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Hydrocolloid-Driven Structure-Property Relationships and pH-Responsive Release of C-Phycocyanin from Alginate-Chitosan Composite Hydrogel Beads

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Abstract

C-phycocyanin (CPC) is a natural blue pigment with high functional value, but its limited stability restricts its use in food systems. This study developed alginate–chitosan composite hydrogel beads containing carrageenan, pectin, or gellan gum to improve CPC retention and control its release under gastrointestinal conditions. Hydrocolloid and chitosan concentrations were optimized using response surface methodology, and the selected formulations were characterized for encapsulation performance, physicochemical properties, morphology, molecular interactions, surface charge, storage stability, and in vitro release. Among the composite systems, alginate–carrageenan–chitosan (Al-Car-Chi) showed the highest CPC encapsulation efficiency (99.69%), whereas alginate–pectin–chitosan (Al-Pec-Chi) gave the highest bead production yield (78.91%). SEM, FTIR, and zeta potential analyses showed clear differences in network organization and interfacial properties among the formulations, indicating that CPC retention was governed by the combined effects of polymer composition rather than by a single physical property. During storage, wet beads deteriorated rapidly at 30°C, while refrigeration at 4°C delayed deterioration. Under simulated gastrointestinal conditions, all formulations limited CPC release to below 2% during the gastric phase. Release increased in simulated intestinal fluid, reaching 41.63% for Al-Chi and only 22.45% for Al-Car-Chi at 240 min. The Korsmeyer-Peppas model provided the most consistent fit to intestinal release data (R^2 = 0.967-0.992), indicating complex non-Fickian transport in the composite systems. Although, hydrocolloid type strongly affected CPC

encapsulation, storage behavior, and release, with Al-Car-Chi providing high initial CPC retention together with relatively slow intestinal release.

Keywords: C-phycocyanin; alginate; chitosan; hydrocolloid; hydrogel beads; gastrointestinal release; release kinetics

1. Introduction

C-phycocyanin (CPC) is a water-soluble blue pigment-protein mainly obtained from *Spirulina* and other cyanobacteria. It is widely studied as a natural blue colorant and a functional food ingredient because of its attractive color and biological properties. However, CPC has limited stability under many food processing and storage conditions. Changes in temperature, pH, and light can affect its protein structure and cause a loss of color and activity. CPC is especially sensitive to acidic conditions and elevated temperatures. These limitations make it difficult to use CPC directly in some food products and have led to increasing interest in methods that can improve its stability [1]. Encapsulation is one possible approach because it can reduce direct contact between CPC and the surrounding environment and may also help control its release.

Hydrogel beads made from food-grade polysaccharides are commonly used to encapsulate sensitive compounds. Sodium alginate is one of the most widely used materials because it can form a gel with calcium ions under mild conditions. This is useful for CPC because high temperature is not required during bead formation. However, calcium-alginate gels can have an open and porous structure, which may allow water-soluble compounds to move out of the matrix. For this reason, alginate is often combined with other polymers. Chitosan is particular interest because its positively charged groups can interact with negatively charged alginate and change the structure of the bead. Previous studies have shown that chitosan can improve the physical stability of alginate-based beads and reduce the release of encapsulated compounds [2]. Recently, Han et al. (2026) reported that chitosan reinforcement improved the structure and sustained release of phycocyanin-loaded alginate beads [3].

Other hydrocolloids can also be added to alginate to modify bead properties. Carrageenan, pectin, and gellan gum are anionic polysaccharides, but they differ in chemical structure, functional groups, viscosity, and gel-forming behavior. These differences may affect bead formation and the organization of the polymer network. Belščak-Cvitanović et al. (2015) showed that adding pectin, carrageenan, or chitosan to alginate changed viscosity, bead

properties, and release behavior [2]. Similar effects have been reported for CPC encapsulation, Alavi et al. (2022) found that adding pregelatinized corn starch to calcium-alginate beads changed their morphology and improved the stability of phycocyanin [4]. Li et al. (2024) presented a CPC encapsulation efficiency of 96.87% using a pectin-alginate composite hydrogel and found that changes in the polymer composition affected the structure of the hydrogel [5].

Carrageenan is also of interest for CPC delivery. Londoño-Moreno et al. (2023) prepared CPC-loaded beads using alginate together with agavins and other polysaccharides, including κ-carrageenan. Encapsulation efficiency increased as more components were added to the matrix, and the most complex formulation reached about 98% and reported that κ-carrageenan contributed to controlled CPC release. These results suggest that adding another hydrocolloid to alginate can affect both CPC retention and later release [6].

The polymer network is also important during gastrointestinal digestion. Alginate-based hydrogels behave differently under acidic and intestinal conditions because pH affects the ionization and hydration of the polymer chains. Londoño-Moreno et al. (2023) showed that polysaccharide-based alginate beads protected CPC during simulated gastrointestinal digestion [6], while Li et al. (2024) reported that pectin-alginate hydrogels improved the protection and bioaccessibility of phycocyanin [5]. Chitosan-reinforced alginate beads have been reported to improve phycocyanin stability and provide sustained release [3]. These studies show that polymer composition is important not only for encapsulation but also for the release pattern under changing pH conditions.

Although several composite materials have been studied for CPC encapsulation, most studies have focused on one polymer combination at a time. Direct comparisons of different hydrocolloids within the same alginate-chitosan bead system are still limited. It is therefore useful to examine how the type of hydrocolloid affects not only CPC encapsulation, but also bead properties, polymer structure, and gastrointestinal release. Combining these characteristics could clarify the structure-property-function link for CPC-loaded hydrogel beads and help explain why various polymer combinations produce diverse outcomes.

This study aimed to develop CPC-loaded alginate-chitosan composite hydrogel beads containing carrageenan, pectin, or gellan gum and to compare their properties and performance. Response surface methodology was used to study the hydrocolloid–chitosan formulations. The selected bead systems were then evaluated for CPC encapsulation efficiency, bead production yield, physicochemical properties, microstructure, polymer interactions, storage stability, and CPC release under simulated gastric and intestinal conditions. SEM, FTIR, and zeta potential

analyses were used to examine differences among the polymer systems, and release kinetics were evaluated during the intestinal phase. This study hypothesized that the type of hydrocolloid would change the alginate-chitosan network and, in turn, affect CPC retention, stability, and release under gastrointestinal conditions.

2. Materials and Methods

2.1 Materials

Spirulina platensis powder source from My Healthy Food Co., Ltd. (Chiang mai, Thailand) was used as the source of C-phycocyanin (CPC). Sodium alginate, pectin, carrageenan, gellan gum, chitosan, and calcium chloride were purchased from Krungthepchemi Company Limited (Bangkok, Thailand) were used to prepare the hydrogel beads. Distilled water was used for solution preparation. NaCl, HCl, KH_2PO_4, and NaOH used for CPC analysis and preparation of simulated gastric and intestinal fluids were of analytical grade.

2.2 Preparation and Determination of C-Phycocyanin Extract

CPC was extracted from *Spirulina platensis* using an ultrasound-assisted method described by Thaisamak et al. (2019). Spirulina powder was mixed with distilled water at a solid-to-liquid ratio of 1:50 (w/v). The mixture was treated in an ultrasonic bath at 40 kHz and 40°C for 10 min. The crude extract was then centrifuged at 8,500 rpm for 10 min at 4°C. The supernatant was collected and used as the CPC extract. CPC concentration was determined by measuring absorbance at 620 and 652 nm using a UV-visible spectrophotometer (UV-1280, Shimadzu, Japan). The extract was stored at -18°C until further use.

2.3 Preparation of CPC-Loaded Alginate Beads

CPC-loaded beads were prepared by extrusion using a BUCHI B-390 encapsulator (BUCHI Labortechnik AG, Switzerland). In the initial screening, CPC extract was mixed with sodium alginate at different concentrations, and the resulting solution was extruded into calcium chloride solution to form calcium-alginate beads. Based on the initial screening, 1.5% (w/v) sodium alginate (Al) and 1.5% (w/v) calcium chloride were used as the base conditions for the subsequent hydrocolloid-chitosan experiments.

The encapsulator was operated at a vibration frequency of 500 Hz, an electrode voltage of 1,500 V. The CPC-alginate solution and calcium chloride solution were used at a volume ratio of 1:1. After gelation, the beads were separated using a sieve and washed twice with distilled water. The remaining calcium chloride solution was collected for determination of non-encapsulated CPC.

2.4 Experimental Design and Optimization of Hydrocolloid–Chitosan Formulations

The effects of carrageenan (Car), pectin (Pec), and gellan gum (Gel) were studied separately in the calcium-alginate system. For each hydrocolloid, the concentrations of the hydrocolloid and chitosan (Chi) were treated as independent variables.

A central composite design (CCD) within response surface methodology (RSM) was used. Each hydrocolloid system consisted of 13 experimental runs. CPC encapsulation efficiency (%EECPC), bead production yield (%EEYield), bead diameter, viscosity, pH, and mechanical properties were measured as responses.

