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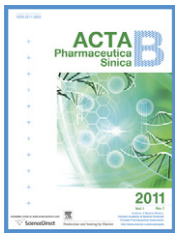

ORIGINAL ARTICLE

Statistically designed enzymatic hydrolysis of an icariin/β-cyclodextrin inclusion complex optimized for production of icaritin

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KEY WORDS

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Box–Behnken statistical design;
Icaritin

Abstract This study focuses on the preparation and enzymic hydrolysis of an icariin/β-cyclodextrin inclusion complex to efficiently generate icaritin. The physical characteristics of the inclusion complex were evaluated by differential scanning calorimetry (DSC). Enzymatic hydrolysis was optimized for the conversion of icariin/β-cyclodextrin complex to icaritin by Box–Behnken statistical design. The inclusion complex formulation increased the solubility of icariin approximately 17-fold, from 29.2 to 513.5 μg/mL at 60 °C. The optimum conditions were predicted by Box–Behnken statistical design as follows: 60 °C, pH 7.0, the ratio of enzyme/substrate (1:1.1) and reaction time 7 h. Under the optimal conditions the conversion of icariin was 97.91% and the reaction time was decreased by 68% compared with that without β-CD inclusion. Product analysis by melting point, ESI-MS, UV, IR, ^{1}H NMR and ^{13}C NMR confirmed the authenticity of icaritin with a purity of 99.3% and a yield of 473 mg of icaritin from 1.1 g icariin.

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1. Introduction

Herba Epimedii, which is derived from the aerial parts of *Epimedium brevicornum*, *E. sagittatum*, *E. pubescens*, *E. wushanense* and *E. koreanum*, is a well-known traditional Chinese medicine[1]. The main constituents of Herba Epimedii are reported to be flavonoids[2], including icariin[3] and icaritin (Scheme 1).

Pharmacological studies showed that icaritin, the aglycone of icariin, enhanced the differentiation and proliferation of osteoblasts[4] and facilitated matrix calcification[5]. It also inhibited human prostate carcinoma PC-3 cells growth[6] and had inhibitory effects on the proliferation of breast cancer cells[7]. Nevertheless, the amount of icaritin in Herba Epimedii is very low, and so the preparation of ample quantities for pharmacological studies is a challenge.

Acid hydrolysis is a common method for preparation of aglycone from glycosides; however, this method requires strict conditions, gives low yields, numerous byproducts and contributes to environmental pollution[8,9]. Indeed, the yield of icaritin from icariin by this technique is poor[10]. Enzyme hydrolysis is widely used for the selective preparation of aglycones from glycosides; hesperidinase and nariginase have been used for this purpose[11–13]. At least one group used snailase to prepare icaritin; Jia et al.[14] found that 48 h was necessary to produce icaritin under the optimum enzymolysis conditions, with a yield of 92%. However, the time required for the enzymatic reaction was relatively long. The reason for the result in this study may be the low solubility of icariin in water[15], which restricted the speed of enzyme hydrolysis.

There are a number of methods used in enzyme hydrolysis, such as promoting the activity of enzymes at high pressure[16] and processing on immobilized biocatalysts[17]. The immobilized enzymes generally present mass transfer limitations and showed lower catalytic activity due to reduced substrate transfer resistance[18]. High pressure added to the process cost. Therefore, these methods were not efficient with poorly water-soluble substrates. Finding an approach to increase conversion efficiency by enhancing the aqueous solubility of poorly water-soluble substrates seems necessary and important.

β-cyclodextrin (β-CD, Fig. 1) is a circular molecule linked by α-1,4-bonds. Hydrophilic outer tails and hydrophobic inner cavities allow β-CD to easily form an inclusion complex with a diversity of molecules to increase their solubility[19–21]. In the pharmaceutical industry β-CD has often been used to enhance the solubility of drugs such as isoproturon[22], celecoxib[23] and many other hydrophobic drugs[24,25], suggesting to us that icariin/β-CD inclusion complex may improve solubility of icariin and consequently increase the enzymatic hydrolysis rate of icariin to icaritin.

