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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 189–197

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

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Fluorescence spectroscopy studies on 5-aminosalicylic acid and zinc 5-aminosalylicylate interaction with human serum albumin

Feng-Ling Cui [a,b], Jing Fan [a,*], Wei Li [a], Yun-Chang Fan [a], Zhi-De Hu [b]

[a] *Key Laboratory of Environmental Science and Engineering for Colleges and Universities of Henan Province, School of Chemistry and Environmental Science, Henan Normal University, Xinxiang, Henan 453002, PR China*

[b] *Department of Chemistry, Lanzhou University, Lanzhou 730000, PR China*

Received 21 May 2003; received in revised form 18 August 2003; accepted 18 August 2003

Abstract

The interaction between 5-aminosalicylic acid (5-ASA) or zinc 5-aminosalylicylate (5-ASZ) and human serum albumin (HSA) was studied by fluorescence spectroscopic technique. The binding constants of 5-ASA or 5-ASZ with HSA were determined at different temperatures under the optimum conditions. The binding sites were obtained and the acting force were suggested to be mainly hydrophobic. The effect of common ions on the binding constants was also investigated. A fluorescence spectroscopy assay of the proteins is presented in the paper. The determination results of the proteins in human serum by this method are very close to those obtained using Coomassie Brilliant Blue G-250 colorimetry, with relative standard deviations of 0.8–2.9%. A practical method was proposed for the determination of 5-ASA or 5-ASZ in human serum samples.

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Keywords: 5-Aminosalicylic acid; Zinc 5-aminosalylicylate; Human serum albumin; Fluorescence spectroscopy

1. Introduction

5-Aminosalicylic acid (5-ASA) [1,2] and zinc 5-aminosalylicylate (5-ASZ) [3] (Fig. 1) are drugs for treatment of ulcerous colonitis and enteritis, with the excellence of high efficiency and low toxicity. The protein is an important chemical substance in human life and the main target of all medicines in organism. Serum albumin is the most abundant of the protein in the circulatory system. It is the major transport protein and capable of binding a range of metabolites, drugs and organic compounds. The remarkable binding property of albumin plays a central role in both the efficacy and the rate of drug delivery. So 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 Bradford [4], Lowry [5], Biuret [6] and Bromocresol Green methods [7]. However, these methods have some limitations. The dye binding

* Corresponding author. Tel.: +86-373-3326335; fax: +86-373-3326445.

E-mail address: fenglingcui@hotmail.com (J. Fan).

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doi:10.1016/j.japna.2003.08.012

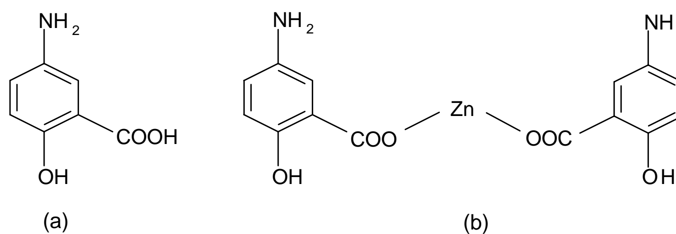

Fig. 1. The structures of 5-ASA (a) and 5-ASZ (b).

method is often 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 which change little or do not change their color on binding cannot be used for the determination of proteins in solution. This limitation in the determination of proteins by spectrophotometry can be overcome by using a fluorescence technique presented in this paper. The fluorescence method is based on the fact that the tryptophan residues in proteins have intrinsic fluorescence. Information about the protein can be obtained by the measurement of intrinsic fluorescence intensity of the tryptophan residues before and after the addition of the drug.

