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Development and *in-vitro* characterization of l-cysteine loaded alginate beads for oral delivery

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ABSTRACT: The purpose of this work is to develop a biodegradable calcium alginate (Ca-alginate) bead formulation to provide controlled release of l-cysteine (Cys) amino acid for oral drug delivery. Ca-alginate bead formulations were prepared successfully and reproducibly using calcium chloride as a cross-linking agent. The beads were then evaluated in terms of morphology, particle size, swellability, thermal behaviors, encapsulation efficiency, and in-vitro drug release at pH 1.2 and pH 7.4. The size of the beads was measured between 2.23±0.11 and 2.41±0.12 mm. In terms of thermal analysis, differential scanning calorimetry was utilized to ensure the encapsulation of Cys into bead formulation. Cys encapsulation was determined as 79.49 %±1.12. Swelling index of blank beads were found to be in a range of 0.31-0.35 in HCl solution at pH 1.2; 0.98-1.08 in PBS buffer at pH 7.4. Alginate beads exhibited controlled release, followed the Weibull model which is consistent with the swelling pattern. The physico-chemical properties of the developed formulation indicate that the formulation is suitable for oral Cys delivery.

KEYWORDS: Ca-alginate bead, sodium alginate; oral drug delivery; l-cysteine; controlled release.

1. INTRODUCTION

Alginate is an anionic polysaccharide, primarily extracted from the cell walls of various species of brown seaweed. β-D-mannuronic acid (M) and α-L- guluronic acid (G) linked by 1→ 4 glycosidic bonds form sodium alginate, which is a linear and amorphous copolymer. The M and G units in the composition of alginate are organized either randomly or non-randomly as heterogeneous or homogeneous sequences. Ionotropic gelation occurs in aqueous solutions of alginates in the presence of di- and tri-valent metal ions such as calcium. Calcium alginate gel beads form via diffusion of Ca^{+2} into alginate droplets and subsequently induction of crosslinking with the guluronic acid residues of alginate molecules. This model is described as the "egg-box" model. As a natural polymer, alginate has received much attention due to its excellent biocompatibility, favorable biodegradability, non-antigenicity, easy gel formation, and low price. As it has high capacity of retaining large quantities of fluid, it is highly suitable as immobilization matrix for various applications such as controlled release drug delivery systems, encapsulation of drugs, genes, living cells and bioactive molecules, wound dressings, dental impression materials, site-specific delivery to mucosal tissue due to bioadhesivity of alginates [1-4]. Using alginate beads to encapsulate peptides, amino acids and proteins provides advantages due to gelation under very mild conditions without the need of high temperatures or chemical crosslinkers. In addition to this, alginate gel beads may be converted to sol by adding chelating agents, such as Na and EDTA [5]. L-cysteine (Cys) is a sulfur containing, non-essential and highly potent antioxidant amino acid that is intra-cellularly synthesized from methionine and serine. Its' antioxidant effect is widely investigated and it has been found that higher reactivity of the thiol groups toward the radical species may be related to the lower ionizing energy of sulfur than that of other atoms in organic molecules [6]. Biological and chemical importance on antioxidant activity of Cys is gaining attention in recent years [7]. Cells utilize Cys for the synthesis of an important tripeptide, glutathione (GSH), which functions as an antioxidant and protects cells and tissues from the deleterious effects of several free radicals [8]. Moreover, Cys is used as a precursor for the synthesis of proteins that are needed by the cells including keratin (hair, skin, and nails are made of) [9]. As a proteinogenic amino acid, Cys is the building block for about 2% of proteins and its ability to form disulfide bridges has crucial importance for protein folding and other specific biological functions [10]. The oral route for drug delivery purposes is the most preferred way since being favorable for sustained and

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controllable drug delivery, easy to administer, applicable for solid formulations, providing painless self-medication and patient compliance. Especially in chronic treatment, oral administration of the drug is the most convenient way for the patient [11]. Regional targeting of drugs in gastrointestinal tract (GI) is also beneficial since it uses significant pharmaceutical advances. Differences in pH through the GI tract provides means for developing delayed release therapies. While gastric environment has a pH between 1.5-2 in the fasted state, it rises to pH 6 in the duodenum and increasing along the small intestine reaches to pH 7.4 at the terminal ileum [12]. As a model amino acid, Cys is known to be highly unstable in air and in aqueous solutions, which easily oxides [13, 14]. The physiological environment of the GI tract can also affect the stability of Cys.

In this work, it has been aimed to prepare and characterize Cys-loaded Ca-alginate gel beads that can be administered orally. It is expected that encapsulation of Cys into alginate gel beads will provide protection for Cys from gastrointestinal environment and delayed amino acid release. Following the encapsulation of Cys into Ca-alginate beads, morphology, thermal behaviors, swelling behaviors, encapsulation efficiency and in-vitro release characteristics of Cys from the beads were analyzed.

