Doctoral Thesis

Magnetic characterisation of iron phases in human brain tissue: Applications to epileptic and tumour tissue

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Publication Date:
2006

Permanent Link:
https://doi.org/10.3929/ethz-a-005273168

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MAGNETIC CHARACTERISATION OF IRON PHASES IN HUMAN BRAIN TISSUE: APPLICATIONS TO EPILEPTIC AND TUMOUR TISSUE

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of
Doctor of Sciences

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2006
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Abstract

Interest in characterizing iron compounds in the human brain has increased as a result of advances in determining a relationship between excess iron accumulation and neurological and neurodegenerative diseases. Furthermore, abnormalities in the expressions of iron and ferritin have been observed in many types of cancer. Iron plays an important role in the brain, but it is potentially toxic if iron metabolism is disrupted. A multidisciplinary approach is required to comprehend the complex relationship between iron and disease. However, the identification and characterisation of iron compounds in human tissue is difficult because the concentrations are very low. Very sensitive magnetic methods, developed in physics and rock magnetic communities to detect magnetic phases with very low concentrations, have been adapted and applied in this work to tissues.

A combination of low-temperature and room-temperature magnetic methods has been employed to characterise iron compounds in brain tissue from patients with mesial temporal lobe epilepsy (MTLE) and in different types of brain tumour tissue among which meningiomas were the prevalent available. Isothermal Remanent Magnetisation (IRM) acquisition was measured at 77 K and at room temperature (~300 K), and the Wohlfarth-ratio S was determined by progressively demagnetizing the IRM. Induced magnetisation was measured continuously at temperatures between 5 K and 300 K after (a) zero-field cooling (ZFC) and (b) cooling in a 50 mT field (FC). Hysteresis loops were measured at 5 K and at room temperature and first order reversal curves (FORC) were measured at room temperature.

Four main magnetic components can be distinguished from these measurements: (a) the diamagnetic matrix, (b) paramagnetic blood, (c) antiferromagnetic ferritin, and (d) ferrimagnetic magnetite (Fe$_3$O$_4$) and/or maghemite ($\gamma$-Fe$_2$O$_3$). Ferritin, the iron storage protein, has an average blocking temperature ($T_b$) at 11 K and exhibits high coercivity, non-saturating hysteresis loops at 5 K. Above 22 K it shows superparamagnetic behaviour. Magnetite and/or maghemite particles in the tissues have a broad grain-size distribution. Some particle sizes are large enough to show stable single-domain behaviour at room temperature, which is recognizable from the shape of the IRM acquisition curves, the open hysteresis loops and the FORC diagrams. A significantly higher concentration of magnetically ordered magnetite and/or maghemite and a higher estimated concentration of heme-iron were found in meningioma tumour tissues. FORC diagrams on meningioma tissue further show that the stable single-domain particles are magnetostatically interacting, implying high local concentrations (clustering) of these particles in brain tumours.

To obtain a deeper insight into the magnetic properties of ferritin, the analysis was extended to commercially available horse spleen ferritin (HoSF). Modeling of the magnetic properties of horse
spleen ferritin is often based on the assumption that they derive from a single-phase core of ferrihydrite \((5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O})\). Hysteresis measurements indicate, however, that the ferritin cores contain at least two magnetic phases. Initial magnetisation curves measured at temperatures between 50 K and 300 K have been modeled using four methods. A model that used a sum of two Langevin functions fitted the data best. The two-phase core model consists of a core phase with a high coercivity that does not undergo saturation and a second core phase with a low coercivity and a saturation field of 300 mT. The high coercivity phase is compatible with antiferromagnetic ferrihydrite, while the low coercivity phase could be magnetite, maghemite, or a mixture of both.

For a better understanding of human brain tissue as a multi-component magnetic system, a simpler proxy is needed. The magnetic properties of a two-component system, consisting of horse spleen ferritin and magnetite nanoparticles (MNP) with an average size of 10–20 nm, have been investigated. ZFC and FC induced magnetisations show very similar results to human brain tissue after subtraction of the magnetic contributions from the diamagnetic tissue matrix and the heme-iron. Moreover, FORC diagrams of the proxy at low temperature are compatible with FORC diagrams of human brain tissue at room temperature.

The results of this study demonstrate that room-temperature and low-temperature magnetic measurements provide a useful and sensitive tool for the characterisation of magnetic iron compounds in human brain tissue. They show that meningiomas contain a higher amount of ordered iron-oxide phases than hippocampal tissue. A further conclusion is that a mixed nanoparticle system can act as a good model for human brain tissue.
Zusammenfassung


Anhand verschiedener magnetischer Methoden wurden die Eisenphasen im Hippokampusgewebe von Patienten mit mesialer Temporallappenepilepsie (MTLE) und in Hirntumorgewebe, meist Meningeomen, in einem Temperaturbereich zwischen Raumtemperatur und 2 K charakterisiert. Isothermische remanente Magnetisierungserwerbung (IRM) wurde bei 77 K und bei Raumtemperatur (−300 K) gemessen und das Wohlfarths-Verhältnis (S) bestimmt, wobei die IRM stufenweise entmagnetisiert wurde. Die induzierte Magnetisierung wurde zwischen 5 K und 300 K nach Kühlung im Nullfeld (ZFC) und nach Kühlung in einem Feld von 50 mT (FC) gemessen. Zusätzlich wurden Hysteresekurven bei 5 K und Raumtemperatur und so genannte „First Order Reversal Curves“ (FORC) bei Raumtemperatur analysiert.

Diese Messungen zeigen die Signale von vier magnetische Komponenten im Hirngewebe, die wie folgt unterschieden werden können: (a) Die diamagnetische Gewebematrix, (b) paramagnetisches Blut, (c) antiferromagnetisches Ferritin, und (d) ferrimagnetische Magnetit- (Fe₃O₄) und/oder Maghemitpartikel (γ-Fe₂O₃). Das Eisenspeicherprotein Ferritin besitzt eine durchschnittliche Blockungstemperatur (Tᵢ) von 11 K und zeigt bei 5 K hochkoerzitive, nicht sättigende Hysteresen. Oberhalb von 22 K verhält es sich superparamagnetisch. Magnetit und/oder Maghemitpartikel im Hirngewebe weisen eine breite Komgrößenverteilung auf. Ein Teil des Partikelspektrums hat eine Größe im Eindomänenbereich und zeigt bei Raumtemperatur stabile Remanenz. Solche Partikel wurden sowohl durch die Form der IRM-Erwerbungskurven, wie auch durch die offenen Hysteresen und die FORC-Diagramme nachgewiesen. In den Meningeomen wurde ein signifikant höherer Anteil an magnetisch geordnetem Magnetit und/oder Maghemit als im Hippokampusgewebe festgestellt. FORC-Diagramme von Meningeomen zeigen auch, dass die stabilen Eindomänenpartikel miteinander magnetostatisch wechselwirken, was auf erhöhte lokale Konzentrationen und eventuelle Verklumpung im Gewebe hinweist.


Chapter 1

INTRODUCTION
1 INTRODUCTION

The investigation of the magnetic properties of human brain tissue may seem exotic, because human tissue is usually not considered to be a magnetic material. However, it is widely known that iron is an important element for the body's metabolism. The human brain is one of the most complex parts of the body and it is strongly dependent on a healthy supply of iron. Excessive accumulation of iron in the brain due to a disruption in iron metabolism may be associated with a variety of diseases. This necessitates a multidisciplinary approach to comprehend the complex relationship between iron and disease. Very sensitive magnetic methods have been developed in physics and rock magnetic communities to detect magnetic phases with extremely low concentrations. These methods can be applied to biologic materials by developing and adapting sample preparation and measuring methods. To understand the relationship between diseases and a disruption in iron metabolism, the iron phases and their possible origins need to be analysed and described accurately. The work in this thesis consists of five individual studies designed to gain a better understanding of the iron compounds present in the brain.

1.1 Iron in the brain

Iron is a central element in the human metabolic system. It is involved in a broad spectrum of biological functions, which include oxygen binding (hemoglobins), oxygen metabolism (oxidase, peroxidases, catalases), as well as electron transfer (cytochromes) (Konemann, et al. 2005). With respect to the human brain, neurons and glia (like all cells) require iron to perform their functions, e.g. for electron transport. Iron serves also as an essential component of numerous cellular enzymes involved in neurotransmitter synthesis. About 10-15 milligrams of iron is introduced daily and absorbed in the blood plasma (Beutler, et al. 2001). More than two-thirds of the iron within the body is in circulating red cells as haemoglobin, and only a few milligrams of iron per day enter or leave the iron storage compartments (Harrison and Arosio 1996). Storage of iron is an essential feature of human iron metabolism, so that it is available for normal neurological function.

The mechanism of iron uptake and regulation in the brain is based on a system mainly composed of transferrin, transferrin receptors and ferritin, which is an important iron storage protein (Qian and Wang 1998). Cells in the brain, unlike cells in other organs, do not have direct access to plasma-borne iron because of the blood brain barrier. The signal from a cell in
the brain that requires iron is sent to endothelial cells lining the blood vessels in the brain that release iron into the extracellular fluid of the brain. In a few selected regions where the blood brain barrier is lacking, iron can enter the brain directly. The blood brain barrier is thought to have evolved to protect the brain from large, harmful hydrophilic compounds. However, it also necessitates the presence of specific transporters to allow iron to cross this barrier. Such a transporter is transferrin, a glycoprotein which is capable of binding two iron atoms (Beard, et al. 1993, Connor 2003, Burdo and Connor 2003). In the endosome (compartment inside the cell) the two iron atoms are separated from the transferrin, which is returned to circulation as apotransferrin. The released iron is incorporated into ferritin. Ferritin can store iron for intracellular use, eventual use by other cells, or for detoxification (Connor and Benkovic 1992).

1.2 Ferritin

Ferritin is an iron-storing protein which serves the function of keeping a reserve supply of iron in a soluble, non-toxic, usable form inside the human body. The protein structure of ferritin is fundamental for its functional ability to keep iron soluble. Ferritin can be detected within any region of the body and any cell type, provided that sufficient iron is present; the amount of ferritin generally varies directly with the iron content.

Ferritin consists of a protein shell and a crystalline core within its cavity. The protein shell has an outer diameter of 120-130 Å (12-13 nm). The central cavity is 50-80 Å (5-8 nm) in diameter (Massover 1993). It is not strictly spherical. The protein shell around the core is known as apoferritin. Each molecule of apoferritin is a non-covalent assembly of 24 polypeptide subunits. The subunits are polymerized with 4-3-2 symmetry to form a hollow polygon. Two types of polypeptides make up the protein shell of ferritin, and these are distinguished as either a heavy-chain (H) or light-chain (L) isoform (Connor, et al. 1995). The H-isoferritin contains a dinuclear ferroxidase site, which converts ferrous iron (Fe\textsuperscript{2+}) to ferric iron (Fe\textsuperscript{3+}) and rapidly sequesters ferric iron, thus decreasing the potential for iron-induced oxidative cell damage. The L-isoferritin lacks the ferroxidase site and is thus relatively slow at sequestering iron. The H isoform is considered to be the major cytoprotectant, whereas the L isoform has the role of promoting mineralisation of iron. The ratio of H/L isoferritins differs among the human organs. In the liver and spleen the L-form predominates, whereas in the heart and red blood cells the H-form is more abundant (Beutler, et al. 2001). In the brain, regional differences in ratios of H/L isoferritins have been determined, but in all regions H-
Isoferritin is more abundant than L-isoferritin. Connor et al. (1995) found that the H-rich ferritin levels increase with age in every region of the brain. L-rich ferritin increases also with normal aging, but the amount of increase is more region-specific.

Apoferitin is converted into ferritin by deposition of iron as an oxide inside the central cavity. Each ferritin molecule can sequester from one to a maximum of about 4500 atoms of iron (Harrison and Arosio 1996). The uptake of iron involves the catalysed oxidation of ferrous iron brought to the molecular surface. The formation of an internal iron-rich core indicates that iron must be able to pass through the protein shell of ferritin, although the exact pathway is unknown. Massover (1993) proposed the following model. First, ferrous iron is bound to apoferritin and oxidized to form solitary ferric atoms, which remain bound to the protein. Small polynuclear clusters of ferric iron start to form in the central cavity and the core grows, either by the addition of oxidized ferric iron or by the oxidation of ferrous iron directly at the crystal surfaces. The iron within the central cavity of ferritin is present as one or more crystallites of ferrihydrite ($9\text{Fe}_2\text{O}_3\cdot5\text{H}_2\text{O}$). The number of crystallites within the cores of different molecules is highly variable; there may be only one crystal or several small crystallites. Not all organisms contain ferritin cores with highly crystalline ferrihydrite, and certain invertebrates show no evidence of crystalline material. Their cores are believed to be poorly crystalline or amorphous. The degree of crystallinity of the ferritin cores is dependent on the source of ferritin and can vary with pathological conditions (StPierre, et al. 1991). Phosphorus has been found within the cores of ferritin, where it coats the ferrihydrite crystallites, but can also be interstitial. For this case a good correlation has been found between the degree of crystallinity of the core in ferritin and their phosphorus content (Mann, et al. 1989, StPierre, et al. 1996). Other metals can become loosely attached to the ferritin core (Joshi and Zimmerman 1988), and such binding may be important for regulation and general detoxification of these elements.

Hemosiderin is a less abundant form for iron storage in the human body. It takes the form of insoluble granules, in contrast to the soluble ferritin form of iron (StPierre, et al. 1987). According to StPierre et al. (1989) hemosiderin can contain peptides that are derived from ferritin and the authors suggest that hemosiderin is a degraded form of ferritin. However, under certain pathological conditions, such as hereditary hemochromatosis, hemosiderin may form independently (Beutler, et al. 2001). Hemosiderin is not associated with well-formed protein cavities and, as such, the particle size distribution may be less restricted in comparison to ferritin. As for ferritin, the structural and compositional characteristics of hemosiderin vary with biological source and pathological condition.
1.2.1 Magnetic properties of ferritin

Ferritin is generally considered to be antiferromagnetic below a "blocking" temperature \( T_B \) and superparamagnetic above \( T_B \). Each core possesses a net magnetic moment arising from uncompensated spins at the surface of the core. Néel (1961a, 1961b, 1962) noted that for grains below a certain diameter uncompensated magnetic moments in sublattices and surface effects lead to an enhanced susceptibility; he named this behaviour superantiferromagnetism. There have been several studies characterizing the magnetic properties of ferritin. Most of these have been made on commercial horse spleen ferritin, which provides an almost ideal monodispersed nanoparticle system (Kilcoyne and Cywinski 1995). The protein envelope keeps the magnetic particles separated by a distance of about 10-12 nm; therefore, interaction effects, which complicate relaxation behaviour, are reduced (Allen, et al. 1998). In the antiferromagnetic state below \( T_B \), horse spleen ferritin exhibits open hysteresis with high coercivity (Makhlouf, et al. 1997) and the hysteresis loop remains open up to high fields (Gilles, et al. 2002). Usually, a distribution of particle sizes and blocking temperatures are found. From induced magnetisation measurements versus temperature after field cooling (FC) and zero field cooling (ZFC), Makhlouf et al. (1997) found a distribution of blocking temperatures between 5 K and 20 K due to the smallest and largest particles, respectively, in the sample. They determined that the average \( T_B \) is 12 K. For artificially reduced ferritin, which has a higher \( \text{Fe}^{2+} \) concentration, the blocking temperature is shifted to higher values (Mohie-Eldin, et al. 1994). The magnetic blocking temperature of a material depends on the degree of crystallinity of the ferritin cores. For ferritin with low crystallinity or with a high level of phosphate present in the core, \( T_B \) is lowered (StPierre, et al. 1996, Chasteen and Harrison 1999).

For superparamagnetic materials whose magnetic ordering temperature (Néel temperature \( T_N \) for antiferromagnetic materials) cannot be determined directly, a method has been used, in which the saturation magnetisation \( M_0 \) is extrapolated as a function of temperature (Makhlouf, et al. 1997, Gilles, et al. 2000, Seehra, et al. 2000). The temperature in which \( M_0 = 0 \) is \( T_N \). For horse spleen ferritin, ordering temperatures (\( T_N \)) between 400 K and 500 K have been estimated.

Very little information is available on magnetic properties of human brain ferritin. Dubiel et al. (1999) examined tissue from globus pallidus. They found that the average \( T_B \) was 8.5 K and the start of ordering was 39.2 K. They did not estimate \( T_N \).
1.2.2 Magnetoferritin

As ferritin has the ability to sequester and store iron, research has been done in removing the native ferrihydrite core from horse spleen ferritin and reconstituting the core by incubation of the apoferritin with other minerals (Gider, et al. 1995). The structure of the protein cage is useful for nanomaterial synthesis (Allen, et al. 2002), since the interior of the protein shell has regions of high charge density, which act as sites for synthetic mineral nucleation. As the shell has a cage-like architecture, the formation of material is size-constrained with narrow size distribution. With this approach, homogenous magnetic nanoparticles of transition metal oxyhydroxides can be synthesized directly (Resnick, et al. 2004). Meldrum et al. (1992) synthesized a magnetic phase within the nanodimensional cavity of horse spleen ferritin by the use of controlled reconstitution conditions. Transmission electron microscopy and electron diffraction analysis indicated that the entrapped mineral particles were 6 nm spherical single crystals of a ferromagnetic oxide, magnetite (Fe$_3$O$_4$) or maghemite ($\gamma$-Fe$_2$O$_3$). This synthesized form of ferritin is called magnetoferritin. Pankhurst et al. (1994) investigated magnetoferritin by Moessbauer spectroscopy and suggested that the mineral form of the cores corresponds to maghemite rather than magnetite.

1.3 Ferrihydrite (5Fe$_2$O$_3$·9H$_2$O)

As the cores of ferritin are mostly ferrihydrite (5Fe$_2$O$_3$·9H$_2$O), the structure and properties of this material are shortly reviewed below. Ferrihydrite is a natural occurring material in soils and sediments or can be synthesized by rapid hydrolysis of Fe(III) solution. The crystal structure of ferrihydrite has a hexagonal lattice with $a = 5.08$ Å (0.58 nm) and $c = 9.4$ Å (0.94 nm) (Harrison, et al. 1967, Towe and Bradley 1967, Quintana, et al. 1987). Due to poor crystallinity, X-ray diffraction patterns show a very broad 2-line or, for relatively well crystallized material, a 6-line pattern, which makes it difficult to obtain accurate structural information. Janney et al. (2000a, 2000b, 2001) investigated the structure of synthetic 2-line (FH2) and 6-line (FH6) ferrihydrite by electron nanodiffraction. For FH2 they found highly disordered material and very few nanocrystals with structures based on hexagonal (ABAB) and cubic (ABC) stacking of close-packed layers of O$_2^-$ and OH$^-$ ions. The structure with cubic stacking is similar to maghemite ($\gamma$-Fe$_2$O$_3$) and has 25% of the iron atoms in tetrahedral sites. The structure with hexagonal stacking consists of double chains of
face-sharing Fe octahedra. FH6 has a more ordered structure, consisting of a double-hexagonal structure (ABAC) based on stacking of close-packed oxygen layers. The fraction of highly disordered material in FH6 is much smaller than in FH2. It is suggested that FH6 is not simply a more crystalline form of FH2.

The average particle size is unusually small and is between 3 nm and 7 nm (Zhao, et al. 1994). Due to its small size the surface of ferrihydrite plays an important role in the phase transformation of ferrihydrite to crystalline hematite ($\alpha$-Fe$_2$O$_3$) and goethite ($\alpha$-FeOOH). A significant percentage of iron ions at the surface of ferrihydrite particles are in the lower coordination sites (tetrahedral sites). After dehydroxylation many spins remain uncoupled. With chemisorbed water molecules, these sites become responsible for the phase transformation. On the other hand, when impurities are present, the sites may be filled with other anions, which in turn block the crystal growth sites, inhibiting the formation of hematite. Zhao et al. (1996) investigated this phenomenon by Moessbauer spectroscopy. They filled the sites with unsaturated coordination by chemisorption of SiO$_4^{4-}$ and found a decrease in the blocking temperature from 100 K to 40 K and a sharpened transition. The results suggest that unpaired spins at the surface are eliminated and a shell of nonmagnetic medium is created around each particle, inhibiting magnetic coupling between the particles. The transformation from ferrihydrite into cubic phases, such as magnetite (Fe$_3$O$_4$) or maghemite ($\gamma$-Fe$_2$O$_3$), is not possible spontaneously but requires organic reductive agents (Campbell, et al. 1997). In addition to the presence of glucose as a reductive agent, temperatures of 300 °C are needed to produce magnetite and/or maghemite.

The magnetic properties of well-crystalline 6-line ferrihydrite between 4 K and 293 K were investigated by Zergenyi et al. (2000). The Moessbauer spectra show a hyperfine superparamagnetic structure at 120 K. The $T_B$ of FH6 was determined from AC susceptibility measurements to be between 86 K and 95 K depending on the AC frequency.

1.4 Magnetite (Fe$_3$O$_4$) and maghemite ($\gamma$-Fe$_2$O$_3$)

Magnetite (Fe$_3$O$_4$) is the most intensively investigated of the naturally occurring iron oxides and the primary magnetic source in biology. It has been reported in a wide range of organisms some of which are of particular interest for studies of the process of iron mineralisation (c.f., Kirschvink, et al. 1985, Mann, et al. 1989 and references therein). Magnetite has an inverse spinel structure with 32 oxygen anions, 16 Fe$^{3+}$ and 8 Fe$^{2+}$ cations per unit cell. Oxygen anions form a face-centered cubic lattice with the Fe$^{2+}$ and the Fe$^{3+}$...
cations in interstitial sites. Within this O$_2^-$ network, there are two sublattices of cation sites. The A sublattice contains eight cations in tetrahedral coordination. The B sublattice is composed of 16 cations in octahedral coordination. The exchange between the cations takes place through an intervening O$_2^-$ anion and this mechanism is called superexchange interaction. The A and B sublattices are coupled antiparallel. Because the B lattice has one Fe$^{3+}$ and one Fe$^{2+}$ for every Fe$^{3+}$ in the A sublattice, the atomic moments for Fe$^{3+}$ cancel, leaving a net magnetisation moment due to the Fe$^{2+}$ cations. Magnetite has a cubic structure with a lattice parameter $a = 8.396$ Å. It undergoes a crystallographic structural change from cubic to monoclinic (Verwey 1939, Yoshida and Iida 1977) at the Verwey transition, which is around 120 K. Impurities and non-stoichiometry can however depress and broaden the Verwey transition cf. (Walz 2002).

Maghemite ($\gamma$-Fe$_2$O$_3$) is the fully oxidized equivalent of magnetite i.e. it contains Fe$^{3+}$ ions only. It also has a cubic inverse spinel structure but with $a = 8.337$ Å it has a slightly smaller cubic cell length than magnetite. To differentiate between magnetite and maghemite, usually X-ray diffraction or Mössbauer spectroscopy is used. No Verwey transition occurs in maghemite. Natural occurring maghemite is most often the oxidation product of magnetite.

