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Interaction of donepezil with human serum albumin on amine-modified magnetic nanoparticles

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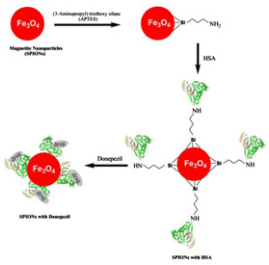
HIGHLIGHTS

- Magnetite nanoparticles were synthesized, and then functionalized.
- Human serum albumin (HSA) was immobilized onto modified nanoparticles.
- Interaction of donepezil with HSA immobilized material was studied.
- Binding mechanism was identified by using Stern Volmer equation.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction between the drug donepezil and human serum albumin (HSA) was examined on the surface of amine modified superparamagnetic iron oxide nanoparticles (SPIONs), which were synthesized by the coprecipitation of ferrous and ferric salts with NH_4OH and then modified with [3-Aminopropyl] triethoxysilane (APTES) to obtain functional amine groups on the nanoparticles' surface. Albumin's binding capacity to APTES-modified SPIONs was estimated by fluorescence spectroscopy. After HSA was bound to the APTES modified SPIONs, donepezil was interacted with the HSA-SPIONs. The binding capacity of the drug was determined using a calibration curve equation that was drawn using fluorescence spectroscopy at 325 and 387 nm, the excitation and emission wavelengths. Thermodynamic parameters were estimated for the interaction of HSA and donepezil on amine-modified SPIONs. Binding was carried out spontaneously via electrostatic interaction. Binding was also examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), scanning electron microscopy (SEM) and zeta-potential measurements.

1. Introduction

Nanotechnology is a critical area of research in the 21st century, and has gained considerable attention [1]. The emerging alliance between nano-material science and the biological and medical sciences is founded upon fundamental scientific principles. Many small molecules that are nanometers in length can be distributed according to physiochemical principles and biological transport processes in living organisms [2]. Material science and medical science offer complementary inputs for living organisms. Polymers, quantum dots, and magnetite nanoparticles have been used for biological applications such as drug delivery, bio-separation, biosensors, diagnostic treatment systems, and protein and DNA immobilization. The use of metal nanoparticles (gold, silver, copper, zinc, iron, etc.) that include active surfaces such as amines, epoxies, and aldehydes is increasingly common in the design of biological materials. Many researchers have tried to improve the binding of biomolecules to nanoparticles due to the strong affinity for their molecular counterparts [3].

Human serum albumin (HSA) is multi-domain macromolecule that acts as the main determinant of plasma oncotic pressure and the main modulator of fluid distribution throughout the body

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[4]. HSA is valuable for its extraordinary ligand binding capacity, providing a depot and carrier for a wide variety of compounds [5]. Accordingly, HSA is used as the main carrier for hormones, enzymes, nutrients, metal ions, and fatty acids, and is effective on the pharmacokinetics of many drugs [6–8]. HSA is a crucial biomarker of many diseases including cancer, Alzheimers, etc. [4]. HSA is also a manageable macromolecule due to its lack of toxicity and immunogenicity, which makes it an ideal candidate for the binding of drugs [9]. The effectiveness of drugs depends on their ability to bind to albumins, so it is important to study the interactions between drugs and this protein [10].

Distributing drugs such as antibiotics without a protein would make it much harder to fight sickness and disease, as albumin allows certain curable substances to halt diseases and illnesses and carries drugs throughout the body. Therefore, drug distribution is controlled by HSA. Depending on a specific drug's affinity for plasma protein, a portion of the drug may become bound to plasma proteins and reach the target tissues by binding with HSA [9].

It is important to research the binding capacities of biological molecules with proteins or drugs. One of the unique features of albumin is its fluorescence property which comes from several amino acid residues such as tryptophan, tyrosine, and phenylalanine. Albumin is intrinsically fluorescent at 280 and 324 nm, the excitation and emission wavelengths, respectively. Indeed, nanoparticle labels have proven to possess high specific activity, and even single binding events can be observed due to the extremely intense luminescence of the particles compared to molecular labels [11].

In this study, super paramagnetic iron oxide nanoparticles (SPIONs) were synthesized by the coprecipitation of ferrous and ferric salts with NH_4OH, and then modified with (3-Aminopropyl) triethoxy silane (APTES) to obtain functional amine groups on the nanoparticle surface. After binding HSA to the modified nanoparticle surface, the interaction between human serum albumin (HSA) and the drug (donepezil) was evaluated. The binding capacity of albumin to APTES-modified SPIONs was estimated by fluorescence spectroscopy. Additionally, the fluorescence quenching mechanism of donepezil by HSA-immobilized SPIONs was explored at different temperatures (296, 299, and 302 K). Thermodynamic parameters (ΔS, ΔG, and ΔH) and binding constants (K) were calculated.