There are some reports in the literatures about the interaction mechanism between drug and the proteins, but not between 5-ASA or 5-ASZ and protein [8–13]. In this paper, the interaction of HSA with the drugs has been studied by fluorescence quenching method. The binding constants were obtained at different temperatures under pH 7.4. According to the mechanism of Föster energy transference, the transfer efficiency of energy and distance between the acceptor 5-ASA or 5-ASZ and HSA were found. The binding sites and main sorts of binding force have been suggested. The effect of common ions on binding constants has been discussed. Based on the binding interaction of the proteins with 5-ASA or 5-ASZ, a method for determination of the proteins was presented. A practical method was proposed for the determination of 5-ASA or 5-ASZ in human serum samples. It is hoped that this study would provide some useful information on the interaction mechanism of the proteins with small molecules.

2. Experimental

2.1. 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 PE 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.

2.2. Reagents

All starting materials were analytical reagent grade and double distilled water was used for all the measurements. 5.0×10^{-4} mol l^{-1} 5-ASA, 2.0×10^{-4} mol l^{-1} 5-ASZ and 0.5 mol l^{-1} NaCl were prepared. HSA (Sigma) was directly dissolved in water to prepare stock solutions (3.0×10^{-3} mol l^{-1}) and then stored at 0–4 °C; 0.1 mol l^{-1} Tris–HCl buffer solution of pH 7.4 and 1.00 mg ml^{-1} common ions solutions were prepared.

2.3. Procedures for fluorescence spectrum measurements

To a 10 ml standard flask, appropriate amount of 5-ASA or 5-ASZ solution, HSA, 2.0 ml Tris–HCl buffer and 2.0 ml of NaCl solution were added, diluted to 10 ml with double distilled water and then shaken. Fluorescence quenching spectra of HSA were obtained at excitation and emission wavelengths of

$\lambda_{ex} = 282$ nm and $\lambda_{em} = 300$–400 nm. In addition, in the presence of common ions, other fluorescence spectra could be obtained.

3. Result and discussion

3.1. Binding constants of HSA and drug

The fluorescence intensity (F) of HSA decreased regularly with increasing concentration of 5-ASA or 5-ASZ, and small red shift was observed for the emission wavelengths if the concentration of HSA was fixed. The fluorescence quenching spectra of HSA at various concentrations of 5-ASA or 5-ASZ are shown in Fig. 2(a) and (b), respectively.

It is suggested from Fig. 2 that a new compound was formed between drug and HSA, which quenched the fluorescence of 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 [14]. The energy transfer was happened by the red shift of emission wavelengths. The possible quenching mechanism can be interpreted by the fluorescence quenching spectra of HSA and the $F_0/F \sim C$ (Stern–Volmer) curves of HSA with 5-ASA (a) or 5-ASZ (b) at different temperatures (16, 26 and 36 °C) as shown in Fig. 3, where F and F_0 are the fluorescence intensities with and without quencher. The results of linear regressions of Fig. 3(a) are: $F_0/F = 0.9576 + 9436.7\ [C_{5\text{-ASA}}]$, at 16 °C; $F_0/F = 0.9676 + 8318.4\ [C_{5\text{-ASA}}]$, at 26 °C; $F_0/F = 0.9622 + 7822.3\ [C_{5\text{-ASA}}]$, at 36 °C, and $F_0/F = 0.9988 + 20{,}542.5\ [C_{5\text{-ASZ}}]$, at 16 °C; $F_0/F = 0.9983 + 19{,}128.8\ [C_{5\text{-ASZ}}]$, at 26 °C; $F_0/F = 0.9881 + 18{,}057.0\ [C_{5\text{-ASZ}}]$, at 36 °C, respectively.

