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Investigation of interactions of Comtan with human serum albumin by mathematically modeled voltammetric data: A study from bio-interaction to biosensing

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ABSTRACT

In this work, voltammetric data recorded at a glassy carbon electrode (GCE) were separately used to investigate the interactions of entacapone (Comtan, CAT) with human serum albumin (HSA). Then, an augmented data matrix was constructed by the combination of voltammetric and spectroscopic data and simultaneously analysed by multivariate curve resolution-alternating least squares (MCR-ALS) to obtain more information about CAT-HSA interactions. The absence of rotational ambiguities in results obtained by MCR-ALS was verified with the help of MCR-BANDS and we confirmed that the results were unambiguous and reliable. Binding of CAT to HSA was also modeled by molecular docking and the results were compatible with those of obtained by recording experimental data. Hard-modeling of combined voltammetric and spectroscopic data by EQUISPEC as an efficient chemometric algorithm helped us to compute binding constant of CAT-HSA complex specie which was in a good agreement with the binding constant value obtained by direct analysis of experimental data. For electrochemical sensing of serum albumin two amperometric measurements were performed to determine HSA in 2–27 nM and 27–70 nM with a limit of detection of 0.51 nM and a sensitivity of 1.84 μA nM^{-1}.

1. Introduction

Entacapone with the brand name of Comtan (CAT, Fig. S1A) is a drug commonly in combination with other medications which is used to treatment of Parkinson's disease [1]. It has been proven that the CAT in combination with levodopa and carbidopa causes levodopa having longer and better effects in the brain which reduces the signs of Parkinson's disease. These effects cannot be observed by levodopa and carbidopa therapy [1].

Human serum albumin (HSA, Fig. S1B) is the most important protein of human blood plasma which acts as a transport molecule for endogenous and exogenous ligands and foreign molecules including dyes and drugs [2–7]. Therefore, HSA has a vital role in free concentration, distribution, metabolism, excretion and interaction with small molecules. The biological activity of a drug is significantly affected by the magnitude and the nature of its interaction with HSA. Weak binding of a drug to HSA causes a short life-time and low distribution of drug while strong binding of a drug to HSA decreases its free concentrations in plasma. Therefore, investigation of interactions of drugs with HSA is very important. On other side, unusual concentration of HSA in serum is an important biomarker for detection of some diseases such as diabetes, cardiovascular disease, kidney disease, etc. Some methods such as capillary electrophoresis, LC-MS/MS, immunoassays and surface-enhanced Raman scattering are usually used to determine HSA. Although, these methods are accurate but are limited by cost and time therefore, developing an efficient, fast and low-cost analytical technique for quantitative determination of a HSA is of great importance in medicine, biology and diagnostics [8,9].

There are many techniques such as capillary electrophoresis [10], NMR [11], FT-IR [12], Uv–Vis spectrophotometry [13], HPLC [14] and electrochemistry [15] which can be used to study drug-protein interactions and extracting relevant information. Some of these methods have been assisted by chemometrics to obtain more reliable information about drug-protein interactions [2–4,16,17]. Protein-drug interaction is an example of complex reactions where drug, protein and their complex product must be simultaneously monitored. Such an analytical task is quite challenging especially when the data are collected from more than one instrumental technique. Therefore, application of an efficient

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chemometric method such as multivariate curve resolution-alternating least squares (MCR-ALS) to solve this problem is very useful [18]. MCR-ALS is a well-known chemometric tool for the analysis of data with different origins and to extract profiles of principal components from composite responses. Obtaining such results can help us to obtain valuable information about the studied system which cannot be obtained by using conventional methods. Therefore, we used MCR-ALS for further analysis of experimental data to obtain more information about CAT-HSA interactions.

The steps of the present study were:

1. Electrochemical data including differential pulse voltammetry (DPV), linear sweep voltammetry (LSV) and cyclic voltammetry (CV), and spectroscopic data including Uv–Vis and fluorescence spectroscopy (F) were separately used to investigate the interactions of CAT with HSA.
2. Electrochemical and spectroscopic data were then combined to construct an augmented data matrix and resolved by MCR-ALS to obtain more new information about CAT-HSA interactions.
3. Electrochemical and spectroscopic data were combined and modeled by EQUISPEC as a hard-modeling chemometric tool to compute binding constant of the complex product.
4. Modeling the binding of CAT to HSA by molecular modeling techniques to verify the results of experimental sections.
5. Sensitive electrochemical determination of electro-inactive HSA by performing two amperometric measurements at the surface of a glassy carbon electrode modified (GCE) by multiwalled carbon nanotubes (MWCNTs)-graphene(Gr)-chitosan (Ch)-ionic liquid (IL).

