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High-Throughput Phase-Distribution Method to Determine Drug-Cyclodextrin Binding Constants

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Abstract

A high-throughput method has been developed to measure drug-cyclodextrin binding constants. It measures the distribution ratio of a drug between a polymer film [polyvinyl chloride (PVC) with 67% (w/w) dioctyl sebacate (DOS)] and a cyclodextrin-containing buffer in a 96-well format. Measurements of distribution ratios at several cyclodextrin concentrations lead to binding constants. Binding constants for econazole with six CDs have been determined in one 96-well microplate with four replications of each condition in 10 h. The $K_{1:1}/10^3 \text{ M}^{-1}$ values are 3.98 ± 0.13 , 3.90 ± 0.22 , 29.3 ± 2.2 , 0.66 ± 0.04 , 1.78 ± 0.30 , 4.08 ± 0.50 , with (2-hydroxyethyl)- β -cyclodextrin, (2-hydroxypropyl)- β -cyclodextrin, 2,6-di-*O*-methyl- β -cyclodextrin, hepta-kis(2,3,6-tri-*O*-methyl)- β -cyclodextrin, α -cyclodextrin, β -cyclodextrin, respectively. It is likely that 1:2 complexes are also formed in some cases. This method has also been applied to study the binding behavior as a function of the drug concentration and pH. Binding weakens at higher drug concentration which may be due to the self-association of the drug. An acidic environment decreases the binding constant of CD with the basic econazole. The formation of the 1:2 complexes is completely suppressed in acid as well. This protocol is faster than the phase-solubility method. Moreover, the material requirement is up to four orders of magnitude lower.

Keywords

cyclodextrin; complexation; binding constant; high throughput technologies; solubility; distribution

INTRODUCTION

Aqueous solubility is one of the fundamental determinants in developing new chemical entities as successful drugs.¹ Multiple formulation techniques exist to increase the apparent solubility of lipophilic compounds without decreasing their optimized potency. These techniques include particle size reduction, pH adjustment, addition of solubilizing excipients, solid dispersion, microemulsification, nanocrystallization, inclusion complex formation, etc.² Cyclodextrins (CDs) are bucket-shaped oligosaccharides produced from starch, with a hydrophilic outer surface and a lipophilic inner cavity. They are able to form water-soluble inclusion complexes with many lipophilic compounds. They are commonly used in pharmaceutical formulations to enhance drug solubility, stability, and bioavailability.^{3,4} To date, there are more than 20 marketed drugs that contain CDs,⁵ and numerous publications are emerging every year

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SUPPORTING INFORMATION Supporting information for this article (time course of distribution equilibrium for econazole in the absence of cyclodextrin in the aqueous phase) is available.

studying the use of CDs for drug formulation and delivery. Although higher order complexes are not uncommon, the simplest and most frequent stoichiometry of drug-cyclodextrin ($S \cdot CD$) complexes is 1:1

The binding constant ($K_{1:1}$) is defined as

$$K_{1:1} = \frac{[S \cdot CD]}{[S][CD]} \quad (1)$$

where $[S]$, $[CD]$, $[S \cdot CD]$ are the concentrations of the free drug, free CD, and drug-CD complex, respectively. For consecutive complexation

The binding constant ($K_{1:i}$) is defined as

$$K_{1:i} = \frac{[S \cdot CD_i]}{[S \cdot CD_{i-1}][CD]} \quad (2)$$

The binding forces within the drug-CD complexes may involve hydrophobic, van der Waals, hydrogen bonding, or dipole interactions.⁶ Depending on the cavity size and functional groups, CDs vary in their ability to form inclusion complexes with specific guest compounds.⁷ The stoichiometry and binding constant are important in any investigation to assess the value of a CD for the formulation of a specific drug.⁸

Grounded on the basic principles of medicinal chemistry, one of the most reliable methods to increase *in vitro* potency of drug candidates is to add lipophilic moieties at appropriate positions of the lead compound.¹ Hence the recent development of combinatorial synthesis and highthroughput technologies has led to an increasing number of poorly soluble compounds from combinatorial libraries being investigated for their therapeutic activities.⁹ To develop a formulation technique that works within the combinatorial screening regimen, it would be beneficial to have a universal protocol that can measure the binding constant of drug-CD complexes in a high-through-put manner. Various techniques exist to measure drug-CD binding and can be generally classified into two groups: separation based and nonseparation based. The former separates the free and complexed drug and quantifies their concentrations. Chromatography,¹⁰ affinity capillary electrophoresis (ACE),^{11–14} dialysis,¹⁵ and electrospray mass spectroscopy (ESI-MS)¹⁶ methods are separation-based techniques. The latter group of methods monitors the change in specific physicochemical properties of the drug or CD upon complexation. This category includes spectroscopy,^{17,18} NMR, conductometry,¹⁹ potentiometry,²⁰ phase-solubility,²¹ and hydrolysis kinetics.^{22,23} Each type of measurement is limited to a certain set of compounds. The most common method to determine $K_{1:1}$ for drug-CD binding is the Higuchi-Connors phase-solubility method.²¹ This method measures the effect of CD concentration on the apparent solubility of the drug; the intrinsic solubility (S_0) and the slope of the solubility versus CD-content diagram are then used to calculate $K_{1:1}$. There are also a few reports using phase-distribution methods to determine binding constants for CD complexes.^{24,25} Although high-throughput protein binding assays are developing rapidly,^{26,}

