Affinity Capillary Electrophoresis Studies on the Influence of Alcohols on the Interaction of β-Cyclodextrin with Non-Steroidal Anti-Inflammatory Drugs

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Summary

The influences of methanol, ethanol, 1-propanol and 1-butanol on the binding constants of β-cyclodextrin (β-CD) with non-steroidal anti-inflammatory drugs such as acemetacin, indometacin, cinmetacin, sulindac and diclofenac sodium and the separation of these drugs were studied by affinity capillary electrophoresis. No obvious effect was observable upon addition of methanol up to 6% (*v/v*) in the running buffer, while the addition of other alcohols at the concentration of 2% resulted in obvious decrease in the binding constants of β-CD with acemetacin, indometacin, cinmetacin and sulindac. With an increasing chain length of added alcohols, all of these changes increased. Upon additions of different alcohols in the running buffer the change of the binding constant of β-CD with diclofenac sodium was inconspicuous. Based on these results, the separation conditions for these drugs were optimized. The presence of 6% methanol in the running buffer containing 3 mM β-CD was helpful to the baseline separation of these drugs. The electrophorograms of these drugs in the presence of ethanol, 1-propanol and 1-butanol showed a worse separation due to the decrease in the binding constants. The methods for the separation of these drugs and the study on the binding constants possess the advantages of easy performance, high speed and low sample consumption.

Introduction

Cycoldextrins (CDs) are cyclic oligosaccharides possessing an interior hydrophobic cavity, which can enclose various kinds of drugs to alleviate undesirable properties of these drug molecules [1] and serve as potent drug carriers in the immediate release and delayed release-formulations [2]. Generally, the drug formulations contain some additives such as buffer salts, anti-microbial agents, surfactants and alcohols [3]. Alcohols are often used as the third components of the CD-drug systems to achieve the different purposes [3, 4–6]. Thus, the impacts of these additives, particularly alcohols, on the binding of CDs with drugs have attracted great attention. Among different cyclodextrin compounds β-CD is the most generally used complexing agent because of its complexing ability [7].

The binding constants of β-CD with drugs have been estimated by spectroscopy such as circular dichroism spectroscopy (CD) [8], ^{1}H-nuclear magnetic resonance (^{1}H-NMR) [9, 10] and steady-state [11–13] and time-resolved [14, 15] fluorescence spectroscopy. These methods are usually used for the system with high-affinity interaction [16]. In addition, molecular modeling [17] and differential scanning calorimetric [8] methods have also been used for this purpose. Recently, capillary electrophoresis (CE) has been extensively used for the estimation of the binding constant due to its advantages of high analytical speed, high efficiency, high resolving power and low sample consumption [18]. Currently, several CE methods are available to measure the binding constant, e.g. affinity capillary electrophoresis (ACE), Hummel-Dreyer method (HD), vacancy affinity capillary electrophoresis (VACE), vacancy peak method (VP) and frontal analysis (FA) [19–21]. Among these methods, ACE is appropriate for the system with a low and medium stability constant ($K < 10^4$ M^{-1}) [22] and a fast formation rate of the complex [7]. This work uses this technique to study the influence of alcohols on the binding constants of β-CD with non-steroidal anti-inflammatory drugs such as acemetacin, indometacin, cinmetacin, sulindac and diclofenac sodium.

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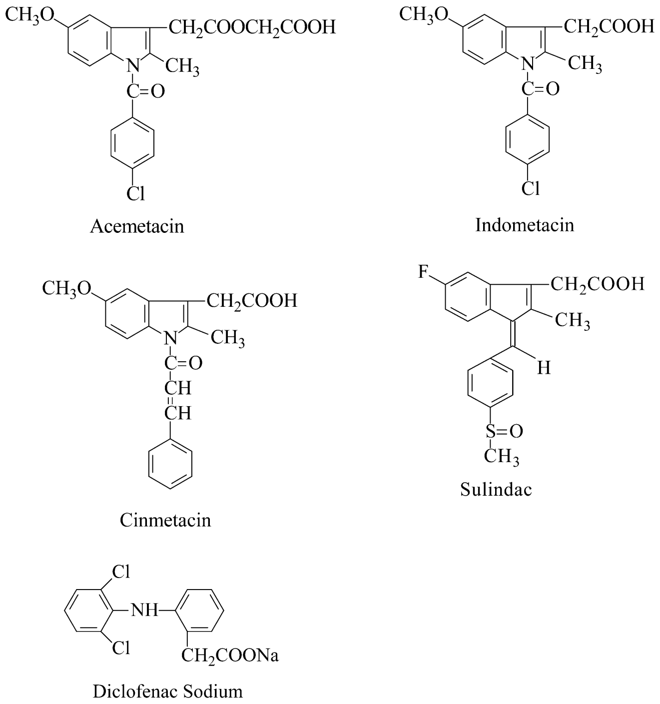

Figure 1. Formulas of the investigated drugs.

