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Optimization of genistein solubilization by κ-carrageenan hydrogel using response surface methodology

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Abstract

κ-Carrageenan was explored to improve genistein solubility by matrix retention. The corresponding maximum value in the efficiency of retention (*Re*) (50.48 mg/100 mg) was achieved when variables were set as: pH 4.76, temperature 52.12 °C and genistein concentration 0.27 mg/mL. The coefficient of determination (R^2) of the response surface regression model presented in this study was 0.9848. The evidences from XRD, DSC and FT-IR attested the amorphous form of genistein in hydrogel matrix. Importantly, the solubility of genistein in hydrogel amorphous form (16.84–34.42 μg/mL) was much higher than that of its free crystalline form (1.89–6.09 μg/mL) over 30–90 °C.

Keywords: Genistein; Carrageenan; Hydrogel matrix; Response surface methodology; Water solubility

1. Introduction

Genistein [4′,5,7-trihydroxyisoflavone (Supplement 1)], the most abundant aglycone form of isoflavone in soybean, is associated with a variety of beneficial health effects. The approved beneficial functions included reducing risk of cardiovascular disease, lowering rates of prostate, breast, and colon cancers [1], and improving bone composition [2,3]. But the application of genistein has been limited by its poor water solubility and bioavailability. Even worse once absorbed in human body, genistein undergoes rapid degradation and excretion within 24 h [4,5].

Genistein is classified in class II (poor soluble/permeable) in the Biopharmaceutical Classification System (BCS) [6]. Especially for this class of substance, solubility enhancement is crucial part of the strategies to improve bioavailability. Many studies have been focused on complexation of genistein with cyclodextrins (CDs) to improve water solubility of genistein. Daruházi et al. [7] reported that genistein formed a supramolecule with both β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD), while it did not form a stable complex with α-cyclodextrin (α-CD). The genistein/γ-CD provided a peak genistein concentration of 27 μg/mL, and the genistein/β-CD was 13 μg/mL, in contrast to the plain genistein alone 3 μg/mL.

Matrix retention is a promising method to solubilize various less-soluble compounds. In this technique, the less-soluble compounds are mixed with a water-soluble carrier through various measures. The commonly used carriers are long-chain polymers, such as carrageenan, polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) [8]. It has been proved that addition of a small amount (0.10%–0.25%, *m/V*), of hydroxypropyl methylcellulose (HPMC) or PVP resulted in significant enhancement of the aqueous solubility of drug, because 30%–50% of drug molecules were bound to the polymers [9]. Dong and Song [10] made a complex of κ-carrageenan with an insoluble drug, which significantly increased the compound's water solubility from 1–2 μg/mL to 30 μg/mL. Nanosizing the complex, solubility of the less-soluble drug increased to 37 μg/mL.

Abbreviations: BCS, biopharmaceutical classification system; CDs, complexation of genistein with cyclodextrins; PVP, polyvinylpyrrolidone; PEG, polyethylene glycol; HPMC, hydroxypropyl methylcellulose; *Re*, the efficiency of retention; BBD, Box–Behnken design; XRD, X-ray diffraction; DSC, differential scanning calorimetry; FT-IR, Fourier transform infrared; SD, standard deviation; ANOVA, analysis of variance; CCC, critical cooperativity concentration.

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Tightly related to the present work, hydrocolloids are mostly used to hold water and provide texture for food. They also have the ability to bind guest molecules and by this influence the characteristics of guest molecules. In a broad sense, the term binding includes adsorption and physical entrapment in colloid matrices, as well as inclusion complexation [11]. Genistein also could be absorbed by hydrocolloids. Pandjaitan et al. [12] reported genistein was enriched in soy protein with the addition of hydrocolloids. But no study has applied κ-carrageenan to improve water solubility of genistein.

The goal of this study was to present a novel approach to prepare κ-carrageenan/genistein matrix with an aim to improve water solubility of genistein. Operational parameters were optimized. Additionally, the XRD, DSC and FT-IR were employed to investigate the characteristics of matrix. We hope this study will be helpful to further exploit for utilization of κ-carrageenan hydrogel to improve solubility of poorly water-soluble compounds.

2. Materials and methods

2.1. Materials

Genistein (4′,5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$, molecular weight 270.24) was obtained from Chengdu Purifa Scientific Ltd. (Chengdu, China), with the purity higher than 98%. Genistein solution was prepared by dissolving in 0.1 mol/L NaOH at 30 °C with genistein concentration ranging from 0.1 mg/mL to 2 mg/mL. κ-Carrageenan was supplied by Tianjing Bodi Chemical Co., Ltd. (Tianjing, China) and employed without any further purification. κ-Carrageenan was dissolved in water (0.2 g, 40 mL) at 80 °C under stirring for 60 min to prepare homogeneous solution and then cooled to desired temperature.

