Simultaneous Determination of Several Antalgic Drugs Based on Their Interactions with β-Cyclodextrin by Capillary Zone Electrophoresis

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Abstract

The binding constants of b-cyclodextrin (β-CD) with antalgic drugs such as naproxen, ketoprofen, ibuprofen, acemetacin, and aspirin are determined by affinity capillary electrophoresis. Based on these interactions, a reliable method for the separation and simultaneous determinations of these compounds in the presence of 5.0mM β-CD in phosphate buffer solution is presented by capillary zone electrophoresis with UV detection at 214 nm for naproxen and 200 nm for the others. The linear ranges for naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine detections are from 2.0 to 800, 2.5 to 1000, 2.5 to 700, 2.5 to 700, 2.0 to 800, and 1.5 to 800 µg/mL, respectively. Their detection limits are 1.0, 0.5, 0.5, 1.5, 1.5, and 1.0 µg/mL at a signal to noise ratio of 3, respectively. This method has been successfully applied to the detections of these drugs in the pharmaceutical formulations (tablets or capsules) and urine samples.

Introduction

Cyclodextrins (CDs) are cyclic nonreducing oligosaccharides with truncated cylindrical molecular shapes (1). Their outside surfaces are hydrophilic, whereas their cavities are hydrophobic (2). They are able to enclose various kinds of drugs in their inner hydrophobic cavities, leading to changes in the physicochemical properties of these drugs (3). Recently, there has been a lot of interest in using CDs as drug delivery media because they lead to an increase of the aqueous solubility, stability, and bioavailability of drugs (4–7). On the other hand, CDs have also extensively been applied to the chiral separation of drugs (8–11). These applications depend on the binding stability of CDs with drugs. Thus, the determination of the binding constants of CDs with drugs is necessary.

Over the last two decades, a number of techniques have been developed to measure the binding constants. The binding constants of β-CD drugs have been estimated mainly by spectroscopic techniques such as circular dichroism (12), ^{1}H-NMR (12–14), and steady-state (15–17) and time-resolved (18–20) fluorescence spectroscopy. These methods are usually used for systems with high-affinity interactions (21). In addition, molecular modeling (22) and differential scanning calorimetric (12) methods have also been used for this purpose. Mura et al. (22) thought the molecular modeling technique was not an accurate method and should be considered only as a preliminary study. Because capillary electrophoresis (CE) possesses the advantages of high speed of analysis, high efficiency, high resolving power, low sample consumption, and a wide range of possible analytes, it is beneficial for the estimation of binding constants (23). Currently, several CE methods are available to measure binding constants [e.g., affinity capillary electrophoresis (ACE), Hummel-Dreyer method (HD), vacancy affinity capillary electrophoresis (VACE), vacancy peak method (VP), and frontal analysis (FA)] (24–26). Among these methods, ACE based on electrophoretic migration data is appropriate for the determination of low and medium stability constants ($K < 10^4 M^{-1}$) (27). Therefore, it is the most suitable method to estimate the binding constant of β-CD with drugs. In addition, this method requires that the analyte must undergo a change in electrophoretic mobility upon complexation; the equilibrium for the complex formation must be much faster than the separation, and sufficient concentrations of both components must be available in the system (28). The binding constants for naproxen, salbutamol, indomethacine, and procaine by β-CD have been determined by ACE with three linear plotting methods (28). Steinbock et al. (29) derived an explicit equation and suggested an experimental method based on ACE to determine the binding constants of the complexes of β-CD with benzoate derivatives.

