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Lysozyme Amyloid Fibrils Doped by Carbon Nanotubes

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Production of new composites for the creation of modern materials with desired properties is the key feature of nanotechnology. Despite the well known advantages of magnetic nanoparticles, the aim of the present study was to synthesize lysozyme amyloid fibrils from hen egg white and subsequently doped this solution with single walled carbon nanotubes and with the magnetite Fe_3O_4 labelled single walled carbon nanotubes. Transmission electron microscopy and polarization optical microscopy were used to obtain the structural and dimensional information about samples. Measurements of magnetic properties indicate the considerable increase of the saturation magnetization for solutions included the magnetite nanoparticles.

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1. Introduction

Carbon nanotubes (CNT) are anisometric particles, where every CNT looks like a graphene sheet rolled up into tubular morphology or some tubes are arranged concentrically [1]. CNT have exceptional physical and chemical stability, low density, unprecedented mechanical properties, optical and semi- to metallic-conductivities [2]. Their response to the applied magnetic field can be diamagnetic, paramagnetic or ferromagnetic that depends on nanotubes chirality, Fermi energy and diameter [3].

Lysozyme amyloid fibrils (LAF) consist of protofilaments, where the polypeptide aggregates are composed predominantly of cross β-sheet structures. β-strands are arranged perpendicular to the constituent axis while the β-sheets are parallel [4]. It was proved that magnetite nanoparticles can interact with LAF [5]. Since hybrid nanomaterials containing carbon nanotubes may open new strategies in various areas as tissue engineering, biosensors, drug delivery, nanoelectronics and many others, a lot of studies deal with the dispersion of nanotubes in lyotropic liquid crystal (LLC) [6–8]. Liquid crystal phases formed by CNT themselves can improve the quality of carbon nanotubes fibers [9]. It is possible to use LAF solution for stabilization of CNT dispersions and for fabrication protein/CNT conjugates, multifunctional conductive coatings for electronic devices or biocompatible sensors and mechanically strong antibacterial films [10, 11]. LAF and CNT are certainly very different materials, but both consist of highly anisotropic molecules. This combination of big contrasts and a few similarities makes the meeting of CNT and LLC so fruitful, for example, LAF-CNT dispersions are used to produce mechanically strong antibacterial films [12]. Bolisetty and Mezzenga have developed inexpensive hybrid composite membrane consisting of amyloid fibrils (β-lactoglobulin) and activated carbon to remove heavy metal ions and radioactive waste from water [13]. In order to use CNT in wide array of applications, they need to have the following characteristics: to be well dispersed and preferably aligned in the composite as well as to interact strongly with surrounding medium, what is not generally simple for their poor solubility and toxicity [9]. However, the meaningful combination of LLC and CNT composite provides novel properties such as optical response, electronic properties, sensing or antiviral activity. The sensitivity of such LLC-CNT composite to magnetic field may be increased by adding magnetite nanoparticles to mixture [14].

In this paper, we provide new insights into the LAF-CNT dispersion, where the interaction between CNT and LAF was studied. Main aim of this study was the possibility to create stable colloid nanomaterial contained of lyotropic liquid crystal formed by LAF and CNT/Fe_3O_4 which can be manipulated by external magnetic field.

2. Matherials and methods

All chemicals were purchased from commercial sources. Hen egg white lysozyme was obtained from Sigma-Aldrich chemical Company. Lysozyme amyloid fibrils (LAF) were prepared by dissolving of lysozyme powder to ultimate concentration 5 pH/ml in 0.2 M glycine-HCl buffer with 2.2 pH and 80 mM NaCl. The prepared solution was heated for 2 hours at 65 °C during continuous stirring. Single-walled carbon nanotubes (SWCNT) were purchased from Cheap Tubes Inc. The synthesis of magnetite-labeled SWCNT (SWCNT/Fe_3O_4) was described in the article by Mitróová et al [15]. Consequently LAF were doped with SWCNT and with SWCNT/Fe_3O_4. The weight concentration of SWCNT and SWCNT/Fe_3O_4 in LAF solution was 10^{-2}.

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The magnetic measurements were performed by a VSM (Vibrating Sample Magnetometer) in a cryogen free high field measurement system from Cryogenic Limited. The studied samples (40 μl) were inserted in sample containers that were hermetically sealed. The diamagnetic signal induced by the container was subtracted from the total measured of signal.

Low voltage transmission electron microscopy (LVTEM) analysis was performed using as Delong Instruments transmission electron microscope at an operating voltage of 5 kV. Samples were prepared by drop casting of nanoparticles dispersions on 400 mesh ultrathin carbon coated copper grids (Ted Pella) and dried.

The pure SWCNTs were observed by high-resolution scanning-transmission electron microscope (TEM) Jeol 2100F with Schottky field emission cathode and accelerating voltage 200 kV. The point-to-point resolution of TEM in standard mode was 0.19.

