International Journal of Pharmaceutics 513 (2016) 404–409

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Alginate nanoparticles protect ferrous from oxidation: Potential iron delivery system

Nuwanthi P. Katuwavila[a,c], A.D.L.C. Perera[a,b], Damayanthi Dahanayake[c], V. Karunaratne[b,c], Gehan A.J. Amaratunga[c,d], D. Nedra Karunaratne[a,b,*]

[a] Post Graduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka
[b] Dept. of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka
[c] SriLanka Institute of Nanotechnology, Mahenwatta, Pitipana, Homagama, Sri Lanka
[d] Electrical Engineering Division, Department of Engineering, University of Cambridge, 9 JJ Thomson Avenue, CB3 0FA Cambridge, United Kingdom

ARTICLE INFO

Article history:
Received 3 July 2016
Received in revised form 31 August 2016
Accepted 18 September 2016
Available online 19 September 2016

Chemical compounds studied in this article:
Sodium alginate (PubChem CID: 5102882)
Ferrous sulphate (PubChem CID: 24393)
Calcium chloride (PubChem CID: 5284359)
Sorbitanmonooleate (PubChem CID: 9920342)
L-Ascorbic acid (PubChemCID: 54670067)

Keywords:
Anemia
Ferrous sulfate
Alginate nanoparticles
Iron loaded nanocomposite
Bioavailability

ABSTRACT

A novel, efficient delivery system for iron (Fe^{2+}) was developed using the alginate biopolymer. Iron loaded alginate nanoparticles were synthesized by a controlled ionic gelation method and was characterized with respect to particle size, zeta potential, morphology and encapsulation efficiency. Successful loading was confirmed with Fourier Transform Infrared spectroscopy and Thermogravimetric Analysis. Electron energy loss spectroscopy study corroborated the loading of ferrous into the alginate nanoparticles. Iron encapsulation (70%) was optimized at 0.06% Fe (w/v) leading to the formation of iron loaded alginate nanoparticles with a size range of 15–30 nm and with a negative zeta potential (−38 mV). The *in vitro* release studies showed a prolonged release profile for 96 h. Release of iron was around 65–70% at pH of 6 and 7.4 whereas it was less than 20% at pH 2.The initial burst release upto 8 h followed zero order kinetics at all three pH values. All the release profiles beyond 8 h best fitted the Korsmeyer-Peppas model of diffusion. Non Fickian diffusion was observed at pH 6 and 7.4 while at pH 2 Fickian diffusion was observed.

1. Introduction

Iron deficiency has become the most common nutritional deficiency today (Horton and Ross, 2003). Since the body requires iron to synthesize its oxygen transport proteins in particular, hemoglobin and myoglobin (Abbaspour et al., 2014), iron deficiency often leads to anemia. Iron deficiency results from insufficient intake of iron from diet and poor utilization of iron from ingested food or a combination of both factors (Gaucheron, 2000). The only proven way to lessen this issue is to increase iron intake, either by providing medicinal iron (supplementation) or by adding iron into the diet (fortification), basically to food staples (such as wheat and maize flour) or condiments (such as soy sauce, fish sauce, sugar and salt) (Cook and Reusser, 1983). Both these approaches heavily utilize ferrous based products (Patil et al., 2012). In spite of the development of newer oral iron preparations, ferrous sulphate still remains the first line of treatment (Martı'nez-Navarrete et al., 2002) mainly due to its low cost and high availability (Patil et al., 2012). However, the ferrous ion based supplements are not always compliant due to adverse gastrointestinal effects such as nausea, vomiting and gastric distress (Saha et al., 2007), hence limiting the benefits of the iron supplementation therapy. In addition, some severe conditions may occur, such as allergic reactions, black and tarry stools, fever and continuing stomach pain (Hosny et al., 2015). Further, its variability is very high in iron absorption, thus effecting the bioavailability (Bregman et al., 2013).

Therefore, there is a need for developing a novel, stable system which is able to increase the iron bioavailability. Previous work on

* Corresponding author at: Dept. of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.
E-mail addresses: nanukanp@yahoo.com (N.P. Katuwavila), chandanip@pdn.ac.lk (A.D.L.C. Perera), damayanthid@slintec.lk (D. Dahanayake), veranjak@slintec.lk (V. Karunaratne), gehanA@slintec.lk (G.A.J. Amaratunga), nedrak@pdn.ac.lk, nedrak37@yahoo.com (D. N. Karunaratne).

http://dx.doi.org/10.1016/j.ijpharm.2016.09.053
0378-5173/

encapsulation techniques for the treatment of anemia with nano drug delivery systems reports the use of ferrous sulphate loaded solid lipid nanoparticles (Zariwala, et al., 2013; Hosny et al., 2015). The prevention of exposure of ferrous directly to the gastrointestinal tract and the slow release property would be advantageous during its oral uptake. Furthermore, the need for high doses of iron to obtain the therapeutic effect can be avoided through slow release, minimizing the side effects associated with conventional oral iron supplements.

Alginate nanoparticles were used by Aynie et al., to protect oligonucleotides from degradation in the presence of serum (Aynié et al., 2009). In their study, they observed that in addition to protecting the oligonucleotides, the alginate nanoparticles efficiently delivered the cargo to the lungs, liver and spleen. Therefore, this ability to protect has been exploited in this study to construct a novel system for delivery of iron in the ferrous form.

