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Determination of stability constant values of flurbiprofen–cyclodextrin complexes using different techniques

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Abstract

Three new experimental approaches for calculating the stability constant (K_{st}) of complexes of flurbiprofen with natural β-cyclodextrin (βCyd) and the hydroxyethyl- (HEβCyd) and the methyl- (MeβCyd) derivatives were tested and compared to the classic phase-solubility procedure: (a) the membrane permeation technique through a lipophilic synthetic membrane permeable to the drug but not to the Cyd molecules, by analysing the permeation profiles with a non-linear least-squares method; (b) the affinity capillary electrophoresis (ACE) technique, where K_{st} were calculated from the relationship between Cyd concentration in solution and drug electrophoretic mobility, using three different linear plotting methods; (c) the molecular modeling technique, based on the relationship between the docking energies and the experimental K_{st} values. The study allowed evaluation of the advantages and limits of each examined method, providing a useful guide for the choice of the most suitable one depending on the kind of host-guest system to be investigated. The K_{st} values obtained with the various techniques were rather different, probably due to the very different experimental conditions required by each one. However, all the methods indicated the methyl-derivative as the most powerful complexing agent for the drug, showing the general trend: K_{st}(MeβCyd) » K_{st}(HEβCyd) > K_{st}(βCyd). Only in the case of the ACE method was an inversion of the trend found between HEβCyd and βCyd; this was probably due to the lower molecular weight of the natural Cyd, which, in this case, became more important in determining the complex electrophoretic mobility than the different affinity of the drug for these two Cyds.

Keywords: Flurbiprofen; Cyclodextrin complexes; Stability constant; Membrane permeation; Affinity capillary electrophoresis; Molecular modeling; Phase-solubility analysis

1. Introduction

Cyclodextrin are well-known cyclic oligomers composed of several D-glucose units bonded by α(1,4) linkages providing an hydrophobic internal cavity able to include various drug molecules, thus forming non-covalent inclusion complexes [1]. Cyclodextrin complexation has been widely used in the pharmaceutical field to improve properties of drugs, such as solubility, dissolution rate, chemical and physical stability and, as consequence, bioavailability, as well as to reduce their irritancy and toxicity [2,3]. The fit of the entire or at least a part of the guest molecule in the cyclodextrin host cavity determines the stability of the inclusion complex and the selectivity of the complexation process. Therefore, the stability constant value of drug–cyclodextrin complexes is a useful index of the binding strength of the complex and is of great importance for the understanding and evaluation of the inclusion complex formation [4]. It is important to accurately determine this parameter, in order to predict changes in the physico-chemical properties of the drug after inclusion in the cyclodextrin cavity and to select the most suitable cyclodextrin-host molecule for a given drug-guest, so that inclusion complexation may be successfully exploited at its best.

Much attention has recently been directed to the development of new rapid and reliable methods for determining this parameter, such as microcalorimetric and chromatographic techniques [5,6], electrophoretic [7–9] and potentiometric methods [10], membrane permeation [11,12] or molecular

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modeling techniques [13,14], as an alternative to the conventional time- and material-consuming phase-solubility method [15].

In the present work, we thought it worthy of interest to test and compare some of these new experimental approaches for calculating the stability constant of complexes of flurbiprofen with both the natural β-cyclodextrin and two amorphous β-cyclodextrin-derivatives and to evaluate their actual effectiveness as alternative methods to the classic phase-solubility one. Flurbiprofen, [2-(2-fluoro-4-biphenyl) propionic acid], a poorly water-soluble anti-inflammatory drug, was selected as guest compound since it was shown that inclusion complexation with cyclodextrins can significantly improve its dissolution properties and reduce its irritative gastric effects [16–18].

The selected methods for the determination of stability constant of flurbiprofen–cyclodextrin complexes were the membrane permeation [11,12], the affinity capillary electrophoresis (ACE) [7–9] and the molecular modeling [13,14] techniques. The first was selected due to the possibility of gaining simultaneously useful information about the permeability properties of free and complexed drug. The ACE method was chosen in virtue of the simple requirements needed for its applicability for binding constant calculation [19], including in particular time- and material-savings. Finally, the molecular modeling technique was utilised because it also enables to obtain the possible geometrical structures of the inclusion complexes. Classic phase-solubility studies according to Higuchi and Connors [15] were performed as reference technique.