The binding constants of β-CD with acemetacin, indometacin, sulindac and diclofenac sodium have been determined by NMR for acemetacin [9] and diclofenac sodium [23], ultraviolet spectroscopic method for sulindac [24] and ACE for indometacin [7], to our best knowledge, however, the value of binding constant of β-CD with cinmetacin and the influence of alcohols on these binding constants have not been reported yet. Based on these results a separation method for these drugs is developed by adding β-CD and methanol in the running buffer. The presence of methanol in the running buffer improves the separation and the additions of ethanol, 1-propanol and 1-butanol make the separation worse because they prevent the binding of these drugs by β-CD. This work provides a simple method to study the influence of alcohol on the bindings of cycoldextrins with drugs with the advantages of easy performance, high speed and low sample consumption.

Experimental

Apparatus and Chemicals

Experiments were performed on an Agilent capillary electrophoresis system (Agilent Technologies Deutschland Gmbh, Waldbronn, Germany) with a 50 cm × 50 µm (i.d.) uncoated silica capillary purchased from Yongnian Optical Fiber Factory, Hebei, China. The effective length of the capillary was 41.5 cm.

β-CD (from Shanghai Biochemical Reagent Co.) was recrystallized before used. Pure powders of acemetacin, indometacin, cinmetacin, sulindac and diclofenac sodium were a generous gift from Nanjing Institute for Drug Control (Nanjing). Their formulas were shown in Figure 1. All other chemicals were of analytical reagent grade. All solutions were prepared with deionized water of 18 MΩ purified from a Milli-Q purification system. The solutions containing 10 mM β-CD, 1.0 mg mL^{-1} acemetacin, diclofenac sodium, and 0.25 mg mL^{-1} indometacin were prepared with deionized water, respectively. 1.0 mg mL^{-1} cinmetacin, and sulindac were prepared with ethanol, respectively. All buffer solutions were filtered through a membrane filter of 0.25-µm pore size.

Conditioning of Capillary

To achieve the maximum reproducibility of migration time, the new capillary was conditioned by continuously rinsing with 1.0 M NaOH for 30 min, 1.0 M HCl for 15 min and deionized water for 10 min. Before each injection the capillary was rinsed with 1.0 M NaOH for 2 min and deionized water for 1 min to avoid precipitation of β-CD in the capillary and then equilibrated with the running buffer for 2 min.

Procedures

Binding Constant Determination

A drug solution was injected to the running buffers containing β-CD with a successive increasing concentration. The migration time of the drug was determined at 25 °C with an ultraviolet detection at 214 nm. The effective electrophoretic mobility of the drug was calculated from its migration time and the time of electroosmotic flow [25]. Then, the binding constant of β-CD with the drug was obtained based on the dependence of its electrophoretic mobility on the β-CD concentration in the running buffer [7].

Separation of Drugs

The separation conditions of these drugs were optimized by studying the effects of voltage and the running buffer on the electrophorogram. The electrophorogram was obtained with an ultraviolet detection at 214 nm and 25 °C by injecting a mixture of these drugs to the running buffer with a pressure of 50 mbar and an injection time of 3 s.

Results and Discussion

Dependence of Migration Time of Drugs on β-CD Concentration

At pH 8.4 these non-steroidal anti-inflammatory drugs are in the anionic deprotonated form, while β-CD is a non-ionic 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 a smaller m_c value than that of m_f, where m_c and m_f is the electrophoretic mobility of drug in its complexed and free forms, respectively. However, the electrophorogram of the drug in presence of β-CD shows only one peak due to the dynamic balance between the drug molecules in the cavity of β-CD and the running buffer. Furthermore the equilibrium for the complex formation is much faster than the separation. With the increasing concentration of β-CD in the running buffer the absolute value of effective electrophoretic mobility of the drug, m_{eff}, decreases according to following equation [7]: $m_{eff} = x_f m_f + x_c m_c$, where x_c and x_f is the fraction of the complexed and free drug, respectively. Here m_{eff} is a negative value. From the equation of $m_{app} = m_{eo} + m_{eff}$, where m_{app} and m_{eo} is the apparent electrophoretic mobility of the drug and the mobility of electroosmotic flow, respectively, m_{app} increases with the decreasing absolute value of m_{eff}. Thus, the migration time of the drug decreases with the increasing concentration of β-CD. As an example, Figure 2 shows the dependence of the migration time of the indometacin on the β-CD concentration.

