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Research Article

Encapsulation and release of a bacterial carotenoid from hydrogel matrix: Characterization, kinetics and antioxidant study

Various natural polymers with hydrophilic properties have been used to form hydrogels for the encapsulation and delivery of nutrients and drugs in food and pharmaceutical industries. Among them, chitosan (ChiHG)- and alginate (AlgHG)- based hydrogels have been extensively explored for delivery of several nutraceuticals in recent years. Release of natural canthaxanthin (CX) obtained from *Dietzia maris* NITD (accession number: HM151403) has been investigated with emphasis on biomedical applications. Significant changes ($P < 0.05$) in degree of swelling (%) and moisture content (% dry basis) were found after encapsulation of bacterial canthaxanthin (BCX), but the gel content remained unchanged. BCX encapsulation efficiency was calculated to be 55.92% and 60.45% in ChiHG and AlgHG, respectively. A noticeable change in heat of fusion (ΔH_m) and melting point (T_m) was recorded in ChiHG and AlgHG after BCX encapsulation. Swelling and BCX release from gel matrix was performed under two different pH (1.2 and 7.4). The results showed that swelling of hydrogel and BCX release was facilitated at higher pH (7.4) than acidic pH (1.2). With regard to the release kinetics data, it was found that BCX is released from both ChiHG and AlgHG in a non-Fickian diffusion transport method. In addition, antioxidant activity of BCX encapsulated hydrogels was found significantly higher ($P < 0.001$) in terms of DPPH, ABTS, nitrite, hydroxyl radical scavenging and reducing power assay. These results indicated that BCX can be successfully encapsulated into a polymeric hydrogel to obtain a dynamic biomaterial that may be used in drug delivery applications in future.

Keywords: Antioxidant activity / Canthaxanthin / Hydrogel / Pharmaceuticals / Release kinetics

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1 Introduction

Carotenoids feature high on the list of food components that are of interest in relation to human health [1]. Microbial carotenoids are gaining worldwide attention due to their strong colour, an-

tioxidant, anticancer and anti-inflammatory and immunomodulatory activities and thus may be used as colour additives for food and feed and are now under consideration for use as health supplements and 'nutraceutical' sectors [1]. A nutraceutical is any substance that is a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease [2]. Canthaxanthin (CX), an orange red keto-carotenoid showed strong antioxidant property [3], thus is showing promising nutraceutical aspects in future. The leading constraint for the carotenoid market is their instability during thermal and oxidative processing [4]. It is also well-known that high temperature and light conditions may promote the isomerization to the cis forms of carotenoids and vice-versa which can affect their biological activities, however, the cis isomers of the carotenoids have less activity than their corresponding all-trans carotenoids [5]. As most carotenoids, CX shows high intrinsic

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Abbreviations: ABTS, 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); AlgHG, alginate based hydrogel; APS, ammonium persulfate; BCX, bacterial canthaxanthin; ChiHG, chitosan based hydrogel; CX, canthaxanthin; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; HRSA, hydroxyl radical scavenging activity; KBr, potassium bromide; NSA, nitrite scavenging activity; PBS, Phosphate buffer saline; RP, Reducing power assay; T_m , melting temperature ($^{\circ}\text{C}$); ΔH_m , heat of fusion

instability and lipophilic behaviour which can cause degeneration of its nutraceutical property. As a result, to handle the carotenoids, stabilized emulsions or encapsulated conditions are now being preferred [6]. The encapsulation process is generally performed by forming a polymeric matrix or coating layer around a particular compound in order to protect its biological activity from environmental factors and enhance its physico-chemical stability. Methods employed are based on preparation of emulsion between the carotenoid and the encapsulating matrix, followed by spray drying of the emulsion. The main disadvantage of these methods is that carotenoid losses occur during the process and the product obtained does not present a good stability [5]. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids [7]. They are receiving prominence in medical field like wound dressing, tissue engineering and drug delivery applications due to its biocompatibility, biodegradability and excellent gel forming properties. Natural biopolymers such as chitosan and alginate are being widely employed to encapsulate various pharmacologically important molecules for localized delivery as their precise ability to carry drugs within their cross linkage properties [8–11]. Fully swollen hydrogel features such as soft and rubbery consistency, low interfacial tension with biological fluid, muco-adhesive and bio-adhesive characteristics which enhance their site specific binding to infected region thus make them appropriate as drug delivery vehicle [11]. However, another known advantage of chitosan or alginate hydrogel is that, they can be modified according to adjacent environments such as pH, temperature, ionic strength, electric current, so on [9] thus making them appropriate for therapeutic modulations.