²⁷ this technique has not extended its application to the determination of drug-CD complexation or drug–small molecule interactions.^{28,29}

Recently, we have developed a high-throughput method that can determine the partition and distribution behavior of drug candidates between a polymer phase and an aqueous phase.³⁰ This method has also been applied to measure intermolecular associations in the polymer phase to screen chiral selectors.³¹ In this article, we report the application of this high-throughput method for the determination of drug-CD binding constants in the aqueous phase. The polymer phase is composed of poly(vinyl chloride) (PVC) and dioctyl sebacate (DOS) at the ratio of 1:2 (w/w). Econazole and miconazole (Chart 1), both antifungal agents, were used as the test drugs. Their abilities to form water-soluble complexes with various CDs were determined under various concentration and pH conditions.

MATERIALS AND METHODS

Materials

(2-hydroxyethyl)- β -cyclodextrin (HE- β -CD), (2-hydroxypropyl)- β -cyclodextrin (HP- β -CD), 2,6-di-*O*-methyl- β -cyclodextrin (DM- β -CD), hepta-kis(2,3,6-tri-*O*-methyl)- β -cyclodextrin (TM- β -CD), α -cyclodextrin (α -CD), β -cyclodextrin (β -CD) were purchased from Sigma–Aldrich (St. Louis, MO) with the highest available purities. Econazole free base was purchased from Molecula Ltd. (Dorset, UK). Miconazole free base was purchased from MP Biomedicals (Solon, OH). HPLC grade tetrahydrofuran (THF) and acetonitrile (AN) were purchased from Aldrich (Milwaukee, WI). PVC (high molecular weight, Selectophore) and DOS (Selectophore) were purchased from Fluka (Ronkonkoma, NY). Water used in all the experiments was purified with a Milli-Q Synthesis A10 system (Millipore, Bedford, MA). Costar polypropylene 96-well microplates (flat-bottom, 330- μ L well volume) and thermal adhesive sealing films were purchased from Fisher Scientific Co. (Pittsburgh, PA).

Equipment

An UltraSpense 2000 microplate dispenser (KD Scientific, Holliston, MA) was used to prepare polymer films in 96-well plates. A Deep Well Maximizer (or BioShaker) (Model M-BR-022 UP, made by Taitec and distributed by Bionexus, Inc., Oakland, CA) was used to speed up the drug distribution kinetics and control the temperature for better reproducibility. An X-LC (Jasco, Inc., Easton, MD) HPLC system was used to determine the econazole and miconazole concentration with a UPLC C₁₈ column (1.0 mm $\times$ 50 mm, particle size: 1.7 μ M, Waters, Milford, MA). The molecular weights of the cyclodextrins in this study were determined by ESI-MS (Agilent HP 1100 LC-MSD).

Buffer Preparation

The phosphate buffer solutions (20 mM, pH 5.8, 7.4, 8.5) were made by mixing appropriate amounts of 20 mM sodium phosphate dibasic solution and 20 mM sodium phosphate monobasic solution. The phosphate buffer (20 mM, pH 1.8) was made by mixing appropriate amounts of 20 mM sodium phosphate monobasic solution and 20 mM phosphoric acid solution.

High-Throughput Phase-Distribution Studies

Chart 2 gives the sequence of operations for the phase-distribution method that measures the binding constants of drug-CD complexes. The plasticized PVC films were prepared in polypropylene 96-well microplates. The detailed process is described elsewhere,³⁰ with the exception that in this study a microplate dispenser rather than a multichannel pipet was used for solution dispensing, which provided higher throughput and better precision. Briefly, an appropriate amount of the PVC, DOS, and drugs were dissolved in THF. Aliquots of 250 μ L