1.4.1 Magnetic properties of magnetite (Fe$_3$O$_4$) and maghemite ($\gamma$-Fe$_2$O$_3$)

The antiparallel coupling of the two unequal sublattices makes Fe$_3$O$_4$ and $\gamma$-Fe$_2$O$_3$ ferrimagnetic. The theoretical net magnetisation moment at 0 K per formula weight is 4.1 $\mu_B$ for magnetite and 2.5 $\mu_B$ for maghemite. The preferred direction for magnetisation – called the easy axis – is along the [111] direction. The saturation magnetisation of magnetite at room temperature is 480 kA/m and the Curie temperature $T_C$ – the temperature at which it loses its spontaneous magnetisation – is 580 °C. For maghemite the saturation magnetisation at room temperature is 380 kA/m and the Curie temperature is 645 °C (Dunlop and Özdemir 1997). However, maghemite is metastable and, when heated, inverts to hematite ($\alpha$-Fe$_2$O$_3$) at temperatures well below the Curie point, which is consequently difficult to measure directly.

Depending on temperature, grain size and grain shape, magnetite and maghemite can occur in three different magnetic states: single-domain, multi-domain and superparamagnetic. The single domain state is characterised by its high remanence and stability. The grain size for which the single-domain state has a lower energy than a two-domain state is called the critical single-domain size. It is around 50-60 nm for equidimensional magnetite particles, but for elongated grains the single domain state can be maintained to much larger sizes. For larger
1.4 Magnetite and maghemite

grains the multi-domain state becomes more favorable. Remanence and coercivity are lower in the multi-domain state.

Below the critical single-domain size, thermal energy approaches the anisotropy energy barrier resulting from crystalline, magnetoelastic or shape anisotropy; this energy barrier is also called the blocking energy. When the thermal energy equals or exceeds the blocking energy, the alignment of the magnetic moment is no longer bound to the easy axis and the magnetic moments become randomized. This thermally activated state is called superparamagnetism. The critical superparamagnetic size for magnetite was experimentally determined to be 25-30 nm (Dunlop and Özdemir 1997). In the superparamagnetic state, particles preserve no remanence. They behave like the atoms in a paramagnetic material, except that their individual particle magnetic moment is large compared to that of a single paramagnetic atom. Superparamagnetic materials reach magnetic saturation in lower applied fields than single-domain material. In a mixture of single-domain and superparamagnetic components, the superparamagnetic component will tend to dominate the induced magnetisation due to its higher magnetic susceptibility.

1.5 Iron related brain diseases

Iron is not only a basic element in the metabolism of normal cells, but also of malignant cells. There have been numerous medical studies elucidating the physiological and clinical features of iron in benign, chronic and malignant disorders (Konemann, et al. 2005). Many of these have linked a dysfunction of the iron storage protein, ferritin, with an imbalance of iron within the cells. For example, Joshi and Zimmerman (1988) report the uncontrolled release of ferrous iron (Fe²⁺) from ferritin due to the presence of a reducing agent and oxygen. The released iron can modify several biomolecules and affect the metabolic processes. Abnormalities in the expressions of iron and ferritin have also been observed in many types of cancer (Arosio and Levi 2002). Enhanced intracellular iron storage by ferritin has been reported in chronic infections and in tumours influenced by proinflammatory cytokines (Konemann, et al. 2005), and evidence has been presented that oncogenes may regulate ferritin expression (Tsuji, et al. 1995). Ferritinaemia in cancer was investigated by Hazard and Drysdale (1977), who found substantially higher levels of H-isoferititin in serum ferritin of cancer patients. According to Mykhaylyk et al. (2005) there is significant increase of stored and chelatable iron in malignant glial brain tumours and peritumoural brain tissue.
Excess brain iron is also consistently observed in neurodegenerative disorders, such as Alzheimer’s disease (AD), Parkinson’s Disease (PD), or Huntington’s disease (HD) (Beard, et al. 1993, Loeffler, et al. 1995, Smith, et al. 1997, Lovell, et al. 1998, Qian and Wang 1998, Bartzokis and Tishler 2000). Loeffler et al. (1995) measured ferritin, iron and its transport protein, transferrin, in different parts of the brain by using immunoassays with antibodies specific to the isoforms and found a significant increase of iron for Alzheimer’s and Parkinson’s diseased tissue. They also found an altered relationship between iron and transferrin, which provides further evidence that a disturbance in iron metabolism may be involved in both disorders.

Fischer et al. (1997) investigated the hypothesis that dementia in Alzheimer’s disease is associated with lower transferrin levels and higher ferritin levels in the brain. They found that ferritin nearly doubled in brains with Alzheimer’s disease and transferrin was decreased. The quantification of ferritin and transferrin was made by immunoradiometry and single radial immunodiffusion, respectively. Bartzokis and Tishler (2000) found similar results by using MRI methods in vivo.

In an initial study, Quintana et al. (2000) analysed ferritin cores extracted from the brains of patients who had Palsy and Alzheimer’s disease using high-resolution transmission electron microscopy (HRTEM) and energy-loss spectroscopy. The main findings were that some of the nanocrystals in the ferritin cores did not consist of the hexagonal ferrihydrite structure generally found in healthy ferritin but rather of a cubic structure similar to magnetite and/or maghemite. The core dimensions of the molecules were similar to those found in healthy tissue (about 5.3 nm), but different crystallographic species, such as ferrihydrite, magnetite/maghemite, and an intermediate cubic phase with a lattice parameter smaller than that of magnetite/maghemite, were detected. In a subsequent study, they detected a wüstite (FeO)-like phase in some of the ferritin cores (Quintana, et al. 2004).

Proper storage and intake of iron by the ferritin protein shell is crucial and a significant number of phases different from ferrihydrite within the cores could indicate a dysfunction. Unbound Fe^{2+} may act as a toxin in the human body, because ferrous ions might contribute to the production of free hydroxyl radicals, leading to cell degeneration (Schafer, et al. 2000, Dobson 2002b). As brain tissue is rich in polyunsaturated fatty acids, it is very susceptible to lipid peroxidation, which can be initiated by Fe^{2+}. Two principal routes have been proposed that might cause the lipid peroxidation: (1) the Fenton reaction, where the iron reacts with hydrogen peroxide producing a hydroxyl radical, and (2) free radical oxidation, which is a peroxidation by means of iron-oxygen complexes. Schafer et al. (2000) reported
1.5 Iron related to diseases

that iron-oxygen complexes are the primary route to the initiation of biological free radical oxidations.

With respect to epilepsy, several investigations have reported that the deposition of iron-containing compounds into limbic structures of rats initiates epileptogenesis followed by chronic spontaneous seizures (Ueda, et al. 1998, Ueda and Willmore 2000, Doi, et al. 2001). All of the rats injected with aqueous FeCl₃ developed isolated epileptiform discharges soon after injection. This suggests that the aqueous iron initiates lipid peroxidation by a free radical mechanism leading to epileptogenesis. Ikeda (2001) studied transferrin saturation in 130 patients with epilepsy and 128 control subjects without epilepsy. He found significantly higher transferrin saturation in the epilepsy group, indicating an iron overload for this group.

1.6 The role of magnetite and maghemite related to diseases in the brain

Magnetite biomineralisation in the human brain was first reported by Kirschvink et al. (1992). Using a superconducting quantum interference device (SQUID) magnetometer, they detected the presence of ferrimagnetic material in frozen autopsy tissue from subjects suspected of having had Alzheimer's disease. Using high-resolution transmission electron microscopy they identified the material as magnetite and/or maghemite. Further magnetic analysis was made by Dunn et al. (1995) on brain tissue from the hippocampus. The hippocampus is made up of sheets of cortex folded into the medial surface of the temporal lobes. It plays a critical role in memory and is a common epileptogenic region. Dunn et al. (1995) made their measurements on tissue from deceased epileptic and non-epileptic subjects, as well as on a sample removed from the epileptogenic zone of a living patient during amygdalohippocampectomy (Wieser and Yasargil 1981). Isothermal remanent magnetisation (IRM) and AF demagnetisation of the saturation magnetisation at 77 K were measured. The crossover point of the IRM curve and AF demagnetisation curve (the Wohlfarth-ratio, S) provides information about magnetostatic interactions between the particles (Wohlfarth 1958, Cisowski 1981). For non-interacting single-domain particles with uniaxial anisotropy, the S-ratio is 0.5 (Wohlfarth 1958). Lower values are attributed to particle interactions, or to superparamagnetic or multidomain influences. The samples in Dunn et al. (1995) had S-ratios below 0.5, suggesting that the magnetic particles were interacting. Clusters of fine opaque particles, 5–10 µm in size, were observed by optical microscopy.

Dobson and Grassi (1996) investigated whether the presence of magnetite was due to a postmortem chemical alteration or contamination or was due to epilepsy. They demonstrated
the existence of ferro- and ferrimagnetic material by applying low temperature magnetic measurements to samples resected by amygdalohippocampectomy from living patients suffering from mesial temporal lobe epilepsy (MTLE). The resected tissue was sealed in HCl-washed quartz glass sample holders and immediately frozen in liquid nitrogen in order to preserve normal brain chemistry. They found that neither the airborne contamination nor the artefacts from cauterisation of blood vessels contribute to the magnetic mineralogy. Therefore the iron-oxides in the human brain tissue were not a consequence of contamination or postmortem changes in brain chemistry.

In a further study, Schultheiss-Grassi and Dobson (1999) measured the remanent magnetic particle concentration in tissues from MTLE patients and in non-pathogenic tissues from cadavers. They did not find any systematic statistical variation or significant difference between the two types of tissue. IRM acquisition curves revealed the presence of a ferrimagnetic material in samples from living epileptic patients and from the hippocampi of non-pathologic cadaver tissue. The concentration was estimated from the intensity of the saturation IRM, and it was found to be in the same range for all samples. They found, however, a systematic variation of the Wohlfarth-ratio, $S$, between the MTLE patients and the non-pathologic tissue. All MTLE patients had $S$-ratios between 0.3 and 0.4, while the cadavers had ratios below 0.3, indicating a possibly higher degree of particle interaction in the latter samples. The presence of superparamagnetic particles in the tissue was determined from the increase in saturation IRM values when measured at 77 K compared to measurements at 273 K. Schultheiss-Grassi et al. (1999) further identified magnetite/maghemite particles extracted from human brain tissue using high resolution transmission electron microscopy (HRTEM), electron diffraction and elemental analysis. A single-crystal diffraction pattern was found by electron diffraction, indicating that the particles were cubic and crystalline. The measured d-spacings were consistent with magnetite or maghemite.

A subsequent study investigated age-related variations in biogenic magnetite levels in the hippocampus, but no significant increasing or decreasing trend of magnetite concentration with age was found (Dobson 2002a). Analyzing males and females separately, Dobson found a trend that suggested increasing magnetite concentration with age in the male population. Magnetite concentration was also investigated in Alzheimer’s disease brain tissue by Hautot et al. (2003). Magnetic properties of the samples were measured using SQUID magnetometry. Separation of the magnetite signal from the diamagnetic signal and the ferritin signal was achieved by hysteresis measurements at low temperatures. The amount of nanoscale biogenic magnetite was shown to be higher in diseased brain tissue than in the control samples. The
discovery of magnetite in the human brain also led to experiments in which the response of epileptic patients to magnetic field stimulation was recorded by electroencephalography (Fuller, et al. 1995, Dobson, et al. 2000b, Dobson, et al. 2000a). These experiments showed that there was an evoked electrical response in the hippocampus, but they were unable to establish whether this was due to anomalous concentrations of magnetite in the epileptogenic focus.

1.7 Objectives and outline of the thesis

The objective of this dissertation is to examine and characterise the iron mineralogy in brain tissue with emphasis on tissues from epileptic patients and from brain tumours. Earlier studies showed that magnetic methods could be used to identify iron oxides and ferritin in the brain (Kirschvink, et al. 1992, Schultheiss-Grassi and Dobson 1999, Dubiel, et al. 1999). However, none of these studies attempted a full characterisation of the magnetic properties and the phases that contribute to them.

The dissertation consists of six chapters. The introductory chapter is followed in chapter II by a detailed overview of general methodology. It describes sample preparation and handling, and the measurement methods used for the different investigations.

Chapter III is concerned with the characterisation of different magnetic contributions to the magnetic measurements and the identification of iron phases. The chapter consists of two papers. The first paper (Brem et al., BioMetals, 2005) describes the identification of ferritin and a ferromagnetic phase (magnetite and/or maghemite) in two human brain tumour tissues. The second paper (Brem et al., Journal of Physics: Conference Series, 2005) represents an extension of the work described in the first paper. In particular, the contribution of heme-iron to the magnetic signal is considered.

Ferritin is one of the important iron phases in brain tissue. It is a natural material and is of interest to physicists, because it is a non-interacting antiferromagnet with nanometer size. There have been numerous magnetic studies on horse spleen ferritin in which the magnetic behaviour of these nanomagnets has been modeled. Most of the published models are not able to fit the experimental data well, particularly in low fields. Chapter IV considers the magnetic properties of horse spleen ferritin alone and in combination with magnetite, with particle size in the superparamagnetic range. This chapter also consists of two papers. The first paper (Brem et al., Journal of Applied Physics, 2006) examines the magnetic properties of horse spleen ferritin as stated. Quintana et al. (2004) have identified cubic iron oxide phases
together with ferrihydrite in the cores of ferritin from human brain and horse spleen. This study models the magnetic signal on the assumption that a second low coercivity phase is present in the cores of commercially available horse spleen ferritin. In the second paper (Brem \textit{et al.}, Physical Review B, 2006), magnetite with particle size between 10 nm and 20 nm has been mixed with horse spleen ferritin to evaluate if this mixed system is an accurate proxy for brain tissue.

Chapter V compares the magnetic properties of non-tumour hippocampal tissue from epileptic patients with meningiomas, which is a particular type of brain tumour (Brem \textit{et al.}, Journal of the Royal Society: Interface, 2006).

The final chapter summarises the main findings in this thesis, and presents a more general discussion of all results. It also provides an outlook of future work that should be pursued.
Chapter 2

SAMPLE PREPARATION AND METHODOLOGY

In this chapter the procedures followed for storing, freeze-drying and preparing the samples for classical rock magnetic measurements are described. These measurements include the acquisition of isothermal remanent magnetisation (IRM), measurement of induced magnetisation as a function of applied field (magnetic hysteresis), as well as the variation of induced magnetisation with temperature, and the determination of first order reversal curves (FORC). For each measurement method a suitable way of mounting the sample in the equipment and different sample holders was necessary. This chapter describes in more detail how the samples were handled, mounted and measured than the descriptions in the relevant sections of the following publications.
2.1 Sample handling and holder material for frozen tissue samples

2.1.1 Storing

The resected samples were immediately frozen in liquid nitrogen. During subsequent transport the samples were kept in a Dewar vessel filled with liquid nitrogen. All samples were then stored in a special freezer at -80 °C, where they can be kept for long-term storage without risking alterations in the tissue. The freeze-dried tissue was kept in a normal freezer at -12 °C for longer storage.

2.1.2 Sample holders

For measuring the frozen tissue samples, suitable holders were needed in order to protect the tissue from contamination, as well as to insulate them thermally and further to insert them into the measuring equipment (e.g. cryogenic magnetometer). The holders have to fulfill the following criteria: (i) they must exhibit a stable magnetic signal at room temperature and at 77 K, (ii) they must be cleanable in hydrochloric acid, and (iii) the samples should be fixed in the holder so that they do not move during the measurements and are not exposed to air. For this purpose teflon holders were designed with a cubic form of 2.5 cm side length. A cylindrical hole with a diameter of 1 cm and a depth of 1.5 cm was drilled into one side. A plug with the same diameter was used to close the holder and at the same time fix the sample inside the holder. The size of the hole was usually big enough for all samples. Some exceptionally large sized samples had to be cut on one side to fit into the hole; this was done with a cleaned ceramic knife.

2.1.3 Cleaning

All glassware and tools, such as ceramic knives and glass pistons, as well as the teflon holders were cleaned in a 30% hydrochloric acid bath during 24 hours. Afterwards they were rinsed with distilled water at least three times. Precautions were taken to maintain a clean working environment and disposable gloves were used for all handling.
2.2 Freeze drying (lyophilisation)

During several magnetic measurements the samples need to be exposed to room temperature conditions for longer periods of time; therefore they cannot be kept in a frozen state. Freeze-drying of biological samples is the most suitable process for conserving tissue against chemical alteration for longer periods. It also prevents changes to the cell or tissue. Freeze-drying is the dehydration of a frozen aqueous material through the sublimation of ice. For a better understanding of this process, the phase diagram of H₂O is shown in Figure 2.1. At the so-called triple point, at 0.00611 bar and 273 K, all the phase lines intersect in a common point. At lower pressures and lower temperatures, there is a direct phase change from the frozen into the gaseous state. These are the conditions which are used for the lyophilisation. Pressure and temperature need to be accurately controlled for an efficient process.

![Phase diagram of H₂O](image)

**Figure 2.1**: Phase diagram for H₂O. The grey area indicates the suitable conditions for the freeze-drying process.

In addition, an ice condenser is needed so that the discharging vapor freezes at the surface and energy must be supplied to the system to initiate the process. Normal ice follows the kinetic gas theory and calculated sublimation rates have been verified experimentally. The sublimation of ice from biological material, however, behaves differently (Edelmann 1986).

Firstly, water molecules must be removed from a highly structured network. After freeze-drying has begun, an increasing layer of dry material inhibits further free sublimation.
Secondly, biological material contains about 5-10% chemically or physically bound water. Finally, the sublimation needs energy which must be transferred to the drying boundary. This transfer is usually hampered by the layer of already dry material. As a consequence, the temperature at the drying boundary may be lower than at the surface of the samples and the sublimation rate may also be lower. Considering these aspects, a procedure consisting of three different steps was established in order to prevent any chemical changes. It is not only crucial to prevent chemical alterations, which could affect the magnetic properties of the material, but lyophilisation is also reported to preserve all morphological properties e.g. the cell structure and antigenic conformation (Stumpf and Roth 1967, Edelmann 1986, Louis, et al. 2000).

The following three-stage procedure was followed using a Christ ALPHA 2-4 lyophilisator:

1. **Freezing**: The tissue was rapidly frozen in liquid nitrogen at 77 K. The rapid freezing is important as it prohibits the formation of larger ice crystals within the tissue. The plate in the freeze drier was pre-cooled down to -40°C, before the samples were placed on the plate in small evaporating dishes filled with liquid nitrogen.

2. **Drying**: The pressure in the chamber was lowered as quickly as possible to 0.07 mbar. This took around 8 minutes. The samples were then dried for 16-24 hours.

3. **Post-drying**: The tissues were finally allowed to warm up to room temperature under a pressure of 0.008 mbar to prevent any condensation of water on the dried sample.

After freeze-drying, the samples were stored in a normal freezer between measurements.

<table>
<thead>
<tr>
<th>Process</th>
<th>Pressure</th>
<th>Temperature [°C]</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Freezing</strong></td>
<td>ambient</td>
<td>-196°C (liquid nitrogen)</td>
<td>Few seconds</td>
</tr>
<tr>
<td><strong>Drying</strong></td>
<td>0.07 mbar</td>
<td>-40°C (shell)</td>
<td>16-24 hours</td>
</tr>
<tr>
<td><strong>Post-drying</strong></td>
<td>0.008 mbar</td>
<td>-40°C to room temperature (shell)</td>
<td>2-4 hours</td>
</tr>
</tbody>
</table>
2.3 Measuring methods

2.3.1 Cryogenic magnetometer

For the acquisition of isothermal remanent magnetisation (IRM) and its subsequent
demagnetisation in alternating magnetic fields (AF), a 3-axis 2G Enterprise Superconducting
Quantum Interference Device (SQUID) in the magnetic laboratory Adlisberg (LNM, ETH
Zurich) was used. The magnetometer is located inside a magnetically shielded room, in which
the Earth's magnetic field is reduced to a few percent of the ambient value. For the
measurements at room temperature and at 77 K, samples were packed into the pre-cooled,
cleaned teflon sample holder described under 2.1.2 and fixed with the teflon plug. For the
measurements at 77 K, the holder containing the sample was put into a Dewar vessel filled
with liquid nitrogen and cooled for at least 15 minutes. All sample transfers between freezer
and measurement equipment or pulse magnetizer were made in liquid nitrogen. To control for
possible contamination in the liquid nitrogen, the teflon holder was measured several times
without the sample after cooling and rewarming. No increase of magnetic signal was observed
due to the liquid nitrogen. The cubic sample holder was designed to fit in the sample handler
of the cryogenic magnetometer. The teflon cube was quickly taken out of the liquid nitrogen
and put into the cryogenic sample handler. This device is loaded automatically, so in order to
adapt the measurement process to cope quickly with frozen tissue samples the software had to
be adapted. Since the sample chamber inside the cryogenic magnetometer is at room
temperature, the control program of the sample handler was altered to speed up the
measurement procedure, which subsequently took only a few seconds. An offset measurement
was done automatically before each measurement. Five measurements lasting three seconds
each were taken for each sample insertion, and the average of the signals was calculated. The
five measurements provide a useful control of the drift of the measurement signal due to
temperature loss or viscous magnetic behaviour. The removal of the sample from the
magnetometer was also programmed in the fast mode and the teflon cube was immediately
put back in liquid nitrogen. For a typical measurement, the samples were out of the liquid
nitrogen for no longer than 25 seconds.

For the measurements at room temperature, the same procedure was followed except
that the freeze-dried samples were used in place of the cold samples. The freeze-dried samples
were left in their original dimensions and were fixed inside the sample holder exactly the
same way as the frozen samples. For the acquisition of isothermal remanent magnetisation
(IRM), a pulsed DC magnetic field was produced by an ASC Scientific Pulse Magnetizer.
Model IM 10-30. Because it is a high-field instrument, the pulse magnetizer cannot be placed inside the shielded room. In order to produce the magnetic pulse, a desired voltage was tuned and stabilized. Then the cubic teflon holder was quickly placed inside the coil in a chosen orientation and the pulsed field was triggered. For the cooled samples this took no longer than five to ten seconds, so they were only briefly out of the liquid nitrogen. Then the samples were transported back to the shielded room for the measurement of magnetisation. The time was determined between triggering the pulse and starting the measurement and was set for all IRM acquisition measurements to be 120 seconds.

For the AF demagnetisation measurements a single initial field pulse was applied to the sample, which was then measured in the magnetometer as described above. The AF demagnetisation steps were programmed manually in the software and the sample was then demagnetized automatically along three orthogonal directions. These measurements were only possible on freeze dried tissue samples at room temperature, because the demagnetisation measurements take 20–30 minutes for 12–15 field steps.

