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Synthesis and engineering of sodium alginate/inulin core-shell nano-hydrogels for controlled-release oral delivery of 5-ASA

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Abstract:-Protection of drug molecules from digestive enzymes and providing their controlled release at the site of intestinal inflammation are the most important aims of oral nano drug delivery systems (NDDSs). Encapsulation and controlled release of 5-aminosalicylic acid (5-ASA), which is a well-known anti-inflammatory drug, for effective treatment of Crohn's disease, ulcerative colitis and inflammatory bowel disease (IBD) were aimed in this study. For the preparation of core-shell structured NDDSs, 5-ASA loaded sodium alginate (Na-Alg) cores with negative surface charge were prepared by calcium chloride crosslinking of Na-Alg and subsequently coating with quaternized inulin (QIn) *via* Coulomb interaction. As a shell layer, QIn was separately synthesized by quaternization of inulin with glycidyl trimethylammonium chloride (GTMAC). While the crosslinked Na-Alg core is responsible for the protection of 5-ASA from acidic pH in stomach, quaternized inulin shell, known to be highly mucoadhesive, was implemented for digestion by the intestinal flora providing controlled release of 5-ASA at inflammation site of small and/or large intestine. Characterization of NDDSs was carried out using GPC, FT-IR, ^{1}H-NMR and SEM techniques. Ideal slow release of 5-ASA was obtained with the from spherical particles having core and shell sizes of 84-100 and 156-198 nm, respectively.

Keywords: Sodium alginate; inulin; nano-hydrogel; oral delivery; 5-ASA, controlled-release.

1. Introduction

Orally administered formulations are the most favorable pharmaceutical dosage forms due to their compatibility to daily life, lower risk of infection and painless utilization. However, poor water solubility and lack of bioavailability of drug molecules along with their instability in the acidic environment of Gastro Intestinal System (GIS) cause to hinder the beneficial properties of this administration route. Furthermore, mucosal secretion of GIS, which is naturally responsible for protecting the epithelial surfaces from pathogens, is another barrier against effective use of oral formulations [1].

Recently, orally administered nano-drug delivery systems (NDDSs) have become promising vehicles for enhancement of bioavailability of drug molecules by providing protection against the acidic environment of GIS and enhanced penetration through intestinal membrane [2]. NDDSs, composed of bio-degradable and bio-compatible materials, use M cells of Peyer's patches, which are less covered by mucosa and more favorable regions for endocytosis. Furthermore, orally administered NDDSs are able to adhere to the intraluminal site of the intestine, where, the payload is released and slowly absorbed to

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the blood circulation [1]. It has been well defined that oral NDDs demonstrate their superior effects in colon targeting therapy [3] especially in Inflammatory Bowel Disease (IBD) [4]. The orally administrated NDDs are required to possess a neutral surface charge with a size less than 200 nm (preferably around 100 nm).

5-Aminosalicylic acid (5-ASA), an anti-inflammatory drug molecule, has indications in the treatment of Crohn's disease and ulcerative colitis. It prevents development of colorectal cancer in patients with IBD [5, 6]. Before the use of 5-ASA, sulfapyridine was used in treatment of Crohn's disease which was replaced by 5-ASA and its derivatives after revealing the truth that 5-ASA is the active moiety of sulfapyridine. Nowadays, some derivatives of 5-ASA are commercially available including mesalazine, olsalazine and balsalazide. However, their long term use is being hampered by some rare but yet serious side effects such as hepatitis, blood dyscrasias, pancreatitis, pleuropericarditis and interstitial nephritis [7].

One major reason for these side effects is the rapid excretion of drug molecules from the intestinal environment which prevents complete absorption of drug molecules from intestinal membrane, compensation of which needs administrations with higher doses. As mentioned above, mucoadhesive nano-drug delivery systems provide adhesion of drug-carrying vehicle to the GIS membrane, where the payload is released in a controlled slow manner and consequently affords constant concentration of drug in plasma. In this way, the constant therapeutic dose of a drug in bloodstream is achieved with single-dose administration [1].

Biodegradable and biocompatible alginates with numerous pharmaceutical applications are linear mucoadhesives with copolymer structure, consisting of α-L-guluronate and β-D-mannuronate residues [8]. Sodium alginate (Na-Alg) has been used in the production of aqueous polymer dispersions for orally delivering biologically active compounds with properties superior to the conventional dosage forms [9]. Na-Alg has a very fast gelation period and it is possible to control this period using $CaCl_2$. Bivalent Ca^{2+} ions could interact with carboxyl and hydroxyl groups of alginate chain to afford a tough and fragile egg-shell model [10]. The interaction between anionic Na-Alg and cationic biodegradable biopolymers can lead to the formation of poly-ionic nano-hydrogels, which offer all above-mentioned advantages of NDDSs in peroral applications.

