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Use of magnetic and fluorescent polystyrene/tetraphenylporphyrin/maghemite nanocomposites for the photoinactivation of pathogenic bacteria

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ABSTRACT

In this work, we initially describe the preparation of magnetic and fluorescent nanocomposites (MF NCs) based on polystyrene/tetraphenylporphyrin/maghemite (PS/TPP/γ-Fe_2O_3). After carrying a series of exploratory experiments, we have found that the composite that presented the highest values of fluorescence intensity and magnetization (S1 samples) was obtained when we employed 1 mL of TPP and 1% of PS. When tested as photosensitizer agents for the inactivation of the pathogenic bacteria *Escherichia coli*, these MF NCs presented excellent antibacterial activity, indicating that they can be promising candidates to inactivate microorganisms dispersed in aqueous solutions. Taking into account this peculiar combination of outstanding properties and simple and low cost synthesis, we suggest that this kind of NC could find widespread use in environmental and biomedical applications.

1. Introduction

In recent years, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) nanoparticles have been extensively studied due to their superparamagnetic behavior, large surface area to volume ratio and low toxicity [1,2]. These interesting properties have allowed the use of these magnetic nanoparticles (MNPs) in different fields of science, mainly in chemical and biological applications [3,4].

However, when used in their pristine state these iron oxide nanoparticles have shown some drawbacks or presented limitations, such as a lack of stability and reduced number of active sites capable of interacting with target molecules (such as heavy metal ions, biomolecules, organic compounds, among others) [5–7]. To overcome these disadvantages, some strategies have been devised towards modifying the surface of the nanoparticles through the inclusion of chemical compounds such as carboxylates, phosphates, sulfates, silica, gold, dextran, polysaccharides, poly(vinyl butyral), poly(methyl methacrylate), polyesters, chitosan, poly(ethylene glycol), and poly(vinyl alcohol) [8–10]. With this, it has become possible to tailor the MNPs to exhibit more stability, an increased biocompatibility and a larger active surface to interact with molecules of interest. Among the many possible applications of functionalized MNPs, one can find examples of their use as controlled drug delivery carriers [11], catalysts [12,13], as components of sensors [14], and as bioseparation [15], MRI contrast [16,17] and hyperthermia [18] agents. Also, they have found a special niche in the area of detection and removal of heavy metals ions [19,20].

In fact, there is a large interest in several areas of science for the production of new magnetic materials that could be equally effective at different simultaneous tasks, of which a typical case are composites that can not only detect the presence of dissolved heavy metal ions but also remove them from aqueous systems [21]. Actually, there is an acute desire in the biomedical field for the development of theranostic materials, i.e., compounds that could be used to help in the diagnosis of diseases (via MRI and/or fluorescent confocal imaging, for instance) [22–24] and at the same time treat (through hyperthermia or drug delivery) them [25,26].

In particular, materials that combine magnetic and fluorescent properties have already found several applications. For example, Chen et al. [27] synthesized photosensitizer loaded magnetic silica

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nanoparticles (NPs) for the photodynamic therapy of tumor cells. As photoactive agent, they have used 2,7,12,18-tetramethyl-3,8-di-(1-propoxyethyl)-13,17-bis-(3-hydroxypropyl), which generates reactive (singlet) oxygen species when irradiated by light of an appropriate wavelength, hence inducing the oxidative damage of malignant cells. In this case, the magnetic property of the MNPs was exploited in transporting them to the specific tissue of interest, while avoiding the diffusion of the MNPs to other parts of the organism, thus improving the effectiveness of the action (the different aspects involved in the in-vivo transport of magnetic particles are nicely discussed in Ref. [28]). Corr et al. [29] reported the synthesis of a material that could be simultaneously used as a MRI contrast agent and as a drug delivery system, a MF NC based on Fe_3O_4, polyhedral octaaminopropylsilsesquioxane ($T_8NH_3^+Cl^-$) and a porphyrin derivative. Choi et al. [30] have shown that MNPs conjugated with a photosensitizer and vancomycin have the ability to capture, remove and selectively kill pathogenic bacteria. More recently, Henke et al. [31] reported the antibacterial use of electrospun polystyrene nanofibers with encapsulated TPP.

In the present work, we describe a new strategy for the preparation of a MF NC that consists in the physical encapsulation of iron oxide NPs and porphyrin molecules in a polystyrene (PS) matrix. To find the optimal conditions under which the resultant composite presents the best compromise between magnetization and fluorescence characteristics, we implemented the structural, magnetic and optical characterization of these MF NCs. Finally, we have shown that these nanocomposites present excellent antibacterial activity when tested against *Escherichia coli*.

