Characterization of the Interaction between 4-(Tetrahydro-2-Furanmethoxy)-N-Octadecyl-1,8-Naphthalimide and Human Serum Albumin by Molecular Spectroscopy and Its Analytical Application

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A novel 4-(tetrahydro-2-furanmethoxy)-N-octadecyl-1,8-naphthalimide (TNN) was synthesized as a spectrofluorimetric probe for the determination of proteins. The effect of different solvents on the spectral characteristics of TNN was investigated. The results showed that TNN displayed dependent solvent polarity properties due to the effect of internal charge transfer. The interactions between TNN and human serum albumin (HSA) were studied by fluorescence and absorption spectroscopy. Fluorescence data revealed that the fluorescence quenching of HSA by TNN was the result of the formation of TNN-HSA complex. The binding parameters of interactions between TNN and HSA at different temperatures were obtained according to the Stern-Volmer equation. The thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS), for the interactions were calculated to be -7.31 kJ mol⁻¹ and 72.75 J mol⁻¹ K⁻¹ according to the van't Hoff equation, indicating that the hydrogen bonds and hydrophobic interactions were the dominant intermolecular force in stabilizing the complex. The effect of TNN on the conformation of HSA was analyzed by circular dichroism and synchronous fluorescence spectroscopy. Furthermore, the results of displacement experiments using warfarin indicated that TNN could bind to site I of HSA. The fluorescence of TNN could be largely quenched by HSA, based on which a new fluorimetric method for detecting HSA in the HCl-Tris buffer solution (pH = 7.4) was developed. The linear ranges of the calibration curves were 0.1–1.84 × 10⁻¹⁰ M for HSA, 0.1–1.30 μM for bovine serum albumin (BSA), 0.2–7.97 μM for γ-globulin, and 0.3–11.3 μM for hemoglobin (Hb), with detection limits (S/N) of 4.48, 0.001, and 0.001, respectively. The effect of metal ions on the fluorescence spectra of TNN in ethanol was also investigated. The method has been applied to the determination of total proteins in human serum samples collected from the hospital and the results were in good agreement with those reported by the hospital.

Index Headings: 1,8-Naphthalimide; Human serum albumin; Fluorescence probe; Circular dichroism; Molecular spectroscopy; Photo-induced electron transfer; Quantitative assay.

INTRODUCTION

Investigations into the interaction between small-molecule drugs and proteins have been attracting much attention because proteins play a fundamental role in sustaining life and are closely related to metabolism, the origin and evolution of life. Serum albumin is the most abundant and the main transport protein in blood plasma. It also plays an important role in transporting and metabolizing many endogenous and exogenous compounds in metabolism. Therefore, studies of the small molecular binding with serum albumin and quantitative determination for serum albumin are significant in the life sciences, clinical medicine, and biochemistry. The traditional spectrophotometric methods for the determination of proteins use dyes as analytical reagents, such as the Lowry method,3 Kjeldahl method,4 Biuret method,5 Coomassie brilliant blue,6 rare earth chelates probes,7 inorganic ions,8 electrochemical method,9 quantum dot,10 resonance light-scattering,11 and surfactant.12 Among these, the fluorimetric methods using covalent and non-covalent probes not only possess high sensitivity and selectivity, but also provide more information for the investigation and determination of proteins, and the advantages in their applications have been summarized in some reviews.13–18

1,8-Naphthalimide and its derivatives, with a strong electron-withdrawing imide group, have been extensively investigated due to their good chemical stability, large Stokes shift, and high fluorescent quantum yield, known to act as laser-active media,19 potential biologically photosensitive units,20 analogies,21 light-emitting diodes,22 solar energy collectors,23 electrofluorescent materials,24 ion probes,25,26 and anti-tumor agents.27 However, to our best knowledge, there are few reports on the determination of proteins using the spectral characteristics of the 1,8-naphthalimide derivatives.27 In this work, the synthesis and photophysical properties of a novel fluorescence probe based on 4-(tetrahydro-2-furanmethoxy)-N-octadecyl-1,8-naphthalimide (TNN) (Scheme 1) are presented, and its wavelength of emission/excitation is 440/365 nm in ethanol, which makes possible its use as a biomolecular probe. The effects of different solvents on the spectral characteristics of TNN were investigated. The interactions between TNN and
human serum albumin (HAS) were studied by spectroscopic methods in physiological condition. The binding parameters and main binding forces were obtained. It was found that the fluorescence intensity of TNN could be quenched greatly by the addition of HAS. Based on this, a sensitive method for the determination of protein was proposed. The method had been employed to determine total proteins in human serum samples.

