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# Physiochemical Properties of the Inclusion Complex of Puerarin and Glucosyl-β-Cyclodextrin

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**ABSTRACT:** Puerarin is a natural isoflavone, found in the Chinese medicinal plant Ge-gen, with many reported health-promoting properties. However, its low water solubility impedes its application in pharmaceutical and functional food products. This study explores the formation of inclusion complex between puerarin and glucosyl-β-cyclodextrin (G-β-CD) to improve the aqueous solubility of puerarin. The complex was prepared by mixing an equal molar mixture of puerarin and G-β-CD for 24 h, followed by freeze-drying. The obtained complex was analyzed by ultraviolet−visible spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, differential scanning calorimetry, X-ray diffractometry, and proton nuclear magnetic resonance spectroscopy. Results showed clearly that the process led to the formation of a supramolecular complex in which the guest molecule, puerarin, was entrapped inside the cavity of the host, G-β-CD. The close association between puerarin and G-β-CD resulted in changes in some of the characteristic spectral, phase-transitional, and morphological properties of puerarin.

**KEYWORDS:** *Puerarin, glucosyl-β-cyclodextrin, inclusion complex, supramolecule, characterization*

## INTRODUCTION

Puerarin (Figure 1) is a natural isoflavone and one of the principal active components found in the traditional Chinese medicinal plant Ge-gen (*Radix puerariae*).[1] Like many other isoflavonoids, puerarin has been found to exhibit strong antioxidant activity with potential beneficial effects to the human body.[2] In recent years, the health effects of puerarin have received a considerable amount of research. Reported health-promoting functions of puerarin include antithrombotic,[3] antihyperglycemic,[4] cholesterol-lowing,[5] anti-inflammatory,[6] anticancer,[7] heptoprotective,[2] and antiosteoporotic[8] effects and the ability to protect pancreatic tissues from oxidative damage[9] and to reduce diabetes-induced damage to retina and improve retinal functions.[10,11] Therefore, puerarin is emerging as a promising bioactive ingredient with potential applications in pharmaceutical, nutraceutical, and functional food products. However, puerarin is sparingly soluble in water (11 mM at 25 °C),[12] which severely restricts its application in medicinal and food formulations.

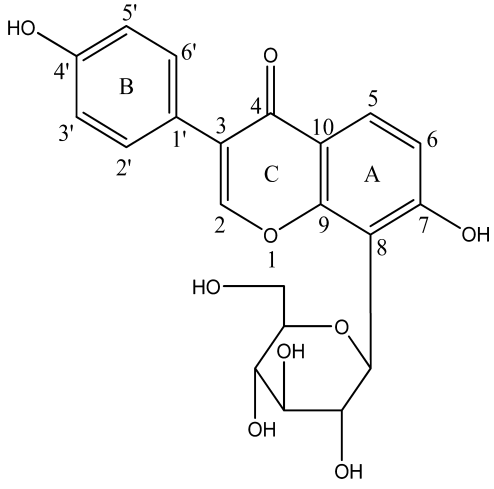


**Figure 1.** Chemical structure of puerarin.

There are several approaches that have been used to improve the solubility of flavonoids, including chemical and enzymatic glucosylation,[13,14] microencapsulation with natural and synthetic polymers,[15,16] and complexation with cyclodextrins (CDs) to form supramolecular structures.[17,18] Among these approaches, complexation with CDs appears to offer a number of advantages, as it does not alter the chemical structure of the flavonoids, nor does it generate toxic waste as chemical glucosylation does, which may raise safety concerns. It also does not introduce synthetic polymers as some microencapsulation does, which may hinder consumer acceptance when used in functional foods. Furthermore, complexation with CDs has been shown to confer protection of flavonoids against thermal and radiation-induced degradation and significantly enhance their antioxidant activity.[18]

Cyclodextrins are a family of molecules comprising several glucopyranoses bound together to form a ring.[19] The distinctive cone-shaped structure of cyclodextrins enables them to entrap hydrophobic molecules to form host−guest complexes. The most common cyclodextrins used as entrapping vehicles are α-, β-, and γ-CDs containing six, seven, and eight glucopyranose units, respectively. Among them, β-CD is the most widely used since its cavity size is suitable for common guests with molecular masses between 200 and 800 g/mol.[20] Glucosyl-β-cyclodextrin