2.3.2 Magnetic properties SQUID magnetometer (MPMS)

For MPMS measurements the samples need to be placed in straws in order to be inserted in the measuring channel, which has a diameter of less than 10 mm. For this reason the freeze-dried samples were broken into pieces with a glass piston and pressed into a plastic straw of 5 mm diameter and 12-13 cm length with teflon pistons. These have a diameter of 4.9 mm and were made specially for this purpose. The sample was pressed into a cylindrical pellet inside the straw, giving a pellet 5 mm in diameter and 2-8 mm in length, depending on the original sample dimensions. The pellet was placed about 9 cm from the upper end of the straw. Above and below the pellet small pinholes were pierced in a ring to prevent the sample from moving by slightly tightening the straw’s diameter. Furthermore, several pinholes were made all over the straw to allow air circulation and in order to guarantee a proper purging of air inside the sample chamber of the MPMS. In contrast to another study (Hautot, et al. 2005), no further packing material was needed. The measurements were made using a Quantum Design MPMS superconcuting quantum interference device (SQUID) magnetometer at the Institute of Geosciences, University of Bremen, at the Chemistry Department, ETH Zurich and at the Institute of Rock Magnetism (IRM), University of Minnesota.
2.3.3 Vibrating sample magnetometer (VSM) and alternating field gradient magnetometer (AGM)

For the measurements in the PMC vibrating sample magnetometer (VSM) at the LNM, ETH Zurich the freeze dried samples were packed into non-soluble gel capsules of 5 mm diameter. Usually the samples had been previously measured in the MPMS, so the pressed pellet described above was simply pressed out of the straw and into the gel capsule, without contacting other surfaces. The gel capsule was closed with its cap and fixed at the end of a straw of about 8 cm length. Pinholes were made all over the straw to avoid an under-pressure inside the straw that might result from the cooling and warming procedure.

The sensitivity of the VSM was not adequate for the weakly magnetic tissue samples, for which the more sensitive alternating field gradient magnetometer (AGM) (Model MICROMAG 2900) at the Princeton Measurement Corporation was needed. Measurements on the AGM were all made at room temperature. The sample holder for the AGM consists of a thin silica rod with a small silica platelet at the end of about 2 mm in diameter. The pressed freeze-dried sample pellets of 5 mm diameter were cut into slices about 1.5–2 mm thick. A very small droplet of silicone grease was applied to the sample holder platelet, the sample slide was placed on it and carefully pressed together. No further holder material was needed.
Chapter 3

CHARACTERISATION OF THE IRON MINERALOGY IN HUMAN BRAIN TISSUE

At the start of this project it was necessary to investigate the measurement techniques needed for the identification of iron phases in brain tissue. This chapter consists of two papers, which illustrate how the different iron components were identified in the brain tissue. The first paper outlines the experimental procedure used to separate three of the four magnetic components that contribute to the magnetic signal in the different measurements. The diamagnetic signal only contributes to the induced magnetisation and — since its contribution is independent of the temperature and linearly proportional to the field — it can be subtracted from the measured signal. The contributions of magnetite, a ferrimagnetic phase, and ferritin, an antiferromagnetic phase, can be identified by using a combination of magnetic methods.

The second paper describes how the magnetic contribution of blood in the tissue can be taken into account. Blood makes a paramagnetic contribution to the induced magnetisation, i.e. it varies inversely with temperature. Appendix A outlines the procedure used for estimating the contribution of blood to measurements of the variation of induced magnetisation with temperature. For hysteresis measurements, it should be noted that the diamagnetic and paramagnetic signals are both linearly dependent on the field; the diamagnetic signal shows a negative slope and the paramagnetic signal shows a positive slope. For the hysteresis experiments, the two components can be compensated with a single correction.
3.1 Characterisation of iron compounds in tumour tissue from temporal lobe epilepsy patients using low temperature magnetic methods

Franziska Brem, Ann M. Hirt, Christian Simon, Heinz-Gregor Wieser & Jon Dobson
Published in BioMetals 18, 191-197 (2005), DOI 10.1007/s10534-004-6253-y

3.1.1 Abstract

Excess iron accumulation in the brain has been shown to be related to a variety of neurodegenerative diseases. However, identification and characterisation of iron compounds in human tissue is difficult because concentrations are very low. For the first time, a combination of low temperature magnetic methods was used to characterise iron compounds in tumour tissue from patients with mesial temporal lobe epilepsy (MTLE). Induced magnetisation as a function of temperature was measured between 2 and 140 K after cooling in zero-field and after cooling in a 50 mT field. These curves reveal an average blocking temperature for ferritin of 10 K and an anomaly due to magnetite at 48 K. Hysteresis measurements at 5 K show a high coercivity phase that is unsaturated at 7 T, which is typical for ferritin. Magnetite concentration was determined from the saturation remanent magnetisation at 77 K. Hysteresis measurements at various temperatures were used to examine the magnetic blocking of magnetite and ferritin. Our results demonstrate that low temperature magnetic measurements provide a useful and sensitive tool for the characterisation of magnetic iron compounds in human tissue.
3.1.2 Introduction

In the brain, iron plays an important role in normal neurological functions such as neurotransmitter synthesis and myelination. It is an essential component of physiological processes, such as oxygen and electron transport, but can also be toxic, acting as a catalyst for the production of free radicals. Many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, are associated with a disruption of iron metabolism (Beard, et al. 1993). Iron-induced epilepsy and electrophysiological responses are described in several studies (Ueda, et al. 1998, Ueda and Willmore 2000) and iron overload may even lead to a predisposition to epilepsy (Ikeda 2001).

The primary iron storage system in most living organisms is in the form of nanoparticles of ferrihydrite \((5\text{Fe}_2\text{O}_3\cdot9\text{H}_2\text{O})\) encapsulated by a spherical protein shell. This iron storage protein, ferritin, maintains iron in an available soluble, non-toxic form. Each protein shell of ferritin consists of 24 polypeptide subunits forming an approximately spherical cage of 12 nm in diameter. Potentially toxic ferrous iron is sequestered by the shell and oxidized in the core. The internal cavity has a diameter of approximately 8 nm and is occupied by the ferrihydrite core which undergoes antiferromagnetic ordering at low temperature and has a net magnetic moment arising from frustrated surface spins due to its small size (Massover, 1993; Harrison & Arosio 1996; Chasteen & Harrison 1999; Seehra, et al. 2000). Many low temperature magnetometry studies have been performed on horse spleen ferritin (Mohie-Eldin, et al. 1994, Makhlouf, et al. 1997, Luis, et al. 1999, Gilles, et al. 2002) and Dubiel et al. (1999) identified ferritin in globus pallidus tissue from the human brain. These authors found that ferritin in the brain had a smaller average core diameter and blocking temperature than ferritin in horse spleen.

Biogenic magnetite is another iron compound that has been identified in human brain tissue (Kirschvink, et al. 1992, Dobson and Grassi 1996, Schultheiss-Grassi and Dobson 1999, Hautot, et al. 2003) and in tumour tissue (Kobayashi, et al. 1997). Magnetite \((\text{Fe}_3\text{O}_4)\) is a ferromagnetic iron oxide with an inverse spinel structure. It contains alternating sublattices of \(\text{Fe}^{2+}\) and \(\text{Fe}^{3+}\) ions, which are antiferromagnetically coupled. The alternation of the two sublattices with unequal numbers of unpaired electron spins leads to magnetite's strong ferrimagnetic magnetisation. There is preliminary evidence that biogenic magnetite is associated with Alzheimer's disease and some studies have suggested that it may be responsible for the triggering of epileptiform activity (Fuller, et al. 1995) and the production of free radicals (Schaf er, et al. 2000). Though the source of biogenic magnetite is still
unknown, it has been suggested that ferritin may be a precursor for magnetite. If the ferritin protein becomes overloaded or if there is a malfunction in the iron transport channels, a mechanism for Fe\(^{2+}\) oxidation is lost (Quintana, et al. 2000, Dobson 2001, Zhao, et al. 2003).

Magnetometry methods, which were developed for investigations of magnetic materials, including sediments and rocks, are effective at detecting small concentrations of magnetic iron compounds within diamagnetic and paramagnetic matrices (Maher and Thompson 1999, Evans and Heller 2003). These methods can provide proxy information on the iron compounds that are present in addition to their concentration, and in the case of magnetite, its particle size. Few studies have detected ferritin in the human brain with magnetic methods. The aim of this study is to identify ferritin and biogenic magnetite in brain tumour tissue from MTLE patients and to determine which magnetic methods are most suitable for the characterisation of these iron oxides in brain tissue.

Figure 3.1: Acquisition of isothermal remanent magnetisation (IRM) at 77 K and 300 K for sample GH, showing a low coercivity phase and saturation between 200 and 300 mT.

3.1.3 Material and Methods

Two brain tissue samples, GH (gemistocytic astrocytoma, WHO grading II) and NU (ganglioglioma, WHO grading I), were resected from patients with MTLE and immediately frozen at \(-80^\circ\)C. Four types of magnetic measurements were made: (1) acquisition of isothermal remanent magnetisation (IRM); (2) thermal demagnetisation of low temperature
3.1.3 Materials and methods

IRM; (3) measurement of induced magnetisation (DC susceptibility) as a function of temperature; and (4) induced magnetisation as a function of field (hysteresis curves). All sample holders, vials, glass ware and surgical instruments used for the sample preparation were soaked in HCl for at least 24 hours and rinsed with distilled water to prevent contamination. Precautions were taken to avoid contaminations, as described in earlier studies (Dobson and Grassi 1996), and all samples were weighed prior to measuring.

Acquisition of IRM is useful in identifying ordered magnetic phases in a material because the measured remanent magnetisation is not affected by diamagnetic or paramagnetic materials in the sample, such as tissue and heme iron. For IRM experiments frozen tissue samples were placed in a pre-cooled teflon holder and put into liquid nitrogen. The IRM measurements were done on a 3-axis 2G Enterprises Superconducting Quantum Interference Device (SQUID) with a sensitivity level of $10^{-12}$ Am$^2$. First, the empty teflon holder was measured at 77 K, and then the tissue was placed in the holder and measured in the same manner. The IRM was acquired in an ASC Scientific Pulse Magnetizer Model 1M-10-30, where the sample was exposed to a pulsed DC field. First, a pulse of 1 Tesla was applied to the sample and the magnetisation was measured. Then, the sample was turned 180° and fields from 10 mT to 1 T were given in the opposite direction.

![Figure 3.2: Field cooled (FC) and zero-field cooled (ZFC) thermal demagnetisation curves of sample GH.](image-url)
After each pulse, the remanent magnetisation in the tissue was measured after 120 seconds. Afterwards, the tissue was removed and the sample holder was demagnetized in a 150 mT AC field, to remove the acquired IRM. The procedure was repeated for the empty teflon sample holder so that the signal of the holder could then be subtracted from the total IRM signal of the holder and sample.

Thermal demagnetisation of low temperature IRM was made on tissue samples that were freeze-dried to remove water. The freeze dried tissue was pressed into a cylindrical pellet within a diamagnetic straw using teflon pistons made for this purpose. Thermal demagnetisation of low temperature IRM was measured with a Quantum Design Magnetic Property Measurement System (MPMS) SQUID Magnetometer at the University of Bremen. The samples were initially cooled down to 2 K either in the absence of a magnetic field (zero-field cooled, ZFC) or in the presence of a strong 5 T field (field cooled, FC). At 2 K a field of 5 T was applied and then turned off to give an isothermal remanent magnetisation. The samples were then heated to 300 K while measuring the magnetic moment in intervals of 2 K. The MPMS is equipped with an internal field compensation coil and has a residual field of less than 100 μT.

Measurements of induced magnetisation as a function of temperature or field were also made on a MPMS SQUID magnetometer at the University of Bremen. In the thermal experiments samples were initially cooled to 2 K, either in the absence of a magnetic field (ZFC) or in the presence of a weak 50 mT field (FC). At 2 K a magnetic field of 50 mT was applied and the sample was then heated to 100 K, with the magnetic moment measured at intervals of approximately 2 K. Sample magnetisation was also measured as a function of field (hysteresis loop) at 5 K, 25 K, 77 K and 300 K, in fields from -7 to 7 T after cooling the samples in zero field.

3.1.4 Results

3.1.4.1 Isothermal Remanent Magnetisation (IRM)

All IRM acquisition curves show a rapid increase in magnetisation in low fields and saturation is reached by 200 mT (Figure 3.1). The shape of the curve is suggestive of the ferrimagnetic minerals magnetite and/or maghemite ($\gamma$-Fe$_2$O$_3$ – an oxidation product of magnetite with slightly smaller saturation magnetisation). By giving the samples a saturation
magnetisation in one direction and then acquiring the IRM in the antipodal direction, it is possible to obtain the coercivity of remanence ($H_c$). The values are 35 mT and 25 mT for GH and NU, respectively, and are approximately the same at 77 K and Room temperature. A difference in saturation remanence of $7.1 \times 10^{-7}$ Am$^2$/kg and $3.7 \times 10^{-7}$ Am$^2$/kg between the IRM at 300 K and the IRM at 77 K can be seen for GH and NU, respectively, and is due to ultrafine particles in the sample that are superparamagnetic at RT but ferromagnetic at 77 K.

![Figure 3.3: Induced ZFC-magnetisation curves as a function of temperature for sample GH between 2 K and 100 K in an applied field of 50 mT. The inset shows the FC- and ZFC curves.](image)

3.1.4.2 Thermal Demagnetisation of Low Temperature Isothermal Remanent Magnetisation (IRM)

The initial IRM intensities of GH and NU at 2 K are $4.5 \times 10^{-4}$ Am$^2$/kg and $3.3 \times 10^{-4}$ Am$^2$/kg, respectively, when cooled in zero field. On heating, there is a sharp decrease in intensity of magnetisation between 2 K and 20 K. The field-cooled samples show a higher initial IRM intensity at 2 K of $6.4 \times 10^{-4}$ Am$^2$/kg for GH and $4.6 \times 10^{-4}$ Am$^2$/kg for NU. Again, there is a sharp decrease of magnetisation on heating between 2 and 30 K. A slight change in slope is seen in the ZFC curve between 10 and 12 K (Figure 3.2). For both samples the FC and ZFC curves superimpose indistinguishably at a temperature between 55 and 60 K.
3.1.4.3 **Induced Magnetisation: DC Susceptibility**

The measurement of the induced magnetic moment as a function of temperature in a constant field after ZFC or FC is a useful method in the characterisation of horse spleen ferritin (Mohie-Eldin, *et al.* 1994, Makhlouf, *et al.* 1997, Friedman, *et al.* 1997, Gilles, *et al.* 2000). Figure 3.3 shows the DC susceptibility as a function of temperature for ZFC and FC. Both curves superimpose above the bifurcation point at approximately 60 K. This point indicates the maximum blocking temperature of the particles in the sample. The FC curve increases rapidly below this temperature, but does not follow the Curie-Weiss law for paramagnetic behaviour. The ZFC curve exhibits two local maxima at 10 and 48 K. The local maximum around 10 K is typical for the magnetic ordering of ferritin in both horse spleen (Makhlouf, *et al.* 1997) and the human brain (Dubiel, *et al.* 1999). The second local maximum at 48 K is typical for pure magnetite (Walz and Kronmüller 1991, 1994, Moskowitz, *et al.* 1998, Muxworthy 1999). There is a very strong increase in the ZFC curve below 6 K, which suggests further blocking of a magnetic phase; this will be discussed below.

3.1.4.4 **Induced Magnetisation: Hysteresis**

The magnetic hysteresis curves are dominated by a strong diamagnetic signal from the tissue also reported by Hautot *et al.* (2003). The diamagnetic signal can be removed from the total signal by subtracting the fit to the curve at 300 K, which is linearly dependent on temperature. Removal of the linear component will also remove any contribution to the magnetisation from paramagnetic materials as they also have a linear, albeit positive response. After the removal of the linear components at 300 K, a weak, closed hysteresis loop remains (Figure 3.4a). The magnetisation is saturated by approximately 200 mT, which is typical for magnetite and/or maghemite. The hysteresis at 77 K is similar to the loop at 300 K, but the intensity of the saturation magnetisation increases from $7.7 \cdot 10^{-5}$ Am$^2$/kg to $7.8 \cdot 10^{-4}$ Am$^2$/kg (Figure 3.4b). This indicates the further ordering in the low coercivity phase. At 25 K, the hysteresis loop has a contribution from a phase with higher coercivity, which can be attributed to the ordering of ferritin (Figure 3.4c). The loop is nearly closed, since the average blocking temperature of the ferritin was determined to be 10 K. Therefore, most of the particles in the sample are already in the superparamagnetic state. The lower coercivity phase is still present. The intensity of the magnetisation at 7 T is $13 \cdot 10^{-3}$ Am$^2$/kg. At 5 K, the hysteresis loop after
ZFC is open and dominated by the ferritin (Figure 3.5). The magnetisation at 7 T is 26·10^{-3} \text{Am}^2/\text{kg} and the ordered phase has a coercivity of about 50 mT.

![Graphs showing hysteresis curves](image)

**Figure 3.4:** Hysteresis curves from -7 T to 7 T of sample GH after subtracting the linear components from the curve. a) Hysteresis at 300 K, b) hysteresis at 77 K, c) hysteresis at 25 K (Note the change in scale).
3.1.5 Discussion

Both brain samples reveal clearly the presence of two magnetic compounds. Room temperature measurements and the measurements at 77 K are dominated by a low coercivity phase, while at low temperature a high coercivity phase is predominant.

The low coercivity phase at room temperature and 77 K is evident in the results of the IRM measurements and the hysteresis curves and is suggested to be magnetite and/or maghemite. The shape of the IRM curves (Figure 3.1), typically showing saturation between 200 and 250 mT, and the small remanent coercivity ($H_{cr}$) between 20 and 30 mT are consistent for either of these materials. Induced magnetisation measurements (Figure 3.3) reveal an anomaly around 50 K that has only been reported for low temperature behaviour in pure magnetite (Walz and Kronmuller 1991, 1994, Moskowitz, et al. 1998, Muxworthy 1999). Assuming that single-domain magnetite is responsible for the magnetisation, an estimate of the magnetite concentration of ordered grains in the tissue can be made from the saturation IRM at 77 K, using a value of 46 Am$^2$/kg for $J_0/2$ (Moskowitz 1993). This value assumes an ensemble of randomly oriented, non-interacting particles. A concentration of 69 ng/g and 82 ng/g can be calculated for samples GH and NU, respectively. These values are comparable to earlier studies on magnetite concentration in hippocampus tissue from epileptic patients (Schultheiss-Grassi and Dobson 1999). It should be noted that the estimated concentration is temperature-dependent due to the presence of superparamagnetic grains. Below 77 K, the high coercivity phase becomes predominant, which can be seen in hysteresis measurements at 25 and 5 K (Figures 3.4c, 3.5). The hysteresis remains closed at 25 K, even though we see a low coercivity phase in the IRM acquisition curves. This is because the magnetisation of the ferritin dominates the induced magnetisation at low temperature. The hysteresis at 5 K shows an $H_{cr}$ of 50 mT. There is a strong increase in the magnetisation intensity of the high coercivity phase with decreasing temperature. Both curves show no saturation at a field of 7 T. FC and ZFC curves from the thermal demagnetisation measurements decrease rapidly between 2 and 30 K and join between 55 and 60 K (Figure 3.2). For the induced magnetisation measurements, the FC and ZFC curves superimpose also at a temperature of 60 K (Figure 3.3). The phase shows ordering between 6 and 12 K that causes the local maximum around 10 K.

Results from all three magnetic methods support ferritin as the high coercivity phase. The increase in the contribution to intensity and the increase in coercivity seen in the hysteresis curves, the local maxima found in the ZFC curves of the induced magnetisation and
Figure 3.5: Hysteresis at 5 K from -7 T to 7 T of sample GH. The inset shows the coercivity field of 50 mT.

thermal demagnetisation and the superposition of the ZFC and FC curves are characteristic for ferritin. Hysteresis measurements are very similar to measurements done for horse spleen ferritin. Perfect antiferromagnets (AFM) do not show hysteresis, therefore the open loops are probably due to the switching of frustrated surface spins at the ferritin core or to a defect moment (Néel 1962). The ordering temperature between 6 and 12 K is also typical for ferritin; horse spleen ferritin has an average blocking temperature from 10 to 20 K (Kilcoyne and Cywinski 1995, Makhlouf, et al. 1997, Friedman, et al. 1997, Gilles, et al. 2000). The only magnetic study on human brain ferritin from globus pallidus by Dubiel et al. (1999) reports an average blocking temperature of 8.5 K. The distribution of particle volume, energy barriers and therefore blocking temperatures is known from many superparamagnetic systems. At the bifurcation point of FC and ZFC curves, the largest particle in the sample starts to be unblocked and the whole sample is superparamagnetic above 60 K. The FC and ZFC curves of both thermal demagnetisation and induced magnetisation superimpose at this temperature. This is in agreement with the results reported for horse- and human spleen ferritin by Allen et al. (2000).

The strong increase of magnetic moment below 6 K might be due to a paramagnetic phase in the tissue, such as heme iron from blood in the tissue. Blood is reported to show no
irregularity in the ZFC curve; the induced magnetisation after FC and ZFC superimpose and show a paramagnetic-like decay (Mosiniewicz-Szablewska, et al. 2003). Similar behaviour of induced magnetisation as a function of temperature has been reported for synthetically formed iron-oxyhydroxide cores inside the protein shell apoferritin using Sol-Gels (Rao, et al. 2001). An open question is whether the behaviour below 6 K can be due to (1) a mutated ferritin core, which forms a different iron phase or (2) an additional crystalline or non-crystalline phase within the ferritin core. The influence of blood on the magnetic properties at low temperature will be the focus of a future investigation.

Our measurements show that it is possible to obtain consistent results from different methods that support two magnetic phases, ferritin and magnetite, in tumour tissue from human brain resected from the mesial temporal lobe from MTLE patients.
3.2 Low temperature magnetic analysis in the identification of iron compounds from human brain tumour tissue

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Published in Journal of Physics: Conference Series 17, 61-64 (2005), DOI:10.1088/1742-6596/17/1/010

3.2.1 Abstract

In the brain, iron plays an important role, but also is potentially toxic if iron metabolism is disrupted. Excess iron accumulation in the brain has been shown to be associated with neurodegenerative diseases. However, identification of iron compounds in human tissue is difficult because concentrations are very low. Three types of magnetic methods were used to characterise iron compounds in tumour tissue from epileptic patients. Isothermal Remanent Magnetisation (IRM) was measured at 77 K and 300 K and reveals a low-coercivity phase with the properties of magnetite or maghemite. Induced magnetisation was measured between 2 K and 300 K after cooling in zero-field and in a 50 mT field. These curves reveal an average blocking temperature of 11 K, which is compatible with ferritin. The results of this study show that the combination of different magnetic methods provides a useful and sensitive tool for the characterisation of magnetic iron compounds in human tissue.
3.2.2 Introduction

Iron is an essential component of physiological processes in virtually all organisms. However, it can also be toxic by acting as a catalyst for the production of free radicals. It has long been known that a disruption in iron metabolism is associated with neurodegenerative diseases (e.g. Beard, et al. 1993, Dobson 2004). Iron-induced epileptic activity as a result of intracranial bleeding and electrophysiological responses are described in several studies, which suggest that iron overload may lead to a predisposition to epilepsy (Ueda, et al. 1998, Ueda and Willmore 2000).

