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Entrapment of β-FeO(OH) nanoparticles in human serum albumin: Preparation, characterization and hemocompatibility

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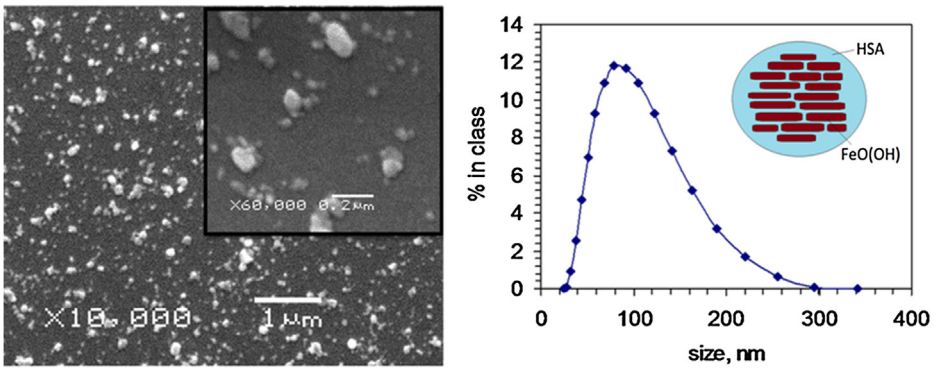
HIGHLIGHTS

- Nanorods of β-FeO(OH) were prepared and characterized.
- The β-FeO(OH) nanorods were entrapped in albumin (HSA).
- The hybrid β-FeO(OH)/HSA nanoparticles were stable in physiological buffer.
- The hybrid nanoparticles did not cause hemolysis in vitro.

GRAPHICAL ABSTRACT



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ABSTRACT

In this article we report the utilization of human serum albumin as a biocompatible stabilizer in preparation of novel hybrid albumin-entrapped ferric oxyhydroxide nanoparticles. For that purpose, positively-charged nanorods of β-FeO(OH) were prepared by controlled hydrolysis of ferric chloride in aqueous medium and entrapped in albumin by desolvation and cross-linking with glutaraldehyde. The obtained hybrid nanoparticles were of average size 84 nm, negatively charged and stable in physiological buffer. Hemocompatibility studies showed that these nanoparticles did not cause hemolysis (tested in vitro), neither had any effects on morphology of blood formed elements, osmotic fragility of erythrocytes and clotting time of blood plasma, which are important prerequisites for their use in biomedical studies.

1. Introduction

Nanocolloids of ferric oxyhydroxide, FeO(OH), find clinical application in treatment of severe iron-deficiency conditions [1–3] and have been proposed as vaccine adjuvants [4–7]. Nanocolloids used in treatment of iron deficiency are usually intended to be administered in relatively large amounts (usually up to 100 mg of iron per injection) via i.v. or i.m. injection, although the i.m. route

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of administration was associated with development of sarcomas [8]. The fate of intravenously injected colloidal ferric hydroxide has been previously studied in rats [9] and humans [10] and showed accumulation in liver, spleen, bone and blood. It is mainly phagocytosed by cells of reticuloendothelial system, including tissue macrophages (in spleen, liver, lymph nodes, etc.) and endothelial cells [11,12]. After dissolution of ferric hydroxide in the relatively acidic microenvironment inside endosomes, ferric ions are transported out of the cell via the ferroportin transporter protein and then become associated with transferrin, further sharing common pathways with normal iron metabolism [11], although transformation into ferritin and hemosiderin has been reported [12]. Normal physiological recycling of hemoglobin iron is performed by phagocytosis of old erythrocytes (that have exposed phosphatidylserine on their surface) mainly by splenic resident macrophages, from where it is exported and associated with transferrin in blood plasma, and transported to erythroblasts in bone marrow, where it is included in synthesis of novel hemoglobin molecules [13–16].

Parenterally administered colloidal particles are known to form adsorption layer of proteins on their surface, known as protein corona, which further largely determines nanoparticle fate and their effect on the organism [17–19]. When non-specific adsorption of immunoglobulin molecules, complement factors and other opsonins takes place, it makes these particles "visible" for macrophages (that have various receptors for opsonin molecules on their plasma membrane, such as Fc-receptors for antibodies) and become phagocytosed [17]. However, the "visibility" of nanoparticles for macrophages and their interaction with complement cascade raises concerns over the effects of these particles on the immune system and inflammatory response [20]. Nanoparticles are known to be able to activate the complement cascade by the classical (via relatively non-specific adsorption of immunoglobulins and activation of C1q) and alternative (via contact activation and adsorption of C3b) pathways [21]. Also, macrophages that have ingested "foreign" nanomaterials are known to secrete cytokines and inflammatory mediators [22]. Therefore, parenterally administered nanocolloids can activate directly the complement cascade and can indirectly cause release of pro-inflammatory cytokines (when phagocytosed by macrophages) that may lead to systemic inflammatory response, associated with pseudo-allergic reactions, fever, muscle pain, headache, gastro-intestinal disorders, neurologic abnormalities, general hypersensitivity and/or anaphylactic reactions [23]. All these have been observed as adverse drug reactions of ferric hydroxide parenteral formulations that are currently in clinical use [24].