Water used throughout the study was double-distilled, and then filtered through 0.22 μm Millipore® GSWP filters (Bedford, USA). Solutions to be analyzed by HPLC were prior filtered through 0.45 μm Sartorius Minisart®-SRP 15 PTFE filters (Germany). The HPLC-grade methanol was purchased from Tianjing Siyou Fine Chemical Co., Ltd. (Tianjing, China). All other reagents were of analytical grade.

2.2. Preparation of genistein–hydrocolloid complex

Complexation was carried out by the acidification of an alkali solution ($NaOH/H_3PO_4$) as previously described [13,14]. When κ-carrageenan solution reached 50 °C, 10 mL of genistein solution was injected. The final concentrations were κ-carrageenan at 4 mg/mL and genistein at 0.2 mg/mL. After stirring for 20 s, the mixture was precipitated by adjusting the pH to 4.7 (±0.1) by using 5% H_3PO_4 and then held for 60 min under gentle stirring at 50 °C. All samples were then centrifuged (14,000 × *g*, 25 min), the supernatant was discarded, and the precipitate was washed twice with an ethanol/water mixture (50:50 *m/m*) and centrifuged as before. The complexes were then freeze-dried.

The single factor experiments were carried out following the same process as above. Final concentration of genistein varied from 0.02 mg/mL to 0.4 mg/mL by altering the concentration of genistein alkali solution. Incubation temperature varied in the range of 30–90 °C, which was the same as hydrocolloid solution reached before genistein solution was injected. Final pH (3–8) and incubation time (5–90 min) were also investigated.

A physical mixture of genistein and κ-carrageenan in the same ratio as the complex was prepared. Genistein and κ-carrageenan were admixed in an agate mortar and pestled to obtain homogeneous blend.

Under the optimal condition the effects of NaCl concentration (10–100 m mol/L) and ethanol concentration (2%–20%, *V/V*) were assessed.

Table 1
Box–Behnken design matrix (in uncoded level of three variables), experimental data and predicted values for three-level-three-factor response surface analysis.

Number[a]	Temperature (°C)	Genistein concentration (mg/mL)	pH	R_e[b]	
	X_1	X_2	X_3	Experimental[c]	Predicted
1	40	0.10	5	24.40 ± 1.31	23.42
2	70	0.10	5	3.45 ± 0.12	2.65
3	40	0.30	5	44.43 ± 1.94	45.24
4	70	0.30	5	47.72 ± 3.83	48.7
5	40	0.20	4	46.95 ± 2.05	48.16
6	70	0.20	4	24.84 ± 0.94	25.88
7	40	0.20	6	28.87 ± 1.02	27.83
8	70	0.20	6	34.03 ± 1.68	32.82
9	55	0.10	4	15.00 ± 1.28	14.77
10	55	0.30	4	47.96 ± 2.73	45.94
11	55	0.10	6	3.29 ± 0.10	5.31
12	55	0.30	6	41.78 ± 0.22	42.01
13	55	0.20	5	48.03 ± 2.79	46.41
14	55	0.20	5	47.12 ± 2.00	46.41
15	55	0.20	5	43.06 ± 2.21	46.41
16	55	0.20	5	43.15 ± 1.75	46.41
17	55	0.20	5	50.71 ± 2.83	46.41

[a] Experiments were conducted in a random order.
[b] The efficiency of retention of genistein with κ-carrageenan (mg genistein/100 mg κ-carrageenan).
[c] Each value represented the mean ± SD ($n = 3$).

2.3. High performance liquid chromatography (HPLC) to quantify genistein content

Genistein content in the matrix was tested by full dissolution of the matrix in 0.1 N NaOH for the release of genistein [13]. Five milligrams of the matrix was incubated in 1 mL of 0.1 N NaOH solutions at 30 °C for 12 h. Because genistein is poorly soluble in water, after this incubation, samples were diluted by phosphate buffer (PBS) to a concentration below their solubility limit. PBS (159 mL, 20 m mol/L phosphate, pH 6.9, and 10 m mol/L NaCl) was added to the solution. Genistein was quantified from the solution by HPLC. The efficiency of retention (*Re*, mg/100 mg) was used to represent genistein content (mg) in 100 mg complex. An LC-20AD HPLC system (Shimadzu, Japan) equipped with a diode array detector (D-M20A) and auto sampler (SIL-20A) was applied to quantify genistein content. HPLC analysis was carried out on an Ultimate 18C reverse phase column (Thermo) 250 mm × 4 mm with 5 μm packing. Samples were eluted at

30 °C at a total flow rate of 1 mL/min composed of 30% solvent A [0.4% phosphoric acid in water (*V/V*)] and 70% solvent B (methanol, HPLC grade). The whole process held for 15 min. The injection volume was 20 μL, and detection was done by UV absorbance at 260 nm [15]. The amount of genistein was quantified by calibration curve using pure genistein as a standard.