It can be found from Fig. 3 that the Stern–Volmer plots are linear and the slopes decrease with increasing of temperature. This indicates the static quenching interaction between HSA and 5-ASA or 5-ASZ. In order to confirm this point, the procedure was assumed to be dynamic quenching. The quenching equation is presented by

$$\frac{F_0}{F} = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

where K_q is the quenching rate constant of the biomolecule, K_{sv} is the Stern–Volmer quenching

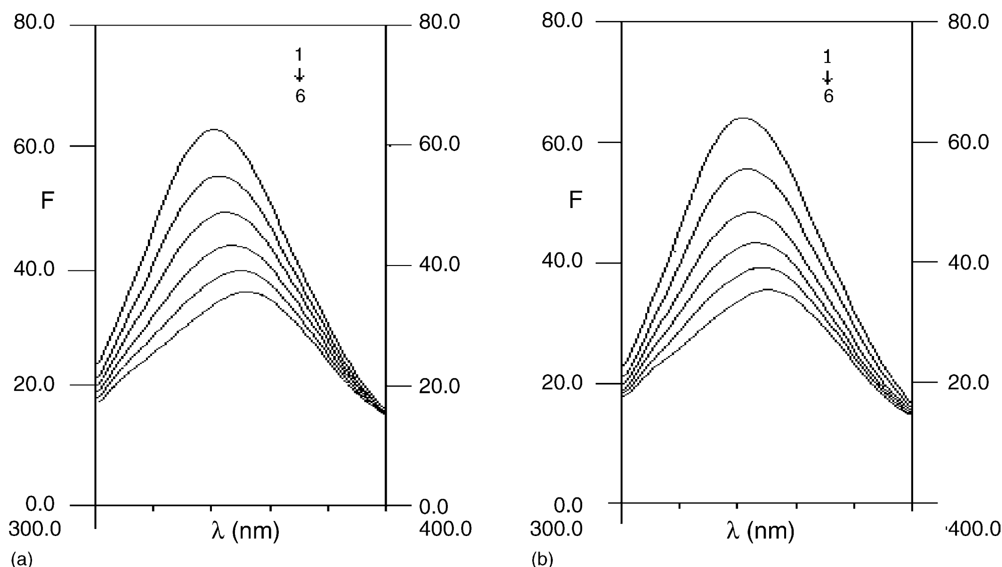

Fig. 2. The fluorescence quenching spectra HSA in the presence of 5-ASA (a) or 5-ASZ (b). $C_{HSA} = 1.5 \times 10^{-6}$ mol l^{-1}; (a) $C_{5\text{-ASA}}$ of 1–6: 0, 2.0×10^{-5}, 4.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 1.0×10^{-4} mol l^{-1}; (b) $C_{5\text{-ASZ}}$ of 1–6: 0, 0.8×10^{-5}, 1.6×10^{-5}, 2.4×10^{-5}, 3.2×10^{-5}, 4.0×10^{-5} mol l^{-1}.

