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Research Article

Quercetin/β-Cyclodextrin Solid Complexes Prepared in Aqueous Solution Followed by Spray-drying or by Physical Mixture

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Abstract. The present study was designed to investigate the influence of operating conditions (temperature, stirring time, and excess amount of quercetin**)** on the complexation of quercetin with β-cyclodextrin using a 2^3 factorial design. The highest aqueous solubility of quercetin was reached under the conditions 37°C/24 h/6 mM of quercetin. The stoichiometric ratio (1:1) and the apparent stability constant (Ks=230 M^{-1}) of the quercetin/β-cyclodextrin complex were determined using phase-solubility diagrams. The semi-industrial production of a 1:1 quercetin/β-cyclodextrin solid complex was carried out in aqueous solution followed by spray-drying. Although the yield of the spray-drying process was adequate (77%), the solid complex presented low concentration of quercetin (0.14%, *w*/*w*) and, thus, low complexation efficiency. The enhancement of aqueous solubility of quercetin using this method was limited to 4.6-fold in the presence of 15 mM of β-cyclodextrin. Subsequently, an inclusion complex was prepared via physical mixture of quercetin with β-cyclodextrin (molar ratio of 1:1 and quercetin concentration of 23% (*w*/*w*)) and characterized using infrared spectroscopy, differential scanning calorimetry, nuclear magnetic resonance spectroscopy, and scanning electron microscopy analyses. The enhancement of aqueous solubility of quercetin using this method was 2.2-fold, similar to that found in the complex prepared in aqueous solution before the spray-drying process (2.5-fold at a molar ratio of 1:1, i.e., 6 mM of quercetin and 6 mM of β-cyclodextrin).

KEY WORDS: β-cyclodextrin; complexation; physical mixture; quercetin; solubility.

INTRODUCTION

One of the main interests associated with cyclodextrins refers to the enhancement of solubility and/or dissolution rate of lipophilic drugs in aqueous media, very often resulting in improved bioavailability (1,2). β-cyclodextrin is the most useful parent cyclodextrin because of its commercial availability, low cost, and cavity size, which is suitable for complexing with aromatic and heterocyclic rings (3).

Quercetin (3,3′,4′,5,7-pentahydroxy flavone) (Fig. 1) belongs to the flavonoid class, naturally occurring in medicinal plants. It is also a frequent component of major dietary constituents, such as onions, apples, red wine, and green tea. Quercetin is also available in the market and can be used in the isolated form. In the literature, quercetin has been described extensively due to its broad biological properties, which are very often related to its antioxidant activity (4). On the other hand, the bioavailability of quercetin and its use in pharmaceutics are limited by its low aqueous solubility (5–10).

Some studies have shown that the association of quercetin with cyclodextrins to form inclusion complexes improves the aqueous solubility (5,11–17) and dissolution rate (16) of the flavonoid. Most quercetin/β-cyclodextrin solid complexes are prepared in liquid media, (aqueous solution submitted to freeze-drying (12,14,18), co-evaporation (11,16), or thin layer (11) methods) or semisolid media (kneading method (11,14,16,19)). However, no study to date has used a factorial design to assess the influence of operating conditions such as temperature, stirring time, and excess amount of quercetin on the complexation of quercetin with β-cyclodextrin. The objective of the present study was to assess these aspects, as well as the possibility of preparing solid complexes using spray-drying or physical mixture methods.

MATERIALS AND METHODS

Materials

Reference quercetin (purity 98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and pharmaceutical grade quercetin was purchased from DEG (São Paulo, Brazil). β-cyclodextrin was supplied by Roquette Frères (Lestrem, France). Potassium bromide was purchased from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Analytical grade methanol was purchased from Vetec (Rio de Janeiro, Brazil). Dimethylsulfoxide-d_6 was supplied by Cambridge Isotope Laboratories (Andover, MA, USA).

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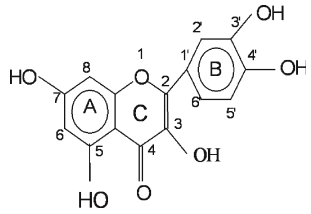

Fig. 1. Quercetin: chemical structure

Methods

Factorial design

A 2^3 factorial design was employed to obtain Higuchi and Connors phase-solubility diagrams (20). Excess amounts of quercetin (3 or 6 mM) were added to 10.0 mL of water or aqueous solutions containing increasing concentrations of β-cyclodextrin (0 to 9 mM). Flasks were covered with aluminum foil to protect quercetin from light. The resulting dispersions were magnetically stirred using an IKA HE4B-thermostated water bath for 24 or 48 h, at 25°C or 37°C. Dispersions were then filtered (25°C) through a 0.45-μm membrane (Millipore HAWP). An aliquot of 4.0 mL of the supernatant was diluted with methanol to 10.0 mL and quercetin content was determined in duplicate by spectrophotometry at 372 nm (Hewlett–Packard 8452A UV-Vis Spectrophotometer). The ultraviolet spectrophotometry method was validated according to the parameters established in the International Conference on Harmonization (21) and in the US Pharmacopoea (22) for specificity, linearity, intermediary precision, repeatability, and accuracy in the concentration range of 1.5 to 12.0 ug mL^{-1}.