2. RESULTS AND DISCUSSION

2.1. Quantification of l-cysteine HCl

Calibration of the method was carried out by measuring a series of standards of diluted stock solutions of Cys using the reaction buffer as described in the Ellman's assay guideline. Absorbance values were plotted against Cys concentrations over a range of 0.25–1.5 mM.

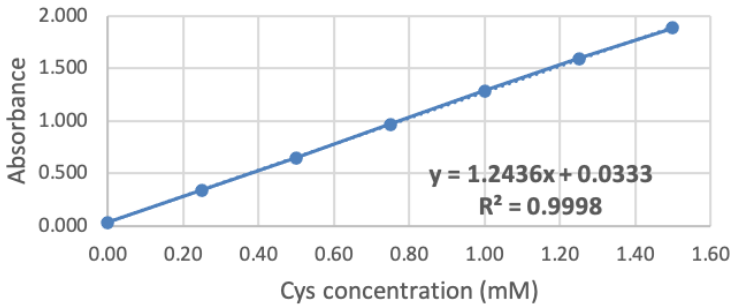

Figure 1. Cys calibration curve with the calibration equation and correlation coefficient.

The linear regression equation was found as y=1.2436x+0.0333 where y is the average absorbance and X is the concentration (mM), with a correlation coefficient of 0.9998. The calibration curve of Cys is represented in Figure 1. Ellman's assay is widely used for quantitation of cysteine in proteins, which is sensitive and accurate enough to quantitate free cysteine levels [15].

2.2. Morphological examination of Ca-alginate beads

Figure 2 shows 24-hour dried blank and Cys loaded alginate beads. The shapes of the beads are almost perfectly spherical with a narrow particle size distribution. Blank alginate beads are transparent whereas Cys loaded alginate beads are semi-opaque, which shows successful encapsulation of Cys into beads. Rehman et al. [16] prepared diclofenac loaded Ca-alginate beads and they showed that while blank loaded bead formulation was clear and transparent, drug loaded formulation had a semi-opaque image similar to our study.

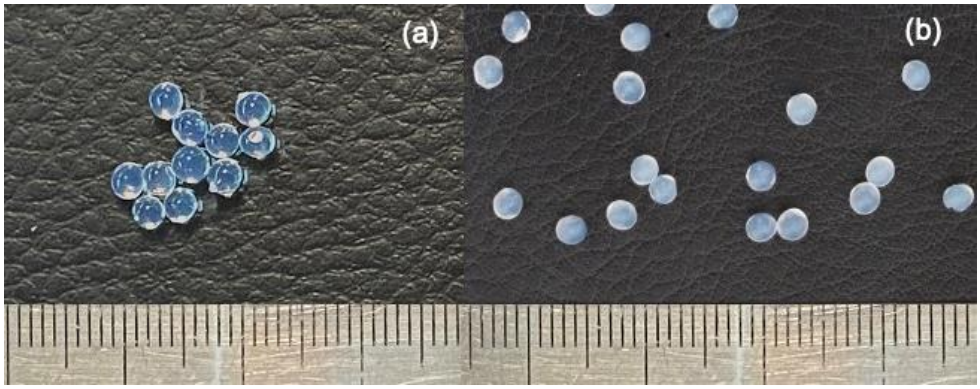

Figure 2. Image of (a) blank and (b) Cys loaded Ca-alginate beads adjacent to a ruler (scale in cm).

As it can be seen from the microscope image (Figure 3) the bead presented a rough surface with characteristic large wrinkles. Although Piras et al. [17] used freeze drying method to minimize drying effects, similar wrinkles were observed on the surface of the bead with scanning electron microscopy.

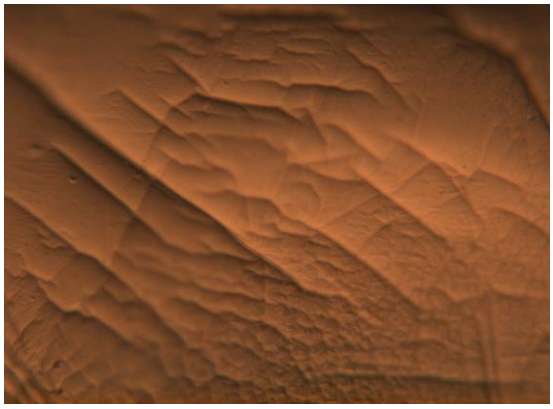

Figure 3. Surface details of the blank Ca-alginate bead formulation obtained by using optical microscope.

