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Magneto-Optical Study Toward Discrimination of Iron Mineral in Human Tissues

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Magnetic optical rotatory dispersion (MORD) of thin selected biological tissues and thin film of composite made from akaganeite mineral and PVA as well as ferritin and their mimetics aqueous suspensions were performed in spectral range 250–650 nm at room temperature. Good correlation between MORD spectra for akaganeite composite film, ferritin and their mimetics aqueous suspensions with spectra of thin slices of human tissue obtained from white matter of the brain and spleen were observed. Comparison suggest a contribution from Fe(III) to MORD spectra of tissues. This preliminary results show that application of MORD spectroscopy to clinical analysis may be useful.

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1. Introduction

Iron is an element of vital importance to human, but at the same time, it is highly toxic in excess. Free ferrous iron (Fe(II)) can promote free radical damage via Fenton reaction [1]. Biological organisms have developed a way of fast scavenging of excess iron by storing them inside ferritin in safe oxyhydroxide mineral form close to ferrihydrite [2, 3]. Discovery of biogenic magnetic nanoparticles (BMN) in the human brain by Kirschvink et al. in 1992 [4] and further studies (e.g. [5]) indicate close relation between neurodegeneration diseases and occurrence of aggregates of magnetically strong nanoparticles (magnetite and/or maghemite). However, origin and function of BMN are still unknown and matter of current research, which was reviewed very recently [6]. MRI methods for the estimation of iron levels in the human brain [7, 8] are the most promising *in vivo* techniques. Also, SQUID susceptometry is being developed for the assessment of iron content in human tissues [9, 10]. Of particular interest are methods allowing the detection and discrimination of magnetic mineral of those nanoparticles, both *in vitro* and *in vivo*. Recently it was shown that magneto-optical method based on the measurement of magnetic linear birefringence (MLB) (Cotton–Mouton effect) followed by that of magnetic circular birefringence (MCB) dispersion (Faraday rotation dispersion, or magneto-optical rotatory dispersion, MORD) can be used to distinguish various magnetic core structure of nanoparticles [6, 11, 12]. Magnetic circular dichroism (MCD) spectroscopy for discrimination between magnetite and maghemite nanoparticles were lately proposed by others [13]. Magneto-optical methods are employed in broad spectrum of application [14] in which recent achievements in field of biomedicine can be included [15–18]. Although application these methods to study of biological tissues have not been often explored presumably because Faraday rotation (FR) is relatively small in a region far from resonance. However, numerus efforts have been made to improve sensitivity which allow to collect broader data set useful for clinical application in near future. For instance Faraday rotation imaging microscopy have been described recently [19]. Preliminary results in reflection mode using polarization-sensitive optical coherence tomography were published for biological tissue models [20, 21]. It is known that ferritin and other iron-proteins are the main storages of non-heme iron in human tissues. This stimulated us to undertake magneto-optical studies in solution-phase (horse spleen ferritin (HSF) and their mimetics in aqueous suspension) and in solid-phase (composite of akaganeite nanoparticles in polyvinyl alcohol (PVA) thin film) and compare this results with biological tissues. The aim of this study is to investigate biological tissues with one magneto-optical method, namely MORD spectroscopy looking for discrimination of non-heme iron valence.

2. Experiment

The white matter (WM) sample from human brain was dissected from a deceased subject without any documented history of neurological disorders where an autopsy was requested by the local health authority. The