2. Material and methods

2.1. Materials

Donepezil hydrochloride was obtained from Ilko pharmaceutical company (Konya, Turkey). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, >99%), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, >99%), (3-aminopropyl) triethoxysilane (APTES), sodium hydroxide (≥97%), hydrochloric acid (37%), tris(hydroxymethyl)-aminomethane (99.8–100.1%), ammonium hydroxide (25% w/w) and ethanol (>99.2%) were supplied from Merck (Germany). Albumin from human serum (97–99%) was obtained from Sigma–Aldrich (USA). Glycine, coomassie brilliant blue, N,N,N',N'-tetramethylethylenediamine (TEMED), ammonium persulfate, sodium dodecyl sulfate, acryl amide, glycerol, bromophenol blue and bisacrylamide were purchased from Sigma–Aldrich (USA). All aqueous solutions were prepared with deionized water via Millipore Milli-Q Plus water purification system. All chemicals were of analytical and molecular grade.

2.2. Functionalization of magnetic nanoparticles by (3-aminopropyl) triethoxysilane

Magnetic nanoparticles were prepared via an improved chemical coprecipitation method [12]. Using this method, 3.1736 g of $FeCl_2{\cdot}4H_2O$ (0.016 mol) and 7.5684 g of $FeCl_3{\cdot}6H_2O$ (0.028 mol) were dissolved in 320 mL of deionized water, such that the mole ratio between Fe^{2+}/Fe^{3+} = 1/1.75. The mixed solution was stirred under N_2 at 80 °C for 1 h. Then, 40 mL of $NH_3.H_2O$ was injected rapidly into the mixture, which was then stirred under N_2 for another 1 h and cooled to room temperature. The precipitated particles were washed five times with hot water and were separated by magnetic decantation. Finally, the magnetic nanoparticles were dried under vacuum at 70 °C. 4.2252 g of SPIONs were sonicated in a 150 mL ethanol/water (volume ratio, 1:1) solution for 30 min to achieve a uniform dispersion. Then 16.1600 g of APTES was added to the solution under N_2 atmosphere at 40 °C for 2 h. The optimal surface modification molar ratio of APTES to Fe_3O_4 was found to be 4:1 [13]. After that the solution was cooled to room temperature. The prepared APTES modified SPIONs were collected with a magnet, and washed with ethanol and then washed three times with deionized water. Finally, the APTES modified SPIONs were dried under vacuum at 70 °C (Scheme 1).

2.3. Protein immobilization

APTES-modified SPIONs were mixed with 0.5 mg/mL human serum albumin in 20 mM Tris–HCl, pH 7.4, and tumbled overnight at 4 °C at a particle concentration range of 5–25 mg/mL. The supernatant was removed and kept at 4 °C for further protein analysis after the HSA bound SPIONs were separated magnetically. Albumin bound nanoparticles were also washed with PBS and ethanol for chemical characterization [13].

2.4. Protein analysis

Protein concentration was determined using a fluorescent method [14]. The remaining protein after albumin binding on APTES modified SPIONs was diluted to appropriate concentrations and scanned by fluorescence spectroscopy. The intrinsic fluorescence of the protein was recorded at 280 and 342 nm, the excitation and emission wavelengths [15]. Unbound protein concentration was measured using the standard curve equation ($y = 32903x + 44.997$) of the HSA. Concentration of the bound protein was estimated by calculation.

2.5. Binding of the drug

100 mM of standard solution of Donepezil was prepared by dissolving it in a solvent mixture of 20 mM Tris–HCl (pH: 7.4) and DMF (90%-10%, v/v). The excitation and emission wavelengths of donepezil were scanned by fluorescence spectroscopy, and the drug was mixed with appropriate amount of HSA immobilized nanoparticles in a Tris buffer. Binding of the drug was calculated using the calibration regression equation ($y = 60.463x + 19.243$) of the drug at the 325 nm and 387 nm excitation and emission wavelengths.

2.6. SDS-PAGE analysis

Samples were examined via SDS-PAGE as described by Laemmli [16]. Briefly, albumin immobilized nanoparticles were mixed with 3xSDS sample buffer and boiled for 15 min to cleavage albumin from magnetic nanoparticles [17]. The samples were then loaded onto an SDS-PAGE gel, run, and stained according to the standard protocol [16].

3. Results and discussion

Functionalized magnetic nanoparticles are widely used in drug delivery. Our group has synthesized Fe_3O_4 nanoparticles with different functional groups such as amine, epoxy, and aldehyde for

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