Particle size of the blank and Cys loaded beads were found as 2.41±0.12 mm and 2.23±0.11 mm, respectively. Particle size distribution of beads may be evaluated as monodisperse. Piras et al. [17] investigated the effect of droplet volume on particle size of the beads. When the volume of liquid added dropwise was changed as 20, 5 and 1 μL, size of the beads was found to be 3.0–3.6, 1.6–2.0 and 0.75–1.0 mm, respectively. Nochos et al. [18] prepared alginate/hydroxypropyl-methylcellulose (HPMC) hydrogel beads with different concentration combinations of Na-alginate and HPMC and it was found that the size of dried beads was in a narrow range between 1.25-1.5 mm.

2.3. Swelling measurements

Swelling measurements of alginate beads were performed using blank beads which were dried for 24-, 36- and 48- hour to see the effect of drying duration on swelling index. The effect of pH on swelling index was also analyzed (Figure 4). It was observed that in HCl solution at pH 1.2, Ca-alginate beads exhibited a swelling index between 0.31-0.35 whereas in PBS buffer at pH 7.4, it was between 0.98-1.08. Comparisons were performed using the Mann–Whitney, non-parametric two-tailed P test. In each case, the *P* value (p<0.05) indicated significant differences in swelling indexes at different pH levels. Swelling of the wet beads may be explained through the absorption of free or bulk water which fills the voids in the polymer network and/or the center of larger pores and macropores [18].

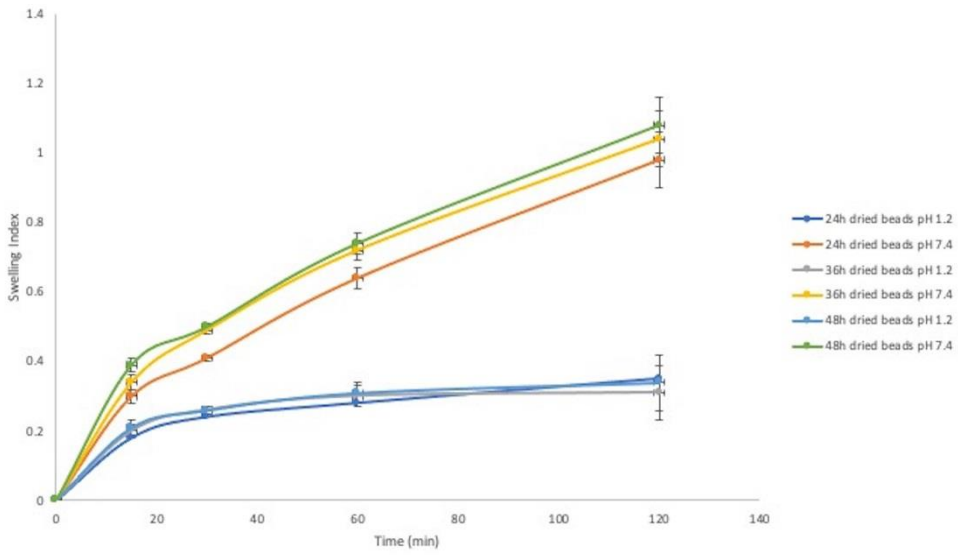

Figure 4. Swelling index changes of Ca-alginate beads.

Swelling process in gel beads takes place with osmotic pressure gradient between the gel bead and the environment. The high osmotic pressure initiates the swelling procedure following the relaxation of polymer network [19]. Swelling index of the Ca-alginate beads in acidic pH is very low, which may be explained by the formation of insoluble alginic acid regions as the solvent penetrates into the dense gel network, as a result of proton-calcium ion exchange [20]. Swelling is an important parameter for drug release process from alginate beads. Gel beads should absorb solvent and swell significantly to achieve drug release. The obtained results showed that dried beads can swell slightly in the acidic environment of stomach, and then when the beads reach to the upper intestine, particles will swell significantly and finally erode into the lower intestine [21].

2.4. Differential scanning calorimetry analysis

The differential scanning calorimetry (DSC) analysis was performed for pure Cys, physical mixture of each component and Cys-loaded alginate beads. Thermograms of three samples are exhibited in Figure 5. The thermogram representing Cys displayed an endothermic peak at 72°C corresponding to its melting point, which complies with that of reported data (Figure 5a).