In the present contribution, ACE is used to study interactions of β-CD with naproxen, ketoprofen, ibuprofen, and acemetacin and to determine their binding constants by a linear plotting method, whereas the binding constant of β-CD with aspirin is determined by a nonlinear curve-fitting method because of an insufficient small change in the μ_{eff} of aspirin at low concentrations upon addition of β-CD. The binding constants of naproxen,

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ketoprofen, ibuprofen, and acemetacin with β-CD obtained are in good agreement with those reported previously (13,30–33). These interactions lead to changes in the electrophoretic mobilities of naproxen, ketoprofen, ibuprofen, and acemetacin in capillary zone electrophoresis (CZE). Based on this, a separation and determination method for naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine is developed by adding 5mM β-CD in the 20mM of phosphate buffer solution (PBS). This method possesses a low detection limit and wide linear range and can be applied to the determination of these drugs in practical samples with a satisfactory result.

Experimental

Reagents

β-CD (Shanghai Biochemical Reagent Co., Shanghai, China) was recrystallized before use. Pure racemates of naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, caffeine, and their formulations (capsules or tablets) were a generous gift from Nanjing Institute for Drug Control (Nanjing, China). All other chemicals were of analytical-reagent grade. The stock solution of 10mM β-CD was prepared with double-distilled water. The solutions containing 1000 µg/mL naproxen, ketoprofen, ibuprofen, aspirin, caffeine, and acemetacin were prepared with 30mM (pH 7.0) PBS, respectively.

Apparatus

Experiments were performed on an Agilent CE system (Agilent Technologies Deutschland Gmbh, Waldbronn, Germany) with a 50-cm × 50-µm i.d. uncoated fused-silica capillary purchased from Yongnian Optical Fiber Factory (Hebei, China). The effective length of the capillary was 41.8 cm.

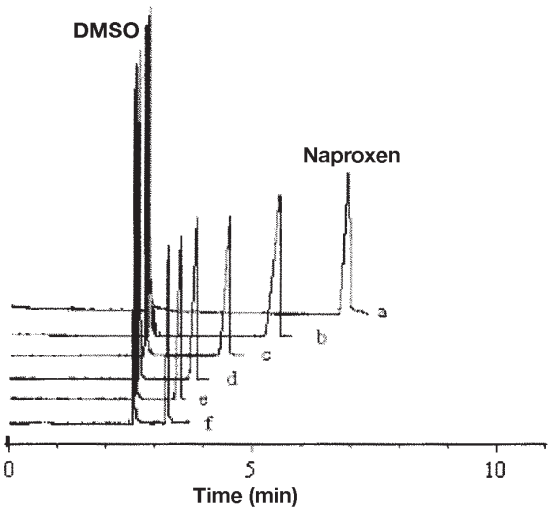

Figure 1. Migration time of naproxen as a function of β-CD concentration in the running buffer: injection of naproxen, 50 mbar, 3 s; voltage, 15KV; temperature, 25°C; UV detection, 214 nm; and buffer, 20mM (pH 7.0) PBS including 0, 0.5, 1.0, 2.5, 5.0, and 7.5mM β-CD for a–f.

Conditioning of capillary

To achieve the maximum reproducibility of the migration time, the new capillary was conditioned by continuously rinsing with 1.0M NaOH for 30 min, 1.0M HCl for 15 min, and double-distilled water for 15 min. Before each injection, the capillary was rinsed with 1.0M NaOH for 2 min, doubled-distilled water for 2 min to avoid the accumulation of β-CD precipitate caused by the use of a saturated β-CD running buffer, and then equilibrated with the running buffer for 3 min.

Results and Discussion

Dependence of migration time of analyte on the concentration of β-CD or drug in the running buffer