of this solution were dispensed by the microplate dispenser to microplates. Evaporation of solvent allowed the formation of polymer films at the bottom of each well. The volume of each film has been calculated to be $\sim 2.5 \mu\text{L}$.³⁰ The concentration of econazole in the films (C_{tot}) is $111 \mu\text{M}$ in the kinetic study, $52.5 \mu\text{M}$ in the investigation of the effect of CD concentration on the distribution of econazole, and $88.8 \mu\text{M}$ in the study of the binding behavior of econazole to HP- β -CD at various pH values. CD-containing aqueous buffers ($200 \mu\text{L}$) at a pH of 1.8, 5.8, 7.4, or 8.5 were then manually dispensed over the films with a multichannel pipet. The plates were covered by adhesive sealing films and incubated in a shaker (500 rpm, 25°C). In order to determine the equilibration time, the concentration of drug extracted into the aqueous phase was measured as a function of time at pH 7.4. Other than for this experiment, all data generated were at equilibrium. To determine the drug concentration, the supernatant from each well was transferred to another plate and injected to the HPLC system by an autosampler. The distribution ratio, D , was then calculated as

$$D = \frac{C_E}{(C_S - C_E) \Phi} \quad (3)$$

Here C_S is the drug aqueous concentration if all the target has been extracted to the aqueous phase, C_E is the drug aqueous concentration at equilibrium, and Φ is the phase ratio (aqueous over polymer).

HPLC Method to Determine Econazole/Miconazole Concentration

To minimize the ionized form, samples in strong acidic solution (pH 1.8) were diluted nine times by 20 mM pH 8.5 %phosphate buffer; samples in weak acidic solution (pH 5.8) were diluted three times by 20 mM pH 8.5%phosphate buffer. The column used was the Waters UPLC C_{18} column. The mobile phase was acetonitrile/ H_2O (65/35), with a flow rate of 0.2 mL/min. The back-pressure was ~ 7000 psi. To ensure reproducibility, the full-load injection mode was used (injection volume $5 \mu\text{L}$; loop volume $1 \mu\text{L}$). Detection was by UV absorbance at 210 nm. The peak area was used for the calibration and determination of sample concentration. The time per analysis is ~ 60 s.

ESI-MS Method to Determine Cyclodextrin Molecular Weights

The mobile phase was acetonitrile/ H_2O (65/35). The samples were introduced into the ESI source at $5 \mu\text{L}/\text{min}$. All experiments were performed in positive ion mode. Typically, a probe high voltage ~ 4000 V was applied between the spray needle and the end plate. The drying gas temperature was 350°C with the flow rate at $10 \text{ L}/\text{min}$. The nebulizer pressure was 29 psig. The mass scanning range was 100–3000 with a resolution of 0.15.

DOS Binding to CDs

A 2.5 mL solution of 10% (w/v) HP- β -CD was equilibrated with pure DOS for 1 day at 25°C . HPLC of the aqueous phase (UPLC C_{18} column, mobile phase 90% AN, 10% W, flowing at $0.13 \text{ mL}/\text{min}$) was used to quantitate DOS in the CD-containing aqueous phase. A standard of DOS had a retention time of 1.7 min. Detection was at 210 nm (absorbance).

RESULTS AND DISCUSSION

Theories to Determine the Stoichiometry n and Binding Constants $K_{1:i}$ ($i = 1$ to n)

The distribution of the free drug between the aqueous phase and the film phase is determined by the partition ratio D_0 :

$$D_0 = \frac{[S]}{[S]_{\text{film}}} \quad (4)$$

where $[S]$ and $[S]_{\text{film}}$ are the free drug concentration in the aqueous phase and film phase, respectively. When the CD is added to the aqueous phase, the drug distribution ratio is

$$D = \frac{[S] + \sum_{i=1}^n [S \cdot CD_i]}{[S]_{\text{film}}} \quad (5)$$

where n is the stoichiometry, $[S \cdot CD_i]$ ($i = 1$ to n) is the drug-CD complex concentration in the aqueous phase in various forms. Dividing Eq. (5) by Eq. (4):

$$\frac{D}{D_0} = 1 + \sum_{i=1}^n \frac{[S \cdot CD_i]}{[S]} \quad (6)$$

From Eq. (2), one obtains

$$[S \cdot CD_i] = [S][CD]^i \prod_{j=1}^i K_{1:j} \quad (7)$$

Inserting Eqs. (7) to (6):

$$\frac{D}{D_0} = 1 + \sum_{i=1}^n [CD]^i \prod_{j=1}^i K_{1:j} \quad (8)$$

It is known that pK_a of econazole is 6.69.²⁸ If system is acidic, the cationic form of econazole should also be considered in the equation as follows. The distribution of the free neutral and cationic drug between the aqueous phase and the film phase is determined by the distribution ratio D_0 ,

$$D_0 = \frac{[S] + [HS^+]}{[S]_{\text{film}} + [HS^+]_{\text{film}}} \quad (9)$$

where $[HS^+]$ and $[HS^+]_{\text{film}}$ are the free neutral and cationic drug concentration in the aqueous phase and film phase, respectively. When the CD is added to the aqueous phase, the drug distribution ratio is

$$D = \frac{[S] + \sum_{i=1}^n [S \cdot CD_i] + [HS^+] + \sum_{i=1}^n [HS^+ \cdot CD_i]}{[S]_{\text{film}} + [HS^+]_{\text{film}}} \quad (10)$$

