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IMPROVED WATER SOLUBILITY OF QUERCETIN BY PREPARING COMPLEXATION WITH CYCLODEXTRINS IN BINARY SOLVENT

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SUMMARY

The antioxidant capacity of polyphenols have been widely used in food and pharmaceutical industries. Quercetin (Quer) is a polyphenolic flavonoid that shows several biological effects such as antioxidant, antitumor, antibacterial and antiproliferative effects both in-vitro and in-vivo. However, the solubility of quercetin in water is poor. Thus, it is essential to improve solubility of quercetin in pharmaceuticals by making its complexation with other compounds. In this study, the synthesis of the 2-hydroxypropyl-β-cyclodextrin complex with quercetin (Quer-HPβCD) in the form of nanoparticles in water-ethanol solvents has been carried out. The results showed that the obtained yield of (Quer-HPβCD) complexation in binary solvent was greater than that in pure water. The highest Y value was 80% in a binary solvent with 20% v/v of ethanol. The composition, morphology, structural and thermodynamic properties of the nanoparticles Quer-HPβCD have been determined. This study demonstrated that using mixed water- ethanol solvent and lyophilization technique was able to produce quercetin nanoparticles with significantly smaller particle size. The nanoparticles have a spherical shape with an average size of about 40-60 nm. The results of the phase solubility diagram showed that in water the solubility of quercetin increased and linearly depended on the concentration of host's molecule while Quer and HP-βCD obtained a 1:1 stoichiometric complex. The stability constant of (Quer-HPβCD) complex was found to be logK = 2.56. The Gibbs energy change of the complexation reaction was found to be -14.60 kJ/mol.

Keywords: *cyclodextrin, inclusion complexation, mixed solvent, nanoparticle, quercetin*

INTRODUCTION

Study on the methods and techniques to improve the solubility of pharmaceuticals has attracted scientists. Main methods used include solid dispersion, microcrystalline, co-crystal, co-precipitation, combined polymers, surface adsorption, complexation, etc. Amongst these techniques, complexation with cyclodextrins has a relatively high efficiency in improving the solubility of drugs. However, the water solubility of native cyclodextrins is low in general, thus interfering the synthetic yield. Semi-synthetic cyclodextrins with hydroxyl groups (-OH) attached on the molecules are frequently employed in many studies to improve the dissolution features and bioavailability of lipophilic drugs. In addition, at the micrometer size, the drug diffused at a slow rate due to enzyme barriers. Biological nanomaterials were recently considered as a proper option as they meet most requirements of a drug delivery system (Misran *et al.*, 2003).

HPβCD is an ether derivative of β-cyclodextrin of the oligosaccharide family with a hydrophobic central cavity and hydrophilic outer surface. Due to the formation of the chair of glucopyranose units, HPβCD molecule is shaped like a truncated cone (Li *et al.*, 2008). Hydroxyl functional groups are oriented on the cone's outer surface, where the primary hydroxyl groups is on the narrow edge of the cone and the secondary is on the wider edge. The cavity of the molecule is covered by a carbon frame and oxygen atoms in the ether of the oligosaccharide. The narrow gap of HPβCD has primary hydroxyl groups and is called the first face. In contrast, the wide gap carries secondary hydroxyl groups and is called the second face. Differences between the primary and secondary hydroxyl groups allow the formation of a selective function on the primary and secondary edges. This is the ideal structure for inclusion complexation with poorly water-soluble drug molecules which are hydrophobic and have been "encapsulated" in the cavity of the HPβCD molecule (Saves *et al.*, 2019).

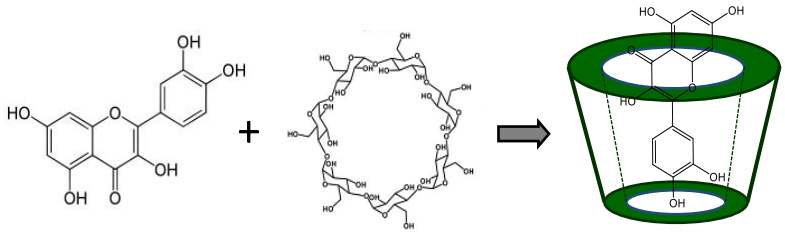

Figure 1. The scheme of inclusion complex formation of Quer and CD.