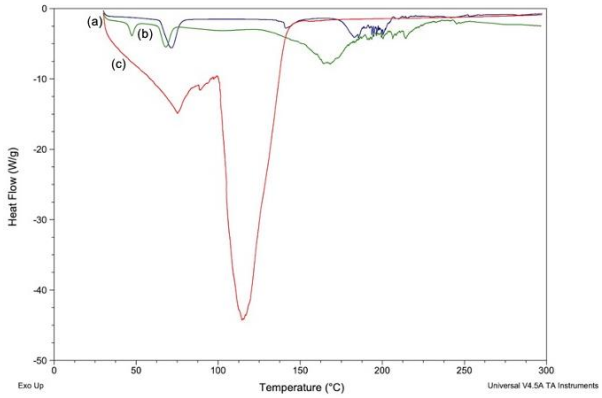

Figure 5. DSC thermograms of (a) Cys, (b) physical mixture of Cys, Na-alginate, $CalCl_2$ (c) Cys loaded alginate beads.

Thermogram of physical mixture of bead components exhibit melting peaks for $CaCl_2$, Cys and Na-alginate at 50, 67, 165°C respectively, which complies with literature [22,23]. Thermogram of Cys loaded alginate beads presented no endothermic peak in the range previously observed with Cys (Figure 5c). This can be explained by the successful encapsulation of Cys into bead formulation in an amorphous state.

2.5. Determination of encapsulation efficiency

Ca-alginate beads were washed three times to analyze encapsulated Cys amount into the beads following the formation of beads. As seen in Figure 6, encapsulation efficiency was found as 79.49 %±1.12 (n=3) after three washing steps.

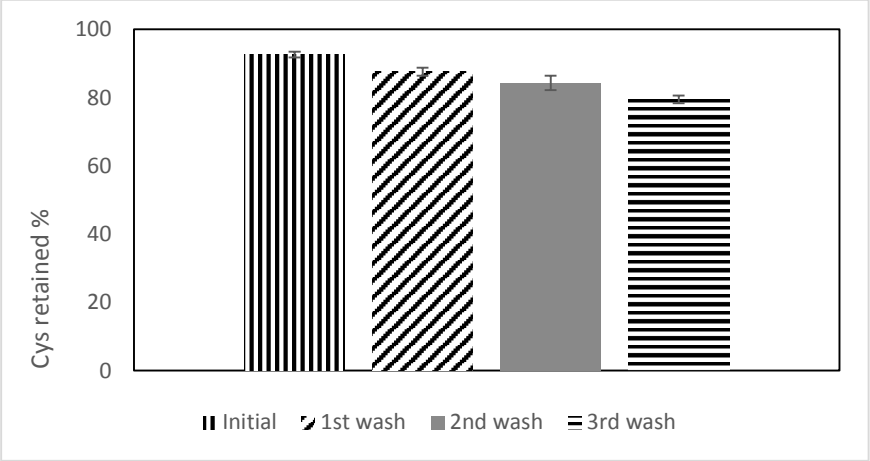

Figure 6. Cys retention in Ca-alginate beads at various stages of bead preparation.

High encapsulation ratio of beads can be explained by small and polar molecular structure of Cys, which facilitates incorporation of Cys into polymer network. Alginate often used for protein delivery including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), bovine serum albumin (BSA), lysozyme, chymotrypsin, myoglobin, cytochrome C due to ease of preparation, biocompatibility, pH sensitivity and high encapsulation efficiency [24]. Sivadas et al. [25] prepared inhalable alginate particles (of an average diameter 3.23±0.25µm) with a high encapsulation efficiency of 97% with the preserved structure and thus bioactivity of BSA. Gu et al. obtained 92.7% encapsulation efficiency of VEGF, which may be attributed by an electrostatic attraction with alginate carboxylate groups [5].

2.6. *In-vitro* drug release studies

The drug release from alginate beads is related to the penetration of dissolution medium into the beads, swelling and dissolution properties of alginate matrix, and the dissolution of the drug subsequent to leaching through the swollen matrix. Figure 7 shows the release profiles of Cys at pH 1.2 and 7.4 from Ca-alginate beads and the free Cys at pH 1.2 and 7.4 which is used as control. The chosen media were used to simulate the condition in gastric and small intestine, respectively. The cumulative Cys release from alginate beads was about 94.33% in phosphate buffer pH 7.4, whereas it was 11.78% in HCl pH 1.2 within 720 min. According to the obtained data, when compared to free Cys, alginate beads provided efficient and sustained drug release through 12 hours.

It is known that bioavailability of Cys is low because it is prone to undergo oxidation in the digestive tract. Ca-alginate bead formulation for oral drug delivery purposes of Cys and other substances, which are susceptible to gastrointestinal tract conditions, will be promising, due to low swelling and low drug release at acidic pH. The drug release kinetics of Cys from alginate beads were calculated by using non-linear regression model of KinetDS. The parameters calculated by these models and the determination coefficients (R^2) obtained are summarized in Table 1. The release kinetic models can be ranked as follows, from best fitted to least: Weibull > Korsmeyer-Peppas > Zero-order > Hickson-Crowell > First order > Higuchi. As can be seen from the regression coefficients, the release kinetics for Ca-alginate beads at two different pH levels are best described by the Weibull model. In literature, there are several studies that experimentally investigated and showed that the release data from swellable polymeric nanoparticles fits best with the Weibull model [26-28].

