Published in final edited form as:

Electrophoresis. 2009 August ; 30(16): 2820–2828. doi:10.1002/elps.200800852.

Combined use of chiral ionic liquid and cyclodextrin for MEKC: Part II. Determination of Binding Constants

Bin Wang¹, Jun He¹, Victoria Bianchi¹, and Shahab A. Shamsi^{1,*}

¹ Department of Chemistry, Center of Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303

Abstract

A competitive inhibition mechanism is proposed to investigate the interactions among 2,3,6-tri-O-methyl- β -cyclodextrin (TM- β -CD), cationic ionic liquid (IL) type surfactants, *N*-undecenoxy-carbonyl-L-leucinol bromide (L-UCLB) and profens (PROFs) using affinity capillary electrophoresis. The apparent binding constant of TM- β -CD to L-UCLB was estimated by nonlinear and linear plotting methods. The binding constants of one representative PROF (e.g., fenoprofen) to TM- β -CD and to L-UCLB were estimated by a secondary plotting approach. The *R*- and *S*-fenoprofen have different binding constant values, resulting in the enantioseparation due to the synergistic effect of the two chiral selectors, TM- β -CD and L-UCLB.

Keywords

TM- β -CD; L-UCLB; Profens; Binding constants; Competitive Binding; Affinity capillary electrophoresis

1 Introduction

Ionic Liquids (ILs) have shown increasing applications in various research areas, including the organic synthesis, electrochemistry, mass spectrometry, and environmental science [1–2]. Separation scientists have paid more attention to ILs as stationary phase and mobile phase modifiers in GC [3–5] and LC, respectively [6–7]. In CE they have been used as pseudostationary phase (PSP) in MEKC, support coatings on the capillary wall, background electrolyte (BGE) in nonaqueous CE, additives as CE buffers with cyclodextrin (CD) for achiral and chiral separation [8]. In addition, the ILs with surfactant properties can form micelles and hence are useful in MEKC. In particular, the chiral ILs micelles is been reported recently for enantioseparation [9].

The inclusion of ILs with CD is a topic, which has generated recent attention in the literature [10–12]. The main reason for studying this system is to evaluate the actual benefits that can be achieved upon addition of CDs to ILs. For example, in CE the addition of ILs would widen the scope of application of CDs for the separation of chiral compounds [10]. The most recent of such studies was reported in 2007 [11–12], where the molar ratio of the complex formed between seven alkyl(methyl)methyl-imidazolium based IL cations and eight neutral CDs was studied by affinity capillary electrophoresis (ACE). This method is based on the changes of

*Corresponding Author: Professor Shahab A. Shamsi, Department of Chemistry, Center of Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303 Phone: 404-413-5513, Fax: 404-413-5551, chesas@langate.gsu.edu.

The authors have declared no conflict of interest.

effective mobilities of the free and bound forms of analytes due to the association-dissociation kinetics between the analyte and ligand [13]. When using ACE, the migration patterns of interacting molecules can be used to identify and quantify specific binding and to estimate binding constants in two steps: (1) the solutes are first separated by CZE, (2) the CE run buffer is doped with a complexing agent, which is specific for the solute. The change in retention time of the solute is recorded before and after the addition of complexing agent [14]. MEKC can also be considered as an ACE method because the partitioning and the electrostatic interactions between the solute and the micelles are the affinity interactions between these two species [13]. However, most of the reactions studied by ACE are non-covalent molecular interactions confined to ligand-receptor or ligand-substrate mode [13].

The goal of this work is to investigate an approach originated from enzyme kinetics, which was used to study the binding constants of L-UCLB to TM- β -CD, PROFs to TM- β -CD, and PROFs to L-UCLB. This approach is applicable based on the assumption that two or more additives have significant interactions with each other. We hypothesize that the IL, L-UCLB act as an “*inhibitor*”, reducing the interaction between TM- β -CD and the PROFs. To test the aforementioned hypothesis, we proposed a general model for the ternary interactions among profens, TM- β -CD and L-UCLB. To the best of our knowledge, this is the first study in which the concepts originated from enzyme kinetics were used to measure the binding constants of $\pm$ PROF drug using a mixture of IL and TM- β -CD as a dual chiral selector.