2. Experimental

2.1. Materials

Pyrrole, benzaldehyde, sodium dodecyl sulfate (SDS), PS (MW = 280 kDa), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and propionic acid were purchased from Sigma Aldrich (USA). Ammonium hydroxide (NH_4OH) was provided by Dinâmica (Brazil). Lactose Broth (LB) and Plate Count Agar (PCA) were acquired from Acumedia (USA) and Merck (Germany), respectively. *E. coli* ATCC 25922 was kindly donated by the supervisors of the microbial inventory of the Departamento de Antibióticos of the Universidade Federal de Pernambuco. All reagents were of analytical purity and used as received, except for pyrrole and benzaldehyde, which were distilled under reduced pressure before use. The water used in all experiments was of ultra-high purity (Millipore).

2.2. Preparation of the MNPs

MNPs were synthesized using the co-precipitation method [32]. Initially, 25 mL of $FeCl_3 \cdot 6H_2O$ (2 M) and 25 mL of $FeCl_2 \cdot 4H_2O$ (1 M) solutions were mixed in a 250 mL round-bottom flask and then stirred for 10 min. Thereafter, 125 mL of a NH_4OH (50 vol.%) aqueous solution was added quickly to the flask. After this, the resulting system was stirred for 2 h. The MNPs were decanted magnetically and the supernatant was removed. Then, the MNPs were washed successively four times with deionized water and, finally, dried at 60 °C in a vacuum oven for 48 h.

2.3. Synthesis of TPP

The 5, 10, 15, 20-tetraphenylporphyrin was synthesized according to the procedure described by Maqueira et al. [33]. Briefly, 0.32 mL of pyrrole (5 mmol) and benzaldehyde (5 mmol) were added to 20 mL of propionic acid and the resulting solution was refluxed during 1 h. Finally, we vacuum filtered and dried the dark solid that was obtained.

2.4. Preparation of MF NC

The procedure used for obtain the MF NC consisted in the following steps: a) 0.01 g of γ-Fe_2O_3 NPs and 0.1 g of SDS were added to 10 mL of deionized water and maintained under intense stirring; b) in other flask, we prepared a solution of PS-TPP (TPP, 2×10^{-5} M) in chloroform (see Table 1); c) subsequently, we mixed the PS-TPP solution and the γ-Fe_2O_3/SDS/water suspension and stirred the mixture to form a pre-emulsion; d) then, this pre-emulsion was ultrasonicated (Ultrasonic Processor Sonics, Vibra-cell, 130 W and 20 kHz, USA) during 3 min to obtain a stable suspension of PS/TPP/γ-Fe_2O_3 in water; e) the suspension was stirred for 24 h, while the flask was left open to allow the evaporation of chloroform, and f) after this period, 20 mL of methanol were added to precipitate the MF NC, which was magnetically decanted (with the help of a handheld magnet), washed with deionized water and dried under vacuum.

We used this procedure to prepare different samples of the MF NCs, by varying the amount of PS and TPP according to the data shown in Table 1. All experiments were performed in triplicate and only the corresponding average values were reported.

2.5. Characterization methods

To learn about the morphology and structure of the freshly prepared nanocomposites, we used a Transmission Electron Microscopy (TEM) Tecnai G^2 Spirit TWIN (FEI, USA). Magnetic measurements were performed in an EV7 Vibrating Sample Magnetometer (MicroSense, USA). We measured the hydrodynamic sizes based on Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS90 (Malvern, UK). Fluorescence measurements were carried out with a Fluorolog Spectrofluorometer (HORIBA Jobin Yvon, USA).

2.6. Antibacterial test

In the anti-bacteriological study, we used an *E. coli* ATCC 25922 suspension for the inoculum, according to the standard 0.5 of McFarland scale [34], which corresponds to a density of 1.5×10^8 bacteria mL^{-1}. We made a first dilution (10^{-1}) of this microbial suspension in sterile water, followed by a second dilution (10^{-2}) using a lactose-broth (LB). Then, 200 μL of the inoculum were deposited in the wells of two ELISA plates, containing 1 mg of MF NC. One of the ELISA plates was stored in the dark, while the other one was exposed to solar radiation during 15 min by using an AM 1.5 filter in a Sun 2000 Class A solar simulator (Abet-technologies, USA). We also used a water filter to avoid heating the sample. Both ELISA plates were incubated in the dark for 48 h at (35 ± 1) °C. After this incubation period, we transferred individual 20 μL aliquots from each well to Petri dishes with Plate Count Agar (PCA), which were also incubated for 48 h at (35 ± 1) °C. The positive control was composed of the inoculum without the MF NC, whereas the pure culture medium was used for the negative control. All antibacterial experiments were performed in triplicate.

3. Results and discussion

We selected PS as a convenient matrix due to its stability and compatibility with the TPP solvent; both of such properties contributed

Table 1
Amounts of TPP and PS employed in the preparation of the MF NCs.

TPP (mL)	PS (%)		
	1	5	10
1	S1	S4	S7
3	S2	S5	S8
5	S3	S6	S9