



Spectrophotometric and electrochemical study of the inclusion complex between β -cyclodextrin and furnidipine

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Abstract

Inclusion complexation between furnidipine (2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-tetrahydrofurfuryl 5-methyl diester), a calcium-channel antagonist, and β -cyclodextrin (β -CyD) was studied in aqueous solution by using both spectrophotometric and electrochemical measurements.

The phase solubility profile was classified as A_L -type, indicating the formation of 1:1 stoichiometric inclusion complex of furnidipine with β -CyD. Based on the spectrophotometric absorbance's variations, a formation constant value, K_f , of 156 M^{-1} was determined.

Electrochemical measurements using chronocoulometric experiments were used for the determination of the diffusion coefficients. In absence of β -CyD, a diffusion coefficient value of $4.32 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ was obtained for furnidipine. The addition of β -CyD produced a decrease of 30% for the diffusion coefficient.

Formation of inclusion complexes of furnidipine with β -CyD was proved to increase more than three times the solubility of furnidipine.

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1. Introduction

Cyclodextrins (CyDs) are cyclic oligomers of six, seven or eight linked α -D-glucopyranose units, denoted α -, β - and γ -CyDs, respectively. These CyDs compounds are well known to form inclusion complexes with a variety of organic and inorganic compounds. This ability is based on the capability of

CyDs to provide a hydrophobic cavity in aqueous solution for both the hydrophobic guest molecule or moieties in the guest molecule [1]. Studies involving inclusion of solute into CyDs are important due to the resulting improvement of aqueous solubility, stability against chemical and photochemical degradation and to the possibility of controlled drug release, which presents many potential applications in drug formulations.

1,4-Dihydropyridines (1,4-DHP) calcium antagonist drugs are a therapeutic class widely used in different cardiovascular conditions such as hypertension

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and angina pectoris [2,3]. The pharmacological activity depends on both, the integrity of the chemical structure and the stereochemistry, which must be retained to develop the optimal interaction with the receptor and thus gets the pharmacological and therapeutic efficacy. 1,4-DHP drugs undergo important chemical changes as a consequence to light exposure. Consequently, this photodecomposition process produce alterations in their activities or potencies and the loss of therapeutic activity. Specifically, there are a lot of studies pointing out the light lability of 1,4-DHP [4–13]. In general terms the chemical modifications as a consequence of the photodecomposition process involve the reduction of the aromatic nitro group to a nitroso group and/or the oxidation of the 1,4-DHP ring to a pyridine ring. Both of these changes involve a lost of the pharmacological effect. One approach in order to improve the photodecomposition resistance of the 1,4-DHP derivatives is the preparation of inclusion complexes with CyD. In fact, there are some studies showing that photochemical stability of 1,4-DHP was increased by the formation of inclusion complexes with CyD [14–16].

Furnidipine, 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-tetrahydrofurfuryl 5-methyl diester (Fig. 1), is a relatively new 1,4-DHP derivative that shares with nifedipine the cardiovascular properties and a chemical structure having the nitro substituent in *ortho*-position of the aromatic ring. As was recently proved, [17] the *ortho*-nitro substitution in 4-nitroaromatic substituted 1,4-DHP produces compounds with high susceptibility to light exposure, consequently furnidipine is highly light sensitive. The study of the photodecomposition of furnidipine revealed [18] that direct exposure to different light conditions produced photodegradation being nitro pyridine and nitroso pyridine derivatives identified as the mainly photodegradation products. Consequently an adequate strategy to avoid the photodecomposition of furnidipine could be the formation of inclusion complex with CyD.

In this scope the aim of this work was the feasibility of formation of an inclusion complex between furnidipine and CyD. Furthermore, the determination of some physico-chemical characteristics as the formation constant and the diffusion coefficient will be carried out by spectroscopic and electrochemical methods.

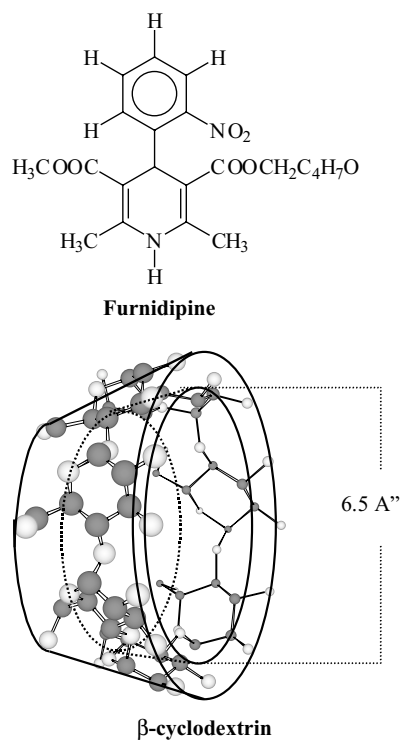


Fig. 1. Molecular structures of furnidipine and β-cyclodextrin.