The primary form of iron storage in most living organisms is in the core of the protein ferritin. Ferritin retains iron in a soluble, non-toxic form. It consists of a protein shell composed of 24 polypeptide subunits that form a spherical cage of approximately 12 nm outer diameter and 8 nm inner diameter. The interior of the shell is occupied by the iron biomineral ferrihydrite (5Fe₂O₃·9H₂O), which undergoes antiferromagnetic ordering at low temperature and has a net magnetic moment that arises from uncompensated surface spins and defects in the crystal structure. The magnetic properties of horse spleen ferritin are well characterised, but those of ferritin in the human brain are not well studied. Biogenic magnetite (Fe₃O₄) is another iron compound that was first identified by Kirschvink et al. (1992) in human brain tissue obtained during autopsies. Dobson and Grassi (1996) determined the magnetite concentrations in tissue removed from the hippocampi of epileptic patients. The source of biogenic magnetite in brain tissue is still unknown, but since ferrous iron (Fe²⁺) is toxic, the fact that magnetite, which contains both ferrous and ferric (Fe³⁺) iron, is present in the tissue raises important questions regarding its possible role in neurodegenerative diseases. We have investigated the usefulness of low temperature magnetic measurements in identifying the different iron phases that are found in epileptic and tumour brain tissue. The ability to identify different iron phases both qualitatively and quantitatively will be necessary to further examine the relationship between iron oxide particles and neurodegenerative disease.

3.2.3 Material and methods

Samples consist of tumour- and hippocampal tissues that were resected from patients with Mesial Temporal Lobe (MTLE) epilepsy. The tissue was immediately frozen at -80°C to prevent any chemical changes in the iron mineralogy. Isothermal Remanent Magnetisation
(IRM) acquisition was imparted with an ASC Impulse Magnetizer and measured both on the frozen tissue at 77 K and on freeze-dried tissue at room temperature on a 3-axis 2G SQUID magnetometer. All measurements were made 120 seconds after the pulse, so only the stable remanent magnetisation was measured. Measurements of induced magnetisation (DC susceptibility) as a function of temperature were made during warming from low temperature after zero-field cooling (ZFC) and after cooling in a 50 mT field (FC) with a Quantum Design MPMS SQUID magnetometer. Induced magnetisation as a function of field (hysteresis curves) was measured at 5 K, 25 K, 77 K and 300 K, also on a Quantum Design MPMS SQUID magnetometer. For these MPMS measurements the samples were first freeze-dried.

Figure 3.6: IRM acquisition at 77 K on different samples of tumour (T) and hippocampus (H) tissue from several MTLE patients, indicated by the sample names.

3.2.4 Results and discussion

3.2.4.1 Isothermal Remanent Magnetisation (IRM)

Acquisition of IRM at 77 K and at room temperature (300 K) shows a low-coercivity phase that is saturated by 200-300 mT, which is consistent with the presence of the ferromagnetic iron oxides magnetite and/or maghemite ($\gamma$-Fe$_2$O$_3$) – an oxidation product of magnetite. Ferritin is not magnetically ordered above 65 K and therefore does not contribute to the remanent magnetic signal. The difference in the intensity of the saturation IRM between
300 K and 77 K is due to the magnetic ordering of superparamagnetic particles of the low-coercivity phase.

The coercivity of remanence ($H_{cr}$) was obtained by giving the samples a saturation magnetisation in one direction and then acquiring the IRM in the antipodal direction. The values for $H_{cr}$ were consistently between 20 mT and 40 mT for all samples (Figure 3.6).

![Figure 3.7: FC and ZFC magnetisations induced in a DC-field of 50 mT in a tumour tissue sample.](image)

**3.2.4.2 Induced DC-magnetisation versus temperature**

Induced magnetisations were measured in a field of 50 mT between 2 K and 300 K after cooling in zero field (ZFC) as well as in a 50 mT field (FC) (Figure 3.7). The curves superimpose above the bifurcation point at 25 K; this indicates the onset of magnetic ordering. The ZFC curve exhibits two local maxima at 11 K and around 50 K. The maximum at 11 K indicates the average blocking temperature of ferritin in the human brain. The second maximum is believed to be due to pure magnetite (Walz and Kronmuller 1991); this will be the focus of further investigations.

There is a strong increase in the ZFC induced magnetisation below 6 K (Figure 3.7), which can be attributed to a paramagnetic phase, such as heme-iron from blood in the tissue. Analyses of blood (Mosiniewicz-Szablewska, et al. 2003) show no peaks due to ordering or phase transitions in the ZFC curve, meaning that the ZFC and FC curves showed identical
paramagnetic-like decay from 2 to 300 K. ZFC and FC induced magnetisations were measured on pure blood samples under the same conditions as for the tumour tissue (Figure 3.8). The subtraction of the 1.7 mg blood signal, which approximates the blood content in the sample, from the measured data reveals that at low temperatures two magnetic contributions can be decomposed: one from heme-iron and the other from ferritin.

![Graph showing ZFC magnetisations induced in a DC-field of 50 mT in tumour tissue, before and after subtraction of the blood signal.](image)

**Figure 3.8:** ZFC magnetisations induced in a DC-field of 50 mT in tumour tissue, before and after subtraction of the blood signal.

### 3.2.4.3 Induced magnetisation versus field (hysteresis)

Hysteresis measurements at different temperatures show the presence of both a low- and high-coercivity phase, which are attributed to magnetite and ferritin, respectively. A strong diamagnetic signal from the tissue as well as the contribution from paramagnetic phases was subtracted, revealing the presence of a low-coercivity phase at 300 K and 77 K. In the hysteresis curves at 25 K and 5 K, the gradual ordering with temperature of the high-coercivity ferritin phase is seen. Hysteresis measurements show an open loop at 5 K with a coercive field of 50 mT. The loop remains open up to high fields, which is typical for antiferromagnetic nanoparticles. More detailed results from these experiments are published elsewhere (Brem, et al. 2005a).
3.2.5 Conclusions

Low temperature magnetic methods were successful in identifying a low-coercivity phase that may be attributed to magnetite and/or maghemite and a high-coercivity phase, ferritin. In addition a paramagnetic signal that is consistent with heme-iron from blood was identified in the tissue sample from the measurement of induced magnetisation as a function of temperature. At present it has not been possible to distinguish if magnetite or maghemite is responsible for the low-coercivity phase. It was not possible to identify a Verwey transition in the induced DC magnetisation curves due to the weakness of the signal; however, the peak in magnetisation found around 50 K has only been identified in magnetite (Walz and Kronmuller 1991). Magnetic methods provide a sensitive tool to examine the role of iron and iron oxide particles in brain tissue, even for very small concentrations.
Chapter 4

MAGNETIC ANALYSIS AND MAGNETIC MODELING OF HORSE SPLEEN FERRITIN AND MAGNETITE NANOPARTICLES

The magnetic properties of nanoparticles often differ considerably from the macroscopic properties of the material in larger particle sizes (Néel 1961b, Rancourt 2001). Surface properties cannot be neglected. The explanation of measured magnetic properties of nanoparticles generally requires a suitable magnetic model. The most commonly used models are based on Néel’s theory of superparamagnetism and the Langevin function (see Appendix B).

At least two magnetic nanoparticle components have been identified in the human brain: the antiferromagnetic cores of ferritin, and nanosized magnetite particles. The goal of this chapter is to obtain a better understanding of the magnetic properties of ferritin and magnetite nanoparticles. Horse spleen ferritin, which is commercially available, is used instead of human brain ferritin. In the first part of Chapter 4, the modeling of the initial magnetisation curves of horse spleen ferritin is described. This is a new approach to the problem: previous studies have treated horse spleen ferritin as a single magnetic phase, consisting of non-interacting ferrithydrate cores. However, hysteresis loops suggest that a second phase is present in commercially available products. In the second part of the chapter, commercial horse spleen ferritin and synthetic, nanosized magnetite particles are characterised by several magnetic methods, separately and also as a mixed system. The magnetic properties of the mixed system are then compared to the magnetic properties of human brain tissue.
4.1 Modeling the magnetic behaviour of horse spleen ferritin with a two-phase core structure

Franziska Brem, Gabriela Stamm & Ann M. Hirt

4.1.1 Abstract

The growth of the nanotechnology industry has led to an increased interest in characterizing magnetic nanoparticles. A natural material with well-defined grain size in the nanoparticle range is commercially available horse spleen ferritin, an iron storage protein. Modeling of the magnetic properties of commercial horse spleen ferritin is often based on the assumption of a single-phase core of ferrihydrite (5Fe₂O₃·9H₂O). Low temperature hysteresis measurements indicate, however, that the ferritin cores contain at least two magnetic phases. Initial magnetisation curves measured at temperatures between 50 K and 300 K have been modeled using four methods. A model that used a sum of two Langevin functions fitted the data 70% better on average than a model that used a single Langevin function. It was also superior to both a random mean orientation model and a model that takes account of crystalline anisotropy. The two-phase model consists of a phase with a high coercivity that does not undergo saturation and a second phase with a low coercivity and a saturation field of 300 mT. The high coercivity phase is compatible with antiferromagnetic ferrihydrite, while the low coercivity phase could be magnetite, maghemite, or a mixture of both. The results from this study are consistent with earlier microscopic studies that characterise horse spleen ferritin as a multiphase system with up to 30% of magnetite/maghemite-like cores.
4.1.2 Introduction

Horse spleen ferritin has received attention in recent years for the modeling of magnetic behaviour of nanoparticles because it is a non-interacting antiferromagnet. Ferritin is the primary protein for iron storage in most living organisms. It consists of a protein shell made up of 24 polypeptide subunits forming a spherical cage that is 8-12 nm in diameter (Chasteen and Harrison 1999). In nature, the interior of the shell is occupied by an iron biomineral, generally described as ferrihydrite ($5\text{Fe}_2\text{O}_3\cdot9\text{H}_2\text{O}$), which undergoes antiferromagnetic ordering between 12 K and 20 K (Makhlouf, et al. 1997, Allen, et al. 2000). The empty protein shell of ferritin (apoferitin) has also been filled synthetically to produce mono-dispersed and highly uniform magnetic nanoparticles (Meldrum, et al. 1992, Resnick, et al. 2004).

The magnetic properties of commercial horse spleen ferritin have been modeled as a single-phase system with superparamagnetic (SP) particle size. Typically, a Langevin function has been used to estimate the magnetisation ($M$) and the average magnetic moment ($\mu_p$) of the ferritin core by assuming a single phase (Kilcoyne and Cywinski 1995, Makhlouf, et al. 1997, Gilles, et al. 2000). These earlier magnetic models are not always completely satisfactory and often show discrepancies in the degree of fit at low fields. Hysteresis loops measured on horse spleen ferritin display a wasp-waisted shape that is indicative of a second phase in the system. Several studies have described the natural core of ferritin as a multi-phase system consisting of mainly ferrihydrite and another phase similar to magnetite and/or maghemite (Towe and Bradley 1967, Quintana, et al. 2000, Cowley, et al. 2000, Janney, et al. 2001). Quintana et al. (2004) found that the proportion of the magnetite/maghemite-like phase is elevated in ferritin from patients with Alzheimer’s disease and supranuclear palsy. But they also found in samples from commercial horse spleen ferritin that up to 30 percent of the cores exhibit a cubic magnetite/maghemite structure.

If only a small amount of a magnetite/maghemite phase is present in the cores of ferritin, it should have a noticeable effect on the magnetic properties of the protein because of the high spontaneous magnetisation and low coercivity of magnetite and maghemite. In this study, initial magnetisation curves were measured in horse spleen ferritin. They were modeled using two Langevin functions: one Langevin function together with a linear high field component accounts for the ferrihydrite component, while a second Langevin function models the superparamagnetic contribution of a magnetite/maghemite phase to the overall magnetic signal.
4.1.3 Model

Antiferromagnetic nanoparticles have a small uncompensated magnetisation arising from (a) defects in the core of the particle, and (b) unpaired surface moments (Néel 1961b). At a given temperature below the Néel temperature ($T_N$), moments of the two sublattices begin to fluctuate and cross the energy barriers, resulting in superparamagnetic relaxation. For the superparamagnetic regime in low fields, Néel has shown that the magnetisation is the sum of two contributions: the first is the susceptibility from the canting of the two sublattices; the second is the magnetisation due to uncompensated moments (Néel 1961c). There have been three approaches to modeling antiferromagnetic particles. The first approach is the modified Langevin model (mLM), which assumes an ensemble of non-interacting isotropic magnetic moments in addition to a linear term $\chi_1 H$ (Kilcoyne and Cywinski 1995, Makhlouf, et al. 1997):

$$M(M_{0,1}, \mu_1, \chi_1) = M_{0,1} \cdot \frac{\text{coth}(\mu_1 H / k_B T) - k_B T / \mu_1 H}{k_B T / \mu_1 H} + \chi_1 H$$

(4.1)

where $M_{0,1}$ is the saturation magnetisation, $\mu_1$ the average magnetic moment of each nanoparticle and $\chi_1$ is the linear susceptibility. In a second approach, Gilles et al. (2000)
describe a random magnetic orientation model (RMO), which takes into account the fluctuation of the spins between the two antiparallel directions along the antiferromagnetic axis. The magnetic moment for the RMO model is given by

\[ M(M_{0,1}, \mu(T), \chi_1) = M_{0,1} \cdot \left[ 0.5 \int_{0}^{\pi} \sin \theta \cos \theta \tanh \left( \frac{1}{k_B T} \frac{\mu(T) H}{k_B T} \cos \theta \right) d\theta \right] + \chi_1 H \quad (4.2) \]

where \( \mu(T) \) is the moment of the uncompensated spins in each core and \( \theta \) is the angle between the random direction of the antiferromagnetic axis and the direction of the applied field. The mLM and RMO models both assume a single material within all ferritin cores. In a third approach, Resnick et al. (2004) modeled \( \gamma \)-Fe\(_2\)O\(_3\) nanoparticles mineralized in ferritin, and added a second order term \( \chi_1 H^2 \) to the Langevin model to account for the presence of crystalline anisotropy (CA). They showed that the CA model gave a better fit to their data than the mLM and RMO models.

If one assumes a contribution to the magnetisation from a magnetite/maghemite phase within the ferritin cores, the above models must be modified to account for a bimorphic system. Magnetite and maghemite are ferrimagnetic, and in order to be accommodated within the apoferritin shell, they must have a small particle size. They behave superparamagnetically and can be modeled by a single Langevin function (Parvin, et al. 2004). A second Langevin function is added to the mLM model for the antiferromagnetic particles to account for the magnetite/maghemite phase. This summation model (SM) is expressed as:

\[ M(M_{0,1}, \mu_1, \chi_1) = M_{0,1} \cdot \left[ \coth \left( \frac{\mu_1 H}{k_B T} \right) - \frac{k_B T}{\mu_1 H} \right] + \chi_1 H + M_{0,2} \cdot \left[ \coth \left( \frac{\mu_2 H}{k_B T} \right) - \frac{k_B T}{\mu_2 H} \right] \quad (4.3) \]

where \( \mu_1 \) is the average magnetic moment for each ferrihydrite particle and \( M_{0,1} \) is the saturation magnetisation of ferrihydrite, \( \mu_2 \) is the average magnetic moment for cores with magnetite/maghemite-like phases and \( M_{0,2} \) is the saturation magnetisation of this phase. Of course, it would be possible to combine two RMO functions or two CA functions, but here we show that the goodness of fit can be improved merely by summing the simplest functions. In order to compare the quality of the SM model to the mLM, the RMO and the CA models, the residuals, root-mean-square of the residuals (RMS), the sum of the least squares \( \Sigma (\text{Res})^2 \) and the empiric correlation coefficient \( R \) of the fits were computed.
4.1.4 Experiment

Three samples were made from a commercial horse spleen ferritin type I (Sigma). Each sample was made from a different batch of horse spleen ferritin, which was purchased over a two-year period. The sample preparation and the magnetic measurements were made within two weeks of purchase and the ferritin was stored and dried at 4 °C to prevent biochemical alteration. After drying, the ferritin was packed into a gel cap for handling. The samples were weighed prior to measurement of their magnetisations with a Quantum Design MPMS SQUID magnetometer. Initial magnetisation measurements were made in fields up to 5 T or 7 T at four or six different temperature steps between 5 K and 300 K. The magnet was re-set to zero before changing to a new temperature. Hysteresis measurements were made on the same MPMS between +5 T and −5 T, or between +7 T and −7 T, at temperatures between 5 K and 300 K.
Figure 4.2: (a) Initial magnetisation curves of horse spleen ferritin at different temperatures. The lines are the fits for model $\Sigma M$. The highlighted data at 50 K and 200 K are fitted by the Langevin model mLm (dotted line) and a sum of two Langevin functions in model $\Sigma M$ (solid line). (b) Residuals (Res) as a function of field for models mLm and $\Sigma M$ at 50 K. (c) Residuals as a function of field for models mLm and $\Sigma M$ at 200 K.
4.1.5 Results and Discussion

Hysteresis measurements at 5 K show a wasp-waisted loop similar to the loop of Makhlouf et al. (1997) (Figure 4.1a). Wasp-waisting suggests the presence of a second phase with lower coercivity (Tauxe, et al. 1996) (Figure 4.1b). At 300 K the magnetisation curve is not linear but shows a weak, closed loop at very low fields, which indicates the presence of a superparamagnetic phase of a low coercivity mineral (Figure 4.1c). These two observations support the TEM results by Quintana et al. (2004) who identified a small percentage of a cubic magnetite/maghemite-like phase in horse spleen ferritin. For this reason it is reasonable to propose a system with at least two contributing magnetic phases that need to be incorporated in the magnetic modeling.

The initial magnetisation curves are shown in Figure 4.2. At low temperature they show a steep gradient and strong curvature, while at higher temperature they become less steep and more linear. The ΣM model fits the data better than the mLM, RMO and CA models, particularly at low fields (Figure 4.2a) and at low temperatures (Figure 4.3). Figures 4.2b and 4.2c show that the residuals are 4.7 times smaller for the ΣM model than for the mLM model. The improvements are most evident at low fields; this again suggests that a low coercivity phase influences the magnetic signal in these low fields. At low temperature (50 K) the residuals for the ΣM model are less than 15% of those for the mLM model. This indicates that the influence of a second phase becomes important at lower temperature. At 250 K and 300 K, the improvement is less obvious; all models fit the curves with better accuracy than at low temperature because both phases are perfectly superparamagnetic at the higher temperatures. For the ΣM model, the best fit to the data in our study is reached at 150 K. Above this temperature, the quality of fit decreases slightly. At 250 K and 300 K all models seem to fit the data equally well, displaying the same trends in the three different samples, although the absolute fits for a single model show slight differences. This may be attributed to minor variations of properties between the ferritin batches, related to different biological sources of the samples.

For the ΣM model we can plot the two modeled phases separately to estimate the saturation fields of the phases (Figure 4.4a). The low-coercivity phase (with parameters \( M_{0,2} \) and \( \mu_2 \)) is seen to have a saturation field of 200-300 mT at 300 K and a slightly higher saturation field at 77 K, which indicates magnetite or maghemite that behave superparamagnetic at 300 K (Figure 4.4b). The second, high-coercivity phase (with parameters \( M_{0,1}, \mu_1, \text{and } \chi_1 \)) shows a steep increase in magnetisation at lower fields and does
not reach saturation in the strongest fields available. The latter feature is typical for an antiferromagnet, such as ferrihydrite (Gilles, et al. 2002).

Table 4.1 provides an overview of all parameters of the different models at temperatures of 50, 100, 150 and 250 K. $M_{0,1}$ decreases with increasing temperature for all models. The Néel temperature of the ferritin core has been commonly estimated by extrapolating $M_{0,1}$.

![Figure 4.3](image)

**Figure 4.3:** The sum of least squares ($\Sigma(\text{Res})^2$) for the four different models as a function of temperature. The $\Sigma M$ model shows the best fit to the measured data, especially at low temperature. At high temperature all models fit the data with better accuracy.

Published values of $T_N$ for ferritin as well as for ferrihydrite vary widely (Makhlouf, et al. 1997, Brooks, et al. 1998, Seehra, et al. 2000), which generates some doubts about the accuracy of this method. In the present study, the extrapolated Néel temperatures are 428 K for the mLm model, 388 K for the RMO model, and 341 K for the CA model. The Néel temperature found for the ferryhydrite cores using the $\Sigma M$ model is 501 K, which is in accordance with more recent studies (Gilles, et al. 2000, Silva, et al. 2005). The magnetic moment $\mu_1$ for the mLm model lies between 350 $\mu_B$ and 400 $\mu_B$. For the $\Sigma M$ model $\mu_1$ is smaller and increases with temperature, similar to observations for ferrihydrite by Seehra et al. (2000) This increase has been attributed to a thermally induced contribution in antiferromagnetic nanoparticles (Morup and Frandsen 2004). However, a recent study has shown that this increase can be due to the magnetic moment distribution (Silva, et al. 2005).
Our modeled values of $\mu_2$ lie between 630 $\mu_B$ and 900 $\mu_B$, which are higher than values for an antiferromagnetic phase. Compared to nanoparticle systems of pure magnetite or maghemite, where values of 2000-9000 $\mu_B$ for nearly defect-free particles are found (Resnick, et al. 2004, Dutta, et al. 2004), the values of $\mu_2$ are relatively small. This may be due to some cores containing amorphous and mixed phases in addition to pure, perfectly grown crystallites, as is common in biological material. The values for the susceptibility $\chi_1$ decrease with increasing temperature for all models and do not show any noteworthy divergence. A difference in $\chi_1$ between the models would not be expected, since $\chi_1$ is influenced largely by the antiferromagnetic ferrihydrite phase. The $\chi_{CA}$ in the CA model has very small values, indicating that the crystalline anisotropy does not appear to be as important here as for the maghemite particles in the study of Resnick et al. (2004) However, its influence can not be neglected, because the CA model obviously enhances the quality of fit compared to the mLm and RMO models.