2 Materials and methods

2.1 Reagents and chemicals

The same as described in Part I [14]

2.2 CE instrumentation

The instrumentation is the same as described in Part I [14]

2.3 CE conditions and methods

2.3.1 Estimation of the binding constant between TM- β -CD and L-UCLB (K_1)—

The stock solution of L-UCLB was prepared by dissolving in 5 mM NaOAc aqueous buffer (pH 5.0) at the concentration of 4.0 mM. The DMSO was dissolved in 5 mM NaOAc buffer (pH 5.0) at the concentration of 0.1 mg/mL. Consequently, 100 μ L of L-UCLB and DMSO solutions were added to a vial and vortexed to make a mixture solution to be injected into the capillary with an injection size of 50 mbar, 20 sec. The CE buffers used in this study were 5 mM NaOAc aqueous buffer (pH 5.0) without TM- β -CD, and the same buffer with TM- β -CD at concentrations ranging from 2.5 mM to 10.0 mM. The capillary rinsing procedure before the first run, the precondition before each run, and the postcondition after each run were generally the same as described in Part I [14] except that the postcondition of 0.1 mM NaOH was 5 min instead of 10 min. The separations were carried out with an applied voltage of +20 kV and a temperature of 16 °C. The direct UV detection of L-UCLB was achieved at 200 nm.

The addition of TM- β -CD in the CE buffer will change the viscosity of the buffer, and thereby affect the mobility of the L-UCLB in the buffer with different concentration of TM- β -CD [11,15]. To study this effect, the relative viscosities of the TM- β -CD buffer were measured by hydrodynamic injection of the sample containing 2.0 mM L-UCLB and 0.05 mg/mL DMSO in 5 mM NaOAc buffer (pH 5.0) with the injection size of 50 mbar, 20 sec. The sample was pushed through the detector with a constant pressure of 50 mbar [11,15]. The viscosity correction factor was estimated using the following equation:

$$\nu = \frac{\eta}{\eta_0} = \frac{t}{t_0},$$

where η_0 is the viscosity of the CE buffer without TM- β -CD, and η is the viscosity of the CE buffer with a certain concentration of TM- β -CD [11,15]. The time t and t_0 represent the time required for the sample plug to move through the injection end of the capillary to the detector in a CE buffer with and without a certain concentration of TM- β -CD, respectively. The sample plugs used to estimate the relative viscosity were monitored at 200 nm.

The shift of the position of the L-UCLB peak relative to the position of the TM- β -CD peak was measured with the increasing concentration of TM- β -CD. The neutral marker, DMSO, was used to indicate the position of TM- β -CD peak and calculate the effective mobilities of free and bound L-UCLB. The binding constant between L-UCLB and TM- β -CD (K_1) was estimated by the linear plotting methods using Microsoft Excel software (version 2007), as listed in Table 1. The plots used were X-reciprocal, Y-reciprocal, and double-reciprocal methods [13]. The values of binding constant K_1 were calculated from the slopes and intercepts of the linear plots, as shown in Table 1 [15–16]. The nonlinear isotherm plot was obtained by a nonlinear regression fitting equation shown in Table 1 [15–16]. The Origin (version 7.5) software was used for the data treatment of nonlinear plotting method [15].

2.3.2 Estimation of the binding constants of fenoprofen to TM- β -CD and fenoprofen to L-UCLB (K_2 and K_i)—The stock solutions of fenoprofen (0.20 mg/mL) and DMSO (0.50 mg/mL) were prepared in 5 mM aqueous buffer of NaOAc at pH 5.0. The mixture solution to be injected into the capillary was made by adding 100 μ L of each stock solution of fenoprofen and DMSO in a sample vial. The mixture was then injected with the injection size of 5 mbar, 10 sec. The concentrations of TM- β -CD ranged from 1.0 to 50 mM. The viscosity correction factors for these buffers were obtained by the same method described in section 2.3.1. The binding experiment for measurement of K_2 was performed under a voltage of +10 kV, temperature of 16 °C with UV detection at 200 nm. The migration time of *R* and *S* enantiomers of fenoprofen was used to estimate the binding constants.

The K_2 value between TM- β -CD and fenoprofen enantiomers were obtained from the same double-reciprocal plotting method described in section 2.3.1. The values of K_2 were different in the absence and presence of L-UCLB. The inhibition constant K_i was estimated from a secondary plot obtained by the method originated from enzyme kinetics [17,18]. The details of inhibition study are explained in section 3.2.