2. Experimental and theoretical considerations

2.1. Chemicals and solutions

HSA, CAT, warfarin (Wr), ibuprofen (Ip), acetic acid, Ch, ethanol, dimethylformamide (DMF), Tris hydrochloride, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)Imide (IL), dimethyl sulfoxide (DMSO), sodium hydroxide, sodium chloride and hydrochloric acid were purchased from Sigma. The MWCNTs were prepared from Ionic Liquid Technologies. The Gr was purchased from PubChem. Other chemicals used in this study were purchased from well-known and legal companies and used as received without any further purification. A Tris buffer solution (TBS) with a concentration of 0.05 M containing 0.025 M sodium chloride to maintain the ionic strength was prepared to control pH at 7.4. A stock solution of CAT and HSA with a concentration of 0.1 M was prepared in 0.05 M TBS and kept in a refrigerator. Stock solutions of Wr and Ip with a concentration of 0.1 M were prepared by in DMSO and kept in a refrigerator. Working solutions used in this study were prepared from their stock solution by appropriate dilutions. 10 mg Ch was dissolved in 100 μL acetic acid and ultrasonicated for 40 min to obtain Ch solution. 50 mg MWCNTs was dissolved in 1 mL DMF containing 100 μL IL, 100 μL Ch solution and 50 mg Gr, and ultrasonicated for 30 min to obtain a homogeneous gel like MWCNTs-Gr-Ch-IL solution. Doubly distilled water (DDW) was used to prepare all the solutions used in this study.

Three human serum samples prepared from a medical diagnostic laboratory and centrifuged at 4500 rpm for 20 min and after discarding the supernatants, 5 mL of each sample was diluted with 45 mL TBS (0.05 M, pH 7.4). Then, each sample was amperometrically analysed towards HSA determination and final concentration of HSA in serum samples was calculated by applying appropriate dilution factors.

2.2. Instruments and softwares

All the electrochemical data reported in this study were recorded by an Autolab PGSTAT302N-high performance controlled by the NOVA 2.1.2 software equipped with an electrochemical cell in which a bare or modified glassy carbon electrode (GCE), a Pt wire and an Ag/AgCl electrode acted as working, counter and reference electrode, respectively. The SEM images were captured by a KYKY-EM 3200 scanning electron microscope. Spectrofluorimetric data were recorded by a Cary Eclipse fluorescence spectrophotometer equipped with a water bath and a 1.0 cm quartz cell. Spectrophotometric data were recorded by an Agilent 8453 Uv–Vis Diode-Array spectrophotometer controlled by the Agilent Uv–Vis ChemStation software and equipped with a 1.0 cm quartz cell. A sigma centrifuge was used to centrifuge the serum samples. An ELMEIRON pH-meter (CP-411) was used to pH adjustments. The molecular structure of the CAT was constructed by Hyperchem package (Version 8.0) and AM1 semi empirical method with Polak-Ribiere algorithm was used to minimize energy until the root mean square gradient of 0.01 kcal mol^{-1}. The known crystal structure of HSA (PDB Id: 1AO6) was downloaded from Brookhaven Protein Data Bank (PDB) and water molecules were removed from its PDB file and hydrogen atoms were added to it. The Molegro virtual docker (MVD) software was used to generate a docked conformation of CAT with HSA. LIGPLOT which is a well-known program for automatically plotting protein-ligand interactions [19], was used to plot the interactions between CAT and HSA. The m-files of MCR-ALS and MCR-BANDS were downloaded from internet [20]. The recorded experimental data was transferred to MATLAB (Version 7.14) environment and smoothed, when necessary. To accelerate exact computing the concentrations of CAT, HSA and their ratio during voltammetric or spectroscopic titrations, a simple homemade MATLAB program was written.

2.3. Fabrication of the modified electrodes

Prior to the modification of GCE, it was well polished with a silky polishing pad and rinsed with DDW. It was then, immersed into a beaker containing ethanol and ultrasonicated for 30 min and finally rinsed with DDW and covered until analysis time. To fabricate HSA/GCE, 10 μL HSA (0.01 M) was dropped onto the surface of the cleaned GCE and left to be dried at room temperature. To fabricate MWCNTs-Gr-Ch-IL/GCE, 10 μL MWCNTs-Gr-Ch-IL was dropped onto the surface of the cleaned GCE and left to be dried at room temperature. Fig. 1A, B and C show the SEM images captured from the surface of bare GCE, HSA/GCE and MWCNTs-Gr-Ch-IL/GCE, respectively. By comparing Fig. 1A and B, it can be clearly observed that HSA has formed a layer on the surface of bare GCE. Fig. 1C shows the formation of a layer of MWCNTs-Gr-Ch-IL onto the surface of bare GCE.

2.4. Procedures

Spectroscopic experiments were performed in a cell with a volume of 3 mL. In each experiment, 2 mL of HSA or CAT with a distinct concentration was poured into the cell and different volumes of HSA or CAT were added into the cell while the total added HSA or CAT was <0.2 mL. Spectroscopic titrations were manually performed where each solution was shaken vigorously and allowed to be equilibrated for 1.0 min and then, its spectrum was recorded.

Voltammetric experiments were performed in a cell with a volume of 5 mL. In each experiment, 4 mL of HSA or CAT with a distinct concentration was poured into the cell and different volumes of HSA or CAT were added into the cell while the total added HSA or CAT was <0.5 mL. Each solution was stirred by an electronic stirrer at 200 rpm for 1.0 min and then, left to be stopped and its voltammogram was recorded.

2.5. Experiments required to construct an augmented data matrix for the analysis by MCR-ALS

Experiment 1 ($\mathbf{D}_{DPV}^{CAT}$): HSA with a concentration of 2.63×10^{-6} M was added to a 5.0×10^{-5} M CAT solution for nineteen times which led us to