Inulin, a natural biopolymer, existing in many edible plants, is a linear polysaccharide, consisted of β-(2,1) linked fructose units [11]. This polysaccharide has attracted the attention of many hydrogel-based studies due to its versatile properties such as possessing functionalizable free hydroxymethyl groups, its stability in upper GIS and degradation only in intestinal environment. Inulinases, produced by *Bifidobacterium spp.* in the colon, are specified enzymes for inulin hydrolysis and thus, inulin based colon-specific drug delivery systems can reach the colon without any degradation by digestive enzymes in the upper part of the GIS system [12-14].

In our previous work, we successfully synthesized thermo-responsive hydrogels, 5-ASA loaded levan-poly(N-isopropyl acrylamide) (NIPA). Levan is a high molecular weight β-(2,6) linked extracellular linear fructan. Its methacrylate derivative was used as a biodegradable cross-linker. Swelling behavior and 5-ASA release profile of the hydrogels significantly varied with composition and temperature [15]. In another work, we prepared pH and temperature-responsive hydrogels for controlled release of 5-ASA using a pH-responsive polysaccharide, chitosan and thermo-responsive NIPA [16]. 5-ASA release profile and hydrogel swelling rate were significantly changed with pH, temperature and hydrogel composition. The tunable release rate of 5-ASA was achieved by changing the hydrogel composition at the pH of small or large intestines.

Based on the above-listed facts, the strategy followed in the current study is to encapsulate 5-ASA molecules in anionic Na-Alg nano-particles (in basic pH) and coat the obtained negatively surface charged nano-particles with quaternized inulin to obtain 5-ASA encapsulated core-shell structured NDDSs. The core-shell structured NDDSs are expected to demonstrate bio-adhesion property due to the presence of high mucoadhesive inulin shell together with their stability in upper GIS and slow degradation in the intestinal environment. It is expected that the encapsulated 5-ASA will be slowly released upon degradation of inulin, exposing to the intestinal membrane and, hence, will present a sustained release of the drug over extended time period into the bloodstream.

2. Experimental

2.1. Materials

5-ASA was purchased from Fluka Chemie AG (Switzerland) and inulin (Molecular weight ≅ 3,500) was kindly supplied by Nante Kimya (Turkey). Na-Alg (Molecular weight ≅ 120,000) was obtained from Nantong Qihai Chemicals International Group. $CaCl_2$, Tween 80 and glycidyl trimethyl ammonium chloride (GTMAC) were purchased from Sigma Aldrich. Phosphate Buffer Saline (PBS) and Na_2CO_3 were supplied by Riedel-de Haen. All chemicals were used as received. The samples were purified by ultrapure (UP) water using dialysis tubing benzoylated with a molecular weight cutoff of 2000 Da, which was supplied by Sigma-Aldrich.

2.1.1 Instrumentation

The degree of quaternization of inulin was determined by using a Varian 600 MHz NMR spectroscopy in D_2O at 25 ^oC. Proton chemical shift values were recorded in ppm downfield from tetramethylsilane (TMS). The molecular weight of inulin in water was determined using Wyatt Dawn Heleos light scattering (LS) instrument. The specific refractive index (dn/dc) of inulin was determined using Wyatt Optilab rex instrument in water. LS data were analyzed by using Zimm plot procedure and the weight average molecular weight of inulin was determined. LS measurements were conducted at 25 ^oC. All the samples were injected through a 200 nm filter. A Thermo Nicolet 6700 Fourier transform infrared spectroscopy (FTIR) with Smart Orbit accessory was used to detect the presence of QIn on the Na-Alg nanoparticles. Drug release studies were performed using a Perkin Elmer Lambda 35 UV-Vis spectrophotometer, which was operated between 200 and 400 nm. The maximum absorbance values of 5-ASA, observed at 330 nm, were converted to concentration using a previously prepared calibration curve. Particle size, particle size distribution and zeta potential values of the emulsion samples were measured using a Microtract Nanowave II model dynamic light scattering instrument installed with a 780 nm wavelength diode laser as a light source. Measurements were performed at 25 ^oC. Scanning electron microscopy (SEM) images were recorded using a HITACHI SU 5000 VP-FEG-SEM&EDS, with Schottky Gun having an acceleration voltage of 30 kV and spot intensity of 10. Before the operation, a drop of emulsion sample was applied on a conductive carbon sheet at room temperature and SEM images were then obtained.

2.2. Methods

2.2.1. Preparation of 5-ASA loaded Na-Alg Core Particles

Na-Alg (3.0 g) was dissolved in 100 mL PBS buffer (pH 7.4). Separately, three different 5-ASA solutions (0.3g, 0.5g, and 0.7g in 10 mL UP water), consisting of 40 µg of Tween-80, were prepared. 5 mL of each 5-ASA solution was mixed with equal amounts of above prepared Na-Alg mixtures. The prepared clear mixture (10 mL) was added dropwise to 25 mL of 2.5% $CaCl_2$ solution, homogenized at 2000 rpm for 10 min, 1000 rpm for 20 minutes and finally at 500 rpm for 30 min. The 5-ASA loaded Na-Alg nanoparticles were characterized by FT-IR and SEM techniques.