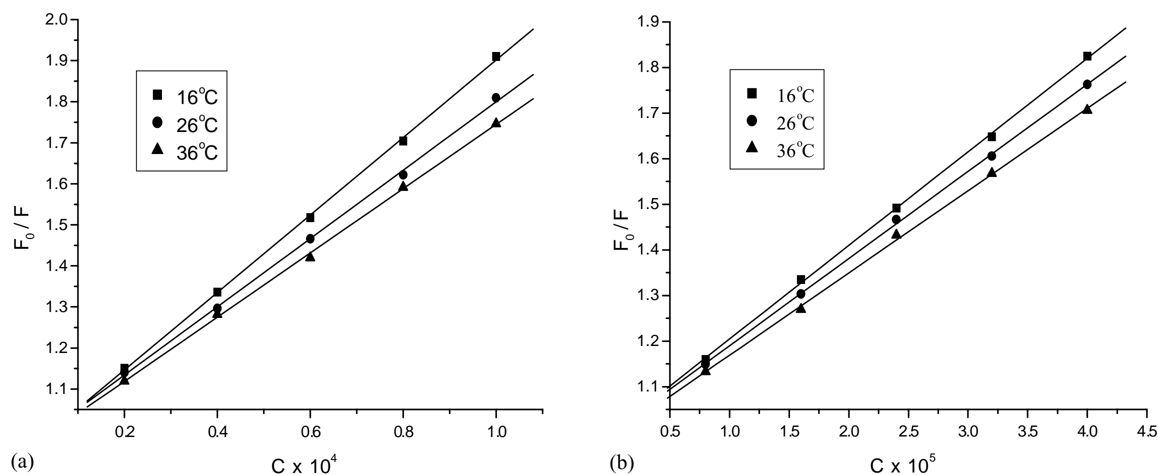

Fig. 3. The Stern–Volmer curves of quenching of HSA with 5-ASA (a) or 5-ASZ (b). C_{HSA}, $C_{5\text{-ASA}}$ and $C_{5\text{-ASZ}}$ are the same as those in Fig. 2.

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 τ_0 is $10^{-8}\,s^{-1}$ [15], K_{sv} is the slope of linear regressions of Fig. 3. According to the 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 $2 \times 10^{10}\,l\,mol^{-1}\,s^{-1}$ [16]. Obviously, the rate constant of protein quenching procedure initiated by drug is greater than K_q of the scatter procedure.

Table 1
The dynamic quenching constants ($l\,mol^{-1}\,s^{-1}$) between 5-ASA (a) or 5-ASZ (b) with HSA

T (°C)	K_q (a)	R (a)	K_q (b)	R (b)
16	9.437×10^{11}	0.9997	2.054×10^{12}	0.9997
26	8.318×10^{11}	0.9995	1.913×10^{12}	0.9998
36	7.822×10^{11}	0.9996	1.806×10^{12}	0.9995

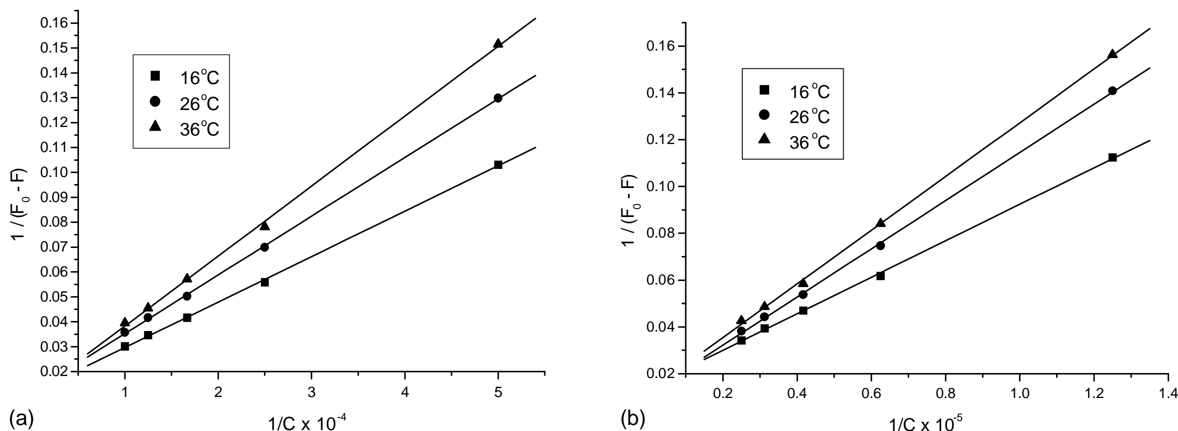

Fig. 4. The Lineweaver–Burk curves of quenching of HSA with 5-ASA (a) or 5-ASZ (b). C_{HSA}, $C_{5\text{-ASA}}$ and $C_{5\text{-ASZ}}$ are the same as those in Fig. 2.

