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Development of a biocompatible magnetic nanofluid by incorporating SPIONs in Amazonian oils

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ABSTRACT

Higher quality magnetic nanoparticles are needed for use as magnetic nanoprobe in medical imaging techniques and cancer therapy. Moreover, the phytochemistry benefits of some Amazonian essential oils have sparked great interest for medical treatments. In this work, a magnetic nanoprobe was developed, allying the biocompatibility and superparamagnetism of iron oxide nanoparticles (SPIONs) with benefits associated with Amazonian oils from Copaiba and Andiroba trees. SPIONs were obtained by two thermal decomposition procedures and different amounts of precursors (iron acetylacetonates). Their characterization was accomplished by Fourier transform infrared spectroscopy, thermogravimetric analysis, transmission electron microscopy (TEM), X-ray diffraction (XRD), Mössbauer spectroscopy and magnetization. The obtained nanoparticles composition and magnetic properties were not affected by the relative proportion of iron(II) and iron(III) in the precursor system. However, when changing the reducing and stabilizing agents the coating layer shows different compositions/relative weight — the more promising SPIONs have a coating mainly composed by oleylamine and an iron oxide:coating wt% ratio of 55:45. Nanoparticles size distributions were very narrow and centred in the average size of 6–7 nm. Cellular assays confirmed the biocompatibility of SPIONs and their effective internalization in human colon cancer cells. Mössbauer/XRD results indicated maghemite as their main iron oxide phase, but traces of magnetite proved to be present. Magnetization saturations of 57 emu/g at 5 K and 42 emu/g at 300 K were achieved. With incorporation of SPIONs into Copaiba and Andiroba essential oils, these values show a 4-fold decrease, but the supermagnetic behaviour is preserved providing the effective formation of a nanofluid.

1. Introduction

The ability to control and drive nanoparticles through the human bloodstream to a specific target by using an external magnetic field is of great interest to biomedical applications [1–3]. The superparamagnetic iron oxide nanoparticles (SPIONs) are a common choice for this purpose. Their superparamagnetism renders them the ability of behaving like a giant paramagnetic atom, granting a fast response to applied magnetic fields with negligible reminiscence and coercivity; it also diminishes the risk of agglomeration [1]. Moreover, SPIONs are usually biocompatible and non-toxic and other characteristics can be added to make them have a broader use. Because of their instability towards oxidation, SPIONs commonly have a protective shell against degradation. This shell may be used to bind specific drugs, proteins, enzymes, antibodies, *etc.*, which can be delivered or may interact at targeted body sites [2]. The use of SPIONs can also greatly increase sensitivity needed to achieve high spatial resolution in magnetic resonance imaging (MRI) [2]. Finally, SPIONs have shown promising results for cancer treatment by hyperthermia [3].

Magnetite (Fe_3O_4) is the preferred iron oxide phase for SPIONs, due to its higher magnetization saturation. It shows ferrimagnetic behaviour when in coarse size and superparamagnetic behaviour below 6 nm [4]. At room temperature (RT), magnetite exhibits a face-centred cubic lattice, with an inverse spinel structure, being the tetrahedral sites (A sites) occupied by Fe^{3+} and the octahedral sites (B sites) distributed between Fe^{2+} and Fe^{3+} [4]. Below the Verwey transition temperature (120 K), the trivalent and divalent iron atoms arrange themselves in a regular pattern giving rise to a normal spinel structure [4]. The mixed valence of iron in magnetite makes it thermodynamically unstable at atmospheric O_2 pressure and, thus, susceptible to oxidation, forming maghemite [5]. The iron oxide intermediates obtained through this process show decreasing amount of Fe^{2+} in its composition,

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accompanied by a decrease in the cell edge [4]. In fact, upon oxidation, magnetite gradually transforms in maghemite, which is also clearly visible by the colour change from black to reddish-brown – therefore SPIONs always have maghemite in their composition. Maghemite (γ-Fe_2O_3) is a ferrimagnetic iron oxide at RT with the same inverse spinel structure as magnetite. Below the critical size of 10 nm, maghemite behaves superparamagnetically [4]. The main difference between their structures is the cation deficient octahedral sites of maghemite (vacancies in the place of divalent iron) that leads to a decrease of the cubic unit cell size [4], which is not easily detected by XRD. Table 1 presents a brief summary of some physicochemical properties that allows distinguishing between these similar and interchangeable iron oxides.

For use in biomedical applications, the control of shape and size of the nanoparticles is of great importance, as well as the ability to prevent agglomeration and achieve a high yield. In this regard, the thermal decomposition/thermolysis method is a convenient approach and is easily applied at large scale. This method is based on the thermal decomposition of organometallic compounds in high-temperature boiling organic solvents containing stabilizing surfactants, under an inert atmosphere. The most often used organometallic precursors are acetylacetonates, being oleic acid and oleylamine the more commonly used surfactants [1]. The ratio between reagents, the reaction temperature and time are decisive for the control of the size and morphology of the obtained nanoparticles. This type of synthesis develops in two steps, nucleation and growth from a supersaturated system, described by La Mer [6]. At low temperature, the dissolved solute increases until reaching a critical supersaturation point, and the nucleation starts to occur. After some time, the nucleation process leads to a decrease in concentration and, at this point, the particle growth starts, which is finally favoured by a change for a higher temperature level (300–400 °C). Concentration will continue to decrease, due to growth mediated by diffusion, until it reaches an equilibrium solubility value.

