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Combined effect of pH and polysorbates with cyclodextrins on solubilization of naringenin

S. Tommasini, M.L. Calabrò*, D. Raneri, P. Ficarra, R. Ficarra

Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università di Messina, Viale Annunziata, 98168 Messina, Italy

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Abstract

pH control and inclusion complex formation are commonly used as solubilization techniques in formulating ionizable drugs. Naringenin is a weakly acid compound with a low water solubility. The role of both ionized and unionized species of naringenin in solution by complexation with β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin was investigated. This combined use of ionization and complexation increases not only the solubility of the unionized naringenin, but also that of the ionized one. This study puts on evidence the role of pH, pK_a and complexation constants in increasing drug total aqueous solubility, determined by the single components in solution, as ionized and unionized naringenin both in free and complexed forms. Moreover, the presence of non-ionic surfactants in the media of complexation gives a positive contribution to the improvement of the solubility of naringenin, alone or in combination with β-cyclodextrin.

Keywords: Naringenin; Modified cyclodextrins; Phase solubility studies; Ionization; Polysorbates

1. Introduction

The rate of absorption of an oral dosage for a molecule having very low solubility either in water or at the stomach or intestine pH values depends on its structural characteristics, in particular, on the extent of ionization, the particle size, the interaction of the molecular moieties with the solvent and the form of crystallization.

Technological expedients widely used in pharmaceutics to overcome solubility problems in oral formulations are proposed, such as to reduce particle size, to modify crystal structure by the formation of various polymorphic forms, to ionize the molecule, to add solubilizing agents or to improve the wettability of the powder. Change in pH is one of the major factors that influence solubility of drugs with ionizable groups.

Naringenin (Fig. 1) is a molecule belonging to the class of flavanones and it is largely studied for its antioxidant activity [1–5], protective effect against the lipid peroxidation [6,7], antiestrogenic [8], hypolipidaemic [9] and antiatherogenic activity [10]. It is nevertheless not used in therapy yet because of its very low aqueous solubility. Inclusion complexes of naringenin with β-cyclodextrin (β-CyD) were prepared in order to improve its dissolution properties, and characterised both in solid state and solution [11]. It is a weak acid compound (pK_a = 6.7) [12], thus showing a greater solubility in basic than in acid media [13].

β-cyclodextrins are cyclic oligosaccharides derived from starch, containing seven D-(+)-glucopyranose units attached by α(1,4) glucoside bonds. They are mainly used in oral and parenteral pharmaceutical formulations as well as in cosmetics and food products, and are generally regarded as essentially non-toxic and -irritant materials [14–18]. Parent β-CyD is the less soluble of the class (1.85 g/ml), while methyl-β-CyD and 2-hydroxy-propyl-β-CyD are greatly soluble (>500 and >600 mg/ml) and more compatible with body fluids.

The non-ionic surfactants are a great class of compounds frequently used in pharmaceutical systems, since their advantages as to compatibility, stability, toxicity and minimal binding to proteins are rather significant [19–22].

Quite water soluble non-ionic surfactants, such as the polyoxyethylene 20 sorbitan esters, polysorbate 20 and 60, were used. They are formed by ether linkages of

* Corresponding author. Tel.: +39 090 6766409; fax: +39 090 355613.
E-mail address: mlcalabro@pharma.unime.it (M.L. Calabrò).

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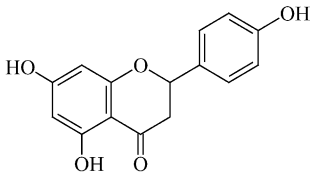

Fig. 1. Chemical structure of naringenin.

