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A study on the photodynamic therapy of photosensitizer-coated magnetic nanoparticles

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One of the important points in the use of magnetic nanoparticles for biomedical applications is the encapsulation of the magnetic material, in order to make it biocompatible, producing a bio-ferrofluid. In this paper, ferrimagnetic particles of Fe_3O_4 were nanometrically synthesized. In order to coat a photosensitizer on the magnetic particles, TSPP was choosed and introduced into the ferrofluid suspension containing magnetite and the adsorption reaction was conducted at 60, 70, and 80 °C. Analytical investigations (UV-Vis, TEM, XRD, TGA) argued the TSPP encapsulated into Fe_3O_4.

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1. Introduction

Today, nanotechnology has developed to a stage, which makes it possible to produce, characterize and specially tailor the functional properties of nanoparticles for clinical applications and diagnostics.

Few experimental procedures are used in order to obtain stable nanomaterials and involve: intercalation processes, the grafting of organic groups onto the surface of inorganic solids, or the entrapment of molecules in organic matrices by sol-gel methods [1]. Porphyrins are synthetic dyes characterized by the specific molecular structure which can serve as photosensitizers in cancer therapy [2]. In order to improve their efficiency of delivery to the specific area of need in a human body, and to minimize the negative side and after effects of dosage, localization and focus medical treatment, such porphyrins have coated on some magnetic particles (ferrofluids).

Ferrofluids are colloidal solutions of iron oxide magnetic nanoparticles surrounded by polymeric layer coated with affinity molecules, such as antibodies, for capturing cells and other biological targets from blood or other fluid and tissue samples. Ferrofluid particles are so small (25–100 nm in radius) that they behave in liquids as a solution rather than suspension. When the coated ferrofluid particles are mixed with a sample containing cells or other targets, they interact intimately and completely. These properties enable the development of specialized reagents and systems with extremely high sensitivity and efficiency and capture.

As one of the most important materials, magnetite (Fe_3O_4) nanoparticles have attracted a lot of attentions [4] for their interesting magnetic properties and potential applications in the fields of sensors [3,8,9], biology [5], pharmacy [6] and diagnostics [7]. However, the tendency to aggregation limits their further applications. Dispersing Fe_3O_4 in a suitable matrix to prevent the aggregation is a new and good alternative, it is simple and the introduction of new substance could also bring new functions for magnetite [10], so this method is attractive.

The aim of this work is to synthesize and to characterize improved ferrofluids (magnetite covered by TSPP) as potential drugs for medical applications.

Photodynamic therapy (PDT) is a non-invasive treatment technique that works along the same lines as the diagnostic method described above; the same photosensitizing drugs are used and the same method of excitation is employed.

2. Experimental part

2.1. Materials and preparation

Meso-tetra(p-sulphophenyl)porphyrin (TSPP) (Fig. 1) has been prepared and purified in the laboratory after the literature receipt [11,12].

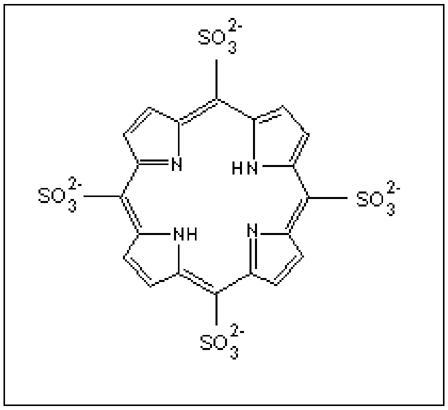


Fig. 1. Chemical structure of TSPP.

Magnetite Fe_3O_4 was prepared by coprecipitation of aqueous iron chloride solution (Fe(II) and Fe(III) with ions ratio $Fe^{2+}/Fe^{3+} = 0.5$ and heated to 50 °C, by quickly addition of excess ammonium hydroxide NH_4OH, followed by strong agitation for 15 minutes. This process leading to a magnetic colloid formation as reaction equation (1):

$$2FeCl_3 + FeCl_2 + 4NH_4OH \xrightarrow[\text{stirring 15 minutes}]{t = 50\ ^{o}C} 4HCl + 4\,NH_4Cl + \underset{\text{magnetic decantation}}{Fe_3O_4\downarrow} \quad (1)$$

The attachment of oleic acid to the surface was done after the formation of magnetite by addition of 15 ml of 20 %wt aqueous solution of oleic acid and 10% ammonium hydroxide. The solution was stirred with a magnetic bar for 30 minutes at 80 °C. Following stirring, the solution was placed on a magnet and washed three times, twice with distilled water and once with ethanol. The solution was dried with nitrogen for two hours and stored for further use.

