Transition metal-substituted cobalt ferrite nanoparticles for biomedical applications

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ABSTRACT

Transition metals of copper, zinc, chromium and nickel were substituted into cobalt ferrite nanoparticles via a sol–gel route using citric acid as a chelating agent. The microstructure and elemental composition were characterized using scanning electron microscopy combined with energy-dispersive X-ray spectroscopy. Phase analysis of transition metal-substituted cobalt ferrite nanoparticles was performed via X-ray diffraction. Surface wettability was measured using the water contact angle technique. The surface roughness of all nanoparticles was measured using profilometry. Moreover, thermogravimetric analysis and differential scanning calorimetry were performed to determine the temperature at which the decomposition and oxidation of the chelating agents took place. Results indicated that the substitution of transition metals influences strongly the microstructure, crystal structure and antibacterial property of the cobalt ferrite nanoparticles.

1. Introduction

Metal-oxide nanoparticles are of interest because of their unique optical, electronic and magnetic properties. Cobalt ferrite ($CoFe_2O_4$) nanoparticles have high permeability, good saturation magnetization and no preferred direction of magnetization. Moreover, they exhibit a high coercivity of more than 5 kOe; a moderate saturation magnetisation of about 80 emu g^{-1}; excellent chemical stability and mechanical hardness; a large magneto-optic effect; a high Curie temperature; and high electromagnetic performance [1,2]. Therefore, cobalt ferrite is a promising candidate material for high-density magnetic recording [3], ferrofluids technology [4], biomedical drug delivery [5], magnetic resonance imaging [6], biocompatible magnetic nanoparticles for cancer treatment [7] and magneto-optical devices [8]. The substitution of the Co^{2+} ion that is present in cobalt ferrite with, for example, Zn^{2+}, Ni^{2+}, Cr^{2+} and Cu^{2+} allows variations in their properties that can be tuned to specific applications.

Vaidyanathan and Sendhilnathan [9] demonstrated that, when Co^{2+} was substituted with Zn^{2+} in $CoFe_2O_4$, the new $Co_xZn_{(1-x)}Fe_2O_4$ nanoparticles exhibited enhanced properties – e.g. excellent chemical stability, high corrosion resistivity, magneto-crystalline anisotropy, magnetostriction and magneto-optical characteristics. Singhal et al. [10] studied nickel-substituted cobalt ferrite nanoparticles produced by an aerosol route. The results showed that the lattice parameter of nanoparticles decreased linearly with an increase in nickel concentration, which could be attributed to the

smaller ionic radius of Ni^{2+} compared to Co^{2+}. Moreover, the increase in nickel concentration decreased the saturation magnetization because the relatively high orbital contribution of the Ni^{2+} to the magnetic moment of Co^{2+} induces large anisotropy.

The magnetic nanoparticles need modification to increase the biocompatibility and bacterial activity before implementation for drug delivery applications. For example, coating a thin silver film onto Fe_3O_4 nanoparticles can improve bacterial activity, as well as benefiting the paramagnetic properties of the nanostructures so that they can be recovered and recycled from the site of action by means of an external magnetic field [11]. Moreover, oleic acid was used as a surfactant coating for Fe_3O_4 nanoparticles, followed by an adsorption coating with four different antibiotics (cephalosporins) [12]. The bacterial activity was tested on two organisms: *Escherichia coli* and *Staphylococcus aureus.* It was observed that, for the same time interval, the inhibition zone diameters for cephalosporins were greater than those for the cephalosporin nanofluid. The nanofluid only acts as a carrier for the antibiotic. Moreover, the small size of the magnetic nanoparticles allows delivery of an antibiotic when targeting certain organs such as the brain and kidney.

On the other hand, most of the transition metal-substituted cobalt ferrite nanoparticles have been studied only for their magnetic property, with few having been applied for biomedical applications. The special properties of magnetic nanoparticles required for biomedical applications demand precise control of particle size, shape, dispersion and conditions that influence these properties. In principle, it is necessary to stabilize the magnetic nanoparticle dispersion in the aqueous environment. Thus, coating the magnetic nanoparticles with a polymer shell, including organic (polyethylene glycol, dextran, chitosan, polyethyleneimine and phospholipids) or

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inorganic (silica) materials, is usually the first step that leads to highly dispersed and high-quality nanoparticles with good biocompatibility [13].

Techniques reported for synthesizing cobalt ferrite nanoparticles include solid-state reaction [14], microemulsion [15], combustion [16], the redox process [17], chemical co-precipitation [18], the hydrothermal method [19] and microwave synthesis [20]. However, sol–gel techniques offer enhanced control over homogeneity, elemental composition and powder morphology. In addition, uniform nanosized metal clusters can be achieved, which are crucial for enhancing the properties of the nanoparticles. These advantages favour the sol–gel route over other conventional preparation methods of ceramic oxide composites [21].

Therefore, the objective of this study is to develop novel multifunctional magnetic iron-based nanoparticles that also exhibit antibacterial properties to fulfil the requirements of a drug delivery system so that the antibiotic concentration could be minimized. For this purpose, we have synthesized transition metal-substituted cobalt ferrite nanoparticles ($Co_{0.5}X_{0.5}Fe_2O_4$ with X = Cu, Zn, Mn and Ni) by the sol–gel process using citric acid (CA) as the chelating agent. We have investigated the effect of substitution of these transition metals on surface morphology, size distribution and antibacterial properties of the synthesized transition metal-substituted cobalt ferrite nanoparticles.