2. Materials and methods

2.1. Materials

Flurbiprofen (FLU) was kindly provided by Montefarmaco (Milan, Italy). β-Cyclodextrin (βCyd) was a gift from Roquette Italia S.p.A. (Cassano Spinola, Alessandria, Italy), methyl-β-cyclodextrin (MeβCyd) and hydroxyethyl-β-cyclodextrin (HEβCyd) with an average substitution degree per anhydroglucose unit DS 1.8 and MS 1.6, respectively, were kindly donated by Wacker-Chemie GmbH (Münich, Germany). Phosphoric acid and dimethylsulfoxide (DMSO) were from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

2.2. Phase-solubility studies

Excess amounts of FLU were added to 10 mL of unbuffered (pH ≈ 4.5) or buffered (pH 5.5) aqueous solutions containing increasing concentrations of Cyd in the 0–25 mM range (MeβCyd and HEβCyd) or in the 0–13 mM range (βCyd) in sealed glass vials. The obtained suspensions were electromagnetically stirred (500 rpm) at constant temperature (25 ± 0.5 or $37 \pm 0.5\,^{\circ}C$) until equilibrium (72 h). Aliquots were then withdrawn, filtered (pore size 0.45 μm) and spectrometrically assayed for drug concentration at 246 nm (Shimatzu UV-1601). The apparent 1:1 stability constants were calculated from the straight line portion of the phase-solubility diagrams, according to Higuchi and Connors [15]. Each test was repeated at least three times (coefficient of variation C.V. < 3%).

2.3. Membrane permeation studies

Permeation rate studies of drug or drug–Cyd complexes were carried out using a Sartorius Mod. SM16750 absorption simulator (Sartorius Membranfilter GmbH, Gottingen, Germany). The donor and acceptor phases were separated by a membrane of cellulose nitrate (thickness 130 μm; pore diameter 0.025 μm; effective permeation area 40 cm^2) impregnated with lauryl alcohol [20] (membrane weight increase 100–110%). The lipophilic membrane was permeable to the drug but impermeable to the Cyd; therefore, only free drug molecules could transfer into the acceptor phase. The donor compartment consisted of 100 mL of water containing or not 25 mM Cyd to which 6 mg drug was added (0.25 mM, i.e. its saturation solubility under the selected experimental conditions). The same volume of water was placed in the acceptor compartment. Both solutions were thermostated at $37 \pm 0.5\,^{\circ}C$ and magnetically stirred (100 rpm). The cumulative amount of drug diffused as a function of time was determined by spectrometrically assaying (as in Section 2.2) its concentration in the acceptor compartment at fixed time intervals. Each experiment was performed at least three times and the results were averaged (C.V. < 5%). The data obtained were elaborated using a non-linear least-squares method of the EASY-OPT program [21] for the calculation of the stability complex constant.

2.4. Affinity capillary electrophoresis (ACE)

Standard stock solutions of FLU (1 mg/mL) and DMSO (1%, v/v) used as electro-osmotic flow (EOF) marker, were prepared in methanol and water, respectively, stored at $4\,^{\circ}C$ and used within one week. Working standard solutions were prepared daily by adding 25 μL of each of the stock solutions directly in a vial and diluting to 500 μL with 5 mM pH 5.5 phosphate buffer, in order to obtain the final desired concentrations for FLU (0.05 mg/mL) and for DMSO (0.05%, v/v).

An Agilent Technologies ^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a UV-visible diode array detector (DAD) and an air thermostating system was used to perform ACE experiments. Data acquisition and signal processing were performed using ^{3D}CE ChemStation software (Rev. A.09.01, Agilent Technologies, Waldbronn, Germany). Experiments were performed on a 33 cm long (24.5 cm effective length) untreated fused silica capillary with an inner diameter of 50 μm and an outer diameter of 375 μm (Composite Metal Services, Hallow, UK) with a

detection window built-in by burning off the polyimide coating on the capillary. Detection wavelength was set at 195 nm. Samples were injected using the hydrodynamic mode by applying a 20 mbar pressure for 3 s, followed by a background electrolyte (BGE) plug (20 mbar, 3 s). A constant voltage of 20 or 25 kV (rise time 0.20 min) was applied with the anode at the inlet and the cathode at the outlet side, holding constant temperature at 37 or 25 °C, respectively. The standard BGE consisted of an aqueous solution of 10 mM phosphoric acid, adjusted to pH 5.5 with 1 M NaOH, containing the appropriate concentration of Cyd (0, 0.5, 1–5 mM).