4.1.6 Conclusion

Magnetic measurements, such as susceptibility and low temperature hysteresis, on different batches of commercial horse spleen ferritin reveal features of a non-single phase core structure. Initial magnetisation curves measured at different temperatures have been fitted by four different models. The best fit was obtained with a model composed of a sum of two Langevin functions, considering the cores of horse spleen ferritin to be a two-phase system. Separation of the two modeled phases reveals the contribution of a high coercivity phase, which is interpreted to be ferrihydrite, and a low coercivity phase similar to magnetite and/or maghemite. These results confirm former TEM observations, which showed that not all the ferritin cores consist of single ferrihydrite crystallites, but that a small fraction of the protein shells encases a magnetite/maghemite-like phase.
TABLE 4.1: Estimated magnetic parameters ($M_0$, $\mu$, and $\chi$) and degree-of-fit parameters ($RMS$, $(1-R^2)$ and $\Sigma(\text{Res})^2$) for the mLm, RMO, CA and SM models at different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Model</th>
<th>$M_0_1$ (emu/g)</th>
<th>$\mu_1$ (emu/T)</th>
<th>$X_1$ (emu/T)</th>
<th>$M_0_2$ (emu/g)</th>
<th>$\mu_2$ (emu/T)</th>
<th>$RMS$ ($\times 10^3$ emu/g)</th>
<th>$\Sigma(\text{Res})^2$ ($\text{emu}^2$)</th>
<th>$1-R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 K</td>
<td>mLm</td>
<td>0.093</td>
<td>350</td>
<td>5.14x10^6</td>
<td></td>
<td></td>
<td>1.790</td>
<td>8.65x10^3</td>
<td>6.81x10^3</td>
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<tr>
<td></td>
<td>RMO</td>
<td>0.724</td>
<td>113</td>
<td>5.44x10^6</td>
<td></td>
<td></td>
<td>2.508</td>
<td>1.70x10^4</td>
<td>9.80x10^3</td>
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<tr>
<td></td>
<td>CA</td>
<td>0.071</td>
<td>454</td>
<td>6.69x10^6</td>
<td>-2.43x10^11</td>
<td>0.735</td>
<td>1.46x10^4</td>
<td>2.65x10^3</td>
<td></td>
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<tr>
<td></td>
<td>SM</td>
<td>0.077</td>
<td>128</td>
<td>4.59x10^6</td>
<td>0.046</td>
<td>631</td>
<td>0.225</td>
<td>1.37x10^4</td>
<td>1.31x10^4</td>
</tr>
<tr>
<td>100 K</td>
<td>mLm</td>
<td>0.080</td>
<td>387</td>
<td>3.67x10^5</td>
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<td></td>
<td>0.875</td>
<td>2.07x10^4</td>
<td>4.88x10^3</td>
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<tr>
<td></td>
<td>RMO</td>
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<td>130</td>
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<td>1.134</td>
<td>3.47x10^5</td>
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<td></td>
<td>CA</td>
<td>0.051</td>
<td>527</td>
<td>5.10x10^5</td>
<td>-1.89x10^11</td>
<td>0.295</td>
<td>2.34x10^4</td>
<td>1.25x10^3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>0.073</td>
<td>177</td>
<td>3.25x10^5</td>
<td>0.033</td>
<td>674</td>
<td>0.141</td>
<td>5.36x10^7</td>
<td>4.39x10^4</td>
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<td>150 K</td>
<td>mLm</td>
<td>0.066</td>
<td>405</td>
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<td></td>
<td>0.420</td>
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<tr>
<td></td>
<td>RMO</td>
<td>0.474</td>
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<td></td>
<td></td>
<td>0.515</td>
<td>7.16x10^5</td>
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<tr>
<td></td>
<td>CA</td>
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<td>556</td>
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<td>-1.44x10^11</td>
<td>0.115</td>
<td>3.60x10^7</td>
<td>7.74x10^4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>0.070</td>
<td>189</td>
<td>2.61x10^5</td>
<td>0.026</td>
<td>671</td>
<td>0.059</td>
<td>9.48x10^8</td>
<td>1.78x10^4</td>
</tr>
<tr>
<td>250 K</td>
<td>mLm</td>
<td>0.045</td>
<td>366</td>
<td>2.24x10^5</td>
<td></td>
<td></td>
<td>0.107</td>
<td>3.09x10^7</td>
<td>3.13x10^4</td>
</tr>
<tr>
<td></td>
<td>RMO</td>
<td>0.299</td>
<td>137</td>
<td>2.35x10^5</td>
<td></td>
<td></td>
<td>0.112</td>
<td>3.39x10^7</td>
<td>3.37x10^4</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>0.031</td>
<td>398</td>
<td>2.68x10^5</td>
<td>-4.06x10^12</td>
<td>0.098</td>
<td>2.57x10^7</td>
<td>1.76x10^4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>0.049</td>
<td>274</td>
<td>2.12x10^5</td>
<td>0.007</td>
<td>633</td>
<td>0.095</td>
<td>2.44x10^7</td>
<td>1.28x10^4</td>
</tr>
</tbody>
</table>
Figure 4.4: (a) Initial magnetisation curves of horse spleen ferritin at 77 K and 300 K and their separation into two co-existing phases using the ΣM model. FH indicates the high-coercivity ferritohydrite phase, and MG indicates the magnetite/maghemite-like low-coercivity phase. (b) Magnetisation of the low-coercivity phase at 77 K and 300 K in lower fields.
4.2 A mixture of ferritin and magnetite nanoparticles mimics magnetic properties of human brain tissue

Franziska Brem, Louis Tiefenauer, Alke Fink, Jon Dobson & Ann M. Hirt

4.2.1 Abstract

Magnetic properties of a two-component system, consisting of horse spleen ferritin (HoSF) which contains a 5-8 nm sized antiferromagnetic ferrihydrite (5Fe₂O₃·9H₂O) core and ferrimagnetic magnetite (Fe₃O₄) nanoparticles (MNP) with an average size of 10-20 nm, have been investigated by using four different methods: induced magnetisation versus (1) temperature and (2) field; (3) AC susceptibility; and (4) first-order reversal curves (FORC). All measurements were done on a mixed system of HoSF and MNP, as well as separately on the individual components. The average blocking temperature ($T_B$) of the mixed system at 50 mT is 15.6 K, which is a shift towards higher temperatures compared to pure HoSF ($T_B = 12$ K). The contribution of the MNP component to magnetic ordering is evident only as a separation of the zero-field-cooled and field-cooled measurement curves. AC susceptibility is dominated by the ferrimagnetic MNP and shows strong frequency dependence. The peak AC susceptibilities can be described by the Vogel-Fulcher law, indicating the influence of interactions within the system. Hysteresis measurements at 5 K show a wasp-waisted shape due to the mixture of a high coercivity phase (HoSF) with a low coercivity phase (MNP). Initial magnetisation curves above $T_B$ can be fitted by a sum of Langevin functions, showing superparamagnetic behaviour of both components. FORC diagrams are effective in illustrating the change from that of blocked MNP particles together with the superparamagnetic HoSF at 20 K to purely superparamagnetic behaviour in both components above 50 K. We conclude that the mixed nanoparticle system is a good model for complex natural samples, such as human brain tissue.
4.2.2 Introduction

Characterisation of magnetic properties can offer valuable information for investigations of the history and function of materials. Natural systems, such as soils, rocks and diverse biological materials, usually contain more than one magnetic component. Furthermore, synthetic materials may also contain more than one magnetic component due to by-products of synthesis or chemical oxidation processes (Schwertmann, et al. 2000, Hirt, et al. 2002). Such mixed systems are rarely described, because measurements of their physical properties are difficult to interpret and various methods of analysis are usually needed to separate and characterise each contributing component. Even more difficult to characterise are mixtures consisting of different nano-sized magnetic particles. One unusual example of such a complex magnetic mixture is tissue from the human brain. The interest in iron metabolism and iron components found in the human brain has increased strongly in the past few years because of their association with neurological and neurodegenerative diseases (Dobson 2001). Human brain tissues contain at least four different magnetic components. The dominant magnetic signal arises from the strongly diamagnetic fatty tissue (the matrix) in which the other components are embedded. The second dominant component is heme-iron from blood, which is near-paramagnetic in its magnetic properties (Slawska-Waniewska, et al. 2004). Ferritin, the third component, which is responsible for the intracellular storage of iron, is present throughout the brain. Ferritin consists of a core of antiferromagnetic ferrihydrite (5Fe$_2$O$_3$·9H$_2$O), 5-8 nm in diameter, surrounded by a protein shell (Chasteen and Harrison 1999), and is superparamagnetic at room temperature. In the past decade magnetite/maghemite (Fe$_7$O$_{12}$/γ-Fe$_2$O$_3$) has been discovered in human brain tissue (Kirschvink, et al. 1992, Schultheiss-Grassi and Dobson 1999). The origin and the formation process of this strongly ferrimagnetic component are still unknown and the subject of intense research. Identification of the different magnetic components is difficult, and magnetic modelling is nearly impossible, since the concentrations of the individual phases and their iron contents are unknown.

To gain a better physical understanding of such multi-component magnetic systems, we measured two known magnetic components separately and as a mixture. This system consists of commercially available horse spleen ferritin (HoSF), which is magnetically well characterised (Makhlouf, et al. 1997, Allen, et al. 2000, Brem, et al. 2006), and albumin coated magnetite nanoparticles (MNP) with 10-20 nm diameter. Both components are superparamagnetic at room temperature. Therefore, measurements at low temperature are
required for a full magnetic characterisation of the mixed system HoSF-MNP. This model will facilitate the characterisation of natural multi-component systems of biological origin.

4.2.3 Experimental Details

The MNP sample was made from 300 mg magnetite nanoparticles with a diameter of 10-20 nm suspended in 10 mM HCl. This material was originally developed as a contrast agent for magnetic resonance imaging (Tiefenauer, et al. 1993, Tiefenauer, et al. 1996). The particles were then coated with 150 mg poly-(D-glutamic acid, D-lysine) hydrobromide (6:4) (wt 20-50 kD, from Sigma) in 100 mM HEPES pH 6.8, washed with 100 mM HEPES pH 7.3 and ultrasonically dispersed. Finally coated particles were incubated for 30 min in 1 ml 0.1% human serum albumin. After washing, the material was lyophilized and the resulting dry powder was mixed with commercial horse spleen ferritin (HoSF, Sigma). The ferritin-magnetite mixture was dried and packed into a gel cap for handling. The samples contain 60-70 mg of HoSF and 0.4-0.5 mg of MNP. These low concentrations were chosen to mimic the assumed natural content in biological samples.

Induced magnetisation was measured as a function of both temperature and field using a Quantum Design MPMS SQUID magnetometer at the University of Bremen and at the Chemistry Department at ETH Zurich. In the thermal experiments, samples were initially cooled to 5 K, either in the absence of a magnetic field (zero field cooling, ZFC) or in the presence of a 50 mT field (field cooling, FC). At 5 K a magnetic field of 50 mT was applied and the induced magnetic moment of the sample was measured at intervals of 2 K during heating to 300 K. The sample magnetisation was also measured as a function of field (hysteresis loops and initial magnetisation curves) at a series of temperatures between 5 and 300 K, in fields of ±5 T after cooling the samples in zero field. AC susceptibility measurements were made at different frequencies between 100 Hz and 10 kHz in a weak applied field of 79.7 A/m (1 Oe) on a Quantum Design Physical Property Measurement System (PPMS) at the Chemistry Department of ETH Zurich. First order reversal curves (FORC) were measured on a PMC vibrating sample magnetometer (VSM) at 20 K, 50 K and 90 K. The FORC data was processed with a Matlab code by Winklhofer and Pike (http://venus.geophysik.uni-muenchen.de/~michael/forcnew/).
Figure 4.5: Induced magnetisation as a function of temperature measured after zero field cooling (ZFC) and cooling in a 50 mT field (FC) of a) pure HoSF, b) pure MNP and c) HoSF-MNP mixed system.

4.2.4 Experimental Results and Discussion

4.2.4.1 Induced magnetisation as a function of temperature

Figures 4.5a-c show the induced magnetisation versus temperature measured in a 50 mT field for the two individual components and the mixed system. Figure 4.5a shows the measurements for pure HoSF, in which the maximum in the ZFC curve at 12 K indicates the average unblocking temperature of the ferritin (Makhlouf, et al. 1997, Seehra and Punnoose 2001). The bifurcation point between ZFC and FC curves at 22 K shows the point in which all
particles are unblocked. For the magnetite nanoparticles (MNP), the average unblocking temperature is at 56 K and the bifurcation point is at 125 K (Figure 4.5b). The shape of the curve resulting from the mixed system (Figure 4.5c) is a combination of the curves in Figs. 4.5a and 4.5b. The average unblocking temperature is at 15.6 K, indicating that the HoSF is the dominant component. The higher unblocking temperature observed for the mixed system compared to the pure HoSF, however, must be due to the magnetite particles. The gap between ZFC- and FC-curves at low temperature is a result of remanent magnetisation acquired when cooling in a field. In the mixed system the distance between the ZFC and FC curves is relatively large in comparison to that of the individual components. The curves are separated below a temperature of 126 K, which corresponds to the final unblocking temperature of the magnetite nanoparticles. The decreasing separation of the curves during heating reveals the decreasing content of blocked particles in the system (Pardoe, et al. 2001).

These results are compared to a natural sample of human brain tissue, which may contain a similar mixed system (Brem, et al. 2005a). The dependence of magnetisation induced in a 50 mT field on temperature is shown in Figure 4.6. The effect of the strong diamagnetic matrix is obvious, since both the ZFC- and the FC-curve show a negative magnetisation above 10 K (Figure 4.6, inset). The diamagnetic signal, however, is independent of temperature and can be separated from other components. At temperatures below 5 K, the uncorrected curves show an increase with decreasing temperature due to a superposed paramagnetic contribution of the heme-iron of blood (Brem, et al. 2005a). The measurements were corrected for a diamagnetic contribution of \(-1.64 \cdot 10^{-4} \text{ Am}^2/\text{kg}\) and a paramagnetic signal of 1.5 mg of freeze-dried blood. The local maximum in the corrected ZFC curve around 11 K is indicative of the average unblocking temperature of ferritin in the brain tissue. The slight gap between the ZFC and FC curves up to around 70 K indicates the influence of a remanent magnetisation from an additional component, which is magnetite/maghemite.
4.2.4.2 AC Susceptibility

Measurements of the in-phase ($\chi'$) and out-of-phase ($\chi''$, quadrature) components of AC susceptibility for the HoSF-MNP system are shown in Figure 4.7a and 4.7b, respectively. Each of the components shows two distinct peaks in the susceptibility curve, which can be assigned to HoSF at lower temperature and to MNP at higher temperature. $\chi'$ displays a strong frequency-dependence for both components. The peak temperatures increase with increasing frequencies while the signal strength decreases at higher frequencies. For $\chi''$ the frequency dependence of the HoSF peak is less obvious, while for the MNP peak the frequency dependence is strong (Figure 4.7b). All values of the peak temperatures are given in Table 4.2. Comparing these to the values of the individual components (shown for 1 kHz in Figures 4.7a,b), $\chi'$ of pure HoSF peaks at slightly lower temperature than the HoSF component in the mixed system. For $\chi''$ the values for pure HoSF are almost identical with the $\chi''$ in the mixed system. The opposite effect is observed for the MNP component. The pure MNP phase peak temperatures of $\chi'$ are significantly higher than for the MNP
4.2.4 Experimental results and discussion

component, which corresponds to a shift towards lower temperatures in the mixed system. A similar shift is seen for $\chi''$ (Table 4.2).

![Figure 4.7](image_url)

**Figure 4.7**: In-phase susceptibility $\chi'$ (a) and out-of-phase susceptibility $\chi''$ (b) as a function of temperature. The HoSF-MNP mixed system was measured at frequencies of 100 Hz, 1 kHz and 10 kHz. The dotted lines indicate (a) $\chi'$ and (b) $\chi''$ for the pure components HoSF and MNP, measured at 1 kHz.
A study of the average blocking temperature of ferrihydrite showed that by coating the nanoparticles with silicon, interaction is partially inhibited leading to a decrease in $T_B$ (Zhao, *et al.* 1996). A similar explanation may be valid for this system, where the magnetite concentration is less than 1% of the sample weight. The small amount of MNP, dispersed within the ferritin, reduces interactions between the MNP particles, thereby shifting the peak to lower temperatures.

An indication that there is still interaction between the MNP particles within the mixed system is seen from the fact that the Arrhenius law $\tau = \tau_0 \exp[E/kT]$ does not properly describe the relaxation peaks of $\chi''$. For systems showing slight particle interactions, it has been proposed that the frequency-dependent peak susceptibility of $\chi''$ would follow the Vogel-Fulcher equation (Shtrikman and Wohlfarth 1981, Zhang, *et al.* 1996, Taketomi 1998, Shim, *et al.* 2006):

$$\tau = \tau_0 \exp[E/k(T-T_0)] \quad (4.4).$$

The measured peak temperatures of the MNP component are in good agreement with the Vogel-Fulcher law (Figure 4.8) and the best-fit model yields $\tau_0 = 2 \times 10^9$, $E/k = 1016$ K and $T_0 = 86$ K. These values for $T_0$ and $E/k$ are very similar to values from other dispersed systems of biocompatible magnetite nanoparticle (Morais, *et al.* 2004). The fact that the measurements can be fitted by the Vogel-Fulcher law indicates that there are slight interactions between the magnetite nanoparticles. $T_0$ can be subtracted from the measured peak temperatures $T_{peak}$ of $\chi''$ to give the theoretical Néel relaxation peak temperatures in the absence of interactions (Figure 4.8).

**TABLE 4.2:** Peak temperatures [K] of the in-phase susceptibility $\chi'$ and the out-of-phase susceptibility $\chi''$ for the mixed system HoSF-MNP and the individual components HoSF and MNP at different frequencies.

<table>
<thead>
<tr>
<th></th>
<th>Mixed system Frequency [kHz]</th>
<th>Individual components Frequency [kHz]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>$\chi'$ (HoSF)</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>$\chi''$ (HoSF)</td>
<td>11.5</td>
<td>12</td>
</tr>
<tr>
<td>$\chi'$ (MNP)</td>
<td>190</td>
<td>197.5</td>
</tr>
<tr>
<td>$\chi''$ (MNP)</td>
<td>145</td>
<td>152.5</td>
</tr>
</tbody>
</table>
4.2.4 Experimental results and discussion

4.2.4.3 Induced magnetisation versus field

At low temperature the hysteresis is dominated by the antiferromagnetic high coercivity HoSF component (Figure 4.9). The MNP component leads to a clear wasp-waisted shape of the hysteresis loop (inset, Figure 4.9) due to the mixing of a low coercivity component, such as magnetite, with a high coercivity component (Tauxe, et al. 1996). The hysteresis at 5 K of the HoSF-MNP system displays a coercive force ($B_c$) of 38 mT compared to values of 120 mT and 20 mT for pure HoSF and MNP, respectively. At higher temperature, $B_c$ decreases from 38 mT (5 K) to 6 mT at 30 K, 3 mT at 50 K, and 0.5 mT at 100 K. At temperatures $\geq 150$ K, the hysteresis is a closed loop and shows purely superparamagnetic behaviour.

Initial magnetisation curves at temperatures above full unblocking of the MNP are shown in Figure 4.10a. They are the result of two different superparamagnetic contributions, such that the initial magnetisation curves must be fitted by a sum of a modified Langevin function and another Langevin function (one for HoSF and one for MNP):
\[ M_{\text{Mix}} = M_{0,\text{MNP}}L_1(\mu_{p,\text{MNP}}H/k_B T) + M_{0,\text{HoSF}}L_2(\mu_{p,\text{HoSF}}H/k_B T) + \chi H \quad (4.5) \]

where \( L = \coth x - 1/x \), \( M_{0,\text{MNP}} \) is the saturation magnetisation of magnetite, \( \mu_{p,\text{MNP}} \) is the magnetic moment per magnetite nanoparticle, \( M_{0,\text{HoSF}} \) is the saturation magnetisation of ferritin, \( \mu_{p,\text{HoSF}} \) is the magnetic moment per ferritin core, and \( \chi \) is the high-field susceptibility.

Using this model gives \( \mu_{p,\text{MNP}} = 6260 \mu_B \) and \( \mu_{p,\text{HoSF}} = 320 \mu_B \). The small value for the horse spleen ferritin agrees with modeled values between 300 \( \mu_B \) and 350 \( \mu_B \) that are reported in the literature (Kilcoyne and Cywinski 1995, Makhlouf, et al. 1997, Seehra and Punnoose 2001).

Fitting the initial magnetisation curves to a sum of two Langevin functions (Eq. (4.5)) allows the total magnetic signal to be divided into the two separate components. An example is given for the data at 250 K in Figure 4.10b. Superparamagnetic MNP particles show saturation below 1 T, whereas “superantiferromagnetic” ferritin (Gilles, et al. 2002) is characterised by a continuous increase of magnetisation with increasing field (Seehra, et al. 2000). The linear part \( \chi H \) becomes weaker at higher temperature (Kilcoyne and Cywinski 1995, Makhlouf, et al. 1997). The signal of the MNP component can be deduced by subtracting the magnetisation of the ferritin and plotting \( (M_{\text{Mix}} - M_{\text{HoSF}} - \chi H) / M_{0,\text{MNP}} \) versus \( H/T \) (inset, Figure 4.10a). It can be seen that the modified, initial magnetisation curves collapse onto a single curve, indicating superparamagnetic behaviour at temperatures above the peak ordering temperature (Dutta, et al. 2004). It should be noted that \( M_{0,\text{Mag}}, M_{0,\text{HoSF}} \) and \( \chi \) are temperature dependent and decrease with increasing temperature (Table 4.3).
4.2.4 Experimental results and discussion

4.2.4.4 First Order Reversal Curves (FORC)

FORC diagrams constitute a powerful method of displaying the grain-size dependence of hysteresis properties of natural and synthetic magnetic materials (Roberts, et al. 2000). FORC measurements for the HoSF-MNP system are displayed in Figs. 4.11a-c. All diagrams show a pronounced density distribution of the microcoercivity around the origin that is due to the superparamagnetic contribution from particles that are unblocked (Pike, et al. 2001). At 20 K the ordered MNP component shows a maximum in the microcoercivity distribution (along $B_c$ axis) at $B_c = 21 \text{ mT}$, indicating the stable single domain state of the MNP component (Figure 4.11a). The vertical spread in this distribution (along the $B_b$ axis) is indicative of particle interaction. Although these particles are coated with a polypeptide and albumin, they still interact and cluster at low temperatures, as suggested by the susceptibility data. The HoSF component is largely responsible for the signal at $B_c = 0$, due to its superparamagnetic behaviour. However some of the distribution along the $B_c$ axis may also be attributed to HoSF, since it is only totally unblocked at 22 K. Continuous unblocking of both components is seen at higher temperature (Figure 4.11b). The maximum of microcoercivity

![Graph showing hysteresis measurements of HoSF-MNP and single components at 5 K. The inset shows the wasp-waisted shape of the hysteresis at low fields.](image)

**Figure 4.9:** Hysteresis measurements of the HoSF-MNP mixed system and the single components at 5 K. The inset shows the wasp-waisted shape of the hysteresis at low fields.
from the MNP shifts towards the origin and the vertical spread decreases, reflecting a decrease in interaction as more magnetite particles are unblocked. The local maximum in coercivity distribution disappears completely at 90 K (Figure 4.11c), where the FORC diagram is only dominated by the superparamagnetic contribution from both MNP and HoSF. The transition to superparamagnetism appears to occur at lower temperature than expected from the induced magnetisation measurements (Figure 4.5c). This is probably due to the different measurement sensitivities of the two instruments. The signal for the FORC measurements was at the lower limit of the VSM, so that a relatively high smoothing factor was applied. This in turn acts effectively as a low-pass filter of the data, which eliminates weaker features (Heslop and Muxworthy 2005).

**TABLE 4.3:** Parameters for the individual components of HoSF and MNP from the fit to a sum of two Langevin functions (Eq. (4.5)) with \( \mu_{p,MNP} = 6260 \mu_B \) and \( \mu_{p,HoSF} = 320 \mu_B \) at temperatures above \( T_B \).

<table>
<thead>
<tr>
<th></th>
<th>150 K</th>
<th>200 K</th>
<th>250 K</th>
<th>300 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M_{0,\text{Mag}} ) [Am²]</td>
<td>4.08</td>
<td>3.84</td>
<td>3.52</td>
<td>3.35</td>
</tr>
<tr>
<td>( M_{0,\text{HoSF}} ) [Am²]</td>
<td>0.88</td>
<td>0.51</td>
<td>0.49</td>
<td>0.23</td>
</tr>
<tr>
<td>( \chi \times 10^9 ) [Am²/T]</td>
<td>5.38</td>
<td>4.59</td>
<td>3.63</td>
<td>2.29</td>
</tr>
</tbody>
</table>

**4.2.5 Concluding remarks**

The mixed system we have studied contains the high coercivity component ferritin and low coercivity component magnetite. In this mixture the ferritin dominates the magnetisation at low temperatures in moderate to high fields, as demonstrated by induced magnetisation and hysteresis analysis. The difference between AC-susceptibility and the induced magnetisation is that HoSF dominates the latter whereas MNP dominates the AC susceptibility. This observation demonstrates the power of using different measuring methods for a two-component system. The small remanent component in the antiferromagnetic HoSF is due to defect moments at the surface of the ferritin core. This is enhanced by the applied DC field of 50 mT. The weak AC field is more efficient, however, in activating the ferrimagnetic particles rather than activating the uncompensated spins.