Numerical optimization was then performed to identify formulations with high CPC encapsulation efficiency. The formulations carried forward for physicochemical and functional characterization were Al-Chi, Al-Pec-Chi, Al-Car-Chi, and Al-Gel-Chi. Sodium alginate and calcium chloride were kept at 1.5% (w/v) in these comparisons.

2.5 Characterization of the CPC beads

2.5.1 Encapsulation Efficiency

CPC encapsulation efficiency (%EECPC) was calculated from the initial amount of CPC added to the formulation and the amount of non-encapsulated CPC remaining in the external solution after bead formation [7]. The CPC concentration in the external solution was determined spectrophotometrically.

$$CPC = \frac{\left(A_{620} - 0.474\, A_{652} \right)}{5.34}$$

$$EECPC\ (\%) = \frac{\left(CPC_{int} - CPC_{unencap}\right)}{CPC_{int}}$$

Where CPC is phycocyanin concentration (mg/ml), $A_{620}A_{652}$ is the absorbance values at 620 and 652 nm, respectively. CPC_{int} is CPC in the initial solution (mg/ml), and $CPC_{unencap}$ is CPC in the $CaCl_2$ bath.

2.5.2 Bead production yield

Bead production yield (%EEYield) was determined from the mass of recovered beads relative to the total amount of material used in the bead-forming process.

$$EEYield\ (\%) = \frac{Weight\ of\ \ CPC_b}{Weight\ of\ \ CPC_{Al\ solution}}$$

Where CPC_b is CPC bead product obtained after processing. $CPC_{Al\ solution}$ is weight of CPC alginate solutions used in the process.

2.5.3 pH and viscosity

The pH and viscosity of the solutions were measured at 25°C using a pH meter (SP-2100, Suntex, Taiwan) and a Brookfield DV2T viscometer (AMETEK Brookfield, USA), respectively. The report specifies 500 mL of solution for viscosity measurements and 50 mL for pH measurements.

2.5.4 Texture properties

Texture properties of the CPC beads were measured following the method described by Lupa et al. (2015) [8]. CPC beads were determined by texture profile analysis using a TA.XTplus Texture Analyzer (Stable Micro Systems, UK) fitted with a P/50 cylindrical probe. The test speed was 5 mm/s, and the beads were compressed to 30%

deformation. Hardness, springiness, cohesiveness, and chewiness were recorded. Samples were maintained at 5 ± 1°C before analysis and were analyzed within 24 h.

2.6 Scanning Electron Microscopy

The morphology of the CPC-loaded beads was examined using a scanning electron microscope (SEM; JSM-5410LV, JEOL, Japan). Freeze-dried beads were prepared for observation of both the external surface and the internal cross-sectional structure. For cross-sectional analysis, the beads were cut or fractured to expose the internal structure. The samples were mounted on SEM stubs using conductive carbon tape and sputter-coated with gold to improve electrical conductivity. The samples were then observed at an accelerating voltage of 10 kV, following methods adapted from Belščak-Cvitanović et al. (2015) and Thinkohkaew et al. (2024) [2, 9].

2.7 Fourier-Transform Infrared Spectroscopy

FTIR analysis was used to examine changes in functional groups and polymer interactions. The method was adapted from Belščak-Cvitanović et al. (2015) and Thinkohkaew et al. (2024) [2, 9]. The CPC beads were freeze-dried, ground into a fine powder, mixed with potassium bromide (KBr), and compressed into transparent pellets. Spectra were recorded using a Nicolet iS5 FTIR spectrometer (Thermo Scientific) over the range of 4,000-400 cm^{-1} at a resolution of 4 cm^{-1}.

2.8 Zeta Potential

The zeta potential of the selected CPC-loaded bead systems was measured using a Delsa Nano C instrument (Beckman Coulter, USA), following the general approach described by Arora et al. (2011) and Atma et al. (2025) [10, 11]. Before measurement, each sample was dispersed to obtain a uniform suspension. Zeta potential was determined from the electrophoretic mobility of the dispersed particles under an applied electric field and calculated using the instrument software. The results were expressed in millivolts (mV).

2.9 Storage Stability

The selected condition of Al-Car-Chi, Al-Pec-Chi, and Al-Gel-Chi beads were evaluated during storage. Samples were packed in transparent or opaque containers and stored at 4°C or room temperature. Samples were evaluated on days 0, 3, 5, 7, 14, and 21. Changes in appearance, odor, bead production yield, CPC encapsulation efficiency, and microbiological quality were monitored. A reference level of 60% was used for both %EECPC and %EEYield when evaluating bead quality during storage.

2.10 Simulated Gastrointestinal Release and Release Kinetics

CPC release was studied under sequential simulated gastrointestinal conditions. The beads were first placed in simulated gastric fluid (SGF) at pH 1.5 for 60 min. They were then transferred to simulated intestinal fluid (SIF) at pH 7.4 and incubated for a further 180 min at 37°C, giving a total digestion time of 240 min. Samples were collected every 15 min. CPC released into the surrounding medium was measured by spectrophotometry, and cumulative CPC release was calculated relative to the initial amount of CPC contained in the beads.

$$Commulative\ CPC\ release\ (\ \%\ ) = \frac{CPC_t}{CPC_0} \times 100$$

where CPC_t is the phycocyanin content at time t, CPC_0 is the initial phycocyanin content in CPC beads. For kinetic analysis, the gastric and intestinal phases were treated separately because they represented different pH environments. Release kinetics were evaluated for the SIF phase only. The start of SIF was set as $t = 0$, and the CPC released during SGF was subtracted from subsequent cumulative values. The intestinal release data were fitted to zero-order, Higuchi, Korsmeyer-Peppas, and Peppas-Sahlin models [3, 12, 13]. Model fit was compared using the coefficient of determination (R^2).

$$Zero\ order: Q = k_0 t$$

$$Higuchi\ model: Q = k_H t^{1/2}$$

$$Kormeyer - Peppas\ model: Q = k_{kp} t^{n}$$

$$Peppas - Sahlin\ model: Q = k_{d} t^{m} + k_{r} t^{2m}$$

where k_0, k_H, k_{kp}, are the model constant for zero-order, Higuchi, Korsmeyer-Peppas, and Peppas-Sahlin models, respectively. k_d, and k_r are the diffusion and relaxation rate. In addition, n is the release exponent indicating of drug release, while m is the release exponent indicating to Fickian diffusion exponent. There are three scenarios for m values, 1) If $m \leq 0.43$, the mechanism is called Fickian and the release is diffusional controlled. 2) If $0.43 < m < 1.0$, the mechanism is called non-Fickian (anomalous) and the release is depending on both of the diffusion and relaxation of the polymer. 3) If m = 1.0, the mechanism is called zero-order release.

2.11 Statistical Analysis

Experimental data were expressed as mean ± standard deviation. Differences among formulations were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. A difference was considered statistically significant at $p < 0.05$. For the response surface experiments, the effects of hydrocolloid and chitosan concentrations were evaluated using response surface methodology (RSM). Release kinetic models were fitted by regression analysis, and model performance was compared using the coefficient of determination (R^2). Kinetic parameters were reported as mean ± standard deviation from replicate experiments.

3. Results and Discussion

3.1 Development and Selection of Hydrocolloid-Chitosan Bead Systems

The development of CPC-loaded beads was first carried out using sodium alginate and calcium chloride as the basic gel-forming system. Sodium alginate concentrations of 0.5-1.5% (w/v) and calcium chloride concentrations were tested to determine suitable conditions for bead formation. Among the conditions examined, the combination of 1.5% (w/v) sodium alginate and 1.5% (w/v) calcium chloride gave the highest CPC encapsulation efficiency, reaching 89.86%. This condition was therefore selected as the base formulation for the next step of the study. The result agrees

with the general behavior of calcium-alginate (Ca-Al) systems, where the alginate concentration and the amount of available Ca^{2+} can affect the formation and strength of the hydrogel network that corresponding to Hadiyanto et al. (2017) which reported that alginate-based encapsulation could provide high retention and improved stability of phycocyanin [14].

After the base alginate system was established, carrageenan (Car), gellan gum (Gel), and pectin (Pec) were studied separately together with chitosan (Chi). Response surface methodology (RSM) was used to examine the effects of hydrocolloid and chitosan concentrations on CPC encapsulation and bead properties. The response surface and contour plots are shown in Fig. 1. The three hydrocolloids gave different response patterns, indicating that each polymer interacted differently with the alginate-chitosan system.

For the Alginate-carrageenan system (Al-Car) (Fig. 1A-C), CPC encapsulation efficiency was affected mainly by chitosan concentration. The quadratic model was significant (p = 0.0359), and for alginate-carrageenan-chitosan system (Al-Car-Chi) showed a significant effect on CPC encapsulation (p = 0.0054). The model explained 77% of the variation in the response ($R^2 = 0.77$). Numerical optimization predicted that 0.794% carrageenan and 1.464% chitosan would give an EECPC of 96.09%, with a desirability value of 0.885 (Table 1). The experimental encapsulation efficiency reached approximately 28-91% under the conditions tested. Bead production yield was also influenced by the formulation, with values ranging from about 60 to 81%. These results suggest that adding carrageenan and chitosan changed not only CPC retention but also the ability of the formulation to form and recover beads. A similar effect of polymer composition on encapsulation has been reported for alginate-based systems containing carrageenan and other polysaccharides [2, 6].

