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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.

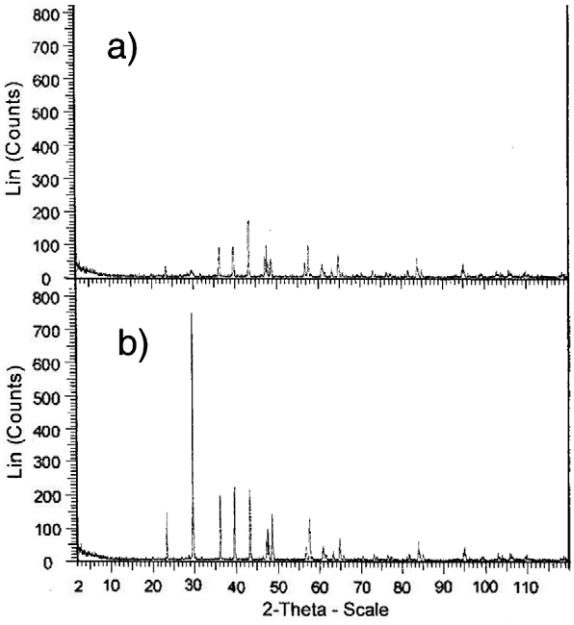

Fig. 2. XRD of (a) pure CMS, (b) CMS–SPIO nanoparticles.

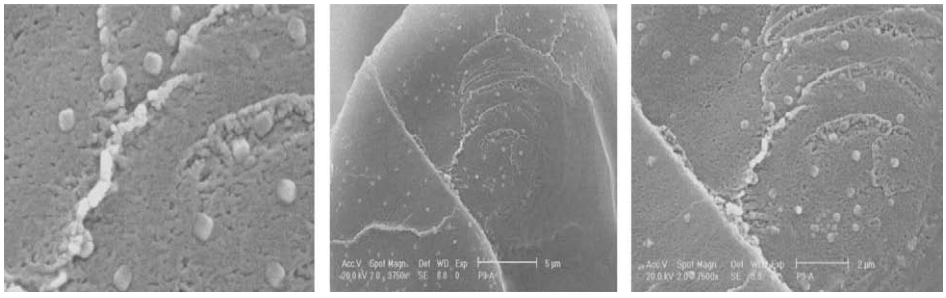

Fig. 1. SEM of CMS/SPIO/(5-ASA) nanoparticles.

2.5. Determination of NP morphology

Scanning electron microscopy (SEM, Philips XL 30 scanning microscope, Philips, the Netherlands) was employed to determine the shape of the produce NPs. Particles were coated with gold under vacuum before SEM.

2.6. X-ray diffraction measurement

The crystallinity of the formed nanoparticles was followed with Philips X-ray diffractometer using Cu K_α radiation (λ = 1.5406 Å) as s function of weight percent inorganic component.

2.7. Fourier transfer infrared (FTIR) measurement

The Fourier transfer infrared (FTIR) spectra of the nanoparticles were recorded on Perkin 810 spectrometer in KBr medium at room temperature, in the region 4000–450 cm^{-1}. X-ray diffraction patterns of the CMS/SPIO nanoparticles.

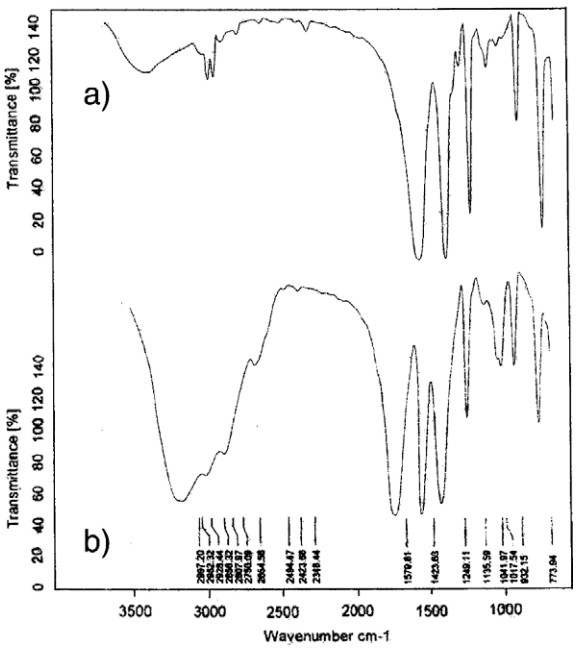

Fig. 3. FT-IR spectrum of (a) pure CMS, (b) CMS–SPIO–drug complex.

2.8. Size and size distribution measurement by ES-SMPS

The particle size and size distribution of the NPs were measured by laser light scattering (Malvern Zetasizer ZS, Malvern, UK). The samples were examined to determine the mean diameter and size distribution. The samples were prepared by suspending the freeze dried NPs in 10 ml of deionized water (10 μg/ml).

2.9. In vitro release study

Drug release from magnetic NPs carried out using a modified dissolution method. The media was a 0.05 M phosphate buffer solution. NP powder (2 mg) was suspended in tubes containing buffer solution of pH 7.4 (10 ml) to simulate physiological pH. The tubes were placed in a shaker bath (Memmert WB14, Germany) at 37 °C.