Two methods are available when ACE is used to determine the binding constant of β-CD with drug. The first method is to inject a drug solution into the running buffers containing β-CD at successively increasing concentrations. With the increasing β-CD concentration, the migration time of the drug decreases. At pH 7.0, the drug is in the anionic deprotonated form, whereas β-CD is a nonionic compound. When the drug is included in the cavity of β-CD, the formed complex has a charge identical to that of free drug. Thus, the charge-to-mass ratio of the complex is less than that of the free drug, leading to $\mu_c < \mu_f$, where μ_c and μ_f are the electrophoretic mobilities of the drug in its complexed and free forms, respectively. With the increasing concentration of β-CD, the effective electrophoretic mobility of drug, m_{eff}, decreases according to following equation (28):

$$\mu_{eff} = s_f\mu_f + s_c\mu_c \qquad \text{Eq. 1}$$

where x_c and x_f are the fraction of the complexed and free drug, respectively. From the equation

$$\mu_{app} = \mu_{eo} + \mu_{eff} \qquad \text{Eq. 2}$$

where μ_{app} and μ_{eo} are the apparent mobility and the mobility of electroosmosis, respectively, μ_{app} increases with the decrease of μ_{eff} because the direction of μ_{eff} is opposite to that of μ_{eo}, and the absolute value of μ_{eo} is larger than that of μ_{eff}. Thus, the migration time of the drug decreases with the increasing concentration of β-CD. Figure 1 shows the change of the migration time for naproxen when the β-CD concentration of the running buffer increases from 0 to 7.5mM.

The second method is to change the drug concentration in the running buffer and inject β-CD solution into the buffer. Contrary to the change of effective electrophoretic mobility of drug upon the addition of β-CD, the effective electrophoretic mobility of β-CD in the complex form is higher than that of free β-CD. Thus, the migration time of β-CD increases with an increasing drug concentration because of the formation of a more negatively charged complex of β-CD with drug.

Determination of the binding constant of β-CD with drug

The effective electrophoretic mobility is determined from the migration times of the analyte, *t*, and the electroosmosis marker,

t_{eo}, according to the following expression (29):

$$\mu_{eff} = \frac{L_c L_e}{V} \cdot \left(\frac{1}{t} - \frac{1}{t_{eo}}\right) \quad \text{Eq. 3}$$

where L_e is the effective length of the capillary, L_c the total length of the capillary, and V the applied voltage.

β-CD usually binds the drug molecule in a binding stoichiometry of 1:1 (13,22,28), thus the relationship between the binding constant K and the effective electrophoretic mobility of the analyte can be described by the following equation (28):

$$\frac{1}{\mu_{eff} - \mu_f} = \frac{1}{(\mu_c - \mu_f) \cdot K} \cdot \frac{1}{[B_0]} + \frac{1}{\mu_c - \mu_f} \quad \text{Eq. 4}$$

where $[B_0]$ is the concentration of β-CD in the buffer. The K value can be obtained from the intercept and slope of the plot of $1/\mu_{eff} - \mu_f$ versus $1/[B_0]$. Our experimental results indicate that the linear plotting method is unsuitable for the inclusion of drug by the lig and at a high concentration in the running buffer. Thus, a nonlinear analysis method is used for this system (29):

$$\mu_{eff} = \left(\frac{\mu_c}{[A_0]}\right) \cdot \left[\frac{[A_0] + [B_0] + (1/K)}{2} - \left(\frac{1}{4}([A_0] + [B_0] + (1/K))^2 - [A_0]\,[B_0]\right)^{\frac{1}{2}}\right] \quad \text{Eq. 5}$$

where and is the initial concentrations of β-CD and drug for the aspirin system, respectively. can be obtained from the plot of μ_{eff} versus $[B_0]$ with the nonlinear curve fitting method.