The antioxidant capacity of polyphenols have been widely used in food and pharmaceutical industries. Polyphenols are compounds of natural origin and exist in plants which have been shown to be extremely effective in antioxidant (Zhang *et al.*, 2016). Polyphenols can protect the body, and then fighting various diseases caused by free radicals. Their common feature is in molecules with aromatic rings (benzene rings), one or two, three ... or many hydroxyl groups (OH) attached directly to the benzene ring. Depending on the quantities and reciprocal positions of OH groups with the chemical framework, the physical, chemical and biological properties will be changed.

Quercetin (Quer), also known as 3,5,7,3',4'-pentahydroxyflavone, is a polyphenolic flavonoid of vegetal origin, which is very abundant in many fruits and vegetables and, in particular, in onions (Demiroglu-Zergeroglu *et al.*, 2016).

Recently, cosolvents have been widely used in the synthesis of organic substances as their function in creating supramolecular pharmacologically active structures. In particular, the hydrogen bond density of water is diminished, and nonpolar solute is "squeezed out" as a result. Previous studies indicated that, a small amount of methanol or ethanol promotes binding of CD with the hydrophobic "guests" (Yoshii *et al.*, 1998). Thus, the aqueous organic solvent mixtures such as water-ethanol, has proven appropriate reaction media in supramolecular chemistry due to specific properties and better ability to dissolve more compounds than pure solvents (Faraji *et al.*, 2009). The availability and diversity of these reaction media is strongly increased from the combination of pure water and alcohol solvents in binary mixtures. In this paper, we synthesized a complex of hydroxypropyl-β-cyclodextrin (HPβCD) and quercetin to improve complexation yield using the water-ethanol solvents. The obtained nanoparticles had the average size of about 40-60 nm and did correspond to the typical size of functional units in living organisms, allowing them to interact effectively with biological molecules, and thus increasing

bioavailability while limiting some side effects due to the long presence on the gastric mucosa.

MATERIALS AND METHODS

Materials

Quercetin and HPβCD were purchased from Sigma-Aldrich, ethanol (EtOH) and dimethylsulfoxide (DMSO) were purchased from Xilong Scientific Co. (China). These reagents were used as received without further purification. All experiments were carried out in distilled water.

Preparation of inclusion complexes

A solid-state inclusion complex between Quer and HPβCD was preparedby in 1:1 molar ratio by dissolving in mixed solvents. Experimental carried out under stirring and thermostating. The solution of HPβCD was added into the Quer solution, 10 mL each time and was stirred by a magnetic stirrer for 24 hours at 25°C. The reaction solution was settled for 48 hours at 4°C to obtain a fine orange precipitate. The precipitate was washed several times with dimethyl sulfoxide (DMSO) and lyophilized. The complexation yield (Y) was calculated as the ratio of the dried complexmass and the sum of the guest and host:

$$Y = \frac{m(Quer - HP\beta CD)}{m(Quer) + m(HP\beta CD)} . 100\% \quad (1)$$

Fourier transforms infrared (FTIR) spectroscopy

Fourier transform IR spectra were recorded on a Nicolet iS10 (Thermo Scientific-USA) spectrophotometer. The spectra were recorded for Quer, HPβCD and (Quer-HPβCD) complex. Samples were prepared in KBr disks with a hydrostatic press at a force of 5.2 T cm^{-2} for 3 min. The concentrations of samples were constant (5% wt).

Morphology characterization of nanoparticles

The morphology of nanospheres was studied by field emission scanning electron microscopy (FE-SEM) using JSM-6510LV (Jeol, Japan). The samples were coated with platinum under vacuum before examination. Surface morphology of unloaded and loaded HPβCD nanoparticles was observed.

