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Interactions between 1-benzoyl-4-*p*-chlorophenyl thiosemicarbazide and serum albumin: investigation by fluorescence spectroscopy

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Abstract—The interactions between 1-benzoyl-4-*p*-chlorphenyl thiosemicarbazide (BCPT) and bovine serum albumin (BSA) or human serum albumin (HSA) have been studied by fluorescence spectroscopy. By the analysis of fluorescence spectrum and fluorescence intensity, it was showed that BCPT has a strong ability to quench the intrinsic fluorescence of both bovine serum albumin and human serum albumin through a static quenching procedure. The binding constants of BCPT with BSA or HSA were determined at different temperatures based on the fluorescence quenching results. The binding sites were obtained and the binding force were suggested to be mainly hydrophobic. The effect of common ions on the binding constants was also investigated. A new fluorescence spectroscopy assay of the proteins is presented. The linear range is 5.36–67.0 μg mL^{-1} with recovery of 101.1% for BSA, and the linear range is 8.28–144.9 μg mL^{-1} with recovery of 102.6% for HSA. Determination of the proteins in bovine serum or in human serum by this method gives results which are very close to those obtained by using Coomassie Brilliant Blue G-250 colorimetry. A practical method was proposed for the determination of BCPT in human serum samples.

1. Introduction

The derivatives of thiourea are new and important organic reagents. In the past several years, a series of saturated, unsaturated fatty hydrocarbon and aryl substituted thiourea compounds have been synthesized and widely used in analytical chemistry.[1–4] Acyl thiosemicarbazide compounds exhibit various biological activities such as antiviral and antifungal.[5] They can be used as insecticides, herbicides and plant-growth regulators.[6] Thiourea is a new kind of drug, and 1-benzoyl-4-*p*-chlorphenyl thiosemicarbazide (BCPT) is a new acyl thiosemicarbazides reagent.

The protein is an important chemical substance in our life and the main target of all medicines in organism. Serum albumin, the most abundant protein in the circulatory system, has been one of the most extensively studied proteins. It is synthesized in the liver, exported as a non-glycosylated protein. Therefore, the quantitative assay of protein is very important in biochemistry and clinical medicine. There are many spectrophotometric methods, usually based on the ability of proteins to bind dyes, for determining the content of proteins in samples. The most frequently used assays are the Lowry,[7] Coomassie Brilliant Blue (CBB),[8,9] Bromophenol Blue [10] and Bromocresol Green [11] methods. However, these methods have their disadvantages in terms of sensitivity, selectivity, stability and simplicity. For example, the Lowry, the Bromophenol Blue and Bromocresol Green methods have relatively poor sensitivity and are severely interfered by substances often found in biological samples. Moreover, the dye binding method is based on the fact that the dyes exist in at least two colored forms, one form being converted into the other after binding to a protein, and then being monitored spectrophotometrically by measuring the change in the absorbance. Hence, dyes whose color do not change significantly on binding to protein cannot be used for the determination of proteins in solution. This limitation can be overcome by using a

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new detection technique—fluorescence spectroscopy described in this paper. The fluorescence method is based on the fact that the tryptophan residues in proteins have intrinsic fluorescence. As the major transport for unesterified fatty acids, proteins are also capable of binding an extraordinarily diverse range of metabolites, drugs and organic compounds. Information about the protein can be obtained by the measurement of intrinsic fluorescence intensity of the tryptophan residues before and after addition of the drug. In recent years, there have been several reports devoted to the study of the interaction mechanism between drug and the proteins.[12–15] The drugs are transported in the blood while bound to albumin. In this paper, the interactions of serum albumin (BSA or HSA) with BCPT have been studied by fluorescence quenching method. The binding constants were obtained at different temperatures in the medium of HCl–Tris (pH = 7.4) buffer solution. According to the mechanism of Föster energy transference, the transfer efficiency of energy and distance between the acceptor BSA or HSA and BCPT were found. The binding sites and main sorts of binding force have been suggested. The effects of common ions on binding constants have been discussed. A novel method is presented for the determination of proteins. The determination of BCPT in serum samples is also proposed based on the binding interaction of the proteins with BCPT.

2. Materials and methods

2.1. Materials

All starting materials were analytical reagent grade and double distilled water was used throughout. BSA or HSA (Sigma) was directly dissolved in double distilled water to prepare their stock solutions (1.0×10^{-4} mol L^{-1}) which were then stored at 0–4 °C. 5.0×10^{-5} mol L^{-1} BCPT, 0.1 mol L^{-1} Tris–HCl buffer solution of pH 7.4 and 1.00 mg mL^{-1} common ions solutions were prepared. Bovine serum samples were obtained from veterinary station of Xiuwu. Human serum samples were provided by the Hospital of Henan Normal University. The serum samples were diluted 1000-fold with doubly distilled water.