In this study, an attempt has been made to exploit natural polymers viz., chitosan and sodium alginate to prepare hydrogels encapsulated with CX. The CX was produced from a gram positive bacterium previously isolated in our laboratory. Hydrogels with encapsulated bacterial canthaxanthin (BCX) were characterized by SEM, FT-IR, DSC and XRD. Release of BCX from gel matrices was analysed by different drug release kinetic models to elucidate probable release mechanism. Antioxidant activity of BCX encapsulated in hydrogel matrix was also done. Encapsulation and controlled release of BCX from any hydrogel matrix has always remained a challenge for researchers. However, some recent study revealed that stable BCX solution may be prepared through emulsification with natural polymers which may enhance the solubility and stability of BCX [6].

2 Materials and methods

2.1 Materials

Chemical for hydrogel preparation such as chitosan (PubChem CID: 3086191, SRL, Mumbai, India, deacetylation degree 90%, viscosity-150 cps), acrylic acid (PubChem CID: 6581, Sigma-Aldrich, USA), Methyl acrylate (PubChem CID: 7294, Sigma-Aldrich, USA), ammonium persulfate (APS) (PubChem CID: 62648, Merck, India), NaOH (PubChem CID: 14798, Merck, India), glacial acetic acid (PubChem CID: 176, Merck, India), Sodium-alginate (PubChem CID: 5102882, Hi-media, India, viscosity- 5.5 ± 2 cps, 100 – 200kg/mol, guluronic acid content

70 %), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (PubChem CID: 6093260, Merck, India) were ACS reagent grade. Chemicals related to antioxidant assays were also purchased from Merck, India (chemical conforms to specifications defined by the Committee on Analytical Reagents of the American Chemical Society). Media ingredients for bacterial CX production viz. D-glucose (PubChem CID: 5793), yeast extract, peptone and sodium chloride (PubChem CID: 5234), were purchased from Hi-media, India. All solvent used in this study were HPLC grade (Sigma-Aldrich, USA).

2.2 Production of carotenoid

Dietzia maris NIT-D (accession number: HM151403) [12] was previously isolated in our laboratory during routine culture of microorganisms and was established as effective canthaxanthin producer [13]. Optimized medium (D-glucose: 15g, Yeast extract: 5g, peptone: 10g, sodium chloride: 5g for 1000 mL distilled water) supplemented with coconut water was used for large scale pigment production at initial broth pH 6.7, 120 rpm and 25°C [3]. Pigment extraction was done according to the procedure of Goswami et al. [12]. Extracted pigment was concentrated using rotary evaporator (Yamato, model RE301) and freeze dried (Digitech, India). Dried BCX was stored at 4°C for further experiments.

2.3 Preparation of CX encapsulated chitosan hydrogel

Chitosan hydrogel (ChiHG) was prepared following the method of Borzacchiello et al. [14] with modifications. Briefly, chitosan powder (10% wt/v) was dispersed in 1% ammonium persulfate (APS) (wt/v). Equal volume of BCX (500µg/ml) was added. Acrylic acid and methyl acrylate mixture (70:30 ratio) was added to the solution and mixed thoroughly. Obtained viscous material was then incubated overnight at 37°C followed by incubation at 50°C for 6 h. Resulting material was swollen in distilled water at room temperature for 2 h. Final product was washed with 5% NaOH (wt/v) for next 72 h. The hydrogel was washed in regular interval. Resulting gel was finally washed with distilled water vigorously and lyophilized. Dried gel was stored at 4°C for further use.

2.4 Preparation of CX encapsulated alginate hydrogel

Alginate hydrogel (AlgHG) was prepared according to the method of Morais et al. [15] with modifications. Briefly, in a 250ml glass beaker (65mm diameter), 5% wt/v sodium alginate solution was prepared and equal volume of ethanol with known concentration of CX (500µg/ml) was added. The mixture was stirred thoroughly and ice cold 1M CaCl_2 was added in a ratio of 1 : 2 (v/v). For further hardening, the newly formed AlgHG was re-suspended into fresh CaCl_2 solution for 24 h at 4°C. Finally, the gel was washed with distilled water and air dried followed by lyophilisation. Obtained dried gel was stored at 4°C for further use.

2.5 Characterization of ChiHG and AlgHG

2.5.1 Moisture content, degree of gelation, encapsulation efficiency and swelling behaviour of BCX hydrogels

Moisture content of the freshly prepared hydrogel was determined by a Moisture Analyzer (Mettler Toledo-HS153, Switzerland). Briefly, 0.5g sample was taken in an aluminium pan and heating programme was set at 105°C. Start weight tolerance was 10%. Switch-off criteria was set as 1mg/50s. Result was recorded as % of moisture content (dry basis).