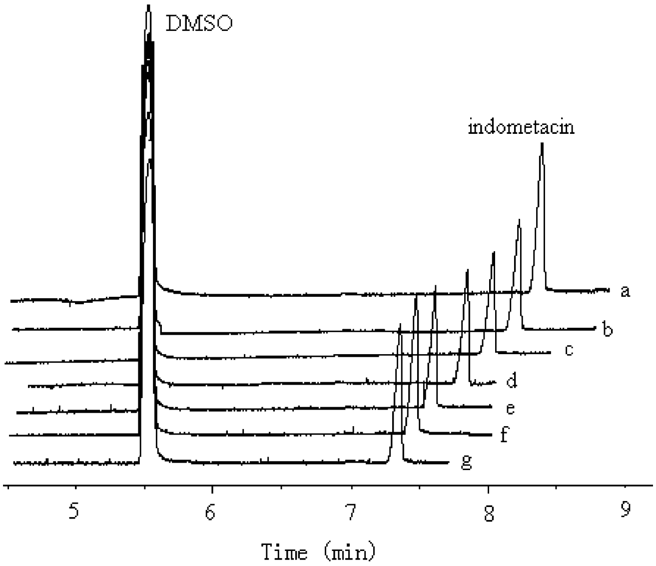

Figure 2. Migration time of indometacin as a function of β-CD concentration in the running buffer. Injection of indometacin: 50 mbar, 3 s; voltage: 15 KV; buffer: 35 mM pH 8.4 borate including 0, 0.2, 0.4, 0.8, 1.6, 2.5, 3.5 mM β-CD for a–g.

Determination of the Binding Constant of β-CD with Drug

β-CD can bind acemetacin [9], indometacin [7], sulindac [24] and diclofenac sodium [26] in a binding stoichiometry of 1:1. Therefore, the relationship between the binding constant K and the change in the effective electrophoretic mobility of the drug can be described by the following equation [7]:

$$\frac{1}{m_{eff} - m_f} = \frac{1}{(m_c - m_f) \cdot K} \cdot \frac{1}{[B_0]} + \frac{1}{m_c - m_f}$$

where $[B_0]$ is the concentration of β-CD in the buffer. The K value can be obtained from the intercept and slope of plot of $\frac{1}{m_{eff} - m_f}$ vs. $\frac{1}{[B_0]}$.

The binding constants of β-CD with acemetacin, indometacin, sulindac and diclofenac sodium in the presence of various alcohols are shown in Table I. The binding constant values of 896 M^{-1} for acemetacin and 76 M^{-1} for diclofenac sodium are comparable with those obtained with NMR spectroscopy [9, 23]. The values of 124 M^{-1} for sulindac and 408 M^{-1} for indometacin are also comparable with that reported in [24] with an ultraviolet spectroscopic method and [7] with the ACE method, respectively. The plot of $\frac{1}{m_{eff} - m_f}$ vs. $\frac{1}{[B_0]}$ for the complex of β-CD with cinmetacin shows a linear graph, thus, it is reasonable to assume the binding stoichiometry of β-CD with cinmetacin is also 1:1. From the intercept and slope of the plot, a binding constant of 334 M^{-1} for cinmetacin by β-CD is obtained.

The results shown in Table I indicate the addition of different alcohols except methanol in the running buffer has an obvious effect on the binding of acemetacin, indometacin, cinmetacin or sulindac by β-CD. In the case of methanol these values of binding constant are close to those in running buffer without presence of alcohol. With the increasing chain length from methanol to 1-butanol, all of the binding constants decrease, which is similar to those reported for the influence of alcohol on the inclusion of naphthalene [3], naproxen [4], 2-naphthol [5] by β-CD. Thus, we conclude that the alcohols with long chain prevent the binding of these drugs by β-CD and the prevention becomes stronger when the chain length of these alcohols increases. These observations have been explained with a competition between alcohols and drugs for the CD cavity [5]. It has been reported that the binding constants of methanol, 1-propanol and 1-pentanol by β-CD are 0.32, 3.7, and 63 M^{-1}, respectively [27]. Obviously, the values increase monotonically with increasing chain length due to the increase in their hydrophobicity. It is reasonable to think that the effects of ethanol and 1-butanal also obey this rule. With increasing chain length, more alcohol molecules occupy the space of the β-CD cavity, thus the binding constant of β-CD with the drug decreases.