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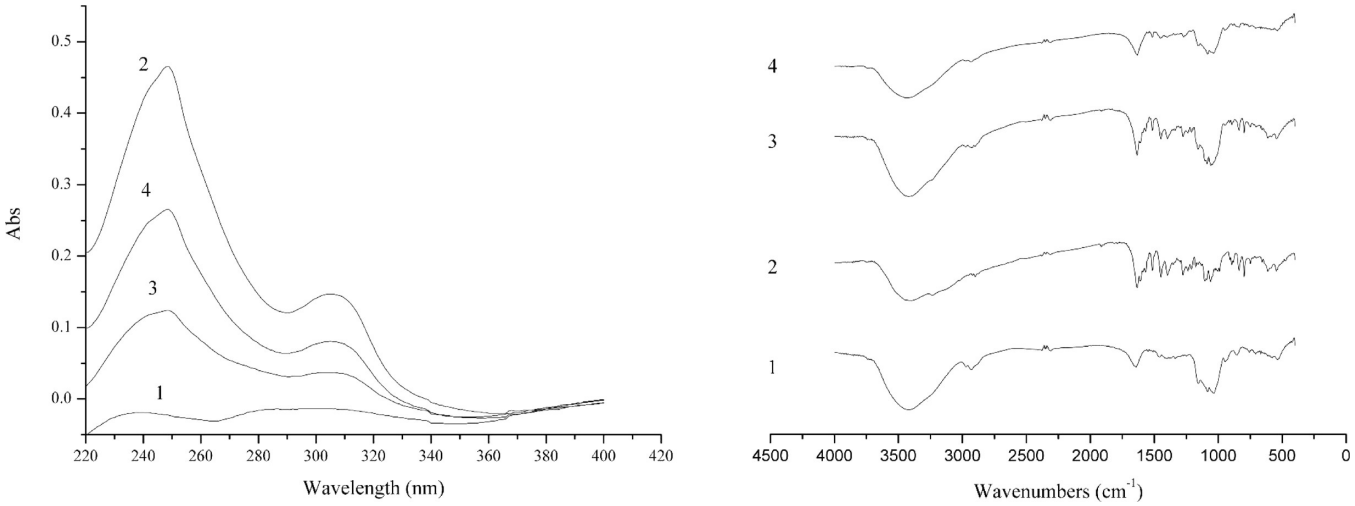


**Figure 2.** (Left) UV and (right) IR spectra of (1) G-$\beta$-CD, (2) puerarin, (3) their physical mixture, and (4) inclusion complex.

(G-$\beta$-CD) is a glucosyl $\beta$-cyclodextrin derivative that has an internal cavity size similar to that of $\beta$-CD but is more water-soluble than the native $\beta$-CD.[21] However, there are very few studies exploring its suitability for forming inclusion complexes with bioactive compounds.

The objective of this study was to investigate the suitability of G-$\beta$-CD as an entrapping agent for forming an inclusion complex with puerarin and to examine the physicochemical properties of the supramolecular structure so formed by several analytical techniques including ultraviolet−visible spectroscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry, scanning electron microscopy, and proton nuclear magnetic resonance spectroscopy.

## MATERIALS AND METHODS

**Chemicals.** Puerarin (>99%) was obtained from Shaanxi Huike Botanical Development Co., Ltd., (Xi'an, China). Glucosyl-$\beta$-cyclodextrin (>99%, MW1297) was purchased from Seebio Biotech, Inc. (Shanghai, China). Other chemicals were of analytical grade unless stated otherwise.

**Preparation of the Inclusion Complex of Puerarin and Glucosyl-$\beta$-Cyclodextrin.** Puerarin (0.416 g, 1 mM) and G-$\beta$-CD (1.297 g, 1 mM) were mixed in 25 mL of distilled water, stirred for 24 h at 30 °C, and filtered through a 0.45 $\mu$m membrane filter to remove undissolved material. The filtrate was freeze-dried (Alpha 1−4, Christ, Germany) and the resultant powdery material was weighed and collected as the inclusion complex of puerarin and G-$\beta$-CD. To determine the amount of puerarin in the complex, 10 mg of the freeze-dried powder was dissolved in ethanol and the absorbance of the solution was measured on a UV spectrophotometer (Purkinje, Beijing, China) at 248 nm and compared with a standard curve of pure puerarin. The influence of G-$\beta$-CD in the complex on the absorbance of puerarin was negligible, as its absorbance in the UV range was very small (see Results and Discussion section). The yield of the inclusion complex was expressed as the percentage of mass recovered as inclusion complex (freeze-dried powder) with respect to the mass of initial materials (puerarin plus G-$\beta$-CD). The inclusion ratio of puerarin was calculated as the mass of puerarin in the inclusion complex over the initial mass of puerarin used for the complexation.

**Preparation of Physical Mixture of Puerarin and Glucosyl-$\beta$-Cyclodextrin.** Puerarin (0.416 g) and G-$\beta$-CD (1.297 g) were mixed thoroughly in a small beaker at room temperature. The obtained product was collected as the physical mixture of puerarin and G-$\beta$-CD.

**Ultraviolet−Visible Spectroscopy.** UV spectra were recorded for G-$\beta$-CD, puerarin, their physical mixture, and the inclusion complex on a model TU1810 scanning UV spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). Each sample was dissolved in water at ambient temperature (25 ± 1 °C). The absorbance of each solution was scanned in the wavelength range 220−400 nm to obtain the UV spectra.

**Fourier Transform Infrared Spectroscopy.** The Fourier transform infrared (FT-IR) spectra of G-$\beta$-CD, puerarin, their physical mixture, and the inclusion complex were collected between 4000 and 400 $cm^{-1}$ on a Tensor 27 infrared spectrophotometer (Bruker, Germany) with 256 scans at a resolution of 4 $cm^{-1}$ by the KBr method. The data were recorded and processed by Opus software (Bruker, Germany) supplied with the instrument.