Before use, a new capillary was flushed with 1 M NaOH and water for 5 min each. Between individual runs, the capillary was rinsed with water (1 min), 1 M NaOH (1 min), 0.1 M NaOH (1 min), water (1 min) and run buffer (6 min). To improve repeatability of migration times and electrophoretic mobilities, buffer vials were replenished after each injection. Each sample was injected in triplicate, apart from the experiments necessary to determine the mobility of the free solute, which were collected in quadruplicate after achieving constancy of the electrophoretic mobility. The measured currents did not exceed 15 μA.

Experimental values of the effective electrophoretic mobilities ($cm^2\ V^{-1}\ s^{-1}$) were calculated as the difference between the apparent mobility in each buffer (with various quantities of Cyds) and that of the neutral marker. The stability constants were calculated using the double reciprocal, the *x* reciprocal and the *y* reciprocal plotting methods with least squares linear regression [9].

2.5. Molecular modeling studies

Modeling of the structures of Cyds and drug, as well as of their complexes, was carried out using the INSIGHT II 2000 program run on Silicon Graphics O2 R10000. The FLU molecule was obtained by the Builder Modules of the INSIGHT II program. The molecular structure of βCyd was generated on the basis of crystallographic parameters provided by the Structural Data Base System of the Cambridge Crystallographic Data Centre. The βCyd-derivatives were built up by adding to the base molecule of βCyd, respectively, 12 methyl (DS 1.8) or 11 hydroxyethyl (MS 1.6) groups. The docking energies of the host-guest inclusion complexes were calculated through the CFF91 Force Field (Discovery Module) opportunely modified [14]. For each complex, the search for the most stable conformation with the best docking energy was performed through stochastic methods based on a molecular dynamic simulated procedure [14].

3. Results and discussion

3.1. Phase-solubility studies

First, the complexing behaviour of FLU with the three examined Cyds was studied by the phase-solubility method, used as reference for the alternative techniques tested. In both media (water and pH 5.5 phosphate buffer) and at both temperatures (25 and 37 °C), the phase-solubility diagrams obtained with increasing βCyd concentration were of B_S type [15], because of the precipitation of an insoluble complex at high carrier concentration (Fig. 1). On the contrary, in the case of both βCyd-derivatives, the diagrams were of A_L type [15], indicative of the formation of soluble complexes of presumable 1:1 stoichiometry.

The apparent 1:1 stability constants, calculated from the straight-line portion of the diagrams at each temperature, are collected in Table 1. The decrease in stability constants, observed in all cases with increasing temperature, indicated the exothermic nature of inclusion complexation. The higher stability constant values of FLU–Cyd complexes observed in the aqueous solution, compared to the pH 5.5 buffered one, were explained with the lower pH values of the unbuffered aqueous solution ($pH \approx 4.5$) in the presence of the acidic drug, i.e. with the higher percent of FLU in the unionized, more lipophilic form, which has a greater affinity for the inclusion complexation [22]. This result was also in agreement with the lower drug solubility in the unbuffered water solution (Fig. 1).

Both βCyd-derivatives were more effective than the natural one, due to their higher water solubility; however, MeβCyd clearly showed greater solubilizing efficiency and complexing power towards the drug. The better performance of this derivative has been attributed to the presence of the methyl groups that increased the hydrofobic region of the macro molecule by capping the edge of the cavity and expanding the location of substrate binding, without causing any structural hindrance to the drug inclusion [23]. On the contrary, some effect of steric blocking, due to the presence of the hydroxyethyl-substituents, which can hamper the inclusion of the guest molecules, could explain the less complexing effectiveness exhibited by HEβCyd.

3.2. Membrane permeation studies

The first alternative method tested in the present work was the permeation through a membrane permeable to the drug but not to Cyds [11,12]. The use of such technique for determining the stability constant of drug–Cyd complexes is based on the fact that the drug permeation rate in the presence of Cyds is dependent on both the Cyd concentration and the time of the permeation, owing to the continuous change of the inclusion equilibrium in the donor phase. In fact, the concentration of the drug in the donor phase decreases because it permeates into the acceptor cell, whereas the Cyd concentration remains constant. Therefore, since only the free drug can pass through the membrane, the permeation rate is related to the stability constant of the complex. The relationship between the drug permeation rate and the stability of the

