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Research paper

Tetracycline-ferrite nanocomposites formed via high-energy ball milling and the influence of milling conditions

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ABSTRACT

High-energy ball milling was used to mediate the formation of nanocomposites containing tetracycline and magnetic nanoparticles. Tetracycline-HCl was ball milled for 1, 3, 5, 15, and 30 h under argon or air atmosphere with preformed $Mg_{0.5}Zn_{0.5}Fe_2O_4$ nanoferrites prepared by glycolthermal method. The structural, thermal, and magnetic properties of these novel materials and the effect of milling atmosphere on composition, crystallinity and cation distribution were then characterized by ICP-OES, DSC/TGA, XRPD, ATR-IR, UV–Vis and Mössbauer spectroscopy. Tetracycline underwent rapid and consecutive metal coordination events in the milling process to yield complexes characterized by bathochromic shifts in its electronic spectra and suppression of electronic absorbance at 365 nm. Changes in stretching vibrations due to the A-ring carbonyl (1616 cm^{-1}), amide II nitrogen (1602 cm^{-1}), and C—O bond (1039 cm^{-1}) indicate Mg-type interactions imposed on the metals. Exothermic oxidation of the drug at 235 °C disappeared after 5 h milling with the nanoferrites, and the composites formed remained thermostable up to 500 °C. Tetracycline-nanoferrites (Tet-NF) are magnetic-ordered materials with a well-defined spinel-type structure. Analysis of the Mössbauer data suggests that the milling time and atmosphere have significant influence on cation distributions in Tet-NF composites.

1. Introduction

Tetracycline (Tet) is an antimicrobial agent, which exhibits activity against a broad range of Gram-positive and Gram-negative bacteria, including obligatory anaerobes. It has been used widely in human and veterinary medicine for over 80 years and remains the standard of care for the treatment for common infections of the upper respiratory and upper gastrointestinal tract. Because its interaction is reversible, tetracycline is generally regarded as bacteriostatic and resistance to the drug is now widespread [1,2]. Tetracycline has a planar structure consisting of four fused rings with hydrophilic groups on one face and hydrophobic groups on the other. The drug possesses four dissociable hydrogen atoms and is therefore an efficient chelating agent that readily coordinates metal ions present in aqueous solution or on solid surfaces [3]. In serum, the drug is transported as a calcium complex in contrast to its intracellular preference toward magnesium complexes which play an important role in its bioactivity and ribosomal binding. Because of their pharmacological importance, the interaction of tetracycline with numerous metal ions has been extensively studied [4–6]. The tetracycline structure is particularly complex, presenting several potential metal-binding sites. Consequently, it may form complexes in a wide range of metal-to-ligand stoichiometries, with different conformations or charged states as well. Although the presence of many metal coordination sites make assignment of the precise binding structure difficult, it has been established that two types of tetracycline-metal bonding commonly take place, that is, calcium-type binding and magnesium-type binding [6]. Coordination of transition metals to this ligand has also attracted attention [7,8], but only a few of these studies concern the interaction of iron oxides with the drug [3,9].

The search for antimicrobial agents not molecularly-recognized by resistance mechanisms is an important strategy in the fight against emerging infectious diseases. Here, an unlike approach at first glance might be the complexation of antibiotics with nanoferrous materials. These complexes may make it possible to modify the biopharmaceutical properties of existing antibiotic drugs like tetracycline into unique, targeting, and effective diagnostic or therapeutic agents. Transition metal ferrites represent an important class of innovative materials with physical properties, which render them suitable for many applications in the next generation of sensors, catalysts, and magnetic storage devices. Although ferrites have been traditionally prepared in bulk, microminiaturization of magnetic and electronic devices has called for advanced materials

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with new forms and dimensions, such as nanocomposites or nanoferrites [10,11]. Nanoferrous alloys are currently being investigated for unique applications in medical imaging, hyperthermia [12], or drug targeting [9,13].

The properties of nanoferrites depend on an extrinsic spinel structure, which describes a cubic close-packed arrangement of oxygen atoms with Fe^{3+} and other metal ions at specific crystallographic sites. Two types of arrangements are present for cationic occupancy and are referred to as A (tetrahedral) and B (octahedral) sites, which may be occupied by different atoms in the system [10,14]. The cations are located at the centers of the tetrahedral and octahedral arrangement of oxygen ions in the lattice. Magnetic properties of "spinel-structured" ferrites will be influenced by the distribution of the metal ions among the available A and B sites. Therefore, dilution with nonmagnetic ions in nanoferrites is expected to affect their magnetic properties. Extensive work has been reported regarding the substitution of impurities in the different ferrite systems in order to improve their basic properties [10,11,14]. However, incorporation of bioorganic molecules into spinel structures, which has not been widely reported, may also be used to modulate its basic properties or its distribution in living organisms. An investigation of antibiotic-nanoferrous inclusion complexes was reported recently [3] with results that confirm magnetic tetracycline microspheres as a potential approach to explore or combat drug resistance infections. Our current report describes the formation of a unique antibiotic-nanoferrous material, its structural, electronic, and thermochemical properties. Ongoing investigations in our group will also determine the pharmacologic and toxicologic activity of these novel materials as potential drug targeting agents.