Dividing Eq. (10) by Eq. (9):

$$\frac{D}{D_0} = 1 + \sum_{i=1}^n \frac{[S \cdot CD_i] + [HS^+ CD_i]}{[S] + [HS^+]} \quad (11)$$

It is known that the equilibrium equation for acid dissociation constant K_a is

$$K_a = \frac{[S][H^+]}{[HS^+]} \quad (12)$$

Inserting Eqs. (7) and (12) into Eq. (11):

$$\frac{D}{D_0} = \frac{[CD]^i \prod_{j=1}^i K_{1:j} + ([H^+]/K_a) \prod_{j=1}^i K_{1:j}^+}{1 + [H^+]/K_a} \quad (13)$$

where $K_{1:j}^+$ is the binding constant of cationic drug and cyclodextrin.

If the system is very acidic,

$$\frac{[H^+]}{K_a} \gg K_{1:j} > 1$$

Eq. (13) will become

$$\frac{D}{D_0} = 1 + \sum_{i=1}^n [CD]^i \prod_{j=1}^i K_{1:j}^+ \quad (14)$$

If the cationic form of the drug is ignored, Eq. (13) will become Eq. (8) again:

$$\frac{D}{D_0} = 1 + \sum_{i=1}^n [CD]^i \prod_{j=1}^i K_{1:j}$$

Plotting D/D_0 versus $[CD]$, the stoichiometry and the binding constants can be obtained from polynomial fitting analyses. In practice, a degree one (linear) fitting should first be performed, assuming that only 1:1 complex forms

$$\frac{D}{D_0} = 1 + K_{1:1} [CD] \quad (15)$$

A proper fitting should give a straight line with a y-intercept of unity, and the slope can report the value of $K_{1:1}$. Otherwise linear regression on a quadratic equation should be carried out, assuming that both 1:1 and 1:2 complexes form:

$$\frac{D}{D_0} = 1 + K_{1:1} [\text{CD}] + K_{1:1} K_{1:2} [\text{CD}]^2 \quad (16)$$

Again, a correct fitting should give a y-intercept of unity, and the $K_{1:1}$ and $K_{1:2}$ values can be obtained from the corresponding coefficients of the polynomial. If the fit is still not satisfactory, fitting analyses with higher degrees should be continued. Note that all the coefficients of the polynomial should have positive values. In this study, the concentration of CD prepared in the aqueous buffer (C_{CD}) is much higher than the drug concentration (C_{S}), hence the drug-CD complexation does not significantly change the free CD concentration, and $[\text{CD}]$ in those equations can be reasonably replaced by C_{CD} .

Experimental Results

A kinetic study was first performed to determine the time needed for the phase distribution of econazole to reach equilibrium. The results are shown in Figure 1. Clearly, 8–9.5 h are enough for all the distribution experiments to be equilibrated. Independent experiments (see Supporting Information) demonstrate that the equilibration time for econazole in the absence of CD in the aqueous phase is about 4 h. Based on the kinetic data, all other distribution experiments were allowed to equilibrate for 10 h.

Instructed by Eq. (8), the effect of CD concentration on the distribution of econazole was then studied. Figure 2 gives the profile of econazole equilibrium concentration versus CD-content in the aqueous phase for six CDs. Each measurement was repeated four times and the corresponding error bar indicates the standard error of the mean (SEM). The SEM values were then used in error propagations to determine the errors of the calculated distribution ratios and D/D_0 values. Apparently, at higher CD concentration, more econazole is extracted to the aqueous phase. For these six CDs, the ability to extract econazole is in the order of DM- β -CD > α -CD > β -CD > HE- β -CD $\approx$ HP- β -CD > TM- β -CD, which is in good agreement with the previous kinetic study (Fig. 1) and reported phase-solubility data of several CDs (α -CD > β -CD $\approx$ HP- β -CD).³² Linear regression on Eq. (16) gives the $K_{1:1}$ and $K_{1:2}$ values, as shown in Table 1. The errors are standard deviations. Some of the fitted curves are shown in Figure 3 and all the coefficients of determination (COD) are listed in Table 1. The econazole-CD binding constants ($K_{1:1}/10^3 \text{ M}^{-1}$) discovered by phase-solubility studies have been reported for α -CD, HP- β -CD, and β -CD, which are 2.63 ± 0.26 , 1.54 ± 0.15 , and 1.42 ± 0.13 , respectively.³² These values are the same order of magnitude as the data in Table 1, however, because the conditions of the experiments differ, the results do not agree quantitatively. Since the pH and choice of buffer species have a great effect on the determination of binding constant,³² the literature values measured in pure water should only be used for qualitative purposes. The $K_{1:2}$ values are larger than zero, indicating the formation of 1:2 complexes. All $K_{1:2}$ values are highly significant as judged by p values. Probabilities that the values of $K_{1:2}$ are different from zero based only on chance are all <0.0001 except for the final entry in Table 1 (β -CD) in which case it is <0.001 . Most studies on imidazole-CD complexation have assumed a 1:1 ratio,^{1,33} but higher order complexes have also been reported.^{34–38} For instance, the stoichiometry of econazole/ β -CD has been published by several groups to be 1:1,^{32,39} while a study has discovered the formation of 2:3 complex.³⁴