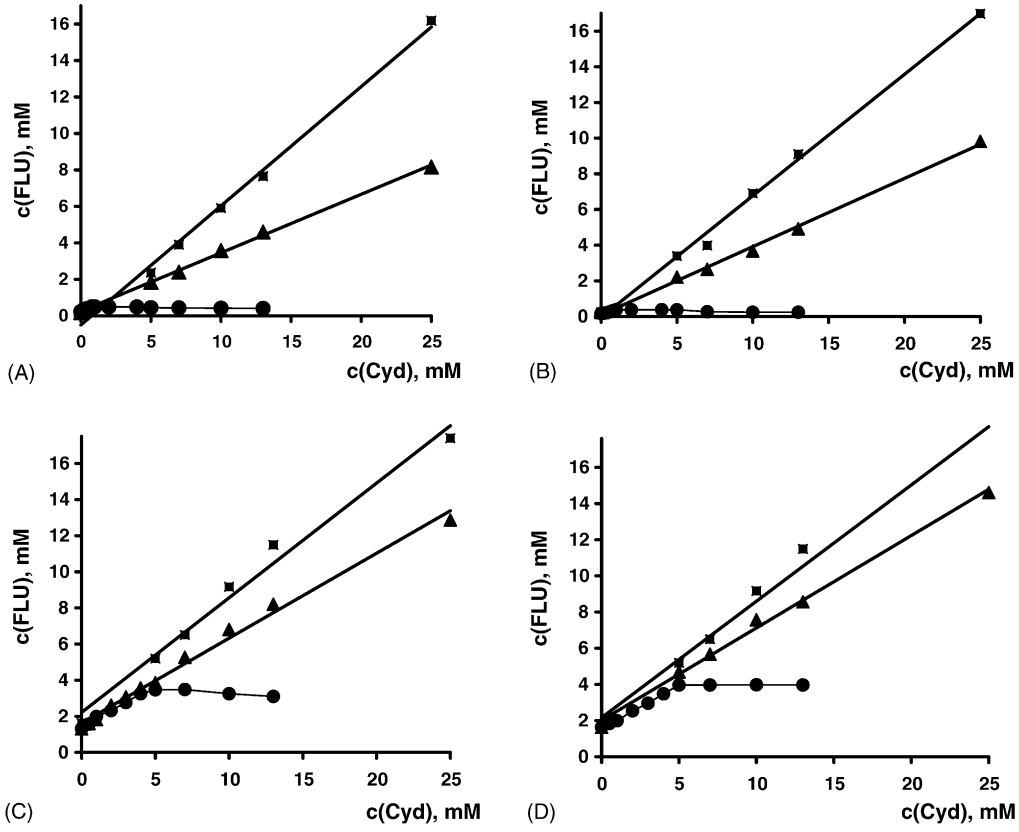

Fig. 1. Flurbiprofen phase-solubility diagrams obtained at 25 °C (left) and 37 °C (right) in aqueous solution (A and B) and pH 5.5 phosphate buffer (C and D) in the presence of βCyd (●), HEβCyd (▲) and MeβCyd (■).

complex with Cyd is given by the following equation [12]:

$$[D_A] = \frac{[D_0]\{1 - \exp(-At)\}}{(2 + K_{st}[\mathrm{Cyd}]_f)}$$

where $A = k(2 + K_{st}\,[\mathrm{Cyd}]_f)/(1 + K_{st}\,[\mathrm{Cyd}]_f)$, D_A is the drug concentration in the acceptor phase, D_0 the concentration in the donor phase at time 0, $[\mathrm{Cyd}]_f$ the concentration of free Cyd in the donor phase, k the drug permeation rate constant, K_{st} the complex stability constant, and t the time. In the adopted experimental conditions, $[\mathrm{Cyd}]_f$ can be considered constant and equal to the total Cyd concentration, being $[\mathrm{Cyd}]_{tot,} \gg [D_0]$ (100:1 molar ratio) [12]. Therefore, K_{st} and k can be calculated by analyzing the data of drug concentration in the acceptor phase as a function of time t, using a non-linear least-squares method of the Easy Opt program [21]. It was not possible to apply this technique to the complexes with βCyd, since its limited water solubility did not permit the high Cyd concentration required by the method to be reached.

Fig. 2 shows the permeation profiles of FLU in the absence or in the presence of the two βCyd-derivatives. The drug alone showed an almost linear permeation profile over time, after an initial lag time of about 15 min required to reach pseudo-steady-state conditions. On the contrary, in the presence of both Cyds, a particular biphasic trend was observed. Initially, the drug permeation rate was higher than that of FLU alone, indicating a favourable effect of the carrier on drug dissolution. However, after about 45 min, a decrease of the FLU permeation rate was observed, as a consequence of the effect of Cyd complexation: in fact, the concentration in the donor phase of free drug (the only one able to permeate through the membrane) decreased as a consequence of the complex formation. The greater the Cyd complexing efficiency, the less the drug passage through the membrane.