Table 1. *In-vitro* release kinetic parameters of Cys from Ca-alginate beads at pH 1.2 and 7.4.

Release Medium pH	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Weibull (R^2)	Korsmeyer-Peppas (R^2)	Hickson-Crowell (R^2)
pH 1.2	0.7632	0.1355	-0.8663	0.9947	0.9944	0.4048
pH 7.4	0.9476	0.1629	-0.5821	0.9999	0.9989	0.6477

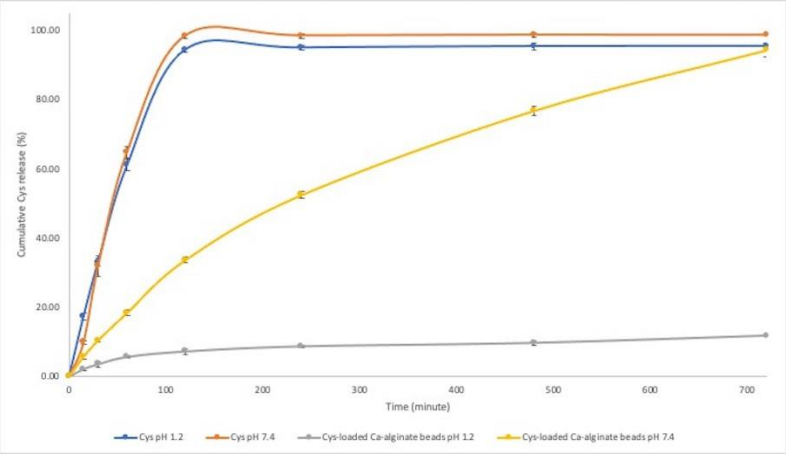

Figure 7. Cumulative release profiles of Cys from calcium-alginate beads in HCl solution pH 1.2 and phosphate buffer pH 7.4. Data are expressed as mean +/− SD. n = 3.

3. CONCLUSION

A delivery system for the controlled release of Cys was designed using Ca-alginate matrix. Besides its antioxidant properties, it has been shown that Cys has other health benefits in a wide range including boosting the immune system [29], lipid biosynthesis and metabolism [30], treatment of diabetes [31], osteoporosis [32], Sjögren syndrome [33], angina pectoris or chest pain [34]. Ca-alginate bead formulations were prepared using ionotropic gelation method successfully. Cys-loaded Ca-alginate beads showed excellent properties of swelling, encapsulation efficiency, morphology, and drug release which could be utilized as a potential drug carrier candidate for oral delivery in the field of biomedicine. Swelling and in-vitro release analysis were performed at two different pH levels, pH 1.2 and 7.4. It has been shown that pH played a crucial role on both of these properties. The results showed that Cys release was low, which may have occured because of the low swelling rate at acidic pH. Alginate beads exhibited controlled release and followed the Weibull model which is consistent with the swelling pattern. Ca-alginate bead formulations might be chosen as a candidate for oral delivery of drugs that are unstable at acidic conditions. Beads could prevent the acidic gastric fluid from destroying the drug carrier, thus avoid liberating the loaded drug.

4. MATERIALS AND METHODS

4.1. Materials

L-cysteine hydrochloride monohydrate and Ellman's Reagent (5,5′-dithiobis(2-nitrobenzoic acid)) were obtained from Sigma-Aldrich (St. Louis, MO). Protanal® LF 10/60 TM Sodium Alginate was purchased from NovaMatrix® Ultrapure Biopolymer Systems (Sandvika, Norway). Calcium chloride (anhydrous) was purchased from Fischer Scientific (Fair Lawn, NJ). All other solvents and reagents used were of analytical grade.

4.2. Methods

4.2.1. Quantification of l-cysteine

Quantification of Cys was performed using Ellman's assay. According to this assay, Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid)), also known as DTNB, is a versatile water-soluble compound for quantification of free sulfhydryl groups in solution. Free sulfhydryl groups in the Cys structure provide ease of quantification. Cys is also a standard solution material in the Ellman's assay protocol. Firstly, reaction buffer (0.1M sodium phosphate, pH 8.0, containing 1mM EDTA) and a set of Cys standards by dissolving Cys at the 1.5, 1.25, 1.0, 0.75, 0.5, 0.25, and 0.0 mM (blank) concentrations in reaction buffer was prepared. A set of tubes containing 50µL of Ellman's Reagent Solution (4 mg/mL) and 2.5 mL of reaction buffer were prepared subsequently. 250µL of each standard solution was added into to tubes that were prepared in the previous step. Tubes were mixed and incubated at room temperature for 15 minutes. Absorbance values were measured at 412 nm with a UV-spectrophotometer. Standard curve was obtained after plotting the values of standards. Using this standard curve, experimental sample concentrations of Cys were determined (n=3) [35, 36].