So far, few papers have reported on enzymatic hydrolysis of β-CD inclusion complexes targeting compound preparation using response surface methodology. The aim of this study was to form an inclusion complex to increase the solubility of icariin and test its use for the preparation of icaritin by enzymatic hydrolysis of icariin/β-CD inclusion complex.

2. Experimental

2.1. Apparatus

The analysis was carried out with an Agilent 1100 HPLC system (Agilent, USA), which consists of an auto sample injection (G1313A), QuatPump (G1311A), column component (G1316A) and photodiode array detector (G1315A). Agilent ChemStation Software (Rev.B.03.02) was used to analyze the data. Enzymatic hydrolysis was carried out in a digital temperature air bath oscillator (Jintan Shuangjie Experimental Instrument Factory, China). A TGL-16H high-speed centrifuge

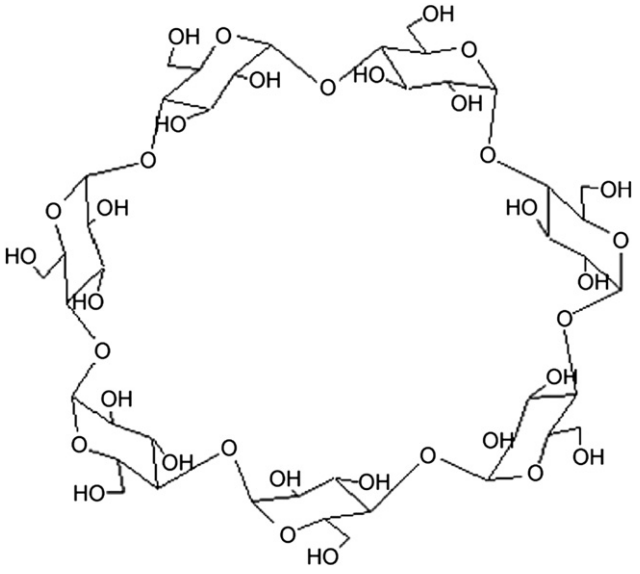
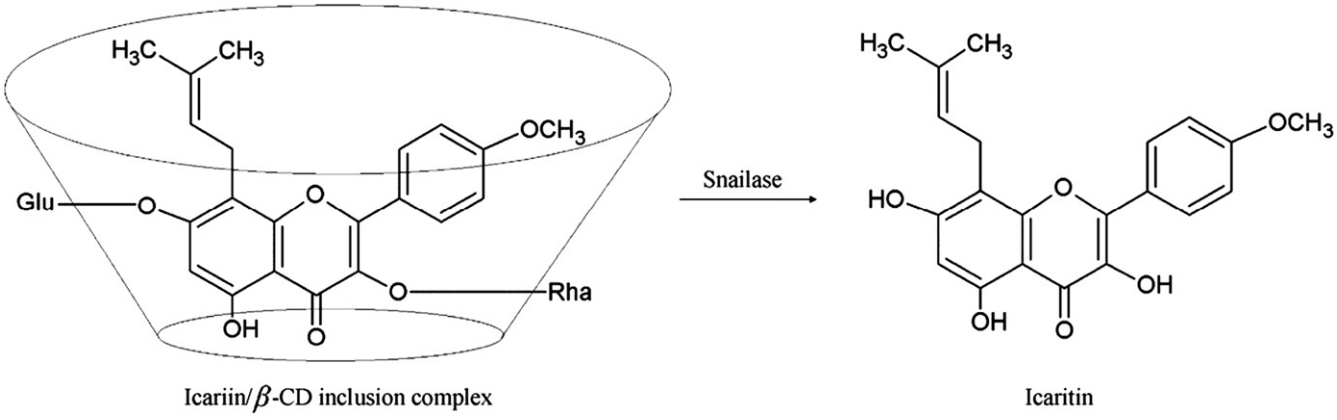


Figure 1 Schematic representation of the β-cyclodextrin molecule.



Scheme 1 Snailase-catalyzed transformation of icariin/β-CD inclusion complex to icaritin.

(Shanghai Precision Instrument Factory, China) was used to prepare the samples.