The alginate-gellan gum system (Al-Gel) (Fig. 1D–F) showed a wider range of CPC encapsulation efficiency, from approximately 32 to 97%. The quadratic model was significant (p = 0.0054), and for alginate-gellan gum-chitosan system (Al-Gel-Chi) had a strong effect on the response (p = 0.0015). The numerical solution gave 0.750% gellan gum and 1.494% chitosan, with a predicted EECPC of 98.44% and a desirability value of 0.902 (Table 1). An interaction between gellan gum and chitosan was also observed (p = 0.0331), showing that the effect of one polymer depended on the concentration of the other. Gellan gum also had a clear effect on solution viscosity and bead size. Thinkohkaew et al. (2024) has been mentioned that gellan gum can change the structure of alginate-based systems and form a denser matrix when combined with chitosan [9].

For the alginate-pectin system (Al-Pec) (Fig. 1G–I), CPC encapsulation efficiency ranged from 62 to 94 %. The quadratic model was significant (p = 0.0151), while the alginate-pectin-chitosan system (Al-Pec-Chi) was the main factor affecting CPC retention (p = 0.0009). The lack of fit was not significant, indicating that the fitted model described the experimental data reasonably well. Numerical optimization selected 1.015% pectin and 1.700% chitosan, with a predicted EECPC of 91.96% and a desirability value of 0.732 (Table 1). Pectin concentration had a clear effect on viscosity, while the mechanical properties of the beads also changed across the experimental region. The result was corresponding to Li et al. (2024) that pectin can modify the structure of alginate hydrogels and improve the encapsulation of phycocyanin [5].

The numerical solutions were then prepared experimentally and used for further characterization. The alginate-carrageenan–chitosan (Al-Car-Chi) and alginate-pectin-chitosan (Al-Pec-Chi) systems gave experimental EECPC values of 96.59 and 90.61%, respectively, which were close to their predicted values of 96.09 and 91.96%. The alginate-gellan gum-chitosan (Al-Gel-Chi) system gave an experimental value of 92.32%, which was lower than the predicted value of 98.44% but remained high compared with the corresponding system without chitosan. These results show that RSM was useful for identifying formulation regions with high CPC retention, although some difference between predicted and experimental values was observed.

The results also indicate that formulation selection should not be based only on the predicted EECPC. Polymer composition may affect bead formation, recovery, viscosity, size, and mechanical properties at the same time. Belščak-Cvitanović et al. (2015) showed that changing the hydrocolloid in an alginate system affected viscosity, bead size, and release behavior in different ways [2]. Additionally, Thinkohkaew et al. (2024) also used RSM to optimize an alginate-gellan gum-chitosan system and showed that several responses should be considered together [9]. As a result, the chosen formulations were further contrasted with the comparable hydrocolloid systems without chitosan (Al-Car, Al-Gel, Al-Pec), alginate alone (Al), and alginate-chitosan (Al-Chi). Their encapsulation performance and physicochemical properties are presented in Table 2 and discussed in the following section.

3.2 Encapsulation Performance and Physicochemical Properties of Selected Bead Systems

The encapsulation performance and mechanical properties of the selected bead systems are shown in Table 2. CPC encapsulation efficiency were clear differences observed among the formulations. The alginate-only beads (Al) showed an EECPC of 58.80%, while the addition of chitosan increased the value significantly to 81.29% (p <

0.05). The same pattern was observed in all hydrocolloid-containing systems. EECPC increased from 66.41 to 90.59% when chitosan was added to the pectin system, from 33.37 to 99.69% in the carrageenan system, and from 43.00 to 92.56% in the gellan gum system.

Among the formulations, Al-Car-Chi showed the highest EECPC, followed by Al-Gel-Chi and Al-Pec-Chi. The strong increase after the addition of chitosan was especially clear in the carrageenan and gellan gum systems. This result suggests that CPC retention was affected not only by the presence of a second hydrocolloid but also by its combination with chitosan. Chitosan can interact with negatively charged polysaccharides such as alginate, carrageenan, gellan gum, and pectin, which may modify the polymer network and reduce the movement of encapsulated compounds during bead formation. Corresponding to Belščak-Cvitanović et al. (2015) which has mentioned about chitosan coating can change the properties of alginate-based beads and reduce the release of encapsulated compounds [2].

The high EECPC obtained with the alginate-carrageenan-chitosan (Al-Car-Chi) system is also consistent with Londoño-Moreno et al. (2023) on CPC-loaded alginate matrices that reported the addition of κ-carrageenan and other polysaccharides to an alginate matrix increased CPC encapsulation and changed its release behavior. The present results also show that carrageenan alone did not improve CPC retention, EECPC increased only when chitosan was included. A similar effect was found for gellan gum, the addition of chitosan increased the EECPC that corresponding to Thinkohkaew et al. (2024) which was mentioned that combining gellan gum with alginate and chitosan changed the structure and performance of the resulting microcapsules. Their results showed that gellan gum contributed to a denser alginate-based matrix when chitosan was present [9].

Bead production yield showed a different order from EECPC (Table 2). Al-Pec-Chi gave the highest EEYield, followed by Al-Car-Chiand Al-Gel-Chi. These values were significantly different from each other ($p < 0.05$). Chitosan also increased bead yield within each polymer system. Mechanical properties did not follow the same pattern as CPC encapsulation. Hardness values ranged from 238.43 to 302.74 N. The differences in hardness were not separated into significant groups in Table 2. This indicates that the large differences observed in EECPC cannot be explained simply by bead hardness. A bead with a higher resistance to compression does not necessarily provide better retention of CPC within its internal polymer network.

Chewiness, in contrast, showed significant differences among the formulations. Al-Gel-Chi and Al had the highest values, and were not significantly different from each other. Al-Pec-Chi showed the lowest chewiness and

was significantly different from the other formulations. Most of the remaining systems were placed in the same statistical group. These results again show that the mechanical response of the beads did not directly follow their CPC encapsulation efficiency.

The pH of the bead-forming systems was affected by polymer composition. Most formulations had pH values close to neutral, while the pectin-containing systems showed clearly lower pH values. The lowest pH was observed for Al-Pec-Chi at 5.95, this may reflect the acidic character of pectin and the pH-dependent ionization of pectin and chitosan. A pH around 5–6 is also within the range reported to favor electrostatic interactions between pectin carboxylate groups and protonated amino groups of chitosan [15, 16].

Viscosity also differed significantly among the formulations (Table 2). The alginate-only system had the lowest viscosity, while Al-Pec-Chi showed the highest value. Similar effects of polymer type and concentration on the viscosity of alginate-based systems have been reported by Belščak-Cvitanović et al. (2015 and Thinkohkaew et al. (2024) [2, 9]. Nevertheless, higher viscosity did not directly correspond to higher CPC encapsulation efficiency. The Al-Pec-Chi system had the highest viscosity, whereas the Al-Car-Chi system showed the highest EECPC. The Al-Gel-Chi system also maintained high CPC retention despite having a lower viscosity. These results suggest that viscosity may influence bead formation, but CPC retention was also controlled by the type of hydrocolloid and its interactions with alginate, chitosan, and calcium ions.

The results in Table 2 show that CPC encapsulation was affected by the overall composition of the bead system rather than by a single physicochemical property. Al-Car-Chi gave the highest EECPC, whereas Al-Pec-Chi had the highest bead production yield and viscosity. Hardness, chewiness, pH, and viscosity showed different patterns from EECPC. These results suggest that CPC retention depended on the combined effects of polymer composition, network formation, and interactions among alginate, hydrocolloid, chitosan, and calcium ions.

3.3 Morphology of CPC-Loaded Hydrogel Beads

The morphology of the freeze-dried CPC-loaded beads was examined by SEM, and clear differences were observed among the formulations (Fig. 2). Both the cross-sectional and surface structures changed when chitosan and the different hydrocolloids were added to the alginate system. In general, all samples showed a porous and irregular structure after freeze-drying, but the arrangement of the pores, walls, and surface folds differed among the formulations.

The alginate-only beads (Al) (Fig. 2A) showed relatively large and irregular internal voids with thin, partly collapsed walls. The surface also appeared folded and layered. After chitosan was added (Fig. 2B), the internal walls appeared more continuous and the surface became more structured, although large cavities were still present. This change was accompanied by an increase in EECPC from 58.80 to 81.29% (Table 2). Chitosan can interact with negatively charged alginate chains and change the organization of the hydrogel network. Similar changes in morphology after chitosan treatment have been reported for alginate-based beads [2].