The drug distribution behavior at various C_{S} values has then been studied and the corresponding equilibrium concentrations versus CD-concentration profiles are shown in Figure 4. The CD used was HP- β -CD and the pH of the aqueous buffer was 8.5. D_0 , $K_{1:1}$, and $K_{1:2}$ values are listed in Table 2. At higher C_{S} , the D_0 value is greater and the $K_{1:1}$ and $K_{1:2}$ values tend to be smaller. This interesting trend may be due to a decreasing drug activity coefficient as concentration is increased, for example, due to favorable drug–drug interactions. This effect

can explain the previous observation that binding constants determined by phase-solubility studies are smaller than by this method, since in phase-solubility experiments, the saturated econazole concentration is much higher than the equilibrium drug concentration in this distribution study.

This method has also been applied in the situation where two mixed drugs are studied at the same time. Since the CD concentration is large with respect to the sum of the drug concentrations, the “multiplex” approach is possible. Miconazole is an analogue of econazole. Both of them were dissolved in the same polymer film. The extraction profile is shown in Figure 5. The values of D_0 , $K_{1:1}$, and $K_{1:2}$ are listed in Table 2. From the data, miconazole is more lipophilic than econazole, and binds more strongly to HP- β -CD. When mixed with miconazole, the distribution ratio of econazole is greater, and the binding constants $K_{1:1}$ and $K_{1:2}$ are smaller, which may be attributable to the decreased drug activity coefficient caused by association with miconazole.

The binding behavior of econazole to HP- β -CD has been studied at various pH values. The drug equilibrium concentrations versus aqueous CD-concentration profiles are shown in Figure 6. The values of D_0 , $K_{1:1}$, and $K_{1:2}$ are listed in Table 3. The $K_{1:1}$ values decrease rapidly in an acidic environment. Econazole is a basic compound and has a pK_a value of 6.69,³⁰ hence it can be concluded that increased ionization decreases its binding constant with neutral CDs, which is consistent with literature observations.³⁹ Moreover, the formation of the 1:2 complexes is suppressed when econazole is ionized, as indicated by the values of $K_{1:2} = 0$ at pH 5.8 and 1.8.

Compared to the phase-solubility method, which can require days for the dissolving of the drug to be saturated, this new technique is faster. Moreover, the drug amounts used in solubility experiments are much greater than in this method. For instance, the highest econazole concentration in a phase-solubility study is ~30 mM,³² but in the phase-distribution experiment, C_S is less than 0.1 mM. In addition, the volume of the CD solution used in a solubility study is typically 10 mL, which is 40 times more than in this distribution experiment. These two factors have led to a 12000-fold decrease in material requirements. The equilibration time is shorter in these phase-distribution studies probably because it does not involve the equilibrium between solid and dissolved drug. For other phase-distribution methods, which study the drug distribution between an organic solvent and an aqueous phase, the solvent-CD complexation may lead to misinterpretation. In addition, entrainment and emulsion can be severe problems for very hydrophobic compounds,⁴⁰ and the handling of small volumes of organic solvent may be difficult.³⁰

In phase-solubility studies, several factors influence the accuracy of the final result. One such factor is the accurate and precise determination of the intrinsic solubility (S_0). Similarly for this approach, the variance of D_0 may lead to misinterpretation of n and $K_{1:i}$ as well. Since the drug concentration is usually low (~0.01–0.1 μ M) when determining D_0 , some error is inevitable. Ways to decrease the measurement error of D_0 have been discussed elsewhere.³⁰ The most important aspect in getting an accurate value for D_0 is the sensitivity and selectivity of the analytical method used to measure the concentrations of the solute. As far as precision is concerned, the 96-well plate approach is beneficial, as it is easy to do repeat measurements. Other potential errors may arise from the distribution process itself. The drug may adsorb to the plate surface. We have determined that this does not occur for a series of compounds ranging in their octanol-water partition coefficients over a logarithmic range of 0.5–3.2.³⁰ Another potential source of error is that DOS may associate with CDs, and thus compete with the drug and lead to inaccurate binding constants. In the experiments described herein, the CDs are in great excess over the drug, so competition is minimized. Nonetheless, we have determined that there is no detectable DOS extracted into aqueous solution containing 10% (w/v) HP- β -CD.

