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The formation of magnetic carboxymethyl-dextrane-coated iron-oxide nanoparticles using precipitation from an aqueous solution

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HIGHLIGHTS

- The carboxymethyl-dextrane coated iron-oxide nanoparticles were synthesized.
- The carboxymethyl-dextrane significantly modifies formation of the spinel nanoparticles.
- The spinel nanoparticles are formed inside the amorphous matrix.
- At approximately 40 °C the matrix decomposes into the suspension of carboxymethyl-dextrane-coated iron-oxide nanoparticles.

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ABSTRACT

The formation of spinel iron-oxide nanoparticles during the co-precipitation of Fe^{3+}/Fe^{2+} ions from an aqueous solution in the presence of carboxymethyldextrane (CMD) was studied. To follow the formation of the nanoparticles, a mixture of the Fe ions, CMD and ammonia was heated to different temperatures, while the samples were taken, quenched in liquid nitrogen, freeze-dried and characterized using transmission electron microscopy (TEM), X-ray diffractometry (XRD) and magnetometry. The CMD plays a role in the reactions of the Fe ions' precipitation by partially immobilizing the Fe^{3+} ions into a complex. At room temperature, the amorphous material is precipitated. Then, above approximately 30 °C, the spinel nanoparticles form inside the amorphous matrix, and at approximately 40 °C the matrix decomposes into the suspension of carboxymethyl-dextrane-coated iron-oxide nanoparticles. The CMD bonded to the nanoparticles' surfaces hinders the mass transport and thus prevents their growth.

1. Introduction

Magnetic nanoparticles have been widely studied for different applications in technology and biomedicine, mainly in relation to their ability to be manipulated by an external magnetic field. In biomedicine, the magnetic nanoparticles can be used both in vitro and in vivo, for the detection, separation and sorting of biomolecules and cells, as magnetic-resonance-imaging (MRI) contrast agents, for magnetofection, and for therapeutic applications like magnetic-fluid hyperthermia and magnetic drug delivery. Of the many different magnetic materials, the magnetic iron oxides, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), are by far the most studied, mainly because they are considered to be nontoxic. Of particular interest are nanoparticles with sizes below 20 nm, where they are in the superparamagnetic state. Such superparamagnetic iron oxide nanoparticles (also known as SPIONs) were also approved by the US Food and Drug Administration (FDA) for in-vivo applications [1].

In a majority of applications the nanoparticles are applied in the form of stable colloidal aqueous suspensions, also called ferrofluids. However, to prevent agglomeration in the aqueous medium, the nanoparticles have to be coated with an organic shell, which provides steric or electrosteric repulsive forces that prevent the nanoparticles from agglomerating. For in-vivo applications, this organic shell should be biocompatible and should enable long blood circulation times. It is also beneficial if the organic shell provides functional groups that enable covalent bonding of the different molecules needed for a specific application (fluorophores, therapeutic agents, targeting agents, etc.) onto the nanoparticles. Of the many different organic molecules that can be used for the stabilization of nanoparticle suspensions, different hydrophilic polymers, such as polyethylene glycol (PEG), polypeptides, and

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polysaccharides, are the most frequently used. Polysaccharide dextran, and especially its derivative carboxymethyl dextran (CMD), have been widely used for the stabilization of aqueous ferrofluids applied in biomedicine. Compared to pure dextran, CMD has some advantages. Additional carboxyl groups bonded to alpha-D-glucopyranosyl monomer units enable better attachment of the organic shell to the SPIONs' surfaces. The free carboxyl groups on the nanoparticle surface can also be used as anchoring points for the covalent bonding of additional molecules needed in a specific application [2]. The CMD-coated SPIONs are already being used as a drug for the treatment of iron anemias [3,4] and as MRI contrast agents [2,5–9]. They were also tested for cell separation [10] and as mediators for magnetic hyperthermia [11–14].

SPIONs can be synthesized by many different methods, including the co-precipitation of Fe^{2+} and Fe^{3+} ions from aqueous solutions using the appropriate base [6,15,16], thermal decomposition [15,17], precipitation in microemulsions [15,18], hydrothermal synthesis [19], and microwave synthesis [20], to mention only the most frequently exploited. By far the most common method for the synthesis of SPIONs is co-precipitation from aqueous solutions. To prepare stable ferrofluids, the CMD is adsorbed onto the pre-synthesized nanoparticles [2,9,12,14,21,22], or the nanoparticles are precipitated in the presence of the CMD [4,6,23]. The adsorption of CMD onto the iron-oxide nanoparticles is attributed to the coordination of the carboxyl and hydroxyl groups of the CMD molecules with iron centres and not just to simple electrostatic interactions [2]. The CMD can also be covalently bonded to the pre-synthesized nanoparticles using reactions between the carboxyl groups of the CMD and the amino groups grafted onto the surfaces of the nanoparticles using carbodiimide chemistry in water [12,24]. Alternatively, the nanoparticles can be coated with dextran, which is subsequently methylcarboxylated using a reaction with chloroacetic acid to form CMD [8].

