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A method for rapid screening of interactions of pharmacologically active compounds with albumin

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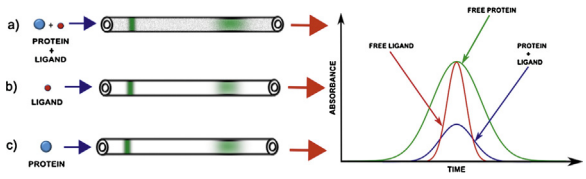
HIGHLIGHTS

- We developed a method for determination of the association constants.
- The method was tested for known selected drugs with albumin.
- The method was applied for interaction of new tropane derivatives with albumin.

GRAPHICAL ABSTRACT



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ABSTRACT

We determine the association constants for ligand–protein complex formation using the flow injection method. We carry out the measurements at high flow rates ($F = 1$ mL min^{-1}) of a carrier phase. Therefore, determination of the association constant takes only a few minutes. Injection of 1 nM of the ligand (10 μL of 1 μM concentration of the ligand solution) is sufficient for a single measurement. This method is tested and verified for a number of complexes of selected drugs (cefaclor, etodolac, sulindac) with albumin (BSA). We obtain $K = 4.45 \times 10^3$ M^{-1} for cefaclor, $K = 1.00 \times 10^5$ M^{-1} for etodolac and $K = 1.03 \times 10^5$ M^{-1} for sulindac in agreement with the literature data. We also determine the association constants of 20 newly synthesized 3β- and 3α-aminotropane derivatives with potential antipsychotic activity – ligands of 5-HT$_{1A}$, 5-HT$_{2A}$ and D_2 receptors with the albumin. Results of the studies reported here indicate that potential antipsychotic drugs bind weakly to the transporter protein (BSA) with $K \approx 10^2$–10^3 M^{-1}. Our method allows measuring K in a wide range of values (10^2–10^9 M^{-1}). This range depends only on the solubility of the ligand and sensitivity of the detector.

1. Introduction

Many biochemical systems such as protein and drug, DNA and drug, antibody and antigen form non-covalent complexes and therefore determination of respective association constants is essential for understanding their interactions [1]. While searching for new antipsychotic drugs it is important to estimate the affinity of a potential drug to plasma protein, because free drug molecules are more likely to cross the blood–brain barrier to reach biological target and exert pharmacological effect. Protein binding can also affect the drug half-life in the body, thus determining its pharmacokinetic profile.

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Here, we present fast and precise flow injection method for rapid determination of drug–protein association constants. We determine the association constants of a large number of potential antipsychotic drugs with BSA.

Traditional methods available for the determination of association constants include the equilibrium dialysis, ultrafiltration, ultracentrifugation and electrophoresis [2]. The most widely used method is the equilibrium dialysis, which is considered as the reference method. However, the dialysis has many disadvantages such as long time of equilibration (usually 12–48 h), problems with volume shift, the Donnan effect and nonspecific adsorption of compounds to the chamber walls [3]. Ultrafiltration needs less time than dialysis, but also suffers from nonspecific binding of reactants to the filter membrane, potential protein leakage and the Donnan effect [4]. The ultracentrifugation is accompanied by physical phenomena such as sedimentation, back diffusion, which hinder the estimation of free fraction of the drug. In capillary electrophoresis the problem with adsorption on the capillary walls often appears [5,6]. There is also a group of methods which does not involve separation of free ligand from the complex. This group of methods includes spectroscopic methods (UV–vis [7], fluorescence [8], infrared (IR) [9], nuclear magnetic resonance (NMR) [10]), isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and surface plasmon resonance (SPR) [11,12]. These techniques also have disadvantages, because they require e.g., changes of physicochemical properties of studied compounds upon binding into a complex. Also the flow injection analysis was used for studying association constants. In this method the calibration of the system is required prior to measurements [13–16]. Each of the presented methods has disadvantages and limitations. Therefore there is still a need for developing new methods.