2. Experimental

2.1. Reagents and solutions

β-Cyclodextrin (β-CyD) was obtained from Fluka and was used without prior purification. Furnidipine was supplied by Chile Laboratory (Santiago, Chile). All the other reagents employed were of analytical grade.

In order to avoid photodecomposition, all solutions containing furnidipine were thoroughly protected from light working in amber vials or by wrapping the vials with aluminum foil.

All the polarographic experiments were obtained after bubble with N_2 by 10 min in the cell before each run. Temperature was kept constant at 25 ± 0.1 °C in all experiments.

2.2. Apparatus

Spectrophotometric measurements were carried out with an ATI Unicam Model UV3, UV-Vis spectrophotometer, using 1 cm quartz cell.

Polarographic and chronocoulometry experiments were carried out using a totally automated BAS-100 voltammetric analyzer attached to a PC computer with proper software for total control of the experiments and data acquisition and treatment. A GCME polarographic stand with a dropping mercury electrode (DME) as working electrode, a platinum wire counter electrode, and a Ag/AgCl as reference electrode were used for the measurements. Chronocoulometric experiments were carried out using glassy carbon as working electrode.

2.3. Methods

Solubility diagrams were obtained according to Higuchi and Connors [19]. Briefly, excess amounts of solid furnidipine (20 mg) were added to 10 ml of 0.1 M Britton–Robinson buffer of pH 7.0 containing various concentrations of β -CyD (0–15 mM). Samples were shaken for 24 h at 37 °C. Then, samples were kept in store for 4 days at 4 °C, and then filtered through a 0.45 μ m membrane filter (Advantec MFS, Inc.). Furnidipine concentration in the filtrate was spectrophotometrically analyzed at 340 nm. The formation constant, K_f , was calculated from the phase solubility diagrams according to the equation:

$$K_f = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the solubility of furnidipine in the absence of β -CyD and the slope means the corresponding slope of the phase solubility diagrams, i.e., the slope of the drug concentration versus CyD concentration graph. The solubility of furnidipine was obtained using the molar absorptivity obtained from a calibration curve obtained in the same experimental conditions.

Polarographic experiments were carried out by keeping constant concentration 1×10^{-5} M of furnidipine in 0.1 M Britton–Robinson (pH 7.0) while varying concentrations of β -CyD (0–15 mM).

Chronocoulometric measurements were performed for 0.11 mM of furnidipine in 30/70:ethanol/Britton–Robinson 0.1 M (pH 7.0) containing various concentrations of β -CyD (0–15 mM).

3. Results and discussion

3.1. Determination of the formation constant of furnidipine β -cyclodextrin complexes by spectrophotometric studies

Fig. 2 shows the effect of adding increasing amounts of β -CyD on the UV-Vis spectrum of furnidipine in 0.1 M Britton–Robinson buffer, pH 7. The concentration of β -CyD was varied in the range 0–15 mM. The spectra indicate an increase in the intensity of the band at 336 nm but no appreciable shift in λ_{max} . The observed increase in the intensity of the absorption band at 336 nm can be ascribed as an increase in the molar absorptivity of furnidipine due to an enhanced solubility obtained as a consequence of the β -CyD addition. The experiments of Fig. 2 clearly showed that the solubility of the drug increased as a function of the β -CyD concentration. In absence of β -CyD, the solubility of furnidipine is $6.2 \mu\text{g ml}^{-1}$, while in presence of β -CyD, the solubility increased to $21 \mu\text{g ml}^{-1}$. The phase solubility profile (Fig. 3) obtained for furnidipine/ β -CyD followed a behavior

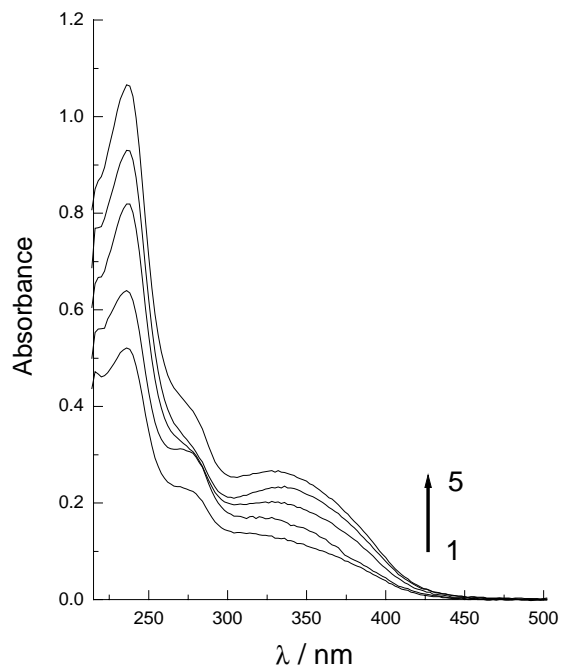


Fig. 2. Absorption spectra of furnidipine in absence (1) and presence of (2) 4, (3) 6, (4) 8 (5) 12 mM of β -CyD.