Phase-solubility study

Phase-solubility diagrams were obtained in triplicate, following the factorial design described above. Quercetin/β-cyclodextrin molar ratios were 1:0, 1:0.5, 1:1, 1:1.5, 1:2, and 1:2.5. Temperature, stirring time, and excess amount of quercetin were set as defined in the factorial design (37°C/24 h/6 mM). The apparent stability constant (Ks, M^{-1}) of quercetin/β-cyclodextrin complexes was calculated based on the phase-solubility diagram according to the following equation:

$$\mathrm{Ks} = \frac{\text{slope}}{\mathrm{So}\ x(1 - \text{slope})}$$

where So is the intrinsic solubility of quercetin (quercetin solubility in the absence of β-cyclodextrin) (M). The enhancement of quercetin solubility in the presence of β-cyclodextrin in the complex prepared in aqueous solution, before the spray-drying process, was also calculated based on the phase-solubility diagram.

Preparation of solid complex in aqueous solution followed by spray-drying

Approximately 10 L of an aqueous solution containing quercetin/β-cyclodextrin complex were prepared following the above-described phase-solubility study procedure, at a molar ratio of 1:1 (6 mM of quercetin and 6 mM of β-cyclodextrin). The solution was spray-dried using a Niro Production Minor atomizer, under the following operating conditions: inlet air temperature, 175°C; outlet air temperature, 99°C; atomizer rotation rate, 10,900 rpm; and feed solution flow, 143 mL min^{-1}. The yield of the spray-drying process was measured as the powder weight percentage obtained at the end of the operation compared with the amount of solid materials (quercetin plus cyclodextrin) present in the sprayed solution. A solid complex sample was dissolved in methanol (1.4 mg mL^{-1}), filtered through a 0.45-μm membrane (Millipore HVLP), and the quercetin content was determined in triplicate by spectrophotometry at 372 nm.

Preparation of solid complex by physical mixture

Accurately weighed amounts of quercetin and β-cyclodextrin were mixed at a molar ratio of 1:1 (6 mM of quercetin and 6 mM of β-cyclodextrin), using a cubic blender (Erweka AR400) at 25 rpm during 30 min. A sample of physical mixture was dissolved in methanol (0.4 mg mL^{-1}), filtered through a 0.45-μm membrane (Millipore HVLP), and the quercetin content was determined in triplicate by spectrophotometry at 372 nm. The enhancement of aqueous solubility of quercetin in the presence of β-cyclodextrin in the solid complex prepared by physical mixture was determined following the above-described phase-solubility study procedure.

Characterization of quercetin/β-cyclodextrin complexes

Quercetin/β-cyclodextrin complexation was characterized by comparing infrared (IR) spectra, differential scanning calorimetry curves, nuclear magnetic resonance spectra, and scanning electron microscopy photomicrographs obtained for quercetin, β-cyclodextrin, and quercetin/β-cyclodextrin solid complexes (prepared by physical mixture or in aqueous solution followed by spray-drying).

Infrared spectroscopy

IR spectra obtained for samples were recorded in a frequency range between 4,000 and 400 cm^{-1}, using a resolution of 4 cm^{-1} and 40 accumulations, in a Shimadzu DR-8001 spectrometer. Discs were prepared by compressing blends corresponding to 1.5 mg of the samples and 150 mg of potassium bromide.

Differential scanning calorimetry

Thermal analysis of the samples was performed using a Shimadzu DSC-60 calorimeter. Samples of 1 to 2 mg were accurately weighed in aluminum pans and crimped. Operating conditions were 10°C min^{-1} of heating rate (25°C to 350°C) and 50 mL min^{-1} of nitrogen gas flow. Temperature was calibrated using indium (mp 157°C) and zinc (mp 420°C) as standards.

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectra obtained for samples were recorded in a Bruker DRX400-Avance spec-

trometer equipped with a 5-mm diameter inverse probe head and a z-gradient coil and operating at 400 MHz, 27°C, using DMSO-d_6 as solvent. Chemical shifts were reported in parts per million, using tetramethylsilane (0 ppm) as internal standard. One-dimensional ^{1}HNMR spectra were obtained under standard conditions. Two-dimensional ^{1}H homonuclear 2D-ROESY spectra were obtained to get insights on the supramolecular geometry of the inclusion complexes (ROESY spinlock pulse=600 ms).