Results and Discussion

3.1 Evaluation of the profen/TM- β -CD/IL interaction system

The PROF enantiomers studied in this work have pK_a values less than 5.0, and the background electrolyte, sodium acetate used in this work, has a pH value of 5.0. Therefore, the PROFs are partially anionic in the CE buffer. During the MEKC separation, the capillary filled with the CE buffer contained three major species: the neutral chiral selector TM- β -CD, the positively charged chiral selector L-UCLB, and negatively charged analytes PROFs. As shown in Figure 1, the interactions among these species could be divided into three parts: (a) the interactions (K_1) between the TM- β -CD and the IL monomers or micelles, (b) the interactions (K_i) between the PROFs and IL monomer or micelles, and (c) the interactions (K_2) between PROFs and TM- β -CD. The monomer of IL might form inclusion complex with TM- β -CD. Note that the IL monomers or micelles are cationic while the PROFs are anionic. Hence, the interactions between these two species might include electrostatic attraction (ion-pair), hydrogen bonding,

and hydrophobic interactions. In addition, the PROFs might also form inclusion complex with TM- β -CD. When a positive polarity was used in CE separation, the L-UCLB monomer or micelles migrated before EOF, TM- β -CD migrated with EOF, and PROFs migrated after EOF. It cannot be ascertained from the schematic (shown in Figure 1) whether the anionic PROFs partition exclusively to the TM- β -CD cavity or whether the PROF-(L-UCLB) complex is included in the TM- β -CD cavity. Nevertheless, the interactions among these species caused the differential binding forces between the racemic pairs of the PROFs and the two chiral selectors TM- β -CD and L-UCLB; thereby the enantioseparation could be achieved.

3.2 Theory of the binding constants and mechanism of separation

As discussed above, L-UCLB is the micelle of a cationic surfactant, while the PROFs are partially anionic in the MEKC buffer. If the interactions between these two species are strong, the PROFs should migrate with ILs and before EOF. However, the PROFs eluted after the EOF, and the migration time of PROF increased with the increasing concentration of ILs. This phenomenon indicates that both TM- β -CD and the ILs played an important role for the enantioseparation. In this section, we propose an inhibition mechanism originated from enzyme kinetics for the interactions of PROF with L-UCLB and TM- β -CD. It is well-known that the enzyme and substrate have specific interaction between each other, and an inhibitor may also bind to the same enzyme [17]. For the competitive inhibition, the inhibitors can exclusively interact with the free enzyme. Hence, the enzyme binds either the substrate or the inhibitor, but not both simultaneously. For non-competitive inhibition, the inhibitor binds with free enzyme and with enzyme-substrate complex simultaneously. In case of uncompetitive inhibition, the inhibitor binds exclusively with enzyme-substrate complex [17].

In our system, we hypothesize that the addition of L-UCLB to the TM- β -CD could decrease the interaction between the PROF and TM- β -CD, thereby inhibiting the formation of PROF-(TM- β -CD) complexes. Moreover, the interaction between the two chiral selectors (i.e., L-UCLB and TM- β -CD) also decreases the binding of the PROF to each of them. During the separation, the PROFs had weaker interactions with both TM- β -CD and ILs due to this inhibition mechanism, which probably resulted in the enantioseparation. The reactions for the competitive inhibition mechanism are shown as follows:

where PROF-CD represents the complex formed between profens and TM- β -CD, PROFIL represents the complex formed between profens and ILs, CD-IL represents the complex formed between TM- β -CD and ILs. Note that the inhibitor L-UCLB has interactions with both PROFs and the TM- β -CD, so it inhibited the chiral interaction between TM- β -CD and PROFs. In addition to competitive inhibition, noncompetitive and uncompetitive inhibitions are also possible. Scheme 2 represents a noncompetitive inhibition in which the L-UCLB binds with the free PROF and with PROF-CD complex.

where PROF-IL and PROF-CD represent the same complexes as mentioned above. However, the PROF-CD-IL represents the ternary complex eventually formed by the three species profen, TM- β -CD, and L-UCLB. The binding of L-UCLB can also occur exclusively with PROF-CD complex, which can mimic uncompetitive inhibition model as shown below in Scheme 3.

In the above Scheme 3, the profen firstly binds to TM- β -CD, and then to L-UCLB. The final product is the PROF-CD-IL, which is essentially the same ternary product as the one shown in Scheme 2.

The double-reciprocal plot (or Lineweaver-Burk plot in biochemistry) can be used to determine the mode of inhibition [17,18]. The double-reciprocal plots for the binding of TM- β -CD and the PROF sample were obtained under several fixed concentrations of the inhibitor L-UCLB, respectively (see section 2.3.2). Overlaying these straight lines showed a pattern that is used

to determine the type of inhibition. For example, the linear plots obtained for competitive inhibition will show the same y intercept values but increasing slope values with the increase of inhibitor concentration. On the other hand, the overlaid plots for uncompetitive inhibition should appear as a series of parallel lines that intersect the y axis at different y values. For the noncompetitive inhibition, both the slope and the y intercept of the double-reciprocal plot will increase with increasing concentration of the inhibitor.