Table 1

Some published formation constants (K_f) for some nitroaromatic compounds complexed with β -cyclodextrin

Compound	K_f (M^{-1})	Method	Reference
Nifedipine	121.9	UV-Vis spectrophotometry	[22]
Nicardipine	49.26	UV-Vis spectrophotometry	[23]
3-Nitrophenol	274	Calorimetry	[24]
4-nitrophenol	350	Calorimetry	[24]
4-Nitrophenol	302	UV-Vis spectrophotometry	[25]
4-Nitrophenolate	933	UV-Vis spectrophotometry	[25]

that fits very well with an A_L -type system [19]. This kind of curves are indicative of the formation of soluble 1:1 type inclusion complex and the corresponding formation constant (K_f) can be calculated by using the above Eq. (1). We have obtained a K_f value of $156 M^{-1}$ for the inclusion complex between furnidipine and β -CyD, suggesting a relatively stable complex. The obtained K_f value was closed to other published values for other related 4-nitroaryl substituted 1,4-dihydropyridine compounds such as nifedipine and nicardipine. Furthermore, the obtained K_f value was related to other nitroaromatic compounds such as nitrophenols (Table 1).

3.2. Electrochemical results

The electrochemical reduction of furnidipine on mercury in aqueous ethanol solutions has been previously studied [20]. According to that study, furni-

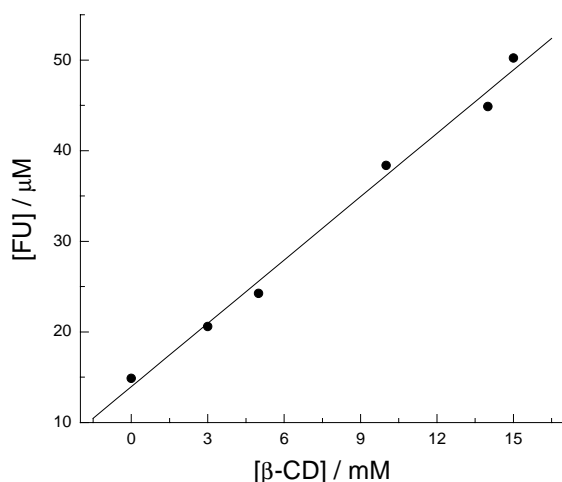


Fig. 3. Phase solubility diagrams for furnidipine with increasing concentrations of β -CyD.

dipine is susceptible to be reduced on the mercury electrode due to the four-electron and four-proton irreversible reduction of the nitroaromatic group to yield the hydroxylamine derivative according to the following overall reaction:



As can be seen in Fig. 4, furnidipine shows a very well resolved polarographic peak, with a peak potential at -600 mV, when was submitted to a differential pulse polarography experiment in aqueous media at pH 7. The addition of β -CyD to a solution of

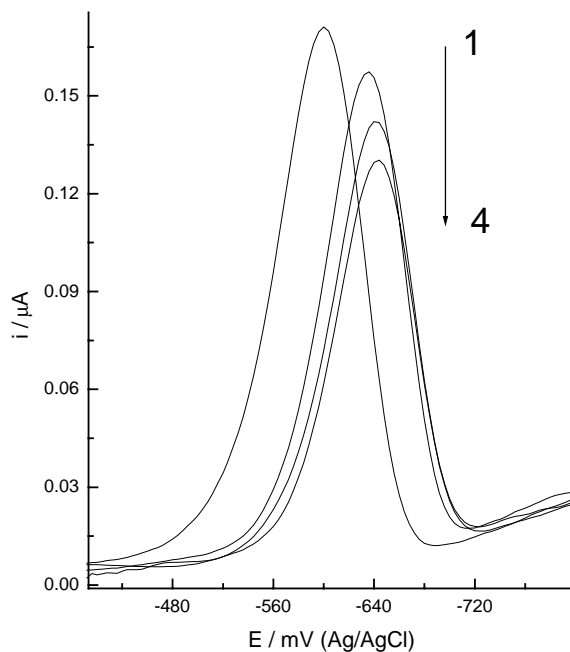


Fig. 4. DPP of 1×10^{-5} M of furnidipine in 0.1 M Britton–Robinson (pH 7.0) in absence (1) and presence of (2) 3, (3) 5, (4) 7 mM β CD. Conditions: scan rate 4 mV s^{-1} , pulse amplitude 50 mV, pulse width 50 ms.

furnidipine causes two main changes in the polarograms. Firstly, the cathodic peak potential (E_{PC}) shifted in a negative direction and secondly, the I_{PC} decreased. These results can be ascribed to the formation of the inclusion complexes according to the equilibrium:



wherein FU means furnidipine and FU/CyD means the inclusion complex between furnidipine and β -CyD.

