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Nano-encapsulation of chicken immunoglobulin (IgY) in sodium alginate nanoparticles: In vitro characterization

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ABSTRACT

Controlled delivery of therapeutic agents by alginate nanoparticles became an attractive issue in the gastric organ. Some therapeutic agents such as proteins could not tolerate in severe condition in the gastrointestinal tract. In the present study, four concentrations of a specific IgY as a prophylactic agent against *E. coli* O157: H7 was entrapped in 0.2% w/v sodium alginate nanoparticles by ionic gelation method. Depending on the IgY concentration entrapment efficacy was 28.31–99.84%. The physico-chemical and structural characteristics of free and IgY-loaded Alg NPs revealed that the individual particles exhibited a spherical shape with a diameter of 45–85 nm, and a negatively charged surface with a zeta potential value of 26–36 mV. In vitro release study showed a high significant difference of released amounts of IgY at 10% and 99.84% in simulated gastric fluid (pH 1.2) and simulated intestine fluid (pH 6.8), respectively. Also, the quality and activity of released IgY from Alg NPs not changed. The cytotoxicity of different concentrations of Alg NPs on the Vero cells was measured. Our results indicated that Alg NPs prepared from 0.2%w/v stock solution could be appropriate candidates for efficient and safe delivery of IgY through the gastrointestinal tract.

1. Introduction

The purpose of pharmaceutical research and development is to design products with ensured safety, quality, and efficacy to treat [1]. Among all aspects of the science, strategies for drug delivery are an important issue to guarantee the best effect of a drug in treatment approaches. Despite numerous applications of conventional drug delivery, it has some uncontroversial disadvantage such as using of high dosage of the drug, unwanted side effects, and several frequencies of drug consumption. Also, the route of drug delivery such as i.m and i.v injections is sometimes undesirable for patient [2]. One of the best approaches for drug delivery without any discomfort and pain for drug recipient is the oral route. Although the condition of gastrointestinal (GI) tract such as very low pH in stomach and high amounts of proteinases in intestine made it difficult especially for protein-based drugs (hormones, growth factors, vaccines and antibodies) [3]. To overcome this physiological problem, several controlled drug delivery systems have been investigated [4,5]. The controlled release formulations offer numerous advantages over the conventional dosage forms which include, reduction in dosage frequency, increase in druguse efficiency, minimizing adverse effects of the drug by localizing it in a particular target area, maintaining the plasma concentration of the drug within therapeutic range [6]. Nanotechnology provides additional parameters on advantages as mentioned above especially when a protein-based drug must be delivered by the oral route. Nanoparticles are promising means, since they have the potential to increase the efficiency of the oral delivery process including protection of encapsulated macromolecules from degradation in the stomach and intestinal lumen, prolonged GI tract residence time by mucoadhesive interactions, and promote epithelial cell targeting and endocytosis [2]. Because of high difference in pH gradient between the acidic environment of the stomach and the more neutral environment of the small intestine, some polymeric nanoparticles such as alginate which exhibit pH-dependent swelling behavior would be appropriate carrier for drug delivery through GI tract [2,7,8]. Alginate (Alg) is a natural unbranched polyanionic polysaccharide of D-mannuronic acid (M) and L-

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guluronic acid (G) isolated from various sources mainly brown algae. Some advantages of this copolymer as a drug carrier in the oral system are excellent cytocompatibility and biocompatibility, biodegradation, sol-gel transition properties, and chemical versatility that make possible further modifications to tailor its properties [1,9]. Alginate hydrogel could be prepared in a simple gelation procedure using of calcium ions [10]. Several studies used of micro and nanoscaled alginate hydrogels for oral delivery of therapeutics such as ibuprofen rifampicin, isoniazid, pyrazinamide, ethambutol, Netrin and Famotidine [11–14]. Prophylaxis mediated by specific chicken egg yolk antibodies (IgY) through oral administration is a promising therapy strategy against gastrointestinal pathogens [15]. *E. coli* O157:H7 is one of these pathogens that several researchers all over the world have been focused on deal with the damages caused by this bacteria and treat them by passive immunization through avian specific antibody [16–19]. The main drawback in oral administration of these antibodies is the sharp decline in their activity when delivery to the gastrointestinal (GI) tract [20]. The Loading of antibody in nanocarriers is an efficient solution to protecting it in GI tract. So, the aim of the present study was to design and preparation and in vitro evaluation of a safe and suitable drug delivery system based on sodium alginate nanoparticle to intestinal delivery and release of specific IgY against colonization of *E. coli* O157: H7.