polyoxyethylene groups between the alcoholic groups. Polysorbates are used as wetting agents at the concentration of 0.1–3% and as solubilizer at the concentration of 1–10% [23], in formulations of oral and parenteral suspensions of water insoluble substances; in both cases, they are used in concentrations greater than the critical micellar concentration (CMC), that is 0.060 g/l for polysorbate 20 and 0.028 g/l for polysorbate 60 [24]. Their micelles are constituted by a polar capsule, the polyoxyethylene chain, faced to the aqueous medium and an apolar core, the hydrocarbon chain, direct toward inside the micelle. They have an anisotropic water distribution within their structure as water concentration decreases from the surface towards the core of the micelle. The position of a molecule within the micelle depends on its polarity. In aqueous systems, non-polar molecules will be solubilized within the micelle core, polar molecules will be adsorbed on the micelle surface and substances with intermediate polarity will be distributed in an intermediate position [25]. On the other side, the longer the hydrocarbon chain in the homologous series of polysorbates, the more efficient the solubilizing power of the surfactant. Therefore, polysorbate 60 is expected to be more efficient as solubilizer than polysorbate 20; when it happens, it indicates that the drug is probably incorporated within the core of the micelle rather than in the capsular portion.

The objective of this study is to evaluate the role of the ionized species of naringenin, a weakly acid molecule, on the improvement of solubility by complexation with parent and modified β-CyDs, and to determine the variation of its solubility given by surfactants, β-CyD alone, or their combined effect, to be able to choose the most suitable kind and amounts of adjuvants in the formulation of this flavanone.

2. Experimental

2.1. Materials

Naringenin (MW = 272.3) was supplied by Sigma–Aldrich S.r.l. (Milan, Italy). β-cyclodextrin (MW = 1135), methyl-β-cyclodextrin (MW about 1312) and 2-hydroxypropyl-β-cyclodextrin (MW about 1400) were purchased from Fluka Chemie (Buchs, Switzerland). Surfactant used were polyoxyethylene 20 sorbitan monolaurate (polysorbate 20) (MW = 1128) and polyoxyethylene 20 sorbitan monostearate (polysorbate 60) (MW = 1312), purchased from A.C.E.F. S.p.A. (Fiorenzuola D'Arba, Italy). Acetonitrile (ACN) of high-performance liquid chromatography (HPLC) grade and methanol of analytical grade were purchased from Merck (Darmstadt, Germany). All solutions were filtered through 0.45 μm Gelman Sciences Acrodisc® LC 13 PVDF syringe filters provided by Merck (Darmstadt, Germany). Solutions at two different pH values (4.0 and 8.0) were prepared. The hydrogen ion concentration (pH 4.0 and 8.0) was obtained, according to current Italian Pharmacopoeia [26], by using solutions of sodium phosphate dibasic 0.2 M and citric acid 0.1 M at the temperature of 25 ± 0.5 °C, purchased from Carlo Erba (Milan, Italy). The water used for solutions was distilled, deionized and filtered through 0.22 μm Millipore filters (Bedford, USA). All other materials were of analytical reagent grade.

2.2. Apparatus

The phase solubility studies of naringenin with different CyDs in media at pH 4.0 and 8.0 were carried out using a Perkin-Elmer series 410 liquid chromatograph equipped with a septumless injector (Rheodyne 7125-075) and a column heater (Perkin-Elmer TC 931). A variable wavelength diode array detector (Perkin-Elmer LC 235) was used. Peak area integration was performed using a chromatographic data system (Perkin-Elmer LCI 100 laboratory computing integrator), with a sensitivity setting of 0.05 AUFS. A Vydac reversed-phase C_{18} column (25 cm × 4.6 mm i.d., particle size 10 μm), thermostated at 25 °C, was used as the stationary phase. The mobile phase was a mixture of acetonitrile–water 80:20 (v/v) at a flow rate of 1 ml/min.

pH of the solutions was measured by a pH-meter Jeanway model 3310, equipped with an electrode which operates in a range of pH from 1 to 14, with an accuracy of ±0.1, in a range of temperature from 0 to 80 °C.

The solubility studies of naringenin performed by using polysorbates 20 or 60 alone and with β-CyD were analysed by a Lambda 45 Perkin-Elmer UV–vis double-beam spectrophotometer, interfaced to a PC for data processing (software: UV-Win Lab, from Perkin-Elmer). Quartz cells with a 10 mm pathlength (Hellma) were employed in the 200–400 nm spectral range (scanning speed 60 nm/min; slit = 2).