4.2.2. Preparation of Ca-alginate gel beads

Sodium alginate and calcium chloride ($CaCl_2$) solutions were prepared in ultra-pure water at concentrations of 2.5 and 3% (w/v), respectively. Then, 5 mL of sodium alginate solution was dropped into 10 mL of $CaCl_2$ solution, from a distance of 10 cm on magnetic stirrer (300 rpm) (IKA® RT 10-WERKE, Germany) using a syringe needle with an internal diameter of 0.34 mm. Beads were stirred for 1 hour. After being hardened, the beads were rinsed with water three times and collected after removal of water. Obtained Ca-alginate beads were dried for 24 hours in desiccator [5, 20].

4.2.3. Preparation of Cys-loaded Ca-alginate beads

Encapsulation of Cys into the Ca-alginate beads was performed by adding Cys (100 mg) to the $CaCl_2$ solution. After the complete dissolution of Cys, Na-alginate solution was added into $CaCl_2$ and Cys containing solution drop by drop using the same syringe. Following 1 hour of magnetic stirring, dispersion medium was separated, beads were washed three times with water. All dispersion mediums were analyzed to determine unloaded Cys amount using Ellman's assay [5, 20].

4.2.4. Morphological studies

The appearance of 24 hours dried blank and Cys loaded beads were observed using a digital camera (Sony α6100, Japan). Surface morphology and particle size were investigated with optical microscope (Leica DFC 320, Germany). Particle size analysis was performed using an ocular and stage micrometer, having an accuracy of 0.01 mm, which is fitted to the microscope. 10x resolution was selected to analyze and determine the diameter of 20 randomly selected beads [37].

4.2.5. Swelling properties

The swelling property of the beads was analyzed using classical gravimetrical method [38]. The 24-, 36- and 48-hour dried beads were accurately weighed and then immersed into 30 mL phosphate buffer solution (PBS, pH 7.4) or HCl solution (pH 1.2) at 37 °C, respectively (n=3). The weight of beads was measured at 15, 30, 60 and 120 min after the absorption of excess surface water with filter paper. Swelling degree was calculated via the following equation:

$$\text{Swelling index} = (W_f - W_0)/W_0 \quad \text{(Eq. 1)}$$

where W0 is the initial weight of the beads and Wf is the final weight after swelling [20, 39].

4.2.6. Differential scanning calorimetry analysis

Thermograms of Cys, physical mixture of Cys, Na-alginate and $CaCl_2$ in the same proportion of the formulation and Cys-loaded alginate beads were analyzed using Q-100 DSC (TA Instruments, USA) to determine their thermal behavior and ensure the encapsulation of Cys into the beads. Five mg sample for each measurement was placed into aluminum hermetic pans and heated from 30 to 300 °C at a scanning rate of 10°C/min [40].

4.2.7. Determination of encapsulation efficiency

Following the preparation of Cys loaded beads, dispersion medium and washing solutions were collected and then analyzed spectrophotometrically using Ellman's assay. Encapsulation efficiency was determined indirectly from unloaded drug in dispersion medium using the following formula:

$$\% \text{ Encapsulation Efficiency} = (W - w/W) \times 100 \quad \text{(Eq. 2)}$$

where, W is the initial drug amount added during the preparation and w is the amount of drug (free unloaded drug) in dispersion medium [18].

4.2.8. In-vitro drug release studies

Drug release experiment was conducted on a magnetic stirrer with gentle mixing (100 rpm) at 37 °C. 300 mg of dried Cys loaded alginate beads were immersed into 50 mL of pH 1.2 HCl solution or pH 7.4 phosphate buffer solution (PBS). Sink condition was maintained throughout the dissolution studies. At regular time intervals 1 mL of sample was taken and measured via UV-Vis spectrophotometer at 412 nm using Ellman's assay method. Meanwhile, 1 mL of fresh release medium was added to the beaker to keep the total volume constant. The same amount of Cys which is encapsulated into the beads were used as controls for both release mediums [39].

4.2.9. Statistical analysis

Data are presented as mean±SD. All statistical analyses were undertaken using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA). *p* value of <0.05 was regarded as statistically significant.