Polarising microscope Nikon Eclipse LV100 was used for performing polarising optical microscopy (POM) to determine the orientation of prepared samples in the absence as well as in presence of external magnetic field (MF). Solutions were dropped on a glass slide and observed under bright field, using a lense at 20×0.40 magnification. The ζ-potentials for individual sample were measured using Zetasizer Nano ZS by Malvern Instruments (Germany).

4. Results and discussion

Three basic samples have been prepared: Lysozyme amyloid fibrils solution (LAF), LAF doped with SWCNT and LAF doped with magnetite Fe_3O_4 labelled SWCNT (Fig. 1). Figure 2 shows magnetization curves of

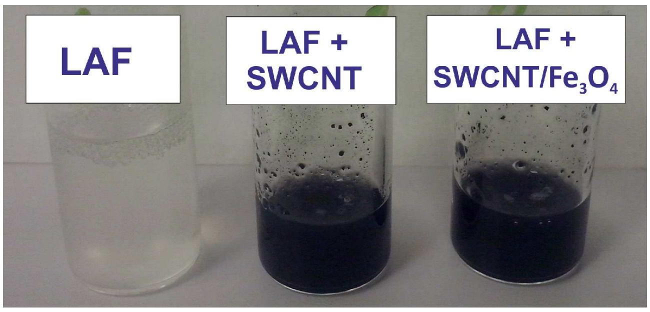

Fig. 1. Pictures of LAF, LAF doped with SWCNT and LAF doped with SWCNT/Fe_3O_4.

LAF, LAF doped with SWCNT and LAF doped with SWCNT/Fe_3O_4. From the observed MF dependence of magnetization it can be seen that for LAF and LAF+SWCNT samples the diamagnetic behavior dominates as the negative magnetization linearly increases with the MF. The weak ferromagnetic behaviour of SWCNT was observed at lower magnetic field. However, the diamagnetic behaviour prevailed at higher magntic field (green curve). This effect is related with SWCNT which are ferromagnetic [3]. On the

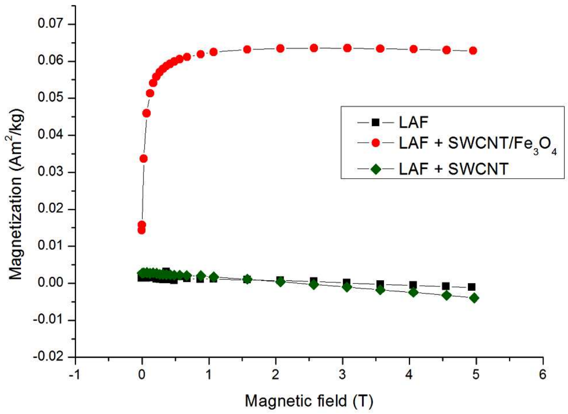

Fig. 2. Magnetization curves of a) LAF, b) LAF+SWCNT, c) LAF+SWCNT/Fe_3O_4.

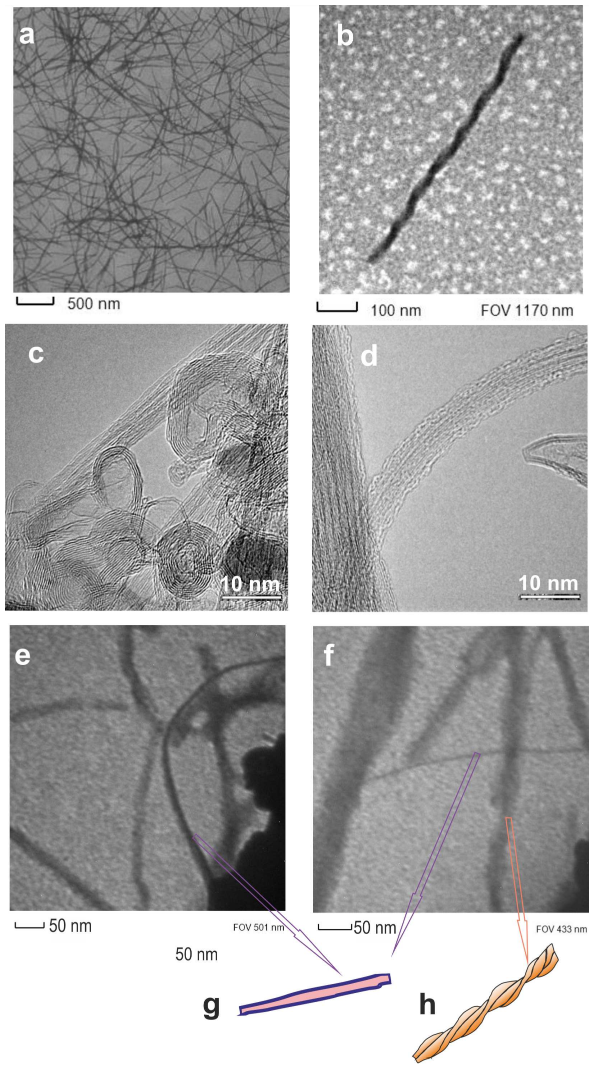

Fig. 3. a) LVTEM image of LAF and b) fibril detail, c) TEM image of SWCNT and d) SWCNT detail e, f) LVTEM images of LAF+SWCNT g) schematic illustration of the SWCNT structure h) schematic illustration of the fibril structure.

