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RESEARCH ARTICLE

Synthesis and In Vitro Evaluation of Iron Cross Linked Alginate Nano Particle for Controlled Drug Release

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ABSTRACT

Highly stable Iron cross linked alginate nano particle was prepared via Ionotropic gelation method using natural honey as stabilizer. The nanoparticles formed were characterized by Fourier Transform Infrared Spectroscopy (FT-IR), which confirmed the formation of iron cross link between alginate chains. Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) imaging revealed the formation of spherical particles of size less than 100 nm. The potential of these nanoparticles for drug delivery application is demonstrated using theophylline as model drug. The ability to release the drug was examined at different physiological conditions. At acidic pH only 15 % of the entrapped drug was released, while is almost 80% when pH raised to 7.4. In vitro cytotoxic evaluation of the nanoparticles in L929 cells showed that the particles are non-toxic. This preliminary study shows that the nanoparticles can be a potential carrier for the pH controlled oral delivery applications.

Introduction

Natural polymers for biomedical applications have undergone extensive investigations in recent years [1]. The non-toxicity, low immunogenicity and biocompatibility of Alginate (ALG), enable it as a versatile polymer in the field of biomedical application [2]. Most of the alginate particles for drug delivery applications reported in literature have milli or micro dimension [3]. In drug delivery particle size have a crucial role and nano particles possess many advantages over micro particles such as easy passage through capillaries, high penetration through cells and tissues, ability to reach the target site and thus improve the efficacy of drugs and reduce toxic side effects. In addition to these properties, they also possess larger specific surface area, higher mechanical strength and show controlled release properties [4,5]. Various methods for the preparation of alginate nanoparticle were reported in the literature including polyelectrolyte complexation, emulsification/internal gelation, solvent evaporation/ diffusion and solvent-casting methods. But they have some limitations like porosity, difficulty to reproduce, precipitation during the evaporation and use of volatile organic solvents [6]. It is found that green chemistry principles could be effectively

applied to the generation of ALG nanoparticle in a safer and greener manner by avoiding organic solvents [7].

Alginate particles prepared by gelation with calcium is most frequently used in drug release studies [8-12]. Release profile is highly sensitive to the composition of the release medium [13-16]. According to the studies of Dainty et al. and Liu et al. calcium cross linked ALG beads were start to disrupt in phosphate buffer solution. This is due to the high affinity of phosphate ions towards ALG than that of calcium [17,18].

Considering all the short comings mentioned above, our aim is to prepare a pH sensitive nano sized stable drug delivery system with good physical properties for oral delivery based on alginate and trivalent cation Fe^{3+} assisted with probe sonication using natural honey as a stabilizer. Theophylline (TH) was chosen as the model drug for studying the drug release profile. The method reported here is fast and in an aqueous environment and avoid the use of toxic organic solvent.

Material and Methods

1. Materials

Sodium alginate of medium viscosity (viscosity of 2% solution, 25°C≈ 3500 cps, Sigma -Aldrich, London), Rifampicin (Himedia Laboratories, Nasik), Ferric chloride (Merck, Germany). Natural honey used in this study was purchased from Kerala Agriculture University.

2. Preparation of nanoparticles

2.1. Preparation of ALG nanoparticles: ALG nanoparticles were prepared by ionotropic gelation method as described in our previous paper with slight modification [19]. Sodium alginate was dissolved in 100 ml deionized water (1%, w/v) containing various concentrations of honey and stirred for 30 minutes. About 20ml ferric chloride solution was added drop wise, stirred and this mixture was sonicated for 5 minutes. Nanoparticles were collected by centrifugation at 3500 rpm for 5 min, washed with deionized water and vacuum dried.

2.2. Preparation of TH loaded ALG nanoparticle: To prepare TH loaded nanoparticles, TH was dispersed into 100 ml aqueous sodium alginate (1 %, w/v) solution containing honey and stirred for 30 minutes. After that 20 ml of ferric chloride solution was added drop wise and stirred. This homogenized mixture was sonicated for 5 minutes. The TH loaded nano particles were collected by centrifugation at 3500 rpm for 5 min, washed with demonized water and vacuum dried.