**Differential Scanning Calorimetry.** Differential scanning calorimetry (DSC) analysis was conducted for puerarin, G-$\beta$-CD, their physical mixture, and the inclusion complex with a Q200 differential calorimeter calibrated with indium (TA Instruments, New Castle, DE). The samples were sealed in an aluminum crimp cell and heated at 10 °C/min from 30 to 300 °C in a nitrogen atmosphere. An empty pan sealed in the same way was used as reference. The data were recorded and processed by Universal Analysis 2000 software (TA Instruments, New Castle, DE).

**Scanning Electron Microscopy.** Scanning electron microscopy (SEM) was performed with a Quanta 200 environmental scanning electron microscope (FEI, Hillsboro, OR). The samples were evenly distributed on SEM specimen stubs with double adhesive tape. The micrographs were obtained with an accelerating potential of 15 kV under low vacuum.

**X-ray Diffractometry.** For X-ray diffractometry (XRD), monochromatic Cu K$\alpha$ radiation (wavelength = 1.540 56 Å) was produced by a D8 Advance X-ray diffractometer (Bruker, Germany). The powdery samples were packed tightly in a rectangular aluminum cell prior to exposure to the X-ray beam. The scanning regions of the diffraction angle, 2$\theta$, were 3−80°, and radiation was detected with a proportional detector.

**Proton Nuclear Magnetic Resonance Spectroscopy.** $^1$H NMR spectra of puerarin and its complex with G-$\beta$-CD were recorded with a 400 MHz Bruker Avance spectrometer at 25 °C. The samples were dissolved in $D_2O$ and degassed by bubbling $N_2$ directly in the NMR tubes. The chemical shifts ($\delta$) were reported as parts per million (ppm) and are referenced to the HOD signals.

## RESULTS AND DISCUSSION

**Preparation of Puerarin/Glucosyl-$\beta$-Cyclodextrin Complex.** Many methods have been explored to prepare inclusion complexes, including coprecipitation, neutralization, kneading, spray drying, freeze-drying, solvent evaporation, ball-milling, and sealed heating.[22] In this study, the freeze-drying method was used to prepare the inclusion complex of puerarin and G-$\beta$-CD, which

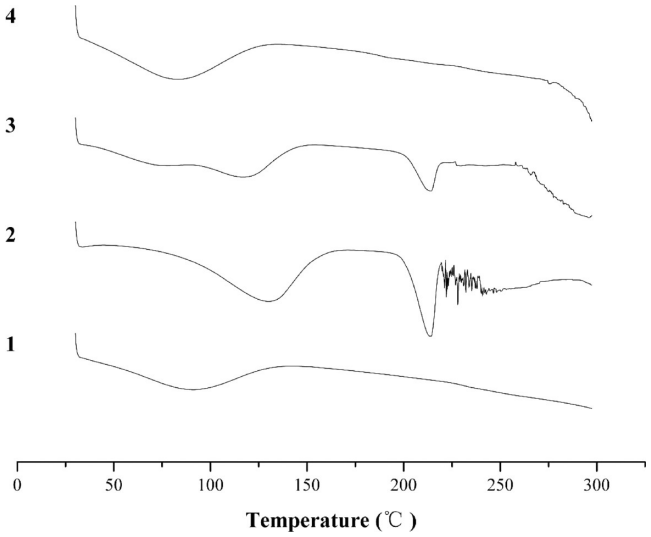


**Figure 3.** DSC curves of (1) G-$\beta$-CD, (2) puerarin, (3) their physical mixture, and (4) inclusion complex.

had the advantage of minimizing potential chemical degradation of the flavonoid and loss of bioactivity. The yield of the inclusion complex was 90% and the inclusion ratio of puerarin (the amount of puerarin in the complex over the initial mass of puerarin used) was 63%, which were better than those obtained by the glucosylation method [13] and comparable to those achieved by the microencapsulation technique.[15]

**UV and IR Analysis.** The UV and FT-IR spectra of G-$\beta$-CD, puerarin, their physical mixture, and inclusion complex are shown in Figure 2. As expected, the UV absorbance of G-$\beta$-CD was very low and did not exhibit any appreciable peak as the molecule does not contain $\pi$-electrons (double bonds) that can absorb energy in the form of ultraviolet light. Puerarin exhibited two characteristic absorption peaks at 248 and 305 nm, which were identical to those of the physical mixture and the inclusion complex of G-$\beta$-CD and puerarin (Figure 2a). The FT-IR spectrum of G-$\beta$-CD showed prominent absorption bands at 3419 $cm^{-1}$ (for O−H stretching vibrations), 2931 $cm^{-1}$ (for C−H stretching vibrations), and 1155, 1083, and 1034 $cm^{-1}$ (for C−H and C−O stretching vibrations). The FT-IR spectrum of puerarin consisted of prominent absorption bands of the hydroxyl group (3397 $cm^{-1}$), the aromatic conjugated carbonyl group (1633 $cm^{-1}$), and the aromatic nucleus (1609, 1568, 1515, and 1448 $cm^{-1}$). The IR spectrum of the physical mixture of G-$\beta$-CD and puerarin displayed a spectral addition effect and was essentially a combination of the spectra of the two molecules. However, in the spectrum of the inclusion complex, several small but characteristic absorption peaks of puerarin between 400 and 1600 $cm^{-1}$ almost disappeared, which was likely due to the vibration of puerarin molecule being restricted, suggesting that it was entrapped in the G-$\beta$-CD cavity. In particular, the small peaks in the proximity of 1450−1600 $cm^{-1}$, which are characteristic of the aromatic nucleus, virtually disappeared in the IR spectrum of the inclusion complex, suggesting that it is