Solutions in solubility phase studies were thermostated to equilibrium by using a Haake C25 bath equipped with a Haake F6 controller which allowed variation of temperature with an accuracy of ±0.01 °C.

Processing of data for the fitting was done using the Statistica® software by Statsoft.

3. Solubility studies

The solubility of naringenin as a function of parent β-CyD, and its derivatives 2-hydroxypropyl-β-CyD and methyl-β-CyD at pH 4.0 and 8.0 was determined. Solutions with concentrations of 0–0.014 M of each CyD in buffers

adjusted to pH 4.0 or 8.0 were prepared. Amounts of naringenin exceeding its solubility were added to saturate each solution. Flasks were sealed to avoid changes in concentration due to evaporation and all solutions were then shaken in a thermostated bath at 25 ± 0.5 °C. After the equilibrium was reached, namely until their concentration did not change with time any more, that is after 72 h, suspensions were filtered and solutions assayed by HPLC, at the wavelength of 285 nm to calculate the resulting concentration of naringenin in solution. The presence of the CyDs did not interfere with the chromatographic analysis. Each data point is the average of three determinations.

The apparent formation constants, K_u, were calculated from the phase solubility diagrams according to the following Higuchi-Connors equation [27] for the unionized substance:

$$K_u = \frac{\text{slope}}{S_u(1 - \text{slope})} \quad (1)$$

where slope is derived from the plot and S_u is the solubility of pure naringenin; besides the apparent formation constant of the ionized form depends on both the pH of the medium and the pK_a of the substance, as the pure naringenin is more soluble in basic media; therefore, it is calculated according to Eq. (2):

$$[S_{tot}] = [S_u] + [S_u] \times 10^{pH-pK_a} + K_u[S_u][CyD_{tot}] + K_i[S_u] \times 10^{pH-pK_a}[CyD_{tot}] \quad (2)$$

where $[S_{tot}]$ is the total concentration of naringenin in solution, S_u is its solubility in neutral solution, $[S_u] \times 10^{pH-pK_a}$ is its solubility as ionized form, K_u and K_i are the apparent formation constants of unionized and ionized naringenin with the CyDs, respectively, and CyD_{tot} is the total concentration of CyDs used [28].

The solubility studies of naringenin with polysorbates 20 or 60 alone and with β-CyD were carried out by means of the same procedure, but following three different approaches.

The first consisted in the addiction of the excess amounts of naringenin to 10 ml tubes containing aqueous solutions of various concentrations of polysorbates 20 or 60 (0–0.100 M); in the second, different concentrations of polysorbates 20 or 60 (0–0.100 M) in presence of a constant concentration of β-CyD (0.008 M), being chosen an intermediate concentration within the range studied, were added to samples of naringenin; in the third, aqueous solutions of various concentrations of β-CyD (0.001–0.014 M) and a constant concentration of polysorbates 20 or 60 (0.020 M) were used.

As for solubility studies carried out with CyDs at different pH, after 72 h, solutions reached the equilibrium; then, an aliquot from each vial was filtered, adequately diluted and analyzed by spectrophotometry at 289 nm to evaluate the concentration of the naringenin dissolved and the relative effect of the solubilizers. It was checked that the presence of the polysorbates did not interfere with the spectroscopic analyses, as they do not absorb in the UV range under study and solutions were diluted to avoid scattering in colloidal solution. The experiments were carried out in triplicate. The solubilizing capacity of the surfactants can be expressed as the mass solubilization ratio (WSR) and the molar solubilization ratio (MSR), i.e. the mass and the moles of naringenin solubilized per mole of surfactant above the CMC, respectively:

$$\text{WSR} = \frac{S_{tot} - S_{cmc}}{C_{surf} - \text{CMC}} \quad (3)$$

and

$$\text{MSR} = \frac{S_{tot} - S_{cmc}}{C_{surf} - \text{CMC}} \quad (4)$$

where S_{tot} is the total apparent solubility of naringenin in micellar solution at the particular surfactant concentration equal to C_{surf}; S_{cmc} is the saturation concentration of naringenin at the CMC of the surfactants, expressed in w/w for the WSR and mol/mol for the MSR [29].