3. Material characterization methods

Fourier Transform Infrared (FTIR) spectra of TH, ALG NPs and TH loaded ALG NPs were recorded between 400 and 4000 cm-1 wavelength range using Schimadzu FTIR model 1801. Surface morphology of the nanoparticle was probed using a JEOL model 1200EX Transmission electron microscope operated at an accelerating voltage at 80 kV.XRD patterns of the samples were recorded by using Brucker D8 Advance diffractometer with monochromatic Cu-Kα1 radiation (λ = 1.5418 Å). The particle size and zeta potential measurement were carried out using a Zetasizer Nano ZSP instrument (Malvern, UK)

4. Entrapment efficiency of ALG-NCC NPs

The concentration of free TH was determined by noting the absorbance of supernatant liquid after removing the nanoparticles at 286 nm, using a UV–VIS spectrophotometer (PerkinElmer Lambda Bio 40). The TH content was obtained from the calibration curve constructed from a series of solutions with known drug concentration. The entrapment efficiency percentage (% EE) was calculated as follows:

$$\% \text{ EE} = \left(\frac{W_a - W_b}{W_a}\right) \times 100 \quad (1)$$

Where W_a is the total weight of the drug fed W_b is the weight of non-encapsulated free drug.

5. In vitro drug release

In vitro release of drug was studied in simulated gastric fluid and intestinal fluid. About 100mg nanoparticles were taken in a dialysis membrane and is placed in simulated fluids. After definite time interval one ml of the solution was taken, separated from the aqueous suspension by ultra-centrifugation at 10,000 rpm for 10 min (SORALL® Bioguge Stratos Ultracentrifuge). The concentration of released TH was monitored by UV–VIS

spectrophotometer (PerkinElmer Lambda Bio 40) at 286nm according to the standard curve of TH. Data were given as mean ± Standard Deviation (SD) based on 3 independent measurements.

6. Drug release kinetics

To understand the release mechanism, the release data were analyzed using Korsmeyer-Peppas equation

$$\frac{Qt}{Q\infty} = kt^n \qquad (2)$$

where Qt /Qα is the proportion of drug released at time t, k is a constant incorporating the characteristics of the particle system that makes up the formulation, and n is the diffusional exponent which is indicative of the transport mechanism.

7. Cytotoxicity evaluation

The cytotoxicity of iron cross linked ALG NP was measured in terms of cell viability using L929 fibroblast by MTT assay. The cells were cultured with DMEM containing 10% FBS and the cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator for 24 h. The medium was then replaced with test solution and incubated at 37°C in a humidified 5% CO_2 incubator for specified time. Later 30 µl of MTT (5mg/ml) was added to the wells and incubated at 37°C in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl DMSO was added to solubilize the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm to determine the optical density (OD). OD of control was obtained from the cells by repeating the above experiment without any sample. Cell viability (%) was calculated using Eq. (3)

$$\text{Cell Viability}(\%) = \left(\frac{\text{OD of sample}}{\text{OD of control}}\right) \times 100 \qquad (3)$$

8. Statistical analysis

Statistical analysis was carried out using Student's t-test. One-way Analysis Of Variance (ANOVA) was used for compare the data and P value of <0.05 was considered significant. The results were expressed as mean ± standard deviation.

Results and Discussion

1. Synthesis and characterization of NP

The aim of the present study was to develop a pH dependent nano particle system which could effectively protect drug from harsh acidic environment of stomach and provide a sustained release in the intestinal condition. The Fe^{3+} cross linked ALG NP was successfully synthesized by ionic gelation technique using natural honey as the stabilizing agent. Here we adopt ionic gelation technique to cross link ALG with Fe^{3+}. From the TEM images (Figure 1) it was found that the obtained nanoparticles has a particle size below 100nm and are spherical in shape. The formation of spherical nano particles is due to the cross linking of carboxyl group of ALG with Fe^{3+} ions.