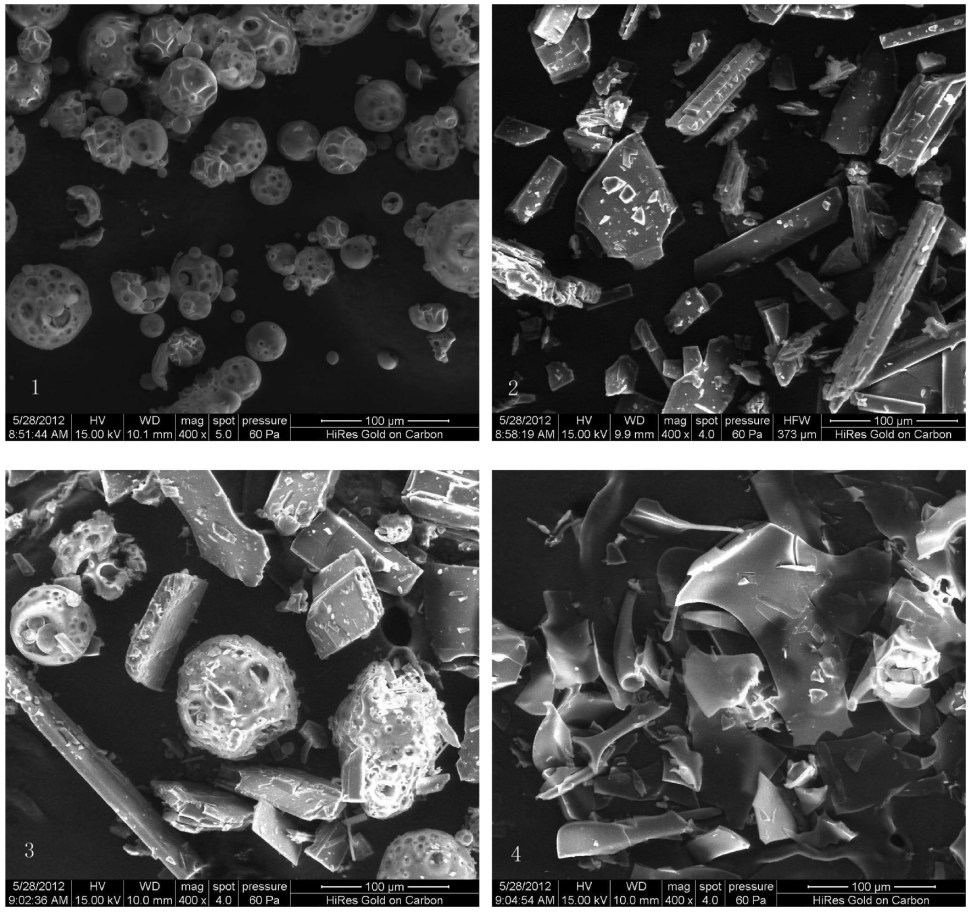


**Figure 4.** Scanning electron micrographs of (1) G-$\beta$-CD, (2) puerarin, (3) their physical mixture, and (4) inclusion complex.