Albumin accounts for 55% of blood proteins (concentration in blood plasma being 35–50 mg/ml), and its major functions include maintaining the osmotic pressure of plasma and transportation of lipids and hormones [25]. Most drugs are transported in the blood stream being associated with albumin soon after their administration and albumin has been used in the design of various drug delivery systems for parenteral administration [26]. Albumin can form nanospheres upon desolvation from aqueous solutions and such nanospheres have been used to encapsulate various small molecule drugs [27] and gold nanoparticles [28]. Surface modification with serum albumin has been known to increase stability of organic [29,30] and inorganic [31,32] nanocolloids. Previous studies have also demonstrated that rapid phagocytosis of nanoparticles by macrophages can be diminished by their association with serum albumin [33].

Our aim in this study was to construct novel hybrid inorganic-biopolymer nanoparticles intended for biomedical applications (such as treatment of iron deficiency) by entrapment of ferric oxyhydroxide nanoparticles in human serum albumin. Such hybrids are expected to be more stable in physiological fluids than bare oxyhydroxide nanocolloids and the biocompatible albumin is expected to provide hemocompatibility required for biomedical studies. For that purpose, we synthesized ferric oxyhydroxide nanoparticles that were entrapped into albumin by desolvation and cross-linking with glutaraldehyde. The obtained hybrid nanocolloids were characterized for morphology, particle size distribution, zeta potential, chemical composition and colloidal stability. Hemocompatibility studies of the obtained hybrids included tests for hemolysis, effects on morphology and osmotic fragility of erythrocytes, and effect on the clotting time of blood plasma after recalcification, which are important prerequisites in biomedical studies of nanocolloids intended for parenteral administration.

2. Materials and methods

2.1. Materials and reagents

Iron(III) chloride hexahydrate (p.a., min 99%, Reag. Ph. Eur.), used for the preparation of iron(III) oxyhydroxide nanoparticles, was from Sigma-Aldrich (Germany). Ammonia solution (p.a., min 25%; density 0.9 g/ml) was from Merck (Germany). Albumin from human serum (lyophilized powder, >96%), absolute ethanol (p.a.) and glutaraldehyde solution (25% in water; p.a.) were from Sigma-Aldrich (USA). Ammonium iron(III) sulfate dodecahydrate (p.a., >99%), used as a standard for determination of iron(III), was from Merck (Germany). All other reagents were of analytical grade. Distilled water was used in all experiments.

2.2. Synthesis of FeO(OH) nanoparticles

2.2.1. Preparation of positively-charged FeO(OH) nanoparticles

Nanoparticles of FeO(OH) were synthesized by controlled hydrolysis of iron(III) chloride in alkaline medium at room temperature (20 °C). For that purpose, concentrated ammonia solution (14–17 M; 4–6 ml) was dropwise added upon intensive stirring to aqueous solution of iron(III) chloride (3 M; 10 ml) until the formation of a small amount of insoluble precipitate; the precipitate was then dispersed by addition of few drops of 0.3 M $FeCl_3$. The obtained dark colored dispersion was sonicated for 10 min and filtered through a porous glass filter (G1). Immediately after preparation, the FeO(OH) nanoparticle dispersion was dialyzed against distilled water (300 ml) for at least 10 h (distilled water was changed each hour) using dialysis tubing membranes with pore size 10–12 kDa (Sigma). (Note: non-dialyzed dispersions were unstable in time due to ripening processes leading to formation of large particles and precipitates.) An aliquot of the dialyzed dispersion was properly diluted for measurement of UV–vis spectra and determination of iron(III) content (see Section 2.4.2). Dispersions were stable upon storage at room temperature for at least few months.

2.2.2. Preparation of negatively charged FeO(OH) nanoparticles

An aliquot of 0.25 ml of the dispersion of positively charged FeO(OH) nanoparticles prepared in Section 2.2.1 (with Fe content ~30 mg/ml) was diluted in distilled water (10 ml) and to this dispersion 10 × PBS (1 ml) was added upon intensive stirring. At this stage, a fine precipitate rapidly formed and then rapidly dissolved. In next few minutes brown precipitate formed which was separated by centrifugation (400*g*; 5 min) and redispersed by sonication in 10 mM NaCl (12 ml). This dispersion was then centrifuged again (18,600*g*; 10 min) and the sediment dispersed by sonication (10 min) in distilled water to obtain an optically clear dispersion of total volume 1.0 ml.

2.3. Preparation of FeO(OH)/albumin nanoparticles

Albumin (10 mg) was dissolved in distilled water (0.5 ml), mixed with freshly prepared and sonicated (for 5 min) dispersion of