2.2.2 Preparation of Quaternized Inulin (Q. Inulin.)

1.0 g of inulin was dissolved in a solution of 0.3 g NaOH in 15 mL UP water. 1.0 mL of Glycidyl trimethylammonium chloride (GTMAC) was injected into the mixture while stirring was continued and the temperature of the mixture was raised to 60 ^oC. These conditions kept constant for 24 hours. At the end of this period the pH was adjusted to neutral using 2% acetic acid. Quaternized inulin (QIn) was dialyzed with distilled water for three days and freeze-dried at -70 ^oC under 0.001 atm vacuum. The conversion was determined by ^1H-NMR spectroscopy.

2.2.3 Preparation of QIn coated NA-Alg nanoparticles

The crosslinked and 5-ASA encapsulated Na-Alg nanoparticles had a negative surface charge and, therefore, they were coated with positively charged QIn *via* Coulomb interaction. For this, to a 10.0 mL of the nanoparticle suspension in UP water, 1.0 mL of QIn solution was added dropwise. The suspension was vortexed for 15 min, freeze-dried and re-suspended with PBS (pH=7.4) for release studies. The SEM analysis was performed to examine the surface morphology, particle size and shape of the nanoparticles. At least three (in general more than five) areas were scanned to obtain an accurate intermediate value for the size measurements of NaAlg/QIn particles. Compositions of the QIn coated NDDSs are given in Table 1.

Table 1. The QIn coated NDDSs compositions

Sample No	Na-Alg		Tween-80		QIn		5-ASA	
	(mg)	(%)	(µg)	(%)	(mg)	(%)	(mg)	(%)
1	216.9	90.4	40	0.0025	12.15	5.01	10.85	4.52
2	210.3	87.6	40	0.0025	12.15	5.01	17.53	7.30
3	204.1	85.0	40	0.0025	12.15	5.01	23.80	9.91

2.2.4 5-ASA release studies

2.0 mL of the re-suspended NDDS dispersion (120 mg/mL) was placed into a Slide-A-Lyzer MINI dialysis microtube (cutoff 3,500 Da, Pierce Rockford, IL). The suspension samples were dialyzed with 45 mL of PBS (pH 7.4) at 37 ºC with gentle stirring. At different time intervals, 3.0 mL of aliquots were taken from the releasing media, which was followed by the renewal of the equal volume of PBS (pH7.4), and analyzed by UV-VIS spectroscopy. The absorbance values were converted to concentration according to a previously prepared calibration curve.

2.2.5 Calculation of encapsulation and loading efficiency

Encapsulation efficiency (EE %) and loading efficiency (LE %) were calculated based on the data obtained from the release studies. As it is well known, the burst release, recorded at the beginning of the study, is due to the release of drug molecules dissolved in the outer aqueous media. The release slowed down by exudation of encapsulated drug molecules to the release media [17]. EE % and LE % are calculated using the below equations:

EE%= (The weight of drug inside of the NDDS/ The initial fed drug)×100 Equation 1
LE%= (The weight of uploaded drug/The weight of NDDS)×100 Equation 2

3. Results and Discussion

3.1. Preparation of 5-ASA encapsulated Na-Alg Nanoparticles

Core-shell structured nano-formulations are one of the most versatile drug delivery platforms. These formulations have many favorable features such that, while the inner core solubilizes the drug, which provides high drug loading capacity and stability, the hydrophilic outer shell can be functionalized with specific functional groups regarding the location where the drug is released. This generally results in longer circulation time without recognition by the macrophages in bloodstream. The gelation properties of Na-Alg has previously been reported for several times [18-20]. For 5-ASA delivery, the inner core was prepared by crosslinked Na-Alg with $CaCl_2$ in the presence of 5-ASA and tween-80 under high speed stirring (2000 rpm) under ambient temperature and pressure. A successful encapsulation of 5-ASA was achieved. Crosslinking reaction mechanism of Na-Alg with bivalent Ca^{2+} ions is given in Figure 1.