2.4. Experiment design

A three-variable, three-level Box–Behnken design (BBD) was employed to optimize the process and evaluate the effects and interactions of the process variables [16]. The independent variables and levels were temperature (X_1, 40, 55 and 70 °C), the genistein concentration (X_2, 0.1, 0.2 and 0.3 mg/mL) and final pH (X_3, 4, 5 and 6), with incubation time for 60 min. Seventeen experiments (Table 1) which included 12 factorial points and 5 center points, were performed in triplicate. Experiments at the center points were conducted for evaluation of the experimental error. A second-order polynomial regression model was used to express *Re* as a function of the independent variables as follows:

$$Y = \alpha_0 + \sum_{i=1}^{3}\alpha_i X_i + \sum_{i=1}^{3}\alpha_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{3}\alpha_{ij} X_i X_j$$

where Y represents the response variables, α_0 is a constant, α_i, α_{ii} and α_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables.

2.5. X-ray diffraction (XRD)

The XRD diffraction patterns of genistein, κ-carrageenan, physical mixture and matrix were recorded with an X'Pert pro X-ray diffractometer equipped with X'celerator as detector (Panalytical, Kassel, Germany). The diffractograms were registered at Bragg angle (2θ) = 5–30° at a scan rate of 5°/min, with step width = 0.02°, and the crystalline nature of the substance was determined by the position of the X-ray diffraction peaks.

2.6. Differential scanning calorimetry (DSC)

Thermal analyses of genistein, κ-carrageenan, physical mixture and matrix were carried out using a differential scanning calorimeter (SDTQ600, TA Instruments, USA). The samples of about 5.5–6.5 mg dry matter were heated at a heating rate of 10 °C/min from 20 °C to 320 °C in the atmosphere of nitrogen, using an empty pan sealed as reference.

2.7. Fourier transform infrared (FT-IR)

FT-IR spectra of genistein, κ-carrageenan, physical mixture and matrix were recorded in an IR Spectrometer (Perkin-Elmer, model 2000, USA) in the wavelength range 450–4000 cm^{-1}. To perform FT-IR measurement, 5 mg of powder sample was dispersed in 200 mg of KBr (pellet procedure). Signal averages were obtained at a resolution of 4 cm^{-1}.

2.8. Solubility

The water solubility of the free genistein or in the form of carrageenan/genistein matrix was determined by a shake-flask method [17]. An excess amount of genistein or matrix was added to 25 mL of distilled water with temperatures ranging from 30 °C to 90 °C. The temperature was controlled by a thermostat (uncertainty of ±0.1 °C) in the shaker. The suspended solution was continuously shaken with 100 rotations per minute for 24 h. After attaining equilibrium, the supernatant liquid was held still for 2 h and filtered through a 0.45 μm membrane which was the same temperature with the samples. The filtered solution was analyzed by an HPLC method. Each measurement was repeated three times.

2.9. Statistical analysis

All the experiments were performed in triplicate. The results were expressed as mean ± standard deviation (SD), and the mean values were considered significantly different at $P < 0.05$ by Duncan's multiple range tests after subjecting to an analysis of variance (ANOVA) processed with SPSS 18.0. The optimal conditions for complexation were estimated through regression analysis and three dimensional response surface plots of the independent variables and each dependent variable which was computed by Design-Expert (version 7.0).

3. Results and discussion

3.1. Effect of physico-chemical conditions on Re of κ-carrageenan with genistein

A study of the rate of the matrix formation was conducted with time varying from 5 to 90 min (Fig. 1A). There was a faster increase in the first 10 min, and the plateau was almost reached in 30 min. It was enough for *Re* to achieve the maximum at 60 min. The complexation of κ-carrageenan with small-molecule compound in most literatures was prepared by dialysis equilibrium which took 24 h to reach equilibrium at least [18]. Although the same method was applied, Cohen et al. [13] spent 24 h on stirring to prepare amylose/genistein complex. In previous study, most of the binding of procyanidins to apple cell wall material occurred in the first 10 min through non-covalent interaction [19]. The molecular interaction of cereal soluble dietary fiber polymers and a model bile salt was almost achieved within 2 h [20].

The effect of pH on *Re* of κ-carrageenan is illustrated in Fig. 1B. *Re* increased with pH from 3 to 5 and a sharp decrease appeared between 6 and 8. The maximum *Re* occurred at pH 5, up to 47.14 mg/100 mg. At pH 8, *Re* was the lowest, which might result from that genistein was readily soluble in alkali conditions. The interactions between amylose helices and genistein molecules decreased at pH 8 resulting in the increase of free genistein in solution [13]. Several studies indicated that the pH had no influence on interactions between κ-carrageenan and small molecule water-soluble amphiphile drugs below 7 [21]. However a great influence of pH on interactions between cell wall material and small molecules was found [22].