Table 1
Stability constants (K_{st}) of complexes of flurbiprofen with βCyd, HEβCyd and MeβCyd in unbuffered water (pH ≈ 4.5) and in pH 5.5 phosphate buffer at 25 and 37 °C obtained from phase-solubility studies

Cyd	K_{st} (M^{-1}) (water, 25 °C)	K_{st} (M^{-1}) (water, 37 °C)	K_{st} (M^{-1}) (phosphate buffer, 25 °C)	K_{st} (M^{-1}) (phosphate buffer, 37 °C)
βCyd	1700	1430	590	540
HEβCyd	2775	2660	680	640
MeβCyd	11570	9660	1480	1085

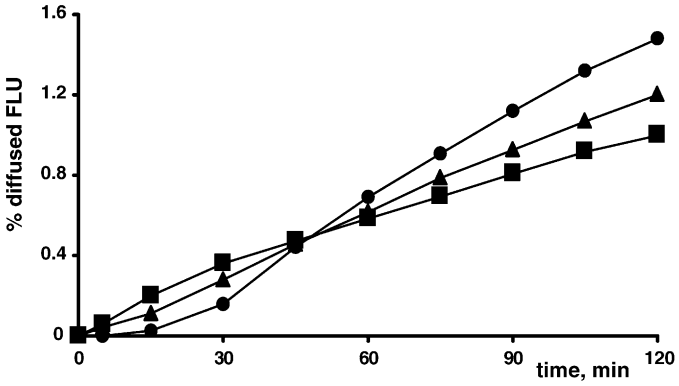

Fig. 2. Permeation profiles of FLU alone (●) and in presence of HEβCyd (▲) and MeβCyd (■) in water at 37 ± 0.5 °C.

The values of permeation rate (k) and complex stability (K_{st}) constants obtained by analyzing the drug permeation data according to the previous equation are listed in Table 2. The K_{st} values showed the same trend of those obtained by the conventional phase-solubility method in unbuffered water at the same temperature (Table 1). Moreover, in the presence of MeβCyd, the drug permeation rate was about 40-fold lower than that obtained in the presence of HEβCyd, thus further confirming the best complexing power of the methyl-derivative towards FLU. These results demonstrated the effectiveness of the membrane permeation technique as a useful alternative method to determine the stability constants of drug–Cyd complexes. Furthermore, with respect to the classic phase-solubility method, it has the additional advantages of requiring only one permeation experiment that lasts a few hours (and not a series of experiments at different Cyd concentrations lasting some days), and to simultaneously provide interesting information about the effect of the Cyd on the drug permeation rate. In this respect, it is important to underline that the observed decrease in drug permeation rate in the presence of Cyd is due to the particular experimental conditions used. In fact when the drug amount in the donor phase is higher than its saturation solubility, an increase of its permeation rate can be observed in the presence of Cyd, due to the very greater solubility and dissolution rate of the complex than the drug alone [16,18]. On the other hand, the main disadvantage of the method is the high Cyd concentration necessary in the donor phase, which prevents or hampers its applicability for poorly soluble or highly expensive Cyds.

Table 2
Flurbiprofen (FLU) permeation rate constants (k) and complex stability constants (K_{st}) of complexes with HEβCyd and MeβCyd determined in unbuffered water at 37 °C by the membrane permeation technique

Sample	k (h^{-1})	K_{st} (M^{-1})
FLU	2.60×10^{-3}	–
FLU-HEβCyd	2.30×10^{-3}	1170
FLU-MeβCyd	5.25×10^{-5}	2720

3.3. Affinity capillary electrophoresis (ACE)

ACE is defined as an electrophoretic separation where the separation patterns are influenced by reversible molecular binding interactions taking place during the separation process. The principle of ACE binding studies is based on the comparison of the solute electrophoretic mobilities in the ligand-free BGE or containing different concentrations of the ligand L [19]. The quantitative analysis of the equilibrium constant for reversible 1:1 solute-ligand complexes is based on the general form of a binding isotherm and on the effective electrophoretic mobilities [7,19]. The electrophoretic mobility of the solute in a BGE containing the ligand is the weighted average of the mobilities of the complexed (μ_c) and the uncomplexed (μ_f) solute:

$$\mu_i = \chi_f \mu_f + \chi_c \mu_c$$

where μ_i is the experimentally measured electrophoretic mobility, χ_c and χ_f the molar fractions of the complexed and uncomplexed solute, respectively.