All of the experiments except the kinetic study were carried out at equilibrium. It is worth noting that, in our experience, solute drug distribution at early times before equilibrium is correlated with the equilibrium concentration. As Figure 1 shows, after only 1 h of equilibration it is already obvious that 10% DM- β -CD is best solubilizing agent for econazole. Although we have not investigated this thoroughly, it seems clear that screening to determine the rank order of the effectiveness of a series of potential solubilizers could be carried out much more rapidly than the equilibrium studies that we have discussed herein.

CONCLUSIONS

We have successfully developed a new method to measure the binding constants of drug-CD complexes in the aqueous phase using high-through-put technologies. This method measures the distribution behavior of a drug between a polymer phase and an aqueous phase in 96-well microplates. With four repeats, distribution ratios of econazole with respect to six CD-containing buffers at four different concentrations can be determined simultaneously. Multivariate linear regression has been established to give the binding constants of econazole to the six CDs respectively. Both 1:1 and 1:2 complexes are found and the calculated $K_{1:1}$ values can be correlated to some literature data from phase-solubility studies. The binding constants of econazole to HP- β -CD have been studied at various drug concentrations and pH conditions. At higher econazole concentrations, the drug-CD binding tends to be weaker, which may due to the self-association of the drug. An acidic environment weakens the binding between econazole with HP- β -CD and suppresses the 1:2 complex formations. Compared to the phase-solubility method, our protocol is much faster. Moreover, the material requirement decreases four orders of magnitude. This method has great flexibility as well, for instance, “multiplex” approaches are possible due to the much lower concentration of the drug relevant to the CD concentration. In addition, this method is possible to be fully automated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Loftsson T, Hreinsdottir D, Masson M. Evaluation of cyclodextrin solubilization of drugs. *Int J Pharm* 2005;302:18–28. [PubMed: 16099118]
2. Das NG, Das SK. Formulation of poorly soluble drugs. *Drug Delivery Report Spring–Summer;2006* : 52–55.
3. Hedges AR. Industrial applications of cyclodextrins. *Chem Rev* 1998;98:2035–2044. [PubMed: 11848958]
4. Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. *Chem Rev* 1998;98:2045–2076. [PubMed: 11848959]
5. Loftsson T, Brewster ME, Masson M. Role of cyclodextrins in improving oral drug delivery. *Am J Drug Deliv* 2004;2:261–275.
6. Hirsch W, Fried V, Altman L. Effect of cyclodextrins on sparingly soluble salts. *J Pharm Sci* 1985;74:1123–1125. [PubMed: 4078713]
7. Bodor N, Buchwald P. Theoretical insights into the formation, structure, and energetics of some cyclodextrin complexes. *J Inclusion Phenomena Macrocyclic Chem* 2003;44:9–14.
8. Rao VM, Stella VJ. When can cyclodextrins be considered for solubilization purposes? *J Pharm Sci* 2003;92:927–932. [PubMed: 12712411]

9. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Methods* 2001;44:235–249. [PubMed: 11274893]
10. Chen J, Ohnmacht CM, Hage DS. Characterization of drug interactions with soluble β -cyclodextrin by high-performance affinity chromatography. *J Chromatogr A* 2004;1033:115–126. [PubMed: 15072296]
11. Schou C, Heegaard NHH. Recent applications of affinity interactions in capillary electrophoresis. *Electrophoresis* 2006;27:44–59. [PubMed: 16315182]
12. Schipper BR, Ramstad T. Determination of the binding constant between alprostadil and alpha-cyclodextrin by capillary electrophoresis: Implications for a freeze-dried formulation. *J Pharm Sci* 2005;94:1528–1537. [PubMed: 15920771]
13. Karakasyan C, Taverna M, Millot M-C. Determination of binding constants of hydrophobically end-capped poly(ethylene glycol)s with beta-cyclodextrin by affinity capillary electrophoresis. *J Chromatogr A* 2004;1032:159–164. [PubMed: 15065792]
14. Rundlett KL, Armstrong DW. Methods for the determination of binding constants by capillary electrophoresis. *Electrophoresis* 2001;22:1419–1427. [PubMed: 11379966]
15. Sideris EE, Georgiou CA, Koupparis MA, Macheras PE. Automated flow-injection serial dynamic dialysis technique in the study of drug binding with cyclodextrins. *Anal Chim Acta* 1994;289:87–95.
16. Beni S, Szakacs Z, Csernak O, Barcza L, Noszal B. Cyclodextrin/imatinib complexation: Binding mode and charge dependent stabilities. *Eur J Pharm Sci* 2007;30:167–174. [PubMed: 17145172]
17. Archontaki HA, Vertzoni MV, Athanassiou-Malaki MH. Study on the inclusion complexes of bromazepam with β - and β -hydroxypropyl-cyclodextrins. *J Pharm Biomed Anal* 2002;28:761–769. [PubMed: 12008156]
18. Koopmans C, Ritter H. Color change of N-isopropylacrylamide copolymer bearing reichardt's dye as optical sensor for lower critical solution temperature and for host-guest interaction with β -cyclodextrin. *J Am Chem Soc* 2007;129:3502–3503. [PubMed: 17328550]
19. Sheehy PM, Ramstad T. Determination of the molecular complexation constant between alprostadil and alpha-cyclodextrin by conductometry. *J Pharm Biomed Anal* 2005;39:877–885. [PubMed: 16126357]
20. Kahle C, Holzgrabe U. Determination of binding constants of cyclodextrin inclusion complexes with amino acids and dipeptides by potentiometric titration. *Chirality* 2004;16:509–515. [PubMed: 15290685]
21. Higuchi T, Connors KA. Phase-solubility techniques. *Advan Anal Chem Instr* 1965;4:117–212.
22. Loukas YL. Inclusion complexation of unstable guests with cyclodextrins: Improved kinetic studies for the calculation of the binding constants in guest-cyclodextrin systems. *Pharm Sci* 1997;3:343–346.
23. Loukas YL, Vraka V, Gregoriadis G. Use of a nonlinear least-squares model for the kinetic determination of the stability constant of cyclodextrin inclusion complexes. *Int J Pharm* 1996;144:225–231.
24. Masson M, Sigurdardottir BV, Matthiasson K, Loftsson T. Investigation of drug-cyclodextrin complexes by a phase-distribution method: Some theoretical and practical considerations. *Chem Pharm Bull* 2005;53:958–964. [PubMed: 16079528]
25. Eli W, Chen W, Xue Q. Determination of association constants of cyclodextrin-nonionic surfactant inclusion complexes by a partition coefficient method. *J Inclusion Phenomena Macrocyclic Chem* 2000;38:37–43.
26. Senisterra GA, Markin E, Yamazaki K, Hui R, Vedadi M, Awrey DE. Screening for ligands using a generic and high-throughput light-scattering-based assay. *J Biomol Screen* 2006;11:940–948. [PubMed: 17092916]
27. Hallikas O, Taipale J. High-throughput assay for determining specificity and affinity of protein-DNA binding interactions. *Nat Protoc* 2006;1:215–222. [PubMed: 17406235]
28. Li S, Sun L, Chung Y, Weber SG. Artificial receptor-facilitated solid-phase microextraction of barbiturates. *Anal Chem* 1999;71:2146–2151.
29. Valenta JN, Dixon RP, Hamilton AD, Weber SG. Enhanced extraction of phenobarbital from serum with a designed artificial receptor. *Anal Chem* 1994;66:2397–2403. [PubMed: 8080112]

30. Chen Z, Weber SG. High-throughput method for lipophilicity measurement. *Anal Chem* 2007;79:1043–1049. [PubMed: 17263333]
31. Chen Z, Yang Y, Werner S, Wipf P, Weber SG. A screening method for chiral selectors that does not require covalent attachment. *J Am Chem Soc* 2006;128:2208–2209. [PubMed: 16478163]
32. Mura P, Faucci MT, Manderioli A, Bramanti G. Multicomponent systems of econazole with hydroxyacids and cyclodextrins. *J Inclusion Phenomena Macrocyclic Chem* 2001;39:131–138.
33. Guillaume YC, Peyrin E. Symmetry breaking during the formation of β -cyclodextrin-imidazole inclusion compounds: Capillary electrophoresis study. *Anal Chem* 1999;71:2046–2052. [PubMed: 10361504]
34. Pedersen M, Bjerregaard S, Jacobsen J, Rommel-mayer Larsen A, Mehlsen Sorensen A. An econazole β -cyclodextrin inclusion complex: An unusual dissolution rate, supersaturation, and biological efficacy example. *Int J Pharm* 1998;165:57–68.
35. Pedersen M. Isolation and antimycotic effect of a genuine miconazole- β -cyclodextrin complex. *Eur J Pharm Biopharm* 1994;40:19–23.
36. El-Barghouthi MI, Masoud NA, Al-Kafawein JK, Abdoh AA. Inclusion complexation of itraconazole with β - and 2-hydroxypropyl- β -cyclodextrins in aqueous solutions. *Russ J Phys Chem* 2006;80:1050–1055.
37. Peeters J, Neeskens P, Tollenaere JP, Van Remoortere P, Brewster ME. Characterization of the interaction of 2-hydroxypropyl- β -cyclodextrin with itraconazole at pH 2, 4, and 7. *J Pharm Sci* 2002;91:1414–1422. [PubMed: 12115841]
38. Miyake K, Irie T, Arima H, Hirayama F, Uekama K, Hirano M, Okamaoto Y. Characterization of itraconazole/2-hydroxypropyl-beta-cyclodextrin inclusion complex in aqueous propylene glycol solution. *Int J Pharm* 1999;179:237–245. [PubMed: 10053216]
39. Pedersen M, Edelsten M, Nielsen VF, Scarpellini A, Skytte S, Slot C. Formation and antimycotic effect of cyclodextrin inclusion complexes of econazole and miconazole. *Int J Pharm* 1993;90:247–254.
40. Poole SK, Poole CF. Separation methods for estimating octanol-water partition coefficients. *J Chromatogr B Anal Technol Biomed Life Sci* 2003;797:3–19.

