Materials Science and Engineering C 48 (2015) 80–85

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Enhancing the versatility of alternate current biosusceptometry (ACB) through the synthesis of a dextrose-modified tracer and a magnetic muco-adhesive cellulose gel

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ARTICLE INFO

Article history:
Received 18 August 2014
Accepted 22 November 2014
Available online 27 November 2014

Keywords:
Alternate current biosusceptometry
Gastric emptying
Bio-magnetic materials
Muco-adhesive gel
Cellulose
Dextrose

ABSTRACT

Alternate Current Biosusceptometry (ACB) is a promising bio-magnetic method, radiation free and easily performed used for gastric emptying exams. Due to development on its sensitivity level, interesting nature, noninvasiveness and low cost it has attracted a lot of attention. In this work, magnetic nanoparticles of Mn–Zn ferrite as well as dextrose-modified nanoparticles were synthesized to be used as possible tracers in ACB gastric emptying exams. In addition, a magnetic muco-adhesive gel was obtained by modifying the ferrite nanoparticles with cellulose. Based on in-vivo tests in rats, we show that the pure ferrite nanoparticles, whose isoelectric point was found to be at pH = 3.2, present a great sensitivity to pH variations along the gastrointestinal tract, while the reduction of the isoelectric point by the dextrose modification leads to suitable nanoparticles for rapid gastric emptying examinations. On the other hand, the in-vivo tests show that the muco-adhesive cellulose gel presents substantial stomach adhesion and is a potential drug delivery system easily traceable by the ACB system.

1. Introduction

Nowadays, there is an increasing perspective for magnetic nanoparticle application in medicine either in diagnosis and treatment of diseases [1–3]. Particularly, the alternate current biosusceptometry (ACB) technique, in which the position of a given magnetic material can be investigated by a first order gradiometric system, has extended such a perspective to the gastrointestinal tract (GIT) [4]. Definitely diagnosis of diseases related to the GIT, such as Chagas disease [5], Parkinson's [6] or Diabetes Mellitus [7] through imaging techniques is typically performed by gastric emptying (GE) scintigraphy, so far considered the gold standard technique [8–10]. However, despite its considerable accuracy, some aspects are still subject to discussion. For instance, the high cost involved in the production of radioisotopes is a barrier for the application of scintigraphy in underdeveloped countries. In addition, the patients are exposed to radioactive doses that, while low, still limit the performance of periodic investigations and require expansive radiation protection policies [11]. The fascinating aspect of ACB is that new developments have allowed a signal-to-noise ratio similar to the one achieved by scintigraphy [12]. Moreover, as a magnetic tracer, normally ferrite particles [13–16], replaces the radioactive one, and a first order gradiometer replaces the scintigraphy apparatus, this imaging technique allows for cheaper and safer examinations. To demonstrate such potential, we have tuned the physical and chemical properties of Mn–Zn ferrite nanoparticles suspensions to show that ACB can be used as an accurate technique for rapid GE exams.

Regarding the application of ACB in the treatment of GIT diseases, such a technique can also allow tracking the position of muco-adhesive gels that have been used as an efficient way to delivery complex molecules while retaining a dosage form at the site of action [17]. To do so, magnetic nanoparticles can be incorporated to the gels and their retention time into the GIT can be evaluated in research laboratories without requiring the euthanasia of animals [18]. Furthermore, by adding magnetic nanoparticles to the muco-adhesive gels one can provide additional possibilities to these materials such as magnetic hyperthermia applications as well as prolonged retention times by magnetic attraction with an external magnet [19]. To prove this prospective, we have prepared a magnetic cellulose based gel, considering the remarkable muco-adhesive properties of this polymer [20], and were able to show that the presence of magnetic nanoparticles does not disrupt the adhesion capacity of the cellulose.

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http://dx.doi.org/10.1016/j.msec.2014.11.059
0928-4931/

2. Materials and methods

2.1. Synthesis of Mn–Zn ferrite nanoparticles by the co-precipitation method

Manganese zinc ferrite of nominal composition $Mn_{1.05}Zn_{0.25}Fe_{1.70}O_4$ was synthesized by the co-precipitation method [21] using $Mn(NO_2)_2$ (Sigma-Aldrich), $Zn(NO_2)_2$ (Sigma-Aldrich) and $FeCl_3$ (Sigma-Aldrich) as starting salts, which were diluted in aqueous solution with the appropriate amounts. The mixture was then dripped onto NaOH (Sigma-Aldrich) solution (0.1 M) under ebullition and vigorously stirred for 120 min. In a typical experiment, 0.4 g of ferrite was prepared in 300 mL of NaOH solution. The resulting black precipitate, after being removed with a magnet, was washed to achieve nanoparticles with neutral pH and dispersed in water in order to obtain a 20 mg/mL suspension.

2.2. Modification of the ferrite nanoparticles with dextrose and synthesis of the magnetic muco-adhesive cellulose gel

Following well-known procedures [22], the modification with dextrose was performed by peptizing 80 mg of ferrite nanoparticles with HNO_3 (Sigma-Aldrich) 0.5%, while an aqueous solution of dextrose (Chemco) was prepared with the pH adjusted to 10. The ferrite was then added to the dextrose solution with a mass ratio of ferrite/dextrose = 1/12, and the resulting suspension stirred for 15 h. Subsequently, the suspension was carefully washed to extract any excess of dextrose and adjusted to a concentration of 20 mg/mL.