Scanning electron microscopy

Photomicrographs obtained for samples were taken at a voltage of 10 kV and ×1,000 or ×3,000 magnification using a JSM 6060 microscope. Samples were mounted on brass stubs using double-sided tape and vacuum-coated with a thin layer of gold.

Statistical analysis

ANOVA was used to evaluate the significance of the results obtained in the factorial design, namely quercetin concentration in the presence of 15 mM of β-cyclodextrin in each phase-solubility diagram. Significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

Factorial Design

Knowledge of different factors that can influence drug/cyclodextrin complexation allows to select the best conditions for preparing complexes with the desirable properties and yield. Many studies assessing quercetin/cyclodextrin complexes (11–14,16,17) using the phase-solubility technique do not mention the exact amount of quercetin added to cyclodextrin solutions; neither do they inform the influence of the operating conditions employed, such as temperature and stirring time. This lack of information about the influence temperature, stirring time, and excess amount of quercetin on quercetin/β-cyclodextrin complexation in aqueous media was the motivation of our investigation employing a 2^3 factorial design.

All the factors evaluated presented a significant influence ($P<0.05$) on quercetin/β-cyclodextrin complexation (Table I); the same was observed when the interaction between temperature and stirring time was analyzed (F^d= 14.9). However, as shown in Fig. 2, the most important factor was temperature: all the curves obtained at 37°C resulted in an increased enhancement of quercetin solubility (continuous lines). Figure 2 also shows the influence of excess amount of quercetin (3 or 6 mM): the complex formation seems to occur faster when the amount is higher (6 mM). In more concentrated conditions, the interaction is easier. Once inside the cyclodextrin cavity, the drug molecule makes conformational adjustments to take maximum advantage of the interaction forces that exist. Thus, the final equilibrium to form the complex can take a long time to be reached (3). In summary, the factorial design showed that the enhancement of quercetin solubility was more significant under the following conditions: 37°C/48 h/6 mM or 37°C/24 h/6 mM. Taking into consideration that stirring time represents a cost factor in semi-industrial production, the operating conditions 37°C/24 h/6 mM were selected for the preparation of the solid complex in aqueous solution followed by spray-drying.

Phase-Solubility Study

The phase-solubility diagram obtained for the quercetin/β-cyclodextrin complex (Fig. 3) showed a linear relationship between increases in the aqueous solubility of quercetin and β-cyclodextrin concentration (R^2=0.978). According to Higuchi and Connors (20), the curve obtained can be classified as type A_L.

The apparent stability constant of a drug/cyclodextrin complex represents the binding strength between the drug and cyclodextrin and has an important influence on the extent of drug release (23). The apparent stability constant determined for the quercetin/β-cyclodextrin complex in our study was 230 M^{-1}, suggesting a relatively weak interaction between both molecules. This value is similar to those reported by Calabrò *et al.* (11), Sri *et al.* (16), Bergonzi *et al.* (14), Lucas-Abellán *et al.* (17), and Pralhad and Rajendrakumar (12): Ks=129 M^{-1}/unbuffered/25°C; Ks= 251 M^{-1}/unbuffered/28°C; Ks=396 M^{-1}/unbuffered/25°C; Ks=398 M^{-1}/pH 4.5/25°C, and Ks=402 M^{-1}/unbuffered/25°C, respectively; on the other hand, it is considerably lower than those reported by Jullian *et al.* (15), Vinadé and Petrovick (5), Zheng *et al.* (13), and Alvarez-Parrilla *et al.* (18): Ks=602 M^{-1}/unbuffered/30°C; Ks=709 M^{-1}/unbuffered/37°C; Ks=

Table I. Results Obtained **Using the** 2^3 Factorial Design **(ANOVA)**

Factor or interaction	*Dof*	SS	MS	F[a]
A (stirring time)	1	1.780×10^{-5}	1.780×10^{-5}	10.95
B (temperature)	1	2.763×10^{-4}	2.763×10^{-4}	170.01
C (quercetin concentration)	1	4.859×10^{-5}	4.859×10^{-5}	29.90
Interaction	1			
A×B	1	2.422×10^{-5}	2.422×10^{-5}	14.90
A×C	1	3.200×10^{-6}	3.200×10^{-6}	1.97
B×C	1	4.800×10^{-6}	4.800×10^{-6}	2.95
A×B×C	1	3.648×10^{-8}	3.648×10^{-8}	0.02
Error	8	1.300×10^{-5}	1.625×10^{-6}	
Total	15	3.880×10^{-4}		

dof degrees of freedom, *SS* sum of squares, *MS* mean square
[a] $F_{(1,8)}$: α 0.05=5.32

