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Synthesis and characterization of superparamagnetic nanoparticles coated with carboxymethyl starch (CMS) for magnetic resonance imaging technique

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ABSTRACT

Magnetic nanoparticles have been proposed for use as biomedical purposes to a large extent for several years. The development of techniques that could selectively deliver drug molecules to the diseased site, without a concurrent increase in its level in healthy tissues, is currently one of the most active areas of cancer research. The conjugate carboxymethyl starch (CMS)/SPIO nanoparticles were prepared by chemical reaction. Several parameters including the drug/polymer ratios in range of 1:14 were examined to optimize formulation. The size distribution and morphology of nanoparticles and *in vitro* release profile in phosphate buffer medium (pH 7.4) during 12 h were then investigated. The magnetic NPs prepared in this study were spherical with a relatively mono-dispersed size distribution. The conjugate carboxymethyl starch (CMS)/SPIO nanoparticles were exhaustively studied as controlled-release systems for parenteral administration of a model drug 5-aminosalicyclic acid (mesalamine) and analyzed using various release kinetic studies.

1. Introduction

The application of small particles in *in vitro* diagnostics has been practiced for nearly 40 years. This is due to a number of beneficial factors including a large surface area to volume ratio, and the possibility of ubiquitous tissue accessibility (Athawale & Rathi, 1997). In the last decade increased investigations and developments were observed in the field of nanosized magnetic particles, term nanoparticles being used to cover particulate systems that are less than 1 μm in size, and normally below 500 nm. Nanoparticles that posses magnetic properties offer exciting new opportunities including improving the quality of magnetic resonance imaging (MRI), hyperthermic treatment for malignant cells, site-specific drug delivery and also the recent research interest of manipulating cell membranes, each of which will be addressed in this paper (Kresse, Pfefferer, & Lawaczek, 2000; Saboktakin, Maharramov, & Ramazanov, 2007a). Iron oxide magnetic nanoparticles tend to be either para magnetic or superparamagnetic, with particles approximately 20 nm being classed as the latter (Honghua & Tiejing, 2005). In most cases superparamagnetic particles (usually Fe_2O_3 and Fe_3O_4) are of interest for *in vivo* applications, as they do not retain any magnetism after removal of the magnetic field (Ratner, 1989). This is important as large domain magnetic and paramagnetic materials aggregate after exposure to a magnetic field. One major hurdle that underlies the use of nanoparticle therapy is the problem of getting the particular site in the body (Saboktakin, Maharramov, & Ramazanov, 2007b). A potential benefit of using magnetic nanoparticles is the use of localized magnetic field gradients to attract the particles to a chosen site, to hold them there until the therapy is complete and then to remove them (David et al., 2001; Hildebrandt et al., 2007). This involved some fairly advanced design of systems for producting these fields. Additionally, such equipment should ideally contain other molecules to show that the particles have been actually located in the appropriate region of the body. Imaging of soft tissue structure of musculoskeletal system has become the domain of MRI due to its superiority over other imaging techniques (Saboktakin, Maharramov, & Ramazanov, 2007c). The technique measures changes in the magnetization of hydrogen protons in water molecules sitting in a magnetic field after a pulse of ratio frequencies has hit them. Protons from different tissues react differently, giving a picture of anatomical structures
(Berry & Curtis, 2003; Tang, Alvarez, & Yang, 2003). These images can be enhanced adding "contrast agents" which sharpen the contrast by affecting the behavior of protons in their proximity. In standard clinical MRI scans contrast agents travel through the bloodstream and tissues, increasing contrast wherever they go (Raghavendra et al., 2003). Although the more commonly used MR contrast media are gadolinium (Gd) chelates, these tend to be nonspecific with rapid accumulation in the liver, thus they only allow a short time imaging window. Colloidal iron oxides therefore play an important role as MRI contrast agents, as superparamagnetic iron oxide particles were the first liver-specific contrast agents used (Simberg et al., 2007). It has been known for many years that the

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inclusion of magnetic particles within tissue enables a very large signal to be obtained from a MRI scanner. To date a wide variety of particles have been produced, differing in size (hydrodynamic particle size varying from 10 to 500 nm) and type of coating material used (such as dextran, starch, albumin, silicones, Poly(ethyleneglycol)). They tend to be classified in two main groups according to their size, as this affects plasma half-life and biodistribution (Andrzej et al., 2007; Saboktakin, Maharramov, & Ramazanov, 2008). The first group are termed SPIOs (superparamagnetic iron oxides)where nanoparticles have a size greater than 50 nm (coated included) and the second type termed USPIOs (ultrasmall superparamagnetic iron oxides) where nanoparticles are smaller than 50 nm . The particle size influences both their physicochemical and pharmacokinetic properties (Li et al., 2005; Thierry, Winnik, Mehri, & Tabrizian, 2003).

2. Materials and methods

2.1. Materials

Starch, monochloroacetic acid was purchased from Merck, Germany, Fe(II) chloride and Fe(III) chloride, 5-aminosalicylic acid were from Sigma-Aldrich, methanol and acetone (analytical grade) were purchased from Merk, Germany. Deionized water was used throughout the experiment . The *in vitro* release measurement was carried out at pH 7.4 at 37 °C in phosphate buffer medium. Sodium dihydrogen phosphate and disodium hydrogen phosphate, used for the preparation of buffer were purchased from Merck, Germany. All other chemicals were of reagent grade.