When the concentration of alcohols increases, the effect of alcohols on the binding becomes greater. At the 1-butanol concentration of 6% (v/v) in the running buffer, the change of the electrophoretic mobility with increasing β-CD concentration is inconspicuous, indicating that these drugs could not be complexed by β-CD in the presence of 6% 1-butanol.

The binding constant of β-CD with diclofenac sodium almost retains at a constant value. It can be seen from Figure 1 that diclofenac sodium possesses different structure from other four drugs. Both its molecular size and the binding constant by β-CD in the buffer without presence of alcohol are smaller than those of other four drugs. Thus, the effect of these alcohols on its binding by β-CD is insignificant.

Separation of Acemetacin, Indometacin, Cinmetacin, Sulindac and Diclofenac Sodium

Effect of β-CD on Separation

The mixture of several non-steroidal anti-inflammatory drugs such as acemetacin, indometacin, cinmetacin, sulindac and diclofenac sodium was introduced into the capillary by a pressure of 50 mbar for 3 s. When 70 mM borate running buffer without presence of β-CD was used, only diclofenac sodium could be separated completely due to its much higher charge-to-mass ratio (Figure 3a), and other four

Table I. Binding constants (M^{-1}) of β-CD with drugs in presence of various alcohols.

Drug	Non-alcohol		2% various alcohols			
	This method	Reference value	Methanol	Ethanol	l-Propanol	l-Butanol
Acemetacin	896 ± 42	1100 [9]	838 ± 27	348 ± 38	183 ± 12	93 ± 23
Indometacin	408 ± 23	423 [7]	415 ± 26	275 ± 13	116 ± 20	101 ± 16
Cinmetacin	334 ± 19	–	323 ± 35	284 ± 19	148 ± 10	61 ± 12
Sulindac	124 ± 16	139 [24]	131 ± 14	125 ± 20	75 ± 14	15 ± 7
Diclofenac sodium	76 ± 11	100.6 [23]	77 ± 13	81 ± 18	64 ± 8	89 ± 11

Buffer: 35 mM pH 8.4 borate; voltage: 15 KV; temperature: 25 °C; injection: 50 mbar, 3s.

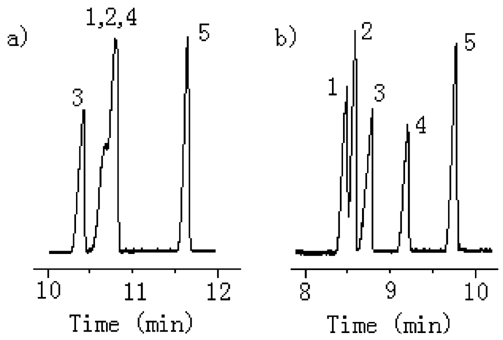

Figure 3. Separation of **1** = acemetacin, **2** = indometacin, **3** = cinmetacin, **4** = sulindac and **5** = diclofenac sodium in the running buffers of a) 70 mM pH 8.4 borate and b) 70 mM pH 8.4 borate including 3 mM β-CD. Voltage: 15 KV.

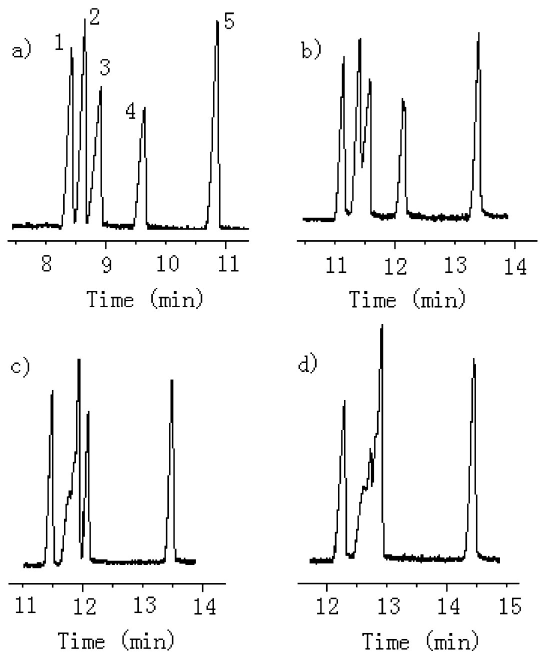

Figure 4. Influence of various alcohols on the separation of acemetacin, indometacin, cinmetacin, sulindac and diclofenac sodium. Voltage: 15 KV; buffer: 70 mM pH 8.4 borate including 3 mM β-CD and 6% a) methanol, b) ethanol, c) 1-propanol or d) 1-butanol; **1** = acemetacin, **2** = indometacin, **3** = cinmetacin, **4** = sulinac and **5** = diclofenac sodium.

