms by reducing the response of their immune systems. This fluid was used as an initial component in the synthesis of the magnetic carrier containing anticancer drug Taxol. It is revealed that quite large amounts of polymer in the fluid structure results in a decrease in the aggregation stability, thus requiring that an optimal polymer content to be chosen.

Figure 1. SANS contrast variation for 1% biocompatible aqueous magnetic fluids with magnetite coated by double layer of lauric (LA) or myristic (MA) acids. Changes in the scattering curves are followed when varying the content of heavy water (D₂O) in the solvent. In insets the effective match points are found from the change in the forward scattering intensity. The revealed aggregation structures in the studied magnetic fluids are schematically shown to the right.

The aggregation stability of magnetic fluids is directly determined by the behavior of stabilizants (surfactants or polymers) in the solvents [10-15]. Thus, in most cases as a first stage of the synthesis the concentrating solutions of fatty acids are added to the medium, where magnetic nanoparticles are produced. Already at this stage the aggregation of fatty acids themselves can affect the final stability of magnetic fluids. SANS makes it possible to reveal such aggregation and follow the transition from
the isotropic solution to liquid crystalline state with the growth in the solute concentration, as it is shown in figure 2 for organic solutions of saturated mono-carboxylic acids with stiff linear structure. It is obtained that the critical concentration of this transition is inversely proportional to the length of surfactant molecules. Such aggregation decreases the number of free surfactant required for adsorption and further stabilization of magnetic nanoparticles, thus affecting the final stability of the magnetic fluid and explaining why longer saturated fatty acids are worse stabilizants as compared to shorter ones. After the SANS analysis of the interaction of acid molecules one comes to conclusion that the discussed transition goes easier in the presence of magnetic particles. In some cases the formation of micelle-type surfactant aggregates can appear in magnetic fluids. It is also reflected in the SANS curves, which makes it possible to describe quantitatively (in terms of micelle size and concentration) this process [14].

Figure 2. Formation of the liquid crystalline phase in bulk organic solutions of mono-carboxylic acids probed in stabilization of magnetic fluids. Experimental SANS curves from the solutions of stearic (C18) (a) and myristic (C14) (b) acids show the isotropic-nematic phase transition with the growth of the volume fraction of the acids in solutions. The scheme at the bottom illustrates the transition into the liquid crystalline state reflecting in the SANS curves.

4. Conclusions
Summarize we can deduce that SANS method can be successfully applied in the structure analysis of various types of magnetic fluids for biomedical applications. The interaction characteristics between surfactant/polymer molecules used in stabilization of magnetic fluids can be also obtained by SANS. Structures of various types of ferrofluids were found and different rate of magnetic surface covering by stabilizants were observed. The formation of the found LC-phase in bulk solutions of mono-carboxylic acids is an important factor, which influences the stabilization efficiency of the studied acids in colloidal solutions of magnetic nanoparticles.
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