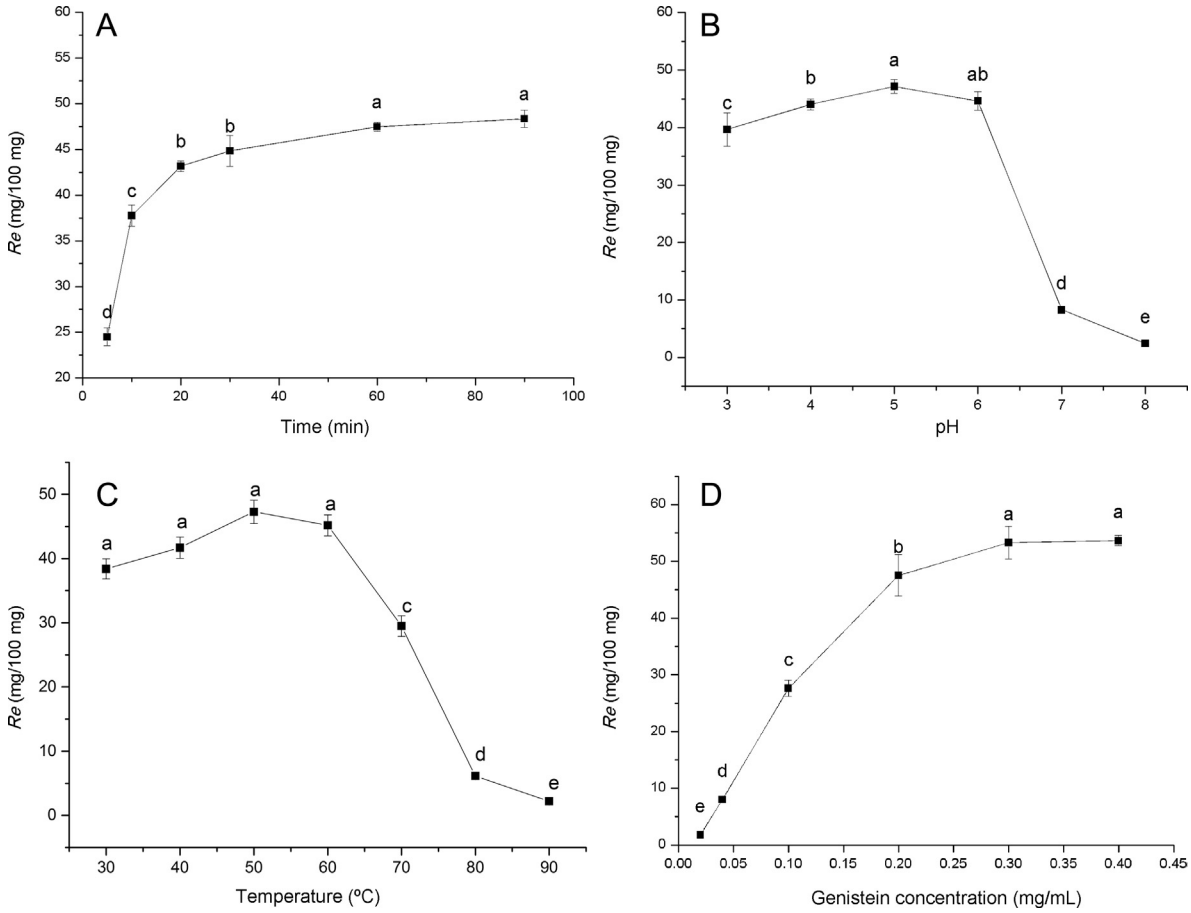

Fig. 1. Effects of time (A), pH (B), temperature (C) and genistein concentration (D) on the efficiency of retention (*Re*) of genistein with *κ*-carrageenan. Different lowercase letters indicate a significant difference at $P < 0.05$. Data shown in mean ± standard deviation ($n = 3$).

Fig. 1C shows the result of temperature influence on *Re* of *κ*-carrageenan with genistein. The *Re* increased until 50 °C and there was a sharp decrease through 60–80 °C. It can be easily inferred that the complexation of genistein with *κ*-carrageenan was not simple endothermic process. And the matrix was not just physical adsorption [23].

At high temperature, *κ*-carrageenan was assumed to have a random coil conformation. On reducing the temperature, it started to form double helices and aggregates that act as knots for the physical junction of the gels. The helix-form of the polymer showed a favorable effect on binding of small molecule compounds. The shorter distance of helix formed led up to a limit to higher hydrophobic interactions between the bound small molecules [24]. The increase of *Re* could partly been explained by the coil-to-helix transition of the *κ*-carrageenan with temperature falling from 80 °C [25]. On the other hand, after the formation of hydrogen bonding, the distance between aromatic rings in genistein and sugar rings in *κ*-carrageenan became shorter. Reducing the distance could enhance the van der Waals interactions [26].