Based on the effective mobilities of the analytes at different β-CD concentrations obtained under the conditions shown in Table I, the binding constants of β-CD with naproxen, ibuprofen, ketoprofen, and acemetacin are calculated with the linear plotting method to be 510, 323, 3660, and 896M^{-1}, respectively. The values of binding constants of β-CD with naproxen and acemetacin are comparable with those reported by fluorescence (30) and NMR spectroscopy (13). The difference between our result and that reported by Bellini et al. (28) for the binding of β-CD with naproxen was caused by the different buffers used. The results for the binding of β-CD with naproxen, ketoprofen, and ibuprofen show obvious differences from those reported by Faucci et al. (22). These values were obtained by a molecular modeling method. As pointed out by the authors of that paper, this is not an accurate method and should be considered only as a preliminary study. Compared with the reported values by the ACE (32) and spectrophotometry methods (33), the binding constant of ibuprofen with β-CD is acceptable. According to the molecular characteristics of β-CD, a binding stoichiometry of 1:2 between ketoprofen and β-CD is possible. However, the binding stoichiometry of 1:1 has been reported (22,31). Here, the plot of $1/(\mu_{eff} - \mu_E)$ versus $1/[B_0]$ shows a good linearity, thus the binding stoichiometry can be thought to be 1:1.

The binding constant of β-CD with aspirin cannot be obtained using the linear plotting method because the change in the μ_{eff} of aspirin, upon the addition of β-CD, is too small to be detected. Thus, we injected 10mM β-CD into the buffer containing 10–60mM aspirin, as shown in Table I, to determine the μ_{eff} of β-CD on the basis of ACE–indirect absorbance detection. The migration time of the negative peak of β-CD increases with the increasing concentration of aspirin. Figure 2 shows the relation between the μ_{eff} and aspirin concentration. With the nonlinear curve fitting according to equation 5, the binding constant of β-CD obtained with aspirin is 193M^{-1}. To our best knowledge, no study on the interaction of β-CD with aspirin has been reported. Aspirin possesses a salicylate structure; the binding constant of β-CD with salicylate has been reported to be 299 or 155M^{-1} obtained with an explicit or simplified expression (29). Thus, it is reasonable to consider our value for the binding constant of β-CD with aspirin to be exact.

Table I. Experimental Conditions and Results for the Estimation of Binding Constants of β–CD with Drugs by ACE*

Drug	Naproxen	Ketoprofen	Ibuprofen	Acemetacin	Aspirin
Sample solution	250 µg/mL napproxen	250 µg/mL ketoprofen	250 µg/mL ibuprofen	250 µg/mL acemetacin	10mM β-CD
Component contained in running buffer	0–7.5mM β-CD	0–5.0mM β-CD	0–3.5mM β-CD	0–3.5mM β-CD	10–60mM aspirin
Electroosmosis marker	DMSO†	DMSO	DMSO	DMSO	water
Detection wavelength	214 nm	200 nm	200 nm	200 nm	254 nm
Parameter in $y = a + bc$‡					
a	1516	0.851	1.017	1.949	–
b	2.972	2.635	0.278	2.175	–
This method	510 ± 34	323 ± 26	3660 ± 147	896 ± 42	193 ± 23§
K(M^{-1})				> 2500 (32)	
Reference value	475 (30)	353 (31)	2900 (33)	1100 (13)	–

* Buffer, 20mM (pH 7.0) PBS; injection, 50 mbar, 3 s; voltage, 15KV; and temperature, 25°C.
† DMSO = dimethylsulfoxide.
‡ c = concentration of β-CD.
§ Nonlinear curve fitting method.

Table II. Calibration Curves and Parameters for Determination of Naproxen, Ketoprofen, Ibuprofen, Acemetacin, Aspirin, and Caffeine at an Interior Marker Concentration of 100 µg/mL

Drug	Parameters in $y = a + bc$ (n = 15, c: µg/mL) a	b	Correlation coefficient	Linear range (µg/mL)	Detection limit at s/n 3 (µg/mL)
Naproxen	0.0006 ± 0.0002	0.0036 ± 0.0001	0.9986	2.0–800	1.0
Ketoprofen	0.0017 ± 0.0005	0.0047 ± 0.0000	0.9998	2.5–1000	0.5
Ibuprofen	–0.0028 ± 0.0006	0.0066 ± 0.0001	0.9992	2.5–700	0.5
Acemetacin	0.0009 ± 0.0002	0.0024 ± 0.0000	0.9997	2.5–700	1.5
Aspirin	0.0027 ± 0.0008	0.0073 ± 0.0001	0.9996	2.0–800	1.5
Caffeine	0.0017 ± 0.0009	0.0054 ± 0.0004	0.9990	1.5–800	1.0