2.2. Materials and reagents

Acetonitrile and methanol were of chromatographic grade (TEDIA, UN). All other reagents were of analytical grade. Ultra-pure water was prepared with Milli-Q water system (Bedford, MA). β-cyclodextrin (average molecular weight: 1135) was purchased from Shanghai Chemical Reagent Company of China Pharmaceutical Group. Snailase was purchased from Baier Di Biotechnology Co., Ltd. Icariin (purity > 98%) and icaritin (purity > 98%) were provided by the Laboratory of Pharmaceutical Preparation (Jiangsu Provincial Academy of Chinese Medicine, China).

2.3. Preparation of the icariin/β-cyclodextrin inclusion complex

The inclusion complex of icariin with β-cyclodextrin at a 1:1 molar ratio was prepared using the dropping method. β-Cyclodextrin was dissolved in distilled water until the solution was saturated. Then icariin, dissolved in 50% (*v*/*v*) ethanol, was added to the saturated β-CD solution drop by drop. The suspension was stirred at 60 °C for 12 h. After standing overnight at 4 °C the suspension was filtered and unincluded drug was removed from the preparation with a 50% (*v*/*v*) ethanol wash. The final icariin-β-CD complex was lyophilized for use.

2.4. Determination of icariin and icariin/β-CD inclusion complex solubility

An excess amount of icariin and icariin/β-CD inclusion complex was added to water and the solution was stirred using a magnetic stirrer for 24 h at 60 ± 0.1 °C, after which the solution was allowed to settle for 2 h. The supernatant liquid was taken and filtered through a 0.45 μm membrane (Chuangwei, China). The first 15% of the filtrate was discarded to avoid any potential loss of the drug due to absorption by the filter and the subsequent filtrate was collected. All procedures were conducted at 60 ± 0.1 °C to avoid precipitation of the drug[26]. A 20 μL aliquot of the resulting solution was analyzed by HPLC in triplicate.

2.5. Differential scanning calorimetry (DSC)

Samples were sealed in aluminum crimp cells and heated at a rate of 10 °C/min from 30 to 450 °C under nitrogen (DSC-60, Shi-Madzu, Japan). The peak transition maximum temperatures of β-CD, pure icariin, the mixture of β-CD and icariin and icariin/β-CD inclusion complexes were compared using a Thermal Analyzer (TA-60WS, Shi-Madzu, Japan).

2.6. Experimental design and analysis

The icariin/β-CD inclusion complex was dissolved in 10 mL distilled water in a 10 mL microcentrifuge tube using a digital temperature air bath oscillator at a constant speed of 100 rpm. For enzymatic hydrolysis of the icariin/β-CD inclusion complex the following factors were considered: pH, temperature, the ratio of enzyme/substrate and reaction time. All samples were tested in triplicate. The Box–Behnken statistical design[27] was used to optimize these reaction factors, as shown in Table 1. To predict the optimal point, the experimental results were fitted to a second-order polynomial function for four factors, using the following equation:

Table 1 Response surface analysis of factors and levels.

Level	Temperature (°C)	pH	Reaction time (h)	Enzyme/substrate
+1	50	5	3	1:2
0	60	7	7	1:1
−1	70	9	11	3:2

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 \quad (1)$$

where Y is the predicted response, b_0 is the model constant, X_1, X_2, X_3 and X_4 are independent variables, b_1, b_2, b_3 and b_4 are linear coefficients, b_{12}, b_{13}, b_{14}, b_{23}, b_{24} and b_{34} are cross-product coefficients, and b_{11}, b_{22}, b_{33} and b_{44} are the quadratic coefficients.

2.7. Purification and analysis of icaritin

Icaritin was generated from the inclusion complex using the optimized conditions for enzymatic hydrolysis. The produced icaritin was extracted from the reaction mixture with ethyl acetate. Subsequently, icaritin was dissolved in methanol and subjected to silica gel column chromatography using $CHCl_3$–MeOH for elution. Fractions containing pure icaritin were combined and icaritin was recovered by drying under reduced pressure. Identity and purity were confirmed by melting point, ESI-MS, UV, IR, ^{1}H NMR and ^{13}C NMR analyses.