For the synthesis of the magnetic muco-adhesive cellulose gel, 1 g of crystalline cellulose (Blanner) was added to an aqueous solution containing 1 g of urea (Sigma-Aldrich) and 1.5 g of NaOH. The resulting solution was vigorously stirred for 15 min at 5 °C leading to a viscous and transparent solution that was added to the ferrite suspension, in a mass ratio of ferrite/cellulose = 1.5/1, and stirred for 30 min. The material was then added to an ammonium sulfate solution (8% w/v), for coagulation as well as cellulose recuperation and stirred for 30 min. Finally the reaction was subjected to slow stirring for a few minutes, and the resulting material was removed with a magnet and washed to achieve neutral pH. The final suspension was adjusted to a concentration of 20 mg/mL, resulting in a viscous black gel.

2.3. Materials characterization

X-ray powder diffraction (XPD) analysis performed using a Rigaku diffractometer (D/MAX-2100/PC), Cu-Kα, 1.54 Å (Ni filter) confirmed the spinel structure in the pure ferrite nanoparticles. Furthermore, based on these data no traces of secondary phases were observed. By means of a Nano ZS zetameter the zeta potential of the magnetic nanoparticles was evaluated as a function of pH by suspending the particles in 0.001 mol/L KNO_3 solution. In addition, to verify the homogeneity and morphology of each synthesized nanoparticle Scanning Electron Microscopy (SEM) measurements were carried out with a FEI, Quanta 200. Finally, the presence of the organic groups in the modified ferrite nanoparticles was evaluated via Fourier Transformed Infra-Red (FTIR) using a Nicolet spectrometer, Nexus 670.

2.4. In-vivo gastric emptying exams

The in vivo exams were performed in 3-month-old male Wistar rats (200–250 g) purchased from the Animals Laboratory, ANILAB (Paulínia-SP, Brazil). The rats were housed in individual cages under controlled temperature (24 ± 2 °C), humidity, and lighting (12-h light/dark cycle) with ad libitum access to a commercial diet (Purina® rat chow Brazil) and tap water. The animals were fasted for 24 h before the procedures, where 1 mL of each material was administered by gavage to different rats. As depicted in Fig. 1(a), two anatomic regions were marked in the rats: i) stomach and ii) cecum. Using the ACB gradiometric system, as shown in Fig. 1(b), we analyzed the tensions generated in these points due to the presence of the magnetic material at 15 minute intervals for 240 min, so that a temporal distribution of the tracer along the animals' tract was obtained. After such a time the animals were euthanized and the amounts of magnetic material retained in their stomachs could be analyzed. These procedures were approved by the CEEA–IBB (Protocol No. 410-CEUA).

3. Results and discussion

Fig. 2(a) shows the SEM images of the pure ferrite nanoparticles, which exhibit agglomerates with mostly spherical shapes and a diameter around 100 nm. The SEM results for the dextrose stabilized material, Fig. 2(b), show the formation of polymeric structures with heterogeneous shapes with the magnetic nanoparticles incorporated into them, while the cellulose gel (Fig. 2(c)) is formed by spherical particles with a diameter around 100 nm. The latter indicates that the gel is formed by magnetic nanoparticles efficiently coated into cellulose capsules, into which drugs and biomolecules could be inserted for controlled release in the gastrointestinal tract (GIT) in future works.

In order to further verify if the ferrite nanoparticles were successfully modified, all specimens were analyzed by FTIR, as depicted in Fig. 3. It is possible to see that the Fe–O characteristic bands at 428 and 571 cm^{-1} [24] are present in the pure ferrite spectra, but hidden by the cellulose, Fig. 3(a), and the dextrose, Fig. 3(b), signals. In both modified materials, however, the presence of the ferrite nanoparticles is confirmed by the presence of the out of plane vibrations from the hydroxyl groups located at 937 cm^{-1} that characterize the nanoparticle surface [25], as well as by the slope in the spectra at low wavenumber values, caused by the nanoparticles scattering [26].

In the case of the gel, Fig. 3(a), a comparison to the spectrum obtained for pure cellulose shows a slight intensity reduction in the C–OH stretching as well as in the CH_2 stretching and symmetric bending modes, located around 1160, 1430 and 2920 cm^{-1}, respectively while, the C═O stretching mode at 1650 cm^{-1} remains almost unchanged. The decrease in the CH_2 mode intensity indicates a reduction in the crystalline level of the cellulose present in the gel in comparison to the pure polymer [27], which can be related to the dissolution of the cellulose in the alkaline solution during the magnetic gel synthesis process described in Section 2.2. The reduction in crystallinity can be taken as a successful step in the synthesis process as it improves the ability of the cellulose to form hydrogen bonds with the GIT walls, which is one of the main features for prolonging the retention time of muco-adhesive materials [28,29].

From the dextrose-modified ferrite spectrum, Fig. 3(b), it is clear that a new mode at 1650 cm^{-1} is observed. This vibration was previously observed for dextrose with an open chain configuration instead of the dominant cyclic chain of the pure sugar [30] and assigned to a C═O stretching mode. This large change in the molecular configuration can be attributed to the dissolution of the polymer in the nanoparticles during modification process as well.

4. In-vivo tests: gastric emptying (GE) exams

4.1. GE using pure ferrite

Fig. 4(a) shows the GE profile (closed dots), as well as the signal in the animal's cecum (open dots) when pure ferrite was administered to the rats. A fast initial emptying is followed by retention of the tracer after 60 min. After 130 min the reduction in the signal in the stomach is once again resolved by the ACB apparatus and shows a slow-down when compared to the initial slope of the