The gellan gum system also showed a clear change after chitosan addition. Al-Gel (Fig. 2C) had an irregular porous cross-section and a rough outer surface, whereas Al-Gel-Chi (Fig. 2D) showed a more compact and folded internal structure with a rough but more continuous surface. This structural change was accompanied by a marked increase in EECPC, from 43.00 to 92.56%. Thinkohkaew et al. (2024) also reported that the addition of gellan gum to alginate, followed by chitosan coating, changed the internal structure of the microcapsules and produced a denser polymer matrix. Although the encapsulated material and preparation conditions were different, the reported trend supports the role of gellan gum and chitosan in modifying alginate-based networks.

For the pectin-containing beads, Al-Pec (Fig. 2E) showed several large internal cavities, while the surface had a clear porous pattern. Al-Pec-Chi (Fig. 2F) retained a porous internal structure, but the walls appeared more interconnected and the surface showed a more continuous honeycomb-like pattern. The addition of chitosan increased EECPC from 66.41 to 90.59%. Li et al. (2024) also showed that pectin can modify the structure of alginate hydrogels and that changes in polymer composition can influence CPC retention [5].

The carrageenan system showed one of the clearest differences between formulations with and without chitosan. Al-Car (Fig. 2G) had a highly porous internal structure with many interconnected voids and a strongly wrinkled surface. In contrast, Al-Car-Chi (Fig. 2H) showed a different internal organization, with denser regions around the cross-section and a more compact, rough outer surface. This formulation also gave the highest EECPC in the study, reaching 99.69%. Londoño-Moreno et al. (2023) similarly reported that adding κ-carrageenan and other polysaccharides to an alginate-based matrix improved CPC retention and changed the behavior of the delivery system [6].

The SEM observations were generally consistent with the differences in CPC retention, especially when each hydrocolloid system was compared before and after chitosan addition. However, the images also show that CPC retention cannot be explained simply by the apparent number or size of pores. For example, both Al-Pec-Chi and Al-

Car-Chi remained visibly porous but showed high EECPC values. This suggests that the continuity, arrangement, and interactions within the polymer network may be as important as the visible pore structure.

It should also be noted that SEM was used only for qualitative comparison in this study. Pore size was not measured, and the freeze-drying step may have changed the original structure of the hydrated beads. Belščak-Cvitanović et al. (2015) has been reported that freeze-drying affected the morphology of alginate-based beads [2]. Therefore, the SEM images support differences in network organization among the formulations, but should not be used to conclude that one formulation had quantitatively smaller pores than another.

3.4 Fourier-Transform Infrared Spectroscopy

The FTIR spectra of the selected CPC-loaded bead systems are shown in Fig. 3. All formulations showed the main absorption bands commonly found in polysaccharide-based hydrogels. A broad band was observed at approximately 3400 cm^{-1}, mainly associated with O-H stretching, while bands around 2930-2970 cm^{-1} were related to C-H stretching. Strong bands in the regions of approximately 1630 and 1420 cm^{-1} were also present and were mainly associated with the asymmetric and symmetric stretching of carboxylate groups in alginate and other anionic polysaccharides.

For the alginate beads, the main bands were observed at approximately 3423, 2935, 1618-1632, 1423, and 1027 cm^{-1}. After chitosan was added, the broad OH region shifted slightly to around 3438 cm^{-1}, while the band in the 1600 cm^{-1} region appeared near 1639 cm^{-1}. A band around 1550 cm^{-1} was also more noticeable in Al-Chi. This region may include contributions from amino and amide groups associated with chitosan. Similar changes in the carboxylate and amino-related regions have been reported for alginate-chitosan systems and are consistent with changes in the local chemical environment caused by electrostatic interactions and hydrogen bonding between the polymers (Karakas et al., 2022; Lemos et al., 2026).

The addition of a second hydrocolloid produced further differences in the spectra. Al-Pec-Chi showed a weak band near 1735 cm^{-1}, which can be related to esterified carboxyl groups of pectin, together with a shift of the main carbohydrate region to approximately 1018 cm^{-1}. In Al-Car-Chi, characteristic bands were observed near 1262 and 932 cm^{-1}. These bands are commonly associated with sulfate groups and 3,6-anhydrogalactose units of carrageenan, respectively. The Al-Gel-Chi spectrum showed a pattern generally similar to the alginate-chitosan system, with small

changes around the carboxylate and amino-related regions. These differences indicate that each hydrocolloid contributed differently to the chemical environment of the multicomponent network.

Although, the FTIR spectra support the presence of interactions among alginate, chitosan, and the added hydrocolloids. Changes in the O-H, carboxylate, and amino-related regions are consistent with ionic interactions and hydrogen bonding within the hydrogel matrix. Similar spectral changes have been reported in alginate systems containing chitosan, pectin, or carrageenan [2, 17, 18]. These interactions may contribute to the differences in network organization observed by SEM and may also help explain why CPC retention differed among the formulations.

However, the observed peak shifts were generally small, and the spectral resolution used in this study was 4 cm^{-1}. In addition, a spectrum of CPC alone was not included for direct comparison. Therefore, the FTIR results should be interpreted as evidence of changes in the polymer environment rather than proof of new covalent bond formation or a specific interaction between CPC and an individual polymer.

3.5 Zeta Potential

The zeta potential values of the CPC-loaded bead systems are shown in Fig. 4. All formulations had negative zeta potential values, indicating that negatively charged groups remained present at the particle-liquid interface. Significant differences were observed among the formulations ($p < 0.05$), showing that the surface charge was affected by polymer composition.

The effect of chitosan was not the same for all systems. For alginate alone, the zeta potential changed from -14.89 to -4.78 mV after chitosan was added, showing a shift toward a less negative surface charge. A similar change was found in the carrageenan system. In contrast, the pectin and gellan gum systems became more negative after chitosan addition. All four pairs showed significant differences between formulations with and without chitosan according to Duncan's multiple range test (Fig. 4). These results indicate that chitosan did not simply neutralize the negative charge of the anionic polysaccharides. Instead, the final surface charge depended on the polymer combination and the charged groups exposed at the particle surface.

The shift toward a less negative value in the alginate-chitosan system is consistent with electrostatic interactions between negatively charged carboxyl groups of alginate and positively charged amino groups of chitosan. Similar behavior has been reported for alginate-chitosan systems [10, 11]. However, the opposite trend observed for the pectin and gellan gum systems suggests that the arrangement of the polymers at the interface was more complex.

The ionization of the different functional groups, as well as pH and polymer composition, can affect the measured zeta potential of polysaccharide systems [16].

No direct relationship was observed between zeta potential and CPC encapsulation efficiency. For example, Al-Car-Chi had the highest EECPC, although its zeta potential was not the most negative, while Al-Gel-Chi showed one of the most negative values. Therefore, surface charge alone cannot explain CPC retention. When considered together with the SEM and FTIR results, the zeta potential data support the view that each hydrocolloid produced a different interfacial and network environment when combined with alginate and chitosan.

It should be noted that zeta potential represents the electrokinetic potential at the slipping plane of dispersed particles rather than the internal charge of the whole hydrogel bead. Thus, the values obtained in this study were mainly used to compare changes in surface charge among formulations and were not used as a direct measure of bead stability.

3.6 Storage Stability

The storage stability of the selected Al-Pec-Chi, Al-Car-Chi, and Al-Gel-Chi composite beads was evaluated under refrigerated and non-refrigerated conditions. At 30°C, microbial growth increased rapidly. Total bacterial counts were too numerous to count in all formulations by day 3, and visible spoilage was observed in all samples by day 5, regardless of packaging type. In contrast, storage at 4°C delayed deterioration and allowed changes in bead and CPC retention to be followed throughout the 21-day storage period. However, bacterial populations were still detected during refrigerated storage, indicating that low temperature slowed microbial changes but did not completely prevent them. Yeasts and molds were generally not detected under refrigerated conditions.

Because rapid spoilage occurred at 30°C, the detailed evaluation of bead and CPC retention was focused on samples stored at 4°C in opaque packaging. The three formulations showed different changes in physical bead retention during storage (Fig. 5A). Al-Pec-Chi showed the highest retention and maintained approximately 90% of its initial bead mass after 21 days. In comparison, Al-Car-Chi and Al-Gel-Chi retained approximately 67 and 57%, respectively. These results suggest that the pectin-containing formulation was more effective in maintaining the physical structure of the beads during refrigerated storage. This difference may be related to differences in polymer network organization, water uptake, and swelling among the composite systems.

A different pattern was observed for CPC retention (Fig. 5B). EECPC decreased progressively in all three formulations with increasing storage time. At day 21, Al-Car-Chi retained the highest EECPC at 41.17%, followed by Al-Pec-Chi at 38.32% and Al-Gel-Chi at 21.65%. Thus, even though Al-Pec-Chi maintained approximately 90% of its bead mass, a considerable loss of CPC occurred during the same storage period. This difference shows that the physical integrity of the bead did not directly represent the ability of the polymer matrix to retain CPC.

