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# Interaction Between Amino Acid Derivatives of Aspirin and Human Serum Albumin

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Aspirin, with simple structure and good effects, is one of the most popular antipyretic and anti-inflammatory drugs. Tested more than a century, aspirin was found enough to treat cancer and cardiovascular disease. However, the side effects of aspirin should not be ignored. In order to overcome these side effects, its mechanism of action and drug-modification were pushed to a hot spot. Three amino acid derivatives of aspirin were synthesised and the interaction with human serum albumin were studied *via* fluorescence spectroscopy. The main binding force between P1, P2 and human serum albumin was mainly van der Waals forces, the interaction of human serum albumin with P3 was mainly electrostatic attraction. Synchronous fluorescence spectroscopy indicated that aspirin and its derivatives have changed the chemical environment of the tryptophan residues, inducing polarity of hydrophobic cavity and stretch of the peptide chain, thus making the protein conformation changed. Amino acid derivatives of aspirin can decrease the fluorescence intensity of human serum albumin, which result from the changing of the chemical environment caused by aspirin and its derivatives.

**Keywords: Aspirin, Amino acid, Human serum albumin, Intercation.**

## INTRODUCTION

For more than four millennia, people have been using aspirin or naturally occurring analogs to treat pain and inflammation[1], but it was only in the 1970s that scientists finally cracked the medicine's biochemical mode of action[2,3].

Aspirin reduces the incidence of and mortality from colorectal cancer (CRC) by unknown mechanisms. It has been reported that aspirin is an inhibitor of mTOR and an activator of AMPK, which could contribute to its protective effects against development of colorectal cancer[4]. Gaynor *et al.*[5] paid attention to how aspirin and salicylate inhibit anti-inflammatory agents and the results indicated that the anti-inflammatory properties of aspirin and salicylate were mediated in part by their specific inhibition of IKK-b, thereby preventing activation by NF-kB of genes involved in the pathogenesis of the inflammatory response. New derivatives were synthesized, for example, NO-donating aspirin (NO-ASA). NO-ASA on colon cancer has been demonstrated *in vivo* and *in vitro*[6-8].

Human serum albumin (HSA) is the most prominent protein components of blood plasma. It serves as transport protein for several endogenous and exogenous ligands as well as for various drug molecules[9]. The interaction between aspirin and human serum albumin shows how aspirin works and what the binding force is. Thus, this kind of research is important.

Also, amino acid derivatives of aspirin were synthesized. Tumor tissue obtain nutrients through competition or even plunder. Therefore, normal cells are inhibited, which brought malnutrition, reduced immunity and metabolic disorders to the patients and even a variety of complications. Combine the amino acid and the molecule anticancer drugs can improve their selectivity for tumor cells and enhance the lipophilic of the drugs, induce the toxicity to the cells[10,11].

## EXPERIMENTAL

All chemicals (for synthesis and HSA extraction) were analytical reagents. Human serum albumin binding experiments were carried out in Tris-HCl buffer (pH 8.2). Human serum albumin purchased from Shanghai YuanYe Biotechnology Company, was used without any purification. All solutions were prepared with doubly distilled water. The structures were determined by Bruker-600 MHz NMR spectrometer and FS spectra were recored on Hitachi F-2700 atomic fluorescence spectrometer.

Three amino acid derivatives of aspirin were synthesized as described in the literature[9] and the structural formulae of theme are shown in Fig. 1.

