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Determination of the kinetic rate constant of cyclodextrin supramolecular systems by high performance affinity chromatography

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ABSTRACT

It is challenging and extremely difficult to measure the kinetics of supramolecular systems with extensive, weak binding ($K_a < 10^5\ M^{-1}$), and fast dissociation, such as those composed of cyclodextrins and drugs. In this study, a modified peak profiling method based on high performance affinity chromatography (HPAC) was established to determine the dissociation rate constant of cyclodextrin supramolecular systems. The interactions of β-cyclodextrin with acetaminophen and sertraline were used to exemplify the method. The retention times, variances and the plate heights of the peaks for acetaminophen or sertraline, conventional non-retained substance (H_2O) on the β-cyclodextrin bonded column and a control column were determined at four flow rates under linear elution conditions. Then, plate heights for the theoretical non-retained substance were estimated by the modified HPAC method, in consideration of the diffusion and stagnant mobile phase mass transfer. As a result, apparent dissociation rate constants of 1.82 (±0.01) s^{-1} and 3.55 (±0.37) s^{-1} were estimated for acetaminophen and sertraline respectively at pH 6.8 and 25 °C with multiple flow rates. Following subtraction of the non-specific binding with the support, dissociation rate constants were estimated as 1.78 (±0.00) and 1.91 (±0.02) s^{-1} for acetaminophen and sertraline, respectively. These results for acetaminophen and sertraline were in good agreement with the magnitude of the rate constants for other drugs determined by capillary electrophoresis reported in the literature and the peak fitting method we performed. The method described in this work is thought to be suitable for other supramolecules, with relatively weak, fast and extensive interactions.

1. Introduction

Cyclodextrins (CDs) have been utilized in pharmaceutical formulations to increase aqueous solubility of poorly soluble drugs and the bioavailability. For cyclodextrin supramolecular systems in aqueous solution, it is assumed that the free and bound drugs exist in a state of equilibrium, which is determined by the equilibrium binding constant (K_a).

The K_a is a useful thermodynamic index to estimate the binding strength between drugs and cyclodextrins. The K_a values for a range of drugs and small molecules have been reported to be less than $10^5\ M^{-1}$, indicating that the interactions between drugs and cyclodextrins are relatively weak [1,2]. A wide variety of organic and inorganic molecules can be included into the hydrophobic cavity of cyclodextrins through noncovalent interactions, indicating the extensive binding of cyclodextrins with small molecules.

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The field of cyclodextrin inclusion is being driven by structural studies, providing information about stoichiometry, geometry, association sites and heterogeneity. Although the kinetics of association and dissociation are fundamental to the formation and the in vivo functions of cyclodextrin supramolecules [3–5], quantitative determination has proved challenging [6–8]. The relaxation time of the supramolecules is short (<1 s) and the high time resolution required is difficult to achieve [9]. As pointed out by Bohne, kinetic studies are necessary to provide the 'movie' in addition to the 'snapshots' taken from structural and thermodynamic measurements (as shown in Eqs. (1) and (2)) [10].

$$D + CD \underset{k_d}{\overset{k_a}{\rightleftarrows}} D - CD \tag{1}$$

$$K_a = \frac{k_a}{k_d} \tag{2}$$

where k_a was the association rate constant and k_d was the dissociation rate constant. These two parameters are also named as on-rate (k_{on}) and off-rate (k_{off}) constants in kinetic studies for drug-target interactions [6–8].

Up to now, few studies have been reported focusing on the kinetics of cyclodextrin supramolecules [11]. Fluorescence correlation spectroscopy (FCS) [12] has been employed to compare the complexation kinetics of pyronines and analyze the individual steps during association and dissociation. However, this method is not applicable in the kinetic study of most drug molecules without fluorescence. Recently, surface plasmon resonance (SPR) [13] and capillary electrophoresis (CE) [14] have also been employed to estimate the rate constants of cyclodextrin-drug interactions. However, the results from the SPR experiments were three orders of magnitude smaller than those from the CE experiments. In addition, the difficulty to detect solutes with low concentrations for SPR and the relatively poor reproducibility for CE also limits their application in interactions with weak to moderate affinities. Therefore, it is of special interest to establish an efficient methodology to measure the kinetics of cyclodextrin supramolecules with extensive, weak binding and fast dissociation.