2. Materials and methods

2.1. Reagent and chemicals

Sodium alginate salt (medium viscosity~3500 cps) potassium dihydrogen phosphate, potassium borate, dimethyl sulfoxide (DMSO) and 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), trypsin–EDTA, penicillin and tetracycline were purchased from Sigma–Aldrich (USA). Hydrochloric acid and ethanol (95%), sodium chloride, calcium chloride, were obtained from Merck (Germany). The cell culture media (DMEM), fetal calf serum (FCS) were provided by Gibco (USA). All chemicals used were of analytical grade (HPLC).

2.2. Cell line and biological molecule

African green monkey kidney (Vero) cell line was purchased from the center of genetic resources (Iran). Purified chicken egg yolk polyclonal antibody (produced against a chimeric antigen consists of virulence factors of *E. coli* O157:H7) was prepared from immunized LSL egg lying hens.

2.3. Preparation of IgY-loaded Alg NPs

Sodium alginate NPs containing specific IgY were prepared by ionic gelation method. First, S-Alg salt (0.2% w/v) was dissolved in DDW by stirring overnight at 4 °C and then passed through 0.45 μm filter (stock solution). Four concentrations (1, 2, 3 and 4 mg) of specific anti-ESI IgY were added to individual 2 ml stirring solutions of Alg within 10 min at room temperature. Finally, fresh $CaCl_2$ solution (0.1% w/v) was extruded dropwisely up to 1500 μl to above mixtures during 10 min in RT in homogenization rate at 1700 rpm for 50 min. The nanoparticles were separated by centrifugation (Sigma, USA) at 11000 rpm for 20 min in 4 °C and supernatants were harvested for the more evaluation. Alginate NPs gels freeze-dried and stored at 4 °C.

2.4. Loading capacity and entrapment efficiency

Loading capacity (LC) and Entrapment efficiency (EE) of IgY in NPs were detected indirectly by determining no-capsulated IgY in the supernatant. For this purpose, the IgY concentration in four supernatants from the previous step estimated via UV spectrometry protein assay. The supernatant of Alg-NPs without IgY was adopted as the blank to correct the absorbance reading value of the IgY-loaded NPs at $\lambda = 280$ nm. The LC and EE values were calculated according to the following equations:

Loading capacity (mg/gr) = (Total amount of IgY (mg)-Free IgY(mg))/(Total amount of Alg NPs(gr))

Entrapment Efficacy (%) = (Total amount of IgY (mg)-Free IgY(mg))/(Total amount of IgY) × 100

2.5. Dynamic light scattering study

The size distribution, particle size and zeta potential of dispersed Alg NPs with and without IgY in the solvent (water) were examined by dynamic light scattering (DLS) technique, using a Zetasizer (Malvern Instrument, UK).

2.6. SEM imaging of samples

The morphology and surface structure imaging of the IgY loaded-Alg NPs conducted using SEM taken with KYKY EM-3200 (China) instrument. The beads were made conductive by sputtering thin coat of gold under vacuum using KYKY SBC12 (China) auto fine coater and then the images were recorded at a 20 kx magnification.

2.7. In vitro release study

Several batches of Alg NPs containing IgY (2 mg) were prepared and after separation from solvent (as described in preparation section), gels were incubated for 0.5, 1, 2, 4, 8 h and 0.5, 1, 2, 4, 8 and 24 h in 1 ml of SGF (simulated gastric fluid, 0.03 M, NaCl without pepsin, pH 1.2) and SIF (simulated Intestinal fluid, 0.05 M, KH_2PO_4 without pancreatin, pH 6.8) buffers, respectively in 37 °C with agitation rate at 70 rpm. After incubation times, gels were precipitated by centrifuge (11000 rpm during 20 min in 4 °C) and IgY releasing from Alg NPs matrices was measured by UV spectroscopy on harvested supernatants. All experiments were performed in triplicate. Studying the in vitro IgY release kinetics was conducted by zero order, first order, Higuchi, and power law models. The adequacy of the delivery profiles of the mathematical models was based on the correlation coefficient value. The study was conducted using the software Graph Pad Prism (Ver. 6).

2.8. Quality and activity of IgY after encapsulation

Same amounts of IgY before and after loading on Alg NPs mixed with sample buffers with and without 2-mercaptoethanol and electrophoresed on 9% SDS-PAGE to comparing the quality and band intensity of samples each other. Also, the activity of IgY after releasing from Alg NPs in comparison with intact IgY was evaluated by indirect ELISA. 5 μg of antigen was coated in 96-well plates overnight at 4 °C and blocked with 5% skim milk in PBS-T (pH 7.4). Plates were washed with PBST and then incubated for 1 h at 37 °C with 100 μl serial diluted IgY (initial concentration of 5 μg) in PBS. Plates were then washed again and incubated with rabbit anti-chicken IgY-HRP (1:2000) (Sigma) for another 1 h at 37 °C. After being washed with PBST and developed with OPD, the absorbance at 495 nm was detected.