Measurement of gel content was done by following the method of Sokker et al. [7] with minor modification. Briefly, required amount of hydrogel samples was extracted in deionized water at 75°C for overnight. Then the gel was placed in 250 mesh stainless steel net and washed with deionized water multiple times. Remaining gel was dried and gel content was measured gravimetrically as

$$\% \text{ of gelation} = \frac{W_f}{W_i} \times 100$$

where W_i and W_f is the initial weight of dry gel and the weight of dry gel after extraction, respectively.

BCX encapsulation efficiency for the hydrogel matrices was determined following the method of Ramyadevi and Rajan [16] using the following formula:

$$\begin{aligned} \text{Encapsulation (\%)} \\ = \frac{(\text{total BCX content} - \text{BCX content in the supernatant})}{\text{total BCX content}} \times 100 \end{aligned}$$

Swelling (SW) of hydrogel was determined by method of Borzacchiello et al. [14].

The degree of swelling was calculated from the following equation:

$$SW = \frac{W_s - W_d}{W_d} \times 100$$

where W_s and W_d are the weight of hydrogel samples at equilibrium swelling state and dry state, respectively. Double distilled water was used at 37°C for this study. SW was done in two types of dissolution medium containing tris-HCl of pH 1.2 (0.1 N HCl) and PBS buffer of pH 7.4 [17].

2.5.2 Determination of surface morphology using SEM

Morphological changes of ChiHG and AlgHG surfaces in native gel form (control) and after BCX encapsulation was investigated with Scanning Electron Microscopy (SEM) (Hitachi, S – 530; Resolution: 5nm; Magnification: 20 – 150, 000×) using an accelerating voltage of 30 kV. The samples were sputtered with gold using gold sputter coater under vacuum before analysis [17].

2.5.3 Thermal property of CX hydrogel using DSC

Thermal analysis was performed using a differential scanning calorimeter (Mettler-Toledo DSC1, Switzerland). Each hydrogel sample (6 – 10 mg) was accurately weighed and placed in an aluminium DSC pan and sealed. The measurements were

performed over the range of 0°C–400°C at a heating rate of 10°C/min [18,19].

2.5.4 Characterization of hydrogel using FT-IR

Fourier Transform Infrared Spectroscopy (FTIR) was done by an IR spectrophotometer (Thermo-Nicolet 6700, USA). Hydrogel samples were subjected to KBr pellet formation using a hydraulic press at 600 kg/cm² prior to FT-IR. Percentage of transmission (%T) of the prepared samples was recorded in a range of 400 – 2000 cm⁻¹.

2.5.5 Characterization of hydrogel using XRD

X-Ray Diffraction (XRD) patterns of BCX encapsulated ChiHG and AlgHG were compared using the X-ray diffractometer (Rigaku Ultima III, USA) with Cu-K β radiation at 2 θ value ranging from 0°–60° with a scanning speed of 0.01°/min.

2.6 Kinetics of CX release from ChiHG and AlgHG

Release of BCX from ChiHG and AlgHG was performed by changing the pH condition from acidic to neutral value [16,17,20]. It was done in two type of dissolution liquid containing tris-HCl of pH 1.2 (0.1 N HCl) and PBS buffer of pH 7.4 which corresponded to gastric fluid pH and intestinal fluid pH, respectively. Required amount of BCX encapsulated ChiHG and AlgHG was taken in enclosed semipermeable bag and placed in a 250 mL glass beaker (65mm diameter) containing dissolution liquid of pH 1.2 for first 15 min and then with PBS buffer solution 7.4 separately [17]. Temperature was maintained at 37°C. Solution from the beaker was taken out at regular interval and BCX concentration was analysed by UV spectrophotometer [9,17]. Release of BCX from ChiHG and AlgHG was calculated from a formula as follows:

$$\text{Cumulative BCX release (\%)} = \left(\frac{\text{released BCX}}{\text{total loaded BCX}} \right) \times 100$$

Possible mechanism of BCX release was determined by fitting the release data into various models like zero-order, where drug dissolution do not disaggregate and product is released slowly. It can be represented by the equation as follows [21]:

$$Q_t = Q_0 + K_0 t$$

where Q_t is the amount of drug dissolved in time t , Q_0 is the initial drug amount present in the solution and K_0 is the zero order release constant. Zero-order kinetic model was obtained by cumulative % of drug released vs. time. In contrast, first order model, release is dependent on drug concentration [22]. The equation is as follows:

$$\log C = \log C_0 - \frac{Kt}{2.303}$$

where C_0 the initial concentration of the drug, C is the drug released at time ' t ' and ' K ' is the first order rate constant. The data obtained were plotted as log cumulative % of drug remaining vs. time which yields a straight line with slope of $-\frac{Kt}{2.303}$. The simplified Higuchi model describes the drug release from planar,