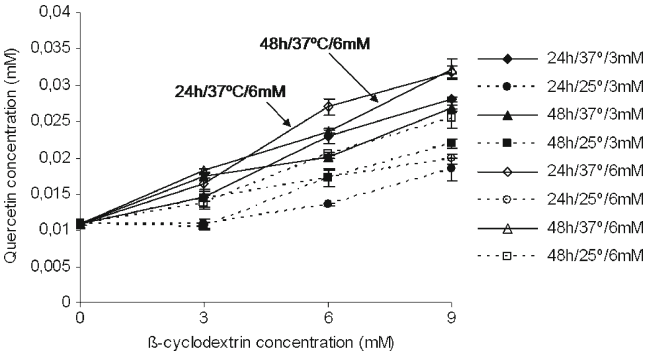

Fig. 2. Phase-solubility diagrams of quercetin/β-cyclodextrin complexes obtained using a 2^3 factorial design (n=2). Stirring time of 24 or 48 h, temperature of 25°C or 37°C and excess amount of quercetin of 3 or 6 mM

1,028 M^{-1}/pH 3.0/24°C and Ks=1,138 M^{-1}/pH 7.0/25°C, respectively. Although it is not possible to explain these differences, the influence of ionization of the quercetin molecule as a result of the media pH employed in those experiments [pKa1=7.0 and pKa2=9.1 (24–26)] cannot be ruled out. The ionized form of the drug usually yields less stable complexes than the unionized form. This is attributed to the increased hydrophilicity of the drug upon ionization, which reduces interaction between the drug and the hydrophobic cavity of cyclodextrin (e.g., Van der Waals and hydrophobic forces), thus, increasing the fraction of free drug molecules in the solution (23). The high apparent stability constant (Ks= 1,028 M^{-1}) reported by Zheng *et al.* (13), obtained in acidic aqueous media (phosphate buffer at pH 3.0), corroborates our hypothesis, while the unexpectedly high apparent stability constant (Ks=1,138 M^{-1}) reported by Alvarez-Parrilla *et al.* (18) at pH 7.0 (phosphate buffer) is probably influenced by the spectroscopic method employed in that study, which usually provides higher values when compared with the phase-solubility method. On the other hand, Lucas-Abellán *et al.* (17) also employed acidic aqueous media (acetate buffer at pH 4.5) and found a lower apparent stability constant (Ks= 398 M^{-1}), which can possibly be explained by the use of an ultrasonic bath for 60 min in the phase-solubility study, a procedure that is well known to provide heat to the system. Since complexation is an exothermic process, the enthalpy of the system decreases during complex formation (23,27). Thus, in our study, in addition to pH value close to 6.0 (unbuffered aqueous media), the temperature of 37°C may also have contributed to the low apparent stability constant found for the quercetin/β-cyclodextrin complex.

Aqueous solubility of quercetin in the absence of β-cyclodextrin was 0.011 mM. In the presence of 15 mM of β-cyclodextrin, the solubility enhanced significantly (4.6-fold; P<0.05). However, as shown in Fig. 3, complexation efficiency was not very high, because relatively large amounts of β-cyclodextrin are necessary to solubilize low amounts of quercetin in an aqueous media. This result can be related to the low apparent stability constant found in the quercetin/β-cyclodextrin complex as well as to the occurrence of polymorphisms, since different intrinsic solubilities of polymorphic forms of quercetin have been reported by Borghetti *et al.* (28). Some strategies aimed at improving complexation efficiency between quercetin and β-cyclodextrin have been previously tested in our laboratory, such as the use of hydrophilic polymers (hydroxypropylmethyl-cellulose (HPMC)) (29). While the association of quercetin with β-cyclodextrin (15 mM) yielded an aqueous solubility enhancement of 4.6-fold, the addition of HPMC (0.1 %, *w*/*w*) to the system resulted in a 6.5-fold enhancement. The association of quercetin with hydroxypropyl-β-cyclodextrin, in the presence or not of HPMC, has been also tested.

In addition to determining the apparent stability constant of drug/cyclodextrin complexes, the phase-solubility diagram can also be used to define the necessary cyclodextrin concentration to obtain the desired drug solubility in aqueous media. In our study, the molar ratio of 1:1 was chosen for the semi-industrial production of quercetin/β-cyclodextrin solid complex in aqueous solution followed by spray-drying. It is worth mentioning that the minimum amount of cyclodextrin necessary to solubilize the drug in the aqueous medium should be used, since its excess can affect complex formation equilibrium and consequently reduce drug bioavailability (1). Moreover, considering the high molecular weight of cyclodextrin, an excess can pose limitations for its incorporation in a vehicle.

Preparation of Solid Complex in Aqueous Solution followed by Spray-Drying

The solid complex was prepared by spray-drying an aqueous solution containing 1:1 quercetin/β-cyclodextrin. The presence of cyclodextrin resulted in low adherence of the powder to the spray-dryer wall, which explains the adequate yield obtained in the spray-drying process (77%). However, quercetin concentration in the solid complex was only 0.14 % (*w*/*w*). Although, in general, spray-drying is not a viable method for drying complexes containing heat-labile drugs (3), a study conducted by Costa (30) demonstrated that quercetin remained stable when submitted to spray-drying temperatures. In addition, no degradation was observed when we analyzed a spray-dried quercetin aqueous dispersion by liquid chromatography (data not shown). Therefore, the low quercetin concentration observed in the solid complex in our study is probably due to the low complexation efficiency between quercetin and β-cyclodextrin.