Table 2
The binding constants ($\mathrm{l\,mol^{-1}}$) between 5-ASA (a) or 5-ASZ (b) with HSA

T (°C)	K (a)	R (a)	K (b)	R (b)
16	$(6.240 \pm 0.057) \times 10^3$	0.9997	$(1.854 \pm 0.058) \times 10^4$	0.9996
26	$(4.941 \pm 0.035) \times 10^3$	0.9998	$(1.220 \pm 0.039) \times 10^4$	0.9998
36	$(3.631 \pm 0.063) \times 10^3$	0.9995	$(1.084 \pm 0.060) \times 10^4$	0.9997

So, this shows that the quenching is not initiated by dynamic collision but from formation of a complex. The static quenching equation is [17]

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

where K is the binding constant of drug and biomolecule, which can be determined by the intercept and slope of the Lineweaver–Burk ($1/(F_0 - F) \sim 1/[Q]$) curves as shown in Fig. 4.

Thus, we can obtain binding constants K of HSA with 5-ASA (a) or 5-ASZ (b) from the intercept and slope of Fig. 4 (intercept $= F_0^{-1}$, slope $= K^{-1}F_0^{-1}$, so $K = F_0^{-1}/\text{slope}$). The results are given Table 2.

It is shown that the binding between HSA and 5-ASA or 5-ASZ is remarkable and the effect of temperature is small. Thus, 5-ASA and 5-ASZ can be stored and removed by protein 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 hydrophobic interaction force, etc [9,18]. 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:

$$\ln\left(\frac{K_2}{K_1}\right) = \frac{\Delta H(1/T_1 - 1/T_2)}{R} \quad (4)$$

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

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

where ΔH, ΔG and ΔS are, respectively enthalpy change, free energy change and entropy change, ΔH and ΔS for the binding interaction between

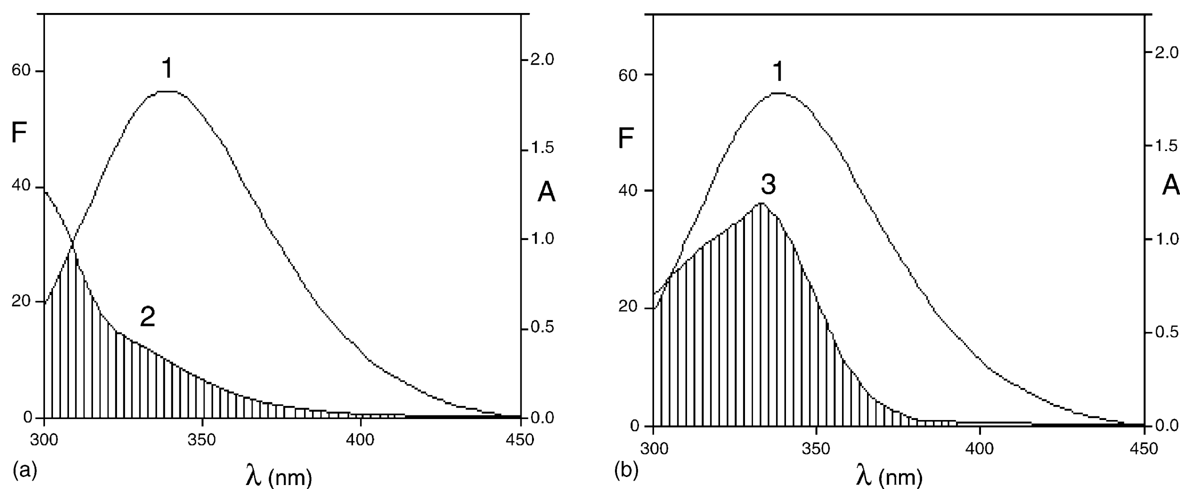

Fig. 5. The overlap of the UV spectrum of 5-ASA (a) or 5-ASZ (b) with the fluorescence emission spectrum of HSA. (1) The fluorescence spectrum of HSA, $C_{\mathrm{HSA}} = 1 \times 10^{-5}\,\mathrm{mol\,l^{-1}}$, F is the fluorescence intensity; (2) the UV absorbance spectrum of 5-ASA, $C_{\text{5-ASA}} = 1 \times 10^{-5}\,\mathrm{mol\,l^{-1}}$, A is the UV absorption intensity of 5-ASA; (3) the UV absorbance spectrum of 5-ASZ, $C_{\text{5-ASZ}} = 1 \times 10^{-5}\,\mathrm{mol\,l^{-1}}$, A is the UV absorption intensity of 5-ASZ.