Expressing the molar fractions in terms of equilibrium concentrations and taking into account the expression for the apparent equilibrium constant K, the following equation is obtained:

$$\mu_i = \frac{\mu_f + \mu_c K[L]}{1 + K[L]}$$

By rearranging this expression, three possibilities are available for linearization and lead to different plotting methods. In the double reciprocal plot, $1/(\mu_i - \mu_f)$ is plotted versus $1/[L]$ and the binding constant is obtained from the intercept/slope ratio. In the y reciprocal plot, $[L]/(\mu_i - \mu_f)$ is plotted versus [L] and K is equal to the slope/intercept ratio. Finally, in the x reciprocal plot of $(\mu_i - \mu_f)/[L]$ versus $(\mu_i - \mu_f)$, the binding constant is equal to −slope [7,9].

All the above-mentioned equations can be expressed in terms of migration times, using the relationship

$$(\mu_i - \mu_f) = \frac{lL}{V}\left(\frac{1}{t_i} - \frac{1}{t_f}\right),$$

where L and l are the total and the effective capillary length, respectively, V the voltage applied, and t_i and t_f the measured and free migration times of the analyte.

Since changes in peak migration times may be due to non-specific effects, such as changes in the electro-osmotic flow, a neutral non-interacting EOF marker should be present in the sample to adjust the analyte migration time [8]. In our case, DMSO was selected as EOF marker and the values of inclusion complex constants were calculated using a pH 5.5 phosphate buffer and setting temperature at 25 and 37 °C as in the previous phase-solubility studies. The concentration of the buffer was lowered to 10 mM to achieve rapid analysis times and avoid Joule effect. With the same aim, the voltage was fixed at 25 and 20 kV, when the temperature was 25 and 37 °C, respectively.