Interaction between the MNP particles appears to be important, even though these particles are coated in albumin. This is seen from (i) the shift in peak temperatures in the mixed system as compared to the individual components in the DC susceptibility data; (ii) the fact that \( \chi'' \) follows the Vogel-Fulcher law; and (iii) the spread along the \( B_b \) axis in the FORC
4.2.5 Concluding remarks

Diagram. The magnetic signals from a natural system may be much weaker than those of the synthetic mixture studied in this work; however, the results from the investigated mixed system, composed of a ferrimagnetic and an antiferromagnetic component, are similar to results from human brain tissues (Brem, et al. 2005b). This observation indicates that such two-component magnetic models are useful for identifying the components of complex natural materials.

Figure 4.10: (a) Initial magnetisation curves as a function of field of the mixed system HoSF-MNP above temperatures required for full unblocking of both components. The inset shows \((M_{\text{mix}} - M_{\text{HoSF}} - \chi H)/M_{\text{MNP}}\) versus \(H/T\). (b) Initial magnetisation curve at 250 K and fit to Eq. (4.5) together with the initial magnetisation of the separated components MNP and HoSF deduced from the fit.
Figure 4.11: FORC diagrams showing the effect of increasing temperature (a) at 20 K, single-domain peak due to MNP and broad superparamagnetic distribution near origin due to HoSF, (b) at 50 K, disappearing of single-domain component and increase of superparamagnetic signal due to conversion of MNP and (c) at 90 K, purely superparamagnetic behaviour of entire mixed system.
This chapter contains the study of a larger series of human brain samples. A set of different magnetic methods, described in the preliminary chapters, was applied to hippocampal tissues and meningioma brain tumour tissues. Four different magnetic compounds are found in each type of tissue and the quantities of each compound can be compared. The comparison between the magnetic properties of these two types of tissue is of great interest, because brain tumour tissue is the result of degenerated cell growth, while hippocampal tissue is not. This study is a direct application of what has been learned from the methodology studies and the preliminary measurements on brain tissue samples, as well as from the detailed measurements on pure components, such as blood, horse spleen ferritin and magnetite nanoparticles. It therefore reflects the findings of the previous two chapters.

In the Appendices C, D and E an overview of all measured samples is given. In addition, the results from preliminary extraction and microscopic studies are presented.
5.1 Magnetic iron compounds in the human brain: A comparison of tumour and hippocampal tissue

Franziska Brem, Ann M. Hirt, Michael Winklhofer, Karl Frei, Yasuhiro Yonekawa, Heinz-Gregor Wieser & Jon Dobson

5.1.1 Abstract

Iron is a central element in the metabolism of normal and malignant cells. Abnormalities in iron and ferritin expression have been observed in many types of cancer. Interest in characterizing iron compounds in the human brain has increased due to advances in determining a relationship between excess iron accumulation and neurological and neurodegenerative diseases. In this work four different magnetic methods have been employed to characterise the iron phases and magnetic properties of brain tumour (meningiomas) tissues and non-tumour hippocampal tissues. Four main magnetic components can be distinguished: the diamagnetic matrix, nearly paramagnetic blood, antiferromagnetic ferrihydrite cores of ferritin, and ferrimagnetic magnetite and/or maghemite. For the first time, open hysteresis loops have been observed on human brain tissue at room-temperature. The hysteresis properties indicate the presence of magnetite and/or maghemite particles that exhibit stable single-domain (SD) behaviour at room temperature. A significantly higher concentration of magnetically ordered magnetite and/or maghemite and a higher estimated concentration of heme iron were found in the meningioma samples. First order reversal curve (FORC) diagrams on meningioma tissue further show that the stable single-domain particles are magnetostatically interacting, implying high local concentrations (clustering) of these particles in brain tumours. These findings suggest that brain tumour tissue contains an elevated amount of remanent iron oxide phases.
5.1.2 Introduction

The importance of iron in the human metabolic system and the role of iron in the cell metabolism have been elucidated in numerous studies. A dysfunction in this role has been associated with various benign and malignant disorders (Konemann, et al. 2005). Abnormalities in iron and ferritin expression have been observed in many types of cancer (Arosio and Levi 2002) and iron overload has been found in association with brain tumours (Kobayashi, et al. 1997, Mykhaylyk, et al. 2005). Kobayashi, et al. (1997) magnetically analysed various human tumour tissues (melanoma, breast, ovary, testicle, sarcoma, meningioma, glioblastoma, astrocytoma and glioma) and found the highest saturation isothermal remanent magnetizations for a glioblastoma and a meningioma. Meningiomas are among the most common occurring types of brain tumours. They evolve from degeneration of cells from the arachnoid membrane and their growth is relatively slow. They are generally considered benign. In this study magnetic iron phases in meningiomas are characterised.

Three main iron components encountered in the human brain are: (1) haemoglobin-bound iron in the blood, which exhibits nearly paramagnetic behaviour (Mosiniewicz-Szablewska, et al. 2003), (2) ferritin, the iron storage protein, and (3) magnetite (Fe$_3$O$_4$) and/or maghemite ($\gamma$-Fe$_2$O$_3$). Ferritin is the primary intracellular iron storage protein and is one of the major proteins of iron metabolism (Arosio and Levi 2002). It consists of a spherical protein shell, 12 nm in diameter, encapsulating a nanoparticle-sized core of antiferromagnetic ferrihydrite ($5\text{Fe}_2\text{O}_3\cdot9\text{H}_2\text{O}$) with a diameter of up to 8 nm (Chasteen and Harrison 1999). Very few magnetic studies have been made on human brain ferritin. A study on the magnetic properties of ferritin from globus pallidus reports that magnetic ordering occurs at 8.5 K (Dubiel, et al. 1999). Magnetite (Fe$_3$O$_4$) was first discovered in the human brain in 1992 (Kirschvink, et al. 1992), but its physiological origin is still open. The possible role of strongly ferrimagnetic magnetite in neurological and neurodegenerative diseases, such as epilepsy or Alzheimer's disease, has been investigated in several studies (Fuller, et al. 1995, Schultheiss-Grassi and Dobson 1999, Hautot, et al. 2003). Independent studies have shown that physiological ferritin cores have a poly-phased structure (Quintana, et al. 2000, Cowley, et al. 2000). Using transmission electron microscopy, Quintana et al. (2004) found a major amount of magnetite/maghemite-like structures and a minor amount of ferrihydrite structures within isolated molecules of pathological ferritin. Furthermore, using in-situ techniques, they identified magnetite-like phases and wüstite-like phases in brain tissue from patients with progressive supranuclear palsy and in hippocampus tissue from patients with Alzheimer's
disease (Quintana, et al. 2006). These findings support the suggestion that ferritin in the human brain might be a precursor for particles of magnetite and/or maghemite with grain sizes that are big enough to carry a remanent magnetisation at room temperature (Dobson 2001).

The specific conditions required for the synthesis of magnetite in human brain tissue have not been identified and it is difficult to synthesize under physiological conditions in a laboratory environment. The individual roles of oxygen, phosphorous, the haemoglobin complex and all other elements present in the brain are insufficiently established and too complex to be reproduced. Magnetic methods are highly sensitive for identifying low concentrations of iron phases and therefore are useful in distinguishing between different iron phases in brain tissue. Brem et al. (2005a) characterised both ferritin and magnetite/maghemite in brain tissue samples by combining different magnetic methods.

In this study, the magnetic properties of twelve meningiomas are analysed with the goal of contributing to a better understanding of the formation of magnetite and/or maghemite and the preferred conditions for its formation in the human brain. To compare these results to non-tumour human brain tissue, twelve hippocampal tissues were collected from patients with mesial temporal lobe epilepsy (MTLE) after selective amygdalohippocampectomies (Wieser and Yasargil 1982, Wieser 1988).

5.1.3 Materials and Methods

The twelve meningiomas (WHO Grade I-II) and twelve hippocampal tissues from mesial temporal lobe epilepsy (MTLE) patients were resected at the Department of Neurosurgery, University Hospital Zurich, and immediately frozen in liquid nitrogen. The two groups are not age-matched and the hippocampal specimens are generally from younger patients (ages ranged from 21-59) than the meningiomas (ages ranged from 59-80). All procedures were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Canton Zurich. All measurements were made on freshly resected tissues; it was not necessary to use tissues from autopsies. This is important, as it excludes possible effects due to post-mortem chemical changes. Precautions were taken to avoid contaminations, as described in earlier studies (Dobson and Grassi 1996), and all samples were weighed prior to measuring. In the frozen state all samples weighed between 200 mg and 1200 mg and their dimensions were on the order of 100-800 mm³. Four types of magnetic measurements were made: (1) acquisition of isothermal remanent magnetisation (IRM) and
Materials and methods

5.1.3 Materials and methods

determination of the Wohlfarth-ratio (S); (2) measurements of induced magnetisation (DC susceptibility) as a function of temperature after zero-field cooling (ZFC) and field cooling (FC); (3) induced magnetisation as a function of field (hysteresis loops) at 5 K and 300 K and (4) first order reversal curves (FORC) at room temperature.

The IRM measurements were made on all 24 samples using a 3-axis, 2G Enterprises Superconducting Quantum Interference Device (SQUID) with a sensitivity level of $10^{-11}$ Am$^2$. The IRM was acquired in an ASC Scientific Pulse Magnetizer Model IM-10-30, in which the sample was exposed to a pulsed DC field, as follows. First, a pulse of 1 T was applied to the sample and the remanent magnetisation was measured. Then, the sample was rotated 180° and progressively increasing fields from 10 mT to 1 T were applied in this opposite direction. This procedure allows for the determination of the coercivity of remanence, $B_{cr}$. After each magnetizing pulse, the remanent magnetisation in the tissue was measured (Brem, et al. 2005a).

All samples were first measured in the frozen state (77 K) and afterwards the tissues were freeze-dried and IRM acquisition was measured at 77 K and 300 K. The comparison of the measurements at 77 K on freeze-dried and frozen tissue served as a control for changes during the freeze-drying process. In order to define the Wohlfarth-ratio (Wohlfarth 1958, Cisowski 1981) on freeze-dried tissues, a saturation IRM was progressively demagnetized in alternating magnetic fields (AF-demagnetisation). This procedure was carried out on six hippocampal tissues and nine meningioma samples.

Induced magnetisation was measured as a function of temperature or field on eight samples from each group of tissue using a Quantum Design Magnetic Property Measurement System (MPMS) SQUID magnetometer at the Institute for Geosciences, University of Bremen, as well as at the Institute of Rock Magnetism, University of Minnesota and in the Chemistry Department, ETH Zurich. Each of the magnetometers is equipped with an internal field compensation coil and has a residual field of less than 100 μT. The measurements were made on freeze-dried tissue, which was pressed into a straw and fixed by small pinholes without other fixation material.

For the induced magnetisation, the samples were initially cooled to 5 K, either in the absence of a magnetic field (ZFC) or in the presence of a weak 50 mT field (FC). At 5 K a magnetic field of 50 mT was applied and the sample was then heated to 300 K, during which the magnetic moment was measured at intervals of no more than 2 K. The magnetisation of freeze-dried tissue was also measured as a function of field (hysteresis loop) at 5 K in fields from -5 T to 5 T after cooling the samples in zero field. Hysteresis measurements and first
order reversal curves (FORC’s) at room temperature were made with a Princeton Measurement Alternating Gradient Magnetometer AGM (Model MicroMag 2900) in fields from -0.5 T to 0.5 T at the laboratory of the Princeton Measurement Corporation. For these measurements the pressed freeze-dried tissue was cut into round, 2 mm thick slices of diameter 4.5 mm. The slices were mounted on a cleaned silica rod with a very small amount of silicone grease. No further packing material was used. Hysteresis measurements were made on twelve slices from seven meningioma samples and on six slices from three hippocampal samples. The FORC analysis was made on nine slices from seven meningioma samples and on four slices from three hippocampal samples. The alternating gradient head is housed in an acoustic attenuation cabinet to reduce noise due to external vibrations. Before measuring, samples were left mounted overnight so that the sample and holder reached the ambient temperature, which reduced the thermal noise level. The hysteresis curve of the empty silica rod serving as the sample holder in the AGM was measured separately, which has a saturation magnetisation $M_s$ of $1.14 (\pm 0.09) \times 10^{-7}$ emu.

![Figure 5.1: Isothermal remanent magnetisation (IRM) acquisition curves at 77 K (filled symbols) and room temperature (open symbols) for meningiomas and hippocampi. All signals are corrected by the weight at 77 K.](image-url)
5.1.4 Results

5.1.4.1 Isothermal Remanent Magnetisation (IRM)

The shapes of the IRM acquisition curves from meningioma and hippocampi are very similar (Figure 5.1). The acquisition shows a rapid increase in low fields and saturation is reached between 200 mT and 250 mT. Magnetic saturation in fields of this size indicates that hematite (α-Fe₂O₃) is not the magnetic carrier; fine-grained hematite may require fields of 2.5 T or more to produce saturation. The shape of the curves and the saturation fields are indicative of the low-coercivity ferrimagnetic minerals magnetite and/or maghemite (Dunlop and Özdemir 1997). The IRM acquisition curves for both tissue types – and consequently the coercivities of remanence $B_{cr}$ – are similar. The average $B_{cr}$ at 77 K is 34.5 ($±$ 3.6) mT for the meningiomas, and 34.9 ($±$ 4.1) mT for the hippocampi (Table 5.1). At 300 K $B_{cr}$ is lower, where it is 28.6 ($±$ 3) mT for the meningiomas and 26.2 ($±$ 5.1) mT for the hippocampi (Table 5.1). These $B_{cr}$ values are typical of the coercivities of remanence found in magnetite and/or maghemite (Dunlop and Özdemir 1997). The IRM characteristics and $B_{cr}$ values suggest that the remanent magnetic phases in both types of tissue have predominantly the same magnetic mineralogy. The strength of signal and the remanent saturation magnetisation, $M_{RS}$, however, vary strongly. For meningiomas the average $M_{RS}$ measured at 77 K is $2.14 (± 0.72)·10^{-5}$ Am²/kg, while for the hippocampi the average $M_{RS}$ at 77 K is $0.22 ± (0.04)·10^{-5}$ Am²/kg. For both types of samples $M_{RS}$ at 300 K is less than $M_{RS}$ at 77 K. The difference in $M_{RS}$ is due to magnetic particles, which are superparamagnetic at room temperature, but whose magnetisations become ordered above 77 K, so that they contribute to the remanent magnetisation at low temperature. The average values for the $M_{RS}$ at 300 K are $1.82 (± 0.67)·10^{-5}$ Am²/kg for meningiomas and $0.11 (± 0.03)·10^{-5}$ Am²/kg for hippocampal tissue (Table 5.1). The percentage of superparamagnetic particles (% SP) in the temperature range between 77 K and 300 K can be expressed by the ratio $(M_{RS}(77 K) - M_{RS}(300 K))/(M_{RS}(77 K))·100$. The average values are 28 ($± 14)$ % for the meningiomas and (37 ± 16) % for the hippocampi.
Determination of the Wohlfarth-ratio (S) is shown in Figure 5.2. For non-interacting single domain particles with uniaxial anisotropy, the S-ratio is 0.5 (Wohlfarth 1958). Lower values are attributed to particle interactions, or to superparamagnetic or multidomain influences. The average S-ratio for the meningioma samples was 0.26 (±0.01), while the mean value for the hippocampi was 0.32 (±0.04). The S-ratios for all measured samples in this study are well below 0.5, which suggests interactions between the particles within the tissue and superparamagnetic effects.

5.1.4.2 Induced Magnetisation: DC Susceptibility as a function of temperature

As brain tissue consists mainly of a diamagnetic fatty matrix with small concentrations of other magnetic components, the method of measuring induced magnetic moment as a function of temperature is very useful (Figure 5.3). The induced magnetisation is composed of the diamagnetic signal, a paramagnetic signal from the heme-iron, as well as ferrimagnetic and superparamagnetic magnetisations. The strength of the diamagnetic contribution,
5.1.4 Results

Results originating from the organic tissue matrix, can be estimated from the temperature-independent magnetisation above 250 K. The diamagnetic signal had an average value of \(-1.62 \pm 0.53\times 10^{-4}\) Am\(^2\)/kg for the meningiomas and \(-2.40 \pm 0.12\times 10^{-4}\) Am\(^2\)/kg for the hippocampi. The magnetic contribution due to the blood has been estimated (Mosiniewicz-Szablewska, et al. 2003, Brem, et al. 2005b), and can be subtracted from the total magnetic signal. For the meningiomas the average estimated concentration of blood in the samples is about five times higher than in the hippocampi. There are 0.1 \((\pm 0.06)\) mg and 0.02 \((\pm 0.009)\) mg per mg freeze-dried tissue in the meningioma and hippocampi samples, respectively. The remaining signal is due to: (i) the superparamagnetic ferritin, which orders magnetically below 22 K, and (ii) an additional remanent (magnetically ordered) phase.

For the characterisation of ferritin, the analysis of induced magnetisation measurements after both zero field cooling (ZFC) and field cooling (FC) is the most common method for obtaining information about the blocking behaviour of the protein cores (Makhlof, et al. 1997, Dubiel, et al. 1999). The maximum of the ZFC curve (Figure 5.3a,b) indicates the average blocking temperature \(T_B\) for the superparamagnetic cores of the protein, which is between 10 K and 12 K for all brain samples. No statistically relevant difference of \(T_B\) can be seen between hippocampi and meningioma samples.

![Figure 5.3: Induced magnetisation as a function of temperature after ZFC (open symbols) and FC (filled symbols), measured in a 50 mT field for (a) meningiomas (Men) and (b) hippocampi (Hipp).](image)

The existence of another magnetically blocked phase is shown by the behaviour of the ZFC and FC curves. For pure ferritin the curves are expected to bifurcate at approximately 22 K. Above this temperature the curves should superpose completely as all particles become superparamagnetic (Makhlof, et al. 1997). For the meningiomas a distinct displacement can
be seen between the ZFC curve and the FC curve (Figure 5.3b). For the hippocampi, the displacement is less obvious, but on closer inspection there is no bifurcation of the curves (Figure 5.3a). Such a displacement has its origin in the coexistence of a blocked phase (Pardoe, et al. 2001), whereby the size of the displacement corresponds to the intensity of the remanent magnetisation of the sample. It thus increases slightly with decreasing temperature between 300 K and 20 K, accompanying the further blocking of magnetite and/or maghemite particles. It shows a very steep increase below 20 K (Figure 5.4) due to the superposition of the ferritin ordering. The magnetisation difference, $M_{\text{FC}} - M_{\text{ZFC}}$, between the FC and ZFC curves was obtained by subtraction; it was then differentiated with respect to temperature to obtain the distribution of the blocking temperature ($T_B$) of the ferritin cores (Figure 5.4, inset) (Hanzlik, et al. 2000, Mamiya, et al. 2005).

![Figure 5.4: Average displacement between the ZFC magnetisation and the FC magnetisation for the hippocampi samples, which corresponds to the remanence curve as a function of temperature. The inset shows the derivative of the remanence curve corresponding to the blocking temperature ($T_B$) distribution as a function of temperature for the hippocampi (Hipp) and the meningiomas (Men).](image)

5.1.4.3 Induced Magnetisation: Hysteresis

At 5 K, the hysteresis measurements are dominated by the ferrihydrite cores of ferritin and show an open non-saturating hysteresis up to high fields (Figure 5.5a). A slight tendency to wasp-waisting can be seen in several samples; this is due to the effect of the low coercivity
5.1.4 Results

phase, magnetite and/or maghemite (Tauxe, et al. 1996). The coercive force $B_c$ averages 23 (± 10) mT and 34 (± 10) mT for meningiomas and hippocampi, respectively (Table 5.2). The remanent magnetisations $M_{RS}$ average $3.9 (± 2.3) \times 10^{-4}$ Am$^2$/kg for meningiomas and $2.4 (± 0.6) \times 10^{-4}$ Am$^2$/kg for hippocampi.

Hysteresis curves measured on the AGM at 300 K are shown in Figure 5.5b (meningioma sample) and Figure 5.5c (hippocampus sample). The hysteresis loops at room temperature are open. Until now, open hysteresis loops have only been observed on human tissue at low temperature, e.g., at 150 K (Hautot, et al. 2003). The room temperature behaviour of ferritin is completely superparamagnetic and does not contribute to the hysteresis loop, which is due to the ferrimagnetic component. $B_c$ is 10.5 mT for the meningioma sample, HA1b, and 9.8 mT for the hippocampus sample, HH (where the sample labelling identifies patients by their initials). The remanent saturation magnetisation $M_{RS}$ and the induced saturation magnetisation $M_S$ for the meningioma HA1b are $5.48 \times 10^{-4}$ Am$^2$/kg and $2.38 \times 10^{-3}$ Am$^2$/kg, respectively. The ratios $M_{RS}/M_S$ and $B_{cr}/B_c$ are 0.23 and 1.93, respectively. For the hippocampus sample HH, $M_{RS}$ is $9.38 \times 10^{-6}$ Am$^2$/kg and $M_S$ is $5.67 \times 10^{-5}$ Am$^2$/kg. The $M_{RS}/M_S$ ratio is 0.17 and the $B_{cr}/B_c$ ratio is 3.5. The average $B_c$ is 8.4 (± 1.8) mT for the meningiomas and 7.7 (± 1.4) mT for the hippocampi, while the average $M_{RS}$ is $1.42 (± 0.99) \times 10^{-4}$ Am$^2$/kg for the meningiomas and $0.07 (± 0.007) \times 10^{-4}$ Am$^2$/kg for the hippocampi (Table 5.2). These average values can not be directly compared to the values of the IRM acquisition, because of the different normalisation. However, the values are in good agreement with each other.
Figure 5.5: (a) Hysteresis at 5 K for the meningioma (Men) and hippocampi (Hipp), (b) hysteresis at 300 K of a meningioma sample (HA1b), and (c) hysteresis at 300 K of a hippocampus sample (HH). Note the difference in scale between (b) and (c). All curves were corrected for linear contributions from the diamagnetic and paramagnetic phases.