2.8. HPLC analysis of icaritin production

Separation was achieved on an Agilent ZORBAX SB-C18 (4.6 mm × 250 mm, 5 μm) column maintained at 30 °C. The mobile phase consisted of water (A) and acetonitrile (B) with linear gradient elution: 0–7 min, 38% B; 7–8 min, 75% B; 8–20 min, 75% B; 20–30 min, 38% B. The flow rate was 1.0 mL/min. The wavelength of detector was set at 270 nm. Due to the variation in the content of icariin and icaritin in the samples, the stock solutions of standards were prepared and diluted with methanol solution for the establishment of calibration curves. Seven concentrations of icariin and icaritin solutions were injected in triplicate. The calibration curves were constructed by plotting the peak areas against the concentration of each analyte. An aliquot of 20 μL from samples were injected into HPLC and the content of icariin and icaritin was calculated using the calibration curves. Precision and accuracy were also tested.

2.9. Calculation of the conversion rate

Sample solutions (100 μL each) were extracted with 900 μL of methanol, vortexed for 30 s and then collected by centrifugation

at 11,000 rpm for 15 min. The supernatant (20 μL) was injected into HPLC. The conversion rate was calculated using following equation:

$$\text{Conversion rate}\ (\%) = [CV/(m_2 \times M_1/M_2)] \times 100\% \quad (2)$$

where C is the concentration of icaritin, V is the volume of icaritin, M_1 is the molecular weight of icaritin, M_2 is the molecular weight of icariin and m_2 is the weight of icariin.

3. Results and discussion

The use of enzymolysis to prepare an active component is of current interest in traditional Chinese medicines. Snailase, an enzyme mixture extracted from the crop and enteron of snails, contains cellulase, pectinase, amylase, protease and other lytic enzymes[28] and is widely used in food processing, cell biology and genetic engineering[29,30]. Snailase will also hydrolyze glycosides to aglycones; however, few examples of this have been reported in traditional Chinese medicines. In this article the optimum conditions for the bioconversion of an icariin/β-cyclodextrin inclusion complex to icaritin using snailase and the Box–Behnken statistical design were obtained.

3.1. Analytical method validation

The analytical method was validated in terms of linearity, precision and accuracy; the calibration curve values are as follows: icariin, $A = 22{,}543C + 29.746$ ($r = 0.9997$), with a linear range of 15.25–1344.2 μg/mL; icaritin, $A = 22{,}602C + 807.96$ ($r = 0.9994$), with a linear range of 12.31–1107.9 μg/mL. The precision (RSD < 5%) and accuracy (RSD < 5%) showed that the HPLC method is precise and accurate enough for the quantitative determination of icariin and icaritin in samples.

3.2. Solubility of icariin and icariin/β-CD inclusion compounds

The water solubility of the icariin/β-CD inclusion complex was higher than that of free icariin. The solubility of icariin increased almost 17-fold from 29.2 to 513.5 μg/mL at 60 °C, demonstrating that the inclusion complex could significantly increase the solubility of icariin.

3.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a rapid and reliable strategy to screen compatibility between icariin and β-CD. Fig. 2 shows the DSC curves of icariin (a), β-CD (b), a physical mixture (c) and icariin/β-CD inclusion compounds (d)[31,32]. The thermogram of icariin exhibits a characteristic peak (242.1 °C). β-CD exhibits a maximum absorption peak at 315.4 °C. The mixture of icariin and β-CD shows two onset temperature peaks that correspond to those for icariin (242.6 °C) and β-CD (310.3 °C). DSC of icariin/β-CD inclusion compounds shows that the endothermal peaks of icariin disappear, demonstrating the formation of an inclusion complex, which differs from that of the individual compounds and the physical mixture.