2.2. Apparatus

All fluorescence measurements were carried out on a FP-6200 spectrofluorimeter (JASCO, Japan) and an RF-540 spectrofluorimeter (Shimadzu, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. A LAMBDA-17 visible ultraviolet spectrophotometer (PE, USA) equipped with 1.0 cm quartz cells was used for scanning the UV spectrum. All pH measurements were made with a pHs-3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass electrode.

2.3. Characterization of BCPT

BCPT was synthesized using solid-state method[16] at room temperature.

Element analysis of BCPT: caculated (%) C, 54.99; H, 3.93; N, 13.75. Found (%), C, 54.78; H, 3.61; N, 13.57. IR (KBr, cm^{-1}): 3357, 3187, 3031, 1673, 1600, 1509, 1400, 1255, 833, 779, 690. ^1H NMR (δ, ppm): 7.45∼8.00 (m, 9H, ArH), 8.97 (s, 1H, NH), 9.60 (s, 1H, NH), 9.85 (s, 1H, NH).

According to the above data, the structure of BCPT was suggested in Figure 1.

2.4. Optimization of experimental conditions

In order to select the optimum conditions, various experimental parameters including medium, pH and temperature were studied with the concentration of BCPT being 2.0×10^{-6} mol L^{-1} in all cases.

2.5. Effect of media

Several buffer solutions (Tris–HCl, NaAc–HAc, Na_2HPO_4–KH_2PO_4, $Na_2B_4O_7$–KH_2PO_4) were tested in the present experiments. It is shown that the sensitivity is higher in Tris–HCl buffer solution than in others. Therefore, Tris–HCl buffer solution was used in the experiments. The influence of volume of Tris–HCl (0.1 mol L^{-1}) upon fluorescence intensity has been investigated in the range 0.5–5 mL. It is found that the sensitivity is higher if 2.0 mL of the buffer is used. Thus, 2.0 mL of 0.1 mol L^{-1} Tris–HCl was considered to be the best choice.

2.6. Effect of pH

The influence of pH was studied in the range of 6.4–8.0. The stable fluorescence intensity for the complex BSA–BCPT or HSA–BCPT could be achieved over the pH ange 6.4–7.5. Therefore, Tris–HCl buffer solution at pH = 7.4 was used in this work.

2.7. Effect of temperature

The binding constants between BSA or HSA with BCPT were determined at different temperatures (15, 25, 35 °C). It was observed that the value of the binding constant was the greatest at 15°, but interaction rate was the slowest at this temperature. Less time was needed when the interaction was at 25 °C than at 15°, and the sensitivity at 25 °C is higher than at 35 °C. Thus the most appropriate temperature for the measurement is 25 °C.

2.8. Methods for fluorescence spectrum and UV absorption spectrum measurements

Under the optimum conditions described above, appropriate amounts of BCPT, BSA or HSA, and 2.0 mL Tris–HCl buffer solution were added to a 10 mL standard flask, diluted to 10 mL with double distilled water and then shaken. Fluorescence quenching spectra of

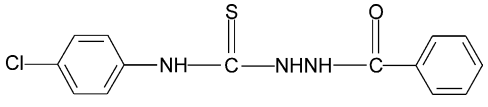

Figure 1. The structure of BCPT.

BSA or HSA were obtained at excitation and emission wavelengths of $\lambda_{ex} = 282$ nm and $\lambda_{em} = 300$–400 nm. In the presence of common ions, other fluorescence spectra could be obtained. In addition, the UV absorption spectrum of BCPT, BSA or HSA were obtained.

3. Results and discussion

3.1. Binding constants of BSA or HSA with BCPT

The fluorescence intensity (F) of BSA or HSA was decreased regularly with increasing concentration of BCPT, and small blue shift was observed for the emission wavelengths if the concentration of BSA or HSA was fixed. The fluorescence quenching spectra of BSA and HSA with varying concentrations of BCPT are shown in Figure 2(a) and (b), respectively.

It is noted from Figure 2 that a complex was possibly formed between BCPT and serum albumin (BSA or HSA) which is responsible to the quenching of the fluorescence of BSA or HSA. In order to confirm the formation of this complex, the absorption spectrum of BSA and HSA in the presence of BCPT are shown in Figure 3(a) and (b), respectively. It is suggested from Figure 3 that the complex was formed BCPT–BSA or BCPT–HSA.