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REFERENCES

[1] Miyazaki S, Kubo W, Attwood D. Oral sustained delivery of theophylline using in-situ gelation of sodium alginate. J Control Release. 2000; 67(2-3): 275-280. [CrossRef]

[2] Hu MH, Zheng GS, Zhao DW, Yu WT. Characterization of the structure and diffusion behavior of calcium alginate gel beads. J Appl Polym Sci. 2020; 137(31): 1-9. [CrossRef]

[3] Jeong C, Kim S, Lee CM, Cho SM, Kim SB. Changes in the Physical Properties of Calcium Alginate Gel Beads under a Wide Range of Gelation Temperature Conditions. Foods. 2020; 9(2): 180. [CrossRef]

[4] Hecht H, Srebnik S. Structural Characterization of Sodium Alginate and Calcium Alginate. Biomacromolecules. 2016; 17(6): 2160-2167. [CrossRef]

[5] Gu F, Amsden B, Neufeld R. Sustained delivery of vascular endothelial growth factor with alginate beads. J Control Release. 2004; 96(3): 463-472. [CrossRef]

[6] Daher B, Vucetic M, Pouyssegur J. Cysteine Depletion, a Key Action to Challenge Cancer Cells to Ferroptotic Cell Death. Front Oncol. 2020; 10: 723-731. [CrossRef]

[7] Miura Y, Honda S, Masuda A, Masuda T. Antioxidant activities of cysteine derivatives against lipid oxidation in anhydrous media. Biosci Biotech Bioch. 2014; 78(8): 1452-14555. [CrossRef]

[8] Yildiz D, Arik M, Cakir Y, Civi Z. Comparison of N-Acetyl-L-Cysteine and L-Cysteine in Respect to Their Transmembrane Fluxes. Biol Membrany. 2009; 26(2): 143-148. [CrossRef]

[9] Sameem B, Khan F, Niaz K,Chapter 2.6 - l-Cysteine, Editor(s): Seyed Mohammad Nabavi, Ana Sanches Silva, Nonvitamin and Nonmineral Nutritional Supplements, Academic Press, 2019, pp. 53-58.

[10] K. M. Amino acids and nitrogen compounds. Nutrient Metabolism: Academic Press; 2003. p. 244-268.

[11] Ozturk-Atar K, Kaplan M, Calis S. Development and evaluation of polymeric micelle containing tablet formulation for poorly water-soluble drug: tamoxifen citrate. Drug Dev Ind Pharm. 2020; 46(10): 1695-704. [CrossRef]

[12] Hua SS. Advances in Oral Drug Delivery for Regional Targeting in the Gastrointestinal Tract-Influence of Physiological, Pathophysiological and Pharmaceutical Factors. Front Pharmacol. 2020; 11: 524-545 [CrossRef]

[13] Turell L, Zeida A, Trujillo M. Mechanisms and consequences of protein cysteine oxidation: the role of the initial short-lived intermediates. Essays Biochem. 2020; 64(1): 55-66. [CrossRef]

[14] Nagy P, Ashby MT. Reactive sulfur species: Kinetics and mechanisms of the oxidation of cysteine by hypohalous acid to give cysteine sulfenic acid. J Am Chem Soc. 2007; 129(45):14082-14091. [CrossRef]

[15] Wright SK, Viola RE. Evaluation of methods for the quantitation of cysteines in proteins. Anal Biochem. 1998; 265(1): 8-14. [CrossRef]

[16] Rehman S, Ranjha NM, Raza MR et al. Enteric-coated Ca-alginate hydrogel beads: a promising tool for colon targeted drug delivery system. Polym. Bull. 2021; 78: 5103-5117. [CrossRef]

[17] Piras CC, Slavik P, Smith DK. Self-Assembling Supramolecular Hybrid Hydrogel Beads. Angew Chem Int Edit. 2020; 59(2): 853-859. [CrossRef]

[18] Nochos A, Douroumis D, Bouropoulos N. In vitro release of bovine serum albumin from alginate/HPMC hydrogel beads. Carbohydr. Polym. 2008; 74(3): 451-457. [CrossRef]

[19] Pasparakis G, Bouropoulos N. Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. Int. J. Pharm. 2006; 323(1-2): 34-42. [CrossRef]

[20] Patel N, Lalwani D, Gollmer S, Injeti E, Sari Y, Nesamony J. Development and evaluation of a calcium alginate based oral ceftriaxone sodium formulation. Prog Biomater. 2016; 5(2): 117-133. [CrossRef]

[21] Manjanna K.M SB, Pramod Kumar T.M. Diclofenac Sodium Microbeads for Oral Sustained Drug Delivery. International Journal of PharmTech Research. 2009; 1(2): 317-327.