We present modified type of the flow method for determination of ligand–protein association constants, which is more flexible than other methods. We use a flow injection method in narrow, coiled capillaries. The method is based on an observation that the effective diffusion coefficient of a ligand flowing in a capillary with the protein depends on the association constant. This method does not require separation of free ligand from the ligand bound in the complex with protein like in size exclusion chromatography, dialysis or electrophoresis [17,18]. The method works for charged as well as uncharged molecules and allows to study a wide group of chemical substances. The method takes only a few minutes for a single measurement and requires small amount of material (1 nM, for one 10 μL injection). The stage of samples preparation is also simple. This method allows changing easily temperature, ionic strength, viscosity or concentration and does not require the calibration like in most flow injection methods.

We conducted a series of experiments (for cefaclor–BSA, etodolac–BSA, sulindac–BSA systems) to test and verify the method and the results showed good agreement with the literature data. We also measured the association constants of the complexes of albumin with 3β- and 3α-aminotropane derivatives (compounds 1–20). The compounds were synthesized in the Department of Drug Technology and Pharmaceutical Biotechnology at the Medical University of Warsaw. The compounds were obtained in a search for a new generation of antipsychotics following the principles of the multitarget strategy. The derivatives were designed for 5-HT_{2A} and D_2 receptors (the basic profile of pharmaceutical activity of atypical antipsychotics) and, additionally, for 5-HT_{1A} receptors [19–25]. With the aim of enhancing cognitive factors and memory, which might be improved by potential antipsychotics [26], the activity of the compounds was expanded to their affinity to 5-HT_{1A} receptors. The role played by the 5-HT_{1A} receptor in the pathomechanism of schizophrenia was related to its regulatory influence on the functions of the prefrontal cortex (PFC), including emotional control, cognitive behavior and working memory [26–31]. The goal of the research was to obtain a new generation of antipsychotics which would display a multireceptor mechanism of action, be devoid of many undesired effects such as extrapyramidal symptoms (EPS), tardive dyskinesia or weight gain in patients and many other effects caused by drugs currently used in the pharmacotherapy of schizophrenia [21,22,32].

The aim of this work was to demonstrate the new method for the association constant measurement. The method was tested and verified for known selected drugs (cefaclor, etodolac, sulindac) interacting with bovine serum albumin. Good agreement with literature data was obtained. Next, the method was applied for determination association constant of new tropane derivatives, compounds 1–20 (5HT_{1A}, 5HT_{2A}, and D_2 receptor ligands) with albumin.

1.1. Determination of the association constant for ligand–protein complex formation in coiled capillaries at high flow rates

The association constant is determined from the experimental values of the diffusion coefficient of ligands and proteins. For measurements of the diffusion coefficient the flow injection method [33,34] is used. The injection zone of the ligand in a cylindrical capillary has a shape of a small cylinder. The cross section of the injection of the sample (shown in Fig. 1) has a rectangular shape. The Poiseuille flow stretches the rectangle into a paraboloid. Therefore the flow forms the gradient of concentration distribution between the center of the capillary and the walls. The gradient of concentration induces perpendicular diffusion which narrows the concentration distribution in the direction of flow. The final distribution of the concentration of the solute has the Gaussian shape at the end of the capillary. The concentration distribution of the solute is obtained by measuring the absorbance of UV–vis light by the sample as a function of time. The width of the concentration distribution is inversely proportional to the diffusion coefficient (details concerning determination of the diffusion coefficient are given in the Supporting information).

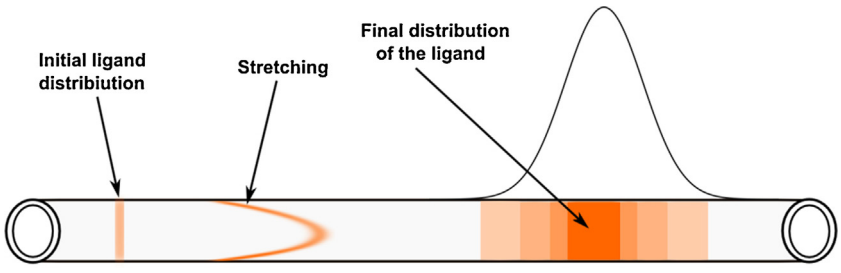

Fig. 1. Dispersion inside a capillary. Initially narrow injection zone is convectively stretched along the capillary and diffusively smeared across the capillary. The concentration distribution of the solute has the Gaussian shape at the end of the capillary.