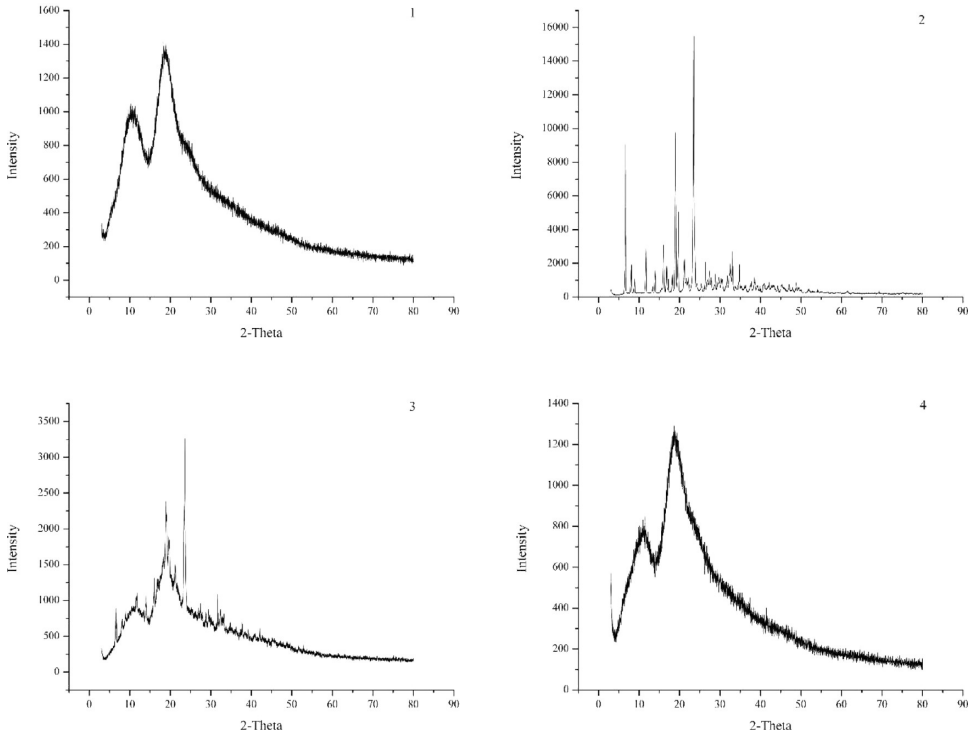


**Figure 5.** XRD patterns of (1) G-$\beta$-CD, (2) puerarin, (3) their physical mixture, and (4) inclusion complex.

**Table 1. Chemical Shift Values of Puerarin before and after Complexation with G-$\beta$-CD at 25 °C**

| | chemical shift (ppm) | | |
|---|---|---|---|
| protons | $\delta_{free}$ | $\delta_{complex}$ | $\Delta\delta = \delta_{complex} - \delta_{free}$ |
| H-2 | 8.13 | 8.18 | 0.05 |
| H-5 | 7.98 | 8.12 | 0.14 |
| H-2′/6′ | 7.29 | 7.40 | 0.11 |
| H-6 | 7.03 | 7.12 | 0.09 |
| H-3′/5′ | 6.92 | 6.96 | 0.04 |

probably the phenyl ring of puerarin that was involved in the inclusion complexation.

**DSC Analysis.** Figure 3 shows the DSC curves of G-$\beta$-CD, puerarin, their physical mixture, and inclusion complex. Owing to its amorphous nature, the thermogram of G-$\beta$-CD displayed a relatively flat line with a broad and shallow peak appearing at about 90 °C. The thermogram of puerarin showed a broad endothermic peak at 137 °C, probably due to the loss of water, and a sharp endothermic peak at 213 °C, corresponding to its melting point.[23] The DSC thermogram of the physical mixture of puerarin and G-$\beta$-CD exhibited combined characteristics of the thermograms of both molecules described above, indicating that no close association formed between the two molecules when the two powders are simply mixed together. In contrast, the DSC thermogram of the inclusion complex exhibited mainly the features of the G-$\beta$-CD curve while the characteristic endothermic peaks of puerarin disappeared entirely, suggesting that an association structure was formed between the two molecules. A similar observation of guest molecule losing its characteristic DSC peaks was also reported for the complexation of ferulic acid with hydroxypropyl-$\beta$-cyclodextrin.[24]

**SEM Analysis.** Scanning electron micrographs of G-$\beta$-CD, puerarin, their physical mixture, and inclusion complex are shown in Figure 4. G-$\beta$-CD appeared as amorphous spheres, while puerarin existed in rectangular crystals. In the electron micrograph of the physical mixture of the two powders, both the characteristic crystals of puerarin and the amorphous spheres of G-$\beta$-CD were found. In contrast, the inclusion complex appeared as irregular particles in which the original morphology of both components disappeared and tiny aggregates of amorphous pieces of irregular sizes were present. These images further demonstrated that when the powders of puerarin and G-$\beta$-CD were simply mixed together, they formed no close association and continued to exist in their original individual forms, whereas when the solutions of the two compounds were freeze-dried, they formed a close association, probably in the form of inclusion complex, in which puerarin no longer exist in the crystal state.

**XRD Analysis.** Powder X-ray diffractometry is a method that can provide insightful information about the complexation between CD and guest molecules. The formation of an inclusion complex between CD and a crystalline guest means that the latter would lose its crystalline nature and, consequently, the diffraction pattern of the complex would not be a simple superposition of those of the two components.[24] Figure 5 shows the XRD patterns of G-$\beta$-CD, puerarin, their physical mixture, and inclusion complex. The XRD pattern of G-$\beta$-CD showed two broad peaks, consistent with its amorphous character,[25] whereas numerous sharp, intense peaks appeared in the XRD pattern of puerarin, confirming its crystalline nature. The XRD pattern of the physical mixture of puerarin and G-$\beta$-CD showed essentially a superposition of the patterns of the two compounds, confirming that no inclusion was formed between them and both retained their original physical characteristics. In contrast, the XRD pattern of the inclusion complex was virtually the same as that of the amorphous G-$\beta$-CD and exhibited none of the characteristic peaks of puerarin. This further shows that when the suspension of puerarin and G-$\beta$-CD was freeze-dried, most of the puerarin had complexed with G-$\beta$-CD matrix and lost its crystallinity as a result.