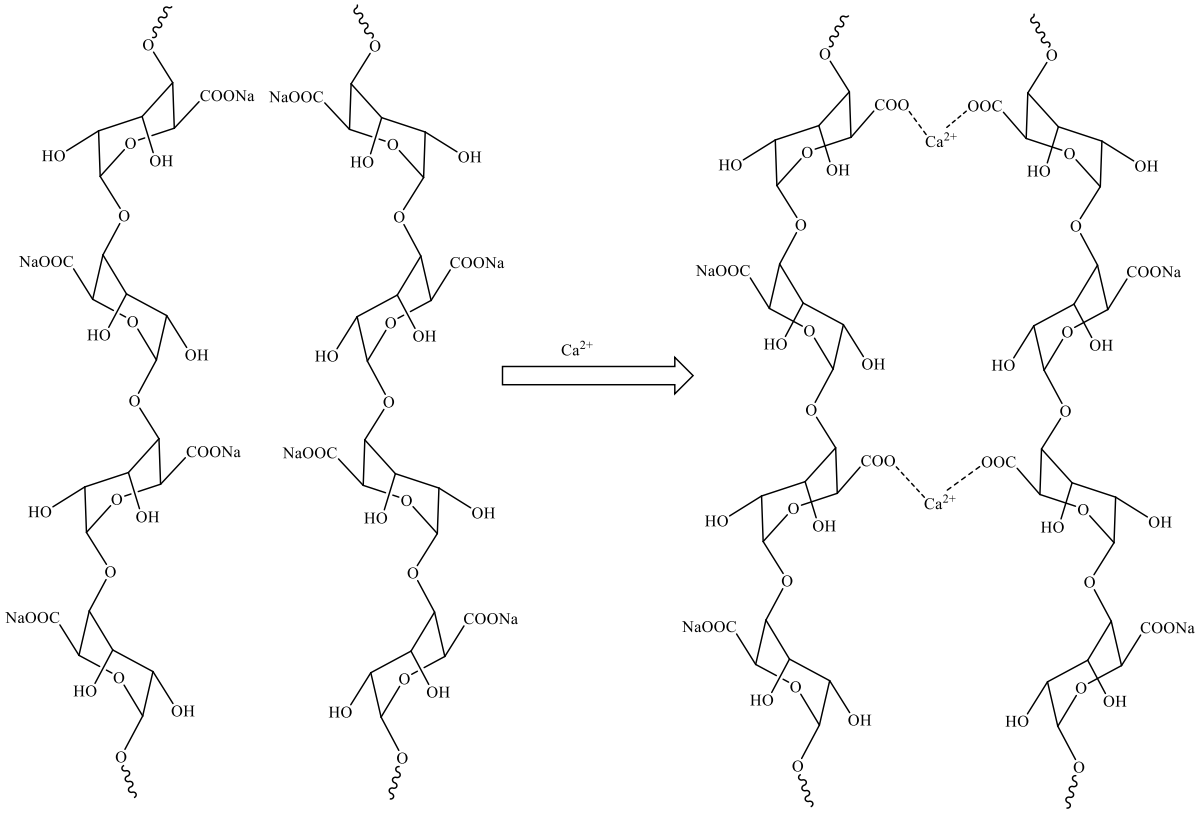

Figure 1. Crosslinking of Na-Alg with bivalent Ca^{2+}

3.2. Preparation of quaternized inulin as outer shell

Inulin was quaternized with glycidyl trimethyl ammonium chloride (GTMAC) to prepare the biocompatible cationic outer shell of the core-shell structured nanoparticles. The reaction mechanism for the synthesis of quaternized inulin is shown in Figure 2.

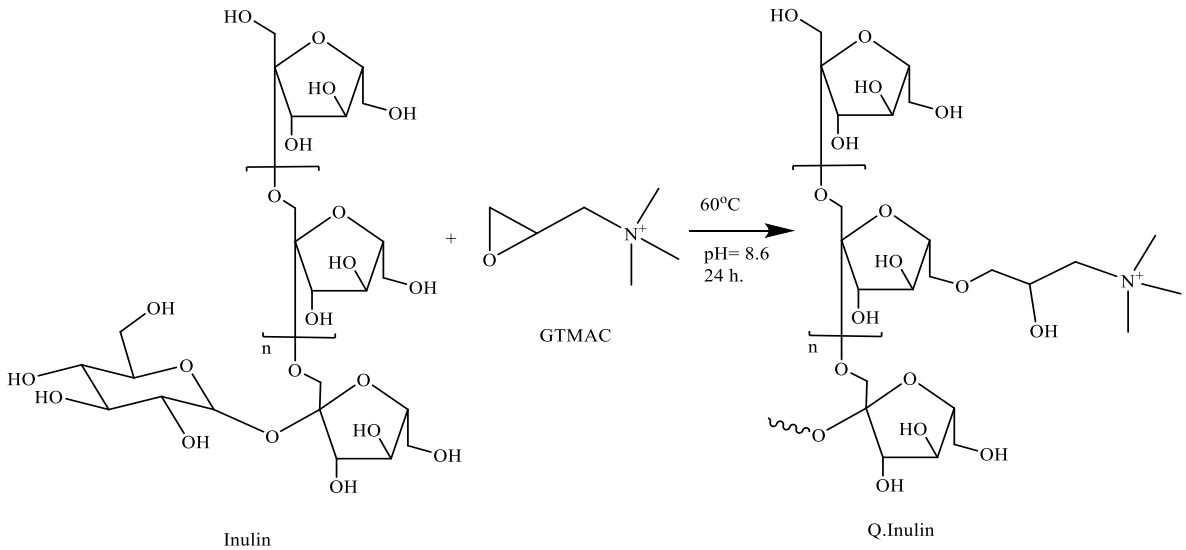

Figure 2. Synthesis mechanism of quaternized inulin which was used as an outer shell of the nanoparticles