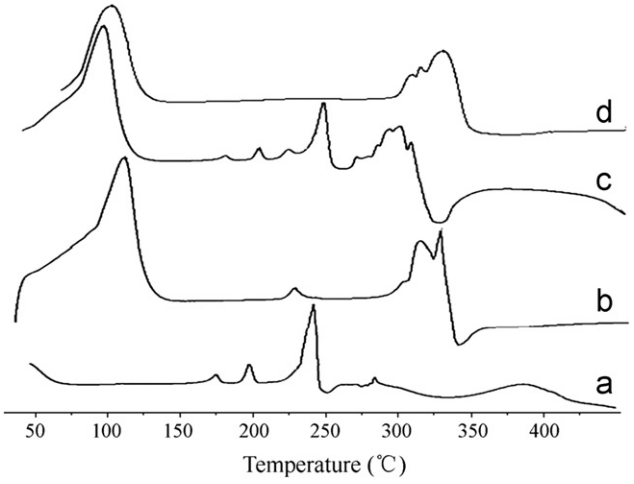


Figure 2 DSC thermograms of icariin (a), β-CD (b), physical mixture (c) and icariin/β-CD inclusion compounds (d).

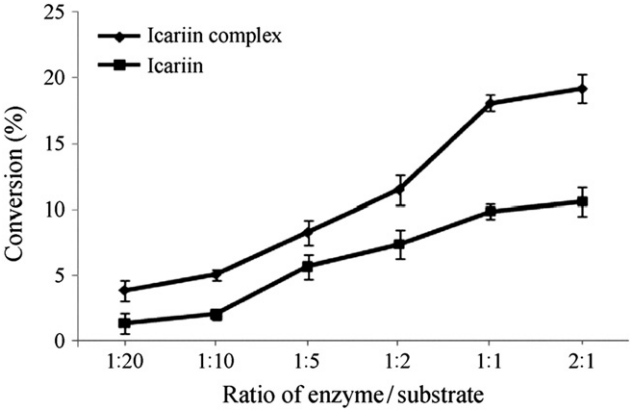


Figure 3 Effect of substrate:enzyme ratio on hydrolysis efficiency of snailase. Substrate was mixed with 5, 10, 20, 50, 100 and 200 mg of snailase (enzyme:substrate = 1:20, 1:10, 1: 5, 1:2, 1:1 and 2:1), respectively. Distilled water was added to the mixture at a final volume of 10 mL. The mixture was incubated at 37 °C for 3 h. Values are expressed as mean ± SD, $n = 3$.

3.4. Optimal conditions for enzymatic hydrolysis

3.4.1. Effect of substrate proportion on hydrolysis efficiency of snailase

Hydrolysis of icariin was performed with different concentrations of snailase (Fig. 3). The hydrolysis increased both with icariin and with icariin/β-CD inclusion. Higher conversion was obtained at a low enzyme concentration. At the ratio of 1:1 (enzyme/substrate), the increase in hydrolysis was 18.1% with the inclusion complex compared to the increase of 9.9% with icariin alone. At all ratios of enzyme/substrate the conversion to icaritin was lower when inclusion complex was not formed. For the ratio of enzyme/substrate, the content of icaritin increased with the rising ratio of snailase/icariin in the mixture and reached its maximum at 1:1. Therefore, the ratio of enzyme/substrate (1:1) was the optimum proportion.

3.4.2. Effect of pH on efficiency of snailase hydrolysis

Fig. 4 shows that for icariin, the optimal pH was 7.0; this differs from the results obtained with arctiin and chitosan[33,34].

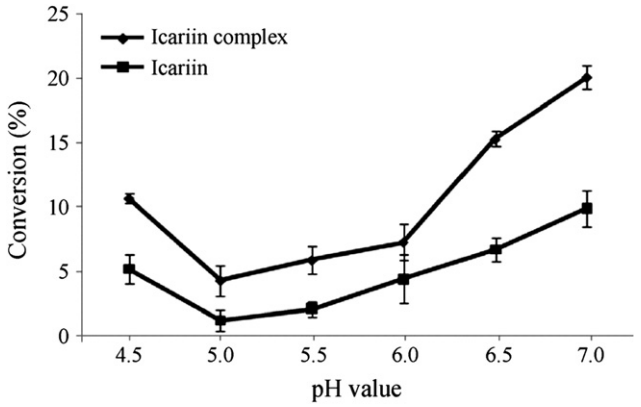


Figure 4 Effect of pH on hydrolytic efficiency of snailase. The reaction mixture contained icariin/β-CD inclusion complex (100 mg of icariin), snailase (100 mg) and glacial acetic acid–sodium acetate anhydrous buffer (10 mL, pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0). The mixture was incubated at 37 °C for 3 h. Values are expressed as mean ± SD, $n=3$.