The experiments were carried out with different genistein concentration (0.02–0.4 mg/mL) in Fig. 1D. A direct steep line appeared while genistein concentration increased from 0.02 mg/mL to 0.2 mg/mL with *Re* increasing from 1.77 mg/100 mg to 47.53 mg/100 mg. A plateau was almost reached when genistein concentration exceeded 0.3 mg/mL. A critical cooperativity concentration (CCC) was introduced in the adsorption isotherms investigated [18]. Below CCC, amphiphile almost did not bind to the *κ*-carrageenan, while above CCC a major portion of the added amphiphile bound to the *κ*-carrageenan immediately. When concentration was less than CCC, The adsorption was due to ionic interactions between the amphiphile and *κ*-carrageenan. Hydrophobic interactions of the bound amphiphile molecules probably were the main force with concentration above CCC. As for genistein, the CCC did not appear. It may be explained that genistein was not amphiphile. In the adsorption of procyanidins to apple cell wall material [19] and tea polyphenols to oat β-glucan [27], the CCC also did not appear.

3.2. Optimization of complexation parameters for genistein with κ-carrageenan

3.2.1. Fitting the model

The operational parameters were optimized using Box–Behnken design combined with response surface methodology. The experiment design and corresponding response data for the complexation are presented in Table 1. The results of analysis of variance, goodness of fit and the adequacy of the model are summarized in Table 2. It was evident

Table 2
Analysis of variance (ANOVA) for the response surface quadratic polynomial model for efficiency of retention of carrageenan and genistein.

Source[a]	Sum of squares	df[b]	Mean square	*F*-value	*P*-Value
Model	3897.272	9	433.0302	50.45	<0.0001*
X_1	149.6765	1	149.6765	17.44	0.0042*
X_2	2303.858	1	2303.858	268.46	<0.0001*
X_3	89.64665	1	89.64665	10.44	0.0144*
X_1X_2	146.8658	1	146.8658	17.11	0.0044*
X_1X_3	185.8887	1	185.8887	21.66	0.0023*
X_2X_3	7.63055	1	7.63055	0.89	0.3771
X_1^2	99.97996	1	99.97996	11.65	0.0112*
X_2^2	560.5507	1	560.5507	65.32	<0.0001*
X_3^2	260.5465	1	260.5465	30.36	0.0009*
Residual	60.07219	7	8.581741		
Lack of fit	16.5781	3	5.526033	0.51	0.6976
Pure error	43.49409	4	10.87352		
Cor total	3957.344	16			

[a] X_1: temperature (°C); X_2: genistein concentration (mg/mL); X_3: pH.
[b] Degree of freedom.
* Significant at $P<0.05$.

that all the three linear (X_1, X_2 and X_3), two interaction terms (X_1X_2, X_1X_3) and three quadratic parameters (X_1^2, X_2^2 and X_3^2) were significant at the level of $P<0.05$ or $P<0.01$. Only one interaction (X_2X_3) was insignificant ($P>0.05$). The coefficients of independent variables determined for the second-order polynomial model are shown in the following equation:

$$Y = 46.41 - 4.33X_1 + 16.97X_2 - 3.35X_3 + 6.06X_1X_2 + 6.82X_1X_3 + 1.38X_2X_3 - 4.87X_1^2 - 11.54X_2^2 - 7.87X_3^2$$

The results of variance (ANOVA) analysis for the model are shown in Table 2 including a good model performance with the correlation coefficient (R^2) value of 0.9848. The calculated model was able to explain 98.48% of the result in the case of *Re*. Exploration and optimization fitted response surface may mislead results, unless the model exhibits a good fit, which makes checking the model adequacy essential. The statistical analysis gave high significant level ($P<0.0001$), attesting excellent goodness of fit of the model. The lack-of-fit *F*-value of 0.51 implied that the lack-of-fit was not significant relative to the pure error. The results indicated that the model could work well for the prediction of the *Re* of κ-carrageenan with genistein [16].

3.2.2. Analysis of response surfaces

The relationship between independent and dependent variables was illustrated by the three-dimensional representation of the response surfaces (Supplement 2A, C and E) and the two-dimensional contours (Supplement 2B, D and F) generated by the model (Supplement 2). Two variables within the experimental range were depicted in three-dimensional surface plots when the third variable was kept constant at zero level. The shapes of the contour plots, elliptical or circular, indicate whether the interactions between the corresponding variables are significant or not. An elliptical contour plot means the interactions between the variables are significant while a circular contour plot means otherwise. It was revealed that the effect of genistein concentration was more significant than temperature and there was a clear optimal level for temperature around 55 °C. When genistein concentration was fixed at 0.2 mg/mL level, the attribute of pH was different when temperature was at lower or higher. The elliptical showed the interaction of pH and temperature was significant. It could draw the conclusion that interactions between genistein concentration and pH were not significant when temperature was fixed at 55 °C. There was a clear optimal level for pH around 4.7, and genistein concentration around 0.26 mg/mL.