The distribution coefficient k_m of naringenin in micelles of the polysorbates is given by the ratio of the solubilized substance in the micelle to that solubilized in the aqueous phase (Eq. (5)).

$$k_m = \frac{[SM]}{[S_0][C_{surf}]} \quad (5)$$

where SM is the concentration of naringenin solubilized into the micelle at the concentration C_m of the surfactant, and S_0 is the solubility of naringenin in water, in absence of the surfactant [30,31].

4. Results and discussion

The solubility studies of naringenin are performed at pH 4.0 and 8.0, in which the contribution of the unionized and ionized forms of naringenin is, respectively, prevalent; the solubility of naringenin as a function of the CyDs at both pH 4.0 and 8.0 showed a diagram with an A_L profile (linear with a slope minor than one), suggesting that naringenin, both in the uncharged and in the charged state, forms soluble complexes in 1:1 ratio, in different extent; the affinity of the unionized naringenin for the hydrophobic cavity of CyDs is higher than that of the ionized form, due to the more hydrophilic character of this last one.

The solubility of naringenin at pH 4.0 as a function of different concentrations of β-CyD, 2-hydroxypropyl-β-CyD and methyl-β-CyD, ranging from 0 to 0.014 M, is shown in Fig. 2. In absence of CyDs the solubility of naringenin at pH 4.0 is very low, as it is about 0.08 mg/ml. When β-CyD is used, an increase in solubility is observed according to data obtained when β-CyD is used as a solubilizer agent in unbuffered water [2]. There is a roughly linear increase in solubility of naringenin, up to 0.62 mg/ml with β-CyD, up to 1.10 mg/ml with 2-hydroxypropyl-β-CyD and 1.99 mg/ml with methyl-β-CyD.

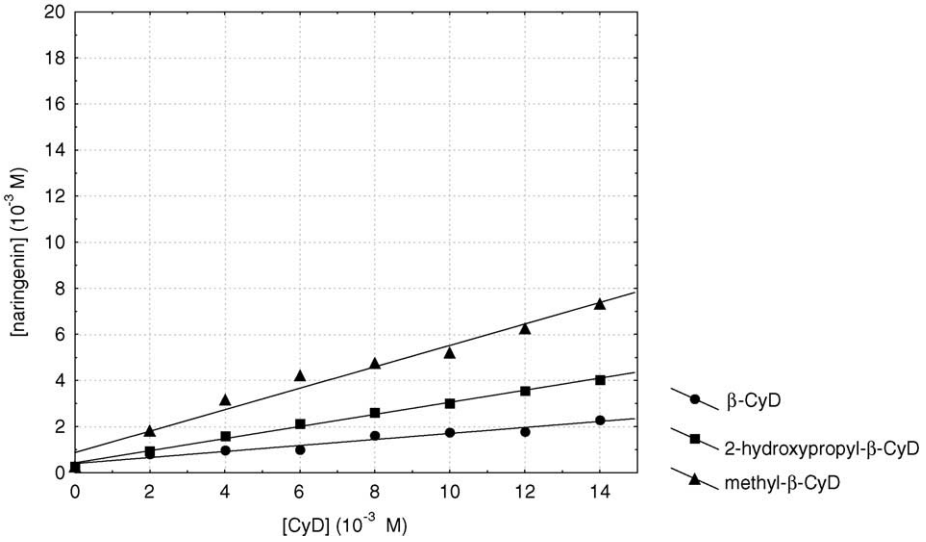

Fig. 2. Phase solubility study of naringenin with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin at pH 4.0.