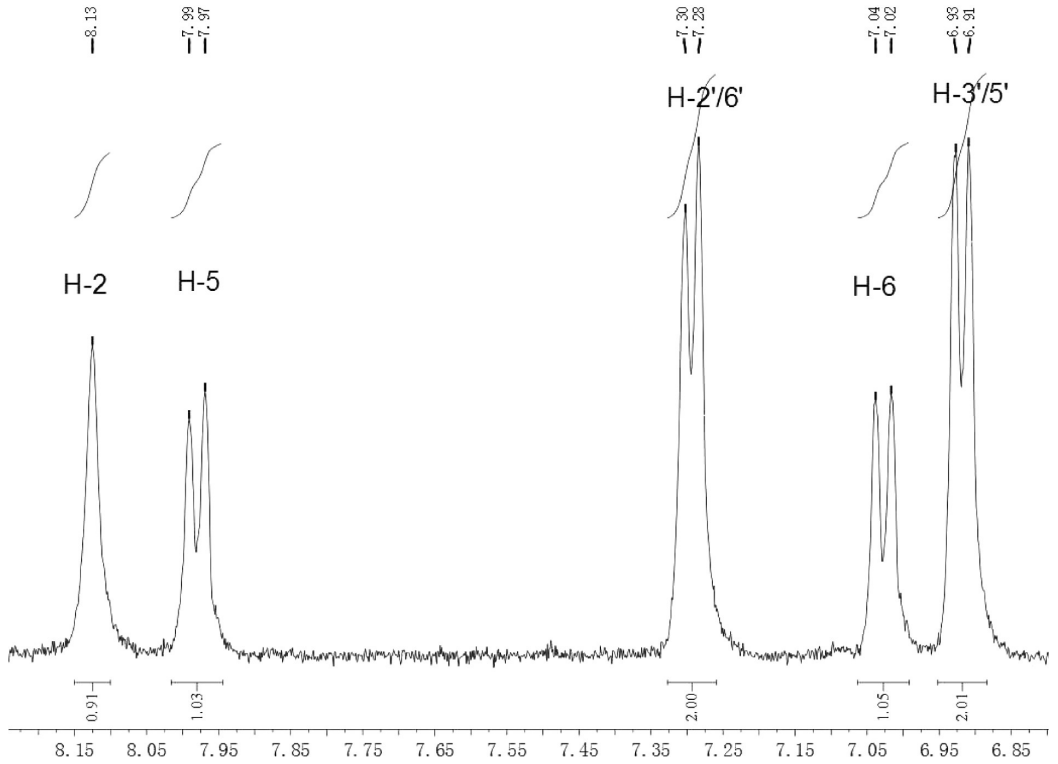


**Figure 6.** Chemical shifts of aromatic protons of puerarin in $D_2O$.

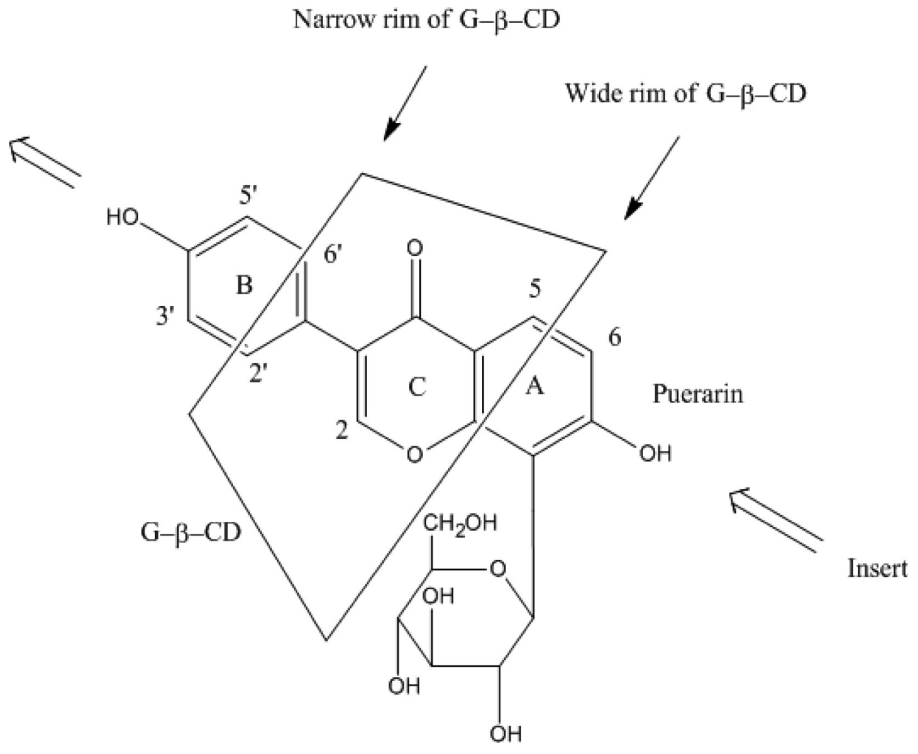


**Figure 7.** Proposed structure for the puerarin/G-*β*-CD inclusion complex.

**$^1$H NMR Analysis.** Further evidence supporting the inclusion of puerarin inside the cavity of G-*β*-CD was obtained by proton nuclear magnetic resonance spectroscopy ($^1$H NMR), which has proved to be very helpful in elucidating the molecular conformation of inclusion complexes between CDs and flavonoids.[26] The formation of inclusion complexes would cause chemical shifts in the $^1$H NMR spectra of the guest and CDs, which could provide valuable clues for deducing the part of the flavonoid molecule that inserts into the CD cavity.[27,18] Table 1 and Figure 6 show the chemical shifts of protons in the isoflavone backbone of puerarin before and after complexation. A downfield shift was observed for all the protons, but especially