The static quenching and dynamic quenching were differentiated by the results at different temperatures. The quenching rate constants decrease with increasing temperature for the static quenching, but the reversed effect was observed for the dynamic quenching.[17] The energy transfer was occurred by the blue shift of emission wavelengths. The possible quenching mechanism can be interpreted by the fluorescence quenching spectra of the proteins and the F_0/F–C (Stern–Volmer) curves of BSA(a) or HSA(b) with BCPT at different temperatures (15, 25, 35 °C) as shown in Figure 4.

It can be found from Figure 4 that the Stern–Volmer plots are linear and the slopes decrease with increasing temperature. This indicates the static quenching interaction between BCPT and BSA or HSA. In order to confirm this point, the procedure was assumed to be dynamic quenching. The quenching equation is presented by

$$F_0/F = 1 + K_q\tau_0\,[Q] = 1 + K_{sv}[Q] \quad (1)$$

where F and F_0 are the fluorescence intensity with and without quencher, respectively. K_q is the quenching rate constant of the biomolecule, K_{sv} is the Stern–Volmer quenching constant, τ_0 is the average lifetime of the biomolecule without quencher, [Q] is the concentration of quencher. Obviously,

$$K_{sv} = K_q\tau_0 \quad (2)$$

Because the fluorescence lifetime of the biopolymer is 10^{-8} s^{-1} ,[18] K_{sv} is the slop of linear regressions of Figure 4. According to eq 2, the quenching constant K_q can be obtained and are listed in Table 1 together with the correlation coefficients. However, the maximum scatter collision quenching constant K_q of various quenchers with the biopolymer is 2×10^{10} L mol^{-1} S^{-1}.[19] Obviously, the rate constant of protein quenching procedure initiated by BCPT is greater than the K_q of the scatter procedure. This means that the quenching is not initiated by dynamic collision but from the formation of a complex. The static quenching equation is presented by:[17]

$$(F_0 - F)^{-1} = F_0^{-1} + K^{-1}F_0^{-1}[Q]^{-1} \quad (3)$$

where K is the binding constant of BCPT with biomolecule, which can be determined by the slope of the Lineweaver–Burk (1/(F_0–F)–1/[Q]) curves as shown in Fig. 5. Thus we can obtain binding constants K of BCPT with BSA (a) or HSA (b) from the intercept and slope of Figure 5 (intercept = F_0^{-1}, slope = K^{-1} F_0^{-1}, so $K = F_0^{-1}$/slope). The results are given in Table 2.

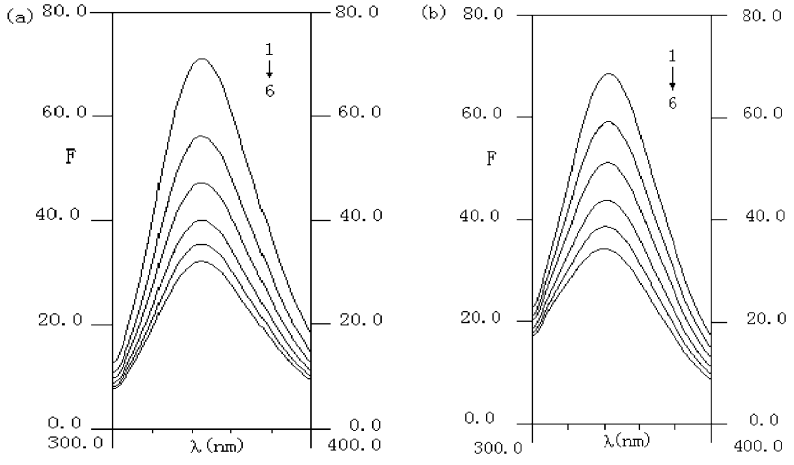

Figure 2. The fluorescence quenching spectrum of bovine serum albumin (a) and human serum albumin (b) at various concentration of BCPT: (a) $C_{BSA} = 0.9\times10^{-6}$ mol L^{-1}; C_{BCPT} of 1–6: 0, 2.0×10^{-6}, 4.0×10^{-6}, 6.0×10^{-6}, 8.0×10^{-6}, 1.0×10^{-5} mol L^{-1}. (b) $C_{HSA} = 1.5\times10^{-6}$ mol L^{-1}; C_{BCPT} of 1–6: 0, 0.4×10^{-6}, 0.8×10^{-6}, 1.2×10^{-6}, 1.6×10^{-6}, 2.0×10^{-6} mol L^{-1}.