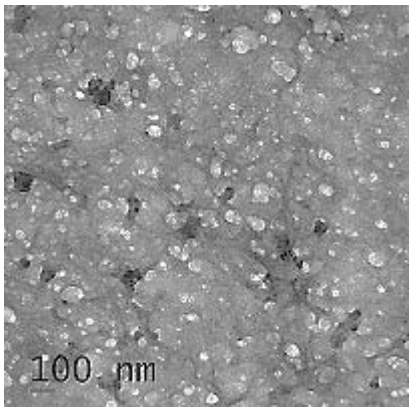


Figure 1: TEM image of Fe ALG NP.

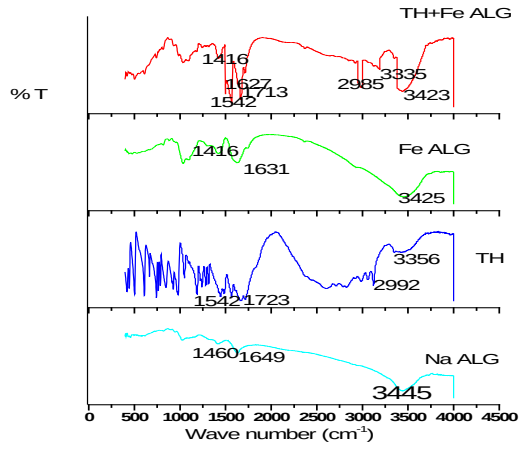


Figure 2: FTIR of a) Na ALG b) TH c) Fe ALG NP d) TH loaded Fe ALG NP.

Most of the ALG nanoparticles reported in the literature have dimensions above 100nm [10,20,21]. It was found

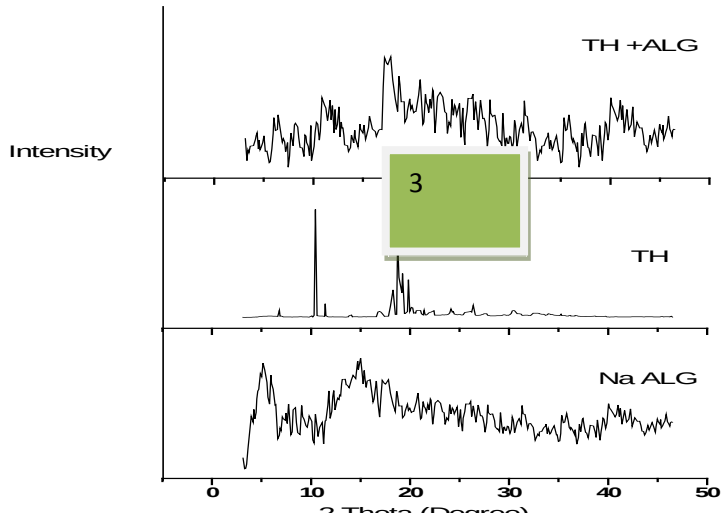


Figure 3: XRD of a) Na ALG b) TH c) TH loaded Fe ALG NP.