Infrared Spectroscopy

IR spectrum obtained for quercetin presented typical molecule bands and peaks: 3,409–3,144 cm^{-1} (O–H),

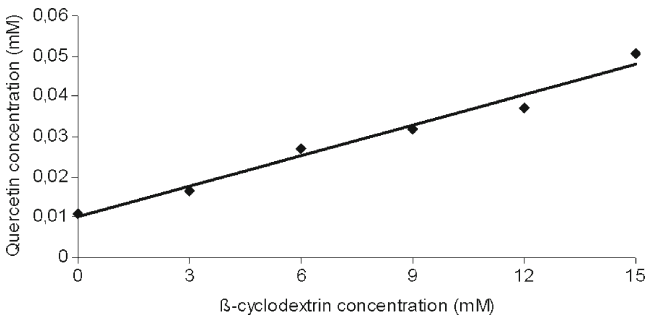

Fig. 3. Phase-solubility diagram of quercetin/β-cyclodextrin complex (n=3). Stirring time of 24 h, temperature of 37°C, and excess amount of quercetin of 6 mM

1,667 cm^{-1} (C=O), 1,610 cm^{-1} (C=C), 1,381 cm^{-1} (C–OH), and 1,264 cm^{-1} (C–O–C; 31–35). IR spectrum obtained for β-cyclodextrin presented a large band and a peak in the region of 2,900 to 3,900 cm^{-1}, a short band between 1,600 and 1,700 cm^{-1} and a large band containing distinct peaks in the region of 900 to 1,200 cm^{-1} (36). IR spectrum obtained for quercetin/β-cyclodextrin solid complex prepared in aqueous solution followed by spray-drying presented the same profile observed for β-cyclodextrin, probably due to the low quercetin concentration in the solid complex. An increased intensity of bands and peaks was also observed. In the IR spectrum obtained for quercetin/β-cyclodextrin solid complex prepared by physical mixture, quercetin bands and peaks overlapped with most of β-cyclodextrin bands and peaks, except for those in the 3,000 cm^{-1} region and between 850 and 1,000 cm^{-1}. The increased intensity of bands and peaks, the presence of a new peak in the 2,500 cm^{-1} region, and the different band shapes observed between 1,000 and 1,150 cm^{-1} when compared with quercetin and β-cyclodextrin spectra suggest an interaction between quercetin and β-cyclodextrin.

Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) curve obtained for quercetin (Fig. 4b) presented two endothermic events: the first one, with an onset temperature of 86°C, corresponds to the loss of bounded water; the other, with an onset temperature of 319°C, is related to the melting point, followed by decomposition (31,37). These events were also observed in the DSC curve obtained for β-cyclodextrin (Fig. 4a), where another endothermic event took place at approximately 220°C corresponding to an irreversible transformation process within the β-cyclodextrin molecule (16). The DSC curve obtained for quercetin/β-cyclodextrin solid complex prepared in aqueous solution followed by spray-drying (Fig. 4d) presented the same thermal profile observed for β-cyclodextrin, probably due to the low concentration of quercetin in the solid complex. However, the endothermic event observed at approximately 220°C for β-cyclodextrin was not detected in the solid complex. The reduction of the endothermic event related to the loss of bounded water can be explained by the elimination of water during the spray-drying process. Finally, in the DSC curve obtained for quercetin/β-cyclodextrin solid complex prepared by physical mixture (Fig. 4c), it is possible to observe the endothermic events corresponding to loss of bounded water in both molecules and the endothermic event related to the irreversible transformation process within the β-cyclodextrin molecule. However, the absence of the endothermic event corresponding to quercetin's melting point suggests an interaction between quercetin and β-cyclodextrin in the physical mixture.