In order to coat a photosensitizer and on the magnetic particles, TSPP was choosed and introduced in a concentration of 5×10^{-4} mol into a aqueous suspension of Fe_3O_4; the adsorption reaction was conducted at 60, 70, and 80 °C. The magnetic suspension included about 2 g of magnetite particles.

2.2. Characterization

The absorption diffuse-reflectance spectra were recorded on a SPECORD M400 Carl Zeiss Jena spectrophotometer with double beam and microprocessor.

Transmission electron microscope (TEM) JEOL 100-CX was used for morphology studies. The aqueous dispersion (one drop) was placed over a copper grid of 400 meshes with carbon film. The droplet was reduced after 5 min with a filter paper to eliminate the excess of nanoparticles. Finally, the sample was air dried prior to placing it in the TEM.

The sample was placed in the furnace of TGA 2950 thermogravimetric analyzer (Du Pont) over an aluminum pan under a nitrogen atmosphere to avoid oxidation. The temperature was varied from 25 to 600 °C with increments of 5 °C per minute.

The crystallinity of both polymer and TSPP was determined by X-ray powder diffraction using a DRON -2 (Cu K_α radiation).

3. Results and discussion

The UV–Vis spectroscopy was also employed to have a first evidence of Fe_3O_4-OA-TSPP formation (fig. 2).

In the wavelength after 330 nm region, a broad featureless adsorption could be observed in all the curves originates primarily from the absorption and scattering of light by magnetic particles and is the characteristic of the absorption band of some iron oxides. Moreover, each deposition cycle adds almost constant increment to the absorbency (at 380 nm), demonstrating the amount of material deposited in each multilayer was almost the same. A strong blue shift for Soret band of TSPP is observed (from 420 nm to 360 nm) as a certain evidence for encapsulation of magnetite into TSPP.

TSPP is an anionic porphyrin, a very large disk-shape molecule which possesses four negative charges the sulfonate groups from the four corners.

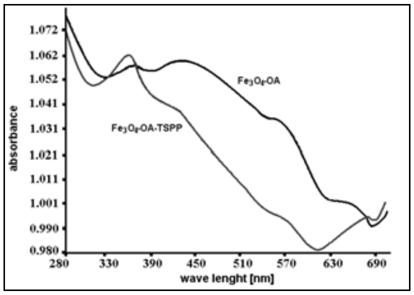


Fig. 2. UV-VIS spectra for Fe_3O_4-OA and Fe_3O_4-OA-TSPP.

In aqueous solutions, at neutral pH, the electronic absorption spectrum of TSPP is typical of free base porphyrins (D_{2h} symmetry) and is characterized by an intense Soret band at arround 420 nm and four Q bands in the 500-700 nm range (the aetio-type spectrum), Table 1.

Table 1. The specific absorption bands of different TSPP forms.

Porphyrinic forms	B band λ/ε x 10^3 (nm/M^{-1}·cm^{-1})	Q bands λ/ε x 10^3 (nm/M^{-1}·cm^{-1})
H_2TSPP^{-4}	412/355	515/130 551/4.5 579/1.9 33/1.01

XRD patterns (Fig. 3) show a wide amorphous background due to the presence of oleic acid and TSPP, and specific peaks can be attributed to nanocrystalline magnetite (Fe_3O_4).

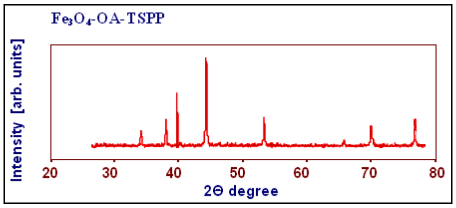


Fig. 3. XRD patern for Fe_3O_4-OA-TSPP.

The magnetite Fe_3O_4 with oleic acid nanoparticles analyzed by TEM showed a spherical shape with a narrow size distribution (Fig. 4). The aggregation present in Fe_3O_4-OA was due to the solvent elimination prior to TEM analysis and to natural clustering of Fe_3O_4-OA. TEM pictures show ellipsoidal particles. A first statistical analysis based on hundred particles per sample lead to an average size of less than 10 nm. The TEM pictures of the ferrofluids with TSPP formed with Fe_3O_4-OA theoretical loading showed a good distribution and a small size of the nanoparticles.