### $^1$H NMR analysis

**P1:** $C_{15}H_{22}N_2O_6$, $^1$H NMR ($D_2O$, 600 MHz, ppm): δ = 1.77-1.79 (m, 7H), 2.23 (s, 3H), 2.87-2.90 (t, *J* = 7.53, 3H),

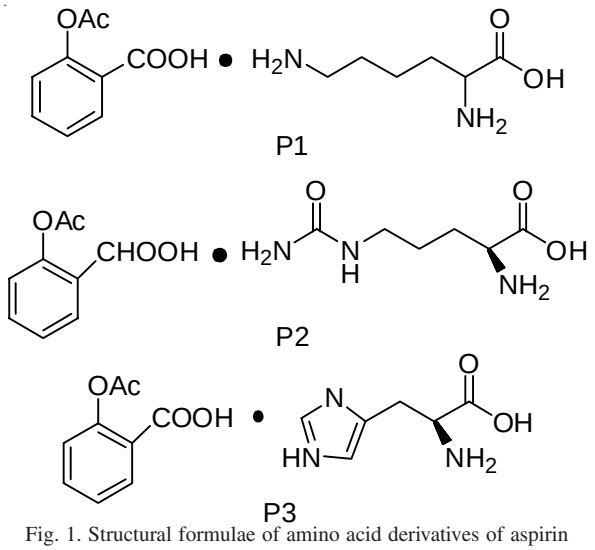



Fig. 1. Structural formulae of amino acid derivatives of aspirin

3.65-3.67 (t, *J* = 6.12, 3H), 4.71 (s, 2H), 7.09-7.10 (m, 1H), 7.30-7.32 (m, 1H), 7.52-7.55 (m, 1H), 7.81-7.83 (m, 1H).

**P2:** $C_{15}H_{22}N_3O_7$, $^1$H NMR ($D_2O$, 600 MHz, ppm): δ = 2.18 (s, 3H), 3.04-3.07 (t, *J* = 6.72, 3H), 3.59-3.61 (t, *J* = 6.12, 1H), 4.68 (s, 11H), 6.98-7.00 (d, *J* = 7.44, 1H), 7.20-7.23 (t, *J* = 7.59, 1H), 7.37-7.39 (t, *J* = 8.49, 1H), 7.57-7.58 (d, *J* = 7.32, 1H).

**P3:** $C_{15}H_{17}N_3O_6$, $^1$H NMR ($D_2O$, 600 MHz, ppm): δ = 1.81-1.90 (s, 3H), 3.15-3.16 (d, *J* = 2.88, 2H), 3.84-3.85 (m, 1H), 6.75-6.79 (m, 4H), 7.20-7.29 (m, 3H), 7.63-7.64 (d, *J* = 8.28, 2H), 8.39-8.47 (m, 2H).

The *in vitro* analysis refer from literature[10]. The successive fixed amount of solution (2 mmol $L^{-1}$, 4 mmol $L^{-1}$, 6 mmol $L^{-1}$, 8 mmol $L^{-1}$, 10 mmol $L^{-1}$) were added to human serum albumin solution and heated at 25 and 37 °C separately for 5 min. The excitation wavelength was 280 nm and the emission wavelength was recorded from 300 to 500 nm.

## RESULTS AND DISCUSSION

The decrease of fluorescence intensity of a fluorophore is referred to as fluorescence quenching. The addition of sample solution to human serum albumin solution resulted in a fluorescence quenching. The quenching intensity were different by sample and concentration and showed in Figs. 2-4. Human serum albumin exhibited a strong fluorescence emission with a peak at 339 nm, which is mainly due to its tryptophan residue, while this complex has no instrinsic fluorescence at around the wavelength.

It is indicated that the decrease of fluorescence was caused by concentration and temperature. When the concentration increased, the fluorescence intensity decreased, same with the temperature. Thus, concentration and temperature have an effect on the fluorescence intensity.