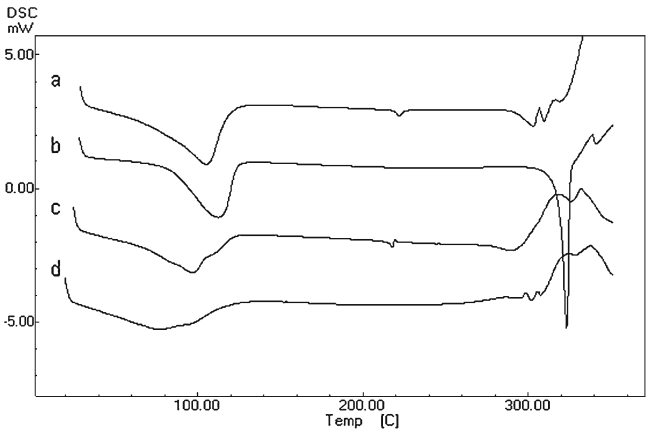

Fig. 4. Differential scanning calorimetry curves of β-cyclodextrin **a**; quercetin **b**; physical mixture **of quercetin with β-cyclodextrin c**; quercetin/β-cyclodextrin solid complex **d**

Nuclear Magnetic Resonance Spectroscopy

^{1}HNMR spectrum obtained for the quercetin/β-cyclodextrin solid complex prepared in aqueous solution followed by spray-drying presented only β-cyclodextrin hydrogen signals (data not shown), probably due to the low quercetin concentration in the solid complex. On the other hand, ^{1}HNMR spectrum obtained for the fresh physical mixture (Fig. 5c) presented hydrogen signals of quercetin and β-cyclodextrin. The comparison between this spectrum and that obtained for quercetin (Fig. 5a) reveals: broadening of –OH signals ($\delta_{5\text{–OH}}$ 12.50, $\delta_{7\text{–OH}}$ 10.80, $\delta_{3\text{–OH}}$ 9.60, $\delta_{4'\text{–OH}}$ 9.33 and $\delta_{3'\text{–OH}}$ 9.30) and loss of resolution in hydrogen signals of the B-ring of quercetin molecule [H-2′ (δ7.69, d), H-6′ (δ7.55, d) and H-5′ (δ6.89, d)] (31,32,35). These differences suggest an interaction between quercetin and β-cyclodextrin in the fresh physical mixture. Nuclear Overhauser Effects (NOEs) between quercetin and β-cyclodextrin hydrogens were not observed in the fresh physical mixture. Thus, a new physical mixture was prepared following the same procedure and then stored during approximately 2 months. The ^{1}HNMR spectrum obtained for the stored physical mixture also presented hydrogen signals of both compounds (Fig. 5b), suggesting an interaction between quercetin and β-cyclodextrin. In the stored physical mixture, NOEs were observed between quercetin hydrogens H-2′ (δ7.69, d), H-6′ (δ7.55, d), H-5′ (δ6.89, d), H-8 (δ6.42, d), and H-6 (δ6.20, d) (31,32,35) and β-cyclodextrin hydrogens H-3 (δ3.89, d) and H-6 (δ3.69–3.80, d) (11) (Fig. 6). These results suggest that the quercetin molecule was located inside the β-cyclodextrin cavity and, consequently, a quercetin/β-cyclodextrin inclusion complex was formed in the stored physical mixture. The findings also suggest that inclusion complex formation between quercetin and β-cyclodextrin by physical mixture is a time-dependent process.

Scanning Electron Microscopy

The photomicrographs of the samples obtained by scanning electron microscopy (SEM) are shown in the Fig. 7. The β-cyclodextrin particles (Fig. 7a) presented a parallelogram shape, whereas quercetin (Fig. 7b) presented columnar crystals. The quercetin/β-cyclodextrin solid complex prepared by physical mixture (Fig. 7c) did not suggest an interaction between both molecules, because quercetin crystals simply covered the surface of β-cyclodextrin particles. The quercetin/β-cyclodextrin solid complex prepared in aqueous solution followed by spray-drying presented amorphous particles with shrunken spherical shape (Fig. 7d), a