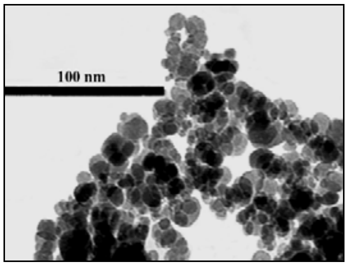


Fig. 4. Surface modified magnetite with oleic acid.

Fig. 5 show Fe_3O_4-OA nanoparticle surrounded by TSPP. Fe_3O_4-OA distribution into TSPP nanoparticles can be appreciated in Fig. 6, where Fe_3O_4-OA is depicted by the black dots. The big dark sphere (inside the dotted circle) manifests the presence of Fe_3O_4-OA. The appearance of clustering is observed in the surrounded TSPP nanoparticles

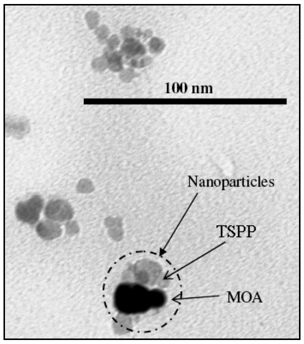


Fig. 5. Nanoparticles formed with TSPP with Fe_3O_4-OA.

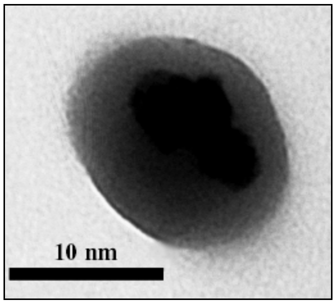
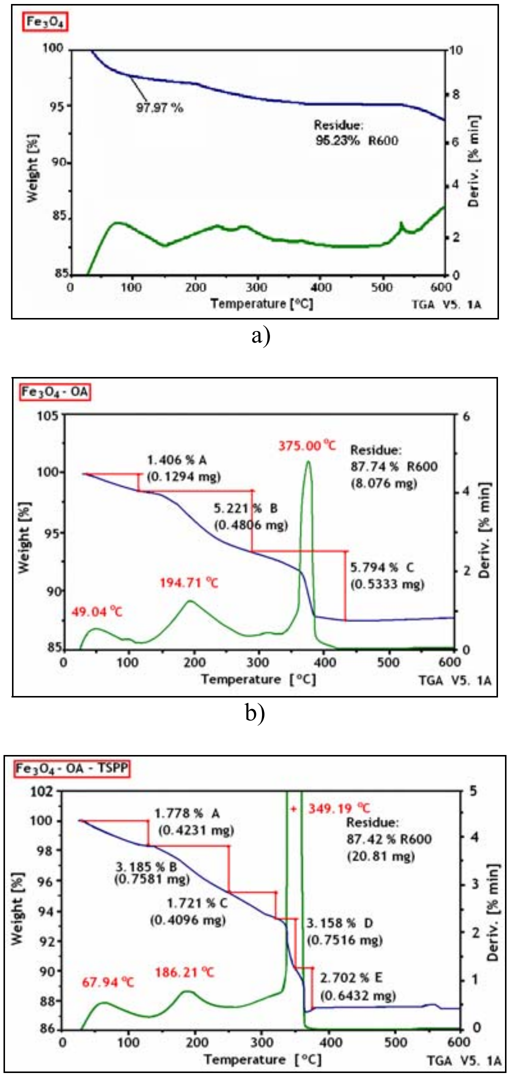


Fig. 6. Fe_3O_4-OA (in the center) covered by TSPP.

The TGA residue for Fe_3O_4-OA at 600 °C was 87.74 wt% (Fig. 7.b). The TGA residue for normal magnetite was 95.23% (Fig. 7.a.) and the residue for Fe_3O_4-OA-TSPP was 87.42% (Fig. 7.c.). The differences were associated with the oleic acid and TSPP presence.



a)

b)

c)

Fig. 7. TGA data for (a) Fe_3O_4, (b) Fe_3O_4-OA and (c) Fe_3O_4-OA into TSPP.

4. Conclusions

Surface modification of magnetite with oleic acid was a useful approach to ensure the entrapment of magnetite into TSPP with high entrapment efficiency. X-Ray diffraction indicated the presence of a phase with crystalline structure consistent with that of magnetite. Transmission electron microscopy (TEM) was used to verify the expected nanoscale size of the particles, providing estimates for the magnetic core diameter ranging from 6 to 13 nm. The TGA indicated some weight differences were associated with the oleic acid and TSPP presence.

Acknowledgements

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