**EXPERIMENTAL**

All the solvents were analytical grade. Octadecylamine, N,N-dimethylethanolamine, 4-bromo-1,8-naphthalic anhydride, and tetrahydro-2-furanmethanol were purchased from Aldrich.

HAS, BSA, hemoglobin (Hb), and γ-globulin purchased from Sigma and used without further purification. Stock solutions of proteins (10.0 μM) were prepared by dissolving in Tris-HCl buffer solutions (0.05 M Tris, 0.15 M NaCl, pH 7.4) and stored at 4 °C.

The working solution of TNN (150 μM) was prepared by dissolving an appropriate amount of TNN in 100 mL anhydrous ethanol; the resulting solution was kept out of light. Doubly distilled water was used throughout.

An EQUINOX 55 Fourier transform infrared spectrometer (Bruker Company, Germany) and AVANCE300 MHz digital superconducting NMR fourier (Bruker Company, Germany) were used. All fluorescence measurements were carried out on an F-7000 fluorescence spectrometer (Hitachi, Japan) equipped with a 150 W xenon lamp. All absorption spectra were recorded at room temperature with a UV-2501PC spectrophotometer (Shimadzu, Japan). A 1.0 cm quartz cell was used for measurements. Elemental analysis data were obtained on a Perkin-Elmer 240c instrument. All pH measurements were made with a PHS-3CF acidity meter (Lei Ci, China).

The synthesis route of 4-(tetrahydro-2-furanmethoxy)-N-octadecyl-1,8-naphthalimide is given in the Supplemental Material, available online.

Three milliliters (3.0 mL) HSA with concentration of 1.0 μM was added to a quartz cell (1.0 cm × 1.0 cm). The TNN solution was then gradually titrated to the cell using a trace syringe (to give a final concentration ranging from 0 to 9.09 μM; total accumulated volume of TNN was less than 20 μL). The fluorescence spectra of the above solutions at different temperatures were collected with the excitation and emission wavelength at 280 and 340 nm, respectively. The width of the emission slit was set to 2.5 nm. The absorption spectra of the above solutions were obtained in the wavelength range of 200 to 400 nm.

Synchronous fluorescence spectra of HSA with various concentrations of TNN were obtained from 200 to 400 nm (Δλ = 15 nm and Δλ = 60 nm) with the emission slit width of 2.5 nm.

Three milliliters (3.0 mL) TNN with concentration of 15.0 μM was added to a quartz cell. The HSA solution was then gradually titrated to the cell using a trace syringe (to give a final concentration ranging from 0 to 14.21 μM; total accumulated volume of HSA was less than 20 μL). The fluorescence spectra were collected with the excitation wavelength at 245 or 365 nm and the emission wavelength at 440 nm.

The circular dichroism (CD) spectra were recorded over a wavelength range of 200 to 260 nm, with three scans averaged for each CD spectra, and the results were expressed in terms of mean residue ellipticity (MRE).

**RESULTS AND DISCUSSION**

The effect of various concentrations of TNN on the fluorescence spectra of HSA was investigated in physiological conditions (pH = 7.4). As seen from Fig. 1, there was no fluorescence emission for TNN in the range measured, and the fluorescence intensity of HSA decreased gradually upon addition of TNN along with a remarkable blue shift, indicating that HSA could interact with TNN, and the intrinsic fluorescence was quenched by TNN. Because TNN was prepared in ethanol, the quenching effect of ethanol on HSA conformation was then evaluated. The ultimate maximum percentages of ethanol in all the solutions were 0.7% (v/v). Results showed that the effect of ethanol on the interaction of TNN with HSA was negligible with the amount used in our experiments. The hypochromatic shift indicated that the chromophore of tryptophan in HSA was placed in a more hydrophilic environment after the addition of TNN.