Fluorescence quenching can be dynamic or static which can be distinguished by their different dependence on temperature. To examine the fluorescence quenching the Stern-Volmer equation was used:

$$F_0/F = 1 + k_q\tau_0[Q]$$

where $F_0$ and F are the fluorescence in the absence and presence of the quencher, $k_q$ is the bimolecular quenching constant, $\tau_0$

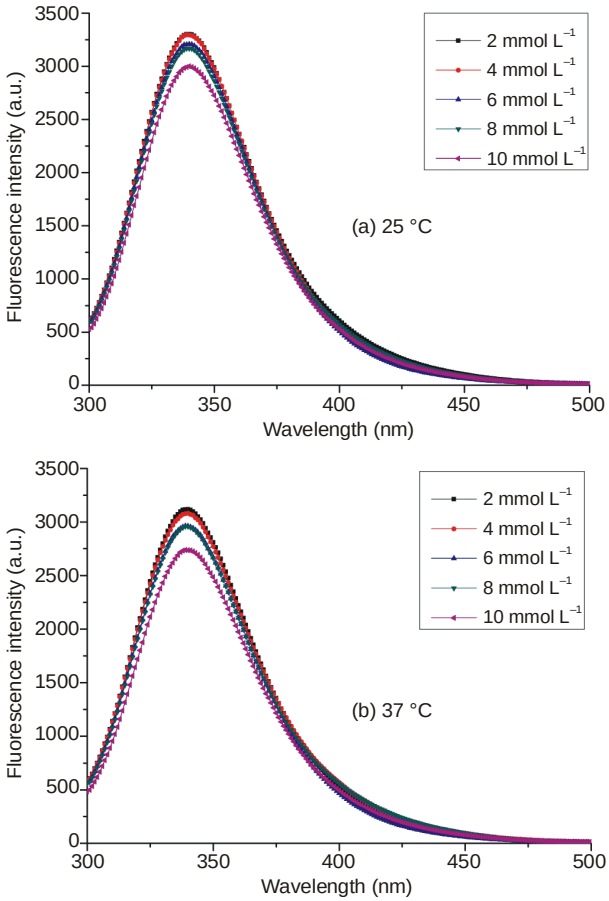



Fig. 2. Effect of P1 on fluorescence of human serum albumin

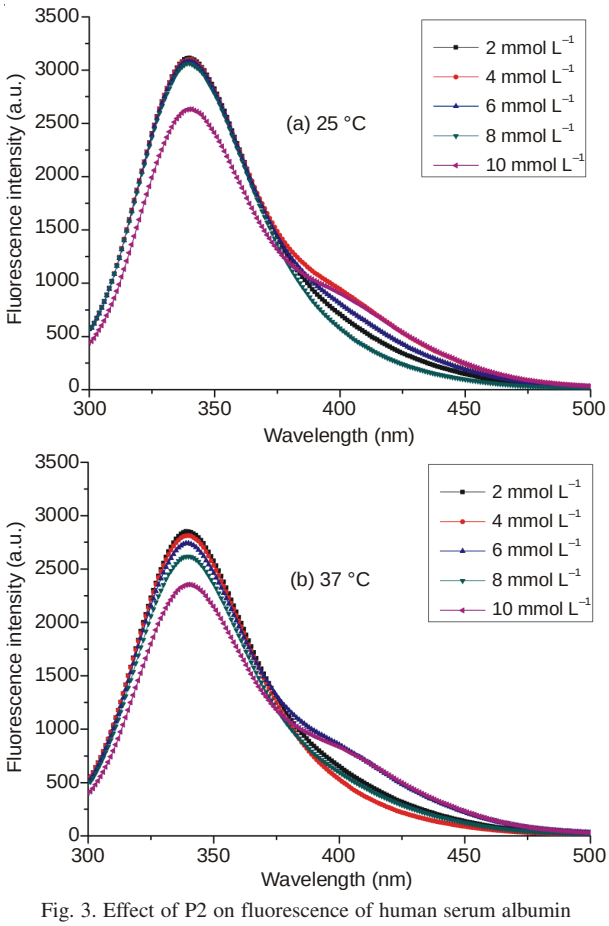



Fig. 3. Effect of P2 on fluorescence of human serum albumin

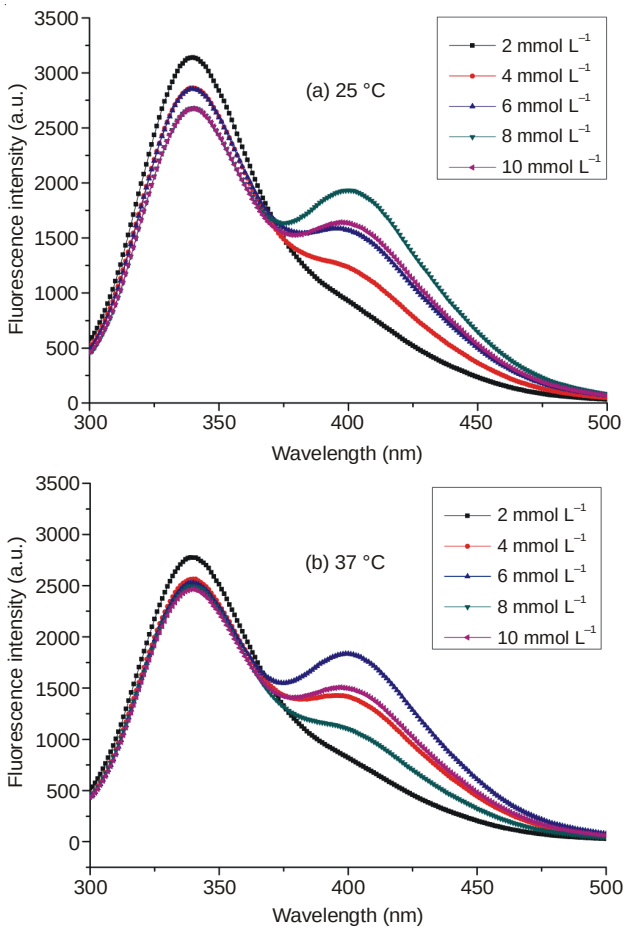



Fig. 4. Effect of P3 on fluorescence of human serum albumin

is the lifetime of the fluorescence in the absence of the quencher, [Q] is the concentration of the quencher.

In order to obtain the binding constant for the interaction between the quencher and human serum albumin from the fluorescence quenching data, the Lineweaver-Burk equation was used.

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

where $F_0$ and F are the fluorescence in the absence and presence of the quencher, K is the binding constant of the system of the quencher and human serum albumin, [Q] is the concentration of the quencher.

Combine Figs. 5 and 6, $K_q$ and $K_{LB}^{-1}$ were obtained (Table-1). It is indicated that fluorescence quenching between P1 and human serum albumin was dynamic, however, the fluorescence quenching between P2, P3 and human serum albumin was static. The binding force between biological macromolecules and small organic molecules such as proteins were mainly hydrophobic forces, van der Waals forces, electrostatic forces and hydrogen bonding interactions, *etc*. When the temperature change is not obvious, the enthalpy ΔH can be regarded as a constant. The ΔS and ΔG can be obtained by the schemes as follows:

$$\ln(k_2/k_1) = \Delta H/R(1/T_1\text{-}1/T_2)$$

$$\Delta G = -RT\ln k$$

$$\Delta S = -(\Delta G\text{-}\Delta H)/T$$

According to the thermodynamic formulae and different temperatures of k values, the thermodynamic parameters of