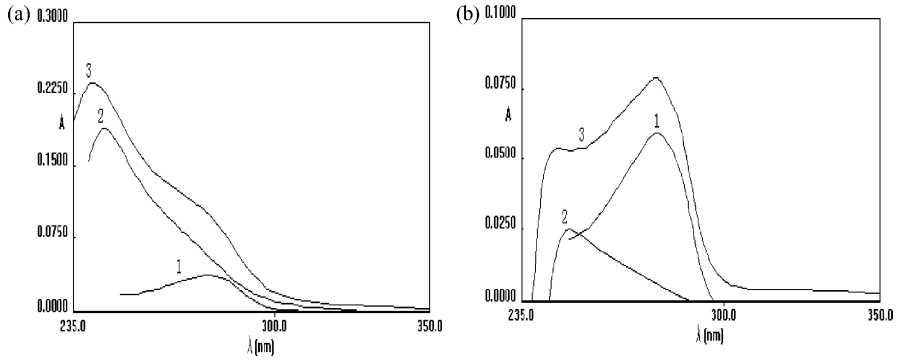

Figure 3. The UV absorption spectrum of BSA (a) and HSA (b) in the presence of BCPT: (a) (1) the UV absorption spectrum of BSA, $C_{BSA}=0.9\times10-^{6}$ mol L^{-1}; (2) the UV absorption spectrum of BCPT, $C_{BCPT}=1.0\times10^{-5}$ mol L^{-1}; (3) the UV absorption spectrum of BSA–BCPT complex, $C_{BSA}=0.9\times10^{-6}$ mol L^{-1}, $C_{BCPT}=1.0\times10^{-5}$ mol L^{-1}; (b) (1) The UV absorption spectrum of HSA, $C_{HSA}=1.5\times10^{-6}$ mol L^{-1}; (2) the UV absorption spectrum of BCPT, $C_{BCPT}=2.0\cdot10^{-6}$ mol L^{-1}; (3) the UV absorption spectrum of HSA–BCPT complex, $C_{HSA}=1.5\times10^{-6}$ mol L^{-1}, $C_{BCPT}=2.0\times10^{-5}$ mol L^{-1}.

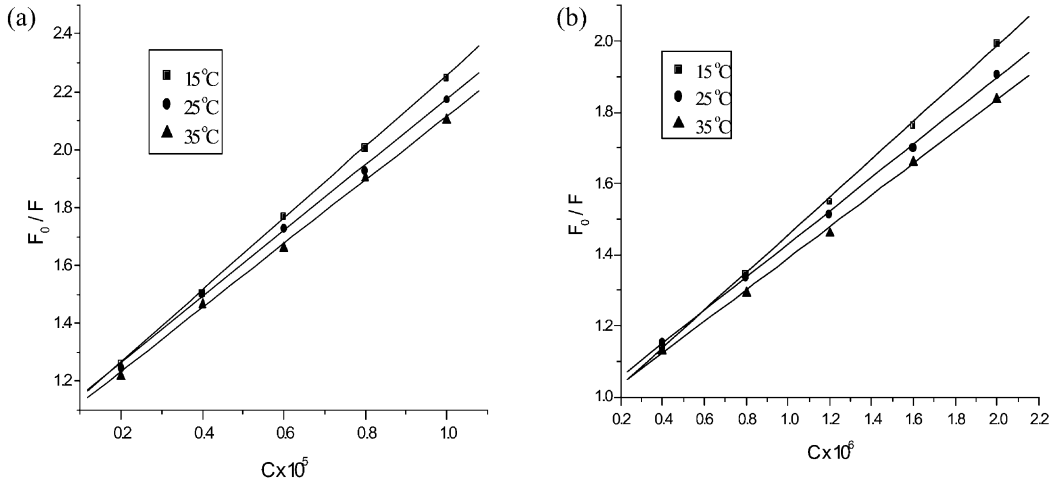

Figure 4. The Stern–Volmer curves for quenching of BCPT with BSA (a) or HSA (b) (a) C_{BSA} and C_{BCPT} are the same as those in Figure 2(a); (b) C_{HSA} and C_{BCPT} are the same as those in Figure 2(b).