Fluorescence quenching can occur by different mechanisms, usually classified as dynamic quenching and static quenching. In order to clarify the quenching mechanism, the Stern-Volmer equation was used to analyze the quenching data:

\[
F_0/F = 1 + K_{SV}[T] = 1 + K_q\tau_0[T]
\]  

where \( F_0 \) and \( F \) are the fluorescence intensities in the absence and presence of quencher (TNN), respectively, \( K_{SV} \) is the


<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$K_{SV}$ ($10^9$ M$^{-1}$)</th>
<th>$K_a$ ($10^9$ M$^{-1}$)</th>
<th>$n$</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
<th>$\Delta S$ (J mol$^{-1}$ K$^{-1}$)</th>
<th>$\Delta G$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>2.23</td>
<td>1.20</td>
<td>1.15</td>
<td>-7.31</td>
<td>72.75</td>
<td>-28.99</td>
</tr>
<tr>
<td>302</td>
<td>2.16</td>
<td>1.17</td>
<td>1.15</td>
<td>-7.22</td>
<td>72.67</td>
<td>-28.92</td>
</tr>
<tr>
<td>306</td>
<td>2.08</td>
<td>1.15</td>
<td>1.14</td>
<td>-7.15</td>
<td>72.65</td>
<td>-28.91</td>
</tr>
<tr>
<td>310</td>
<td>1.98</td>
<td>1.07</td>
<td>1.14</td>
<td>-7.13</td>
<td>72.63</td>
<td>-28.89</td>
</tr>
</tbody>
</table>

Stern–Volmer quenching constant, and [T] is the concentration of TNN, $K_a$ is the bimolecular quenching rate constant, and $K_q = K_{SV}/n_0$. $\tau_0$ is the average lifetime of the molecule without any quencher, and the fluorescence lifetime of the biopolymer is $10^{-8}$ s.\textsuperscript{30} The Stern–Volmer plots of $F_0/F$ versus [T] at different temperatures are shown in Fig. S1 (in the online Supplemental Material), and the calculated $K_{SV}$ values are presented in Table I. The values of $K_{SV}$ were decreased with increasing temperature and the values of $K_q$ were much larger than the maximum scattering collision quenching constant ($2 \times 10^{10}$ M$^{-1}$ s$^{-1}$), suggesting that the quenching was initiated by the static quenching.\textsuperscript{31} According to Sharma, dynamic quenching affected the excited states of molecules rather than the absorption spectra of dye.\textsuperscript{32} As seen from Fig. 2, the absorbance of HSA was increased with the addition of TNN and the absorption spectra maximum shifted towards shorter wavelength region, indicating that the complex of HSA–TNN was formed. This result reconfirmed that the quenching of HSA was initiated by static quenching.

The binding constants and numbers of binding sites of interaction of TNN and HSA could be determined by the following equation:\textsuperscript{33}

$$\log\left(\frac{[F_0 - F]}{F}\right) = \log K_a + n \log [T]$$  \hspace{1cm} (2)

where $K_a$ is the binding constant, $n$ is the number of binding sites per HSA, and $F_0, F,$ and [T] have the same meanings as in Eq. 1. The values of $K_a$ and $n$ could be calculated by the intercept and slope by plotting $\log \left(\frac{[F_0 - F]}{F}\right)$ versus $\log [T]$ (intercept = $\log K_a$, slope = $n$) (Fig. S2), and the corresponding results are listed in Table I. The decreasing trend of $K_a$ with increasing temperature is in accordance with the dependence of $K_{SV}$ on temperature as mentioned above, indicating that the binding between TNN and HSA was moderate and the TNN–HSA complex was reversible.\textsuperscript{34} The values of $n$ were approximately equal to 1, indicating that there was one binding site in HSA for TNN.