different geometrics and porous system [21]. The equation is as follows:

$$Q = K_H \times t^{\frac{1}{2}}$$

where Q is the cumulative amount of drug released and K_H is the Higuchi dissolution constant. The data obtained were plotted as cumulative % drug release vs. square root of time. Korsmeyer–Peppas is one of the kinetic models where drug release is analysed from both swelling and non-swelling polymeric matrix [23]. The working formula of this model is as follows:

$$\frac{M_t}{M_\infty} = K \times t^n$$

where $\frac{M_t}{M_\infty}$ is the cumulative amount of drug released at time t , K is the kinetic constant and n is the diffusion exponent indicative of the mechanism transport of the drug through the polymer. The data was plotted as log cumulative % of drug release vs. log time. Hixon–Crowell model is based on the idea that particles regular area is proportional to the cube root of its volume [21]. The working equation is as follows:

$$W_0^{\frac{1}{3}} - W_t^{\frac{1}{3}} = \kappa \times t$$

where W_0 is the initial amount of drug in the dosage form, W_t is the remaining amount of drug in the dosage form at time t and κ is Hixon–Crowell rate constant incorporating the surface-volume relation. Data obtained were plotted as cube root of drug % remaining in matrix vs. time. Kitazawa's model reveals changes in the release rates of a system releasing its content under sink condition [21]. Kitazawa's simplified equation is expressed as:

$$\log \frac{C_s}{C_s - C_t} = \frac{K't}{2.303}$$

where C_s and C_t are amount of drug dissolved at infinite time and at time t , respectively, K' is the dissolution rate constant derived from the slope of the regression line plotted as $\log \frac{C_s}{C_s - C_t}$ vs. time. Baker–Lonsdale model is a derivation of Higuchi model which describes the drug release pattern if a spherical matrix is developed. The equation is as follows:

$$f_1 = \frac{3}{2} \left[1 - \left(1 - \frac{M_t}{M_\infty} \right)^{\frac{2}{3}} \right] \times \frac{M_t}{M_\infty} = k_t$$

where $\frac{M_t}{M_\infty}$ is the cumulative amount of drug released at time t , k is the release rate constant which corresponds to the slope. Data obtained from *in vitro* drug release studies were plotted as $[d(\frac{M_t}{M_\infty})]/dt$ with respect to the root of time inverse. Power law is a semi-empirical equation that describes drug release from polymeric system [22]. The equation is as follows:

$$\frac{A_t}{A_\infty} = k_p t^n$$

where A_t and A_∞ are the absolute cumulative amount of drug released at time ' t ' and infinite time respectively, k_p is a constant incorporating structural and geometric characteristics of the device and ' n ' is the release exponent.

The values of R^2 , n (release exponent) and k (rate constant) were determined and compared.

2.7 Antioxidant activity of BCX encapsulated ChiHG and AlgHG

Antioxidant property of BCX encapsulated hydrogels was performed according to Norajit et al. [18] with minor modifications. Briefly, lyophilized hydrogel sample was ground to a powdered form (0.5 g) with the help of liquid nitrogen and mixed with 5 ml of methanol followed by vigorous stirring for 1h and then centrifuged at 6000g for 15 min. Obtained methanolic supernatant was subjected to different free radical scavenging methods viz., DPPH assay, ABTS assay, nitrite scavenging activity (NSA), hydroxyl radical scavenging activity (HRSA) and reducing power (RP) activity [3, 18, 24, 25].

2.8 Statistical analysis

All experiments were performed in triplicates. Data were expressed as the mean $\pm$ standard deviation (SD) of their values. Analysis of variance (ANOVA) was performed followed by Tukey's test using SPSS 16 software (SPSS Inc., Chicago, USA). A value of $P < 0.05$ was considered significant [4].