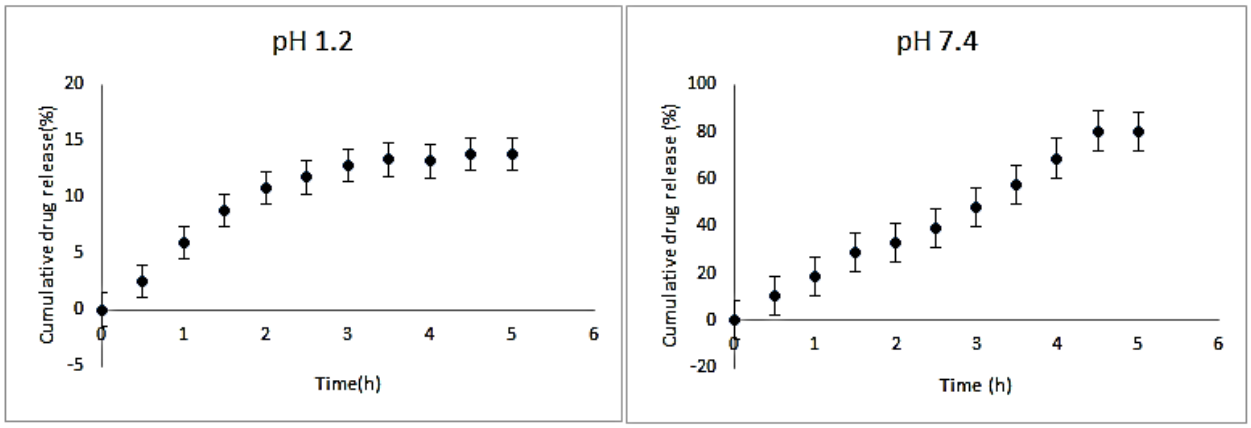


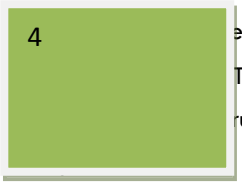
Figure 4: Cumulative TH release at a) pH 1.2 and b) 7.4 (Data presented as mean ± SD, n = 3).

Table 1: Optimization parameters for Fe^{3+} cross linked ALG NP formulation.

Formulation codes	Fe^{3+} Concentration (%, v/v)	TH:ALG	Surfactant (honey) concentration (%, v/v)	Drug entrapment efficiency %(%EE)	Particle size(nm)	Zeta potential(mV)
F1	0.5	1:4	1	33.82±1.53	85±14	-27.4±2.4
F2	**1**	**1:4**	**1**	**35.58±1.85**	**80±20**	**-25.4±3.1**
F3	1.5	1:4	1	31.73±1.92	110±15	-26±1.5
F4	0.5	1:2	1	23.66±2.89	82±18	-26±2.5
F5	1	1:2	1	26.98±1.98	89±14	-27±2.7
F6	1.5	1:2	1	28.5±2.58	105±1.98	-26.4±1.4
F7	0.5	1:6	1	25±2.92	90±2.85	-26.7±2.4
F8	1.0	1:6	1	27.5±2.05	88±1.56	-25.9±1.7
F9	1.5	1:6	1	24±2.43	107.5±2.71	-25.7±1.65
F10	0.5	1:2	2	-	Precipitation	-
F11	1.0	1:2	2	-	Precipitation	-
F12	1.5	1:2	2	-	Precipitation	-
F13	0.5	1:4	2	-	Precipitation	-
F14	1.0	1:4	2	-	Precipitation	-
F15	1.5	1:4	2	-	Precipitation	-
F16	0.5	1:6	2	-	Precipitation	-
F17	1.0	1:6	2	-	Precipitation	-
F18	1.5	1:6	2	-	Precipitation	-

that the particle whose sizes below 100nm are better absorbed in the intestinal track [22]. The application of sonication help to reduce the particle size and this observation was according to the literature report [23]. The presence of honey which act as a stabilizer and surfactant also help to reduce the particle size [24]. The effects of process variables like TH-ALG ratio and surfactant concentration on the formation of nanoparticle were analyzed and optimized on the basis of their particle size, zeta potential and effect on the drug entrapment efficiency. The drug entrapment efficiencies (%) of various formulations were within the range of 23.62 ± 1.45 to 35.58 ± 1.75 % as shown in Table 1. The particle size ranges from 80±15nm to 110± 24 nm and the zeta potential from -27±1.4 mV to -25.4 ±1.5 mV. The suitable conditions for nanoparticle preparation were optimized as 1% surfactant concentration, 1 % cross linking agent and 1:4 TH: ALG ratio. This optimum formulation has the maximum entrapment efficiency (35.58±1.85 %), minimum particle size (80±20 nm) and maximum stability. The negative zeta potential is due to the presence of carboxylate group of ALG.

The Fourier Transform Infrared (FTIR) spectra of the sodium alginate, TH, blank Fe^{3+} cross linked ALG NP and TH loaded Fe^{3+} cross linked ALG nanoparticles were given in Figure 2. The absorption peaks at 3445 cm-1 in the spectra of the sodium alginate is indicative of stretching vibrations of OH bond. The bands around 1649 and 1460 cm^{-1} were assigned to asymmetric and symmetric stretching vibrations of carboxylic carbonyl groups .Upon iron cross linking process there is an obvious shift of this carbonyl stretching vibration to lower wave number (1649 to 1631cm^{-1}, 1460 to 1416 cm^{-1}). This is due to the decrease in the double bond character of carboxylic carbonyl groups during the cross linking [25]. Theophylline has shown the characteristic band at 3356 cm^{-1} due to amide N-H stretching vibration. The bands at 2992, 1723 and 1542 cm^{-1} are due to C-H stretching, C=O stretching vibrations and C=N stretching vibrations respectively. These characteristics peaks of TH have also appeared in TH loaded NP. This indicates the successful encapsulation of TH in ALG matrix. Fig.3 describes the XRD patterns of sodium ALG, TH and the TH loaded ALG NP. It was seen that the characteristic peaks of TH at 2 θ of 7.1, 12.4, 14.2236, 25.3 and



