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Research Paper

Fabrication of folic acid magnetic nanotheranostics: An insight on the formation mechanism, physicochemical properties and stability in simulated physiological media

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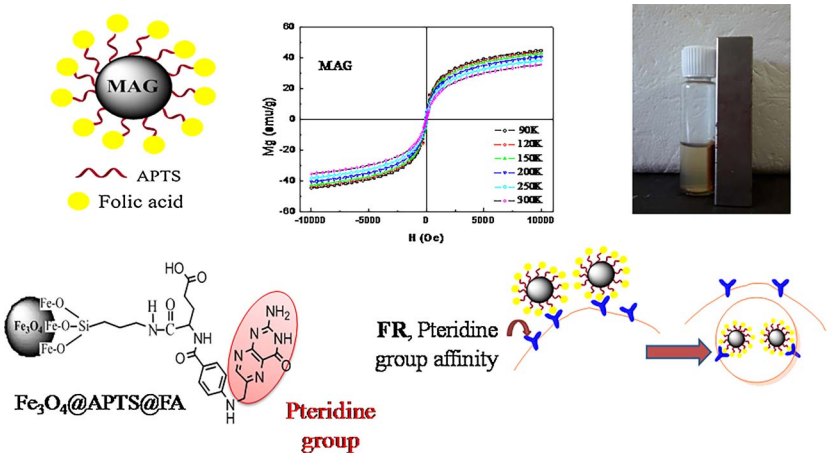
GRAPHICAL ABSTRACT



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ABSTRACT

Nanodevices based on magnetite functionalized with folic acid (FA) with improved properties to be employed as theranostics in various types of cancer are here proposed. Two methodologies for FA incorporation were explored aiming to reach suitable loading efficiency as well as adequate stability of nanosystems in physiological media. To this end, simple adsorption and covalent binding of FA and some experimental conditions derived from both procedures were studied. A thorough physicochemical characterization was performed using all the formulations. The mechanism of the interaction between FA and magnetite nanoparticles (MNPs) was elucidated from characterization results supported by theoretical studies using spin-polarized density functional theory (DFT). Both data coincide in that the selective functional group of FA (pteridine group) remained available after FA binding MNPs. Such studies also demonstrated that any of FA carboxylate groups could be available to potentially link other molecule (i.e therapeutic agents). Besides, other issues that are not normally accomplished in reported articles were included; i.e the stability according to two different criteria: size evolution (expressed as hydrodynamic diameter) as a function of time in aqueous media; and the capacity FA retention in PBS, pH = 7.4. Recovered data indicated that the samples are stable at least 15 days in water and 4 h in buffer without significant modifications of their properties. The feasibility of these formulations to interact with simulated physiological fluid was also assayed. The results revealed that protein corona was formed around all the tested formulations leading to more stable nanodevices in terms of their hydrodynamic sizes and size evolution

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along the time. To complete the theranostic characteristic, Doxorubicin was added to the MNPs@FA by physical adsorption, to provide the therapeutic function. The satisfactory incorporation was verified by FTIR spectroscopy.

1. Introduction

With the advance of nanotechnology, a large variety of nanocarriers has been optimized in terms of their in vivo performance and their ability to reach specific sick regions in the body. In this regards multifunctional nanosystems have emerged providing both inputs: therapeutic and diagnostic in an unique device, actually known as theranostics [1,2]. In this concern, it is possible to combine therapeutic strategies such as nucleic acid delivery, chemotherapy or hyperthermia with one or more imaging functionalities, such as magnetic resonance imaging (MRI), for both, in vitro and in vivo, studies. Moreover, with the implementation of this type of technology it would be possible to obtain information not only of an initial diagnosis, but also theranostics would serve for monitoring the progress of the pathology in real time, giving light on the efficiency of a medical treatment [3,4]. Currently, the most studied pathologies regarding the application of theranostics are oncological diseases because of their social impact and the benefits offered by these novel technologies [5–8]. Magnetic nanoparticles based on iron oxides (MNPs), i.e magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), have been of great interest in several nanomedicine fields. They have been successfully employed in MRI and hyperthermia treatments due to their magnetism (or superparamagnetism), biodegradability and relatively low toxicity [9,10]. In spite of the wide information regarding to biomedical applications of MNPs, their practical implementation has not been concreted yet. In fact, only a few iron oxide based nanosystems are currently available in the market [11] and any of them is devoted to therapeutic non theranostics issues. This inconsistence is, in part, because MNPs may accumulate at reticuloendothelial sites such as the liver, spleen and kidney. In consequence, insufficient uptake at tumor sites would decrease the therapeutic benefits of the administrated drug dose, and non-specific association with healthy tissues would lead to toxic side effects. This limitation prevents drug-loaded nanoparticles from achieving the potential therapeutic effects they might otherwise attain.