3 Results and discussion

3.1 Properties of ChiHG and AlgHG

3.1.1 Moisture content, degree of gelation, entrapment efficiency and swelling behaviour of hydrogels

Physico-mechanical properties viz., moisture content, degree of gelation and CX-encapsulation efficiency of the prepared hydrogel are shown in Table 1. Moisture content and degree of swelling (after 60 min) of ChiHG and AlgHG encapsulated with BCX had significantly higher ($P < 0.05$) values than their respective control. The higher degree of moisture content may be due to increased porosity and fibril structure which resulted after BCX encapsulation, which was later confirmed by other studies such as SEM, DSC, so on. A nutraceutical when ingested passes through the elementary canal where it faces lower pH (1.2 at stomach) and higher pH (7.4 at intestine) consecutively. This robust pH fluctuation may result physico-chemical changes or bioactivity loss of ingested product. Hence, to observe possible effect of gastric fluid pH and intestinal fluid pH respectively, two different pH (1.2 and 7.4) was tested for hydrogel swelling. Swelling behaviour of both ChiHG and AlgHG are shown in Fig. 1. Results showed that both gels undergo swelling better at pH 7.4 than acidic pH 1.2 ($P < 0.05$). Swelling ability of hydrogel is mostly facilitated due to specific functional groups (e.g. carboxyl group), which eventually attract water molecules. At pH 1.2, carboxylate group changes to acid form and most carboxymethyl groups are in the form of $-\text{COOH}$ that is less ionized, resulting in less swelling than at pH 7.4 [18].

However, at pH 7.4, carboxyl groups become more ionized, generating a repulsive force within gel matrices which eventually cause the hydrogel to swell. This type of observation is in agreement with Muhamad et al. [17] where they found a swelling of around 155% and 128% at pH 7.4 and pH 1.2, respectively during beta carotene release from modified cellulose beads. In

Table 1. Moisture content, degree of gelation, and encapsulation efficiency and DSC parameters of hydrogels. Values are average of three experimental set $\pm$ SD

Hydrogel samples	Moisture content (% dry basis)	Degree of gelation	Entrapment efficiency	H_m ($J g^{-1}$)	T_m ($^{\circ}C$)
<i>ChiHG</i> – control	44.14 $\pm$ 0.0035	97.35 $\pm$ 0.0002	—	149.31 $\pm$ 0.0001	121.79 $\pm$ 0.0004
<i>ChiHG</i> – BCX	50.66 $\pm$ 0.0067	97.03 $\pm$ 0.0028	55.92 $\pm$ 0.0032	121.79 $\pm$ 0.0015	117.98 $\pm$ 0.0018
<i>AlgHG</i> – control	69.48 $\pm$ 0.0097	98.71 $\pm$ 0.0024	—	170.08 $\pm$ 0.0012	172.21 $\pm$ 0.0011
<i>AlgHG</i> – BCX	75.59 $\pm$ 0.0024	98.24 $\pm$ 0.004	60.45 $\pm$ 0.0024	109.87 $\pm$ 0.0005	127.83 $\pm$ 0.0008

Figure 1. Swelling property of hydrogel in pH 1.2 and 7.4 (ctrl: Control, test: BCX encapsulated).

another study with riboflavin release from alginate hydrogel, Abd El-Ghaffar et al. [9] found around 130% swelling at pH 7.2 compared to negligible swelling at pH 1.2. With this regard our experimental results were significantly higher in swelling percentage during BCX release. After encapsulation of BCX, degree of swelling increased which may be due to the functional groups present in BCX that interacted with the gel matrices in a similar way. However no significant difference ($P > 0.05$) in gel content was found in both hydrogels encapsulated with BCX and their respective controls. Encapsulation efficiency of BCX was found to be relatively higher in AlgHG than in ChiHG. Encapsulation efficiency of BCX in AlgHG and ChiHG was calculated to be 60.45% and 55.92%, respectively. This may be due to more apparent interaction of alginate gel matrix with BCX than chitosan.

3.1.2 Determination of surface morphology using SEM

Physical appearances of prepared ChiHG and AlgHG, before and after BCX encapsulation are shown in Fig. 2 (A1-4, B1-4). Scanning electron micrographs of AlgHG and ChiHG, before and after BCX encapsulation are also shown in Fig. 2 (C1-2, D1-2). The surface of both ChiHG and AlgHG without BCX encapsulation was smooth and homogenous; however, after encapsulation of BCX, the gels showed heterogeneous surface with porous and fibril appearance. This could be a reason for the difference in both moisture content and swelling degree of BCX entrapped hydrogels compared to their controls. Micro-phase separation between hydrogel and BCX may be responsible for the porous and fibril appearance of ChiHG and AlgHG after carotenoid en-

Figure 2. AlgHG freshly prepared (A1 & A2) and lyophilized (A3 & A4); ChiHG freshly prepared (B1 & B2) and lyophilized (B3 & B4); gel surface morphology by SEM (C1: AlgHG control, C2: AlgHG-BCX, C3: ChiHG control, C4: ChiHG-BCX).

capsulation. In 2010, Norajit et al. [18] found no significant difference after encapsulation of ginseng extract into alginate based hydrogel film with their respective control. However, our experimental observations were in agreement with Abd El-Ghaffar et al. [9] where they found micro pores and large wrinkles after encapsulation of riboflavin into pH sensitive sodium alginate hydrogels.