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tissue specimen was stored in 4% buffered formaldehyde. The sample from human spleen was dissected from a healthy subject undergoing splencotomy due to traumatic spleen rupture. Several slices (3−5 μm) were cut from cubic centimeter blocks using a ceramic knife and Microtome (Leica) and were then transferred on optical quality quartz glass support. HSF, iron dextran solution and PVA were purchased from Sigma-Aldrich. Other akaganeite (FeOOH) aqueous suspension were obtained from American Regent and LEK Companies – more details about this compounds can be found elsewhere [12]. The akaganeite type nanoparticle suspended in water and mixed with about 10% PVA/water solution. A few drops of this solution were dried on glass substrate producing a film 50−100 μm of high optical transparency and low scattering. The thickness of films were measured using induction Gimeter with precision of 1 μm. Wavelength dependence of FR i.e. MORD spectra were measured using upgraded polarimeter P 2000 (JASCO) equipped with an HBO 200 W lamp and double prism monochromator which allows for measurements with sensitivity of 0.001°. Contribution from support/solvent and natural optical activity were subtracted. Taking this and other sources of possible errors into account total accuracy is lower and estimated to be below 0.01°. MORD spectra in the spectral range between 250 – 650 nm were performed at room temperature using a homemade solenoid which fit the polarimeter. The magnetic field could be switch from –3 to 3 kOe. A program written in LabVIEW was used for synchronizing the measurement sequence and data collection. The repeatability of the MORD results were within the estimated accuracy. Absorption UV-VIS spectra were measured using computer enhancement spectrophotometer Specord M40 (Zeiss).

3. Results and discussion

Several measurements of MORD spectra were made at room temperature for all aqueous suspension i.e. ferritin, iron dextran (Sigma – S and American Regent – AR), iron sucrose and iron polymaltose (LEK) for a well-defined concentration of iron — c^{Fe} (see Fig. 1). The increase of c^{Fe} shifts the Faraday rotation edge to higher wavelengths while the decrease of c^{Fe} shifts observed edge to lower wavelengths region (not shown). The spectral dependence of those substances shown on Fig. 1 are similar with negative FR edge about 400 nm and without any characteristic feature especially in region from about 400 nm to 550 nm where bands related to ferrous iron could be observe as was reported, e.g. [11]. This is consistent with the fact that core of studied suspension are only built from oxyhydroxide type mineral with approximated formula FeOOH [12, 22]. Consequently those substances are an appropriate model of compound with ferric iron only. As may be expected solid - phase i.e. polymer film of akaganeite and PVA show no evidence for any major spectral changes in comparison to solution – phase as may be seen in Fig. 2. Optical density of

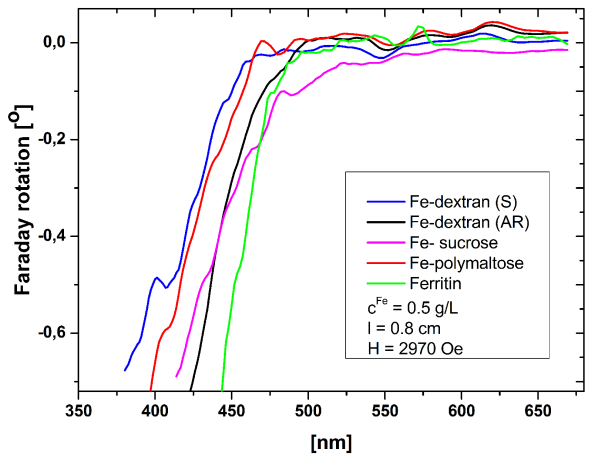

Fig. 1. Wavelength dependence of the Faraday rotation for HSF and akaganeite nanoparticles aqueous suspension.

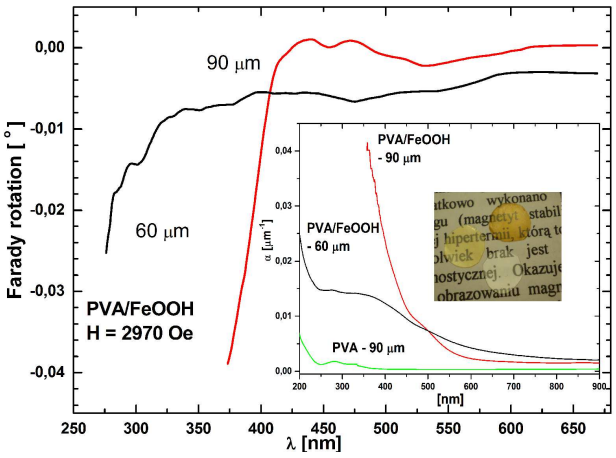

Fig. 2. Wavelength dependence of the Faraday rotation thin films of composite PVA/FeOOH of different thickness. The inset show absorption spectra and picture of composite polymer thin films of different thicknesses with and without iron mineral.