Table 1. The dynamic quenching constants (L mol^{-1} s^{-1}) between BCPT and BSA (a) or HSA (b)

T (°C)	K_q (a)	R (a)	K_q (b)	R (b)
15	$(1.239\pm0.015)\times10^{13}$	0.9998	$(5.362\pm0.020)\times10^{13}$	0.9996
25	$(1.138\pm0.029)\times10^{13}$	0.9992	$(4.674\pm0.020)\times10^{13}$	0.9995
35	$(1.112\pm0.026)\times10^{13}$	0.9993	$(4.479\pm0.036)\times10^{13}$	0.9993

It is shown that the binding between BCPT and BSA or HSA is remarkable and the effect of temperature is small. Thus, BCPT can be stored and removed by the proteins in the body.

3.2. Determination of the acting force

The acting forces between a drug and a biomolecule may include hydrogen bond, van der Waals force, electrostatic force and hydrogen bond interaction force and so on[20] Because the temperature effect is very small, the interaction enthalpy change can be regarded as a constant if the temperature range is not too wide. Therefore, from the following equations:

$$\ln(K_2/K_1)=\Delta H(1/T_1-1/T_2)/R \quad (4)$$

$$\Delta G=\Delta H-T\Delta S \quad (5)$$

$$\Delta G=-\mathrm{RT}\ln K \quad (6)$$

where ΔH, ΔG and ΔS are, respectively, enthalpy change, free energy change and entropy change, ΔH and ΔS for the binding interaction between BCPT and BSA are calculated to be -2.80 KJ mol^{-1} and 88.53 J mol^{-1} K^{-1}, and those for the binding interaction between BCPT and HSA are -12.10 KJ mol^{-1} and 62.21 J mol^{-1} K^{-1}, respectively. In other words, enthalpy change $\Delta H<0$ and entropy change $\Delta S>0$. So it can be deduced that the acting forces are mainly a hydrophobic interaction.[21]

3.3. Energy transfer between BCPT and BSA or HSA

The overlap of the UV absorption spectra of BCPT with the fluorescence emission spectra of BSA (a) or HSA (b) is shown in Fig. 6. According to Föster's non-radiative energy transfer theory,[22] the energy transfer will happen under conditions: (i) the donor can produce fluorescence

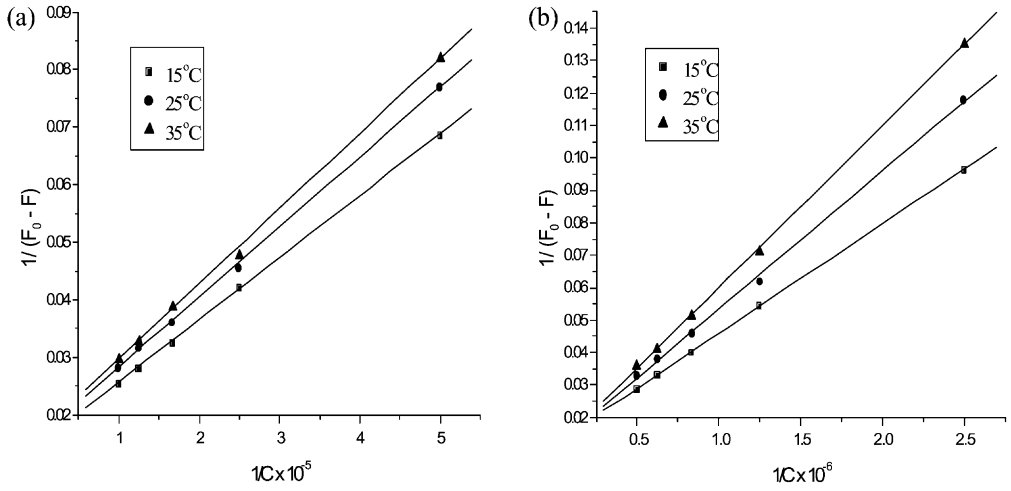

Figure 5. The Lineweaver–Burk curves for quenching of BCPT with BSA (a) or HSA (b): (a) C_{BSA} and C_{BCPT} are the same as those in Figure 2(a); (b) C_{HSA} and C_{BCPT} are the same as those in Figure 2(b).