Although the synthesis of the coated SPIONs with their precipitation of the Fe^{3+}/Fe^{2+} ions from an aqueous solution in the presence of CMD has been known for a long time and has also been used in industry, controlling its properties remains a challenge. One of the reasons for the uncontrollable and inconsistent results of the synthesis procedure is the lack of understanding of the involved mechanisms and kinetics. The presence of CMD significantly changes the mechanisms and kinetics of the iron-oxide nanoparticles' formation. In this work, we studied the formation mechanisms of SPIONs during precipitation in the presence of CMD using transmission electron microscopy (TEM), X-ray diffractometry (XRD) and magnetometry.

2. Experimental

2.1. Materials

Iron (III) chloride hexahydrate (ACS) and iron (II) chloride (Regent grade, 99%) were purchased from Alfa Aesar. The exact content of Fe in the salts was measured using inductively coupled plasma mass spectroscopy (ICP/MS, Perkin–Elmer Sciex ELAN DRC-e). The NH_4OH (aq) (Fluka, p.a., 25%), and HCl (p.a., Riedl-de-Haën) were used as received. The CMD was the product of Lek Pharmaceuticals d.d., synthesised from Dextran 10 with an apparent average molar mass of 12 kDa, corresponding to a polymer of 62 alpha-D-glucopyranosyl monomer units that are carboxymethylated (-CH_2COOH groups) by 27%.

2.2. Synthesis

The CMD-coated nanoparticles (NP-CMD) were synthesized using the co-precipitation of Fe^{3+}/Fe^{2+} ions from an aqueous solution in the presence of CMD. A total of 3.80 mmol of $FeCl_3 \cdot 6H_2O$, 1.90 mmol $FeCl_2$ (anhydrous), and 1.04 g of CMD were dissolved in 50 mL of water that was acidified with HCl in a three-neck round-bottomed flask equipped with a condenser and purged with Ar for 1 h. The pH of the solution was below 1. Exactly 10 mL of concentrated aqueous ammonia (25%), previously purged with Ar, was quickly added to the solution to set the pH value to 10.5. Then, the mixture was heated at a rate of 2 K/min to 80 °C under a blanket of Ar gas. After 2 h at the final temperature, the mixture was aerated by bubbling air and further maintained at the temperature for another 3 h. The product was cleaned of dissolved ions using ultrafiltration on a membrane with a MW cut-off index of 10,000 Da. The final stable suspension of the CMD-coated iron-oxide nanoparticles was marked as NP-CMD.

For comparison, nanoparticles without the addition of CMD were synthesized using the same procedure (sample NP) and by co-precipitation at room temperature (the heating step was omitted) (sample NP-25).

In the presence of the CMD the nanoparticles NP-CMD are formed in a complex, sluggish process at elevated temperature. To study their formation mechanism, the reaction mixture was heated to different temperatures and then maintained at a constant temperature for 24 h, while the samples were taken at different times after reaching the constant temperature, quenched in liquid nitrogen and stored in a refrigerator at below − 24 °C. Finally, the frozen samples were freeze-dried (lyophilizer LIO-5P, Kambič Laboratory Equipment). Apart from the fast cooling, submerging in liquid nitrogen provides an inert atmosphere, preventing changes to the samples. The samples were marked as “NP-CMD-xx-yy”, where “xx” and “yy” denote the temperature and time of the sample extraction, respectively.

Without CMD, the nanoparticles form already at room temperature. For comparison, samples were therefore also taken for various times after the precipitation of the iron ions without CMD at the room temperature. The samples are denoted as “NP-25-yy”, where “yy” denotes the time of the sample extraction.

2.3. Characterization

The content of inorganic phase (iron oxide) in the samples was determined using simple thermogravimetry. The hydrodynamic size distributions of the particles in the suspensions were measured using a dynamic light scattering (DLS) Fritsch Analysette 12 DynaSizer. The design of the apparatus enables measurements in a thin film of the suspension, which significantly reduces the probability of multiple scattering of the detected light and thus enables measurements in relatively concentrated, as-prepared suspensions.

The freeze-dried samples taken at different stages of the synthesis procedure were characterized using XRD, TEM and magnetometry. The XRD spectra were taken using a Siemens D5000 diffractometer. The average nanoparticle size (the mean size of the coherently scattering crystal region) was obtained from refinements of the XRD spectra ($2\theta = 20°–60°$) using the Debye-Scherrer method [25]. The X-ray diffraction line-profile was fitted using the crystallographic program Topas2R 2000 from Bruker AXS. The fitting of the line-profile was based on a convolution approach (Pawley method) [26] in which the line shape is composed of the Cu-Kα emission profile, the dimensions of the diffractometer and the physical variables of the sample [27]. For the TEM investigations the dried samples were re-dispersed in water. A drop of the suspension was dried on a copper-grid-supported, transparent, carbon foil. The transmission electron microscopy (TEM) in combination with energy-dispersive x-ray spectroscopy (EDXS) was performed using a field-emission electron-source TEM JEOL 2010F equipped with an EDX spectrometer (EDXS-ISIS300; Oxford Instruments)