3.2.1 FTIR

Comparative FTIR analysis of Inulin and QIn was performed to confirm the quaternization reaction (available in Supporting Information, Figure S1). In the FTIR spectrum of native Inulin, a broad band in the range of 3500–3200 cm^{-1} is due to the -OH stretching absorption of fructose units (Figure S1-purple line). The strong bands, observed at 950, 1000 and 1100 cm^{-1} are due to the symmetric glycosidic C-O-C bending vibration of fructose units. The bands between 2800 cm^{-1} and 3100 cm^{-1} were attributed to the C-H stretching absorption of inulin. Similarly, those characteristic absorption bands of native inulin can be seen in the FTIR spectra of QIn (Figure S1-red line). In addition to the characteristic peaks of native inulin, a strong new band appeared at 1557 cm^{-1} was assigned to the C-N stretching absorption of quaternary ammonium units. This band was not observed in FTIR spectra of native inulin and evidenced the successful quaternization of native inulin. [21]

3.2.2 NMR

Further characterization of QIn was carried out using ^{1}H-NMR spectrophotometry (See Supporting Information, Figure S2). Figure S2-A and S2-B show the ^{1}H-NMR spectra of native inulin and QIn, respectively. In Figure S2A, the ^{1}H-NMR peaks of native inulin (ppm): 4.13(H4), 3.97 (H5), 3.79 (H3), 3.70 (H6) and 3.59 (H1). In Figure S2-B, ^{1}H-NMR peaks of QIn (ppm): 4.13(H4), 3.97 (H5), 3.79 (H3), 3.70 (H6), 3.59 (H1) and 3.11 (H8). From the integration of the peaks at 3.11 ppm (H8) and 4.13 (H4), the quaternization degree of native inulin was calculated to be 30%.

3.2.3 LS results

Figure 3 shows the Zimm plot of Inulin. The number average molecular weight and the specific refractive index (dn/dc) values of native inulin were determined to be 3,560 g/mol and 0.131 mL/g, respectively, in water at 25 ºC.

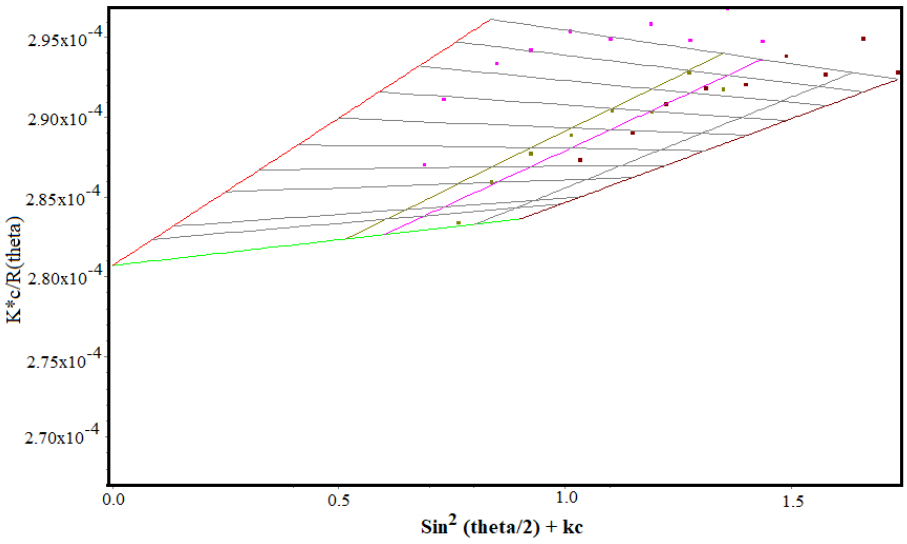

Figure 3. Zimm plot of native inulin
rms radius (Rz): 17.5±23.1 nm and 2nd virial coefficient: (4.755±53.174)e-4 mol mL/g² in water at 25 ºC.

3.3. Characterization of 5-ASA loaded core-shell structured nanoparticles

In our previous study, we reported that sterically stable nanoparticles could be formed by Coulombic interaction between different charged core and shell structures [22]. 5-ASA loaded Na-Alg core particles have a negative surface charge. The schematic diagram of the Coulombic assembly of core-shell structured nanoparticles is presented in Figure 4. The final product contains 5-ASA in the QIn covered core. Bivalent calcium ions crosslink the alginate chains, providing tunable gelation period and stability to the core part of the NDDSs.