3.3 Determination of TM- β -CD/L-UCLB binding constant (K_1)

To investigate the mechanism of inhibition, the binding constant between TM- β -CD and L-UCLB was first measured by the conventional linear plotting methods used in most of the published work related to ACE [19–23]. As shown earlier in Part I (Figure 2) [14] the migration time of the EOF shifted with increasing concentration of L-UCLB, which is due to the increase in viscosity of the MEKC buffer. Therefore, we had to allow for viscosity correction when calculating the L-UCLB effective mobilities (see section 2.3.1). Table 1 shows the four equations used to estimate the binding constant K_1 between TM- β -CD and L-UCLB after multiplying the mobilities with the viscosity correction factor (v). In the equations shown in Table 1, μ_f is the effective mobility of the free form of L-UCLB without TM- β -CD, μ_i is the effective mobility of the L-UCLB in equilibrium with TM- β -CD in serial CE buffers containing increasing concentrations of TM- β -CD. The μ_c is the effective mobility of the TM- β -CD/L-UCLB complex form at saturating conditions, which is difficult to measure experimentally but can be easily obtained from any standard nonlinear regression software [15–16]. The v is the viscosity correction factor, as we described earlier. The effective mobilities of L-UCLB (μ_f and μ_i) were calculated at different concentrations of TM- β -CD.

The four equations shown in Table 1 are equivalent in their algebraic forms. However, the experimental precision on the dependent and independent variables will affect the correlation differently [13]. This difference will be dependent on whether the variables are placed in the numerator or denominator of the equation. For instance, the effect of the precision on variable [TM- β -CD] will alter when the data are transferred to $1/[\text{TM-}\beta\text{-CD}]$ for plotting in the X-reciprocal and double-reciprocal methods. Moreover, it is worth mentioning that X-reciprocal, Y-reciprocal, and double-reciprocal methods can not measure the actual mobilities of the complexed L-UCLB cation (μ_c), whereas the nonlinearized regression of isotherm equation can provide the value of μ_c using any standard software [13,15–16]. In general, a good estimation of μ_c can be obtained by measuring the limited effective mobility of the L-UCLB cation at very high TM- β -CD concentration [13,15–16].

The data treatment of the free and complex mobility as well as the viscosity correction is tabulated in Table 2. Upon increasing the concentration of TM- β -CD, an increase in migration time of L-UCLB cation was observed. This relationship indicates a decrease in charge-to-mass ratio of L-UCLB once complexed with TM- β -CD. The time difference between the peaks of L-UCLB and TM- β -CD (the same peak as neutral marker) decreased gradually with the increasing concentration of TM- β -CD, and the binding constant was estimated from this time difference.

Figure 2A–C shows the linearized representations (X-reciprocal, Y-reciprocal, double-reciprocal plots, see Table 1 for equations of the 1:1 binding isotherm for (L-UCLB)-(TM- β -CD) complex). Note that the negative deviation in linearity on the y-axis at the x-axis maxima (low TM- β -CD concentration) of the double-reciprocal plot (Figure 7C) represents the concentration of TM- β -CD at which sample overloading is present. Although the correlation using X-reciprocal method (Figure 2A) was only moderate, the high degree of correlation obtained by Y-reciprocal and double-reciprocal plots (Figure 2B and 2C, respectively) suggests that 1:1 complexation model is acceptable [13].

The binding constants (i.e., K_1) values between TM- β -CD and L-UCLB are listed in Table 3. The deviations of K_1 values were from the errors of different plotting methods [13]. The non-linear isotherm plot of L-UCLB mobility is shown in Figure 2D. As shown, the corrected effective mobility of L-UCLB reached a plateau when the CE buffer contained more than 10 mM of TM- β -CD. The value of K_1 calculated was $1093 \text{ M}^{-1} \pm 123$, which is consistent with the values obtained from the linear plotting methods. In general, the value of K_1 obtained in Table 3 was around 1000 M^{-1} , which is comparable with the binding constants between TM- β -CD and imidazole based ILs [24]. These reported K_1 values implied that TM- β -CD and L-UCLB have relatively high interaction with each other.

