Determination of Drug Plasma Protein Binding by Solid Phase Microextraction

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ABSTRACT: The plasma protein binding of drugs has been shown to have significant effects on the quantitative relationship between clinical pharmacokinetics and pharmacodynamics. In many clinical situations, measurement of the total drug concentration does not provide the needed information concerning the unbound fraction of drug in plasma, which is available for pharmacodynamic action. Therefore, the accurate determination of unbound plasma drug concentrations is important in understanding drug action. Many methodologies exist for determining the extent of plasma protein binding, but different methods produce a rather wide range of results for the same compound at the same concentration level. The solid phase microextraction (SPME) method reported in the present study attempts to eliminate many experimental variables that could lead to the lack of reproducibility, such as the variable content of organic solvent or ionic strength in plasma, pH shifts, and volume shifts. Five well-known drugs were chosen to study plasma protein binding: ibuprofen, warfarin, verapamil, propranolol, and caffeine, with high, intermediate and low binding properties. Dilution of plasma with isotonic PBS or incubation with 10% CO_2 in the atmosphere was found to compensate for changes in pH during incubation. The data obtained using these pH-controlled methods correlate well with the average values of plasma protein binding found in the literature. SPME, which uses an extraction phase that dissolves or adsorbs the drug of interest and rejects proteins, overcomes several limitations of currently available techniques and is a thermodynamically sound method, since the measurements are always performed at equilibrium. Compared to other methods, SPME offers several advantages: small sample size, short analysis time, possibility to automate, and ability to directly study complex samples. J Pharm Sci 95:1712–1722, 2006

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INTRODUCTION

Determining the amount of drug binding to plasma proteins is an essential step in both drug discovery and in clinical phases of drug development. Binding of drugs to plasma proteins is important in understanding the pharmacokinetics and pharmacodynamic relationship of a drug.[1–3] Therefore, plasma protein binding (PPB) is normally recognized as an important factor in assessing drug disposition, efficacy, and safety.[4] In the early drug development stage, the knowledge of drug protein binding property is essential in extrapolating preclinical animal data to predict the drug's efficacy and toxicity in human subjects. Moreover, plasma protein binding propensity of a drug also affects the amount of drug available to

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diffuse into target tissues, for example brain,[5–7] the calculation of *in vivo* hepatic clearance,[8] and the interpretation of the drug's bioavailability.[1]

Although the main drug-binding proteins are albumin and alpha 1-acid glycoprotein, plasma contains many other proteins; consequently, there is a high probability that many small molecules will exhibit some levels of binding. To determine the extent of PPB, the molecule should be tested directly in a protein-binding assay using plasma or serum. This is a critical step in characterizing the distribution of a small molecule with respect to the plasma compartment.[5,9]

The investigation of binding parameters has received significant attention since its importance was recognized at the beginning of the 20th century. Different aspects of drug-protein interactions have been reviewed, including their molecular nature, biological functions, pharmacological significance, as well as methodological approaches applied and their potential shortcomings.[10,11] Several methods have been developed to measure the free concentration of drugs, and most involve the physical separation of free and bound fractions followed by conventional analysis. Examples of separation techniques include equilibrium dialysis, ultrafiltration, ultracentrifugation, and gel filtration. These techniques are usually time consuming, can suffer loss of analyte to membranes, can generate errors due to protein leakage or Donnan effects, and can create a shift in concentrations and binding equilibrium during separation.[9,12,13] Recently developed chromatographic methods based on columns with immobilized human serum albumin[9,14,15] allow only the assay of the fraction of drugs bound to albumin, and not to whole plasma, in conditions that are very different from physiological ones. The same is true about methods based on electrospray ionization mass spectrometry (ESI-MS), which work only with certain buffer solutions and when the combination ratio between receptor and ligand is 1:1. Capillary electrophoretic (CE) methods have been applied successfully for both 1:1 and 1:n combination ratios between receptor and ligand, but they are restricted as well to certain buffer solutions, a single protein at a time, and do not allow precise control of the temperature.[12,16,17] An interesting technique, rarely mentioned even in review articles, consists of measuring the partitioning of the drug between plasma and blood cells and the partitioning between buffer and blood cells; the ratio of the two partition coefficients yields the free fraction.[18] A recent advancement consists of using solid-supported lipid membranes instead of blood cells, but the method is still cumbersome.[19] Whereas chromatographic methods used for the assay of free concentrations and binding constants assume a very fast equilibrium between ligand and receptor, ultrafiltration and ultracentrifugation techniques assume a slow equilibrium so the free fraction can pass through a membrane without shifting the equilibrium in the other compartment. Of the methods applied so far for the study of binding constants, only dialysis, distribution in blood cells, ultracentrifugation, and ultrafiltration allow the determination of binding ratio in plasma. Since these methods have their own limitations, new technologies for fast and accurate determination of plasma protein binding must be developed.

Solid phase microextraction (SPME) has been applied to the determination of binding constants or free concentrations,[20–23] but only in the simple case of an interaction between a drug and a specific protein, or when negligible extraction conditions were met. As a result of its simplicity, reliability, flexibility, and possibility to eliminate solvent usage during sample preparation, SPME has become a well-known technique for analysis of volatile and semivolatile substances. Recently, this sample preparation method has begun to receive increasing attention for applications involving nonvolatile polar compounds in biological matrices.[24–26] SPME as a new sampling alternative not only provides simplicity but also allows the investigation of supplementary parameters, like the binding constant and the free concentration of analyte in a sample. ESI-MS, CE, and chromatographic methods for the determination of plasma protein binding work only when the samples are dissolved in a select few buffer solutions, but the SPME method seems not to be limited by the type of extraction media and the concentration range, with the selection of a suitable extraction phase (chemical composition, volume, and length).

Compared to other methods, SPME offers several advantages: small sample size, short analysis time, possibility to automate, and ability to directly study complex samples (e.g., whole blood). The study of binding equilibria is no longer restricted to certain buffer solutions, but can be performed in any "natural" environment, by suitable choice of experimental conditions. With the introduction of new extraction phases (restricted access materials,[27,28] molecularly imprinted polymers,[29] fibers with immobilized