The decrease in EECPC during storage may result from more than one process. Water can enter the hydrogel network and promote swelling, which may allow the encapsulated compound to diffuse through the polymer matrix. Similar behavior has been reported for alginate-based hydrogel beads, where swelling and diffusion through the polymer network affect the retention and release of encapsulated compounds [2]. In addition, CPC itself is sensitive to environmental conditions, including temperature, light, and pH, because changes in its protein–chromophore structure can reduce its stability during storage [19]. Therefore, the decrease in EECPC may reflect both the loss of CPC from the hydrogel matrix and degradation of CPC remaining within the system. The better performance under refrigerated conditions is also consistent with previous studies on phycocyanin stability. Buecker et al. (2022) reported that lower storage temperature improved the stability of phycocyanin–carrageenan complexes compared with storage at higher temperature [20].

3.7 CPC Release under Simulated Gastrointestinal Conditions

The cumulative release profiles of CPC from Al, Al-Chi, Al-Pec-Chi, Al-Car-Chi, and Al-Gel-Chi beads under simulated gastrointestinal conditions are shown in Fig. 6. During the first 60 min in simulated gastric fluid (SGF, pH 1.5), CPC release remained very low for all formulations. At 60 min, cumulative release was 1.61% for Al, 0.66% for Al-Chi, 0.85% for Al-Pec-Chi, 1.71% for Al-Car-Chi, and 0.87% for Al-Gel-Chi. Thus, all bead systems limited CPC release to below 2% during the gastric phase.

The low release in SGF may be related to the behavior of alginate and the other anionic polysaccharides under acidic conditions. At low pH, protonation of carboxyl groups can reduce electrostatic repulsion between polymer chains and limit swelling of the hydrogel network. This may decrease the movement of CPC through the matrix. Chitosan may also contribute to the compact structure through electrostatic interactions with negatively charged polysaccharides. Similar protection under simulated gastric conditions has been reported for alginate-based composite systems containing chitosan and other hydrocolloids [6, 9].

After the beads were transferred to simulated intestinal fluid (SIF, pH 7.4), CPC release increased in all formulations, but the release profiles differed according to polymer composition. At 120 min, corresponding to 60 min after exposure to SIF, cumulative CPC release reached 12.13% for Al, while lower values were observed for Al-Chi, Al-Pec-Chi, Al-Car-Chi, and Al-Gel-Chi at 4.11, 7.09, 5.66, and 2.43%, respectively. The slower release from the composite systems during the early intestinal phase suggests that the additional polymer components provided greater resistance to CPC diffusion than alginate alone.

The increase in release after transfer to SIF may be associated with changes in the ionization and hydration of the polymer network. At the higher pH, deprotonation of carboxyl groups can increase repulsion between polymer chains and promote water uptake and network expansion. Changes in the Ca^{2+}-crosslinked alginate network may also increase the permeability of the beads. These processes could facilitate CPC diffusion from the hydrogel matrix. Similar pH-dependent release behavior has been reported for alginate-based delivery systems containing additional polysaccharides [5, 6, 9].

Clear differences among the formulations were observed toward the end of the intestinal phase. At 240 min, Al-Chi showed the highest cumulative CPC release at 41.63%, followed by Al-Gel-Chi at 26.43%, Al-Pec-Chi at 25.80%, Al-Car-Chi at 22.45%, and Al at 21.87%. The higher release from Al-Chi shows that the addition of chitosan alone did not necessarily produce the strongest barrier during the intestinal phase. In contrast, the addition of a second hydrocolloid together with chitosan reduced the final release compared with Al-Chi. Among the ternary systems, Al-Car-Chi showed the lowest cumulative CPC release. This formulation also showed the highest initial CPC encapsulation efficiency, indicating that the carrageenan-containing network provided both high initial CPC retention and relatively slow release during simulated intestinal conditions.

3.7.1 Release Kinetics under Simulated Intestinal Conditions

The release kinetics of CPC during the simulated intestinal phase were evaluated using zero-order, Higuchi, Korsmeyer-Peppas, and Peppas–Sahlin models (Table 3). The SIF phase was analyzed separately because the transition from SGF to SIF produced a clear change in the release profile. The kinetic models were therefore used to compare CPC transport behavior among the different polymer systems under intestinal conditions.

Among the models tested, the Korsmeyer-Peppas model gave the most consistent fit across all formulations, with R^2 values ranging from 0.967 to 0.992. This model has been widely used to describe solute release from polymeric

matrices and has also been applied to phycocyanin-loaded alginate systems. Ritger and Peppas (1987) showed that the release exponent n depends on matrix geometry, with representing Fickian diffusion for spherical systems. Previous studies have also used the Korsmeyer–Peppas model to describe phycocyanin release from alginate-based hydrogel systems and polysaccharide beads (Londoño-Moreno et al., 2023; Buliga et al., 2024).

For the Al beads, the release exponent was $n = 0.591$. This value falls within the anomalous or non-Fickian region for a spherical matrix, indicating that CPC release was likely influenced by both molecular diffusion and changes in the polymer matrix. This interpretation was also supported by the Higuchi model, which gave a high R^2value of 0.981 for Al. Thus, diffusion appeared to make an important contribution to CPC transport through the alginate matrix. A strong fit to the Higuchi model has also been reported for phycocyanin release from an alginate-based hydrogel, where Buliga et al. (2024) obtained an $R^2$of 0.9997 at pH 7.45.

In contrast, the chitosan-containing formulations showed much higher $n$values: 1.626 for Al-Chi, 1.072 for Al-Pec-Chi, 1.322 for Al-Car-Chi, and 1.548 for Al-Gel-Chi. For spherical matrices, values above approximately 0.85 are generally associated with Super Case II-type transport. These high values suggest that CPC release from the composite beads was more strongly influenced by polymer relaxation, swelling, or structural changes in addition to diffusion. At the same time, the Higuchi fit decreased to R^2values of 0.644-0.816 for the composite systems, further indicating that a simple diffusion-controlled model was less able to describe their release behavior. However, swelling and matrix erosion were not measured directly in this study; therefore, the high $n$values were interpreted as evidence of complex non-Fickian transport rather than definitive proof of a specific mechanism.

The zero-order model provided additional information on the release pattern. Al-Pec-Chi and Al-Car-Chi showed relatively high zero-order fits, with R^2values of 0.981 and 0.964, respectively. Among all formulations, Al-Car-Chi had the lowest zero-order rate constant ($k_0 = 0.109$), while Al-Chi showed the highest value ($k_0 = 0.219$). These results agree with the cumulative release profiles in Fig. 6, where Al-Car-Chi showed relatively slow CPC release among the ternary systems, whereas Al-Chi showed the highest cumulative release at 240 min. The zero-order parameters were therefore used mainly to compare release rates and patterns rather than to assign a physical release mechanism.

The Peppas-Sahlin model also gave high R^2values, ranging from 0.962 to 0.992. This model was originally developed to separate the contributions of Fickian diffusion and polymer relaxation through the $k_d$and $k_r$terms, respectively (Peppas and Sahlin, 1989). However, negative $k_d$values were obtained for all chitosan-containing

formulations, and some $k_r$estimates showed large standard deviations. These parameter values limit a direct physical interpretation of the relative contributions of diffusion and relaxation. For this reason, the Peppas-Sahlin results were treated as supporting information rather than being used to quantify the individual release mechanisms.

The differences among models also highlight the effect of polymer composition on CPC transport. Buliga et al. (2024) reported that phycocyanin release from a comparatively simpler alginate-based hydrogel was best described by the Higuchi model, supporting a strong diffusion-related contribution. In the present study, the high Higuchi fit observed for Al was consistent with this behavior, whereas the addition of chitosan and secondary hydrocolloids reduced the suitability of the Higuchi model and increased the Korsmeyer-Peppas release exponent. This difference suggests that adding additional polymers created a more complex matrix in which CPC release was influenced by changes in the network as well as diffusion. Studies on CPC-loaded alginate–polysaccharide beads under simulated gastrointestinal conditions have likewise shown that polymer composition can modify CPC retention and release behavior (Londoño-Moreno et al., 2023).

From the results, the kinetic analysis supports the release profiles shown in Fig. 6. The Al system showed a stronger diffusion-related contribution, whereas the chitosan-containing composite systems exhibited more complex non-Fickian transport. Among the ternary formulations, Al-Car-Chi combined the lowest zero-order release constant, a high Korsmeyer–Peppas fit ($R^2 = 0.989$), and relatively low cumulative CPC release. These findings indicate that the type of hydrocolloid incorporated into the alginate-chitosan matrix affected not only the amount of CPC released but also its transport behavior under simulated intestinal conditions.