3.2.3. Validation of the model

The optimized results showed that the highest *Re* of κ-carrageenan with genistein could reach 52.92 mg/100 mg under the following conditions: pH 4.76, temperature 52.12 °C and genistein concentration 0.27 mg/mL. The suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. In the optimal conditions, the experimental *Re* was 50.48 ± 2.35 mg/100 mg, which was consistent with the predicted value 52.92 mg/100 mg (relative error 4.67%). Although genistein was not a charged amphiphile, the complexation with κ-carrageenan resulted in a huge *Re* by the acidification of an alkali solution method. Reportedly, the maximum binding capacity of κ-carrageenan was 51:49 (*m/m*) for bupropion HCl, 63:37 (*m/m*) for diltiazem HCl, 67:33 (*m/m*) for metoprolol tartrate, and 50:50 (*m/m*) for tramadol HCl [28].

3.3. Effect of ethanol and NaCl concentration

Under the optimal condition (temperature 52.1 °C, pH 4.8 and genistein concentration 0.27 mg/mL), the effect of NaCl concentration (10–100 mol/L) and ethanol concentration (2–20%, v/v) is showed in Fig. 2. When the NaCl concentration increased no significant variation occurred (Fig. 2A). The result probably suggested that the electrostatic interaction between genistein and κ-carrageenan was negligible [19]. On the other hand, about 20 m mol/L Na^+ already exists when genistein alkali solution was injected. The following addition of NaCl could not affect *Re* significantly.

The *Re* decreased with increasing ethanol concentration (Fig. 2B). Compared with control (0 ethanol concentration), 2% ethanol brought a significant reduction with the *Re* 39.17 mg/100 mg. No significant difference appeared between 5% and 10% ethanol concentration corresponding to the *Re* 27.71 mg/100 mg and 24.80 mg/100 mg, respectively. Obviously 20% had the lowest *Re* (17.81 mg/100 mg). We have not done any experiments at a higher ethanol concentration, because higher ethanol concentrations lead to an insolubleness of κ-carrageenan completely. When ethanol concentration varied in the range 10–40%, a sharp reduction was investigated for procyanidins binding to apple cell wall material [19]. No further effect beyond 40% was detected. Ethanol is non-polar solvent. Decreasing the polarity by addition of ethanol could disrupt hydrophobic interactions. Ethanol is also a hydrogen bond acceptor, which could disturb the interactions between two compounds by hydrogen bond [29]. By addition of ethanol

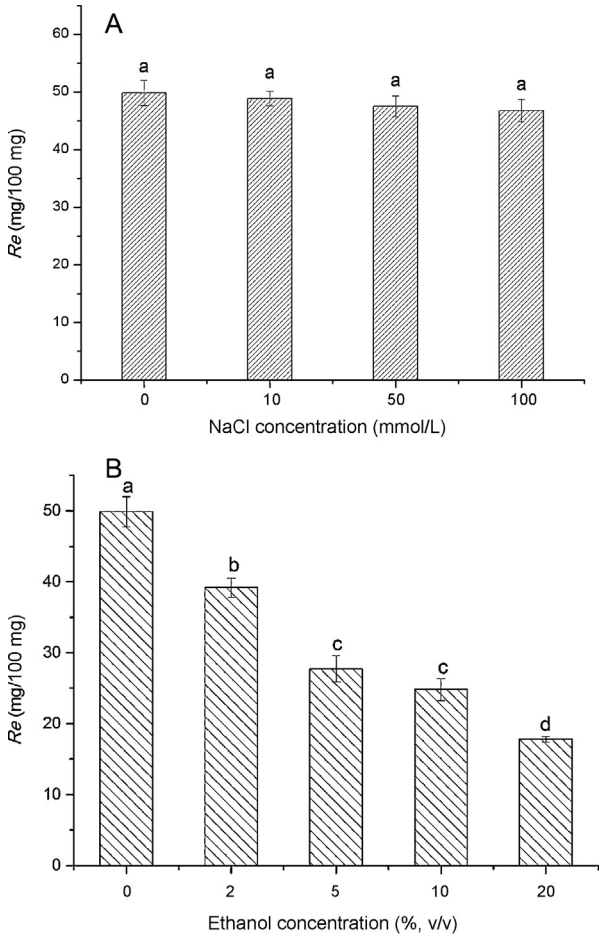

Fig. 2. Influence of NaCl (A) and ethanol (B) concentration on the efficiency of retention (*Re*) of genistein with κ-carrageenan. The matrix system was composed of 40 mL of 4 mg/mL κ-carrageenan concentration and 10 mL of genistein in 0.1 N NaOH with final pH 4.76, genistein concentration 0.27 mg/mL, and at 52.12 °C incubated for 60 min. Different lowercase letters indicate a significant difference at $P < 0.05$. Data shown in mean ± standard deviation ($n = 3$).

the *Re* strongly decreased probably because the hydrophobic interactions and hydrogen bond were bated.