The warfarin binding site was the best characterized drug binding site, designated as site I, and was located in subdomain IIA near tryptophan.\textsuperscript{35} The binding site of TNN in HSA was investigated with warfarin as a site I marker using the fluorescence titration method. The concentrations of warfarin and HSA were stabilized at a molar ratio of 10:1, and TNN was gradually added to the warfarin–HSA mixtures. As seen from Fig. 3, warfarin had a weak fluorescence at 400 nm when excited at 295 nm, and the fluorescence intensity was increased along with a red shift after the addition of HSA, suggesting that warfarin bound to a single site in HSA.\textsuperscript{36} The fluorescence intensity of the warfarin–HSA mixtures was decreased (about 68%) upon addition of TNN, indicating that the binding capacity of warfarin at the primary binding site of HSA was reduced. The result suggested that TNN binding occurred at the Trp 214 proximity, located in subdomain IIA of the albumin structure (the warfarin binding pocket).\textsuperscript{37} The effect of warfarin on the fluorescence of TNN and the effect of TNN on the fluorescence of warfarin were investigated (Fig. S3). Results showed that the interaction of warfarin with TNN could be neglected in the study of site markers.

Generally, a small molecule binds to a macromolecule by the following four binding modes: hydrogen bonds, van der Waals forces, hydrophobic interactions, and electrostatic interactions. The thermodynamic parameters of the binding reaction are the main evidence for confirming binding mode. If the enthalpy change ($\Delta H$) does not vary significantly in the temperature range studied, both the $\Delta H$ and entropy change ($\Delta S$) can be evaluated using the van’t Hoff equation:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$  \hspace{1cm} (3)

The free energy change ($\Delta G$) is calculated from the following relationship:

$$\Delta G = \Delta H - T \Delta S$$  \hspace{1cm} (4)

$K$ is analogous to the binding constant $K_a$. The $\Delta H$ and entropy change ($\Delta S$) were calculated from the slope and intercept of the
van't Hoff relationship (Fig. S4), and the corresponding results are listed in Table I. The values of \( \Delta G \) and \( \Delta H \) were negative, and the value of \( \Delta S \) was positive, indicating that the binding process was spontaneous and the hydrogen bonds and hydrophobic interactions played a dominant role in the binding of TNN to HSA.\(^{38}\) It should also be noted that \( \Delta H \) is relatively small (\(-7.31 \text{ kJ mol}^{-1}\)), indicating weak enthalpy effects in the binding process, and the results conform to the assumption.

Synchronous fluorescence spectra are a useful method for evaluating the conformational changes of HSA.\(^{39}\) When the wavelength interval (\( \Delta \lambda \)) is 15 nm or 60 nm, the synchronous fluorescence offers the characteristics of tyrosine or tryptophan residues.\(^{40}\) As seen from Fig. 4b, the maximum excitation wavelength of tryptophan residues had a slight blue shift (from 276 to 272 nm), while no obvious wavelength shift of tyrosine residues (Fig. 4a) was observed. The results indicated that the tryptophan residue was placed in a more hydrophobic environment and hydrophobicity was increased. Thus, the interactions caused a minor conformational change of tryptophan residue micro-regions.

Circular dichroism is a sensitive technique to determine the conformational changes in the protein upon interaction with the ligand. To further verify that the secondary structure change of HSA occurred in the binding reaction, CD was utilized (Fig. 5). The CD spectra of HSA exhibited two negative bands in the ultraviolet (UV) region at 208 and 222 nm, characteristic of an \( \alpha \)-helical structure of protein.\(^{41}\) The binding of TNN to HSA caused a decrease in band intensity without significant shift of the bands, indicating that TNN bound with the amino acid residues of the main polypeptide chain of HSA and destroyed their hydrogen bonding networks. It was evident that interaction of TNN with HSA caused a slight conformational change of the protein, leading to a loss of \( \alpha \)-helical stability.\(^{42}\)

The basic photophysical properties of 1,8-naphthalimide derivatives depend basically on the polarization of 1,8-naphthalimide molecule due to the electron donor–acceptor interaction occurring between the substituent at the C-4 position and the carbonyl groups from the imide structure of the chromophoric system, which could be affected by the environmental effect of the media upon the interaction.\(^{43,44}\) The absorption and fluorescence spectra of TNN (Fig. S5) in solvents of varying polarity were investigated. Table II summarizes the spectral characteristics of TNN in these solvents: the maximum absorption (\( \lambda_{\text{A}} \)) and fluorescence maxima (\( \lambda_{\text{F}} \)), the fluorescence intensity (\( I_{\text{F}} \)), and the Stokes shift (\( \Delta \lambda \)). As seen from Fig. S5a and Table S1, with the increase of solvent polarity, the absorption band shifted from 361 nm to 366 nm, indicating that TNN exhibited a positive solvatochromism. As the polarization of the dye molecule depends strongly on the solvent’s polarity as well as to the specific interactions of dye–solvent causing a change in the polarization of the dye chromophoric system, the fluorescence intensity and Stokes shift are also parameters. It was also seen