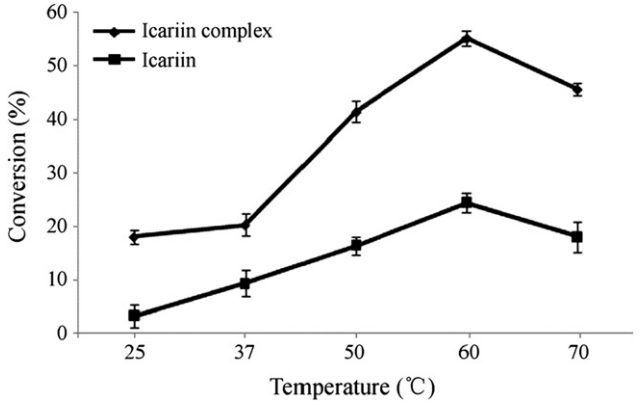


Figure 5 Effect of temperature on the snailase-catalyzed hydrolysis. The reaction mixture contained icariin/β-CD inclusion complex (containing 100 mg icariin), snailase (100 mg) and distilled water (10 mL), and was incubated at 25, 37, 50, 60 and 70 °C for 3 h. Values are expressed as mean ± SD, $n=3$.

The reason may be that snailase is a compound enzyme mixture, which includes cellulase, pectinase, amylase, protease and other enzymes. Moreover, the pH 7.0 was probably not the optimal pH for all the enzymes contained in snailase. This also shows that the conversion to icaritin increased remarkably when the inclusion complex was formed. The conversion to icaritin with the inclusion complex reached a maximum value of 20.1% (in 3 h reaction) when the buffer pH was 7.0 and the transformation rate increased by more than 100%. When icariin was included within β-CD, the solubility was significantly enhanced, which may explain the increased transformation rate.

3.4.3. *Effect of temperature on the snailase catalyzed hydrolysis*

The optimal temperatures are different among various substrates of snailase. It hydrolyzed ginsenoside-Rb_1 and chitin at 40 °C[35] and 25 °C[36], respectively. In this study, 60 °C was most suitable for the reaction, as demonstrated in Fig. 5. The conversion to icaritin increased remarkably when the reaction temperature was increased from 25 °C, and reached its maximum at 60 °C, then decreased slowly with increasing temperature. A temperature of 60 °C was optimal for snailase both with icariin alone or the inclusion. At 60 °C, the activity of the snailase with the inclusion was significantly superior to the snailase with icariin alone. The conversion was increased by almost 125% (from 24.5% to 55.2%).

3.4.4. *Effect of reaction time on hydrolysis*

Icariin was hydrolyzed by the snailase in isolation and in complex with β-CD (Fig. 6). Substantial hydrolysis over the 7-h time-course was obtained with both icariin reaction systems at a relatively constant rate. The conversion was also superior with the inclusion complex as compared to that of icariin alone. After 3 h, the conversion was increased by almost 125% (from 24.5% to 55.2%), and after 7 h, it was increased by 68% (from 58.4% to 97.9%). The best reaction time is 7 h according to the result (Fig. 6). Therefore, the reaction time of icariin hydrolysis to icaritin was significantly shorter than that reported in the literatures[12,14].

3.4.5. *Process optimization*

According to the results of the optimization study, the ratio of enzyme/substrate (1:1), pH of 7.0, temperature of 60 °C and reaction time of 7 h were chosen as the conditions of the Box–Behnken statistical design to optimize the production process. Results were obtained by an HPLC analysis after the reactions were carried out under the chosen conditions. The analysis was repeated in triplicate. The coefficients of the response surface model given in Eq. (1) were evaluated by fitting to the experimental data, where the responses and variables were fitted with multiple regressions. Table 2 shows the experimental conditions and results for the icaritin production and yield according to the factorial design. A good fit was obtained and no outliers were observed. An analysis of variance (ANOVA) indicated that the model was highly appropriate for the prediction, owing to its high F value (54.35) and small significance P value (0.0001). Moreover, a statistical analysis revealed no lack of fit, and the coefficient of determination (R^2) of the model was 0.9819, indicating an adequate precision and that the model is suitable for navigating the design space (Table 3).