2. Methods and procedures

2.1. Sample preparation

The chelating agent, CA gel, was prepared by dissolving CA powders in distilled water (5% w/v) at 70 °C. The chelating agent solutions were kept at 70 °C for 5 h or until the solution became clear. Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$), iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$), copper nitrate ($Cu(NO_3)_2 \cdot 6H_2O$), nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$), manganese nitrate ($Mn(NO_3)_2 \cdot 4H_2O$) and zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$) powders with Fe:Co:(Zn, Cu, Ni, Mn) molar ratios of 2:0.5:0.5 were dissolved into the chelating agent solution under magnetic stirring. The sol–gel reaction was continued for 3 h and then the temperature increased to 80 °C for 10 h or until the gel dried into powder form. Finally, all samples were sintered at 800 °C for 4 h and then ground using a mortar and pestle to form a nanoparticle.

2.2. Characterization

Elemental composition analyses of the transition metal-substituted cobalt ferrite nanoparticles were performed using energy-dispersive X-ray spectroscopy (EDX) and phase analyses were carried out using X-ray diffraction (XRD). Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were conducted in air to determine the temperature for the decomposition and oxidation of the chelating agent. Surface wettability, an indicator of the surface interaction of the metal oxide with a given solvent and a measure for biomedical applications, was measured using the water contact angle technique (WCA-FTA200). The surface roughness of all nanoparticles was measured using an AlphaStep-D-120 Stylus Profiler (KLA-Tencor Instruments). The morphology of the nanoparticles was observed using field emission scanning electron microscopy (FESEM; ZEISS SUPRA 40 VP).

The antimicrobial activity of transition metal-substituted cobalt ferrite nanoparticles was tested against the Gram-negative bacterium E. coli and the Gram-positive bacterium S. aureus. Both types of bacterial strain were obtained from the American Type Culture Collection (ATCC), USA. The bacteriological test series were carried out according to the modified ASTM E2180-07 (Standard Test Method for Determining the Activity of Incorporated Antimicrobial

Agent(s) in Polymeric or Hydrophilic Materials). All tests were performed on solid agar plates with different transition metal-substituted cobalt ferrite nanoparticles. E. coli and S. aureus were grown aerobically at 37 °C overnight with shaking (200 rpm) in an ordinary broth medium. The optical density (OD) of the overnight culture (OVN culture) was measured at 600 nm with an ultraviolet spectrometer and diluted with lysogeny broth (LB) to achieve an OD of 0.1. The diluted OVN culture was then seeded into 20 ml of LB (in a 50 ml Falcon tube) and incubated at 37 °C on the shaker for 2 h to obtain an OD of 0.3. At OD = 0.3, the diluted OVN culture and transition metal-substituted cobalt ferrite nanoparticles were further incubated for 24 h and 100 μl of these mixtures were spread on an agar plate using an L-shaped spreader.

The inoculated plates were incubated at 37 °C and the number of colonies on the Petri dishes were counted after 24 h. The colony-forming units were calculated by multiplying the number of colonies by the dilution factor. The survival percentage was used to evaluate the antimicrobial effect of particles, which is defined by the formula [22]:

$$\text{Survival \%} = \frac{\text{Colony number of treated bacteria}}{\text{Colony number of control bacteria}} \times 100 \qquad (1)$$

For the modified Kirby–Bauer method [23], an equal amount of transition metal-substituted cobalt ferrite nanoparticles obtained from CA were coated on filter papers. All samples were placed on the E. coli growth LB agar plate and incubated overnight at 37 °C. The zone of inhibition was measured.

Prior to each bacterial attachment experiment, a fresh bacterial suspension of OD = 0.2 was prepared from E. coli and S. aureus cells grown in LB at room temperature ($\sim$22 °C) for 24 h. All nanoparticles were attached to the glass slides using double sided tape. A 3–5 ml aliquot of bacterial suspension was poured into sterile Petri dishes, where the glass slides (one per Petri dish) were completely immersed and left to incubate for 12 h at room temperature ($\sim$22 °C). After incubation all of the slides were washed with deionized water and left to dry in air. This experimental approach allowed bacterial attachment to be performed under identical conditions for each microscope slide. A FESEM–ZEISS SUPRA 40VP was used to obtain high-resolution images of the bacterial cells. Just before imaging, all slides were gold coated to achieve better conductivity.

3. Results and discussion

3.1. Differential scanning calorimetry and thermogravimetric analyses

DSC and TGA curves of synthesized transition metal-substituted cobalt ferrite nanoparticles ((a) $Co_{0.5}Cu_{0.5}Fe_2O_4$, (b) $Co_{0.5}Zn_{0.5}Fe_2O_4$, (c) $Co_{0.5}Mn_{0.5}Fe_2O_4$ and (d) $Co_{0.5}Ni_{0.5}Fe_2O_4$) obtained from CA as a chelating agent are shown in Fig. 1. The TGA curve exhibits two distinct weight loss steps and the DSC curve presents one exothermic and one endothermic peak over 25–500 °C. The first weight loss step in the range of 25–150 °C, which was accompanied by an endothermic broad peak around 150 °C in the DSC curve, arises due to the loss of residual moisture in the powder. The second weight loss step in the range of 200 °C and the sharp exothermic peak around 200 °C are associated with the combustion of nitrates and the oxidation of carbon from the CA to form carbon dioxide. No weight loss was observed above 300 °C, implying the presence of transition metal-substituted cobalt ferrite nanoparticles in this temperature range.

3.2. FESEM/EDX analyses

SEM micrographs were analysed to investigate the grain structure of the nanoparticles and assist in understanding the