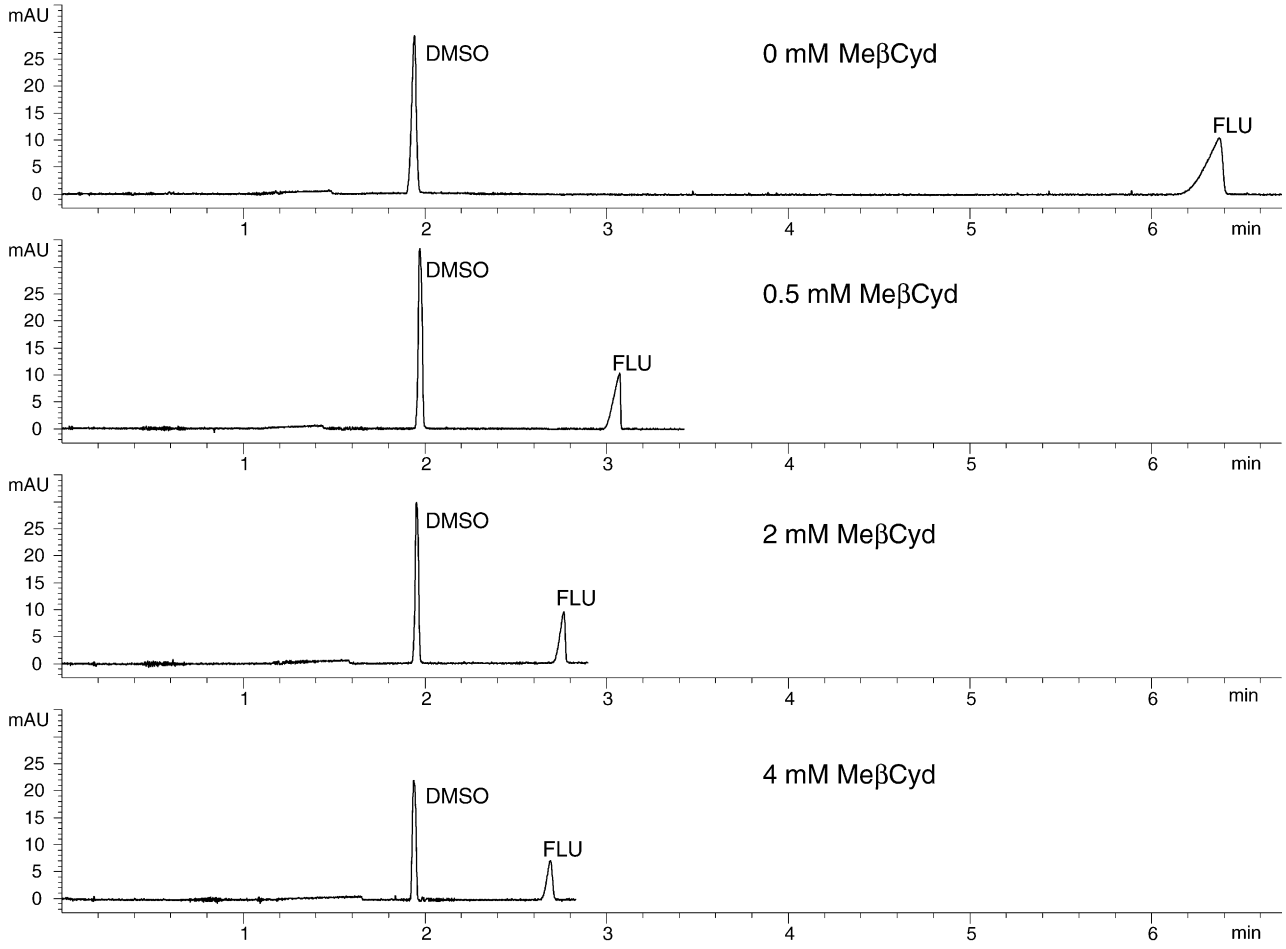

Fig. 3. Electropherograms of flurbiprofen (FLU) in pH 5.5 phosphate buffer at 37 °C in the presence of increasing concentrations of MeβCyd.

At first, the same range of Cyds concentrations was chosen corresponding to the linearity range found in the phase-solubility studies, namely 0–5 mM for βCyd and 0–25 mM for MeβCyd and HEβCyd. However, in the case of the derivatized Cyds, the K_{st} values obtained by the *y* reciprocal plot did not agree well with those obtained by the other plotting methods. Moreover, a deviation from the linearity for the double reciprocal and the *x* reciprocal plots could be noticed. Thus, the same range of concentrations was set for all the Cyds (0–5 mM) in order to avoid these effects.

Table 3
Stability constants (K_{st}) of complexes of flurbiprofen with MeβCyd, HEβCyd and βCyd obtained by affinity capillary electrophoresis in pH 5.5 phosphate buffer at 25 and 37 °C with the three different plotting methods

	25 °C				37 °C			
	a[a]	b[a]	R^2	K_{st} (M^{-1})	a[a]	b[a]	R^2	K_{st} (M^{-1})
MeβCyd								
Double reciprocal fit	0.783	7520	0.9910	9600	0.8857	6494.7	0.9927	7330
Y reciprocal fit	7506.9	0.8131	0.9999	9230	6491.3	0.8967	0.9999	7240
X reciprocal fit	−9514.7	1.2661	0.9890	9510	−7295.5	1.1238	0.9911	7300
HEβCyd								
Double reciprocal fit	1.9757	7442.4	0.9918	3770	1.8121	6201.1	0.9978	3420
Y reciprocal fit	7461.3	1.951	0.9999	3820	6153.6	1.9092	0.9999	3220
X reciprocal fit	−3778.1	0.5075	0.9891	3780	−3394.8	0.5482	0.9966	3390
βCyd								
Double reciprocal fit	1.3833	8262.4	0.9917	5970	1.7614	7328.5	0.9969	4160
Y reciprocal fit	8204	1.5021	0.9998	5460	7260.4	1.9054	0.9996	3810
X reciprocal fit	−5878.6	0.7127	0.9881	5880	−4115.5	0.5625	0.9938	4120

[a] Parameters of the regression equation: $y = ax + b$.

The FLU–Cyd anionic complex has a negative self-mobility which is lower, in absolute value, if compared to the one of the free anion, which corresponds to a decrease of the migration time with Cyd concentration. This is illustrated in Fig. 3, which shows the electropherograms of the samples containing FLU in the presence of increasing concentrations of MeβCyd in the buffer.

The linearity data and the values of K_{st} obtained are reported in Table 3. The values of K_{st} obtained from the different linear plotting methods were all consistent, assuring a reliable K_{st} estimation; moreover, the assumption of the 1:1 binding stoichiometry is supported by the good linearity of the plots (all the R^2 values were in the range from 0.9881 to 0.9999).

When comparing the binding constants of the different Cyds, MeβCyd was found to be undoubtedly the best complex forming compound, and the observed trend was $K_{st}(\text{Me}\beta\text{Cyd}) \gg K_{st}$ $(\beta\text{Cyd}) > K_{st}(\text{HE}\beta\text{Cyd})$. The trend observed with ACE achieved only a partial agreement with that of the phase-solubility studies: $K_{st}(\text{Me}\beta\text{Cyd}) \gg K_{st}(\text{HE}\beta\text{Cyd}) > K_{st}$ (βCyd).