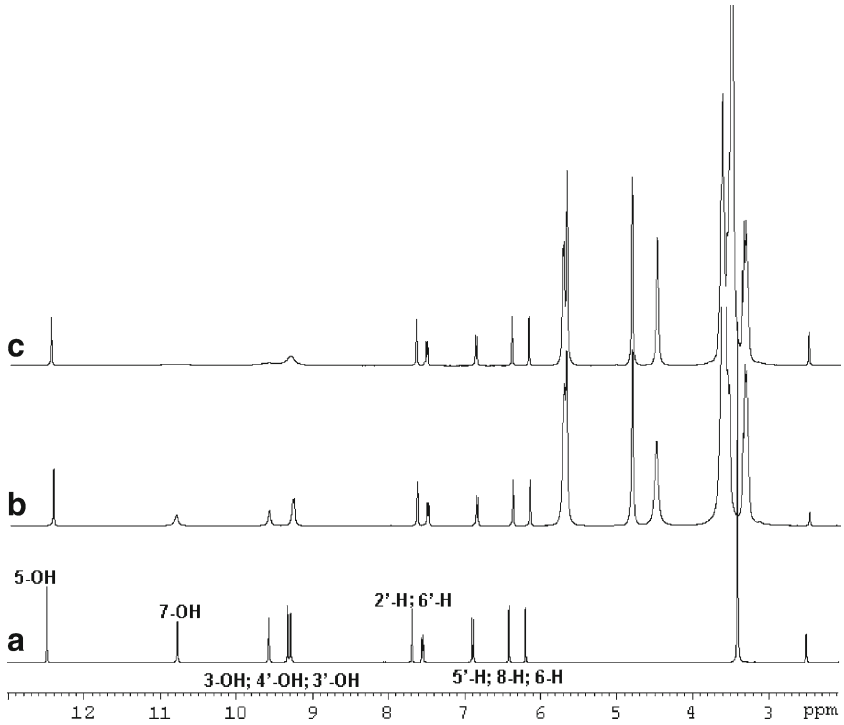

Fig. 5. [1]HNMR spectra of quercetin **a**; stored physical mixture of quercetin with β-cyclodextrin **b**; fresh physical mixture of quercetin with β-cyclodextrin **c**

finding that is in accordance with the studies conducted by Calabrò *et al.* (11), Pralhad and Rajendrakumar (12), and Sri *et al.* (16), which employed X-ray powder diffractometry to demonstrate that drug/cyclodextrin solid complexes exist in amorphous state. However, our study could not suggest an interaction between quercetin and β-cyclodextrin, because it was impossible to differentiate between the morphology of solid complex and that of the isolated compounds.

Taken together, our IR, DSC, NMR, and SEM analyses suggest the formation of a quercetin/β-cyclodextrin solid complex by physical mixture, a different method when compared to others previously described, which employ liquid or semisolid media (5,11–19). According to Zheng *et al.* (13) who elucidated the mode of interaction between quercetin and β-cyclodextrin using a molecular modeling study, the B-ring, the C-ring, and part of the A-ring (except C_6) of quercetin are positioned inside the β-cyclodextrin cavity. In the present study, indications of interaction between quercetin and β-cyclodextrin in the solid complex prepared in aqueous solution followed by spray-drying were absent both on the NMR (due to the low quercetin concentration) and SEM analyses (due to the impossibility to differentiate between the morphology of solid complex and that of the isolated compounds).

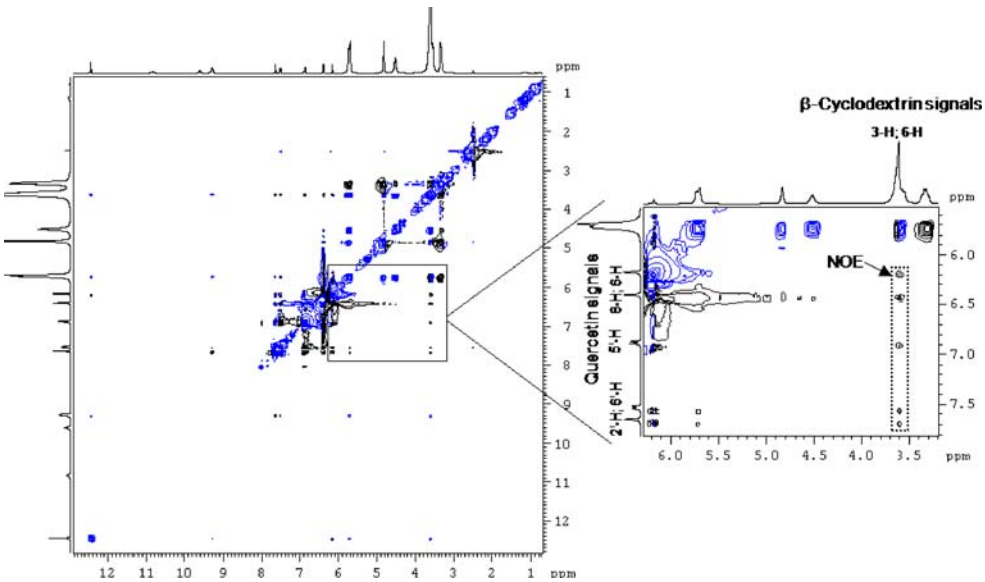

Fig. 6. 2D-ROESY spectrum of stored physical mixture of quercetin with β-cyclodextrin