3.1.3 Determination of thermal property using DSC

Thermal behaviour of prepared hydrogels before and after BCX encapsulation is shown in Table 1. Heat of fusion (ΔH_m) of ChiHG and AlgHG before BCX encapsulation was found to be 149.31 $J g^{-1}$ and 170.08 $J g^{-1}$ respectively. Melting point (T_m) of ChiHG and AlgHG control was found to be 121.79 $^{\circ}C$ and 172.21 $^{\circ}C$ respectively. However these control values were different from previous published reports. This may be due to variation in polysaccharide source and plasticizer used along with modification done in experimental protocol [18]. From Table 1 it is evident that after encapsulation of BCX into ChiHG and AlgHG, both ΔH_m and T_m decreased significantly ($P < 0.05$). The reduction in these thermal properties may be attributed to the large number of unsaturated bonds present in BCX which affect the overall chain mobility of hydrogels. Norajit et al. [18] reported a varying range of ΔH_m (158.64 – 250.78 $J g^{-1}$) and T_m (101.3 – 108.6 $^{\circ}C$) after encapsulation of ginseng extract into alginate hydrogel film, however their result showed ΔH_m decreased significantly with respect to control samples but T_m remained unaltered.

Figure 3. FT-IR profile of the prepared hydrogels. A: *Chi HG* control, B: *Chi HG-BCX*, C: *Alg HG* control, D: *Alg HG-BCX*.

3.1.4 Characterization of hydrogel using FT-IR

The IR spectrum of prepared hydrogels is shown in Fig. 3. In control ChiHG, (Fig. 3A) a strong peak at 1646 cm^{-1} , 1243 cm^{-1} and 1068 cm^{-1} indicate the presence of chitosan polysaccharide in the gel. Sharp peaks at 1457 cm^{-1} , 1411 cm^{-1} and 636 cm^{-1} are typical of sodium polyacrylate [14]. After encapsulation of BCX, absorption bands for polyacrylates shifted to 1560 cm^{-1} , 1458 cm^{-1} , 633 cm^{-1} and 563 cm^{-1} (Fig. 3B). Presence of chitosan was showed by its broad peak at 1068 cm^{-1} . A typical sharp and strong peak at 865 cm^{-1} and 563 cm^{-1} may be attributed to C-H bending (alkene) or $\text{C}=\text{C}$ bending (di-substituted *trans*). In case of AlgHG, control sample showed (Fig. 3C) clear sharp bands at 1139 cm^{-1} , which was due to the saccharide backbone. Moderate bands at 995 cm^{-1} and 944 cm^{-1} were due to $\text{C}=\text{C}$ bending (alkene) whereas peaks at 835 cm^{-1} and 627 cm^{-1} were assigned to C-H bending (alkene). BCX encapsulation in AlgHG showed (Fig. 3D) a strong sharp peak at 1632 cm^{-1} , 1419 cm^{-1} and 1316 cm^{-1} which principally attributed to $\text{C}=\text{O}$ (conjugated ketone), $\text{C}=\text{C}$ (unsaturated ketone) and -CH_2 (alkane) stretch respectively. Medium sharp peaks at 1245 cm^{-1} and 1029 cm^{-1} was obtained due to -C-O (ester) group stretching. Polysaccharide present in the BCX encapsulated AlgHG was accountable for the peaks obtained at 1125 cm^{-1} and 1090 cm^{-1} . Clear peaks at 903 cm^{-1} and 945 cm^{-1} were assigned to $\text{C}=\text{C}$ (alkene) bending whereas peaks at 822 cm^{-1} and 549 cm^{-1} was due to the C-H bending. These results are in accordance with the previous reports obtained while working with chitosan and alginate based hydrogels [7, 9, 10, 14]. FT-IR analysis showed the successful BCX encapsulation in ChiHG and AlgHG.

3.1.5 Characterization of hydrogel using XRD

X-ray diffraction (XRD) is one of the common techniques used to examine the physico-chemical association and degree of crystallinity of unknown solids. Data obtained in this procedure are collected and analysed in the form of diffraction patterns for most intense d value (inter-planer spacing) and relative intensities (I/I_0). A lower 2θ value indicates larger inter-planer spacing, while an increase in the number of high-angle reflections indicate higher molecular state order [26]. A wide range of protuberance in the diffraction pattern of hydrogel over a large range of 2θ suggests that key material of the hydrogel is present in amorphous state. Generally a loosely crystalline material shows reflections broadness, high noise and low peak intensities [26].