Phase solubility diagram

The phase-solubility diagram was obtained according to the Higuchi and Connors method (Higuchi *et al.*, 1998). An excess amount of Quer was added to 25 mL of deionized water containing increasing amounts of HPβCD, the initial concentration of HPβCD is changed in the range of 0÷ 9mM. The corresponding thermodynamic equilibrium conditions were reached by shaking the tubes for 24 h at 25±1°C. Using UV-Vis spectrophotometer (S80, Biochrom, UK) to determine concentrations of the dissolved quercetin at 375 nm. Samples were filtered through a membrane with a 0.45 µm pore diameter. The binding stability constant (K_s) of the complex was calculated from the phase–solubility diagrams according to the equation:

$$K_s = \frac{slope}{S_0(1 - slope)} \quad (2)$$

Where S_o is the solubility of guest at 25°C in absence of HPβCD and slope means the corresponding slope of the phase–solubility diagrams, i.e., the slope of the guest molar concentration versus HPβCD molar concentration graph.

RESULTS AND DISCUSSION

Determination of obtained yield of complex (Quer-HPβCD)

Complex of Quer with HPβCD was obtained in water-ethanol solvents with different volume fraction of ethanol. The volume fraction of ethanol in the mixed solvent is limited by a low solubility of HPβCD in media with high content of non-aqueous solvent. The obtained complexation yield is shown in Table 1.

As reported in Table 1, obtained yield of Quer-HPβCD complexation in binary solvent greater than in pure water. The highest Y value at region of 15-25 % v/v of EtOH is probably caused by the reagent solvation changes at the initial additions of EtOH to water. Influence of binary H_2O-EtOH solventon the obtained yield of complexation of cyclodextrins have been observed previously (Pham *et al.*, 2019). Such effects could be explained by strengthening of the three-dimensional spatial network as water hydrogen bonds occur addition of an organic solution to the solvent (Krestov *et al.*, 1984).

Table 1. Complexation yield (Y) at different composition of solvents (X_{EtOH}) of HPβCD with Quer.

% EtOH,v/v	0	10	15	20	25	30	50
Y(Quer-HPβCD)	38	50	73	80	70	66	52

The results of Fourier transform infrared spectroscopy analysis

In the IR spectrum of Quer (Fig. 2), the valence vibrations of the carbonyl (C=O) bonds and the C=C bonds in the aromatic ring with maxima at 1662 cm^{-1} and 1613 cm^{-1}, respectively, were registered. The main differences in the IR spectra of Quer and complex were the shift and decrease in the vibration intensities of C=O and C=C bonds due to the partial penetration of Quer molecule inside in the cavity HPβCD.

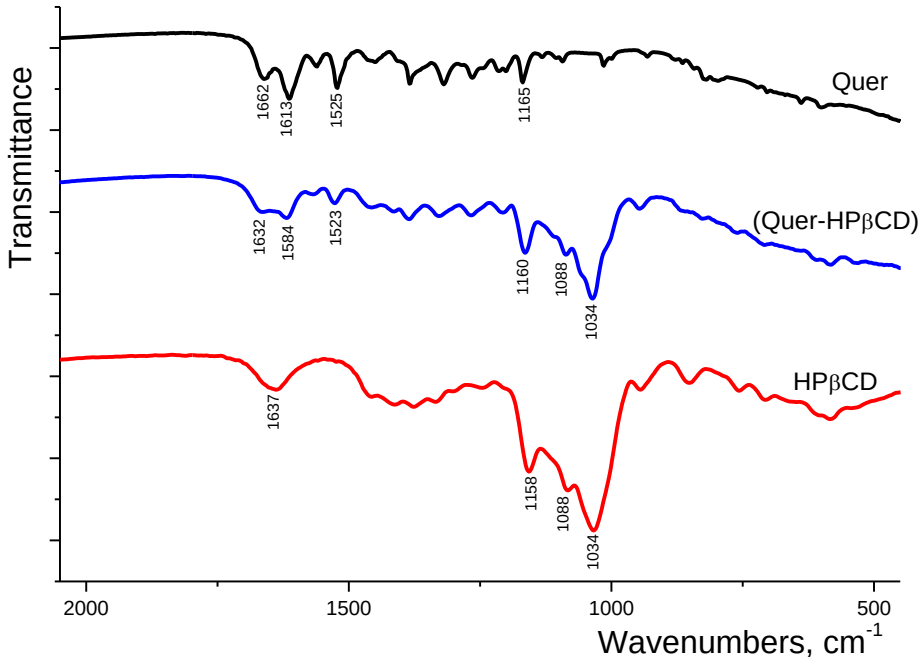

Figure 2. FT-IR spectra of raw Quer, HPβCD and (Quer-HPβCD) complex.