2. Materials and methods

2.1. Materials

Tetracycline hydrochloride was purchased from Frankel Chemicals (South Africa). All starting materials anhydrous $FeCl_3$, $MgCl_2$ and $ZnCl_2$ with >99% purity were used as supplied (Sigma–Aldrich). Purified reagent water (Millipore Corp.) was used throughout the preparation of the composites. Argon gas with 99.999% purity and air were used to provide the milling atmospheres in airtight grinding jars. All other chemicals used were analytical or HPLC grade.

2.2. Preparation of the tetracycline-nanoferrites (Tet-NF)

Tet-NF compounds were reacted with single-phase high purity $Mg_{0.5}Zn_{0.5}Fe_2O_4$ nanoferrites via mechanical milling using a Retsch planetary ball mill (model PM 400) under argon or air atmosphere. The mill was operated at 200 rpm with a ball-to-sample mass ratio of 20:1. The preparation schedule of the compounds is given in Fig. 1. Before milling, the single-phase ferrites were synthesized by the glycolthermal method [15] using high purity metal chlorides and ammonium nitrate as starting materials. Stoichiometric amounts of chlorides were mixed in deionized water for about 0.5 h. Ammonium nitrate solution was then slowly added to the chloride mixture under rapid stirring until a pH of about 9 was achieved. The precipitate was then washed several times with deionized water until no detectable chloride ions were indicted as confirmed using a standard solution of $AgNO_3$. The washing process was performed in a centrifuge by spin/decanting. Fifty milliliter (50 mL) aliquots of deionized water were used to remove impurities until the decanted solution tested negative for chlorides. The clean wet precursors were thereafter dispersed in 300 mL of ethylene glycol under rapid stirring; then placed in a

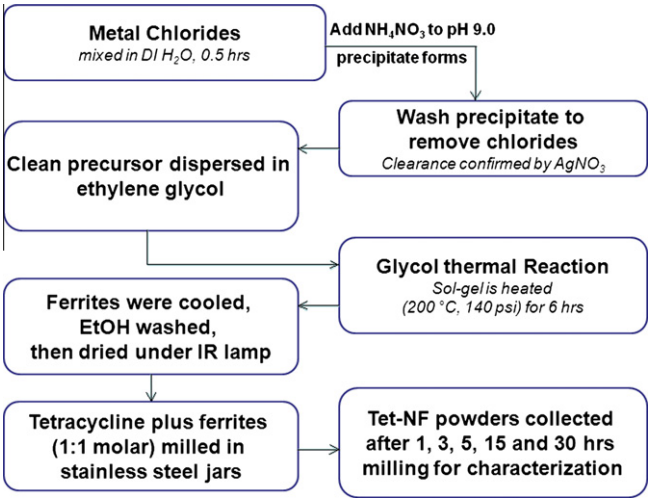

Fig. 1. Schematic illustration of the preparation of the tetracycline-ferrite nanocomposites (Tet-NF). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

500 mL pressure vessel (model PARR 4843). The pressure vessel was heated to 200 °C, and the pressure monitored to 140 psi and held for 6 h. The cooled product was again washed with deionized water and finally with ethanol. The recovered paste was dried under an infrared lamp and homogenized using an agate mortar and pestle. A 1:1 molar ratio of this nanoferrite product and tetracycline-HCL was then placed in separate hardened stainless steel milling jars and milled with stainless steel balls under argon or air atmospheres for 1, 3, 5, 15, and 30 h. Intermediate samples were collected from each jar to characterize the Tet-NF composites formed.

2.3. X-ray powder diffraction (XRPD)

X-ray powder diffraction patterns were recorded using a monochromatic beam of Co Kα radiation (λ = 1.7903 Å) on a Phillips X Pert diffractometer (PANalytical B.V. Twentepoort, Netherlands). The positions of the X-ray tube and detector were checked by using zero point definition of the 2θ scale. The measurements were performed in symmetrical reflection mode at 40 kV and 40 mA. The powder samples (Tetracycline-HCL, nanoferrite product, and milled Tet-NF) were each scanned in the angular range of 2θ = 20–90° with a step size of 0.01° and a count time of 1 s per step.

2.4. Infrared (IR) spectroscopy

Infrared spectra of tetracycline-HCl, nanoferrite product, and milled Tet-NF were recorded on an Alpha Platinum FTIR spectrophotometer (Bruker, Bryanston, ZA). The instrument was configured with a Pike ATR sample cell, including a diamond crystal with a scanning depth up to 2 μm. Pelletized sample powders were applied to the surface of the crystal then locked in place with a "clutch-type" lever before measuring transmittance. Each of the spectra was collected in the range 4000–400 cm^{-1} at 2 cm^{-1} resolution.

2.5. Differential Scanning Calorimetry (DSC)/Thermogravimetric Analysis (TGA)

DSC/TGA thermograms of tetracycline-HCl, nanoferrite product, and milled Tet-NF composites were obtained using a TA Instruments SDT-Q600 thermal analyzer (AMS Laboratory Technologies