ed its crystalline behavior and was TH loaded ALG NP. This provides ug is amorphous in nature in the has the advantage of better dispersion and easy release from the matrix.

2. In vitro drug release

The release of TH from Fe^{3+} cross linked ALG NP was studied in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (7.4) and shown in Figure 4. At pH 1.2 only 15% of TH was released in 5 h. This slow release maybe due to the formation of insoluble alginic acid by the protonation of carboxylate group of ALG. As a result strong hydrogen bonding interaction formed and keeps three dimensional network structures which is responsible for the low TH release. At pH 7.4 the NP showed a profound increase of TH and almost 80% of the entrapped TH was released in 5 h. This is due to the partial ionization and electrostatic repulsion of carboxyl group which increase the swelling degree and hence higher drug release. The stability of ALG NP can be explained on the basis of the extended three dimensional cross linking between Fe^{3+} and ALG [26]. In vitro drug release shows pH dependent drug release behavior of the nanoparticles. In oral delivery the drug is well protected in gastric fluid in stomach, and show a profound increase in drug release in intestinal condition, which is a primary criteria for the oral formulation.

In order to understand the drug release mechanism, the obtained data were fitted with Korsmeyer-Peppas equation and the mechanism was analyzed on the basis of value of diffusion constant 'n'. For a spherical system $n \leq 0.43$ indicates Fickian release and $n = 0.85$ indicates a purely relaxation-controlled delivery which is referred to as Case II transport. Intermediate values $0.43 < n < 0.85$ indicate an anomalous behavior (non-Fickian kinetics corresponding to coupled diffusion/polymer relaxation) been observed [27,28]. The value of n is

found be 0.81, which indicated an anomalous behavior of release kinetics.

3. Cytotoxicity evaluation

Cytotoxic evaluation is very important for a material for biomedical application. In vitro cytotoxicity of the obtained nano particles were evaluated by MTT assay. As seen in Figure 5, the L929 cell viability after being treated with the NP was more than 80% even when the concentration was up to 100 µg/ml, indicating the drug carrier did not have significant cytotoxicity and possess excellent biocompatibility. This study demonstrates that the ALG nanoparticle can be used as a safe drug carrier.

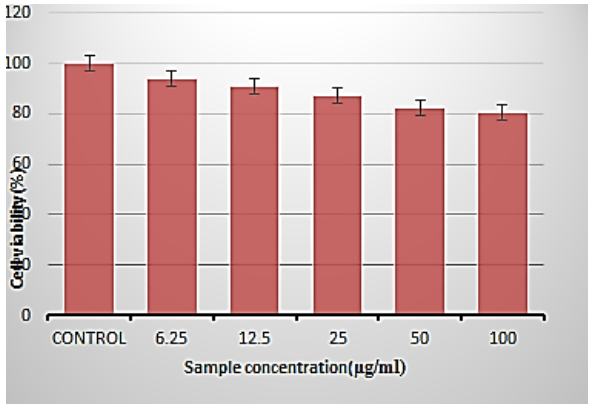


Figure 5: The cell viability of L929 cells treated with ALG nanoparticle.

Conclusion

In this study, iron cross linked alginate nanoparticles were prepared and characterized. The analysis carried out by TEM and DLS revealed that the particles have size below 100nm. The potential of the developed nanoparticles as a drug carrier was investigated using theophylline as a model drug and observed a pH dependent, drug release. The values obtained for the release parameters indicate an anomalous transport mechanism. In vitro cyto toxicity studies proved its biocompatible nature. The studies suggest that the developed nanoparticles could be successfully explored for drug delivery applications.

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