Morphology of complex (Quer-HPβCD)

Surface morphology of loaded and unloaded HPβCD nanoparticles was depicted in Fig. 3. FE-SEM image of Quer (Fig. 3A) revealed small aggregates or flakes throughout surface. While in micrograph of (Quer-HPβCD) complex (Fig. 3B), nanoparticles illustrated

highly porous and rough surfaces. However, the shape of the nanoparticles is inconsistent, and tending to agglomerate. Their size varies from a few nm to more than 100 nm. SEM micrographs of Quer loaded lyophilized cyclodextrin (Fig. 3C). So, lyophilization technique plays important role in formation porous surface and in the size of nanoparticles. As shown in Fig. 3C the nanoparticles have a spherical shape with an average size of about 40-60 nm.

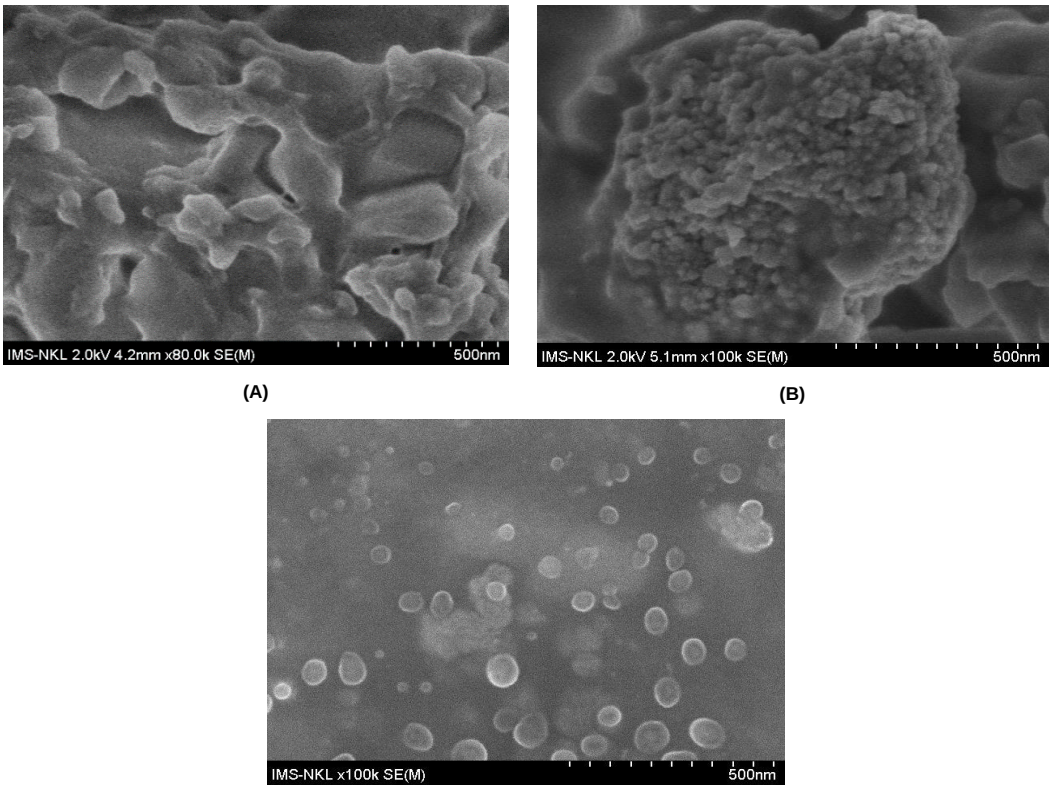

Figure 3. Scanning electron micrographs of quercetin (A), (Quer-HPβCD) complex without lyophilization (B), and (Quer-HPβCD) nanoparticles with lyophilization (C).

Phase solubility diagrams

Phase solubility diagrams are often used in supramolecular chemistry for determining the stoichiometry and stability constant of complexes. Phase solubility diagrams that were introduced by Higuchi and Connors, show how the total drug solubility changes with increasing host concentration.