2.2. Synthesis of carboxymethyl starch (Athawale & Rathi, 1997)

Starch (M = 9500 g mol^{-1}, 1 g) and NaOH (1.2 g) were suspended in isopropanol/H_2O (85/15; 22 ml) and heated to 60 °C. Monochloroacetic acid (1.5 g) was added slowly and the mixture was stirred for 2 h at 60 °C. After cooling to room temperature, the organic solvent was removed under reduced pressure and the aqueous phase was neutralized with acetic acid. Cold MeOH (30 ml) was added and the solution was kept at 4 °C overnight. After drying of the precipitate at high vacuum carboxymethyl starch (Athawale & Rathi, 1997) (1.5 g) was obtained. Titration of starch-methylcarboxylate (Athawale & Rathi, 1997) (57 mg) with 0.1 M HCl (2.6 ml, 0.26 mmol) and bromophenol blue in acetone/H_2O (1:1, 10 ml) resulted in 3.3 mmol COO^- g^{-1}. Therefore, on average, degree of substitution of carboxymethyl starch (Athawale & Rathi, 1997) is 0.33 (DS = 0.33).

2.3. Synthesis of carboxymethyl starch–iron oxide particles (Kresse, 2000)

Carboxymethyl starch (Athawale & Rathi, 1997) (0.5 g) and $FeCl_3{\cdot}6H_2O$(35 mg) were solved in H_2O (4 ml) and nitrogen was flushed for 1.5 h. $FeCl_2{\cdot}4H_2O$ (14 mg) was added, followed by aqueous ammonia (100 μl) in two portions while the mixture was kept under nitrogen. The solution turned black and was heated to 80 °C for 100 min. After the mixture was cooled to room temperature, the ammonia was remove by flushing the solution with nitrogen over 10 min. Freeze drying led to the desired particles (Saboktakin et al., 2007a) (0.55 mg), which are stable at 4 °C for at least 1 year and were used for all further experiments. Titration of the resulting particle (18 mg) with 0.1MHCl (0.85 ml, 85 μmol) and bromophenol blue in acetone/H_2O (1:1, 10 ml) resulted in 3.3 mmol COO^- g^{-1}. The particle size-distribution experiments were carried out as described above.

2.4. Electrostatic binding of 5-ASA to [2] particle (Saboktakin et al., 2007a)

Mesalamine [5-aminosalicylic acid, (5-ASA)] (0.33 mg, 0.23 mmol) and particle (M, 2000) (1.0 mg, 3.3 mmol COO^-, 20 eq.) were dissolved in H_2O (500 μl) and the solution was shaken for 12 h at room temperature. To purify the product an ultrafiltration device was used for centrifugation and after concentration the sample was washed with H_2O (3 × 2 ml). Size-distribution experiments were carried out as described above.

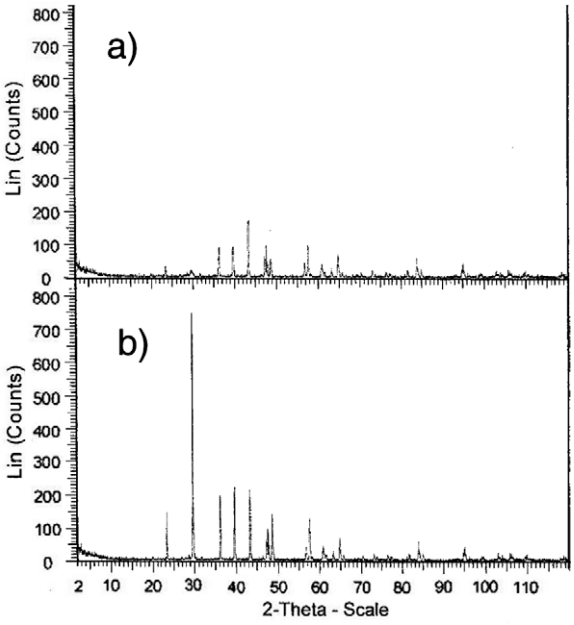

Fig. 2. XRD of (a) pure CMS, (b) CMS–SPIO nanoparticles.

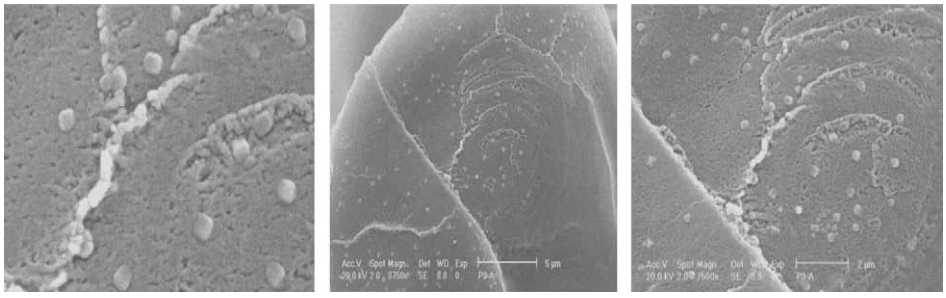

Fig. 1. SEM of CMS/SPIO/(5-ASA) nanoparticles.