4. Conclusion

This study demonstrated that the type of hydrocolloid incorporated into alginate-chitosan hydrogel beads strongly influenced CPC encapsulation, structural characteristics, storage behavior, and gastrointestinal release. Among the selected formulations, Al-Car-Chi showed the highest CPC encapsulation efficiency, whereas Al-Pec-Chi provided the highest bead production yield and the best physical bead retention during refrigerated storage, indicating that high CPC retention and high physical stability did not necessarily occur in the same formulation. SEM, FTIR, and zeta potential analyses further showed that the different hydrocolloids changed the organization and interfacial properties of the polymer network, although CPC retention could not be explained by a single property such as viscosity, hardness, morphology, or surface charge. During storage, 4°C was more suitable than 30°C, where rapid

microbial growth and spoilage occurred within the first few days. At 4°C, the beads maintained their physical structure for longer, but EECPC decreased progressively, with Al-Pec-Chi showing the highest bead retention and Al-Car-Chi retaining the highest CPC level at day 21. Under simulated gastrointestinal conditions, all formulations effectively limited CPC release in SGF to below 2%, while release increased after transfer to SIF. At 240 min, Al-Chi showed the highest cumulative release, whereas Al-Car-Chi showed the lowest release among the ternary systems. The Korsmeyer–Peppas model gave the most consistent fit to the intestinal release data, with the alginate-only system showing a stronger diffusion-related contribution and the chitosan-containing composite systems showing more complex non-Fickian transport. These findings suggest that the choice of secondary hydrocolloid can be used to adjust the performance of alginate-chitosan beads for CPC delivery, although further studies on swelling, erosion, longer-term storage, bioaccessibility, and application in real food systems are still needed.

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Author Contributions

Phirunrat Thaisamak: Conceptualization, Methodology, Funding acquisition, Formal analysis, Supervision, Writing - original draft. **Kantharakorn Taengrit**: Investigation, Data curation. **Jiungluk Runglert**: Investigation, Data curation. **Kittikoon Wannasawad**: Writing - review and editing. **Janejira Phakawan**: Conceptualization, Supervision, Investigation, Writing - review and editing.

Declaration

During the preparation of this manuscript, the authors used ChatGPT for language editing and grammatical improvement in selected sections. All content was reviewed and revised by the authors, who take full responsibility for the final manuscript.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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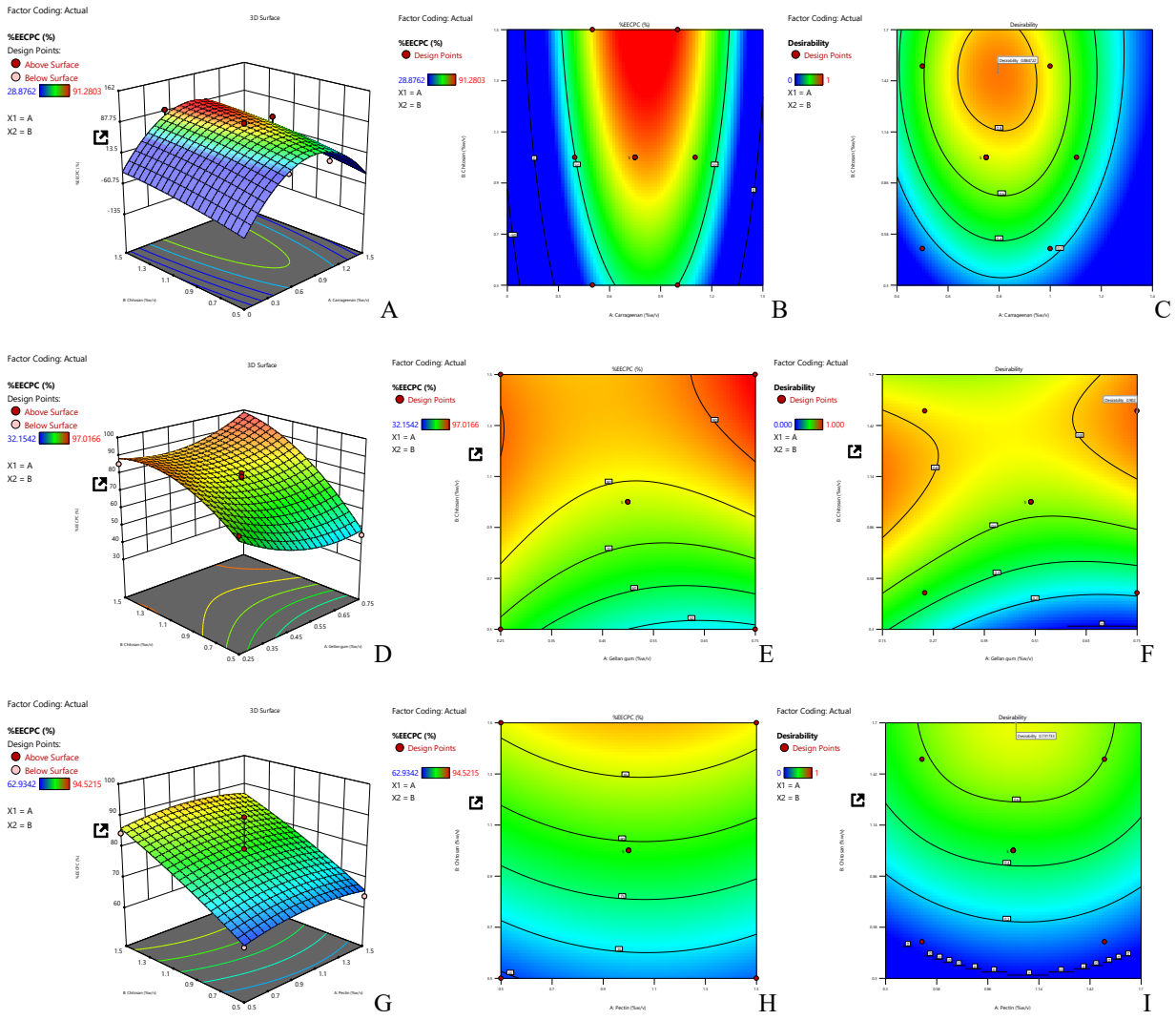


Fig. 1 Response surface, contour, and desirability plots for optimization of CPC-loaded alginate-hydrocolloid–chitosan beads. (A-C) Alginate–carrageenan–chitosan system, (D-F) alginate-gellan gum-chitosan system, and (G-I) alginate-pectin-chitosan system.

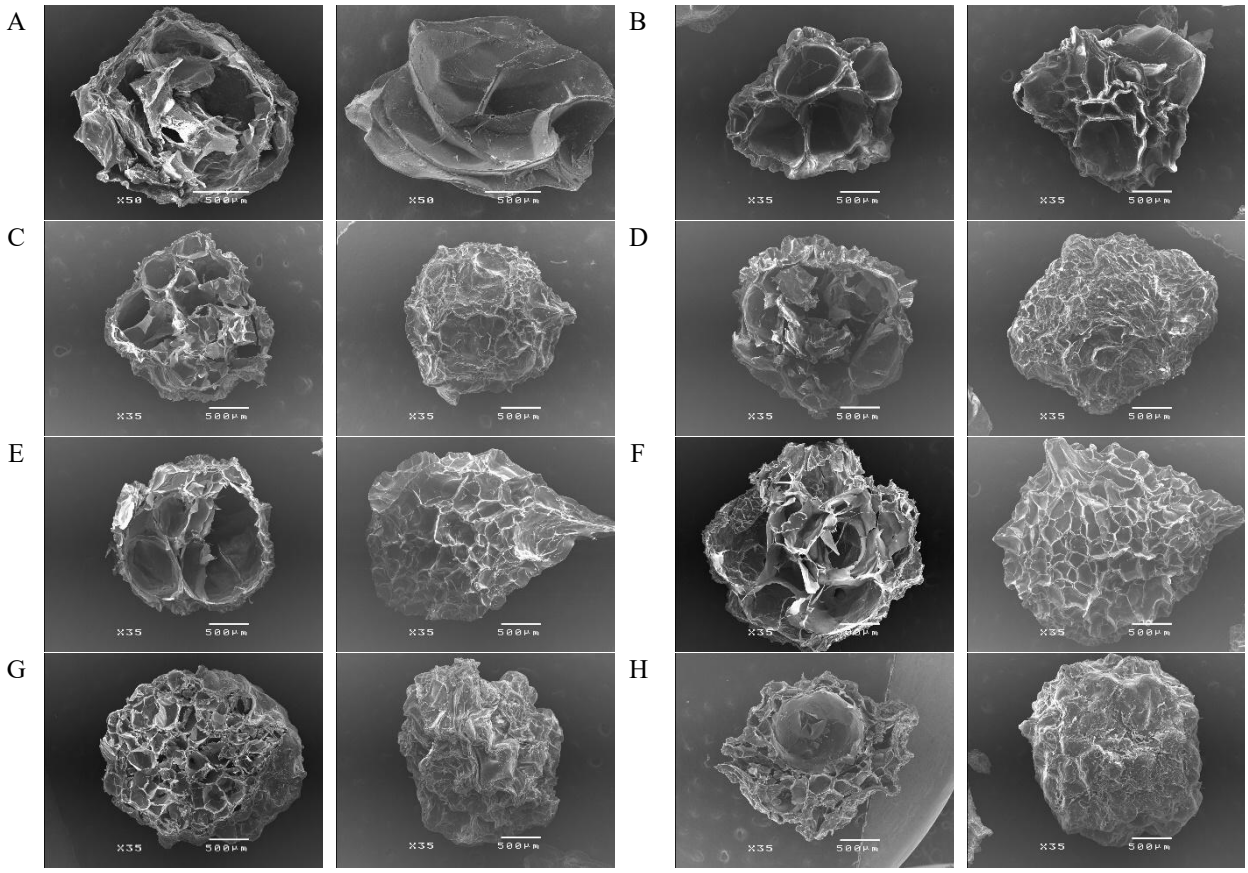


Fig. 2 SEM micrographs of freeze-dried CPC-loaded hydrogel beads showing cross-sectional (left) and surface (right) morphologies: (A) Al, (B) Al-Chi, (C) Al-Gel, (D) Al-Gel-Chi, (E) Al-Pec, (F) Al-Pec-Chi, (G) Al-Car, and (H) Al-Car-Chi