When basic media is used to solubilize naringenin, a different behaviour is observed. In absence of CyDs, the solubility of naringenin at pH 8.0 is higher than at pH 4.0, as it is 0.35 mg/ml; this is due to the ionization of naringenin in basic media as it tends to easily solubilize, giving pale yellow coloured solutions. Also in this case, there is a roughly linear increase in solubility of naringenin, up to 1.81 mg/ml with β-CyD, up to 2.53 mg/ml with 2-hydroxypropyl-β-CyD and 4.12 mg/ml with methyl-β-CyD (Fig. 3). The solubility of pure naringenin is greater at pH 8.0 than at pH 4.0 and a similar trend after CyDs complexation is observed. In fact, the solubility of the complex is proportional to the product of the complexation constant with the solubility for both the unionized and ionized solutes.

CyD inclusion is a molecular phenomenon in which usually the whole or part of a molecule interacts with the cavity of the CyD by means of non-covalent interactions, such as Van der Waals forces, hydrophobic interactions and other low energy ones. So, as more hydrophobic and thus apolar the molecule is, as more stable complexes are formed; the unionized species will form a more stable complex with CyDs, and we can assume that in acid solutions, it is primarily responsible for the formation of the complex and the subsequent increase in solubility. But though the ionized naringenin has a smaller formation constant, it shows greater water solubility and an even greater increase in solubility with increasing concentrations of CyDs, when compared with that of the unionized species. It happens because a change in pH, that causes solute ionization, will increase the intrinsic solubility of naringenin in water, and greater concentrations of a substance in solution can favour the formation of complexes; in our case, this event prevails on the

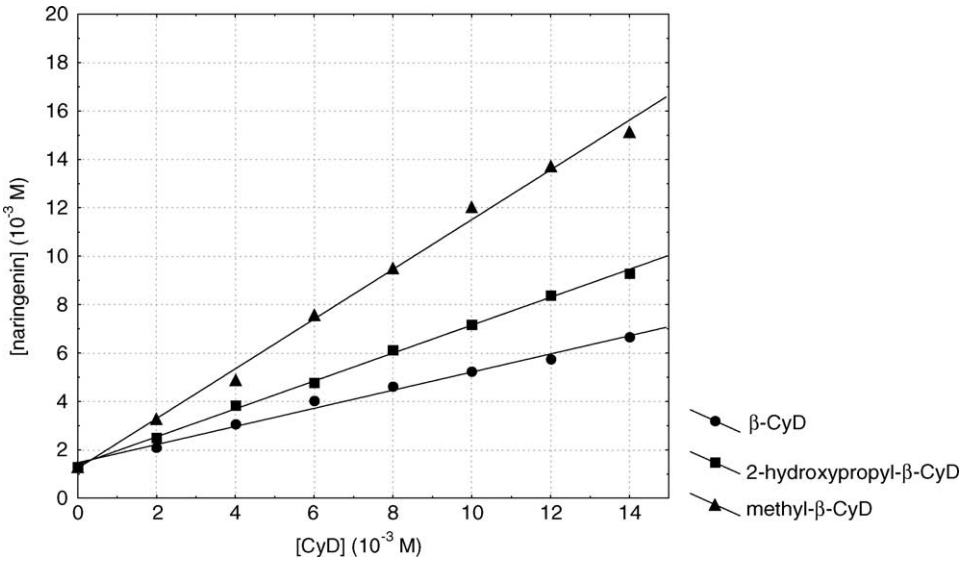

Fig. 3. Phase solubility study of naringenin with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin at pH 8.0.

Table 1
Formation constants for unionized (K_u) and ionized (K_i) naringenin

Cyclodextrin (CyD)	K_u (M^{-1})	K_i (M^{-1})
β-CyD	379.01	19.23
OH-propyl-β-CyD	832.82	44.16
Methyl-β-CyD	999.15	356.03

$pK_a = 6.7$.

Table 2
Micellar parameters indicating interactions of naringenin with polysorbates 20 and 60

Parameter	Polysorbate 20	Polysorbate 60
WSR (g/g)	0.67	1.08
MSR (mol/mol)	0.16	0.22
k_m (M^{-1})	595.59	826.47

negative contribution of the lower stability of ionized complexes, thus leading to such an increase in the solubility of naringenin.