5-ASA and HSA are found to be $-2.03\,\mathrm{kJ\,mol^{-1}}$ and $64.75\,\mathrm{J\,mol^{-1}\,K^{-1}}$, and those for the binding interaction between 5-ASZ and HSA are $-3.07\,\mathrm{kJ\,mol^{-1}}$ and $68.32\,\mathrm{J\,mol^{-1}\,K^{-1}}$, 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 binding interaction [19].

3.3. Energy transfer between HSA and drug

The overlap of the UV absorption spectrum of 5-ASA (a) or 5-ASZ (b) with the fluorescence emission spectrum of HSA is shown in Fig. 5. According to Föster's non-radiative energy transfer theory [20,21], the energy transfer will happen under these conditions: (1) the donor can produce fluorescence light; (2) fluorescence emission spectrum of the donor and UV absorbance spectrum of the acceptor have more overlap; (3) 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, i.e.

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

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 overlap integral of the fluorescence emission spectrum of the donor, J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,

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

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

$$E = \frac{1 - F}{F_0} \tag{10}$$

J can be evaluated by integrating the spectra in Fig. 5. It has been reported for HSA that, $k^2 = 2/3$, $\phi = 0.118$, $n = 1.336$ [17]. Based on these data, we found $R_0 = 2.81$ nm and $r = 3.59$ nm for 5-ASA, and those for 5-ASZ are $R_0 = 2.66$ nm and $r = 3.84$ nm. So the distance between 5-ASA or 5-ASZ and tryptophan residue in HSA is 3.14 and 2.81 nm, respectively. Obviously, they are lower than 7 nm after interaction between HSA and 5-ASA or 5-ASZ. These accord with conditions of Föster's non-radiative energy transfer theory, indicating again the static quenching interaction between HSA and 5-ASA or 5-ASZ.

3.4. The effect of other ions on the binding constants

The fluorescence intensity was almost unchanged before and after the addition of common ions at $\lambda_{em} = 340$ nm. The effect of common ions on the binding constants was investigated at 26 °C. The results are listed in Table 3. It is shown that the binding constants between the protein and 5-ASA or 5-ASZ increased in the presence of common ions, implying stronger binding between HSA and 5-ASA or 5-ASZ. 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, 5-ASA and 5-ASZ can be stored and removed better by protein.

3.5. Analysis of the protein and the drug

3.5.1. The experimental methods for determination of the protein and the drugs

The protein and the drugs are measured separately by measuring the fluorescence of the protein quenched by the drugs.

3.5.1.1. For the proteins. Under the optimum conditions, the concentration of 5-ASA or 5-ASZ was kept unchanged. The fluorescence intensity was measured before and after the addition of 5-ASA or 5-ASZ with the concentration of the protein changed. The liner relationship between the difference in fluorescence intensity ΔF (with and without 5-ASA or 5-ASZ) and

Table 3
The binding constants ($l\,mol^{-1}$) between HSA and 5-ASA (a′) or 5-ASZ (b′) at 26 °C in the presence of common ions