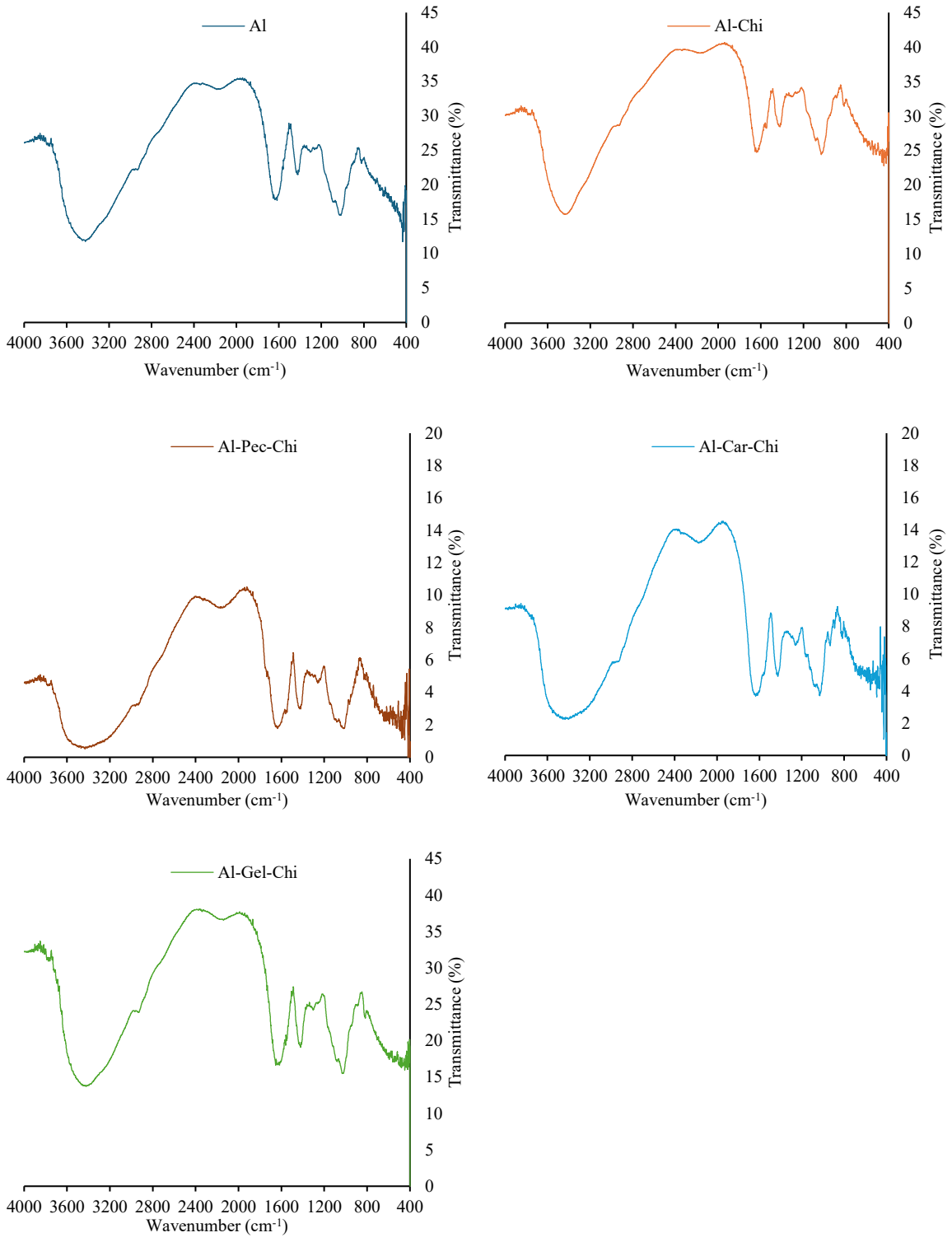
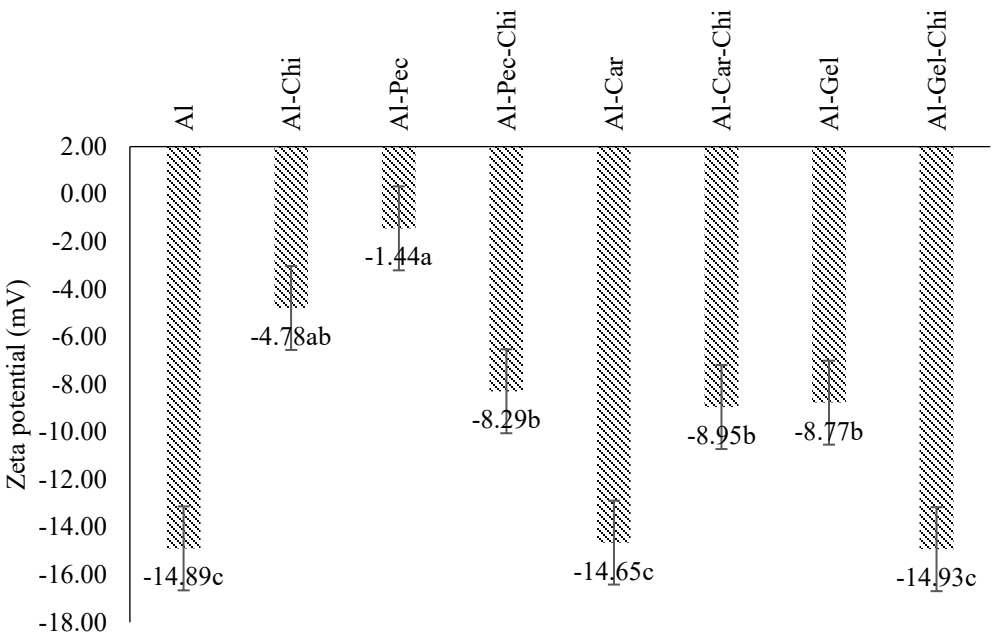


Fig. 3 FTIR spectra of CPC-loaded alginate-based hydrogel beads: Al, Al-Chi, Al-Pec-Chi, Al-Car-Chi, and Al-Gel-Chi.



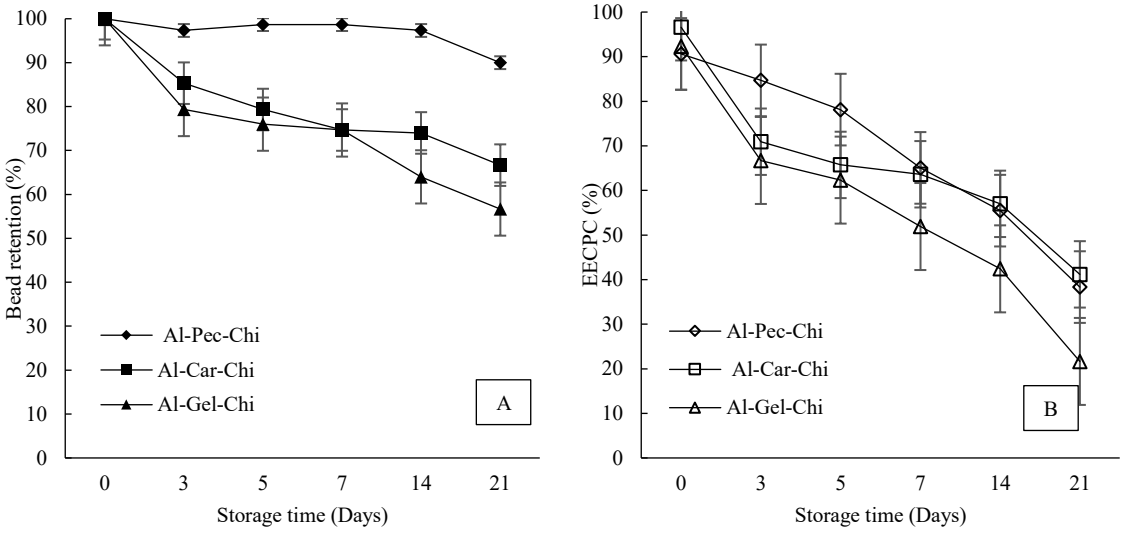
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687 **Fig. 4** Zeta potential of CPC-loaded alginate-based hydrogel bead systems with and without chitosan.