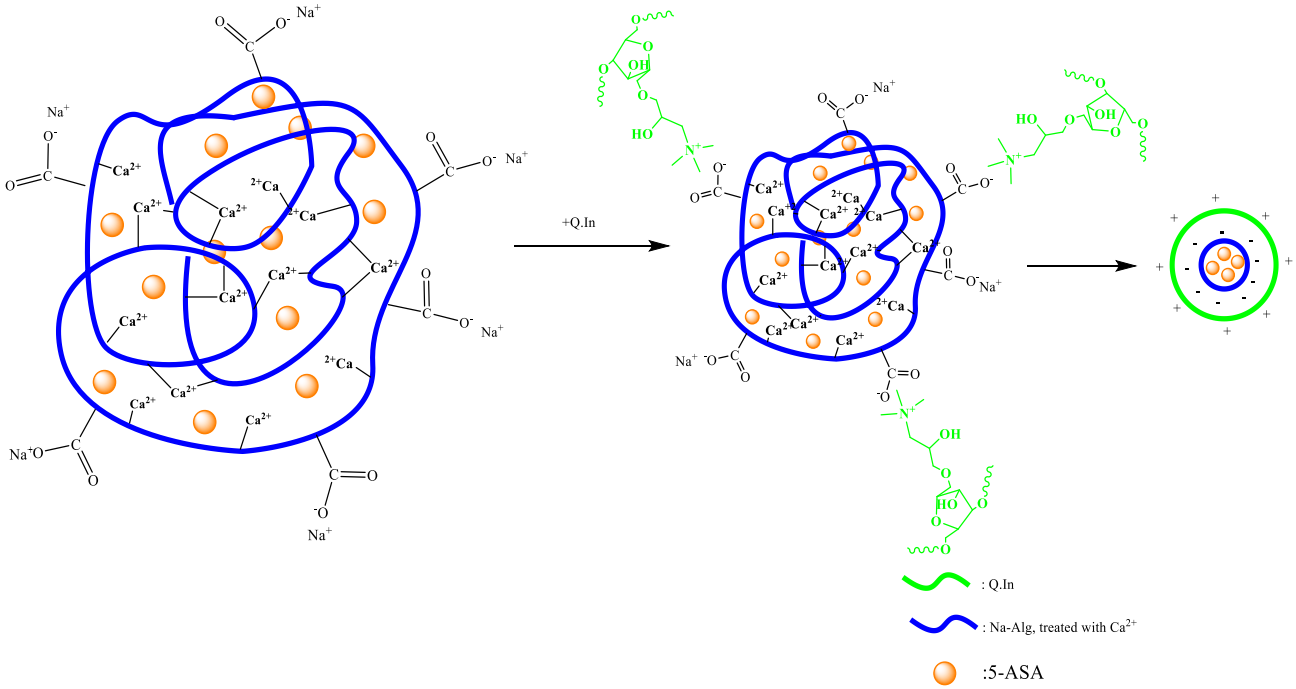

Figure 4. Schematic formation of 5-ASA loaded core-shell structured NDDSs

3.3.1 SEM

The NDDSs consisted of 5-ASA were analyzed with SEM to determine their size and shape before and after coating. As it could be seen in Figure 5a and 5c, both Na-Alg core and the final product, 5-ASA uploaded Na-Alg/QIn, demonstrate a very homogenous spherical shape, which is ideal for drug delivery. Figure 5b and 5d show the particle size measurements assessed on SEM, revealing the enlargement of particles from ≈100nm to ≈180 nm upon their coating with QIn in the final product.

The use of inulin in treatment of GIS disorders is a very well established phenomenon in recent nano-medicine studies. This polysaccharide is known for its protective effect on 5-ASA as it is unaffected by human alimentary enzymes [23, 24]. Although 5-ASA is absorbed in proximal small bowel, [25] effective treatment of IBD becomes possible only if 5-ASA is successfully transported to the site of colonic inflammation. Inulin is the best known for the release of 5-ASA away from proximal small bowel at the site of inflammation [23]. Furthermore, it has been shown that inulin itself has some beneficial effects in treatment of different GIS diseases including Crohn's [24, 26-28]. Thus, it is an ideal carbohydrate polymer for 5-ASA delivery.