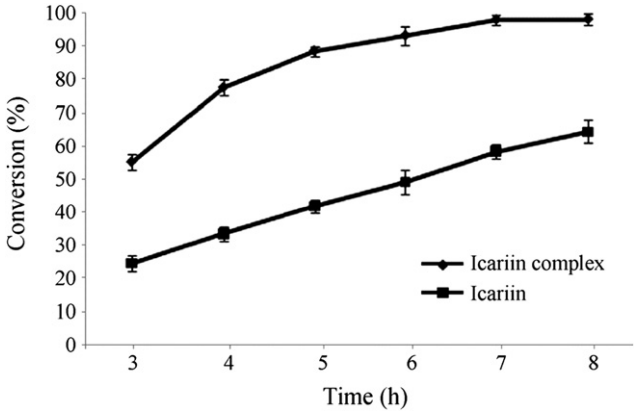


Figure 6 Effect of reaction time on hydrolysis. The reaction mixture contained substrate, snailase (100 mg) and distilled water (10 mL). It was incubated at 60 °C for 3, 4, 5, 6, 7 and 8 h. Values are expressed as mean ± SD, $n=3$.

Table 2 Operating variables, levels and experimental data used in the Box–Behnken design.

Run	Temperature (°C)	pH	Reaction time (h)	Enzyme/substrate	Conversion (%)
1	70	7	7	1:2	59.04
2	60	9	3	1:1	32.57
3	60	7	3	1:2	35.23
4	60	9	7	3:2	71.54
5	60	9	11	1:1	60.84
6	60	7	11	3:2	95.74
7	50	7	7	1:2	42.73
8	60	9	7	1:2	43.64
9	50	7	7	3:2	79.11
10	60	7	11	1:2	51.72
11	60	7	3	3:2	52.96
12	50	9	7	3:2	40.27
13	70	7	11	1:1	76.48
14	60	5	11	1:1	21.67
15	50	5	7	1:1	19.07
16	70	5	7	1:1	19.39
17	70	7	3	1:1	39.37
18	70	7	7	3:2	85.22
19	50	7	11	1:1	63.28
20	60	5	3	1:1	15.08
21	50	7	3	1:1	39.33
22	60	5	7	1:2	17.13
23	60	5	7	3:2	22.29
24	70	9	7	1:1	51.16
25–29	70	7	7	1:1	97.09

Table 3 Analysis of variance for quadratic model.

Source	Sum of squares	d*f*	Mean Square	*F*-value	*P*-value
Model	22118.24	14	1579.87	54.35	<0.0001
Residual	406.96	14	29.07		
Lack of fit	405.05	10	40.51	84.97	<0.0003
Pure error	1.91	4	0.48		
R^2	0.9819				

The statistical analysis also showed that all the four factors had a significant effect on the enzymatic hydrolysis of icariin/β-CD inclusion compounds into icaritin (Table 4); however, the most relevant variable ($P<0.05$) regarding the icaritin yield was the reaction time (RT). After eliminating the nonsignificant interaction coefficients (*P*-value > 0.05), the reduced model is expressed as follows:

$$Y = 97.09 + 3.91X_1 + 15.45X_2 + 12.93X_3 + 13.11X_4 + 5.42X_2X_3 + 5.69X_2X_4 + 6.57X_3X_4 - 19.03X_1^2 - 44X_2^2 - 22.8X_3^2 - 13.79X_4^2 \quad (3)$$

where X_1, X_2, X_3 and X_4 represent the temperature (°C), pH, reaction time (h) and the ratio of enzyme/substrate, respectively.