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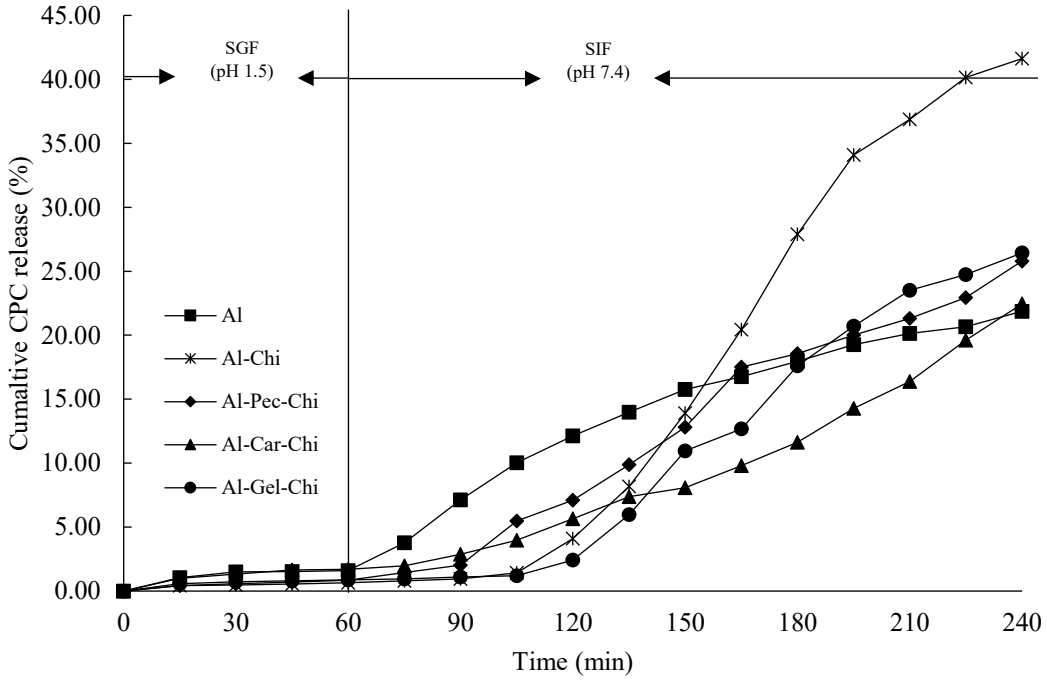
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692 **Fig. 5** Changes in (A) bead retention and (B) CPC encapsulation efficiency (EECPC) of Al-Pec-Chi, Al-Car-Chi,

693 and Al-Gel-Chi composite beads during storage at 4°C in opaque packaging for 21 days.



694

695 **Fig. 6** Cumulative CPC release from Al, Al-Chi, Al-Pec-Chi, Al-Car-Chi, and Al-Gel-Chi hydrogel beads under

696 simulated gastrointestinal conditions. Beads were exposed to simulated gastric fluid (SGF, pH 1.5) for the first 60

697 min, followed by simulated intestinal fluid (SIF, pH 7.4) until 240 min.

698

699

700 **Table 1** RSM model statistics and numerical optimization of hydrocolloid-chitosan formulations for CPC
701 encapsulation

Hydrocolloid system	Model *p*-value	Significant model terms for EECPC	R^2	Hydrocolloid at numerical optimum (% w/v)	Chitosan at numerical optimum (% w/v)	Predicted EECPC (%)
Alginate-carrageenan-chitosan (Al-Car-Chi)	0.0359	Chitosan (p = 0.0054)	0.77	0.794	1.464	96.09
Alginate-gellan gum-chitosan (Al-Gel-Chi)	0.0054	Chitosan (p = 0.0015); hydrocolloid × chitosan (p = 0.0331); gellan gum² (p = 0.0405)	0.87	0.750	1.494	98.44
Alginate-pectin-chitosan (Al-Pec-Chi)	0.0151	Chitosan (p = 0.0009)	0.82	1.015	1.700	91.96

702

Table 2 Composition, encapsulation performance, and physicochemical properties of selected CPC-loaded alginate-based hydrogel beads.

Formulation	EECPC (%)	EEYield (%)	Hardness (N)	Chewiness	pH	Viscosity (cP)
$Al_{1.5}$	58.80±0.00^{f}	44.40±0.15^{f}	295.93±52.86	7.97±0.29^{a}	7.66±0.10^{ab}	1878.00±16.37^{f}
$Al_{1.5}$-$Chi_{1.5}$	81.29±0.08^{d}	62.04±0.09^{e}	302.74±1.57	6.59±0.44^{b}	7.74±0.08^{a}	2455.33±62.42^{d}
$Al_{1.5}$-$Pec_{1.01}$	66.41±0.02^{e}	67.89±0.59^{d}	238.43±61.01	5.90±0.07^{b}	6.10±0.11^{d}	2666.00±72.81^{c}
$Al_{1.5}$-$Pec_{1.01}$-$Chi_{1.70}$	90.59±0.08^{c}	78.91±0.05^{a}	248.24±33.26	4.66±0.01^{c}	5.95±0.02^{e}	4217.67±109.09^{a}
$Al_{1.5}$-$Car_{0.79}$	33.37±0.01^{h}	43.32±0.05^{g}	246.64±18.44	6.02±0.02^{b}	7.32±0.06^{c}	2712.33±18.82^{c}
$Al_{1.5}$-$Car_{0.79}$-$Chi_{1.46}$	99.69±0.05^{a}	72.01±0.65^{b}	286.46±2.94	6.59±0.54^{b}	7.39±0.07^{c}	3345.67±25.72^{b}
$Al_{1.5}$-$Gel_{0.75}$	43.00±0.10^{g}	42.34±0.05^{h}	242.18±63.27	6.73±0.26^{b}	7.59±0.03^{b}	2072.67±12.50^{e}
$Al_{1.5}$-$Gel_{0.75}$-$Chi_{1.49}$	92.56±0.04^{b}	69.69±0.06^{c}	263.20±34.88	8.15±0.51^{a}	7.66±0.03^{ab}	2471.67±5.77^{d}

Note:

- Values are expressed as mean ± standard deviation.
- Means within the same column followed by different superscript letters are significantly different according to Duncan's multiple range test ($p < 0.05$).
- Al: sodium alginate; Chi: chitosan; Pec: pectin; Car: carrageenan; Gel: gellan gum; EECPC: C-phycocyanin encapsulation efficiency; EEYield: bead production yield.
- Sodium alginate and $CaCl_2$ concentrations were fixed at 1.5% (w/v) for all formulations.
- $Al_{1.5}$ represents 1.5% (w/v) sodium alginate, while $Al_{1.5}$-$Pec_{1.01}$-$Chi_{1.70}$ represents a formulation containing 1.5% (w/v) sodium alginate, 1.01% (w/v) pectin, and 1.70% (w/v) chitosan.

Table 3. Kinetic parameters of CPC release from alginate-based hydrogel beads during the simulated intestinal phase

Model	Parameter	Al	Al-Chi	Al-Pec-Chi	Al-Car-Chi	Al-Gel-Chi
Zero-order	k_0	0.142 ± 0.007	0.219 ± 0.014	0.144 ± 0.003	0.109 ± 0.003	0.139 ± 0.008
	R^2	0.851	0.890	0.981	0.964	0.908
Higuchi	k_H	1.610 ± 0.027	2.299 ± 0.288	1.566 ± 0.112	1.181 ± 0.100	1.467 ± 0.171
	R^2	0.981	0.644	0.816	0.762	0.667
Korsmeyer–Peppas	k_p	1.047 ± 0.134	0.010 ± 0.008	0.101 ± 0.035	0.023 ± 0.007	0.009 ± 0.006
	n	0.591 ± 0.027	1.626 ± 0.158	1.072 ± 0.071	1.322 ± 0.067	1.548 ± 0.138
	R^2	0.992	0.967	0.983	0.989	0.971
Peppas–Sahlin	k_d	0.188 ± 0.375	-4.969 ± 0.758	-2.523 ± 0.402	-3.078 ± 0.565	-4.133 ± 0.585
	m	0.298 ± 0.006	0.433 ± 0.009	0.361 ± 0.006	0.351 ± 0.010	0.391 ± 0.008
	k_r	-0.361 ± 0.675	1.644 ± 2.827	0.026 ± 1.041	0.995 ± 1.443	1.092 ± 1.813
	R^2	0.992	0.972	0.988	0.962	0.971

Note: k_0, zero-order release constant; k_H, Higuchi release constant; k_p, Korsmeyer-Peppas release constant; n, release exponent; k_d, diffusion contribution constant; k_r, polymer-relaxation contribution constant; m, Peppas-Sahlin release exponent.