the film could be modulated by its thickness and/or concentration of iron mineral (see inset on Fig. 2). This film could find application as model compound to use in low-temperature, high-field magneto-optical studies using different mineral which can be found in human organs. Figure 3 provides MORD spectra of human tissues from brain (white matter) and spleen. We observe a good qualitative correlation between MORD spectra for akaganeite composite film and ferritin and their mimetics aqueous suspensions with spectra of thin slices of both human tissue, which suggest a contribution from Fe(III). Contribution of heme-iron is negligible as may be notice from MORD and especially, optical absorption spectra presented on Fig. 3 where positons of these bands are marked by doted vertical line. Absorption spectra show also bands with the maximum at about 280 nm,

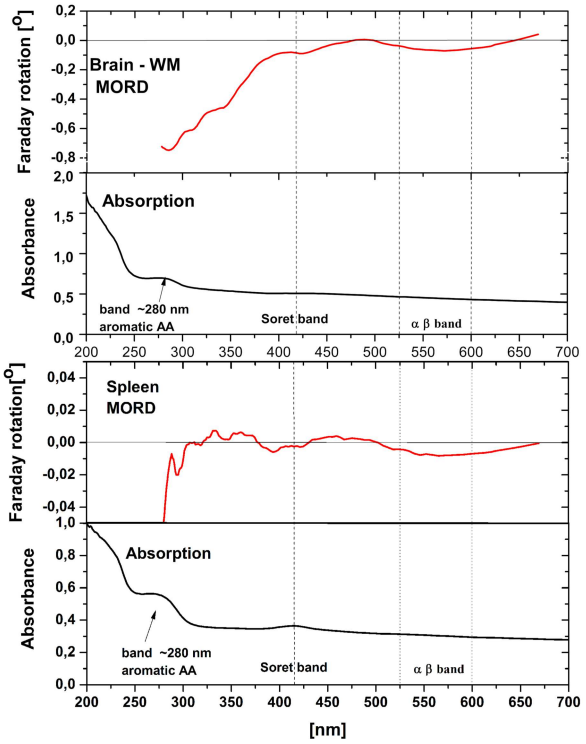

Fig. 3. Wavelength dependence of the Faraday rotation and absorption spectra for human brain and spleen tissues – upper and lower panel respectively, H = 2350 Oe. Bands position related to heme-iron and aromatic amino acids are shown by dotted vertical line and arrow respectively.

which can be ascribed to aromatic amino acids present in proteins of the tissues. However, their contribution to MORD spectra seems to have opposite sign because most of protein will have positive sign of FR [23], although their position do not interfere directly with possible ferrous iron bands position. Fixation of tissues with formalin and/or paraffin were neglected in present study but their contribution to shape of spectra need to be evaluated more extensively. Briefly, obtained preliminary results are promising, however, more information is warranted to better quantitative understanding magneto-optical properties of biological tissues.

4. Conclusions

Our results provide a comparative qualitative study of HSF and their mimetics in solution- and solid-phase with human tissues obtained from brain and spleen. Further work on correlation of magneto-optical method with other technique are under the way. All studied MORD spectra do not show spectral features related with ferrous non-heme iron (Fe(II)). Spectral behavior of studied materials were correlate with ferric iron (Fe(III)) of oxy-hydroxide mineral which constitute ferrihydrite core of ferritin and akaganeite nanoparticles. These preliminary results suggest that described method with further developments and collection of more data could be a promising tool in clinical analyzes, not exclusively in the discrimination of valence of iron.

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