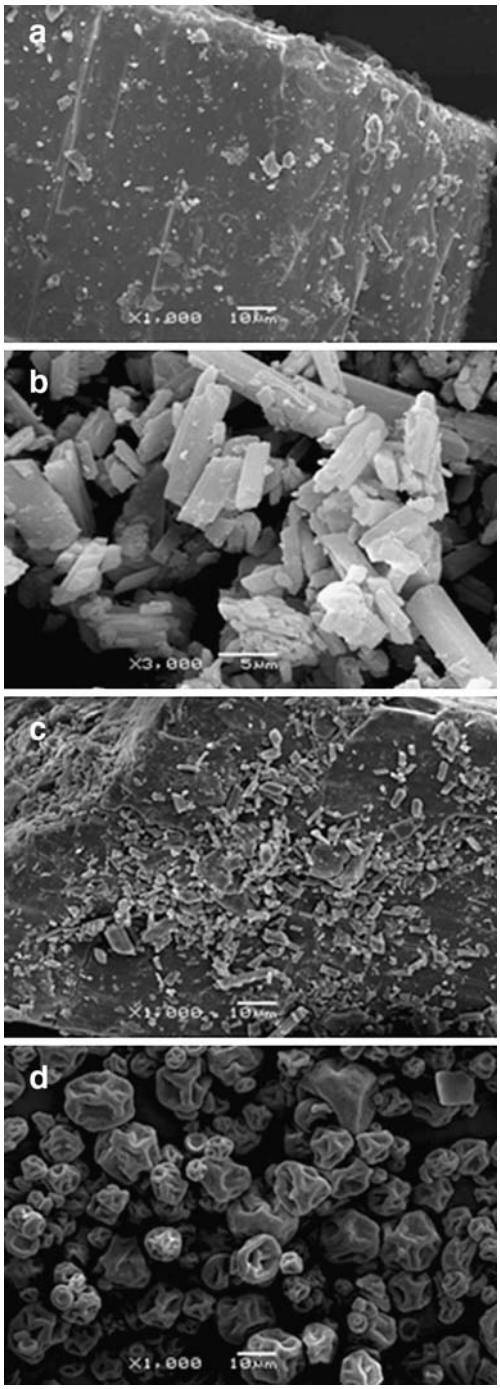

Fig. 7. Photomicrographs obtained by scanning electron microscopy of β-cyclodextrin **a**; quercetin **b**; physical mixture of quercetin with β-cyclodextrin **c**; quercetin/β-cyclodextrin solid complex **d**

Drug/cyclodextrin complexation in physical mixtures has been previously reported by Del Valle (3). According to that author, drug/cyclodextrin complexes can be formed either in liquid or solid state by simply adding the drug to cyclodextrin and mixing both compounds together. The mixing time required to complete complexation and the effectiveness of the preparation method depend on the nature of the drug and cyclodextrin. In the present study, although the solid complex prepared by physical mixture showed a higher concentration of quercetin (23 %, *w*/*w*), complexation efficiency, measured by the enhancement of quercetin solubility, was similar to that found in the complex prepared in aqueous solution before the spray-drying process: 2.2- and 2.5-fold, respectively, at a quercetin:β-cyclodextrin molar ratio of 1:1 (6 mM of quercetin and 6 mM of β-cyclodextrin).

Aqueous solution is the method most widely used in the preparation of drug/cyclodextrin complexes. In the liquid state, more cyclodextrin molecules are available for complexation when compared with solid-state preparations, in which only surface molecules of the cyclodextrin particles can form a complex with the drug. On the other hand, the main disadvantage associated with the aqueous media method regards scale-up. Because of the limited solubility of β-cyclodextrin, large volumes of water have to be used. Container capacity, time and energy for heating and cooling, and treatment of undissolved solids may generate cost impacts. Moreover, the aqueous solution method is not a continuous process because it depends on solvent removal (3).

In the present study, the physical mixture was prepared in a cubic blender, equipment commonly used in industrial scale. Physical mixture preparation in a mortar, as described by Pralhad and Rajendrakumar (12), Sri *et al.* (16), and Alvarez-Parrilla *et al.* (18), is only possible in laboratory scale. The preparation of drug/cyclodextrin solid complexes by physical mixture method has the advantage of being a one-step process, where neither water nor heat are necessary, resulting in a lower cost when compared with aqueous solutions (3).

CONCLUSIONS

The present report is the first to demonstrate the influence of the operating conditions for quercetin/β-cyclodextrin complexation in aqueous media using a 2^3 factorial design. The best conditions were 37°C/24 h/6 mM of quercetin (stoichiometric ratio of 1:1 and Ks=230 M^{-1}). Enhancement of aqueous solubility of quercetin was limited to 4.6-fold in the presence of 15 mM of β-cyclodextrin. Although the yield of the spray-drying process of 1:1 quercetin/β-cyclodextrin complex produced in aqueous solution was adequate (77 %), the solid complex presented a low concentration of quercetin (0.14 %, *w*/*w*) and, thus, low complexation efficiency. This is also the first study to report the formation of 1:1 quercetin/β-cyclodextrin inclusion complex by physical mixture, using different analytical techniques. The physical mixture contained 23 % (*w*/*w*) of quercetin and presented an enhancement of quercetin solubility of 2.2-fold, similar to that obtained in the complex prepared in aqueous solution before the spray-drying process (2.5-fold) when quercetin and β-cyclodextrin concentrations were 6 mM (molar ratio of 1:1). Our findings suggest that the physical mixture method is an adequate alternative method for the preparation of quercetin/β-cyclodextrin solid complexes, since it is a one step process as well as neither water nor heat are necessary.

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