other hand, the presence of magnetite nanoparticles in LAF+SWCNT/FE_3O_4 manifests the paramagnetic behaviour. The saturation magnetization of the sample LAF+SWCNT/Fe_3O_4 is 0.0632 Am^2/kg. Figure 3 a presents LVTEM image for undoped LAF. The LVTEM image (Fig. 3b) shows detail structure of one fibril. One can see here the helical structure of prepared fibrils. The structure of pure SWCNT was observed by TEM (Fig. 3c,d). Figure 3e and f illustrate the LVTEM images of LAF+SWCNT that confirm the presence of fibrils and nanotubes in sample. In this case, it can be seen the smooth surface of SWCNT comparing to surface of the fibril. The schematic illustrations of the SWCNT and fibril structure are shown in Fig. 3g and h, respectively.

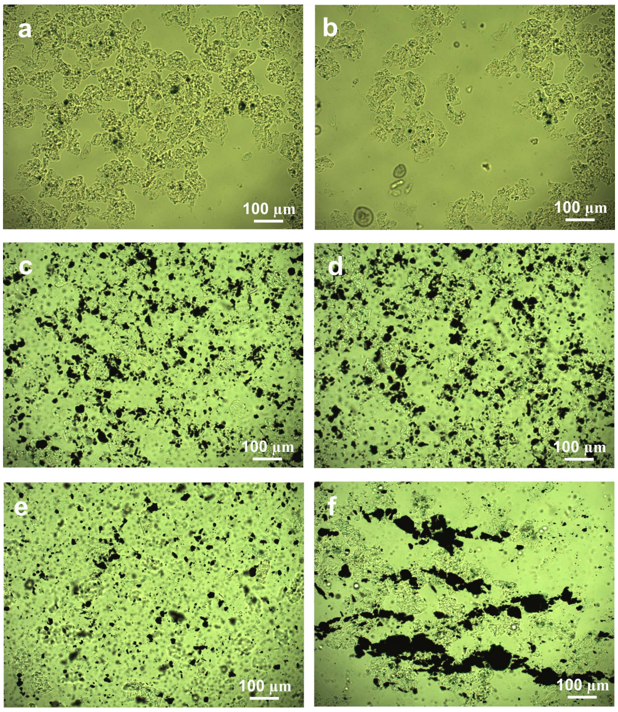

Fig. 4. POM images of a) LAF, b) LAF in external magnetic field of 1.5T c) LAF+SWCNT, d) LAF+SWCNT in external magnetic field of 1.5T, e) LAF+SWCNT/Fe_3O_4 f) LAF+SWCNT/Fe_3O_4 in external magnetic field of 1.5 T.

POM images were obtained for undoped LAF (Fig. 4a), LAF+SWCNT (Fig. 4c) and LAF doped with magnetite labelled single walled carbon nanotubes (Fig. 4e). Subsequently, all this samples were exposed to external magnetic field 1.5 T. In the case of LAF doped with SWCNT/Fe_3O_4, magnetite particles were oriented along the magnetic field direction (Fig. 4f), while LAF doped with SWCNT particles were organized randomly (Fig. 4d). No orientation was observed for undoped LAF solution (Fig. 4b). This suggests that in the case of presence magnetite nanoparticles, it is possible to order such nanocomposite to the direction of the magnetic field. It was confirmed that magnetic field can orient LAF+SWCNT/Fe_3O_4 solution into its direction due to presence of the bonded magnetic particles. The obtained POM images are in good accordance with magnetization measurements.

According to the Zeta potential measurements, Zeta potential of all samples was positive what suggests that interaction between LAF and CNT is due to van der Waals forces. The corresponding values are summarized in the Table I.

The Zeta potential [mV] measurements. TABLE I

LAF	SWCNT	LAF+SWCNT	LAF+SWCNT/Fe_3O_4
61.1	26.9	51.3	48.7

5. Conclusions

In this work characterisation of nanocoposite LAF+SWCNT and LAF+SWCNT/Fe_3O_4 was described.

The results of this research show the possibility for creating hybrid materials based on LAF and CNT. Due to the presence of magnetic particles, the prepared solution exhibit magnetic activity that may lead to new potential applications. Combination of these types of nanomaterials opens new possibilities in wide field of use.

Acknowledgments

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