The dependence of the optical density of drug solutions on its concentration was obtained, and the calibration graph was constructed by UV-Vis spectroscopies. The calibration equations is used for calculating soluble concentrations of Quer in solvent, containing HPβCD. Fig. 4 presented an example of UV-Vis absorption spectra of quercetin solutions in the absence of HPβCD (curve a) and in the presence of increased HPβCD concentrations (curves 1-5) in the range of 1÷8 mM. It can be seen that in the range of 300 ÷450 nm, Quer exhibited the absorption maximum (curve a), which was detected around 370 nm, and may be considered as transiting in the single benzene ring (Buchweitz M *et al.*, *2016*). Curves 1-5 in the Fig. 4 showed that the

inclusion complex formation of Quer with HPβCD led to the bathochromic effect: the absorption maximum was shifted towards longer wavelength (375 nm).

The phase solubility diagram for the (Quer-HPβCD) complexation was presented in Fig. 5.

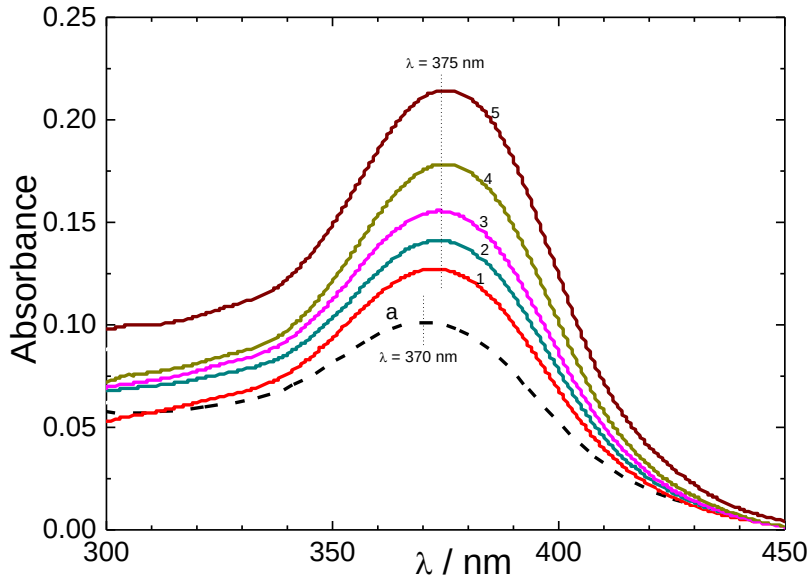

Figure 4. UV-Vis spectra of quercetin solutions in the absence of HPβCD (curve a) and in the present of HPβCD solution with increased concentrations (curves 1-5).

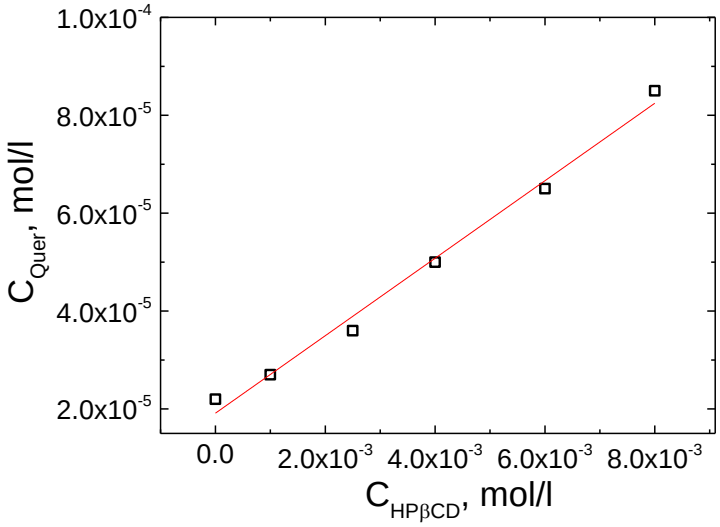

Figure 5. Phase-solubility diagrams for the binary complex and Quer- HPβCD in water.