3.4. Characteristic of genistein–carrageenan complex

3.4.1. X-ray diffraction (XRD)

The characteristic of carrageenan/genistein matrix was investigated in the results of XRD (Fig. 3A). In this research, genistein, κ-carrageenan and their physical mixture were used as references. The pure genistein showed reflection corresponding to the Bragg angles (2θ) at 7.5°, 12.8°, 14.0°, 14.3°, 15.0°, 16.8°, 18.1°, and 22.6°. There was a strong reflection for κ-carrageenan at 28.5°. The physical mixture of κ-carrageenan and genistein did retain most of their diffraction patterns. Just the peak of genistein at 16.8° weakened in physical mixture. The carrageenan/genistein matrix showed a diffraction pattern different from that of pure genistein, κ-carrageenan and their physical mixture. The carrageenan/genistein matrix showed reflection corresponding to the Bragg angles (2θ) at 14.5° and 28.5°. Compared with κ-carrageenan the reflection at 28.5° was impaired

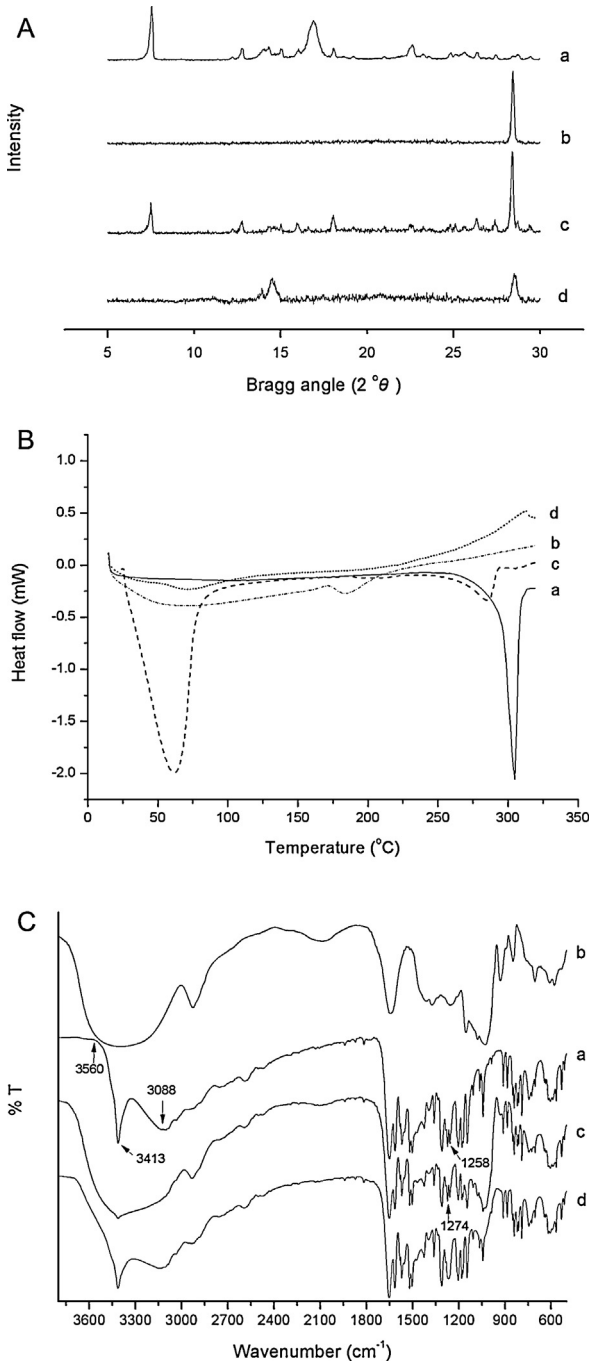

Fig. 3. Instrumental characterization of the interaction between genistein and κ-carrageenan by XRD (A), DSC (B) and FT-IR (C). a: genistein, b: κ-carrageenan, c: genistein–carrageenan physical mixture and d: carrageenan/genistein matrix.

while the Bragg angle at 14.5° was reinforced. These diffractograms indicated that genistein in matrix might lose its crystal, and the orderly structure of κ-carrageenan was abated.

3.4.2. Differential scanning calorimetric (DSC)

The carrageenan/genistein matrix was further characterized by the DSC results (Fig. 3B). As can be observed in Fig. 3B, genistein presented a unique endothermic peak at 305 °C, characteristic of melting point, in agreement with the previous literature [30]. The physical mixture presented a broad band at

around 65 °C. This was characteristic of losing water. The intensity of the peak corresponding to the melting point of genistein was reduced and its position displaced the same with curcumin and PVP physical mixture [31]. While in carrageenan/genistein matrix, the melting point of genistein can be no longer observed. The absence of melting peaks in the DSC thermogram indicated that genistein lost its crystal and become amorphous [30]. It was probably that during preparation the random polymer chain in κ-carrageenan created the irregular structures, which adsorbed genistein due to non-covalent bond [10].