Separation of naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine

The mixture of several antalgic drugs (naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine) was introduced into the capillary by a pressure of 50 mbar for 3 s. In 20mM (pH 7.0) PBS, these drugs could not be separated without the presence of β-CD. Upon the addition of 5mM β-CD to the PBS, the baseline separation of these drugs could be achieved by applying a voltage of 15KV at 25ºC (shown in Figure 3). As mentioned, the addition of β-CD resulted in a change of the mobility of drug because of its inclusion by β-CD. The change was related to the binding ability, producing different migration times of these drugs and achieving their baseline separation. From Figure 3, the efficiency for the final optimized CE separation was estimated to be from 310,000 to 780,000 plates/m, which was located in the range of general separation efficiency. The slightly broad peaks were possibly caused by the very slight differences in binding of the chiral drugs, which resulted in partial chiral separation.

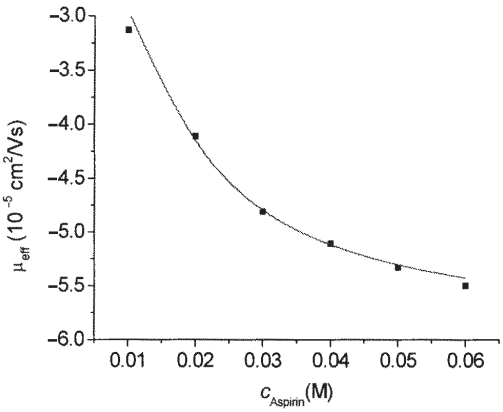

Figure 2. Effective mobility of β-CD as a function of aspirin concentration in the running buffer: injection of 10.0mM β-CD, 50 mbar, 3 s; voltage, 15 KV; temperature, 25°C; UV detection, 200 nm; and buffer, 20mM (pH 7.0) PBS.

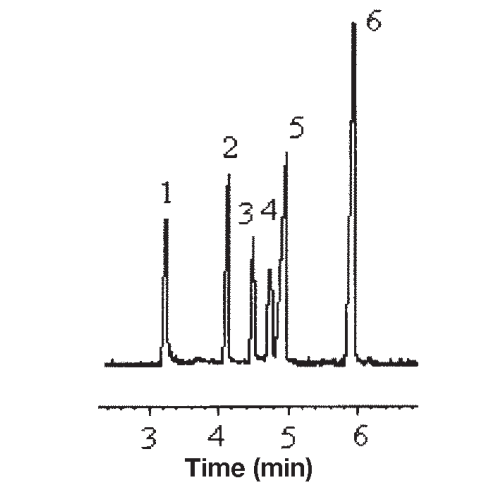

Figure 3. Separation of naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine: buffer, 5.0mM β-CD in 20mM (pH 7.0) PBS; injection of mixture, 50 mbar, 3 s; voltage, 15KV; temperature, 25°C; UV detection, 214 nm; and peaks 1–6: caffeine, ibuprofen, acemetacin, ketoprofen, naproxen, and aspirin.

Calibration curves for naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine

For analysis purposes, the UV detection for naproxen was performed at a wavelength of 214 nm and the others at a wavelength of 200 nm. The CZE peak area of each drug was in direct proportion to its concentration over a certain concentration range. Table II gives their linear regression equations together with the standard deviations, correlation coefficients, linear ranges, and detection limits. The linear ranges for naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine detections were from 2.0 to 800, 2.5 to 1000, 2.5 to 700, 2.5 to 700, 2.0 to 800, and 1.5 to 800 µg/mL, respectively. Their detection limits were 1.0, 0.5, 0.5, 1.5, 1.5, and 1.0 µg/mL at a response three times the