The apparent formation constant, K_u, for the neutral substance can be calculated using Eq. (1), while the total solubility of naringenin dependent on the pH is described by Eq. (2), the K_i for the ionized form can be calculated from Table 1.

Moreover, the effect of some non-ionic surfactants on naringenin is studied by three different experimental conditions. The equilibrium solubility of naringenin increases linearly with increasing concentrations of surfactant due to a larger number of micelles in solution depending on the concentration of surfactant (Fig. 4). When surfactant is above the CMC, polar and non-polar solutes are partitioned between the polar bulk aqueous phase and the non-polar portions of the micelle, and the solubilization is due to the interactions within the hydrophobic core of the micelles. The degree of solubilization of naringenin is calculated by Eqs. (3) and (4), and is 0.67 g/g for polysorbate 20 and 1.08 g/g for polysorbate 60 (Table 2). As CMC for polysorbates 20 and 60 is really a small value relating to the concentrations here used, the terms S_{cmc} and CMC itself can be approximated to 0, as $S_{tot} \gg S_{cmc}$ and $C_{surf} \gg$ CMC.

Micellar partition coefficients, k_m, of naringenin in micelles of the polysorbates are calculated by Eq. (5), giving the measure of the interaction degree of the flavanone with the surfactants, and are shown in Table 2. Moreover, it is worthy noting that polysorbate 60 has a lower HLB than polysorbate 20 [23], and as it is the most effective as solubilizing agent, this shows that also HLB is an important parameter and suggests that the hydrocarbon chain, i.e. the micellar core plays the most important role in the solubilization of naringenin besides surfactants with low HLB tend to form larger micelles that can physically contain a greater number of molecules of naringenin.

In the second approach, when β-CyD is added to the micellar systems, in a constant concentration, an increase of apparent solubility of naringenin is evident. Such a behaviour puts on evidence that polysorbates do not compete with naringenin for the cavity of β-CyD; on the contrary the solubility of naringenin would have decreased. Therefore, the contribution of the two solubilizers could be addictive or synergic. Fig. 4 shows that the total solubility of naringenin increases linearly with polysorbate concentration, in a greater extent by also adding β-CyD; for both the polysorbates the slopes are similar, that points out an addictive effect. In this case, total concentration of naringenin is given by Eq. (6):

$$[S_{tot}] = [S_0] + K_u[S_0][CyD_{tot}] + k_m[S_0][C_{surf}] \quad (6)$$

In the third approach, when polysorbate concentration is maintained constant, at 0.020 M, the prevalent solubilizer effect of the surfactant is evident. At first, in absence of

