KINETIC STUDIES OF DRUG-PROTEIN INTERACTIONS USING HIGH-PERFORMANCE AFFINITY MICROCOLUMNS AND PEAK PROFILING

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Introduction

Affinity chromatography is a type of liquid chromatography that employs biologically-related interactions for the separation and analysis of specific analytes within a sample. High-performance affinity chromatography (HPAC) is an adaptation of affinity chromatography that combines the selective nature of affinity chromatography with the small, rigid support particles of HPLC systems, given a system that is capable of withstanding high pressures and flow rates that its low-performance counterpart.

Human serum albumin (HSA) is a major transport protein in blood for carrying various endogenous and exogenous compounds throughout the body. This protein can greatly influence drug distribution and can play a major role in affecting drug absorption, distribution, metabolism, and excretion. Binding to HSA also allows hydrophobic drugs to be more soluble in blood and increases the overall lifetime of a drug before it is metabolized. Protein binding in blood plays an important role in the pharmacokinetics and pharmacodynamics of many drugs. For instance, warfarin and carbamazepine are two common drugs that are known to have significant binding to HSA.

Rate constant measurements for drug-protein interactions involve HSA are important for many drugs in describing and predicting the behavior of these agents in the circulation and in the body. Approaches that have been previously used in HPAC to measure these dissociation rate constants have included band-broadening methods, such as techniques based on plate height and peak profiling measurements, and peak fitting methods.

In this study, the dissociation rate constants for carbamazepine, verapamil, diazepam, disopyramide and lidocaine with HSA were determined by using HPAC along with affinity microcolumns and peak profiling. The peak profiling method was used to compare the elution profiles for each drug and a non-retained species on an HSA column and control column over a broad range of flow rates, with the resulting data then being used to estimate the rate constants for each of the injected drugs with HSA.

Theory of Peak Profiling

The following equation can be used for the single binding site by an analyte in an HPAC column.

$$H_R\text{-}H_M=\frac{2uk}{k_d(1+k)^2}=H_k \qquad (1)$$

H_R: total plate height measured for the analyte on a column containing a binding agent
H_M: total plate height measured on the same column for a non-retained solute
k: retention factor for the analyte
u: linear velocity of the mobile phase
H_k: plate height contribution due to stationary phase mass transfer

For a system following a two-site binding model:

$$H_R\text{-}H_M=\frac{uk}{(1+k)^2}\left(\frac{2\alpha_{HSA}}{k_{d,HSA}}+\frac{2\alpha_n}{k_{d,n}}\right)=\frac{2uk_{HSA}}{k_{d,HSA}(1+k)^2}+\frac{2uk_n}{k_{d,n}(1+k)^2} \qquad (2)$$

$k_{d,HSA}$: dissociation rate constant for the analyte with HSA
k_{HSA}: retention factor for the analyte due to the interactions of analyte with HSA
α_{HSA}: fraction of the total retention factor due to the binding of analyte with HSA
$k_{d,n}$: dissociation rate constant for analyte due to non-specific binding sites
k_n: retention factor for analyte due to non-specific binding
α_n: fraction of the total retention factor due to non-specific binding

Methods

In this study, the control and HSA columns were 1 cm and 2.5 cm × 2.1 mm i.d. The mobile phase was pH 7.4, 67 mM potassium phosphate buffer. The samples for each drug and sodium nitrate (used as a non-retained solute) were prepared in this mobile phase. All band-broadening measurements were carried out using five replicate injections. The final peak profiling experiments were performed by injecting 20, 15, 10, 7.5, and 5 µM carbamazepine verapamil, diazepam, lidocaine or disopyramide and 20 µM sodium nitrate onto the HSA column, control column and union at flow rates ranging from 0.25 to 1.25 mL/min at 37°C and 25°C.

The final solute concentrations were selected because of their ability to provide linear elution conditions on the microcolumns. The retention time and variance of each chromatographic peak was obtained by using Peakfit 4.12 along with an exponentially modified Gaussian (EMG) fit and the linear progressive baseline plus residual options of this program.