XRD patterns of prepared ChiHG and AlgHG (control and BCX encapsulated), are shown in Fig. 4. In ChiHG control, predominant d value was obtained as $4.02\text{ \AA}$, $3.26\text{ \AA}$ and $2.82\text{ \AA}$ at 2θ value of 22.1° , 27.3° and 31.6° respectively. After BCX encapsulation additional peak at 45.5° with a d value of $1.98\text{ \AA}$ was obtained. This may be due to the encapsulation of BCX. In AlgHG control, predominant d value was obtained as $2.78\text{ \AA}$, $2.68\text{ \AA}$ and $2.67\text{ \AA}$ at 2θ value of 32.1° , 33.3° and 33.4° respectively. After BCX encapsulation, a peak similar with ChiHG-CX was obtained at 45° (2θ) with a d value of $2.01\text{ \AA}$. Non-significant changes in XRD pattern of both BCX encapsulated hydrogels and respective controls revealed that BCX might exist dispersed in a non-crystalline state within the polymeric matrix and forming an amorphous complex with intermolecular interaction occurring within the polymer matrix.

Figure 4. XRD pattern of the prepared hydrogels. A: *Alg HG* control, B: *Alg HG*- BCX, C: *Chi HG* control, D: *Chi HG*-BCX.

3.2 Kinetics of CX release from *ChiHG* and *AlgHG*

The drug release performance of a hydrogel depends on hydrogel materials, network conformation and drug loading mechanism which must be accompanied with the properties of the drug (e.g. hydrophobicity, charge) and its mechanism of action (sustained drug release versus rapid, high exposure) [11].

Release of BCX from hydrogel matrix was slower in acidic pH compared to high pH. This can be explained from the observations of swelling study where higher pH (7.4) facilitates more swelling of hydrogel than lower pH (1.2). High degree of swelling after BCX incorporation could be a major factor in controlling BCX release from gel matrix which corresponds to enhanced electrostatic repulsion through network porosity of cross-linked hydrogels [27]. At higher pH, dissolution liquid could diffuse more efficiently inside the gel matrix resulting effectual discharge of BCX. Interaction of hydrogel matrix with BCX probably facilitated some changes in mechanical property of gel allowing *ChiHG* and *AlgHG* to hold BCX for longer period. Similar observation was recorded by Muhamad et al. [17] during β -carotene release from genipin-cross-linked kappa-carrageenan/carboxymethyl cellulose beads.

Mathematical modelling of drug release can improve the process for designing drug loading matrices. Different parameters e.g. dissolution medium, permeability of the polymer to disso-

lution medium, drug solubility, molecular size of the drug etc. may affect drug release behaviour significantly [22]. The values of R^2 (correlation coefficient), n and k obtained from different kinetic model during BCX release from *ChiHG* and *AlgHG* are shown in Table 2. k is the constant incorporating the structural characteristics of the matrix and n is the release exponent, indicative of the drug release mechanism. The non Fickian release or anomalous transport of drug occurred when the n values lies between 0.43 and 0.85, which corresponds with coupled diffusion/polymer relaxation [27, 28].

Results showed that, mechanism of CX release from gel matrices was primarily based on non Fickian diffusion transport as suggested by Korsmeyer – Peppas model (R^2_{ChiHG} : 0.9598, R^2_{AlgHG} : 0.9647) with a n value > 0.5 [16] for both chitosan and alginate hydrogel. Malakar et al. [28] found a diffusion exponent (n) ranged between 0.9211 and 1.1855 at the time of controlled release of insulin from a transferosomal gel matrix. Bassi and Kaur [19] observed similar result ($n = 1.19$) at the time of nystatin release from tamarind seed polysaccharide. Kinetics values of Baker – Lonsdale model (R^2_{ChiHG} : 0.9539, R^2_{AlgHG} : 0.9678) and Power Law (R^2_{ChiHG} : 0.9598, R^2_{AlgHG} : 0.9641) model suggested that BCX might be releasing anomalously through capillary fracture of heterogeneous polymer matrix in case of spherical formulation [16, 21, 22].

Table 2. Release kinetic model parameter for hydrogels after encapsulation of BCX

	ChtCX			AlgCX		
	<i>k</i>	<i>n</i>	<i>R</i> ²	<i>k</i>	<i>n</i>	<i>R</i> ²
Zero Order	6.3069	—	0.9016	6.8368	—	0.9004
First Order	0.1678	—	0.7614	0.1860	—	0.8145
Higuchi	20.423	—	0.9472	22.634	—	0.9576
Korsmeyer – Peppas	—	0.723	0.9598	—	0.778	0.9647
Hixon – Crowell	0.1349	—	0.9318	0.1545	—	0.9402
Kitazawa	0.5310	—	0.8065	0.3977	—	0.9204
Baker – Lonsdale	0.1617	—	0.9539	0.1545	—	0.9678
Power Law	0.1666	—	0.9528	0.2211	—	0.964

Figure 5. Free radical scavenging profile of prepared hydrogel [control1: only alginate hydrogel]; control2: only chitosan hydrogel.