Results indicated that in water, the solubility of Quer increased significantly and linearly depended on the concentration of HPβCD solution (Fig. 5). Particularly, the water solubility of Quer was $2.2 \cdot 10^{-5}$ mol L^{-1}, that was 4 times higher in the solution, containing HPβCD with concentration $8 \cdot 10^{-3}$ mol L^{-1}. Thus, phase solubility diagram for this system is the

A_L type, which corresponds to the 1:1 stoichiometric ratio between guest and host molecules (Higuchi *et al.*, 1998). The slope of the line is 0.00792 with $R^2 = 0.989$. The stability constant of the complex had an important influence on the extent of drug release (Stella VJ *et al.*, 1999). The stability constant determined for the Quer-HPβCD complex in our study was found to be 363 M^{-1}. It was similar to those reported by Federica (Federica *et al.*, 2017) and Pralhad (Pralhad *et al.*, 2004).

The change in the Gibbs energy of the complexation was calculated by the equation:

$$\Delta_r G = -2.303RT\log K \quad (3)$$

The Gibbs energy of the complexation were found to be $\Delta_r G$ = -14.60 kJ/mol, which are agreed with the literature data (Federica *et al.*, 2017; Pralhad *et al.*, 2004).

CONCLUSIONS

This work showed that the nanoparticles of Quer were synthesized using HPβCD and the lyophilization technique. The nanoparticles had a spherical shape with an average size of 40-60 nm. The solubility of Quer significantfly improved in the presence of HPβCD and were linearly increased in the solution of increasing amount of the macrocycle. Obtained yield of complex was improved using mixtures of water and ethanol. Its maximum values were observed in solvent with ethanol content in the range 15-25 % v/v. Results of FTIR analysis showed that the main differences in the IR spectra of Quer and complex were the shift and decrease in the vibration intensities of C=O and C=C bonds due to the partial penetration of Quer molecule inside in the cavity HPβCD. The stability constants of the (Quer-HPβCD) complex in water were found to be log$K$ = 2.56.

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NGHIÊN CỨU CẢI THIỆN ĐỘ TAN CỦA QUERCETIN TRONG NƯỚC BẰNG CÁCH TẠO PHỨC VỚI CYCLODEXTRIN TRONG DUNG MÔI HỖN HỢP

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TÓM TẮT

Khả năng chống oxy hóa của polyphenol đã được ứng dụng rộng rãi trong các ngành công nghiệp thực phẩm và dược phẩm. Quercetin (Quer) là một trong những flavonoid polyphenolic có nhiều tác dụng sinh học như hoạt tính chống oxy hóa, chống ung thư, kháng khuẩn và tác dụng chống đông máu cả in-vitro và in-vivo. Tuy nhiên, độ hòa tan của quercetin trong nước kém làm giảm sinh khả dụng. Bởi vậy, nghiên cứu các phương pháp và kỹ thuật để cải thiện độ tan của quercetin là hết sức cần thiết và thu hút được sự quan tâm của các nhà khoa học. Tạo phức với cyclodextrin là phương pháp đem lại hiệu quả cao trong việc cải thiện độ tan của dược chất. Trong nghiên cứu này, phức hợp của 2-hydroxypropyl-cyclodextrin với quercetin (Quer-HPβCD) có kích thước nano đã được tổng hợp trong dung môi hỗn hớp ethanol-nước. Kết quả cho thấy, hiệu suất tổng hợp (Y) phức (Quer-HPβCD) trong dung môi hỗn hợp lớn hơn trong nước tinh khiết. Giá trị hiệu suất cao nhất đạt được là 80% trong dung môi hỗn hợp chứa 20% v/v ethanol. Thành phần, hình thái cấu trúc và tính chất nhiệt động của phức hợp (Quer-HPβCD) đã được nghiên cứu. Sử dụng dung môi hỗn hợp nước-ethanol và áp dụng kỹ thuật đông khô có thể tạo ra các hạt nano có dạng hình cầu với kích thước trung bình khoảng 40-60 nm. Kết quả phân tích giản đồ pha của quá trình hòa tan cho thấy, trong nước độ tan của quercetin tăng tuyến tính theo chiều tăng nồng độ của HPβCD. Kết quả phân tích cũng chứng minh Quer và HP-β-CD tạo phức hợp với tỉ lệ hóa học 1:1. Hằng số bền của phức (Quer-HPβ-CD) đạt được giá trị logK = 2,56. Biến thiên năng lượng Gibbs của phản ứng tạo phức đạt được giá trị -14,60 kJ / mol.

Từ khóa: *Cyclodextrin, inclusion complexation, mixed solvent, nanoparticle, quercetin*