![Fig. 4. Synchronous fluorescence spectra of HSA in the presence of various concentrations of TNN: (a) \( \Delta \lambda = 60 \text{ nm} \); (b) \( \Delta \lambda = 15 \text{ nm} \). The concentrations of HSA and TNN are the same as in Fig. 1.](image)

![Fig. 5. CD spectra of TNN-HSA system at pH 7.4 at room temperature. \( c(\text{HSA}) = 1.0 \text{ \mu M} \); \( c(\text{TNN}) = 0.1 \text{ \mu M} \); (A-D) 0, 3.85, 6.54, and 9.09, respectively.](image)

**Table II.** Analytical parameters of fluorimetric method for the determination of different proteins (\( n = 6 \)).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Linear range (( \mu M ))</th>
<th>Linear regression equation (( C = \mu M ))</th>
<th>Correlation coefficient</th>
<th>Limit of detection (3( \sigma ), 10(^{-10} \text{ M} ))</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSA</td>
<td>0.1–14.2</td>
<td>( F_{560}F_{245} = 0.54 + 0.11C )</td>
<td>0.9976</td>
<td>1.37</td>
<td>1.23</td>
</tr>
<tr>
<td>BSA</td>
<td>0.2–13.0</td>
<td>( F_{560}F_{245} = 0.62 + 0.17C )</td>
<td>0.9988</td>
<td>1.84</td>
<td>1.10</td>
</tr>
<tr>
<td>( \gamma )-Globulin</td>
<td>0.1–9.7</td>
<td>( F_{560}F_{245} = 0.43 + 0.32C )</td>
<td>0.9983</td>
<td>3.14</td>
<td>2.44</td>
</tr>
<tr>
<td>Hb</td>
<td>0.3–11.3</td>
<td>( F_{560}F_{245} = 0.36 + 0.49C )</td>
<td>0.9973</td>
<td>6.88</td>
<td>2.31</td>
</tr>
</tbody>
</table>

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from Table II that the fluorescence intensity of TNN was decreased and the Stokes shift was increased with increasing polarity of solvents. According to Andrea et al., formation of intramolecular charge transfer (ICT) is favored in polar solvents and results in enhanced solvent relaxation processes and a more pronounced Stokes shift. Thus, in most cases, ICT states were more likely to relax by nonradiative processes than by fluorescence emission, resulting in low fluorescence intensities of TNN in polar solvent. The results implied that TNN was sensitive to the polarity of solvent and was expected to act as a fluorescent probe for biomolecules such as protein.

A three-dimensional fluorescence contour map of TNN in ethanol is shown in Fig. S6. The effect of different concentrations of HSA on the excitation spectra (λ<sub>ex</sub> = 440 nm) and emission spectra (λ<sub>ex</sub> = 245 or 365 nm) of TNN were studied (Fig. 6). As seen from Fig. 6, the fluorescence intensity of TNN decreased upon increasing amounts of HSA, along with a slight red shift. The ratio of emission spectral intensity (F<sub>365</sub>/F<sub>245</sub>) was in proportion to the concentrations of HSA, based on which the working curve for detecting HSA was obtained and is shown in Fig. 7. The analytical parameters of this method for determination of HSA, BSA, Hb, and γ-globulin were listed in Table II. In comparison with other fluorimetric methods (Table S2), this method had merits of more sensitive fluorimetry for proteins.

The effect of interfering substances with different concentrations on the determination of HSA was studied. The results are listed in Table III. It can be seen that the water-soluble amino acids and most of the cations did not interfere or only interfered slightly under the permission of ±5.0% relative error, whereas Fe<sup>3+</sup>, Cu<sup>2+</sup>, and Mg<sup>2+</sup> produced obvious interference.