Ions	*K* (a′)	*R* (a′)	*K* (a′)/*K* (a)	*K* (b′)	*R* (b′)	*K* (b′)/*K* (b)
K^+	$(6.166 \pm 0.015) \times 10^3$	0.9999	1.248	$(1.271 \pm 0.056) \times 10^4$	0.9990	1.042
Ca^{2+}	$(5.312 \pm 0.060) \times 10^3$	0.9997	1.075	$(1.304 \pm 0.024) \times 10^4$	0.9999	1.069
Cu^{2+}	$(1.611 \pm 0.063) \times 10^3$	0.9989	0.326	$(0.283 \pm 0.034) \times 10^4$	0.9993	0.232
Zn^{2+}	$(5.144 \pm 0.059) \times 10^3$	0.9996	1.041	$(1.244 \pm 0.039) \times 10^4$	0.9994	1.020
Ba^{2+}	$(8.332 \pm 0.058) \times 10^3$	0.9998	1.686	$(1.298 \pm 0.037) \times 10^4$	0.9998	1.064
Mg^{2+}	$(5.514 \pm 0.058) \times 10^3$	0.9998	1.096	$(1.278 \pm 0.058) \times 10^4$	0.9990	1.046
Al^{3+}	$(1.063 \pm 0.062) \times 10^3$	0.9993	0.212	$(1.769 \pm 0.059) \times 10^4$	0.9990	1.450
Br^-	$(5.239 \pm 0.064) \times 10^3$	0.9993	1.060	$(1.452 \pm 0.063) \times 10^4$	0.9990	1.190
F^-	$(1.214 \pm 0.067) \times 10^3$	0.9990	0.252	$(1.409 \pm 0.045) \times 10^4$	0.9992	1.155
$CO_3{}^{2-}$	$(6.045 \pm 0.070) \times 10^3$	0.9990	1.223	$(1.151 \pm 0.060) \times 10^4$	0.9989	0.943
$SO_4{}^{2-}$	$(5.030 \pm 0.085) \times 10^3$	0.9989	1.018	$(1.678 \pm 0.038) \times 10^4$	0.9993	1.375
$PO_4{}^{3-}$	$(6.705 \pm 0.072) \times 10^3$	0.9990	1.357	$(1.457 \pm 0.035) \times 10^4$	0.9994	1.194

concentration of the protein is applied in the determination of the protein.

3.5.1.2. For the drugs. Under the optimum conditions, the concentration of the protein is kept unchanged with different concentrations of the 5-ASA or 5-ASZ. The proposed method was applied to the determination of the drug by using the procedure described in the fluorescence spectrum measurements section. The linear relationship between fluorescence intensity and the concentration of 5-ASA or 5-ASZ, as shown in Eq. (3), is used in the determination of the drugs.

3.5.2. Optimization of experimental conditions for the determination of the protein and drugs

In order to select an optimized analytical system, various experimental parameters including medium, pH and temperature were studied with the concentration of each drug being $2 \times 10^{-5}\,mol\,l^{-1}$ in all cases.

3.5.2.1. 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 experiment. The sensitivity is higher in Tris–HCl buffer solution than in others. So, Tris–HCl buffer solution was used in the experiment. The influence of concentration of Tris–HCl ($0.1\,mol\,l^{-1}$) upon fluorescence value was investigated in the range of 0.5–5 ml. It was found that the sensitivity was higher if 2.0 ml of the buffer was used. Therefore, 2.0 ml of $0.1\,mol\,l^{-1}$ Tris–HCl was considered to be the best choice.

3.5.2.2. Effect of pH. The influence of pH was studied in the range of 6.0–8.0. The stable fluorescence intensity for the complex HSA-5-ASA or HSA-5-ASZ could be achieved over the pH range 6.4–7.9. Therefore, Tris–HCl buffer solution at pH 7.4 was used in this work.

3.5.2.3. Effect of temperature. The binding constants between HSA with the drugs were determined at different temperatures (16, 26 and 36 °C). It was observed that the value of the binding constant was the greatest at 16 °C, but interaction rate was the slowest. Less time was needed when the interaction was at 26 °C than at 16 °C, and the sensitivity at 26 °C is higher than at 36 °C. Thus, the most appropriate temperature for the measurement is 26 °C.