The nanoparticles resulting from this method have a hydrophobic coating, which avoids the oxidation/degradation of these particles by exposure to the environment. However, this coating requires the search for a biocompatible liquid in which the particles can form a stable dispersion, *i.e.* a magnetic nanofluid. Natural oils can be an interesting alternative as dispersing medium.

Natural oils extracted from trees of Amazonian rainforest, specifically oils from Copaiba (*Copaifera* spp. species) [7] and Andiroba (*Carapa guianensis* species) [8] trees, have been the target of research since the XIXth century and in the first decades of the century XXI, due to their unique characteristics and for being biodegradable and coming from renewable resources. In fact, their physicochemical, biological and biomedical properties enable their application in the paint and varnish industry, cosmetics industry and as phytomedicines in folk medicine, in various regions of Latin America [9–13].

Copaiba oil is produced in the wood of the *Copaifera* spp. trees, belonging these trees to the *Leguminosae* family that has several species. It is extracted directly from the trunk through holes to the storage channels. Photochemical studies showed that the molecular composition of Copaiba oil comprises two groups, which are diterpenes acids (8%) and sesquiterpenes hydrocarbons (90%) [9]. Conversely, other parts of the tree, such as leaves, bark and branches, have other molecular components [10]. A total of 28 diterpenes acids were identified in the Copaiba oil, the majority being kauranes (kaurenoic acid), clerodanes (hardwickiic acid and kovalenic acid) and labdanes (copalic acid); the sesquiterpenes hydrocarbons are 72 in total, being the most representative β-caryophyllene, α-copaene, zingiberene, caryophyllene oxide, α-humulene and α-*trans*-bergamotene [11]. These are the volatile components constituting the essential part of Copaiba oil, and these terpenes have recently attracted a growing interest, and studies have been made that show their high potential as anti-inflammatory, anti-allergic and antimicrobial agents [9,11,13,14]. More specifically, β-caryophillene has also shown great potential as antioxidant and anti-carcinogenic [12,13].

On the other hand, the vegetable oil commonly known in Brazil as Andiroba is widely used in traditional medicine as anti-inflammatory agent, analgesic spray, insect repellent and other applications [15–21]. The Andiroba oil is extracted from the fruit seeds of *C. guianensis* trees, belonging this species to the *Meliaceae* family [16,18,21]. This family is native of South America, West Indies and South Africa. The oil composition, determined by photochemical studies, includes triterpenes and tetraterpene compounds. Alkaloids and limonoids compounds are found in leaves, branches and trunks of *C. guianensis* tree [17–21]. Tetraterpenes and triterpenes are 6α-acetoxygedunin, 7-deacetoxy-7-oxogedunin, andirobin, gedunin, methyl angolensate and 6a-acetoxy-epoxy-azadiradione compounds. However, the Andiroba oil is mostly composed by fatty acids: oleic acid (~57%), myristic acid (~18%), palmitic acid (~9%) and linoleic acid (~9%) [19–21]. The high therapeutic potential of the Andiroba oil has been recognized to be connected to tetra and triterpenoids molecular compounds [15].

In this work, we will develop a biocompatible magnetic nanoprobe using SPIONs and Amazonian essential oils. For the synthesis of SPIONs, two procedures of thermal decomposition will be implemented, inspired in earlier works [22,23], as well as the testing of different

Table 1
Physicochemical properties of magnetite and maghemite (compiled from [4]).

Mineral	Magnetite (Fe_3O_4)	Maghemite (γ-Fe_2O_3)
Colour	Black	Reddish-brown
Density ($g \cdot cm^{-3}$)	5.18	4.87
Crystallographic structure	Cubic	Cubic or tetragonal
Cubic cell dimension (nm)	0.8396	0.8347
Curie temperature (K)	850	820–986
Magnetization saturation at 300 K (emu/g)	92–100	60–80

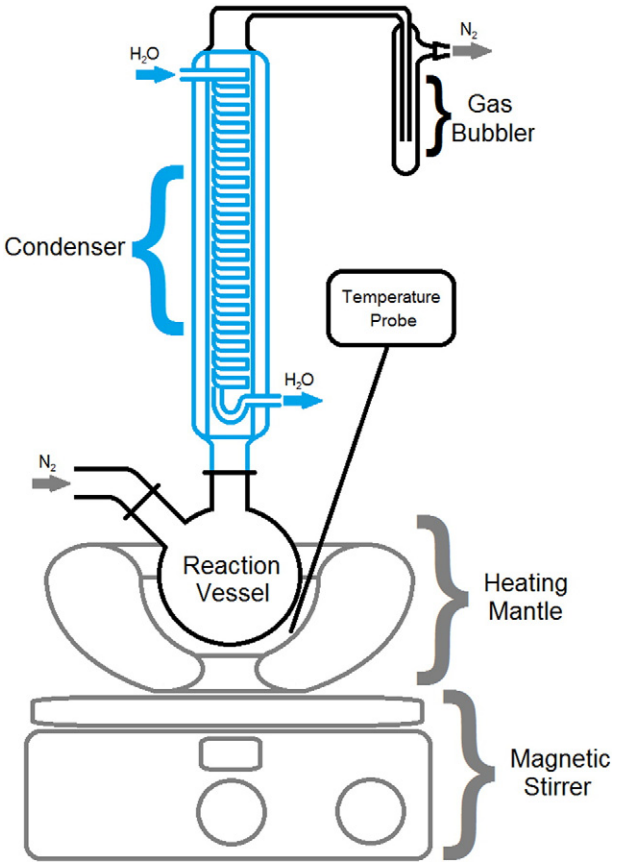

Fig. 1. Experimental assembly for the syntheses. (Colour on-line).

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