3.4 Determination of profen/TM-B-CD binding constant K_2

The second step was to measure the binding constants of one representative PROF (fenoprofen) to TM- β -CD. This experiment was conducted together with the experiments to measure the binding constant of fenoprofen to L-UCLB. The measurements of K_2 and K_i were achieved in one series of experiments by double-reciprocal plotting method. The TM- β -CD concentration in the CE buffers ranged from 1.0 mM to 50.0 mM. Note that in Figure 3A and 3B, the linear plot without L-UCLB (i.e., 0.0 mM L-UCLB) has the smallest slope compared to the other linear plots in the presence of various concentration of L-UCLB. Hence, the slopes of the linear plots in Figure 3 increased as the concentration of L-UCLB increased. Because K_2 is obtained from the ratio of slope/intercept, therefore, the values of K_2 decreased with the increasing concentration of L-UCLB in the CE buffer. The values of K_2 were calculated from these linear regression plots and are shown in Table 4. For the *R*-fenoprofen, the K_2 decreased from $598 \pm 26 \text{ M}^{-1}$ to $198 \pm 22 \text{ M}^{-1}$ upon increasing the concentration of L-UCLB up to 0.8 mM, and then slightly increased to $215 \pm 19 \text{ M}^{-1}$ when the concentration of L-UCLB is further increased 1.0 mM. For the *S*-fenoprofen, the K_2 decreased from $625 \pm 22 \text{ M}^{-1}$ to $207 \pm 24 \text{ M}^{-1}$ and then increased slightly to $226 \pm 21 \text{ M}^{-1}$. The abnormal increase of K_2 upon increasing L-UCLB from 0.8 mM to 1.0 mM is probably caused by the experimental errors that will be explained in the following section (i.e., section 3.2.3). Nevertheless, for both enantiomers, the minimal K_2 values showed at 0.8 mM L-UCLB. This trend implied that the L-UCLB in the buffer interacted with fenoprofen, which in turn reduced the affinity between fenoprofen and TM- β -CD. The increasing amount of L-UCLB in the buffer will cause the decreasing affinity between fenoprofen and TM- β -CD.

3.5 Determination of profen/L-UCLB binding constant K_i

The third step was to estimate the binding constants of fenoprofen to L-UCLB. We respectively added L-UCLB into the 5.0 mM NaOAc buffer at concentrations of 0.0, 0.2, 0.5, 0.8, and 1.0 mM, which were considered the concentration of inhibitors, L-UCLB. Next, the double-reciprocal plots were generated at each fixed concentration of L-UCLB. Figure 4A and 4B showed the pattern of double-reciprocal plots for *R*- and *S*-fenoprofen, respectively. The *R*-squared values of these lines were better than the one for the binding between TM- β -CD and L-UCLB, because 10 kV was applied instead of 20 kV. Table 4 listed the values of slopes and intercepts of these linear regression plots. For the *R*-fenoprofen (columns 2 and 3), the slope values changed from 13.4 ± 0.5 to 37.0 ± 1.6 , while the y-intercept values range from 7134 ± 696 to 8392 ± 155 . The %RSD of the y-intercept values is about 6.0%. In Figure 4, the error bars on the data points were removed for clarity of presentation. The 95% confidence interval (95% CI) of the data point in double reciprocal plot at a fixed concentration of 1.0 mM TM- β -CD but variable concentration of L-UCLB (i.e., 0.8 and 1.0 mM), were $\pm 4820 \text{ V} \times \text{s} \times \text{cm}^{-2}$ and $\pm 3961 \text{ V} \times \text{s} \times \text{cm}^{-2}$, respectively. These 95% CI values are much greater than the ones for the other data points of the respective plots. The deviation of the intercept values (especially for the plot at 0.8 mM L-UCLB) were due to the inaccuracy and irreproducibility of the migration times of fenoprofen at low concentrations of TM- β -CD. For example, under the conditions of 0.8 mM or 1.0 mM L-UCLB, and the concentrations of TM- β -CD at 0.0 or 1.0 mM in the CE

buffers, it took more than 2 hrs for the fenoprofen peaks to elute out and caused greater error on the migration time than at other concentration of L-UCLB. However, this relatively high deviation is acceptable in the literature of biochemistry when determining the type of inhibition [25]. The data for *S*-fenoprofen showed the same trends for the slopes and intercepts with the greatest deviation at 0.8 mM L-UCLB. As we discussed in section 3.2, shown in Figure 4 and Table 4, we believe that the pattern of the double-reciprocal plots with increasing slope values but constant intercept values suggest that the inhibition happened between L-UCLB and the (TM- β -CD)-(fenoprofen) complex is similar to the enzyme-substrate-inhibitor systems [25], and mainly it is a type of competitive inhibition.

The data generated in Table 4 can be used to obtain the inhibition constant between L-UCLB and fenoprofen (K_i) from a secondary plot of which the x-axis is the concentration of the L-UCLB and y-axis is the slope values of the double-reciprocal plots obtained from Table 4 [18,25]. Here the inhibition constant is the dissociation constant of the (L-UCLB)-(fenoprofen) complex [17,18]. As shown in Figure 4, the value of the x-intercept of the linear plot was the negative value of the inhibition constant K_i [18], which was $4.81 \pm 1.18 \times 10^{-4}$ M for *R*-fenoprofen, and $4.73 \pm 1.18 \times 10^{-4}$ M for *S*-fenoprofen. However, strictly speaking, the inhibition constant we obtained was an apparent inhibition constant.