3.5.3. Precision, limit of detection and working curve

3.5.3.1. For the protein. Under the conditions described above, the working curve was obtained. The relationship between the difference in fluorescence intensity ΔF (with and without 5-ASA or 5-ASZ) and concentration of the protein is found to be linear in the range of 2.07–94.2 $\mu g\,ml^{-1}$ for HSA (with 5-ASA) and 1.04–144.9 $\mu g\,ml^{-1}$ for HSA (with 5-ASZ), with the correlation coefficients 0.9986 and 0.9991 ($n = 6$), respectively. The detection limit, as defined by IUPAC [22], is determined to be 1.14 $\mu g\,ml^{-1}$ HSA with 5-ASA and 0.87 $\mu g\,ml^{-1}$ HSA with 5-ASZ, respectively. The relative standard deviations are 3.1% HSA with 5-ASA and 2.6% HSA with 5-ASZ, respectively,

Table 4
Determination results of the proteins in samples

Human serum sample number	This method ($n = 6$) (mg ml^{-1})	Recovery (%)	RSD (%)	CBB method ($n = 6$) (mg ml^{-1})	Recovery (%)	RSD (%)
1 (with 5-ASA)	60.2	98.9	0.8	61.1	101.9	1.5
2 (with 5-ASA)	74.6	102.6	1.9	72.9	102.3	2.7
3 (with 5-ASA)	83.7	101.4	2.3	85.6	98.9	2.5
4 (with 5-ASZ)	76.3	103.4	2.9	73.2	102.6	2.3
5 (with 5-ASZ)	88.9	97.6	2.6	87.5	101.4	1.9
6 (with 5-ASZ)	102.2	101.1	2.4	104.4	102.0	2.0

Table 5
Determination results of 5-ASA or 5-ASZ in samples ($n = 6$)

Human serum sample	Initial (μg)	Added (μg)	Found (μg)	Recovery (%)	RSD (%)
5-ASA	0	7.66	7.49	97.72	2.66
	0	15.31	15.16	102.3	2.41
	0	22.97	23.96	98.62	0.97
5-ASZ	0	8.11	7.92	102.4	2.32
	0	16.23	15.29	98.4	1.02
	0	24.34	26.72	102.0	1.76

which were obtained for 11 replicate determinations of 1.0×10^{-6} mol l^{-1} HSA solutions.

3.5.3.2. For the drugs. Under the optimum conditions described above, linear relationships were found between fluorescence intensity and concentration of the 5-ASA or 5-ASZ in the range 0.32–27.6 μg ml^{-1}(5-ASA in HSA) and 0.24–32.5 μg ml^{-1}(5-ASZ in HSA), with the correlation coefficients 0.9988 and 0.9987 ($n = 6$), respectively. The detection limit is determined to be 0.16 μg ml^{-1} (5-ASA) and 0.14 μg ml^{-1} (5-ASZ), respectively. The relative standard deviations are 2.2% (5-ASA) and 2.6% (5-ASZ), respectively, as obtained from 11 replicate determinations of 1.0×10^{-6} mol l^{-1} HSA solutions.

3.5.4. Analysis of samples

The protein concentration in the samples from a hospital was very high, so they were diluted to the detectable range for the determinations. Fresh Human serum samples (obtained from a hospital) were diluted about 1000 times in this work. The concentration of the protein in these 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 [4].

The amount of drug in human serum (each 0.3 ml) is determined by a standard addition method. The results are shown in Table 5. It can be seen that the present method has good reproducibility and high sensitivity.

4. Conclusions

It is shown that a complex was formed between the drug and HSA by hydrophobic binding interactions. Based on this phenomenon, a practical method for the rapid and simple determination of the proteins is provided. This is the first reported procedure for the determination of proteins and the amount of 5-ASA or 5-ASZ in human serum. These experimental and theoretical data could be a useful guide for efficient drug synthesis.

Acknowledgements

This work was supported by the National Natural Science Foundation of China and partially financed

from the Natural Science Foundation of Henan Province.

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