3. Results and discussion

Fig. 1 shows the SEM of CMS/SPIO/mesalamine (5-ASA) nanoparticles that synthesized by chemical reaction. This nanoparticle is very sensitive to the temperature that due to the interaction electron and sample. Scanning electron micrography images were obtains from a diluted solution of the nanocomposite particle. The white spots are drug nano particles. The SEM image shows the presence of 5-ASA spherical particles in polyfunctional dendrimeric matrix, which are homogeneously distributed throughout the composites, which is also confirmed from ^{1}H-NMR studies.

The ability of the CMS/SPIO to form a complex with drugs depends on the nanoparticles, electrostatic interactions between the nanoparticles and the drug, and the ability of the drug to form a conjugate with the nanoparticles through chemical bonding. Therefore, it is possible to manipulate the incorporation process for a given drug by appropriate selection of the nanoparticles and the surface functionality. One might expect that the mesalamine with the carboxylic group may form a complex.

Fig. 2a and b show X-ray diffraction pattern of pure CMS and CMS–SPIO nanoparticles, respectively .Diffraction of CMS–SPIO nanoparticles have a strong peak at about $2\theta = 28.92°$, which is a characteristic peak of CMS–SPIO nanoparticles. Studies on XRD patterns of nanoparticles are scarce in the literature.

The Fig. 3a and b show the FT-IR spectrum of pure carboxymethyl starch (CMS) and CMS–SPIONs, respectively, where the % of transmittance is plotted as a function of wave number (cm^{-1}). The wide peak around 3411 cm^{-1} is attributing to the O–H stretching vibrations of CMS. The peaks at 1597 and 1417 cm^{-1} attribute to the COO^- unsymmetrical and symmetrical stretching vibration respectively.

The measurement of nanoparticles size, electrospray (ES) was used in combination with a scanning mobility particle sizer

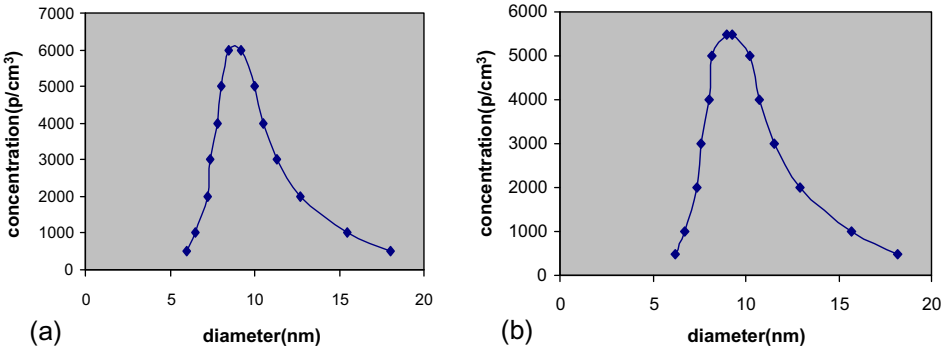

Fig. 4. The particle size distribution of (a) CMS–SPIO nanoparticle, (b) CMS–SPIO nanoparticles–5-ASA.

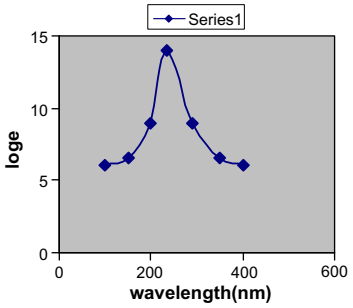

Fig. 5. UV spectra of CMS-SPIO–drug complex (λ_{max} = 235 nm).

(SMPS). The solution containing nanoparticles was sprayed in the ES with subsequent solvent evaporation. The size distribution of the generated aerosol was determined with the SMPS, resulting in a distribution of particles around 8.4 nm assuming singly charged particles (Fig. 4).

For learn of effect of the nature and size of the drug in drug delivery, we study drug release of the CMS/SPIO nanoparticles containing 5-ASA as a pharmaceutically active compound as a function of time. The concentration of 5-ASA released at selected time intervals was determined by UV spectrophotometry, (λ_{max} = 235 nm), respectively. In order to study potential application of nanoparticles containing 5-aminosalicylic acid as pharmaceutically active compounds, we have studied the drug release behavior the polymers under physiological conditions. The concentration of drug in released at selected time intervals was determined by UV spectrophotometry (Fig. 5). Important parameter for increasing of diffusion coefficient is decreased of particle size. It appears that the degree of drug release polymer depends on their particle size. In the other hand, the chemical structure of the drug too is an important factor in hydrolytic behavior of polymeric prodrugs. The high different hydrolysis rate 5-ASA at pH 7.4 can be related to the functional groups along the drug. 5-ASA contains both amine (basic) and carboxylic acid (acidic) functional groups. This factor ultimately result in an increase hydrophilicity of 5-ASA in pH 7.4.

4. Conclusion

We have demonstrated that carboxymethyl starch as the coating material for SPIONs to achieve the stabilization and drug delivery of ferrofluid. The CMS-coated SPIONs of about 10 nm diameter having a core–shell structure with magnetic core and polymeric shell have been successfully prepared. The FT-IR experimental results proved that the CMS is adsorbed onto the surface of SPIONs through the hydrogen bonding between polar functional alcohol groups of CMS and hydroxylated and protonated surface sides of the oxide. Hence the resultant nanoparticles possess an excellent solubility and stability in ferrofluid. Therefore, CMS as a coating material not only prevented the aggregation between SPIONs in physiological medium but also provided a capacity to be delivered in cancer tissue specifically, which suggests the potential utility of CMS-coated SPIONs as a contrast agent for cancer diagnosis.

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