Table 2. The binding constants (L mol^{-1}) between BCPT and BSA (a) or HSA (b)

T (°C)	K (a)	R (a)	K (b)	R (b)
15	$(1.353\pm0.018)\times10^5$	0.9999	$(2.697\pm0.015)\times10^5$	0.9999
25	$(1.290\pm0.023)\times10^5$	0.9997	$(2.488\pm0.019)\times10^5$	0.9994
35	$(1.254\pm0.033)\times10^5$	0.9993	$(1.950\pm0.017)\times10^5$	0.9998

light; (ii) fluorescence emission spectrum of the donor and UV absorbance spectrum of the acceptor have more overlap; (iii) the distance between the donor and the acceptor is approach and lower than 7 nm. The energy transfer effect is related not only to the distance between the acceptor and the donor, but also to the critical energy transfer distance, that is:

$$E = R_0^6/(R_0^6 + r^6) \tag{7}$$

where r is the distance between the acceptor (A) and the donor (D), R_0 is the critical distance when the transfer efficiency is 50%, which can be calculated by

$$R_0^6 = 8.8 \times 10^{-25} k^2 \phi n^{-4} J \tag{8}$$

where k^2 is the spatial orientation factor of the dipole, n is the refractive index of the medium, ϕ is the fluorescence quantum yield of the donor, J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,

$$J = \Sigma F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda/\Sigma F(\lambda)\Delta\lambda \tag{9}$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength λ, $\varepsilon(\lambda)$ is the molar absorptivity of the acceptor at wavelength λ. The energy transfer efficiency is given by

$$E = (1 - F)/F_0 \tag{10}$$

J can be evaluated by integrating the spectra in Figure 6. It has been reported for BSA that, $k^2 = 2/3$, $\phi = 0.10$, $n = 1.45$.[23] Based on these data, we found $R_0 = 2.13$ and $r = 2.65$ nm for BSA, and $k^2 = 2/3$, $\phi = 0.118$, $n = 1.336$, $R_0 = 2.12$ nm, $r = 2.60$ nm for HSA. So the distance between BCPT and tryptophan residue in BSA or HSA is 2.65 and 2.60 nm, respectively. Obviously, they are

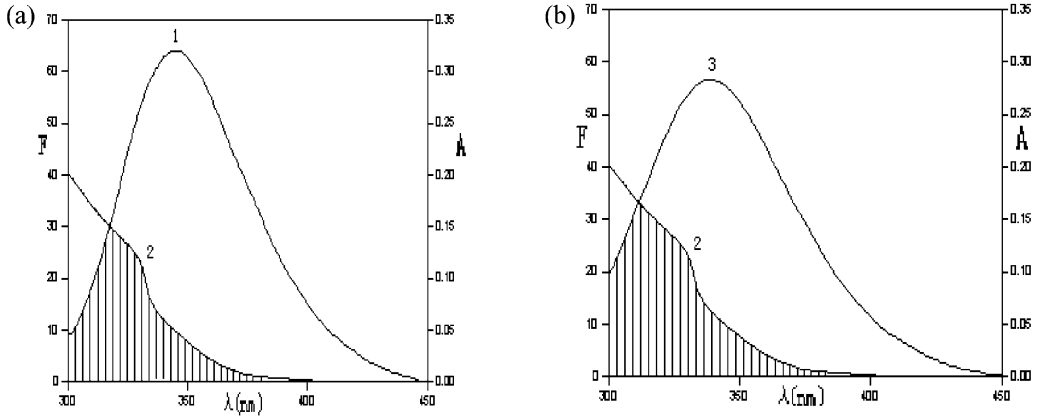

Figure 6. The overlap of the UV absorption spectrum of BCPT with the fluorescence emission spectrum of BSA (a) or HSA (b): (1) the fluorescence spectrum of BSA, F is the fluorescence intensity, $C_{BSA} = 1\times10^{-5}$ mol L^{-1}; (2) the UV absorbance spectrum of BCPT, $C_{BCPT} = 1\times10^{-5}$ mol L^{-1}, A is the UV absorption intensity of BCPT; (3) the fluorescence spectrum of HSA, $C_{HSA} = 1\times10^{-5}$ mol L^{-1}.

lower than 7 nm after interaction between BCPT and BSA or HSA. These accord with conditions of Föster's non-radiative energy transfer theory, indicating again the static quenching interaction between BCPT and BSA or HSA.

3.4. The effect of other ions on the binding constants

The effect of common ions on the binding constants was investigated at 25 °C. The results are summarized in Table 3. It is shown that the binding constants between the protein and BCPT increased in the presence of common ions, implying stronger binding between BCPT and BSA or HSA. The higher binding constant obtained in the presence of metal ions might be resulted from the interaction of metal ion with the drug to form a complex, then the complex interacted with the protein. Thus, prolonging the storage time of the drug in blood plasma and enhancing the maximum effectiveness of the drug. Therefore, in the presence of common ions, BCPT can be stored and removed better by the proteins.