From entire kinetic modelling and the data represented in Table 2, it was evident that, Korsmeyer – Peppas model fitted best with the chitosan and alginate based hydrogel with a R^2 value of 0.9598 and 0.9647 respectively. In this model, the value of n characterizes the release mechanism of drug. In case of cylindrical tablets, $0.43 \leq n$ corresponds to a Fickian diffusion mechanism, $0.43 < n < 0.85$ to non Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport [27, 28]. Hence, a n value of 0.723 and 0.778 for chitosan and alginate based hydrogel clearly suggest that the BCX was released from the hydrogel matrix by a non Fickian diffusion transport method.

3.3 Antioxidant activity of ChiHG and AlgHG entrapped with BCX

Free radical scavenging activities of ChiHG and AlgHG with and without BCX are shown in Fig. 5. Results show that methanol extract of BCX-hydrogels had significantly high ($P < 0.05$) scav-

enging activity in terms of DPPH, ABTS, NSA, RP and HRSA ($P < 0.05$) assay system. However no significant difference ($P > 0.05$) was found in the radical scavenging activity when BCX was encapsulated in ChiHG and AlgHG. DPPH is a nitrogen centred radical with an unpaired electron which is scavenged by proton radical quenching molecules. ChiHG-BCX and AlgHG-BCX showed 46.28% and 48.93% DPPH inhibition ($P < 0.001$). This may be due to the hydrogen donating ability of BCX present in the hydrogel [3]. ABTS⁺ is also a nitrogen centred synthetic radical which can be neutralized by giving an electron. ChiHG-BCX and AlgHG-BCX showed 30.67% and 32.88% ABTS inhibition ($P < 0.001$) which may be due to the electron donating propensity of carbonyl functional group present in BCX.

Nitrite compound is extensively used in food colouring, processing and preservation. However, they can generate nitrosamines which have significant threat to animal health [3, 25]. Strong nitrite scavenging power ($P < 0.001$) of ChiHG-CX and AlgHG-CX (41.17% and 44.92% inhibition respectively) was probably due to the hydroxyl group present in CX which inhibited the formation of nitrosamine by donating hydrogen.

Capability to convert oxidized ferric form to reduced ferrous form of iron (Fe) is generally regarded as reducing power of a molecule and it is an index to measure the antioxidant activity of the molecule [18]. ChiHG-BCX and AlgHG-BCX showed an optical absorbance of 0.2816 and 0.3062 at 700nm, respectively ($P < 0.001$). Hydrogen-donating abilities of BCX may be responsible for such strong reducing power of the hydrogels [3,25].

Hydroxyl radical is the reactive form of stable hydroxide ion which can cause significant impairment to living cells. Hydroxyl radical reacts with aromatic group of biomolecules to generate hydroxycyclo-hexadienyl and peroxy radical by elimination of water molecules thus leading to molecular damage [24]. High hydroxyl radical scavenging activity ($P < 0.001$) was observed by ChiHG-CX and AlgHG-CX in HRSA assay (89.51% and 91.27% respectively). Reducing power and hydrogen atom donating ability of BCX probably played synergistic role to facilitate the strong HRSA activity.

4 Concluding remarks

Present investigation demonstrate that bacterial canthaxanthin (BCX) can be successfully encapsulated into polymeric gel system for controlled release. Successful encapsulation of BCX into alginate and chitosan hydrogel was achieved with significant difference in degree of swelling and moisture content, however gel content remained unaffected. Surface morphology, thermal property, intra molecular bonding pattern and crystallographic structure was not significantly affected by BCX encapsulation. Kinetics study showed that BCX from both alginate and chitosan hydrogel released anomalously through *non Fickian* diffusion transport method. Radical scavenging activities revealed that encapsulated BCX provides strong antioxidant property to both type of hydrogel. Hence both alginate and chitosan based hydrogel may be useful for controlled release of BCX in terms of pharmaceutical aspect.

Practical application

This study evaluates a simple process to encapsulate and release carotenoids. Foremost limitations of such processes are intrinsic instability, lipophilic behaviour and isomerization effect leading to loss of nutraceutical properties. However, data obtained from the present study will be helpful to design delivery systems for natural carotenoids viz. canthaxanthin in such a way, so that the bioactive molecule could retain its strong antioxidant property after encapsulation. Insight into the hydrogel characterization and release kinetics data obtained from gastric fluid pH and intestinal fluid pH will be helpful to formulate delivery system for the carotenoids from pharmaceutical aspect.

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5 References

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