A recent strategy to achieve efficient tumor targeting is to conjugate drug carriers with specific ligands able to recognize molecular receptors on the surface of cancer cells. Among different targeting ligands, folic acid (FA), represents an attractive option to modify MNPs because it is a low cost, biocompatible, and non inmunogenic molecule. Moreover, FA is of high importance for the synthesis of new cells since it is necessary for the biosynthesis of DNA bases. Because of this, FA receptors (FR) with high affinity are overexpressed in several types of cancer cells [12]. Nanocarriers conjugated with FA may be targeted to tumor cells and internalized through receptor-mediated endocytosis.

Although abundant works may be found in open literature devoted to the preparation of FA modified Fe_3O_4 MNPs because of the abovementioned issues [13,14], some aspects related to the interaction mechanisms as well as the stability of the prepared nanosystems are missing. It is important to highlight that those aspects are crucial to the in vivo implementation of FA-based systems.

Different pathways have been reported to achieve nanocarriers conjugated FA. From them, the most widely used protocol involves a covalent linkage with, generally, an aminated nanoparticle's surface. For example, Huang et al. designed a nanoplatform for delivery of drugs based on polymers coated SPIONs modified with FA. In such work, the nanoparticles obtained by thermal decomposition of $Fe(acac)_3$, were functionalized directly with FA employed dimethylsulfoxide (DMSO), 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride(EDC) and *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS) in only one step. Polyethilene imine (PEI) was the source of amino groups to conjugate with FA [13]. On the other hand, Yang et al., functionalized nanoparticles of chitosan with FA using the same reagents for carrying 5-aminolaevulinic acid (5-ALA) and to determine its targeting and uptake efficiency in different human colorectal cancer cell lines (HT29 and Caco-2) by folate receptor-mediated endocytosis [14]. Patel et al. prepared core-shell hybrid iron oxide-zinc oxide nanoparticles conjugated folic acid for applications as a photosensitier (PS) in photodynamic therapy. In this case, they first conjugated FA with *N*-hydroxysuccinimide (NHS) and then the product reacted with amino group from the nanocarriers cover with tetraethylorthosilicate (TEOS) and (3-aminopropyl)triethoxysilane (APTS) [15].

The aim of this contribution is to prepare MNPs modified with folic acid with improved properties to be employed as theranostics to the treatment and diagnostic of various types of cancer. To do this, an initial in deep study is presented devoted to find an efficient incorporation procedure for the FA molecules to the MNPs surface. It is important to remark that even when vast information about these methods may be found in open literature, the most of them include a large number of reactants without a clear justification on the role of each one on the nanocarriers structure. Here we intend to simplify the procedure by employing the minimum amount of steps and reactants to link FA on the MNPs surface.

The focus was not only on achieving high levels of loaded FA, but mainly in reaching a stable formulation able to retain the FA as well as their physicochemical properties. The mechanism of MNPs@FA interactions is studied aiming to elucidate if the potentially selective functional groups remained surface exposed. To develop this issue experimental data and computational tools have been combined. The behaviour of MNPs@FA in a media simulating the blood plasma is also presented as an initial stage to assess the viability of these nanosystems. Size, aggregation trend and stability were the analyzed parameters.

Besides, preliminary results regarding to the incorporation of an oncological drug, Doxorubicin, are also presented. On this way, this research may be considered as a necessary prior study to the design of a nanoplataform susceptible of being in vivo assayed.

2. Materials and methods

2.1. Materials

All reagents and solvents were of analytical grade and used without further purification. Ferric chloride hexahydrate (99.99%) and sodium dodecyl sulfate (SDS) was provided by Biopack (Argentina). Ferrous sulphate heptahydrate (99.99%) was provided by Mallinckradt Chemical Works (USA). Sodium hydroxide and acetic acid (AA) (28–29%) were purchased from Cicarelli (Argentina). Absolute ethanol was provided by Quimicor (Argentina). (3-aminopropyl)triethoxysilane (APTS) was provided byAvocado Research chemicals (United Kingdom). *N*,*N*′-diciclohexilcarbodiimida (DCC) was purchased from Fluka (Germany). Folic acid (FA), dimethylsulfoxid (DMSO) and Doxorubicin hydrochloride (Doxo) were purchased from Sigma Aldrich (Germany). Bovine serum albumin (BSA) was provided by Winer Lab, and Ringer Solution (RS) was obtained from ROUX-OCEFA S.A (Argentina). Distilled water with conductivity about 5.00 μS was employed.

2.2. Methods

2.2.1. Synthesis of MNPs

Magnetite nanoparticles (MNPs) were synthesized by co-