The effect of metal cations on the fluorescence spectra of TNN in ethanol was investigated and presented in Fig. S7. It can be seen that the fluorescence intensity of TNN was decreased with the addition of metal cations, which was different for every metal cation. On the other hand, a bathochromic shift of the fluorescence maxima was observed. These changes could be explained by the formation of a

### Table III. Effect of interfering substances.

<table>
<thead>
<tr>
<th>Foreign Substance</th>
<th>Concentration (μM)</th>
<th>Relative error (%)</th>
<th>Foreign Substance</th>
<th>Concentration (μM)</th>
<th>Relative error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>50</td>
<td>1.34</td>
<td>Lactose</td>
<td>40</td>
<td>3.21</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>50</td>
<td>2.51</td>
<td>Chitosan</td>
<td>10</td>
<td>5.18</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>50</td>
<td>-3.24</td>
<td>Trehalose</td>
<td>30</td>
<td>-2.98</td>
</tr>
<tr>
<td>Mg&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>50</td>
<td>4.64</td>
<td>Sucrose</td>
<td>30</td>
<td>-1.22</td>
</tr>
<tr>
<td>Ni&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>50</td>
<td>-4.31</td>
<td>VB1</td>
<td>20</td>
<td>4.34</td>
</tr>
<tr>
<td>Pb&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>40</td>
<td>-3.10</td>
<td>Pepsase</td>
<td>25</td>
<td>3.85</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>40</td>
<td>2.33</td>
<td>Trypsophan</td>
<td>5</td>
<td>6.01</td>
</tr>
<tr>
<td>Ag&lt;sup&gt;+&lt;/sup&gt;</td>
<td>40</td>
<td>4.87</td>
<td>Tyrosine</td>
<td>9</td>
<td>3.52</td>
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<tr>
<td>Cr&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>40</td>
<td>3.14</td>
<td>Glycine</td>
<td>10</td>
<td>4.34</td>
</tr>
<tr>
<td>Mn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>30</td>
<td>4.87</td>
<td>Proline</td>
<td>10</td>
<td>4.65</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>15</td>
<td>9.74</td>
<td>Serine</td>
<td>10</td>
<td>3.78</td>
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<tr>
<td>Mg&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>10</td>
<td>11.02</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;</td>
<td>30</td>
<td>4.89</td>
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<td>Fe&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>10</td>
<td>8.59</td>
<td>SO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>20</td>
<td>2.88</td>
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<tr>
<td>Glucose</td>
<td>40</td>
<td>-3.91</td>
<td>Cr&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>40</td>
<td>3.79</td>
</tr>
</tbody>
</table>
complex between the metal cations and TNN. In the case of TNN, the oxygen at the 1,8-naphthalimide C-4 position acted as a fluorophore-donating moiety, whereas the oxygen in the tetrahydrofururyl fragment acted as a receptor unit. The electron-donating ability was strengthened by the interactions between metal cations and TNN, resulting in a red shift in the emission. This was different from the 1,8-naphthalimide fluorophores with one electron-donating group, which showed the fluorescence enhancement through the inhibition of photo-induced electron transfer (PET) in the presence of metal ions and weakness of the quenching between cations and the electron-deficient fluorophore. Thus, the interactions between TNN and serum albumin were weakened in the presence of Fe$^{3+}$, Cu$^{2+}$, and Mo$^{6+}$.

Fresh human serum samples were obtained from Fourth Military Medical University and diluted 1000-fold. HSA was selected as a control sample to determine the protein in human serum samples, and the results are listed in Table IV. It could be seen that the results obtained by this method coincide with the data provided by clinical physicians. Therefore, this method could be applied to the determination of total protein in human serum with satisfactory results.

CONCLUSION

In this work, a novel 4-(tetrahydro-2-furanmethoxy)-N-octadecl-1,8-naphthalimide (TNN) has been synthesized as a fluorescent probe for detecting proteins. The interactions between TNN and HSA were investigated by spectroscopic methods including fluorescence spectroscopy and circular dichroism. At the same time, the interaction between TNN and HSA lead to the remarkable fluorescence intensity decrease and the decrease was proportional to the concentration of HSA in a certain range. Based on this, a new fluorimetric method has been developed to determine protein. The detection limits reached the level of 10$^{-10}$ M$^{-1}$. This method was satisfactorily utilized in actual sample determination. These experimental data might be available for analytical application of TNN as a biological stain.

ACKNOWLEDGMENTS

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