[22] Lozano-Vazquez G, Lobato-Calleros C, Escalona-Buendia H, Chavez G, Alvarez-Ramirez J, Vernon-Carter EJ. Effect of the weight ratio of alginate-modified tapioca starch on the physicochemical properties and release kinetics of chlorogenic acid containing beads. Food Hydrocolloid. 2015; 48: 301-311. [CrossRef]

[23] Barreneche C, Fernandez AI, Cabeza LF, Cuypers R. Thermophysical characterization of sorption TCM. Enrgy Proced. 2014; 48: 273-279. [CrossRef]

[24] Hariyadi DM, Islam N. Current Status of Alginate in Drug Delivery. Adv Pharmacol Pharm Sci. 2020; 2020: 1-16. [CrossRef]

[25] Sivadas N, O'Rourke D, Tobin A, Buckley V, Ramtoola Z, Kelly JG, Hickey AJ, Cryan SA. A comparative study of a range of polymeric microspheres as potential carriers for the inhalation of proteins. Int J Pharm. 2008; 358(1-2): 159-167. [CrossRef]

[26] Ashrafi H, Azadi A. Chitosan-based hydrogel nanoparticle amazing behaviors during transmission electron microscopy. Int J Biol Macromol. 2016; 84: 31-34. [CrossRef]

[27] Jafari-Aghdam N, Adibkia K, Payab S, Barzegar-Jalali M, Parvizpur A, Mohammadi G, et al. Methylprednisolone acetate-Eudragit((R)) RS100 electrospuns: Preparation and physicochemical characterization. Artif Cell Nanomed B. 2016; 44(2): 497-503. [CrossRef]

[28] Liu CK, Wu YG, Zhao LY, Huang XZ. Preparation of acetylsalicylic acid-acylated chitosan as a novel polymeric drug for drug controlled release. Int J Biol Macromol. 2015; 78: 189-194. [CrossRef]

[29] Ghezzi P. Role of glutathione in immunity and inflammation in the lung. Int J Gen Med. 2011; 4: 105-113. [CrossRef]

[30] Hook VYH, Reisine TD. Cysteine proteases are the major beta-secretase in the regulated secretory pathway that provides most of the beta-amyloid in Alzheimer's disease: Role of BACE 1 in the constitutive secretory pathway. J Neurosci Res. 2003; 74(3): 393-405. [CrossRef]

[31] Jain SK. L-Cysteine supplementation as an adjuvant therapy for type-2 diabetes. Can J Physiol Pharm. 2012; 90(8): 1061-1064. [CrossRef]

[32] Ji HJ, Liu YK, Zhao XL, Zhang M. N-acetyl-L-cysteine enhances the osteogenic differentiation and inhibits the adipogenic differentiation through up regulation of Wnt 5a and down regulation of PPARG in bone marrow stromal cells. Biomed. Pharmacother. 2011; 65(5): 369-374. [CrossRef]

[33] Walters MT, Rubin CE, Keightley SJ, Ward CD, Cawley MI. A double-blind, cross-over, study of oral N-acetylcysteine in Sjogren's syndrome. Scand J Rheumatol Suppl. 1986; 61: 253-258.

[34] Horowitz JD, Henry CA, Syrjanen ML, Louis WJ, Fish RD, Smith TW, et al. Combined Use of Nitroglycerin and N-Acetylcysteine in the Management of Unstable Angina-Pectoris. Circulation. 1988; 77(4): 787-794. [CrossRef]

[35] Schmitz T, Grabovac E, Palmberger TE, Hoffer MH, Bemkop-Schnurch A. Synthesis and characterization of a chitosan-N-acetyl cysteine conjugate. Int J Pharm. 2008; 347(1-2): 79-85. [CrossRef]

[36] Branigan E, Pliotas C, Hagelueken G, Naismith JH. Quantification of free cysteines in membrane and soluble proteins using a fluorescent dye and thermal unfolding. Nat Protoc. 2013; 8(11): 2090-2097. [CrossRef]

[37] Iswandana R, Putri KSS, Wulandari FR, Najuda G, Sari SP, Djajadisastra J. Preparation of Calcium Alginate-Tetrandrine Beads Using Ionic Gelation Method as Colon-Targeted Dosage Form. J App Pharm Sci. 2018; 8(05): 68-74. [CrossRef]

[38] Rasmussen MR, Snabe T, Pedersen LH. Numerical modelling of insulin and amyloglucosidase release from swelling Ca-alginate beads. J Control Release. 2003; 91(3): 395-405. [CrossRef]

[39] Yin ZC, Wang YL, Wang K. A pH-responsive composite hydrogel beads based on agar and alginate for oral drug delivery. J Drug Deliv Sci Tec. 2018; 43: 12-18. [CrossRef]

[40] Smrdel P, Bogataj M, Podlogar F, Planinsek O, Zajc N, Mazaj M, et al. Characterization of calcium alginate beads containing structurally similar drugs. Drug Dev Ind Pharm. 2006; 32(5): 623-633. [CrossRef]