3.6 The binding constant of the enantiomers

In the experiments discussed above to determine the values of K_2 and K_i , the sample used was the racemic mixture of fenoprofen. The separation of the racemic pair was based on the different binding constants between the enantiomers and the two chiral selectors TM- β -CD and L-UCLB. Figure 5 showed the double-reciprocal plots for *R*- and *S*-fenoprofen at a fixed concentration of 0.5 mM L-UCLB using various concentrations of TM- β -CD ranging from 0.0 mM to 50.0 mM. Clearly at low concentration (i.e., below 5 mM TM- β -CD) the regression trendlines for the two enantiomers overlapped, suggesting no chiral resolution. When the concentration of TM- β -CD is greater than 5 mM, the y axis values of the *R*- and *S*-fenoprofen at the same concentration of TM- β -CD deviated from each other with the increasing concentration of TM- β -CD. Table 4 showed the K_2 values for *R*- and *S*-fenoprofen at different concentration of L-UCLB. The K_2 values for *S*-fenoprofen were always greater than *R*-fenoprofen at all concentration of L-UCLB ranging from 0.0 to 1.0 mM. This trend implied that the interaction between *S*-fenoprofen and TM- β -CD was stronger compared to the one between *R*-fenoprofen and TM- β -CD. The addition of L-UCLB did not change this trend, but increase the chiral resolution, which gradually changed from no resolution to partial resolution and then baseline resolution. This trend is consistent with the enantioseparation of profens shown in Part I [14].

4 Concluding remarks

The concepts of enzymatic reactions were successfully applied to determine the mode of inhibition of L-UCLB. Using fenoprofen as a model compound that strongly interacts with TM- β -CD, a series of double reciprocal plot showed a trend of increasing slope values with almost a constant intercept. This trend is in accordance with the observation described in the literature [25], suggesting that the inhibition observed in chiral separation of PROFs is a competitive inhibition. Future studies will be focused on the combined use of the same dual chiral selector (i.e., TM- β -CD and L-UCLB) for the separation of neutral compounds.

Acknowledgments

The present work was supported by the National Institute of Health (2R01-GM062314) and the Petroleum Research Fund (47774-AC7).

List of abbreviations

CD	cyclodextrin
L-UCLB	<i>N</i> -undecenoxy-carbonyl-L-leucinol bromide
PROF	profen
TM-β-CD	2,3,6-tri-O-methyl- β -cyclodextrin

References

1. Wassercheidt, P.; Weldon, T. *Ionic Liquids in Synthesis*. Wiley-VCH; New York: 2003.
2. Visser AE, Swatloski RP, Rogers RD. *Green Chem* 2000;2:1–4.
3. Anderson JL, Ding R, Ellern A, Armstrong DW. *J Am Chem Soc* 2005;127:593–604. [PubMed: 15643883]
4. Anderson JL, Armstrong DW. *Anal Chem* 2003;75:4851–4858. [PubMed: 14674463]
5. Anderson JL, Armstrong DW. *Anal Chem* 2005;77:6453–6462. [PubMed: 16194112]
6. He L, Zhang W, Zhao L, Liu X, Jiang S. *J Chromatogr A* 2003;1007:39–45. [PubMed: 12924549]
7. Kaliszan R, Marszall MP, Markuszewski M, Baczek T, Pernak J. *J Chromatogr A* 2004;1030:263–271. [PubMed: 15043278]
8. Shamsi SA, Danielson ND. *J Sep Sci* 2007;30:1729–1750. [PubMed: 17623452]
9. Rizvi SA, Shamsi SA. *Anal Chem* 2006;78:7061–7069. [PubMed: 17007537]
10. Qi S, Cui S, Chen X, Hu Z. *J Chromatogr A* 2004;1059:191–198. [PubMed: 15628141]
11. Francois Y, Varenne A, Sirieix-Plenet J, Gareil P. *J Sep Sci* 2007;30:751–760. [PubMed: 17461117]
12. Francois Y, Varenne A, Juillerat E, Villemain D, Gareil P. *J Chromatogr A* 2007;1155:134–141. [PubMed: 17222416]
13. Neubert, RHH.; Ruttinger, H., editors. *Affinity capillary electrophoresis in pharmaceuticals and biopharmaceuticals*. Marcel Dekker; New York: 2003.
14. Wang B, He Jun, Shamsi SA. *Electrophoresis*. 2009in Press
15. Karakasyan C, Tavern M, Millot M. *J Chromatogr A* 2004;1032:159–164. [PubMed: 15065792]
16. Rundlett KL, Armstrong DW. *J Chromatogr A* 1996;721:173–186.
17. Copeland, RA. *Enzymes: a practical introduction to structure, mechanism, and data analysis*. Vol. 2. Wiley-VCH; New York: 2000.
18. Smith, HJ.; Simons, C. *Enzymes and their inhibition: drug development*. CRC press; Boca Raton: 2005.
19. Gomez FA, Avila LZ, Chu Y, Whitesides GM. *Anal Chem* 1994;66:1785–1791. [PubMed: 8030787]
20. Rundlett KL, Armstrong DW. *Electrophoresis* 1997;18:2194–2202. [PubMed: 9456034]
21. Rundlett KL, Armstrong DW. *Electrophoresis* 2001;22:1419–1427. [PubMed: 11379966]
22. Heegaard NHH, Nissen MH, Chen DDY. *Electrophoresis* 2002;23:815–822. [PubMed: 11920866]
23. Heegaard NHH. *Electrophoresis* 2003;24:3879–3891. [PubMed: 14661223]
24. Mwongela SM, Numan A, Gill NL, Agbaria AR, Warner IM. *Anal Chem* 2003;75:6089–6096. [PubMed: 14615986]
25. Barkhimer TV, Kirchhoff JR, Hudson RA, Messer WS. *Anal Biochem* 2005;339:216–222. [PubMed: 15797561]

