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Multianalyte determination of the kinetic rate constants of drug–cyclodextrin supermolecules by high performance affinity chromatography

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ABSTRACT

The kinetics of the dissociation is fundamental to the formation and the in vivo performance of cyclodextrin supramolecules. The individual determination of the apparent dissociation rate constant ($k_{d,app}$) using high performance affinity chromatography (HPAC) is a tedious process requiring numerous separate studies and massive data fitting. In this study, the multianalyte approach was employed to simultaneously measure the $k_{d,app}$ values of three drugs through one injection based on the investigation of the dependence of drug–cyclodextrin interaction kinetics on the mobile phase composition. As a result, the $k_{d,app}$ values increased when decreasing the ion strength, increasing the ionization of drugs and adding extra organic solvents. The values of $k_{d,app}$ for acetaminophen, phenacetin and S-flurbiprofen estimated by the multianalyte approach were 8.54 ± 1.81, 5.36 ± 0.94 and $0.17 \pm 0.02\ s^{-1}$, respectively, which were in good agreement with those determined separately (8.31 ± 0.58, 5.01 ± 0.42 and $0.15 \pm 0.01\ s^{-1}$). For both of the single and multiple flow rate peak profiling methods, the results of the multianalyte approach were statistically equivalent with that of the single compound analysis for all of the three drugs ($p > 0.05$). The multianalyte approach can be employed for the efficient evaluation of the drug–cyclodextrin kinetics with less variance caused by cyclodextrin column bleeding.

1. Introduction

It is challenging and extremely difficult to measure the kinetics of supramolecular systems with extensive, weak binding (the equilibrium binding constant $K_a < 10^5\ M^{-1}$), and fast dissociation, such as those composed of cyclodextrins and drugs [1]. Up to now, few studies have been reported focusing on the kinetics of cyclodextrin supramolecules. The lack of fluorescence for most drug molecules, the difficulty to detect solutes with low concentrations and the relatively poor reproducibility have limited the application of traditional techniques like fluorescence [2], surface plasmon resonance [3] and capillary electrophoresis to study the kinetics of cyclodextrin supramolecules [4].

With advantages of rapid speed, high precision and ease of automation, the high performance affinity chromatography (HPAC) is possibly one of the best techniques to measure 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 [5,6] and peak profiling method [7–10]), peak decay [11–13] and split peak [14,15] methods, to study the kinetics of drug–human serum albumin (HSA) and antibody–antigen interactions [16,17]. Good correlation with results reported for the same analytes by other methods has been obtained.

They modified the data analysis of the peak profiling method for the corrections of stagnant mobile phase mass transfer and the non-specific binding between drugs and the support, and

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examined the apparent dissociation rate constants of carbamazepine and imipramine from HSA columns [8–10]. Based on their modified peak profiling, another modified peak profiling HPAC method based on the correction of the plate height for the non-retained substance was employed to determine the apparent dissociation rate constant of acetaminophen and sertraline–cyclodextrin supramolecules in our previous study [18]. The apparent dissociation rate constants ($k_{d,app}$) of 1.82 ± 0.01 and $3.55 \pm 0.37\ s^{-1}$ were estimated for acetaminophen and sertraline respectively at pH 6.80 and 25 °C with multiple flow rates, which were in good agreement with the magnitude of the rate constants for other drugs determined by capillary electrophoresis reported in the literature [4].