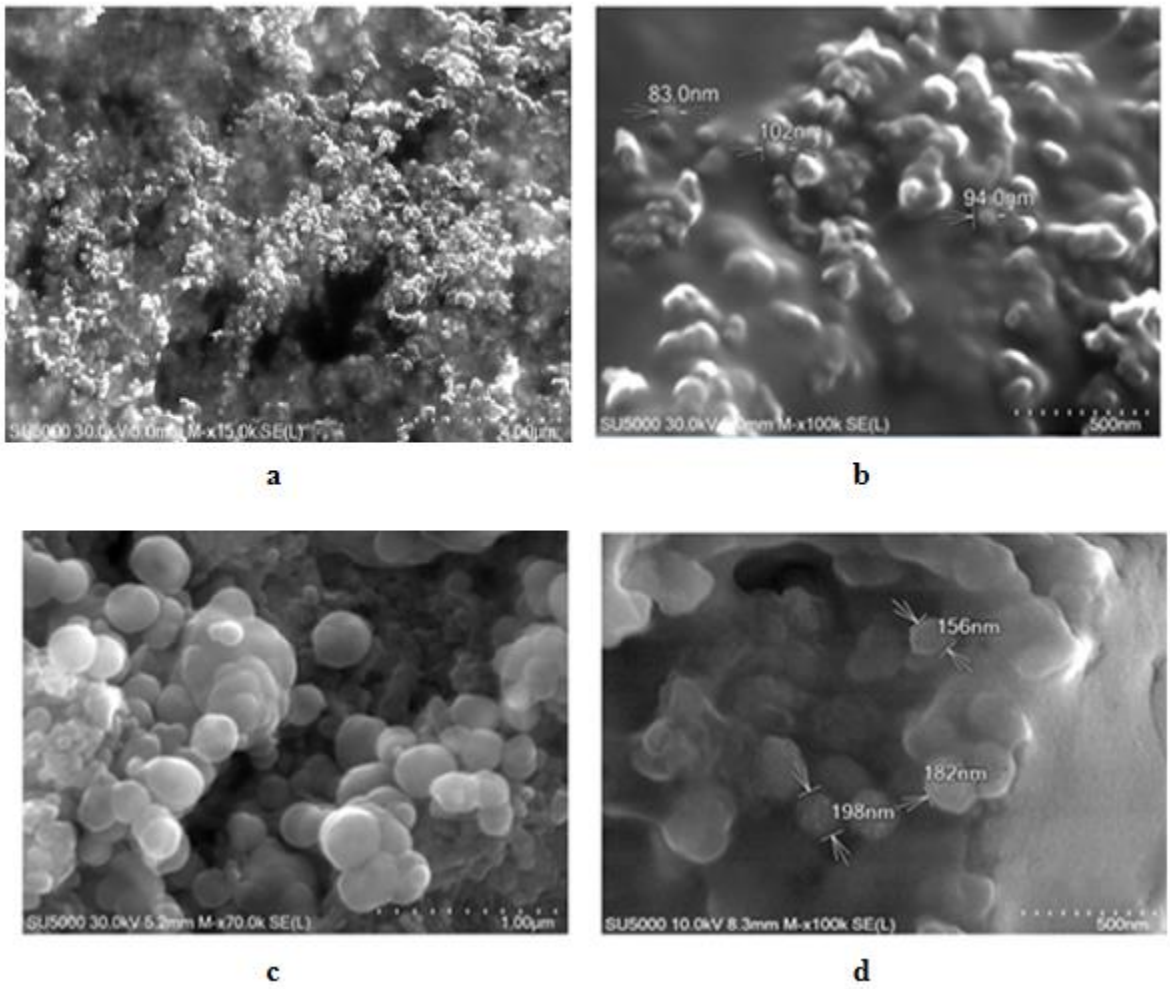

Figure 5. Assessment of particle shape and size of the NDDSs using SEM (composition 3 in Table 1). a: an overview of spherical shape of Na-Alg core, b: measurement of size of Na-Alg core demonstrated their size as 84-100nm. c: an overview of spherical shape of QIn coated final product and d: size measurement of the 5-ASA loaded final product, their size is in the range of 156-198nm

3.3.2 5-ASA release

5-ASA release from the NDDSs was followed by UV-VIS spectroscopy to measure specific absorption peak of 5-ASA at 330 nm. The change in absorbance values with time was converted to concentration with the use of a previously prepared calibration curve, and 5-ASA release profile of the NDDSs in PBS (pH 7.4) was prepared. To mimic the behavior of NDDSs in the intestinal environment, PBS was chosen as the release media at 37 ºC. The release assay was conducted for 72 hours and the samples were gathered from outer media every hour at the first six hours and 24th, 48th and 72nd hours. Figure 6 shows the 5-ASA release profile of the sample no 3 (in Table 1). The synthesized NDDSs showed a fast release at initial 6 hours, which is probably due to the burst release of drug absorbed on the NDDSs core surface [29]. This may indicate the fact that the outer QIn core is able to carry a part of the pay-loaded 5-ASA. After 6 hours, when the entrapped drug molecules start leaving the nano-particles, a very slow release is observed. This is a desired behavior for drug delivery systems engineered as controlled release dosage forms.