5.1.4.4 First Order Reversal Curves (FORC)

The FORC data were obtained from slices of several meningioma and hippocampi samples. The variation of magnetisation between different tissues is large, as was the case for the IRM acquisition and the hysteresis measurements. Therefore, different smoothing factors were necessary, depending on the weakness of the sample signal. In Figures 5.6a-d FORC diagrams of four different samples (3 meningiomas and 1 hippocampus) are compared. The parameter $B_c$ corresponds to the coercivity distribution and the parameter $B_h$ corresponds to
5.1.5 Discussion

The results of these magnetic measurements illustrate that human brain tissue contains four major magnetic components: diamagnetic tissue, nearly-paramagnetic blood, antiferromagnetic ferrihydrite cores of ferritin, and ferrimagnetic magnetite and/or maghemite. The magnetic detection of these components is in good agreement with reported results from X-ray absorption spectroscopy on brain tissue (Mikhaylova, et al. 2005, Collingwood, et al. 2005). Meningioma and hippocampal samples show equivalent diamagnetic properties. Measurements of induced magnetisation as a function of field and temperature show that magnetic behaviour of ferritin is approximately the same for both types of tissue. IRM acquisition shows that meningiomas contain approximately ten times more ferrimagnetic particles than the hippocampi. This difference is also expressed in a displacement between the ZFC and FC curves in the induced magnetisation and in wasp-waisted hysteresis curves at 5 K. The open hysteresis loops and FORC analysis at room temperature are compatible with single-domain behaviour in magnetite and/or maghemite, while smaller, superparamagnetic particles would reveal closed loops e.g. (Morales, et al. 1999). Magneto-mineralogical studies in fine-grained magnetite have shown that the transition from superparamagnetic (SP) to single-domain (SD) behaviour occurs at grain sizes around 25-30 nm (Dunlop and Özdemir 1997).
Figure 5.6: First order reversal curves (FORC) measured at room temperature for four different tissue samples: (a) meningioma HA1a, (b) meningioma HA1b, (c) meningioma (HM2b) and (d) hippocampus HH. The section below each figure presents the profile along the $B_c$-axis.
The hysteresis and FORC analyses show that, in addition to the SD-component, there is also a superparamagnetic component from smaller grains. The superparamagnetic signal dominates some samples, especially the weaker hippocampi. S-ratios were all below 0.5 for both tissue types, which suggests that the ferrimagnetic grains are interacting. FORC diagrams further suggest that these particles are not homogeneously distributed throughout the tissue, but cluster in distinct areas. To assess the role of magnetostatic interactions in our samples, it is helpful to compare the FORC diagrams for the meningiomas with FORC diagrams for a synthetic reference sample, a concentrated SD magnetite powder sample (Pan, et al. 2005). The half-width fields in our meningioma samples range from 6 mT to 10 mT, measured along vertical sections through the isolated peak of the FORC distribution, which corresponds well with the 8.2 mT for the synthetic SD magnetite powder sample. This similarity strongly suggests that the SD particles in the meningioma samples form local clusters rather than occurring isolated. In the case of the hippocampus sample it is more difficult to specify the coercivity value through which to draw a profile yielding the half-width field because the FORC distribution does not show an isolated maximum any more but increases monotonously towards the left margin of the diagram. At 10 mT, roughly where the peaks of the FORC distributions for the other samples are located, we obtain a half-width of 14 mT.

The saturation magnetisation deduced from the hysteresis loop at room temperature of the meningiomas exceeds $M_{RS}$ of the hippocampi, expected from the observed difference in the IRM measurements. In the study by Kirschvink et al. (1992), the magnetite concentrations in meninges, which were calculated from saturation IRM, were an order of magnitude higher than those from the cerebrum and the cerebellum. Furthermore, the magnetite/maghemite concentrations measured in this study are comparable to the values in meningioma measured by Kobayashi et al. (1997). It is known that brain iron burden increases with aging (Burdo and Connor 2003, Bartzokis, et al. 2004), which theoretically could influence our results. However, no correlation of magnetite and/or maghemite content with age was found in either type of tissue. In addition, a comparison of results from the two youngest meningioma patients with the results from the oldest hippocampi patients revealed the same statistically significant difference as for the average values from all patients.

The average estimated blood content in the meningiomas is higher than in the hippocampi. The relation between blood supply within the tissue and the formation of magnetite and/or maghemite is unknown. However, the broad particle size distribution, shown by the difference of $M_{RS}$ in the IRM acquisition, the increasing displacement in the ZFC-FC
measurements, the ratios $M_{R5}/M_S$ and $B_{ce}/B_c$ in conjunction with the different FORC diagrams suggest that particle growth starts at nanometric size and different growth states are detected within all tissue samples. In the hippocampi the sizes are smaller, while in the meningioma the conditions may be more favorable for growth of larger particles. Such a process would support the idea that ferritin behaves as a precursor for magnetite and/or maghemite growth (Dobson 2001, Quintana, et al. 2004). In the present study however it has been shown that the formation of strongly ferrimagnetic particles is favored in the meningiomas compared to hippocampal tissue.

5.1.6 Conclusions

The magnetic methods described in this study show that human meningioma brain tumour tissues contain an order of magnitude higher concentration of ferrimagnetic particles than non-tumour hippocampi. Although these tumours are well vascularized, it is not yet known whether blood supply plays a role in this higher concentration. Iron distribution in the brain is heterogeneous. However, the formation rate of ferrimagnetic magnetite and/or maghemite appears to be higher in the tumour tissue. FORC diagrams provide insight into room-temperature coercivity distributions in human brain tissue and can be used to assess the role of magnetostatic interactions as proxy of magnetic particle concentration. We observed a pronounced vertical spread in the FORC diagram implying high local concentrations of magnetic remanence carriers. This information may provide insights into differences in iron metabolism in tumour versus non-tumour tissue. Our magnetic results demonstrate that these methods, in combination with medical research, can lead to a better understanding of iron physiology in the human brain.
TABLE 5.1: Averaged values for the remanent saturation magnetisation ($M_{\mathrm{RS}}$), coercivity of remanence ($B_{\mathrm{c}}$), the percentage of superparamagnetic particles (% SP) and the Wohlfarth-ratio (S) with ± 95% confidence limits (in parentheses) from IRM acquisition at 77 K and 300 K (corrected by weight of the sample at 77 K). The data are for twelve meningiomas and twelve hippocampal tissues, except for the S-values, which are for eight meningioma and eight hippocampi.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$M_{\mathrm{RS}}$ (77 K)</th>
<th>$B_{\mathrm{c}}$ (77 K)</th>
<th>$M_{\mathrm{RS}}$ (300 K)</th>
<th>$B_{\mathrm{c}}$ (300 K)</th>
<th>% SP ($\times 10^5$)</th>
<th>S $(\times 10^5)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningiomas</td>
<td>2.14 (± 0.72)</td>
<td>34.5 (± 3.6)</td>
<td>1.82 (± 0.67)</td>
<td>28.6 (± 3.0)</td>
<td>28 (± 14)</td>
<td>0.26 (± 0.01)</td>
</tr>
<tr>
<td>Hippocampi</td>
<td>0.22 (± 0.04)</td>
<td>34.9 (± 4.1)</td>
<td>0.11 (± 0.03)</td>
<td>26.2 (± 5.1)</td>
<td>37 (± 16)</td>
<td>0.32 (± 0.04)</td>
</tr>
</tbody>
</table>

TABLE 5.2: Averaged values for the blood content, the average blocking temperature ($T_{\mathrm{B}}$), coercive force ($B_{\mathrm{c}}$) and the remanent saturation magnetisation ($M_{\mathrm{RS}}$) with ± 95% confidence limits (in parentheses) from induced magnetisation measurements as a function of temperature and hysteresis measurements at 5 K and 300 K (corrected by freeze-dried weight of the sample). The data are for eight meningiomas and eight hippocampal tissues, except for the $B_{\mathrm{c}}$ (300 K) and $M_{\mathrm{RS}}$ (300 K), which are for twelve meningioma slices and six hippocampi slices.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Blood content</th>
<th>$T_{\mathrm{B}}$</th>
<th>$B_{\mathrm{c}}$ (5 K)</th>
<th>$M_{\mathrm{RS}}$ (5 K)</th>
<th>$B_{\mathrm{c}}$ (300 K)</th>
<th>$M_{\mathrm{RS}}$ (300 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningiomas</td>
<td>0.10 (± 0.060)</td>
<td>11.7 (± 0.4)</td>
<td>23 (± 10)</td>
<td>3.9 (± 2.3)</td>
<td>8.4 (± 1.8)</td>
<td>1.42 (± 0.990)</td>
</tr>
<tr>
<td>Hippocampi</td>
<td>0.02 (± 0.009)</td>
<td>10.9 (± 0.4)</td>
<td>34 (± 10)</td>
<td>2.4 (± 0.6)</td>
<td>7.7 (± 1.4)</td>
<td>0.07 (± 0.007)</td>
</tr>
</tbody>
</table>
Chapter 6

SUMMARY AND OUTLOOK
6.1 Summary

The goal of this thesis was the identification and characterisation of iron compounds in human brain tissue. Several magnetic measurement methods have been applied to obtain a clearer insight into the different magnetic phases present in the human brain. The sample preparation, such as freeze-drying, and the methodology had to be adapted for all measuring methods, because different techniques at room temperature and low temperature are needed to identify the distinct iron compounds in the brain tissue. The three magnetic components investigated in the first study (Chapter 3) are the diamagnetic tissue matrix, antiferromagnetic ferritin, and ferrimagnetic magnetite and/or maghemite particles. In the second study (Chapter 3) the investigations of nearly-paramagnetic heme-iron from blood is added.

The diamagnetic tissue matrix is the dominant component in all measured brain samples, however, from the magnetic point of view, it is the least interesting. The diamagnetic fraction varies from sample to sample, but no significant difference between different types of tissue can be seen. Furthermore, the diamagnetic signal can not be interpreted to obtain an indication of disruption in iron metabolism or to identify a relation with diseases. From the technical point of view, however, the diamagnetic tissue matrix is the most challenging component, because it overwhelms the weaker signals from the other magnetic components in all the magnetic measurements, except in the acquisition of IRM.

The nearly-paramagnetic signal from the heme-iron atoms in blood shows a steep increase in magnetisation below 30 K and tends to overprint the magnetic contribution from the thermal blocking of antiferromagnetic cores of ferritin. The amount of heme-iron within the samples is only an estimated value, but it reveals information about how well each sample was supplied with blood. Higher blood content was measured in the meningioma samples than in hippocampal tissue. The role of heme iron in the formation of ferrimagnetic iron-oxide particles might not be negligible.

The iron storage protein ferritin is of both medical and physical interest. The amount of ferritin can not be exactly determined by the magnetic methods. However, no significant differences are observed between meningiomas and hippocampal tissue when their magnetisations at the average blocking temperature in the ZFC induced magnetisation curves are compared. The question arises whether hemosiderin is present in the tissue samples. The issue is not addressed in this thesis, because it is not possible to access this phase separately by the applied magnetic techniques. If there is hemosiderin in the samples, it is measured
6.1 Summary together with the ferritin signal and can not be separated magnetically. Extensive and careful microscopic work with electron diffraction might resolve this question.

The existence of ferrimagnetic magnetite and/or maghemite particles has been established by all magnetic techniques used in this work. An estimate for the concentration of magnetite in tissue has been made from the remanent saturation magnetisation in Chapter 3 by assuming randomly oriented, non-interacting particles. The later results from the S-ratios and FORC diagrams measured on hippocampi and meningiomas contradict the assumptions, clearly showing interactions between the particles. We can however assume, that interactions would lower the remanent saturation magnetisation (Afremov and Panov 1995), therefore the estimate is a minimum value for the particle concentration. For the meningiomas an average minimum concentration of 465 ng/g can be determined, while for the hippocampal tissue the average minimum concentration is 48 ng/g. The latter value is in good agreement with the study of Schultheiss-Grassi and Dobson (1999). The broad grain-size distribution of the ferrimagnetic particles tends to reinforce the hypothesis that ferritin acts as a precursor for magnetite and/or maghemite. This hypothesis does not state that the ferrihydrite core is chemically changing into magnetite particles, but rather that the magnetite-like cores of the ferritin grow to larger particles. The endogeneous chemical processes are still unclear.

The magnetic properties of human brain tissue are partly dominated by nanoparticle behaviour. The cores of ferritin and the magnetite and/or maghemite particles, which show superparamagnetic behaviour at room temperature, influence all induced magnetic measurements (c.f. ZFC-FC magnetisation, hysteresis loops and FORC analysis). The need for a better understanding of the magnetic behaviour of nanoparticles led to in the magnetic measurements on horse spleen ferritin (Chapter 4). Horse spleen ferritin is a well-studied natural nanomagnet, however magnetic measurements reveal a multi-phase core. This is in agreement with microscopic studies of ferritin (Quintana, et al. 2004). Horse spleen ferritin can be modeled more realistically with a two-phase core model, where one phase corresponds to ferrihydrite and the other to magnetite and/or maghemite. The particle sizes of the cores are smaller than 10 nm. Experimental results on brain tissue showed a broader grain size distribution of the ferrimagnetic phase, therefore a proxy system with a mixture of horse spleen ferritin and magnetite nanoparticles with 10-20 nm in diameter was characterised (Chapter 4). Both components behave superparamagnetically at room temperature, as verified by fitting the observations to a sum of two Langevin-functions. Furthermore, the magnetite component shows distinct relaxation behaviour that follows a Vogel-Fulcher law and
indicates interactions between the magnetite particles. This was also verified by FORC analysis at lower temperatures.

The agreement of the magnetic properties of the two-component proxy with human brain tissue is demonstrated in several measurements: The ZFC and FC induced magnetisations show similar features after the subtraction of the diamagnetic tissue matrix and the heme-iron component. Hysteresis at 5 K shows wasp-waisting for both the proxy and brain tissue, and the FORC analyses at low temperature of the proxy are directly comparable to the FORC diagrams at room temperature for brain tissue. The main differences are due to grain-size distributions; while the proxy contains only magnetite particles with sizes up to 20 nm, the grain-size spectra in brain tissue contains some larger particles. This difference is directly revealed by the hysteresis loops at room temperature. The proxy shows pure superparamagnetic behaviour at room temperature, while human brain tissue shows an open hysteresis loop with single-domain magnetite and/or maghemite behaviour, slightly elongated by superparamagnetic contributions.

As a result of the difficulty of obtaining control tissue, the original intention of comparing tissue from epileptic patients with non-pathogenic tissue had to be changed to a comparison of tumour tissue and non-tumour tissue (Chapter 5). Only three measured hippocampal control tissues from autopsies were analysed, however no difference could be found between their magnetic properties and those from MTLE-hippocampi; the number of samples was too small for statistical comparison. It has been shown that meningiomas contain a larger amount of ordered magnetite and/or maghemite particles than found in hippocampal tissue. This indicates larger particle sizes in tumour tissue; a higher degree of interactions was measured in the meningiomas. Furthermore, compared to the hippocampal tissue, a generally higher average remanent saturation magnetisation has been found in all other types of brain tumour tissue except in the astrocytomas. For some types, however, the difference was not statistically significant (Appendix C). This opens the question whether there are differences in iron metabolism in tumour tissue compared to non-tumour tissue.

No clear distinction could be made between magnetite and maghemite as the carrier of the low-coercivity ferrimagnetic phase. Presumably, there is a mixture of both phases present in the brain tissue. Maghemite does not show a low-temperature transition like the Verwey transition at 120 K in magnetite, and no low-temperature transition was observed in this study while measuring magnetic moment versus temperature. It is known, however, that magnetite particles do not always exhibit a Verwey transition. It is evident only in multidomain grain sizes or finer grains with equidimensional grain shape. To investigate this point further,
samples of magnetotactic bacteria ("Magnetospirillum gryphiswaldense") that were analysed in the late stages of this thesis, showed clear single domain magnetite behaviour and no Verwey transition. In order to distinguish whether the fine nanoparticles in brain tissue are magnetite or maghemite, HRTEM analysis and electron diffraction are necessary. This is the subject of ongoing research. The microscopic analysis is in itself complicated. For extraction of the particles from the tissue, a suitable solvent is needed, which is able to dilute the fatty organic material but does not chemically change the structure of the particles. So far, only a limited number of microscopic analyses have been made, and the preliminary results are in agreement with magnetite.

Generally, the study shows that a single magnetic method is not ideal for characterizing the magnetic multi-component system that is brain tissue. The combination of different magnetic methods is advantageous for a more precise description of the iron compounds in human brain tissue. The agreement between the data of different methods is very good. IRM acquisition, hysteresis measurements at room temperature, and coercivity distribution in the FORC analysis all show single-domain magnetite and/or maghemite behaviour in approximately the same range of signal strength. Slight variations may be due to different sensitivity of instruments and different sample sizes. The difference between the IRM acquisition at 77 K and room temperature, the increasing displacement between ZFC and FC magnetisation curves with decreasing temperature, and the peak of the signal around the origin in the FORC diagrams all indicate the broad grain-size distribution and the strong presence of temperature-dependent superparamagnetic grains. Furthermore, S-ratio and FORC analysis are both in good accordance, indicating interaction and clustering of the ordered magnetite and/or maghemite particles within the samples.

The complexity of iron metabolism and the role of iron in the brain cannot be solved by magnetic methods alone. A more multidisciplinary research is necessary to elucidate the heterogeneous distribution of iron in the brain, the impact of aging, or the harming influence of iron oxides and their direct relationship with disease in the brain, to list a few examples. More research data from investigations of iron in the brain are needed for a reliable medical interpretation of the data in this study. Magnetic measurements are one element in a continuous research field, but they have the potential to allow for a better identification and quantification of the different iron phases in the human brain.
6.2 Outlook

Additional magnetic measurements could be made that are advantageous for a good characterisation of the iron compounds in the human brain, but for many of them technical obstacles would have to be overcome. The weakness of all samples, together with the overwhelming diamagnetic signal, pose a difficult challenge, and at the same time most sets of measuring equipment are pushed to the limits of their sensitivity.

IRM acquisition at temperatures down to 5 K would give further insight into the grain size distribution within the tissue. Three possible types of equipment would be suitable for this measurement: (i) A cryogenic magnetometer with a built-in cryostat, but at present only one non-commercial prototype exists; (ii) an alternating gradient magnetometer (AGM) with a built-in cryostat. However, building the cryostat into the AGM increases the coil spacing and decreases thereby the sensitivity of the instrument; (iii) MPMS measurements, where the coils would have to be reset after each temperature. The rest-field remains a problem, because it cannot be perfectly controlled. As a further experiment, AC-susceptibility measurements as a function of temperature at different frequency could reveal the exact blocking behaviour of the different components. This method proved to be suitable at very low temperatures for human brain tissue, but at temperatures above 40 K there is still room for improvement. Hysteresis measurements and FORC analysis on the AGM at low temperatures would be suitable to access the coercivity distribution and the particle interactions as a function of temperature. Heating the samples up to 700 °C (above the Curie temperatures of magnetite and maghemite) could possibly help to differentiate between magnetite and maghemite. However, this method is destructive of the samples and the burning process of all the organic material might cause undesirable chemical reactions.

As it is not realistic to extract all four magnetic components out of the brain tissue samples for separate investigations, measurements on the mixed-system proxy can be expanded. New samples with an added blood component and the diamagnetic matrix, simulated by adding a synthetic diamagnetic material, could be investigated. The different roles of the four components can be elucidated and by varying the concentrations, more information about interaction behaviour can be obtained.

The difficulties with electron microscopy have been mentioned above. Some in-situ techniques have been reported, such as X-ray absorption spectroscopy (Mikhaylova, et al. 2005, Collingwood, et al. 2005). New analyzing techniques are now slowly becoming available. One example is the PPMS-AFM, where the physical properties measurements
system (PPMS) is coupled with an atomic force microscope (AFM). Such analysis on tissue samples would reveal magnetic properties between 4 K and 400 K together with high resolution images at nanoscale. The main difficulty for this instrument would probably be the sample preparation of brain tissue, as the instrument is designed for magnetic thin films and superconductors. The method can only be used on samples with limited surface roughness, and very flat slices of organic material are difficult to prepare.

In order to investigate the precursor role of ferritin as the source of cubic iron-oxides and to investigate under which circumstances the magnetite and/or maghemite form from ferrihydrite, chemical processes under different conditions and with various parameters – such as oxygen and phosphorous concentration – could be simulated in a laboratory environment, for example by pumping blood through a system containing ferritin. In addition, the preferred conditions, under which apoferritin shells take in the iron and form a cubic magnetite and/or maghemite phase instead of ferrihydrite in the core, could be investigated.

With respect to medical aspects, more measurements are needed on different types of tumour tissue and on tissues from different regions of the brain so as to obtain statistically significant results for all types of tissue. A systematic age-matching of all groups is also needed. Furthermore, it is worth to investigate which tumours are responsible for epileptic seizures and to correlate these findings with the iron-oxide phase content. An investigation of ferritin cores from tumour tissue could be of interest to clarify whether the ferritin cores of meningioma tissue contain a higher amount of cubic phases. Little has as yet been reported on developing in-vivo techniques for early detection of disruptions in iron metabolism, except for magnetic resonance imaging (MRI). There are, however, existing SQUID sensor technologies to investigate inhaled iron oxide particles in the lungs of living patients, which might eventually be adapted to the human brain (Möller, et al. 2006). However, this method has thus far only been able to detect particle sizes around 1 μm.
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A. Appendix I Chapter 3

A.1 Induced magnetisation versus temperature for pure blood samples

As the human brain is an organ that is well-supplied by blood, the presence of heme-iron is expected in all brain tissue samples. The Fe$^{2+}$ ions in the haemoglobin complex have been reported to show reversible paramagnetic behaviour (Slawska-Waniewska, et al. 2004). Human blood has been analysed magnetically by electron paramagnetic resonance (EPR) and by measurements of induced magnetisation as a function of temperature (Mosiniewicz-Szablewska, et al. 2003); the latter study also showed a "paramagnetic" behaviour. Measurements of induced magnetisation versus temperature show that the signals of blood and ferritin overlap at low temperature and the distinct ferritin signal can only be distinguished by subtraction of the blood signal as described in Chapter 3.

Blood measurements on two self-donated blood samples were analysed using the same procedure as for the human brain tissue. Induced magnetisation was measured as a function of temperature on freeze-dried samples using a Quantum Design Magnetic Property Measurement System (MPMS) SQUID magnetometer at the Institute of Rock Magnetism, University of Minnesota. For the induced magnetisation profiles, the samples were initially cooled to 2 K, either in the absence of a magnetic field (ZFC) or in the presence of a weak 50 mT field (FC). At 2 K a magnetic field of 50 mT was applied and the sample was then heated to 300 K, during which the magnetic moment was measured at intervals of no more than 2 K. The result is shown in Fig. A.1. The ZFC and the FC curve coincide completely. The decay of the magnetisation shows a paramagnetic behaviour to a first order. To test whether the magnetisation of blood follows a Curie law, which states that the magnetisation (M) is inversely proportional to temperature (T), the 1/M values were plotted against temperature. A perfect paramagnet would show a linear dependence of 1/M on T with a gradient equal to C$^{-1}$ (where C is the Curie constant) (Seehra and Punnoose 2001). As seen in the inset in Fig. A.1 a distinct curvature in the 1/M curve is present at lower temperature. Therefore, the behaviour of blood is not purely paramagnetic and for this reason the magnetic behaviour of blood is always referred to as "nearly paramagnetic" in this thesis. By knowing the exact weight of the blood samples, an estimate of the blood content in all measured
samples could be made and subtracted from the overall signal. It should be noted that the blood signals in these studies are in good agreement with previous values (Slawska-Waniewska, et al. 2004).