large shifts occurred for 5- and 2′/6′-protons, which was probably due to their strong interaction with the hydroxyl groups in the narrow and wide rims of G-$\beta$-CD. It is therefore probable that the complex was formed in the following manner: the molecule of puerarin enters the cone of G-$\beta$-CD through the wide rim with part of its B ring extruding outside of the cone (Figure 7). In this structure the 3′- and 5′-H of the B ring would be relatively distant from the narrow rim of the G-$\beta$-CD cone with resultant weak interactions with the hydroxyl groups of the rim and, consequently, small chemical shifts. The 2′- and 6′-H of the B ring, on the other hand, would be relatively close to the narrow rim with stronger interactions with its hydroxyl groups, resulting in larger chemical shifts for these two protons. Similarly, the 5-H of the A ring would be closer to the wide rim than the 6-H, with consequent stronger interactions with the hydroxyl groups and greater chemical shift. Finally, the 2-H would be imbedded inside the cone and rather distant from the functional groups in the interior, with a consequently small chemical shift. The proposed structure of the puerarin/G-$\beta$-CD complex would thus satisfactorily explain the different chemical shifts occurring for the protons of puerarin and would suggest that the puerarin molecule was deeply inserted into the cavity of G-$\beta$-CD.

In conclusion, in the present study we successfully prepared the inclusion complex of puerarin and G-$\beta$-CD with good yield via the freeze-drying method. Evidence obtained by UV, IR, DSC, SEM, XRD, and $^1$H NMR analyses demonstrated clearly that the inclusion process led to the formation of a supramolecular complex in which the guest molecule, puerarin, was entrapped inside the cavity of the host, G-$\beta$-CD. The close association between puerarin and G-$\beta$-CD resulted in changes in some of the characteristic spectral, phase-transitional, and morphological properties of puerarin. Future research could examine the chemical, biological, and processing properties of the supramolecular complex and explore their applications in pharmaceutical and functional foods.

## AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Fang, Q. Some current study and research approaches relating to the use of plants in the traditional Chinese medicine. *J. Ethnopharmacol.* **1980**, *2*, 57−63.

(2) Guerraa, M. C.; Speronia, E.; Broccolia, M.; Canginia, M.; Pasinib, P.; Minghettib, A.; Crespi-Perellinob, N.; Mirasolib, M.; Cantelli-Fortia, G.; Paolinia, M. Comparison between Chinese medical herb *Pueraria lobata* crude extract and its main isoflavone puerarin: Antioxidant properties and effects on rat liver CYP-catalysed drug metabolism. *Life Sci.* **2000**, *67*, 2997−3006.

(3) Yeung, D. K. Y.; Leung, S. W. S.; Xu, Y. C.; Vanhoutte, P. M.; Man, R. Y. K. Puerarin, an isoflavonoid derived from *Radix puerariae*, potentiates endothelium-independent relaxation via the cyclic AMP pathway in porcine coronary artery. *Eur. J. Pharmacol.* **2006**, *552*, 105−111.

(4) Hsu, F.-L; Liu, I.-M.; Kuo, D.-H.; Chen, W.-C.; Su, H.-C.; Cheng, J.-T. Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. *J. Nat. Prod.* **2003**, *66*, 788−792.

(5) Yan, L.-P.; Chan, S.-W.; Chan, A. S.-C.; Chen, S.-L.; Ma, X.-J.; Xu, H.-X. Puerarin decreases serum total cholesterol and enhances thoracic aorta endothelial nitric oxide synthase expression in diet-induced hypercholesterolemic rats. *Life Sci.* **2006**, *79*, 324−330.

(6) Kim, K. M.; Jung, D. H.; Jang, D. S.; Kim, Y. S.; Kim, J. M; Ha-Na Kim, H.-N.; Surh, Y.-J.; Kim, J. S. Puerarin suppresses AGEs-induced inflammation in mouse mesangial cells: A possible pathway through the induction of heme oxygenase-1 expression. *Toxicol. Appl. Pharmacol.* **2010**, *244*, 106−113.

(7) Yu, Z.; Li, W. Induction of apoptosis by puerarin in colon cancer HT-29 cells. *Cancer Lett.* **2006**, *238*, 53−60.

(8) Michihara, S.; Tanaka, T.; Uzawa, Y.; Moriyama, T.; Kawamura, Y. Puearin exerted anti-osteoporotic action independent of estrogen receptor mediated-pathway. *Jpn. Nutr. Sci. Vitaminol.* **2012**, *58*, 202−209.

(9) Xiong, F.-L.; Sun, X.-H.; Lu, G.; Yang, X.-L.; Xu, H.-B. Puerarin protects rat pancreatic islets from damage by hydrogen peroxide. *Eur. J. Pharmacol.* **2006**, *529*, 1−7.

(10) Xuan, B.; Zhou, Y.-H.; Yang, R.-L.; Li, N.; Min, Z.-D.; Chiou, G. C. Y. Improvement of ocular blood flow and retinal functions with puerarin analogs. *J. Ocular Pharmacol. Ther.* **1999**, *15*, 207−216.