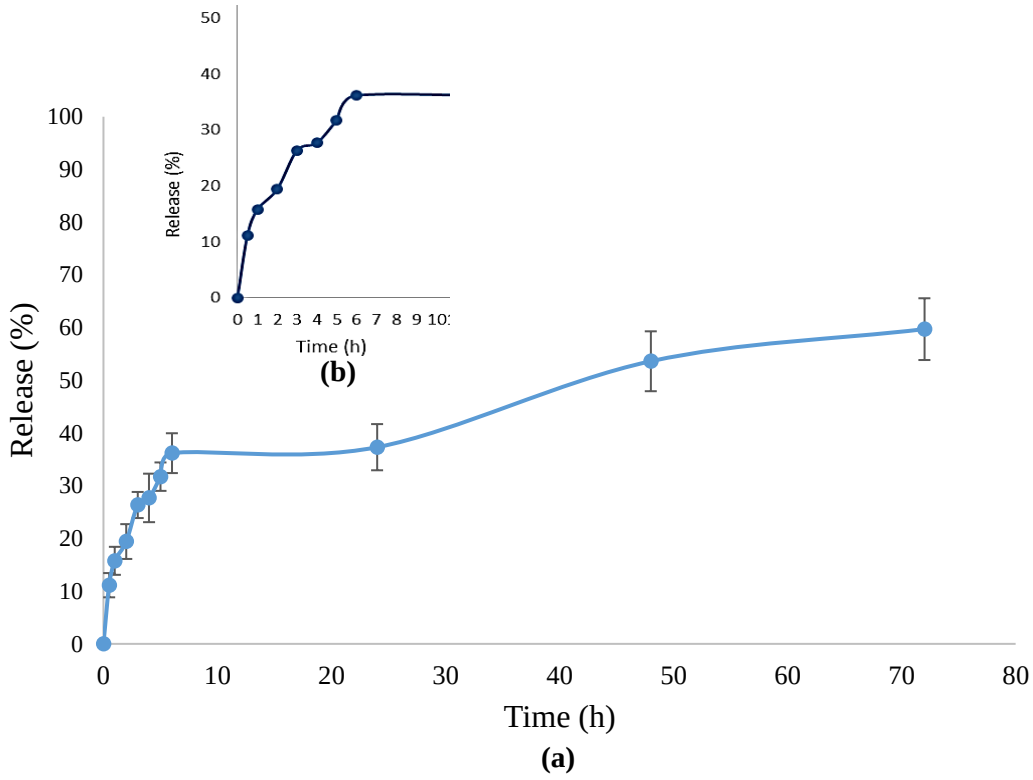

Figure 6. 5-ASA release profile of **(a)** the sample no 3, as described in Table 1 and **(b)** the extended image of release profile of the first hours, used in the calculation of loading efficiency

Although the release ratio of 5-ASA from the designed NDDSs is so low in this essay, it needs to be considered that the digestion of inulin in the bowel will cause the release of the remaining portion of drug in GIS. Inulin, escaping from gastric enzymes is fermented by coeco-colonic flora to gases, lactic acid and short-chain fatty acids, which are beneficial for GI health [24, 30]. Degradation of inulin in the bowel will expose the 5-ASA carrying Na-Alg core particle to the intraluminal site of the intestine, where bio-adhesion of alginate chain takes place and the payload is slowly released.

Alginate has previously been used for 5-ASA encapsulation in several studies [6, 31, 32]. Lin et al. have coated 5-ASA loaded Ca-Alg with different polymers, and, similar to our results, shown the decrease in the release rate of 5-ASA from alginate core upon coating with polymeric chains [32]. The study and results reported by Mladenovska et al. [6] however, is the most similar study to our currently reported data. In their successful attempt for colon-specific delivery and controlled release of 5–ASA, it has been shown that in pH 1.2 (stomach) the protonated alginate (to alginic acid) shows swelling properties, which prolong the drug release [33]. Meanwhile, it has been hypothesized that protonated 5-ASA could electrostatically bond to dissociated carboxyl groups of alginate resulting in even more prolonged release of drug molecules. Probably this hypothesis could explain the very low ratio of the release of drug from ASA-Alg-In, obtained in our study.

Previously, Hartzell etal.[23] prepared a conjugated derivative of 5-ASA with inulin to obtain a controlled release system for 5-ASA and demonstrated that although a very little release of 5-ASA from the drug delivery system was observed *in vitro* (similar to that of our NDDSs) during fermentation with human fecal microbiota, the drug was effectively released from its conjugate with inulin. Based on this observation, we could hypothesize that in our engineered NDDSs, in which 5-ASA is physically entrapped not chemically, the release of the payload is even more effective than previous studies.

3.3.3 Calculation of encapsulation and loading efficiency

As it could be seen in the release profile of 5-ASA from the core-shell structured NDDSs, 10% of the uploaded drug is released at the first hour of the study. The release study was conducted on the

3rd formulation (Table 1), in which the 5-ASA was 23.8mg. Thus, 2.38 mg of the drug was present at the outer aqueous media and caused a burst release effect, and the rest was encapsulated either in the Na-Alg core or in the QIn shell. Based on these data and the values on Table 1, EE% and LE% were calculated as 90% and 9.9%, respectively.

4. Conclusion

To provide controlled release of 5-ASA after peroral administration, quaternized inulin coated Na-Alginate nanoparticles were synthesized, in which 5-ASA was successfully encapsulated, and slow release was obtained in the intestinal pH. Particle size and shape were appropriate for oral drug delivery, and coating the oral delivery system with inulin could provide some additional advantages for treatment of intestinal disorders such as IBD.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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