Figure 1. Schematic description of the interaction system involving TM- β -CD, anionic PROFs (A^-), and chiral cationic IL-type surfactants.

Figure 2. The linearized plots (A–C) and non-linearized plot (D) used to estimate the binding constant K_1 between TM- β -CD and L-UCLB. Each data point is the average value of triplicate runs. The error bars represent 95% confidence intervals (95% CI).

Figure 3.

The double-reciprocal plots used to study the type of inhibition in TM- β -CD/L-UCLB/profen system. A: *R*-fenoprofen, B: *S*-fenoprofen. CE conditions: 5 mM NaOAc, pH 5.0 buffer; fused silica capillary, 64.5 cm total length, 56.0 cm from inlet top to detector, and 50 μ m i.d.; separation temperature 16°C; applied voltage +10 kV. Pressure injection: 5 mbar, 10 sec. Each data point is the average value of triplicate runs. The error bars were removed for clarity of presentation. The average 95% CI of the data points is $\pm 407 \text{ V} \times \text{s} \times \text{cm}^{-2}$ for *R*-fenoprofen (A), and $\pm 424 \text{ V} \times \text{s} \times \text{cm}^{-2}$ for *S*-fenoprofen (B).

Figure 4.

The secondary plot used to estimate the apparent inhibition constant K_i . The y-axis is the slope values of the double-reciprocal plots obtained from Figure 3. A: *R*-fenoprofen, B: *S*-fenoprofen. The x-intercepts marked with circles are the negative values of K_i for the two enantiomers.

Figure 5.

The comparison of double-reciprocal plots for the K_2 calculation of *R*- and *S*-fenoprofen in the presence of 0.5 mM L-UCLB. A: *R*-fenoprofen, B: *S*-fenoprofen. Each data point is the average value of triplicate runs. The error bars were removed for clarity of presentation. The average 95% CI of the data points is $\pm 228 \text{ V} \times \text{s} \times \text{cm}^{-2}$ for *R*-fenoprofen (A), and $\pm 229 \text{ V} \times \text{s} \times \text{cm}^{-2}$ for *S*-fenoprofen (B).

Scheme 1.
competitive inhibition

Scheme 2.
noncompetitive inhibition:

Scheme 3.
uncompetitive inhibition:

Table 1Equations used to estimate K_1 between TM- β -CD and L-UCLB

Plotting method	Regression equation	Calculated K_1
Nonlinear isotherm	$v\mu_1 - \mu_f = K[\text{TM} - \beta - \text{CD}](\mu_c - v\mu_1) = \frac{(\mu_c - \mu_f)K[\text{TM} - \beta - \text{CD}]}{1 + K[\text{TM} - \beta - \text{CD}]}$	Nonlinear regression by software
X-reciprocal	$\frac{v\mu_1 - \mu_f}{[\text{TM} - \beta - \text{CD}]} = -K(v\mu_1 - \mu_f) + K(\mu_c - \mu_f)$	-slope
Y-reciprocal	$\frac{[\text{TM} - \beta - \text{CD}]}{v\mu_1 - \mu_f} = \frac{1}{\mu_c - \mu_f} = [\text{TM} - \beta - \text{CD}] + \frac{1}{K(\mu_c - \mu_f)}$	slope/intercept
Double-reciprocal	$\frac{1}{v\mu_1 - \mu_f} = \frac{1}{K(\mu_c - \mu_f)} \frac{1}{[\text{TM} - \beta - \text{CD}]} + \frac{1}{\mu_c - \mu_f}$	intercept/slope