3.5. Precision, limit of detection and working curve

3.5.1. For the proteins. Under the conditions described above, the working curve was obtained. The relationship between the difference in fluorescence intensity ΔF (with and without BCPT) and concentration of the protein is found to be linear in the range of 5.36–67.0 μg mL^{-1} for BSA and 8.28–144.9 μg mL^{-1} for HSA, with the correlation coefficients R (BSA) = 0.9997 and R (HSA) = 0.9992 ($n = 6$), respectively. The detection limit, as defined by IUPAC,[24] is determined to be 2.14 μg mL^{-1} (BSA) and 3.12 μg mL^{-1} (HSA), with the recovery of 101.1% (BSA) and 102.6% (HSA), respectively. The relative standard deviations are 1.96% (BSA) and 2.07% (HSA), respectively, which were obtained for 11 replicate determinations of 1.0×10^{-6} mol L^{-1} BSA or HSA solutions.

3.5.2. For the BCPT. Under the above optimum conditions, linear relationships were found between the difference in fluorescence intensity and concentration of the BCPT in the range 0.536–46.9 μg mL^{-1} (in BSA) and 0.621–41.4 μg mL^{-1} (in HSA) with the correlation coefficients R (in BSA) = 0.9994 and R (in HSA) = 0.9990 ($n = 6$), respectively. The detection limit is determined to be 0.32 μg mL^{-1} (in BSA) and 0.33 μg mL^{-1} (in HSA), respectively. The relative standard deviations are 1.97% (BSA) and 2.61% (HSA), respectively, as obtained from 11 replicate determinations of 1.0×10^{-6} mol L^{-1} BSA or HSA solutions.

Table 3. The binding constants (L mol^{-1}) between BCPT and BSA (a′) or HSA (b′) at 25°C in the presence of common ions

Ions	K (a′)$\times10^{-5}$	R (a′)	K (a′′)/K (a)	K (b′)$\times10^{-5}$	R (b′)	K (b′)/K (b)
Na^+	1.304±0.051	0.9989	1.011	3.020±0.030	0.9999	1.214
K^+	1.579±0.028	0.9990	1.224	3.154±0.035	0.9998	1.268
Ca^{2+}	1.824±0.045	0.9989	1.414	2.749±0.028	0.9999	1.105
Ba^{2+}	1.863±0.023	0.9995	1.444	6.210±0.032	0.9998	2.495
Mg^{2+}	1.905±0.040	0.9993	1.476	2.660±0.030	0.9995	1.069
Al^{3+}	1.997±0.034	0.9994	1.548	4.643±0.039	0.9992	1.866
Cl^-	1.691±0.021	0.9998	1.310	2.982±0.043	0.9989	1.986
Br^-	1.516±0.054	0.9989	1.175	4.018±0.027	0.9998	1.615
F^-	1.509±0.036	0.9997	1.233	6.637±0.039	0.9990	2.667
CO_3^{2-}	1.871±0.036	0.9994	1.450	4.420±0.033	0.9990	1.777
SO_4^{2-}	1.331±0.042	0.9991	1.032	2.566±0.037	0.9993	1.031
PO_4^{3-}	2.453±0.043	0.9989	1.902	5.526±0.040	0.9990	2.221

4. Analysis of samples

Fresh serum samples were diluted about 1000 times in this work. The concentration of the proteins in bovine serum and human serum samples were determined with our proposed method. Table 4 lists the real content of the protein in the samples, which are very close to those obtained using the Coomassie Brillant Blue G-250 (CBB) method.[10]

The amount of BCPT in bovine serum and human serum (each 0.3 mL) could be determined by a standard addition method. The results are listed in Table 5. It can be seen that the present method has good reproducibility and high sensitivity.

Table 5. Determination results of BCPT in samples ($n = 6$)

Sample	Initial (μg)	Added (μg)	Found (μg)	Recovery (%)	RSD (%)
Bovine serum	0	12.23	11.20	97.68	2.76
	0	24.46	23.25	98.07	1.93
	0	36.69	38.70	102.1	1.02
Human serum	0	12.23	13.02	101.9	1.99
	0	24.46	22.97	98.12	2.20
	0	36.69	38.61	103.6	1.98