3.4.3. Fourier transform infrared (FT-IR)

The result of FT-IR of the characteristic of carrageenan/genistein matrix is showed in Fig. 3C. As can be seen in Fig. 3C, the infrared spectrum of genistein shows several characteristic peaks. The most prominent are at 3413 cm^{-1} and ~3088 cm^{-1}, representing O–H and aromatic C–H stretching vibrations, respectively. The vibrational stretching frequency of C=O, C=C, C–O–C and C–C appears at 1650, 1615, 1320–1150, 1260–1000 cm^{-1}, respectively [32].

A change in band at 3700–3100 cm^{-1} was obvious for carrageenan/genistein matrix, compared with spectra of genistein and physical mixture. It was sharper than that of physical mixture, but wider than that of genistein, indicating a mid content of free –OH groups. It may be explained that the interaction of –OH in κ-carrageenan was diminished because of the formation of hydrogen bonding between genistein and κ-carrageenan [33]. The peak at 2924 cm^{-1} corresponding to $-CH_2$ asymmetric stretching vibration weakened in matrix. Coalescent and band enlargement were distinct at 1274 cm^{-1} and 1258 cm^{-1} corresponding to the C–O stretching. These suggested an amorphization of the carrageenan/genistein matrix [34]. The bands located at 1106 cm^{-1}, 1063 cm^{-1} and 1043 cm^{-1} had been enhanced, whereas the bands at 1077 cm^{-1} and 1027 cm^{-1} disappeared, indicating that the ring C–H in plane bending vibration diminished. These can be probably due to the interaction between hydroxyl groups of genistein and 3 oxygen atoms of the sulfate groups in κ-carrageenan. The interaction may alter the spatial structure of genistein and κ-carrageenan.

3.5. Specific solubility of genistein in carrageenan/genistein matrix

The specific solubility of genistein in carrageenan/genistein matrix at different temperatures is exhibited in Fig. 4. The specific water solubility of genistein in matrix increased from 1.89 μg/mL to 16.84 μg/mL at 30 °C, a 8.9-fold increase compared with pure genistein. A significant increase appeared at 50 °C for carrageenan/genistein matrix (26.33 μg/mL), a 7-fold increase compared with genistein (3.59 μg/mL). When temperature reached 90 °C, the solubility (34.42 μg/mL) was increased 5 times compared with free genistein (6.09 μg/mL). Following the increase of temperature, the specific water solubility of genistein increased significantly. As demonstrated in the results of XRD, DSC and FT-IR, genistein lost its crystalline structure in κ-carrageenan/genistein matrix. Compounds had higher solubility in an amorphous state than in a crystalline structure [10]. As

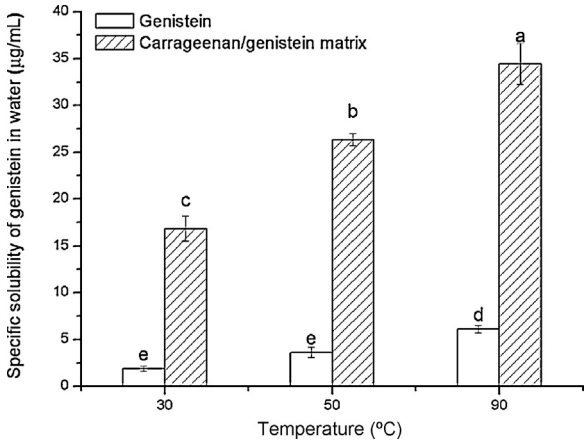

Fig. 4. Specific solubility of genistein as a form carrageenan/genistein matrix in water as a function of temperature. Different lowercase letters indicate a significant difference at $P < 0.05$. Data shown in mean ± standard deviation ($n = 3$).

temperature increased, the chain of κ-carrageenan in matrix dispersed in solution, which probably increased the water solubility of genistein.

4. Conclusion

Solubility is one of most important factors that affect bioavailability of poorly water-soluble genistein which limits broad utilization in food and pharmacy. In this study, a novel approach to prepare carrageenan/genistein matrix was investigated. A three-variable, three-level Box–Behnken design was applied to enhance *Re* of κ-carrageenan. The optimal conditions determined were as follows: pH 4.76, temperature 52.12 °C and genistein concentration 0.27 mg/mL. Under these optimal conditions, a maximum genistein fraction 50.48 mg/100 mg could be achieved. The results of XRY, DSC and FT-IR approved that genistein in matrix lost its crystal structure. Hydrogen bond and hydrophobic interactions may exist, confirmed by the effect of ethanol concentration. Remarkable increase of genistein specific solubility in matrix was studied at different temperatures. Further study should be carried out to elucidate the effect of carrageenan molecular weight on specific solubility of genistein. The mechanism of molecular interaction between carrageenan and genistein should be testified in future. It is also worthy to investigate the rule of genistein releasing in gastrointestinal tract and the bioavailability of genistein *in vivo* in the form of matrix.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fshw.2013.06.001.

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