**Figure A.1:** ZFC and FC induced magnetisation as a function of temperature in a 50 mT field of freeze dried blood samples.
B. Appendix I Chapter 4

B.1 Superparamagnetism and the Langevin theory

Exchange coupling between the atomic magnetic moments results usually in ferromagnetic, ferrimagnetic or antiferromagnetic ordering. In any of these cases, there is a magnetic anisotropy energy associated with the direction of the spin system which can be either of crystalline or shape (magnetostatic) origin. There can be more than one easy direction for the spin system to lie along, and these easy directions are separated by magnetic anisotropy barriers. If the volume of a magnetic particle is small enough or the temperature high enough, thermal fluctuations will be sufficient to overcome the anisotropy energy barrier causing a spontaneous reversal of magnetisation. This is called superparamagnetic relaxation and the relaxation time \( \tau \) can be expressed by the Néel-Arrhenius relation:

\[
\tau = \tau_0 \cdot \exp \left( \frac{E_b}{k_B \cdot T} \right),
\]

where \( E_b \) is the anisotropy energy barrier and \( \tau_0 \) is the characteristic time of a system and is of the order of \( 10^{-9} \) to \( 10^{-11} \) s. Because of the inverse temperature dependency, for high temperatures, the relaxation time \( \tau \) is small; for low temperatures it is large. The temperature below which the magnetic moment has relaxation times that are long compared to observation times is called the blocking temperature \( (T_B) \). Above the blocking temperature the magnetic particle exhibits unstable behaviour similar to paramagnetism.

In zero-field an ensemble of particles will approach thermal equilibrium and the net magnetic moment will average to zero. In an applied field, there will be a net statistical alignment of magnetic moments. The fraction of the total magnetisation that will be aligned by the field and at a specific temperature \( T \) is given by the Langevin function, which is described below.
B.1.1 Langevin theory

A system contains \( N \) atoms or ions with a magnetic moment \( \mu \) per unit of volume. Let the angle between an atom \( i \) and the field \( H \) be \( \theta_i \). The magnetisation \( J \) in the direction of the field is

\[
J = \mu \cdot \sum_{i=1}^{N} \cos \theta_i = N \cdot \mu \cdot \overline{\cos \theta},
\]

where \( \overline{\cos \theta} \) is the average value of all \( \cos \theta_i \) and is determined by the rules of static mechanics in a field. The derivation is as follows. The potential energy \( E_{\text{pot}} \) of a dipole with moment \( \mu \) in an applied field \( H \) is:

\[
E_{\text{pot}} = \mu \cdot H \cdot \cos \theta,
\]

The probability \( p(\theta) \) of a magnetic moment having angle \( \theta \) to the field is, according to Boltzmann-statistics, given by

\[
p(\theta) = \exp \left( \frac{-E_{\text{pot}}}{k_B T} \right), \quad (\text{where } k_B = \text{Boltzmann-constant}).
\]

Because all directions of \( \mu \) are possible, \( \overline{\cos \theta} \) is given by

\[
\frac{J}{J_{\text{max}}} = \overline{\cos \theta} = \frac{\int_0^\pi \cos \theta \cdot \exp \left( -\frac{\mu H}{k_B T} \cdot \cos \theta \right) \cdot \sin \theta \, d\theta}{\int_0^\pi \exp \left( -\frac{\mu H}{k_B T} \cdot \cos \theta \right) \cdot \sin \theta \, d\theta} = \coth x - \frac{1}{x} = L(x),
\]

where \( x = \frac{\mu H}{k_B T} \) and \( L(x) \) is called the Langevin function.

\( L(x) \) is dependent on the field \( H \) and on temperature \( T \). It describes the orientation behaviour of dipoles in a magnetic field against the thermal disorder. For small values of \( x \), \( L(x) \approx x/3 \) and
\[ \frac{J}{J_{\text{max}}} = \frac{\mu H}{3k_B T} \]  

(B.6)

equation B.6 is similar to the temperature-dependence of a paramagnetic material. Thus, a single domain grain at temperatures above its blocking temperature cannot preserve any remanence and its behaviour is analogous to paramagnetism except that the magnetic moment \( \mu \) is not due to a single atom but due to a particle with several thousands of atoms. This gives a much higher susceptibility than found in ordinary paramagnetism and the effect is called superparamagnetism.
C. Appendix I Chapter 5

C.1 Comparison of all samples

In the previous chapter emphasis was placed on hippocampal tissue and meningioma brain tumour tissue. Beside these types of tissue, a range of other brain tumour tissue was obtained from Prof. Dr. Karl Frei from the Department of Neurosurgery at the University Hospital Zurich. On all samples, isothermal remanent magnetisation (IRM) acquisition was first measured at 77 K to obtain a first impression of remanent phases within the tissue samples. An overview of all samples and their remanent saturation magnetisations ($M_{RS}$) and coercivities of remanence ($H_{cr}$) at 77 K is given in Table V.1. From all groups with three or more samples of one type, average magnetisations with their 95% confidence limits were calculated. The results are shown in Figure C.1 and Table C.1. The average $M_{RS}$ for the neurinomas is the highest, and that from the astrocytomas is the lowest. The average $M_{RS}$ of the hippocampi is smaller then those from the gangliogliomas, the haemangiomas, the neurinomas, the glioblastomas and, as described before, the meningiomas. It is only slightly higher than the $M_{RS}$ of the astrocytomas.

These results have to be interpreted with care because of the different numbers of samples and their statistical significance. In Figure C.1 the wide spread of values is shown by the black bars for the 95% confidence boundary. For all types of tissue, where the confidence boundary extends into the negative magnetisation, the average is not statistically significant. For the gangliogliomas, the neurinomas and the glioblastomas, the spread of the magnetisations from only three or four samples is too large to give a statistically meaningful average value of $M_{RS}$.

Though the results are not statistically significant for all types of tumours, there is a tendency toward stronger remanent magnetisation signals in brain tumour tissue compared to hippocampi. Meningiomas seem to exhibit consistently high $M_{RS}$ values. However it is of potential interest to analyse the same number of samples for the other five tumour groups and to evaluate whether the significance improves with a higher number of samples.
### TABLE C.1: Averaged values for the remanent saturation magnetisation ($M_{rs}$) with ± 95% confidence limits from IRM acquisition at 77 K.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Number of samples</th>
<th>Averaged $M_{rs}$ [Am$^2$/kg]</th>
<th>± 95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningiomas</td>
<td>12</td>
<td>$2.15\times10^{-5}$</td>
<td>± $0.72\times10^{-5}$</td>
</tr>
<tr>
<td>Hippocampi</td>
<td>13</td>
<td>$2.04\times10^{-6}$</td>
<td>± $0.44\times10^{-6}$</td>
</tr>
<tr>
<td>Astrocytomas</td>
<td>3</td>
<td>$1.93\times10^{-6}$</td>
<td>± $1.35\times10^{-6}$</td>
</tr>
<tr>
<td>Gangliogliomas</td>
<td>3</td>
<td>$9.67\times10^{-6}$</td>
<td>± $14.3\times10^{-6}$</td>
</tr>
<tr>
<td>Haemangiomas</td>
<td>4</td>
<td>$6.03\times10^{-6}$</td>
<td>± $3.86\times10^{-6}$</td>
</tr>
<tr>
<td>Neurinomas</td>
<td>4</td>
<td>$2.22\times10^{-5}$</td>
<td>± $2.40\times10^{-5}$</td>
</tr>
<tr>
<td>Glioblastomas</td>
<td>4</td>
<td>$7.74\times10^{-6}$</td>
<td>± $11.6\times10^{-6}$</td>
</tr>
</tbody>
</table>

**Figure C.1:** Average remanent magnetisations ($M_{rs}$) and ± 95% confidence limits (black bars) for all tumour types with three or more samples (number of samples in brackets).
### TABLE C.2: Remanent saturation magnetisation $M_{rs}$ and coercivity of remanence $H_{cr}$ from IRM acquisition at 77 K.

<table>
<thead>
<tr>
<th>Name</th>
<th>$M_{rs}$ 77 K [Am$^2$/kg]</th>
<th>$H_{cr}$ 77 K [mT]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meningioma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA1b</td>
<td>6.70E-05</td>
<td>39.7</td>
</tr>
<tr>
<td>DL</td>
<td>4.42E-05</td>
<td>26.5</td>
</tr>
<tr>
<td>LBS</td>
<td>2.85E-05</td>
<td>33.5</td>
</tr>
<tr>
<td>HM2</td>
<td>2.64E-05</td>
<td>32</td>
</tr>
<tr>
<td>HA1</td>
<td>2.46E-05</td>
<td>40</td>
</tr>
<tr>
<td>VE</td>
<td>1.78E-05</td>
<td>32.5</td>
</tr>
<tr>
<td>KF</td>
<td>1.16E-05</td>
<td>44</td>
</tr>
<tr>
<td>SA1</td>
<td>1.05E-05</td>
<td>37</td>
</tr>
<tr>
<td>NB</td>
<td>1.03E-05</td>
<td>37</td>
</tr>
<tr>
<td>CH2</td>
<td>7.53E-06</td>
<td>36</td>
</tr>
<tr>
<td>BW2</td>
<td>5.20E-06</td>
<td>38</td>
</tr>
<tr>
<td>SG2</td>
<td>4.11E-06</td>
<td>32</td>
</tr>
<tr>
<td><strong>Hippocampi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SG1</td>
<td>4.10E-06</td>
<td>40</td>
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<td>GS4</td>
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<td>35</td>
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<td>GS2</td>
<td>1.26E-06</td>
<td>43</td>
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<td>HH</td>
<td>1.05E-06</td>
<td>28.5</td>
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<tr>
<td>ER</td>
<td>1.02E-06</td>
<td>44</td>
</tr>
<tr>
<td>MW</td>
<td>3.00E-07</td>
<td>32</td>
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<td><strong>Astrocytomas</strong></td>
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<td>GH</td>
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<td>BB1</td>
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<tr>
<td><strong>Oligoastrocytomas</strong></td>
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<td>PA</td>
<td>1.86E-06</td>
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<td><strong>Gangliogliomas</strong></td>
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<td>KL</td>
<td>2.47E-05</td>
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<td>CJ</td>
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<td>AO</td>
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<td>NH</td>
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<td>HT</td>
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<td>MK</td>
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<td><strong>&quot;Arterielles Gefäss&quot;</strong></td>
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D. Appendix II Chapter 5

D.1 AC susceptibility

AC susceptibility is the term used for magnetic susceptibility measured in alternating magnetic fields. AC-magnetic susceptometry is suitable for the magnetic study of superparamagnetic properties because measurements in different frequencies allow for an adjustable time scale of measurement. AC-susceptibility measurements yield information about the magnetisation dynamics, which are not obtained in DC measurements. In addition, very small magnetic fields are used, minimizing the effect of external DC fields on the particle moment relaxation. In DC measurements the applied field suppresses the relaxation process in the particles and the blocking temperature $T_B$ is shifted to lower temperatures. AC-magnetic susceptometry exploits on the Néel-Arrhenius relation reported in Appendix B. The AC-susceptibility $\chi$ is measured as real (in-phase) and imaginary (quadrature) components, denoted $\chi'$ and $\chi''$, respectively.

AC-susceptibility measurements as a function of temperature were used to characterise the mixed system of horse spleen ferritin and magnetite nanoparticles described in Chapter 4. Low-field AC-susceptibility measurements are reported to be suitable for a distinction between ferritin and hemosiderin (Allen, et al. 2000). However, the measurements in this report were made on ferritin extracted from horse spleen, dugong liver and human liver, and on hemosiderin extracted from dugong liver and human liver. Another study describes AC-susceptibility measurements on rat muscle tissues after iron dextran injection (Lazaro, et al. 2005).

AC-susceptibility measurements on human brain tissue have not been reported until now. The main difficulty posed for AC-susceptibility is that of detectability, due to the low concentrations of ferritin and hemosiderin, and also of superparamagnetic magnetite and/or maghemite particles. Measurements are often near the detection limit of the instrument. This is the main reason why ZFC and FC induced magnetisation measurements are currently used for characterising antiferromagnetic and ferrimagnetic nanoparticles. For one haemangioma in this study, HT, a relatively high ferritin signal in the ZFC induced measurements was seen. AC-susceptibility measurements were made on this haemangioma with the MPMS at the
Institute of Rock Magnetism, University of Minnesota. The frequencies used were 0.1 Hz and 1 Hz; these are very low, but more suitable for weak samples. The measurements were made between 4 K and 150 K, but above 50 K they had to be discontinued, because the signal to noise ratio became too small.

Figure D.1 shows the in-phase and out-of-phase susceptibility of sample HT. The maxima of the in-phase susceptibility are at 12.5 Hz for 0.1 Hz and at 14 K for 1 Hz corresponding to the average blocking temperatures ($T_B$) of brain ferritin. In comparison with the AC-susceptibility, the $T_B$ of 11 K in the induced measurements is slightly shifted to lower temperature due to the induced field. The increase at temperatures below 5 K is due to the nearly-paramagnetic heme-iron contribution. The out-of-phase susceptibility only shows the blocking-process of the antiferromagnetic cores of ferritin. There is no superimposed signal from paramagnetic phases. $\chi''$ becomes very low at temperatures above 24 K, indicating that the ferritin cores are completely unblocked above this temperature. This temperature corresponds to the bifurcation point of the ZFC and FC magnetisation curves in the induced measurements.

No further AC-susceptibility measurements have been possible on other samples due to their weak magnetisations.
Figure D.1: AC-susceptibility measurements as a function of frequency and temperature of sample HT. (a) In-phase susceptibility. (b) Out-of-phase susceptibility.
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E. Appendix III Chapter 5

E.1 Microscopy

The magnetic analysis has shown that most of the samples contain magnetite and/or maghemite particles, which display stable single domain behaviour at room temperature (c.f., IRM acquisition, hysteresis loops and FORC analysis). This indicates that these particles have a critical grain size $\geq 25$ nm (Dunlop and Özdemir 1997). Particles of this size and smaller are observable by transmission electron microscopy (TEM). Kirschvink et al. (1992) and Schultheiss-Grassi et al. (1999) report on magnetite particles extracted from human hippocampal tissue and identified by high resolution TEM, electron diffraction and elemental analysis.

The extraction of the particles from the brain is not trivial, because the particles are embedded in the fatty tissue matrix, which needs to be dissolved, since it can prohibit good TEM images. This requires a suitable solvent that can eliminate the organic material but not alter the chemical properties of the extract.

A first attempt to extract particles from hippocampal and tumour tissue was made at the University of Keele, Stoke-on-Trent, UK with Dr. Joanna Collingwood. The procedure for extraction is as follows. The sample was first ground to a fine powder in liquid nitrogen with a mortar and pestle. The powder was then dispersed in ultra-pure, magnetically cleaned, distilled water and dispersed ultrasonically for 1 hour. The extraction line consisted of two flasks, a tube system, a peristaltic pump (flow rate 7.5 ml/min) and a trap and probe (Figure E.1). Each sample was run for 2-3 days. Two extracts were produced: one was collected in the trap and the second on a sheathed magnetized probe. The material collected in the trap was rinsed several times and ultrasonically cleaned for at least 40 minutes. The material on the probe, which consisted of finer particles, was also rinsed and ultrasonically cleaned. Both tubes, together with the trap and probe, were then set on a large magnet for several hours, before the glassware was removed. All extracts were magnetically concentrated by setting a strong magnet at the side of the collecting tubes and the solution could be pipetted. A drop of the solution from both the probe and trap from each sample was air-dried on a carbon-coated copper grid.
A different method was used in a second attempt at extracting particles from brain tumour tissue. This was done in cooperation with Dr. Damien Faivre at the Max Planck Institute for Marine Microbiology in Bremen, Germany. Protease K was added to the freeze dried tissue and centrifuged. The supernatant was then re-suspended in a buffer solution (Hepes and EDTA), centrifuged again, and put in a magnetic column. The final solution was put on a carbon-coated copper grid.

![Diagram](image)

**Figure E.1:** Extraction line as built up by Dr. Joanna Collingwood, University of Keele, UK.

TEM analysis on the grids from the first extraction showed only one rather large particle with angular shape (Figure E.2 (inset)). The resulting diffraction pattern (Figure E.2) shows well-crystalline behaviour and the measured d-spacings are comparable to d-spacings measured by Quintana *et al.* (2000).

Preliminary TEM analyses from the second extraction are shown in figures E.3-E.6. There are several clusters of small particles between 5 nm and 30 nm in size (Figure E.4),
together with organic material. The presence of organic matter indicates that the solvent did not completely eliminate the tissue matrix. In the upper part of the picture, some distinct angular-shaped particles can be identified (Figure E.5). Scatter area electron diffraction (SAED) was made on some of the clusters of particles. These show distinct d-spacings that are compatible with magnetite (Figure E.3, Table E.1). High resolution TEM would be useful for better definition of SAED patterns in this material, so that more grains could be examined to confirm the identification of magnetite rather than maghemite.

Figure E.2: Diffraction pattern of particle shown in inset (first extraction method).  
Figure E.3: Diffraction pattern of clustered particles in Figure E.4 (second extraction method).

Figure E.4: Magnetic extract from method 2.  
Figure E.5: Magnification of upper part from Figure E.4 showing angular shaped particles.

An energy dispersive X-ray analysis (EDXA) was made on the extracted particles in order to investigate whether iron is present. Figure E.6 indicates the presence of iron and
oxygen as expected for iron-oxides, but it also shows phosphorous and sulfur, both common elements in organic materials. The presence of copper and carbon is due to the TEM grids.

![Energy dispersive X-ray analysis (EDXA) of extracted particles from method 2 (Figure E.4).](image)

**Figure E.6:** Energy dispersive X-ray analysis (EDXA) of extracted particles from method 2 (Figure E.4).

**TABLE E.1:** Lattice spacing for the magnetic extraction in Figure E.2 and Figure E.3 and reference values with direction in parentheses.

<table>
<thead>
<tr>
<th>Magnetic extract (Figure E.2)</th>
<th>Fe$_3$O$_4$ (In courtesy of M. Hanzlik, München)</th>
<th>Maghemite (In courtesy of D. Faivre, Bremen)</th>
<th>Magnetic particle (Figure E.3)</th>
<th>Fe$_3$O$_4$ (Quintana et al. 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.296</td>
<td>0.2967 (220)</td>
<td>0.2953 (220)</td>
<td>0.242</td>
<td>0.2424 (222)</td>
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<tr>
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<td>0.2532 (311)</td>
<td>0.25177 (313)</td>
<td>0.419</td>
<td>0.4198 (200)</td>
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<tr>
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<td>0.20886 (400)</td>
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<td>0.162</td>
<td>0.1616 (511)</td>
<td>0.16073 (513)</td>
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<td>0.148</td>
<td>0.1485 (440)</td>
<td>0.14758 (440)</td>
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Acknowledgements

A long list of people helped me directly and indirectly in the preparation of this thesis during the last three years and I would like to take the opportunity to thank all of them. Many people were contacted by me for information or concrete help with sample preparation and equipment for making the various measurements and all of them reacted positively and responded spontaneously to my questions and requests. They helped me to make my measurements or furnished me with the necessary material, although the subject was new to them or seemed exotic or strange. For me, this was one of the most enriching and positive aspects of this three-year project.

First of all, I would like to express my gratitude to my direct supervisor Dr. Ann M. Hirt, who offered the position on her project to me. She was always extremely supportive and open to my ideas, letting me have the freedom I needed, but always there for questions and discussions, and always taking a lot of her time for me and my problems. I am grateful for her indefatigable commitment to this work; I know I kept her busy!

I further wish to thank Prof. Jon Dobson and Prof. Dr. med. Heinz-Gregor Wieser for the valuable ideas they gave me, and for being so supportive and positive about this work. Without their help, I would never have had enough samples to measure. I thank Prof. Alan G. Green for accepting to chair my thesis committee. Furthermore I would like to thank Prof. Dr. Karl Frei and Prof. Dr. med. Yasuhiro Yonekawa and Dr. med. Christian Simon for supplying the tissue and for their friendly help and discussions and Prof. Dr. Jean-Marc Fritschy and Dr. Fabienne Loup from the Institute of Pharmacology at the University of Zurich for supplying brain tissue from mice and for being very open to this work.

With regard to my measurements, I thank Dr. Thomas Frcdrichs, who introduced me to the MPMS, and shared his extensive working experience. He and the team from Bremen were hugely supportive colleagues during the three weeks I spent at their institute. I thank Dr. Mike Jackson, Peat Solheid and Jim Marvin from the IRM in Minnesota for giving me the opportunity of a visiting fellowship and for their invaluable help with the delicate measurements. Further, I am very grateful to Alois Weber from the ETH Chemistry department for his reliable willingness to measure my samples. A superb new measuring method was kindly placed at my disposal by Harry Reichard and Anthony Cumbo from the Princeton Measurements Corporation, Princeton, where I was fortunate to spend one week in their lab. I am deeply grateful for their generosity and support. For the evaluation and interpretation of FORC data I received important help from Dr. Michael Winklhofer from München. I thank him for his contribution and interesting E-mail discussions.

For the magnetite particles, I thank Dr. Louis Tiefenauer from PSI, Villigen, who also contributed a lot to the mixed system paper and two interesting meetings at the PSI. I also thank Dr. Alke Fink from the EPF Lausanne for sending me particles. For the microscopic work, I thank Dr.
Joanna Collingwood from the University of Keele, UK, Dr. Damien Faivre from the Max Planck Institute, Bremen, Dr. Marianne Hanzlik from TU München, and Dr. Elisabeth Müller from the ETH Physics department. Furthermore, I thank Martin Wälti from ETH Microbiology department for the use of the freeze-drier.

I thank my two excellent semester students, Gabriela Stamm and Davina Pollock, for daring to carry out a project with me and for the successful outcome of their efforts. I am grateful to André Blanchard, Elisabeth Läderach, Sabine Räss and Chrissy Chatzidis for helping me with material support, administrative work, but also for enjoyable conversations.

From the magnetic group I am deeply grateful to Pascall Rosselli and Claudio d’Addario for supporting me in the Lab, sound advice about holder techniques, for always keeping the Cryo running and adapting the software for me. I thank Prof. William Lowrie for his constant interest in this work, his correction and proof-reading support and for sound advice about statistics and physics. I further thank Dr. Andreas Gehring for hearty discussions and for being so communicative, enlivening room O3. I thank my great colleges from O3, Volkmar, who always solved acute computer problems for me, being so patient with my temperament and listening to me or switching on classical music, and Hakon, exchanging Panini pictures with me and teaching me that a good scientist can have a chaotic desk!

Furthermore I would like to thank my non-ETH friends, Coco, Fränzi, Corinne and Susanne for always keeping contact during my new projects, exchange years, traineeships or travels, and for talking with me about real life instead of science.

I am deeply grateful to my family: My parents for always being so positive and supportive concerning my projects, and for their loving care and motivation; and my brothers and sisters for teasing me about becoming a “doctor ETH”, and for many happy hours gardening and working in the vineyard or watching football.

Finally, I thank my boyfriend Mark. With his good humour and his balanced personality he makes my life easier. His realistic way of thinking and his honesty have been very healthy for me for many years.
Curriculum Vitae

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