drugs could not be separated well, though the electrophorogram showed one peak for cinmetacin. The peaks for acemetacin, indometacin and sulindac showed a complete overlap. The addition of 3 mM β-CD in the running buffer improved obviously the separation (Figure 3b). The electrophorogram showed five peaks corresponding to five drugs in the mixture. As mentioned above, the addition of β-CD resulted in a change of the mobility of these drugs due to the formation of β-CD-drug complexes. This change was related to the binding ability of these drugs to the cavity of β-CD, producing different migration times of these drugs. However, a cooperation of some factors such as the binding ability, molecular structure and molecular weight resulted in the baseline separation of acemetacin from indometacin could not be realized.

Effect of Methanol on Separation

Organic additives such as alcohol, acetone and acetonitrile have been proved to be useful to improve the separation [28]. The general mechanism of the improvement is due to the effects of organic modifiers on the viscosity and conductivity of running buffer, the electroosmotic flow and the effective electrophoretic mobility of analyte [29]. In absence of β-CD the addition of methanol in the running buffer did not improve the separation of these drugs except a slight change in their migration times. The change in the migration time was due to the effect of methanol on the viscosity of the running buffer, the electroosmotic flow and thus the apparent electrophoretic mobility of these drugs [29]. Upon addition of methanol in the running buffer containing 3 mM β-CD, the separation of five non-steroidal anti-inflammatory drugs was further improved. With increasing concentration of methanol the separation of indometacin from acemetacin became better. When the concentration of methanol was at 6% (v/v) the electrophorogram of the mixture showed a baseline separation (shown in Figure 4a). Obviously, this improvement resulted from the effect of methanol on the apparent electrophoretic mobility of the complexes. Higher concentrations than 6% would result in a negative effect on the electrophorogram due to a larger change in the viscosity and polarity of the running buffer, which made the separation worse. Thus the presence of 6% methanol was necessary for the complete separation of these drugs.

Effects of Ethanol, 1-Propanol and 1-Butanol on Separation

The effects of ethanol, 1-propanol and 1-butanol on the separation of the mixture in presence of β-CD were shown in Figure 4b–d, respectively. With increasing chain length of alcohols the separability of these drugs became worse systematically. When the running buffer contained 6% 1-propanol the electrophorogram of the mixture showed a shape similar to that without presence of β-CD and alcohol. These phenomena resulted from the changes in both the viscosity and polarity of the running buffer and the binding constants of these drugs by β-CD. All of the binding constants of acemetacin, indometacin, cinmetacin and sulindac by β-CD were less than 200 M^{-1} in presence of 2% 1-propanol (Table I). At a high concentration of 1-propanol these values of binding constant would further decrease and more drug molecules were in their free form, which eliminated the improvement in the separation by addition of β-CD. In the case of 1-butanol the binding constants became less, thus the electrophorogram showed a worse separation.

Conclusions

Added ethanol, 1-propanol or 1-butanol shows an obvious impact on the binding ability of β-CD with some non-steroidal anti-inflammatory drugs. With the increasing chain length of the alcohols the binding constants of acemetacin, indometacin, cinmetacin and sulindac by β-CD decrease. Addition of methanol does not affect the inclusion of these drugs by β-CD. The presence of β-CD improves greatly the separation of these drugs and diclofenac sodium. The addition of 1-propanol or 1-butanol eliminates the improvement due to the competition between alcohol molecules and drug molecules for the CD cavity. The presence of methanol in the running buffer can further improves the separation of five drugs due to its effect on the electrophoretic mobility of these β-CD-drug complexes. A baseline separation can be achieved at the methanol concentration of 6%. This work provides the optimal conditions for the separation and detection of non-steroidal anti-inflammatory drugs by capillary electrophoresis and indicates that ACE technique is a simple method to evaluate the influence of alcohol on the binding constant of β-CD with drug.

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