The change in the E_{PC} reveals that furnidipine molecules were reduced with more difficulty, when they were included in the inclusion complex. Probably this enhanced difficulty is due to that the electroactive nitro group is located inside the cavity of β -CyD hindering the interaction with the electrode. As furnidipine molecule have a clear hydrophobic site, i.e., the nitroaromatic group, is highly probable that the hydrophobic microenvironment from β -CyD would be a very good place for this moiety. On the other hand, the decrease of the peak current can be ascribed as a diminution of the diffusion current due to a diminution of the apparent diffusion coefficient of the furnidipine included in the complex with β -CyD, when compared with the apparent diffusion coefficient of furnidipine alone. This difference between apparent diffusion coefficients was confirmed by electrochemical measurements. In fact, the chronocoulometric method is a very good alternative for the determination of the diffusion coefficients of furnidipine and its inclusion complex. In Fig. 5, different chronocoulometric curves showing the effect of add different quantities of β -CyD to a solution containing furnidipine is showed. From these results we can observe a notorious dependence of the charge–time relationship as a consequence of the addition of β -CyD. According to the theory [21] the chronocoulometric curves obey the following equation:

$$Q = \frac{2nFAC(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \quad (4)$$

wherein Q_{dl} is the double-layer charge (integration of charging current), Q_{ads} is the Faradaic component given by the reduction of adsorbed species. Therefore, from the slope of the linear plots obtained between Q and $t^{1/2}$ the diffusion coefficient can be determined. Specifically, in the case of furnidipine in the absence of β -CyD, a diffusion coefficient value

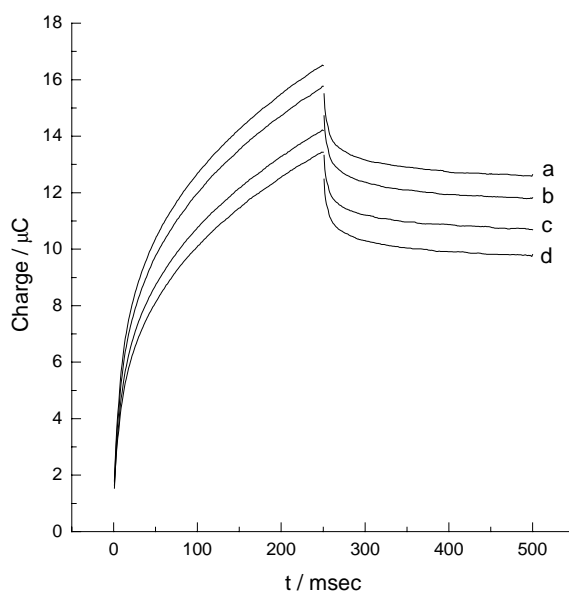


Fig. 5. Chronocoulometric response for 0.11 mM of furnidipine in ethanol/Britton–Robinson 0.1 M (pH 7.0):30/70, in absence (a) and presence of (b) 4, (c) 10, (d) 15 mM β -CyD.

Table 2

Diffusion coefficients of furnidipine inclusion complexes with β -cyclodextrin

β -CyD (mM)	Diffusion coefficient ($\times 10^6 \text{ cm}^2 \text{ s}^{-1}$)
0	4.3 ± 0.1
4	3.8 ± 0.1
10	3.3 ± 0.1
15	3.1 ± 0.1

of $D = 4.32 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ was determined. As the drug is incorporated into the cavity, its diffusion coefficient is reduced due to the larger complex formed. Therefore, the observed decrease in furnidipine diffusion confirms the drug incorporation as the β -CyD concentration increases. Table 2 shows the diffusion coefficients in presence of different concentration of β -CyD.

4. Conclusions

From the above results it may be concluded that β -CyD forms 1:1 type inclusion complexes with furnidipine and the obtained formation constant was 156 M^{-1} .

The effect of β -CyD on the polarographic behavior of furnidipine can be summarized by both a negative shift in the cathodic peak potential and a peak current decrease. From these changes we can assume that the nitroaromatic group on the furnidipine molecule was located inside the cavity of β -CyD and a diminution of the diffusion coefficient of furnidipine as a consequence of the formation of an inclusion complex with β -CyD.

The new finding that furnidipine forms inclusion complexes with β -CyD would permit establishing the formation of an inclusion complex as a good alternative to increase the solubility.

A further challenge in this topic will be to prove that complexation of furnidipine with β -CyD hinder the photodecomposition of the drug such as has been previously proved for other related 1,4-DHP derivatives [14–16].

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