This behaviour can be partially explained by the fact that, even though the binding with FLU imparts the same negative charge to the two complexes, the major determinant of electrophoretic mobility in CE is the charge/mass ratio. The complex with βCyd has a lower mass, thus likely resulting in a higher negative mobility (in absolute value) than the one with HEβCyd. Probably, the affinity of FLU for the two Cyds was not different enough, and the mentioned effect may become important and lead to an inversion of the trend between the apparent K_{st} values with the two Cyds.

This may represent a limit of the method, together with its applicability only for ionizable molecules; however, by contrast, short analysis times, high reproducibility of the results and minute amounts of sample necessary are its main advantages, and make it particularly suitable in the study of large cyclodextrins [24].

3.4. Molecular modeling

Molecular modeling studies were performed on the basis of the relationship between the docking energy of drug–Cyd complexes and their corresponding stability constants, previously demonstrated by some of us [13,14]. According to the CFF91 Force Field, the docking energies were calculated as the sum of the Van Der Waals and electrostatic interaction energies. However, some parameters of this force field were suitably modified, in order to more correctly describe the behavior of drug–Cyd complexes in aqueous solutions and maximise the correlation of the function relating docking energy and complex stability constant [14]. The previously developed theoretical model was then used to predict the stability constant of FLU–Cyd complexes.

The predicted K_{st} values were 650, 930 and 1495 M^{-1} for the FLU complexes with βCyd, HEβCyd and MeßCyd, respectively. A satisfying agreement with the experimental results obtained by the previous methods was found, revealing the same trend $K_{st}(\text{Me}\beta\text{Cyd}) \gg K_{st}(\text{HE}\beta\text{Cyd}) > K_{st}(\beta\text{Cyd})$ as in solubility and permeation studies.

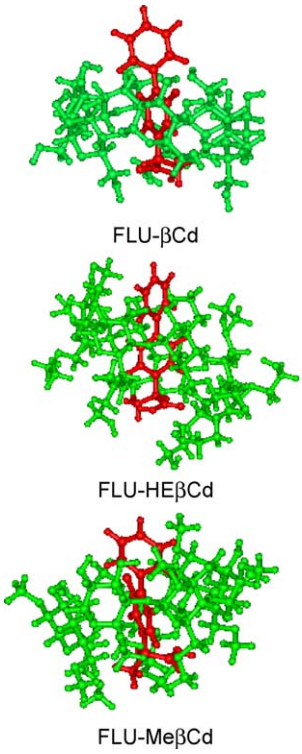

Fig. 4. Computer-generated inclusion complexes of flurbiprofen (FLU) with βCyd, HEβCyd (MS 1.6) and MeβCyd (DS 1.8) at 0 K.

This approach also allowed the determination of the possible tridimensional structure of the different FLU–Cyd inclusion complexes (Fig. 4). The proposed method has only a predictive value; however, it seems to be a useful tool in preformulation studies to perform a preliminary rapid screening aimed at quickly selecting the best set of Cyds to experimentally examine for the inclusion complexation of a given drug.

4. Conclusions

All the techniques tested in the present work can be considered effective alternative methods for calculating the stability constants of FLU–Cyd complexes, making it possible to overcome the drawbacks of the time-consuming conventional phase-solubility method. However, none emerged as an ideal method. Rather, each one has its advantages and limits, which have to be taken into account when selecting the most suitable method(s) to use depending on the host-guest system(s) to be investigated. In particular: the permeation technique is a fast and simple method useful for simultaneously obtaining drug permeation data, but it requires very high Cyd concentration in the donor phase; ACE provides a rapid and selective tool for analysing drug–Cyd complexes, requiring minute amounts of samples, but it is applicable

only to guests in the ionised form; molecular modeling has only a predictive value and is suitable for a preliminary rapid screening before experimental determinations. Moreover, the K_{st} values obtained with the different techniques were rather different, probably due to the differences in the experimental conditions required by each one, confirming the complexity of the factors influencing the determination of the stability constant of the inclusion complexes. On the other hand, this is further demonstrated by the largely different values reported in the literature for the FLU–βCyd complex stability constant [16,18,25–27].

However all the examined methods indicated the methyl-derivative as the most powerful complexing agent for the drug, by observing this general trend: K_{st}(MeβCyd) » K_{st}(HEβCyd) > K_{st}(βCyd). Only in the case of the ACE method was an inversion of the trend found between HEβCyd and βCyd, and it was attributed to the lower molecular weight of the FLU complex with the natural Cyd, which became in this case more important in determining the complex electrophoretic mobility than the different affinity of the drug for such Cyds.

Acknowledgment

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