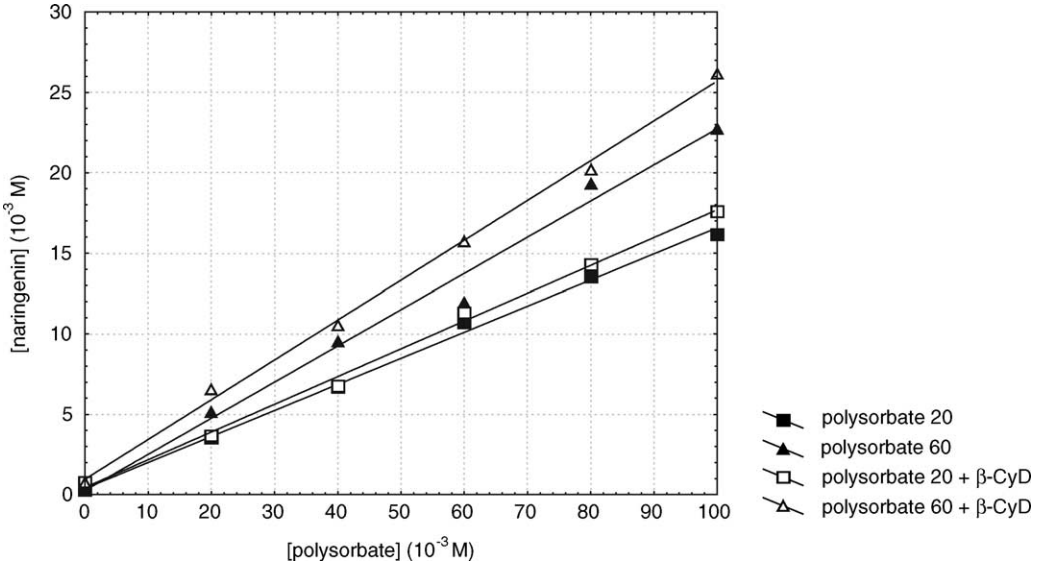

Fig. 4. Solubility plot of naringenin given by polysorbates in absence or with β-cyclodextrin.

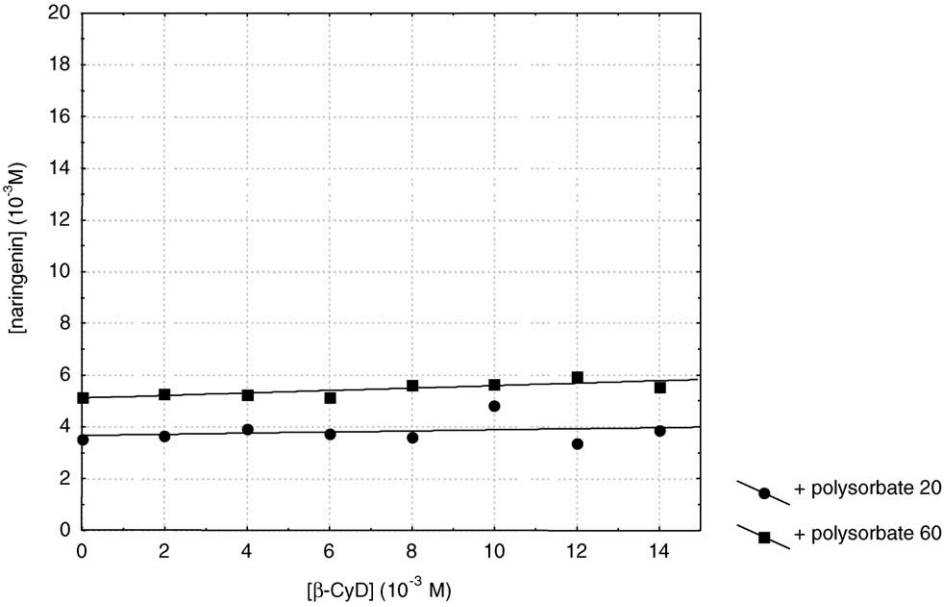

Fig. 5. Solubility plot of naringenin given by β-cyclodextrin with polysorbates 20 and 60.

β-CyD, as the concentration of naringenin goes from 0.08 to 4.40 and 6.19 mg/ml with polysorbates 20 and 60, respectively. Then, when increasing concentration of β-CyD are added, just a light increase in solubility of the naringenin to 4.79 and 7.13 mg/ml is observed (Fig. 5).

5. Conclusions

This solubility study puts on evidence that the combination of pH variation and complexation with β-CyDs allows to increase the naringenin solubility, and this can be useful for producing stable formulations for weakly ionizable drugs.

The solubility of naringenin increases linearly as a function of β-CyDs concentration and evidences the positive effects of the derivations as to parent β-CyD.

The differences between values obtained for apparent formation constants of the unionized and the ionized forms of naringenin indicate that the complexation degree is greater when the drug is in the neutral state, while ionized naringenin shows lower complexation degree due to its more hydrophilic characteristics. On the other side, this last one is more soluble in basic medium; so any pH variation that increases the concentration of a free ionized drug will also increase the concentration of ionized drug complexed.

The study of combination of two commonly used solubilizers such as β-CyD and polysorbates evidences that there is no competition of polysorbates with naringenin for the cavity of β-CyD. Further, both β-CyD and polysorbates 20 and 60 operate an additive effect to take naringenin in solution, with a prevalent solubilizing contribution of the surfactant as to the β-CyD.

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