Figure 1. Percent of econazole extracted as a function of time in the presence of six CDs; 20 mM phosphate buffer pH 7.4 at 20°C.

Figure 2.
Effect of CD concentration on econazole equilibrium concentration in the aqueous phase; 20 mM phosphate buffer pH 7.4 at 25°C.

Figure 3. Multivariate linear regression results for fitting D/D_0 versus CD concentration. Twenty millimolar phosphate buffer pH 7.4 at 25°C.

Figure 4. Effect of HP- β -CD concentration on econazole equilibrium concentration in the aqueous phase (pH 8.5 phosphate buffer, 20 mM at 25°C) at three different initial concentrations of drug in the membrane.

Figure 5. Effect of HP-β-CD concentration on econazole and miconazole concentrations in the aqueous phase (pH 8.5 20 mM phosphate buffer at 25°C). Both drugs were initially present in the membrane.

Figure 6.
Effect of HP- β -CD concentration on econazole equilibrium concentration in the aqueous phase (pH varies, 20 mM phosphate buffer at 25°C).

Chart 1.
Structures of econazole (1) and miconazole (2).

Chart 2.
Schematic illustration of the preparation and use of polymer films in 96-well plates.

Table 1
Binding Constants of Econazole With Six CDs in 20 mM pH 7.4 Phosphate Buffer at 25°C

Do	Cyclodextrin	M.W. (g/mol)	$K_{1:1}$ (10^3 M^{-1})	$K_{1:2}$ (M^{-1})	COD
(1.20±0.09)×10(T ⁻⁵)	HE-β-CD	~1480 ^a	3.98±0.13	4.9±0.5	0.9989
	HP-β-CD	~1580 ^a	3.90±0.22	10.0±1.9	0.9976
	DM-β-CD	~1330 ^a	29.3±2.2	57.7±8.7	0.9982
	TM-β-CD	1429.54	0.66±0.04	53.9±3.7	0.9994
	α-CD	972.84	1.78±0.30	256±44	0.9997
	β-CD	1134.98	4.08±0.50	47.6±12.1	0.9956

^a Randomly substituted. Their average molecular weights are determined by ESI-MS.

Table 2
Binding Constants of Econazole and Miconazole With HP- β -CD at Various Drug Concentrations in 20 mM pH 8.5 Phosphate Buffer at 25°C

Drug	C_{tot} (μ M)	D_0	$K_{1:1}$ ($10^3 M^{-1}$)	$K_{1:2}$ (M^{-1})	COD
Econazole	44.4	$(3.19 \pm 0.26) \times 10^{-6}$	1.91 ± 0.11	8.0 ± 0.9	0.9998
	88.8	$(3.74 \pm 0.08) \times 10^{-6}$	1.81 ± 0.02	6.0 ± 0.02	0.99999
	88.8^a	$(5.01 \pm 0.21) \times 10^{-6}$	1.57 ± 0.02	3.9 ± 0.1	0.99998
	177.6	$(5.43 \pm 0.44) \times 10^{-6}$	1.28 ± 0.03	4.4 ± 0.2	0.99995
Miconazole	80.0^b	$(5.35 \pm 1.43) \times 10^{-7}$	4.2 ± 0.44	8.8 ± 0.9	0.9994

^aMixed with miconazole.

^bMixed with econazole.

Table 3Binding Constants of Econazole With HP- β -CD at Various pH Values

pH	D_0	$K_{1:1}$ (10^3 M^{-1})	$K_{1:2}$ (M^{-1})	COD
1.8	$(2.67 \pm 0.16) \times 10^{-3}$	0.207 ± 0.001	0	0.9932
5.8	$(6.09 \pm 0.48) \times 10^{-4}$	0.654 ± 0.006	0	0.9996
7.4	$(1.20 \pm 0.09) \times 10^{-5}$	2.02 ± 0.34	6.8 ± 0.7	0.9921
8.5	$(3.74 \pm 0.08) \times 10^{-6}$	1.81 ± 0.02	6.0 ± 0.02	0.99999