Encapsulation protects ferrous ions interacting with other materials and prevents the direct contact of ferrous with gastrointestinal lumen, thus reducing the possible adverse effects (Hosny et al., 2015; Xia and Xu, 2005). Alginate biopolymer system has promising properties (Sosnik, 2014) and is safe to be used as an oral carrier for iron. Alginic acid and sodium alginate have turned out to be the most extensively explored mucoadhesive biomaterial with good cytocompatibility and biocompatibility (Lee and Mooney, 2012; Sarei, et al., 2013). One major advantage of using alginate in oral delivery formulations is their property of being in solid like structure at gastric conditions due to the formation of alginic acid. Hence, it protects the encapsulant inside the core (Draget and Taylor, 2011). Also, alginate beads dissolve under neutral and basic pH values which is more effective in iron delivery since the absorption of iron occurs mainly in the duodenum in which the pH is around 7.0–8.5 (Draget and Taylor, 2011).

In the current study, we have examined the potential of alginate nanoparticles for oral iron delivery. The aim of our study was to formulate alginate nanoparticles to encapsulate ferrous sulphate and evaluate the release kinetics of iron in pH varying buffer solutions with a view to examining its potential as a nano-biopolymeric carrier of Fe^{2+}.

2. Materials and methods

2.1. Materials

Low viscosity sodium alginate, ferrous sulphate, calcium chloride, Sorbitanmonooleate (span 80) and L-ascorbic acid were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other reagents were of analytical grade and used directly. Snake skin dialysis tubing (MWCO 3500) was purchased from Thermo Scientific USA.

2.2. Preparation of ferrous loaded alginate nanoparticles

The nanoparticles were prepared by ionic gelation based on methods described previously (Daemi and Barikani, 2012).The pH of a 0.3% w/v solution of sodium alginate (40.0 mL) was adjusted to around 5 and then stirred with span 80 for 2 h at 60 °C to obtain a homogeneous mixture. This sodium alginate solution was stirred with 50.0 mL of ferrous sulphate solution in the presence of ascorbic acid where the ratio of ferrous sulphate to ascorbic acid was always 15:1 and with varying iron concentrations (1%, 2% and 3%, w/w alginate). The above complex was gelated by drop wise addition of 40.0 mL of $CaCl_2$ solution (0.1%w/v) while stirring at a high speed for 1 h. The nanoparticle suspension was refrigerated overnight and centrifuged at 9000 rpm for 45 min to obtain the nanoparticle pellet.

2.3. Characterization of ferrous loaded alginate nanoparticles

2.3.1. Particle size and zeta potential measurements

The average particle size and size polydispersity of the nanoparticles dispersed in distilled water were determined by dynamic light scattering technique at 25 °C using a particle size analyzer (Zetasizer Nano ZS, Malvern Instruments, UK) at a fixed scattering angle of 90°. The zeta potential of nanoparticles was measured using the Zeta potential analyzer (Zetasizer Nano ZS, Malvern Instruments, UK). All measurements were performed in triplicate.

2.3.2. Fourier transform infrared spectroscopy (FTIR) characterization and thermal analysis

FTIR spectra of sodium alginate, alginate nanoparticles and iron loaded alginate nanoparticles were obtained with a Bruker Vertex 80 IR spectrometer (Germany) at a resolution of 4 cm^{-1} from 4000 to 400 cm^{-1}. Thermal decomposition of sodium alginate, alginate nanoparticles and ferrous loaded alginate nanoparticles were analyzed using a SDT Q600 thermogravimetric analyzer (TA Instruments, USA) from 25 °C to 800 °C using a ramp rate of 10 °C/min in air.

2.3.3. Transmission electron microscopic (TEM) imaging

A drop of ferrous loaded alginate nanoparticle dispersion was placed on a holey carbon Cu grid and allowed to dry at room temperature. Then nanoparticles were imaged using a high resolution transmission electron microscope (TEM) (JEM 2100, JEOL, Japan) operated at accelerating voltage 200 kV.

2.3.4. Electron energy loss spectroscopy(EELS) study of ferrous loaded nanoparticles

EELS spectrum of ferrous loaded alginate nanoparticles was acquired with an EELS spectrometer (EELS Gatan, Quantum 963, USA) attached to the TEM with the energy resolution of 0.05 eV/channel in STEM spectral imaging mode. EELS spectra of $FeCl_3$ and $FeSO_4$ standards were acquired for a comparison study.

2.4. Determination of encapsulation efficiency

The amount of incorporated ferrous in the nanoparticles was determined by thiocyanate colorimetry. The supernatant obtained after centrifugation was subjected to oxidation with 0.15 mol dm^{-3} of $KMnO_4$ in acidic medium to convert all ferrous ions to ferric ions since ferrous ion does not form a coloured complex with thiocyanate. Next, the oxidized supernatant was complexed with 1 mol dm^{-3} potassium thiocyanate solution and the absorbance was measured at 490 nm using the UV–vis spectrophotometer (SHIMADZU, UV-3600, UV–Vis–NIR). Then, the concentration was calculated from a calibration plot obtained for ferric ion standard solution. Percentage encapsulation efficiency was calculated as follows.

$$\%\ \text{Encapsulation efficiency} = \frac{\text{Amount}_{\text{total}} - \text{Amount}_{\text{supernatant}}}{\text{Amount}_{\text{total}}} \times 100\% \quad (1)$$

2.5. In vitro release study of iron loaded alginate nanoparticles

The release characteristics of iron from alginate nanoparticles were studied in pH 7.4, 6 and 2 solutions. The iron loaded alginate nanoparticles were dispersed in 5.00 mL of buffer solution and trapped inside a dialysis membrane and this was immersed in 25.00 mL of buffer solution at 37 °C with mild agitation. Aliquots