In comparison, with advantages of good speed, high precision and ease of automation, HPAC is possibly one of the best techniques to study the kinetics of interactions with weak to moderate affinities. Since 1980s, Hage's group has employed chromatographic techniques based on HPAC, including the band broadening (plate height method [15,16] and peak profiling method [17–20]), peak decay [21–23] and split peak [24,25] methods, to study the kinetics of drug–human serum albumin (HSA) and antibody–antigen interactions [26,27]. These methods can be applied to perform kinetic studies for systems that range from weak-to-moderate affinities to those with high affinities, and good correlation with results have been reported for the same analytes by other methods can be obtained.

The band broadening measurements have been used to examine the rate constants of interactions between R-, S-warfarin and D-,L-tryptophan with HSA over a range of temperatures [15,16]. The peak profiling method has been used with data acquired at single flow rates [17] or at multiple flow rates [18] to determine the dissociation rate of L-tryptophan from HSA. Modified data analysis for the corrections of stagnant mobile phase mass transfer and the non-specific binding between drugs and the support have also been developed and used to examine the dissociation kinetics of carbamazepine and imipramine from HSA columns [18–20]. The peak decay and split peak methods have been employed to examine the kinetics of drugs–HSA, α_1-acid glycoprotein (AGP) interactions and antibody–antigen interactions [21–26].

However, there are no reports as far as we are aware about the application of HPAC in the kinetic study of cyclodextrin–drug interactions. In consideration of the requirement of the precise measurement of peak variances on multiple columns for band broadening studies, small columns and high flow rates for peak decay and split peak methods, the peak profiling method has gained great interest in recent kinetic studies of solute–protein binding [28,29].

In this study, the kinetics of cyclodextrin–drug interactions, with extensive, weak binding and fast dissociation, was investigated by the modified peak profiling method of HPAC techniques. Acetaminophen and sertraline were used as candidates with different binding affinities to represent the interactions of drugs with β-cyclodextrin. The principal objectives were therefore (i) to develop a modified HPAC method for the kinetic measurement of cyclodextrin–drug interactions. Considering the extensive affinity of cyclodextrins to most small molecules, a correction was made to estimate the plate height for theoretical non-retained substances;(ii) to estimate dissociation rate constants of β-cyclodextrin–acetaminophen (sertraline) interactions at a single flow rate and multiple flow rates and to investigate the non-specific binding of drugs to the control support; and (iii) to evaluate the accuracy of the method by comparing the results of this work with those obtained using the CE and the peak fitting method.

2. Theory

2.1. Peak profiling theory

The peak profiling method is a useful chromatographic tool to study the kinetics of biological reactions. The theoretical derivation for this approach was first reported in 1975 by Denizot and Delaage [30]. In the peak profiling method, the retention times and variances were measured on an affinity column for both a retained solute and a non-retained substance using linear zonal elution. Initially, the peak profiling method was carried out at a single flow rate and the apparent dissociation rate constant ($k_{d,app}$) was estimated as follows.

$$k_{d,app} = \frac{2t_M^2(t_R - t_M)}{\sigma_R^2 t_M^2 - \sigma_M^2 t_R^2} \tag{3}$$

where t_R and σ_R^2 are the retention time and variance of the peak for the retained solute on an affinity column, while t_M and σ_M^2 are the retention time and variance of the peak for the non-retained substance on the same affinity column. In Eq. (3), it was assumed that all sources of band broadening other than stationary phase mass transfer were either negligible or the same for the retained and non-retained species.

A modified form of the peak profiling method at multiple flow rates was further developed [28,29], as shown in Eq. (4).

$$H_R - H_M = \frac{2uk}{k_{d,app}(1+k)^2} \tag{4}$$

where H_R is the plate height of the retained solute on an affinity column, and H_M is the plate height of the non-retained substance on the same column. The term k is the retention factor for the retained solute on the affinity column. The term u is the linear velocity of the mobile phase. The value of $k_{d,app}$ can be determined by plotting $(H_R - H_M)$ versus $(uk)/(1+k)^2$, which would result in a linear relationship with a slope inversely related to $k_{d,app}$.

If the interaction of solutes with the inert column support cannot be regarded as negligible, additional studies should be carried out on the control column, to determine the mass transfer contributions due to processes other than interactions between analytes and the stationary phase. Hage's group also derived the multi-site