In addition to the effects of the shown variables on the icaritin yield, the contour plots also indicated several optimal variable combinations that can be selected by a manufacturer to obtain a higher yield. Thus, according to the optimization based on the contour plots, the recommended conditions were the ratio of enzyme/substrate (1:1.1), pH (7.0), temperature (60 °C) and reaction time (7 h), which predicted an icaritin yield of 98.35%.

Table 4 Regression coefficients of variables.

Variable	Coefficient estimate	d*f*	Standard error	Prob > *F*
b_0	97.09	1	2.41	
b_1	3.91	1	1.56	0.025
b_2	15.45	1	1.56	<0.0001
b_3	12.93	1	1.56	<0.0001
b_4	13.11	1	1.56	<0.0001
b_{12}	2.64	1	2.70	0.3436
b_{13}	3.29	1	2.70	0.2425
b_{14}	−2.55	1	2.70	0.3602
b_{23}	5.42	1	2.70	0.0489
b_{24}	5.69	1	2.70	0.0434
b_{34}	6.57	1	2.70	0.0287
b_{11}	−19.03	1	2.12	<0.0001
b_{22}	−44.00	1	2.12	<0.0001
b_{33}	−22.80	1	2.12	<0.0001
b_{44}	−13.79	1	2.12	<0.0001

3.5. *Preparation of icaritin under optimum conditions*

The time needed to generate icaritin decreased by 85.4% as compared with that in literature[14], with an efficiency of up to 97.91%. The inclusion complex of icariin containing 1.13 g of icariin was mixed with 1.02 g snailase in 100 mL of water in triplicate. The buffer was subsequently incubated in an HH-4 digital constant temperature water bath at 60 °C for 7 h. The final reaction mixture was extracted with ethyl acetate for three times, and the combined ethyl acetate fractions were evaporated to dryness under reduced pressure. HPLC analysis showed that 97.9% of icariin in the inclusion complex was hydrolyzed to icaritin. To isolate icaritin, the residue was dissolved in 10 mL methanol and subjected to silica gel column chromatography using $CHCl_3$–MeOH for elution. Finally, 473 mg of icaritin was obtained with the purity of 99.3%. The identification of icaritin was carried out by melting point, ESI-MS, UV, IR, ^{1}H NMR and ^{13}C NMR and compared with the data given in references[37,38]. The results are as follows: yellow needle crystal, molecular formula: $C_{21}H_{20}O_6$; mp 205–207 °C; ESIMS, *m*/*z* (%): 369 $[M+H]^+$; UV_{max}: 270 nm; IR(KBr) $\nu_{max}cm^{-1}$: 3319, 1655, 1603, 1511, 1461, 1385, 1257, 1227, 838; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 146.65(C_2), 136.39(C_3), 176.70(C_4), 160.95(C_5), 98.30(C_6), 161.72(C_7), 106.09(C_8), 153.99(C_9), 103.54(C_{10}), 122.96(C_1'), 129.64(C_2'), 114.56(C_3'), 158.77(C_4'), 114.56(C_5'), 129.64(C_6'), 55.85(C-OMe) 21.67(C_1''), 124.05(C_2''), 131.47(C_3''), 25.90(C_4''), 18.30(C_5''); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.374(1H, s, C_5-OH), 10.737(1H, s, C_7-OH), 9.475(1H, s, C_3-OH), 8.10(2H, d, J=8.7 Hz, H-2′,6′), 7.10(2 H, d, J=5.7 Hz, H-3′,5′), 6.306(1H, s, H-6), 5.183(1H, t, J=6.7 Hz, H-2′), 3.851(3H, s, 4′-OMe), 3.433(2H, d, J=6.7 Hz, H-1″), 1.684(3H, s, H-4″), 1.61(3H, s, H-5″).

4. Conclusions

The conditions chosen in this study significantly increased the hydrolysis rate and shortened the reaction time for the generation of icaritin from icariin. The incorporation of icariin into β-CD complexes increased the solubility of icariin and without protecting the glycosidic bond from hydrolysis by snailase. This study clearly demonstrates an improvement in the speed of enzyme hydrolysis for the preparation of icaritin from icariin in high yield and suggests that this approach may be effective with other poorly water-soluble drugs.

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