Table 4. Determination results of the proteins in samples

Sample (no.)	This method ($n = 6$) (mg mL^{-1})	Recovery (%)	RSD (%)	CBB method ($n = 6$) (mg mL^{-1})	Recovery (%)	RSD (%)
Bovine serum 1	60.6	97.9	1.96	61.1	102.0	3.07
Bovine serum 2	74.0	103.2	2.76	72.9	103.3	4.56
Bovine serum 3	87.7	97.6	2.09	85.6	97.9	3.67
Human serum 1	75.1	103.3	2.97	73.2	103.2	4.06
Human serum 2	89.7	98.3	3.01	87.5	102.3	3.43
Human serum 3	106.1	102.6	2.72	104.4	102.2	3.31

5. Conclusion

The study of the interaction of the newly synthesized reagent, BCPT, and BSA or HSA showed that a complex was formed between BCPT and serum albumin by a hydrophobic binding interactions. Based on this phenomenon, a new method for the rapid and simple determination of the proteins is provided. The method is easy to operate and is reliable, practical, and simple. This is the first reported procedure for the determination of the amount of BCPT in bovine serum and human serum. It can be used to determine the proteins containing sulfur. The BCPT might be developed to a new thiourea drug in the future. These experimental and theoretical data could be a useful guide for efficient drug synthesis.

Acknowledgements

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References and notes

1. Ma, D. L.; Cui, F. L.; Xia, D. S.; Wang, Y. L. *Anal. Lett*. **2002**, *35*, 413.
2. Ma, D. L.; Li, Y.; Ma, K. B.; Li, J. P.; Chen, J. G.; Yan, J. W.; Wang, Y. L. *Talanta* **2001**, *53*, 937.
3. Ma, D. L.; Li, Y.; Li, Q. J.; Wang, Y. L. *J. Chin. Chem. Soc*. **2001**, *48*, 1111.
4. Ma, D. L.; Ding, G. S.; Wang, C. X.; Zhang, L.; Wang, Y. L. *Anal. Lett*. **2000**, *33*, 2533.
5. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem*. **1951**, *193*, 265.
6. Crowin, A. H.; Rein Heimer, J. D. *J. Am. Chem. Soc*. **1951**, *73*, 114.
7. Zhang, Z. Y.; Chen, L. M. *Chem. Res. Appl*. **1991**, *3*, 3.
8. Bradford, M. M. *Anal. Biochem*. **1976**, *72*, 248.
9. Zor, T.; Selinger, Z. *Anal. Biochem*. **1996**, *236*, 302.
10. Flores, R. *Anal. Biochem*. **1978**, *88*, 605.
11. Rodkey, F. L. *Clin. Chem*. **1965**, *11*, 478.
12. Gelamo, E. L.; Silva, C. H. T. P.; Imasato, H.; Tabak, M. *Biochimi. Biophys. Acta* **2002**, *159*, 484.
13. Jiang, C. Q.; Gao, M. X.; He, J. X. *Anal. Chim. Acta* **2002**, *452*, 185.
14. Klajnert, B.; Bryszewska, M. *Bioelectrochemistry* **2002**, *55*, 33.
15. Kitamura, K.; Mano, H.; Shimamoto, Y.; Tadokoro, Y.; Tsuruta, K.; Kitagawa, S. *Freseius J. Anal. Chem*. **1997**, *358*, 509.
16. Li, J. P.; Luo, Q. F.; Wang, Y. L.; Wang, H. *J. Chin. Chem. Soc*. **2001**, *46*, 73.
17. Chen, G. Z.; Huang, X. Z.; Xu, J. G.; Zheng, Z. Z.; Wang, Z. B. *The Methods of Fluorescence Analysis*, 2nd ed.; Science Press: Beijing, 1990; p 112.
18. Lakowica, J. R.; Weber, G. *Biochemistry* **1973**, *12*, 4161.
19. Ware, W. R. *J. Phys. Chem*. **1962**, *66*, 445.
20. Nie, L. H.; Zhao, H. C.; Wang, X. B.; Wang, X. *J. Beijing Normal University (Nat. Sci.)* **2001**, *37*, 87.
21. Ross, P. D.; Subramanian, S. *Biochemistry* **1981**, *20*, 3096.
22. Yang, P. *The Guide to Bioinorganic Chemistry*; Xi'an Jiaotong Press: Xi'an, 1991; p 164.
23. Shang, Z. C.; Yi, P. G.; Yu, Q. L.; Lin, R. L. *Acta Phys. Chim. Sin*. **2001**, *17*, 48.
24. Irving, H. M. H. N.; Freiser, H. In T. S. Wet, Ed. *IUPAC Compendium of Analytical Nomenclature*, *Definitive Rules*; Pergamon: Oxford, 1987.