(11) Hao, L.-N.; Zhang, Y.-Q.; Shen, Y.-H.; Wang, Z.-Y.; Wang, Y.-H.; Zhang, H.-F.; He, S.-Z. Effect of puerarin on retinal pigment epithelial cells apoptosis induced partly by peroxynitrite via Fas/FasL pathway. *Int. J. Ophthalmol.* **2010**, *3*, 283−287.

(12) Wang, L.-H.; Cheng, Y.-Y. Solubility of puerarin in water, ethanol, and acetone from (288.2 to 328.2) K. *J. Chem. Eng. Data* **2005**, *50*, 1375−1376.

(13) Bertrand, A.; Morel, S.; Lefoulon, F.; Rolland, Y.; Monsana, P.; Remaud-Simeona, M. *Leuconostoc mesenteroides* glucansucrase synthesis of flavonoid glucosides by acceptor reactions in aqueous-organic solvents. *Carbohydr. Res.* **2006**, *341*, 855−863.

(14) Jiang, J.; Yuan, S.; Ding, J.; Zhu, S.; Xu, H.; Chen, T.; Cong, X.; Xu, W.; Ye, H.; Dai, Y. Conversion of puerarin into its 7-O-glycoside derivatives by *Microbacterium oxydans* (CGMCC 1788) to improve its water solubility and pharmacokinetic properties. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 647−657.

(15) Sansone, F.; Picerno, P.; Mencherini, T.; Villecco, F.; D'Ursi, A. M.; Aquino, R. P.; Lauro, M. R. Flavonoid microparticles by spray-drying: Influence of enhancers of the dissolution rate on properties and stability. *J. Food Eng.* **2001**, *103*, 188−196.

(16) Oidtmann, J.; Schantz, M.; Mäder, K.; Baum, M.; Berg, S.; Betz, M.; Kulozik, U.; Leick, S.; Rehage, H.; Schwarz, K.; Richling, E. Preparation and comparative release characteristics of three anthocyanin encapsulation systems. *J. Agric. Food Chem.* **2012**, *60*, 844−851.

(17) Liu, B.; Li, W.; Nguyen, T. A.; Zhao, J. Empirical, thermodynamic and quantum-chemical investigations of inclusion complexation between flavanones and (2-hydroxypropyl)-cyclodextrins. *Food Chem.* **2012**, *134*, 926−932.

(18) Nguyen, T. A.; Liu, B.; Zhao, J.; Thomas, D. S.; Hook, J. M. An investigation into the supramolecular structure, solubility, stability and antioxidant activity of rutin/cyclodextrin inclusion complex. *Food Chem.* **2013**, *136*, 186−192.

(19) Koontz, J. L.; Marcy, J. E.; O'Keefe, S. F.; Duncan, S. E. Cyclodextrin inclusion complex formation and solid-state characterization of the natural antioxidants $\alpha$-tocopherol and quercetin. *J. Agric. Food Chem.* **2009**, *57*, 1162−1171.

(20) Wang, J.; Cao, Y.; Sun, B.; Wang, C. Physicochemical and release characterisation of garlic oil-$\beta$-cyclodextrin inclusion complexes. *Food Chem.* **2011**, *127*, 1680−1685.

(21) Yoshikiyo, K.; Sugimoto, M.; Aso, Y.; Takahashi, T.; Matsui, Y.; Yamamoto, T. The effect of glucosyl-$\beta$-cyclodextrin on the hydrogen-deuterium exchange rate constant of the peptide bonds of chicken egg white lysozyme in a $D_2O$ solution. *J. Mol. Struct.* **2008**, *888*, 375−378.

(22) Yamada, T.; Imai, T.; Ouchi, K.; Otagiri, M.; Hirayama, F.; Uekama, K. Inclusion complex of 3,9-bis(N,N-dimethylcarbamoloxy)-5H-benzofuro[3,2-c] quinoline-6-one (KCA-098) with Heptakis(2,6-di-O-methyl)-β-cyclodextrin: Interaction and dissolution properties. *Chem. Pharm. Bull.* **2000**, *48*, 1264−1269.
(23) Li, Y.; Yang, D. J.; Zhou, W.; Chen, S. B.; Chen, S. L. Recrystallization of puerarin using the supercritical fluid antisolvent process. *J. Cryst. Growth.* **2012**, *340*, 142−148.
(24) Wang, J.; Cao, Y.; Sun, B.; Wang, C. Characterisation of inclusion complex of trans-ferulic acid and hydroxypropyl-β-cyclodextrin. *Food Chem.* **2011**, *124*, 1069−1075.
(25) Zeng, J.; Ren, Y.; Zhou, C.; Yu, S.; Chen, W. Preparation and physicochemical characteristics of the complex of edaravone with hydroxypropyl-β-cyclodextrin. *Carbohyd Polym.* **2011**, *83*, 1101−1105.
(26) Lasonder, E.; Weringa, W. D. An NMR and DSC study of the interaction of phospholipid vesicles with some anti-inflammatory agents. *J. Colloid Interface Sci.* **1990**, *139*, 469−478.
(27) Kim, H.; Choi, J.; Jun, S. Inclusion complexes of modified cyclodextrins with some flavonols. *J. Incl. Phenom. Macrocycl. Chem.* **2009**, *64*, 43−47.