Although the influences of the drug concentration and flow rate on the kinetic rate constants determination have been investigated in prior study about drug–HSA interactions [7–10] and in our previous study about drug–cyclodextrin interactions [18]. And the effect of mobile phase composition on the binding kinetics of D-,L-tryptophan on HSA-immobilized column has been studied [6], much less is known about the effects of the mobile phase composition on the $k_{d,app}$ determination of the drug–cyclodextrin interactions. As regards the effects of the mobile phase composition on the drug–cyclodextrin interaction, buffers are known to be included into the cyclodextrin cavity. As the buffer concentration increases, solute peaks become sharper and the retention time decreases. For example, ammonium nitrate can strongly bind with the primary hydroxyl groups in cyclodextrin and can be used to reduce solute retention for strongly retained analytes. For pH, a combination of pH adjustment and complexation with cyclodextrin has been successfully used to solubilize compounds. Normally, the more lipophilic form of a given drug molecule has a greater affinity to the hydrophobic cyclodextrin cavity than the ionized form. Thus, the unionized form has a higher binding constant value. However, ionization of a poorly water-soluble drug will increase the solubility and if the increase in solubility is greater than the decrease in binding constant, then an increase in the complexation efficiency will be observed [19]. Organic solvents increase aqueous solubility of nonpolar drugs by reducing the hydrogen bond density in the aqueous mixture. The tendency of the drug molecule to enter the cyclodextrin cavity decreases with decreasing polarity of the complexation media, or the drug–cyclodextrin complexation may be hampered by competition of solvent with the drug for a space in the cavity. In addition, the organic solvent molecules may participate in the complexation through the formation of drug–cyclodextrin–solvent ternary complexes. Thus, the organic solvents can both increase and decrease drug–cyclodextrin interaction and the effect is concentration dependent [20]. Thus information on the effects of mobile phase composition on $k_{d,app}$ values would be useful in determining accurate estimation of $k_{d,app}$ and the drug–cyclodextrin interactions, especially for ionized drugs [21].

In addition, the determination of $k_{d,app}$ for different drugs individually is time consuming due to the large number of repeated chromatography tests required coupled with massive data fitting. The difficulties are often exacerbated due to the limited lifetime of the cyclodextrin column (CD column). There is an increasing interest in the concurrent administration of multiple substrates to characterize the molecular interactions, the multianalyte approach, or the so called “cocktail” approach [22]. Generally, the multianalyte approach of chromatography testing has been investigated as a screening tool for potential in vivo drug-drug interactions [23,24]. Compared to the individual administration of specific probes in multiple studies, simultaneous administration of multiple in vivo probes of drug metabolizing enzymes offers very distinct advantages. The most obvious advantage of the multianalyte approach is to minimize the confounding influence of inter-individual and intra-individual variability over time and the program cost [25]. There are also other potential benefits, such as simultaneous assessment of multiple interactions in the same study, and minimization of the effect of intra-individual variability. For above reasons, especially to ensure the minimization of the error caused by column bleeding of the CD column, it is important to investigate the application of the multianalyte approach in the kinetic studies of drugs–cyclodextrin supermolecules.

In this study, the effects of the mobile phase composition on the kinetics of drug–cyclodextrin supramolecular system will be considered using the non-steroidal anti-inflammatory drug S-flurbiprofen as the model drug. The investigation will be carried out by estimating the apparent dissociation rate constants for this system through our previously reported modified peak profiling methodology, as performed for mobile phases over different buffer types, buffer concentration and buffer pH, and the percentage of acetonitrile added in the mobile phase. Then, the multianalyte approach will be validated by simultaneous measurement of the $k_{d,app}$ values for the selected three drugs through one injection, using acetaminophen, phenacetin and S-flurbiprofen as model drugs. The results will then be compared with that determined by the conventional method testing single compound by individual measurement.

2. Theories

2.1. Peak profiling theory

For the peak profiling method [26], 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, assuming that all sources of band-broadening other than stationary phase mass transfer were either negligible or the same for the retained and non-retained solutes [26].

$$k_{d,app} = \frac{2t_M^2(t_R - t_M)}{\sigma_R^2 t_M^2 - \sigma_M^2 t_R^2} \quad (1)$$

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.

A modified form of the peak profiling method at multiple flow rates was further developed [7–10], as shown in Eq. (2).

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

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 $k_{d,app}$.

Hage’s group also derived the multi-site model to calculate k_d using mass balance (as shown in Eq. (3)) and examined the multisite interactions of carbamazepine [9], imipramine [9], phenytoin metabolites [10] with HSA.

$$H_R - H_M = \frac{uk}{(1+k)^2}\left[\frac{2\alpha_1}{k_d} + \frac{2\alpha_{control}}{k_{d,control}}\right] \quad (3)$$

where α_1 is the fraction of the total retention factor that is due to the solid phase-drug interactions on the affinity column and $\alpha_{control}$ is the fraction of the total retention factor that is due to the nonspecific binding of the retained solutes on the control column. The