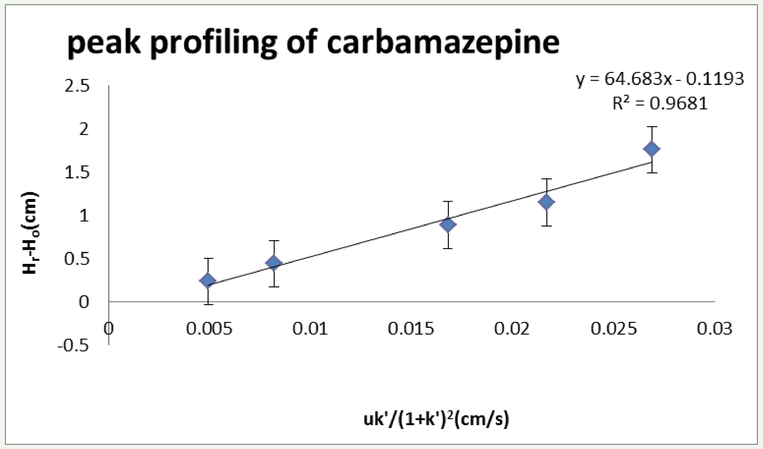
Results

Table 1. Retention and plate height data for injections of disopyramide on a 1cm HSA column at 25° C (under linear elution conditions)

Flow rate (mL/min)	Retention factor, k′	uk′/ (1+k)²	H_r - H_m
0.25	2.97 (±0.25)	0.0265	0.0059
0.50	2.79 (±0.30)	0.0504	0.0146
0.75	2.45 (±0.13)	0.0828	0.0156
1.00	2.08 (±0.40)	0.107	0.0624
1.25	2.02 (±0.07)	0.136	0.0691

The final $k_{d,HSA}$ value that was obtained was 3.19(±0.24) s^{-1}.

Figure 1. Peak profiling plot for carbamazepine on a 1cm HSA column at 25° C



The final $k_{d,HSA}$ that was found from the slope of the above plot was 0.028 (±0.025) s^{-1}.

Table 2. Retention and plate height data for injection of carbamazepine on an 1cm HSA column at 25° C (under linear elution conditions)

Flow rate (mL/min)	k′	uk′/ (1+k)²	Hr-Hm
0.25	3.14 (± 0.37)	0.00495	0.240
0.5	4.05 (± 0.37)	0.00821	0.445
0.75	3.67 (± 0.37)	0.0168	0.890
1	3.55 (± 0.37)	0.0217	1.150
1.25	3.39 (± 0.37)	0.0269	1.760

In this project, for 2.5 cm x 2.1 mm i.d. microcolumn, carbamazepine was used as the drug model, and the final k_d that was obtained was 0.031 (±0.120) s^{-1}. This value has small difference compared with the k_d obtained from 1cm x 2.1 mm i.d. microcolumn (0.028 (±0.025) s^{-1}).

Table 4. Dissociation rate constants for different drugs using 1cm HSA column at 37° C and reference values

drug	k_d 37° C	Reference values
Verapamil	0.28 (± 0.10)	0.38 (± 0.05)
Diazepam	0.45 (± 0.04)	0.44 (± 0.02)
Disopyramide	0.28 (± 0.16)	Not reported
lidocaine	0.35 (± 0.14)	Not reported
carbamazepine	0.51 (± 0.15)	0.67 (± 0.04)

Conclusion

- The goal of this study was to examine the binding of drugs with HSA by using affinity microcolumns with HPAC and the peak profiling method. Dissociation rate constants were measured under linear elution conditions and at several temepratures for various drugs.
- The peak profiling method was used with both 1 cm and 2.5 cm long HSA microcolumns. The measured dissociation rate constants gave small differences for the different lengths of columns from the results of carbamazepine. This indicated that the measured dissociation rate constants were not related to the length of the column.
- The results for 37° C gave good agreement for the drugs with the results from other methods but do vary with temperature.
- The use of affinity microcolumns is attractive for this work because they need much smaller amounts of protein than more traditional HPLC column and they provide shorter analysis times.
- The peak profiling method described in this work is not limited to carbamazepine, verapamil, diazepam, lidocaines and disopyramide but could also be used as a fast method for studying the kinetics of other drug-protein interactions.

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