Table III. Results for Determination of Naproxen, Ketoprofen, Ibuprofen, Acemetacin, Aspirin, and Caffeine in the Pharmaceutical Formulation (Tablets or Capsules)

Drug	Method of pharmacopoeia	Drug (mg dosage unit^{-1}) Average found (n = 3)	RSD (%)	Deviation from the method of pharmacopoeia (%)
Naproxen	99.2	97.7	1.2	98.7
Ketoprofen	50.5	49.6	1.6	98.2
Ibuprofen	50.0	51.3	1.8	102.6
Acemetacin	24.7	24.2	2.3	98.0
Aspirin	99.4	98.9	1.3	99.5
Caffeine	49.3	50.9	1.9	103.2

Table IV. Recovery Assays for the Spiked Urine Samples

Drug	Spike (µg/mL)	Average found (n = 3) (µg/mL)	RSD (%)	Average recovery (n = 3) (%)
Naproxen	5	4.76	2.6	100.2
	10	10.5	1.8	
	20	20.9	2.1	
	50	47.9	1.2	
Ketoprofen	5	4.80	2.4	98.7
	10	9.98	2.7	
	20	19.4	1.9	
	50	51.0	2.1	
Ibuprofen	5	5.25	1.9	102.8
	10	9.92	1.3	
	20	20.8	2.4	
	50	50.1	1.8	
Acemetacin	5	5.05	3.1	99.3
	10	10.2	2.7	
	20	19.5	2.3	
	50	51.5	1.5	
Aspirin	5	5.20	2.9	102.0
	10	9.82	3.4	
	20	20.9	2.3	
	50	49.2	1.9	
Caffeine	5	4.84	2.8	99.8
	10	10.4	2.6	
	20	19.5	1.8	
	50	51.9	2.1	

noise level, respectively. In comparison with the results for naproxen, ketoprofen, ibuprofen, and caffeine obtained with reversed-phase high-performance liquid chromatography (34), this method gives a lower detection limit and much wider linear range; at the same time, it also keeps the advantages of CZE.

Determination of naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine in their pharmaceutical formulations and the urea samples

Contents of naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine in their pharmaceutical formulations (capsules or tablets) were determined with the present method. The results are listed in Table III. The relative standard derivation (RSD) of triplicate determinations of each drug in each selected formulation was less than 2.3%, displaying a good precision. The deviations of the found contents from the claimed values in their corresponding formulations were between 98.0–103.2%. Thus, this method is very acceptable for practical application.

The recovery assays were performed in the spiked urine samples. Urine samples were spiked in triplicate with six antalgic drugs at three levels (10, 20, and 50 µg/mL) that were within the working linear range of these drugs. The average recovery was calculated as the mean value obtained by spiking the analyte studied at three levels. The RSD of triplicate determinations of each drug in the spiked urine sample was less than 3.4%. The average recovery for each drug was from 98.7% to 102.0% (given in Table IV), indicating a good accuracy. Thus, the method is reliable.

Conclusion

The binding constants of β-CD with antalgic drugs such as naproxen, ketoprofen, ibuprofen, and acemetacin are determined by the ACE method based on the effect of β-CD on their mobility, whereas that of aspirin is determined based on its effect on β-CD mobility. The obtained binding constants are comparable to those reported previously. The complexation results in a change of the electrophoretic mobility of these antalgic drugs. The difference in the binding ability produces the different migration times of these drugs, which has been used to achieve the baseline separation for simultaneous determinations of naproxen, ketoprofen, ibuprofen, acemetacin, aspirin, and caffeine. This proposed detection method shows a low detection limit, wide linear range, reliable accuracy, and good precision and could be applied to the analysis of practical sample. This work proves that β-CD is indeed a good additive to improve the separation of these drugs that possess similar mobility in absence of β-CD and thus provides a promising method for the separation and determination of other drugs.

Acknowledgments

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