Table 2
Calculation of the mobility values and viscosity correction factors for K₁

[TM-β-CD] (mM)	t(i) (min)	t(m) (min)	$\mu(i) = (1/t(i) - 1/t(m)) \times L_d \times L_r / V$ (cm ² × V ⁻¹ × s ⁻¹ × 10 ⁻⁴)	t ₀ (min)	v = t/t ₀
0.00	7.60 7.68 7.66	12.22 12.60 12.64	1.524 ± 0.024	15.93	1.000
[TM-β-CD] (mM)	t(i) ^a (min)	t(m) ^b (min)	$\mu(i) = (1/t(i) - 1/t(m)) \times L_d \times L_r / V$ (cm ² × V ⁻¹ × s ⁻¹ × 10 ⁻⁴)	t _c (min)	v = t/t ₀
2.50	8.16 7.93 7.72	11.46 11.10 10.71	1.078 ± 0.015	16.75	1.051
3.00	7.50 7.60 7.83	10.26 10.50 10.88	1.082 ± 0.009	16.15	1.014
3.50	7.52 8.10 8.13	10.25 11.35 11.48	1.071 ± 0.008	16.08	1.009
4.00	8.04 9.06 9.61	11.30 13.39 14.62	1.075 ± 0.004	15.96	1.002
4.50	9.22 8.71 9.63	13.61 12.61 14.49	1.057 ± 0.012	15.94	1.000
5.00	9.15 9.03 9.09	13.32 12.99 13.33	1.033 ± 0.017	16.04	1.007
5.50	10.00 10.57 9.41	15.17 16.42 14.02	1.030 ± 0.019	16.10	1.011
6.00	8.63 8.18 8.53	12.20 11.41 11.93	1.023 ± 0.017	16.16	1.014
7.00	8.30 8.51 8.59	11.43 11.92 12.05	1.004 ± 0.011	16.57	1.040
8.00	9.04 8.53 9.14	12.86 11.87 13.07	0.991 ± 0.001	16.74	1.050
9.00	9.02 8.92 9.12	12.85 12.62 12.94	0.986 ± 0.011	16.63	1.044
10.00	9.04 9.50 10.05	12.74 13.89 14.94	0.982 ± 0.018	16.40	1.029

^at(i): the migration time of L-UCLB.

$b_{t(0)}$: the migration time of neutral marker DMSO.

c_t : the elution time used to calculate the viscosity correction factor.

Table 3The estimated values of K_1 by linear plotting methods

Plotting method	Regression equation	K_1 value
Nonlinear isotherm	$y = -0.05536x/(1+1093x)$	$K_1 = 1093 \pm 123 \text{ M}^{-1}$
X-reciprocal	$y = -0.9534x - 0.054$	$K_1 = -(-0.9534 \pm 0.110) \times 1000 = 953 \pm 110 \text{ M}^{-1}$
Y-reciprocal	$y = -18280x - 15.51$	$K_1 = (-18280 \pm 322)/(-15.51 \pm 1.97) = 1179 \pm 151 \text{ M}^{-1}$
Double-reciprocal	$y = -17.433x - 17902$	$K_1 = (-17902 \pm 351)/(-17.433 \pm 1.537) = 1027 \pm 93 \text{ M}^{-1}$

The standard deviations for non-linear regression and linear regression data were calculated by Origin 7.5 and MS Office Excel 2007 software, respectively.

Table 4
The estimated values of K_2 for *R*- and *S*-fenoprofen from double-reciprocal plots.

[L-UCLB] (mM)	Slope for <i>R</i> form ($V \times s \times M \times \text{cm}^{-2}$)	Intercept for <i>R</i> form ($V \times s \times \text{cm}^{-2}$)	K_2 for <i>R</i> form (M^{-1})	Slope for <i>S</i> form ($V \times s \times M \times \text{cm}^{-2}$)	Intercept for <i>S</i> form ($V \times s \times \text{cm}^{-2}$)	K_2 for <i>S</i> form (M^{-1})
0.00	13.4±0.5	8018±173	598±26	13.1±0.4	8205±139	626±22
0.20	17.6±0.4	8392±155	477±14	17.3±0.4	8595±138	497±14
0.50	20.9±0.2	8162±71	391±5	20.6±0.2	8397±67	408±5
0.80	36.0±1.9	7134±696	198±22	35.7±2.0	7394±734	207±24
1.00	37.0±1.6	7966±614	215±19	36.6±1.8	8280±662	226±21