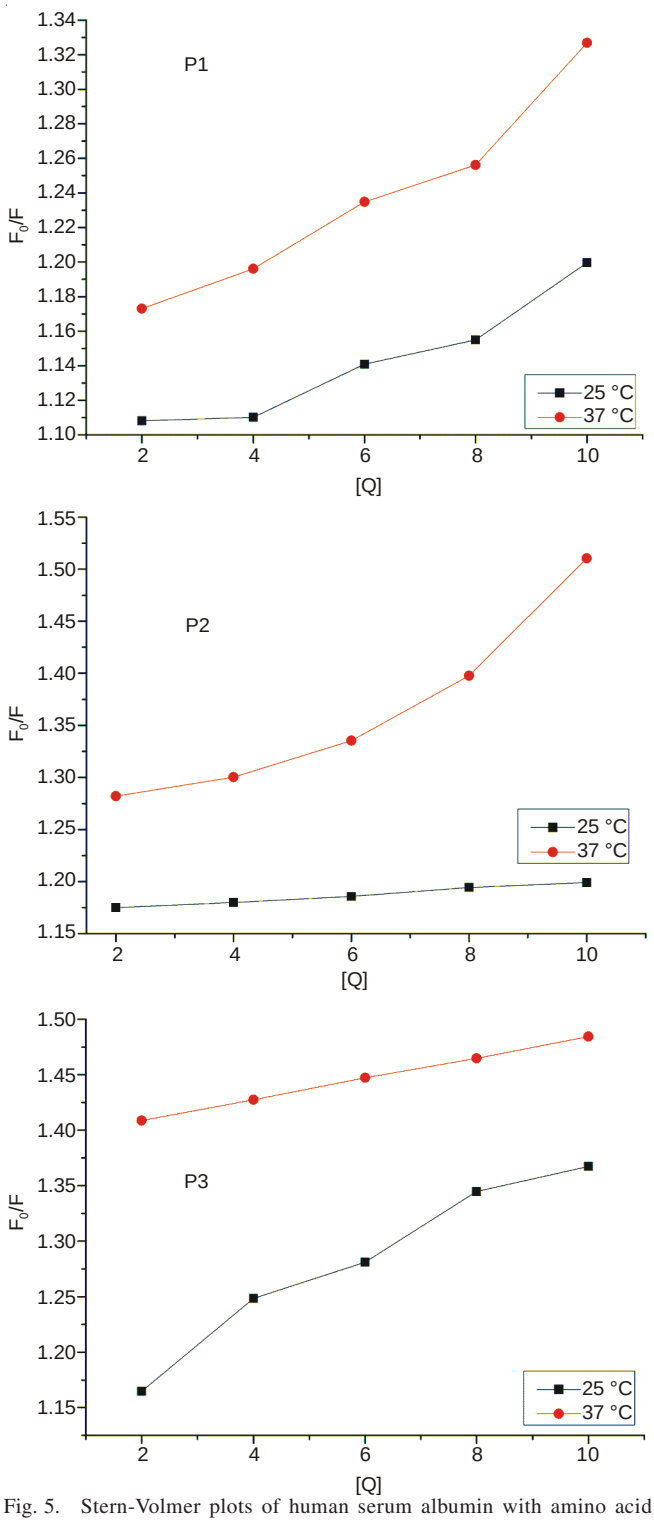



Fig. 5. Stern-Volmer plots of human serum albumin with amino acid derivatives of aspirin at 25 and 37 °C

TABLE-1
$K_q$ AND $K_{LB}^{-1}$ OBTAINED BY FITTING THE CURVES

| Pharmaceutical | Temp. (°C) | $K_q$ | | $K_{LB}^{-1}$ | |
|---|---|---|---|---|---|
| P1 | 25 | $1.1\times10^{10}$ | 0.90167 | $2.7\times10^{4}$ | 0.9868 |
| | 37 | $1.8\times10^{10}$ | 0.95511 | $6.1\times10^{4}$ | 0.91596 |
| P2 | 25 | $2.3\times10^{10}$ | 0.95677 | $1.2\times10^{3}$ | 0.90377 |
| | 37 | $7.2\times10^{9}$ | 0.97483 | $7.0\times10^{3}$ | 0.93379 |
| P3 | 25 | $3.2\times10^{10}$ | 0.95189 | $3.6\times10^{3}$ | 0.95678 |
| | 37 | $2.94\times10^{10}$ | 0.99955 | $2.6\times10^{3}$ | 0.97490 |

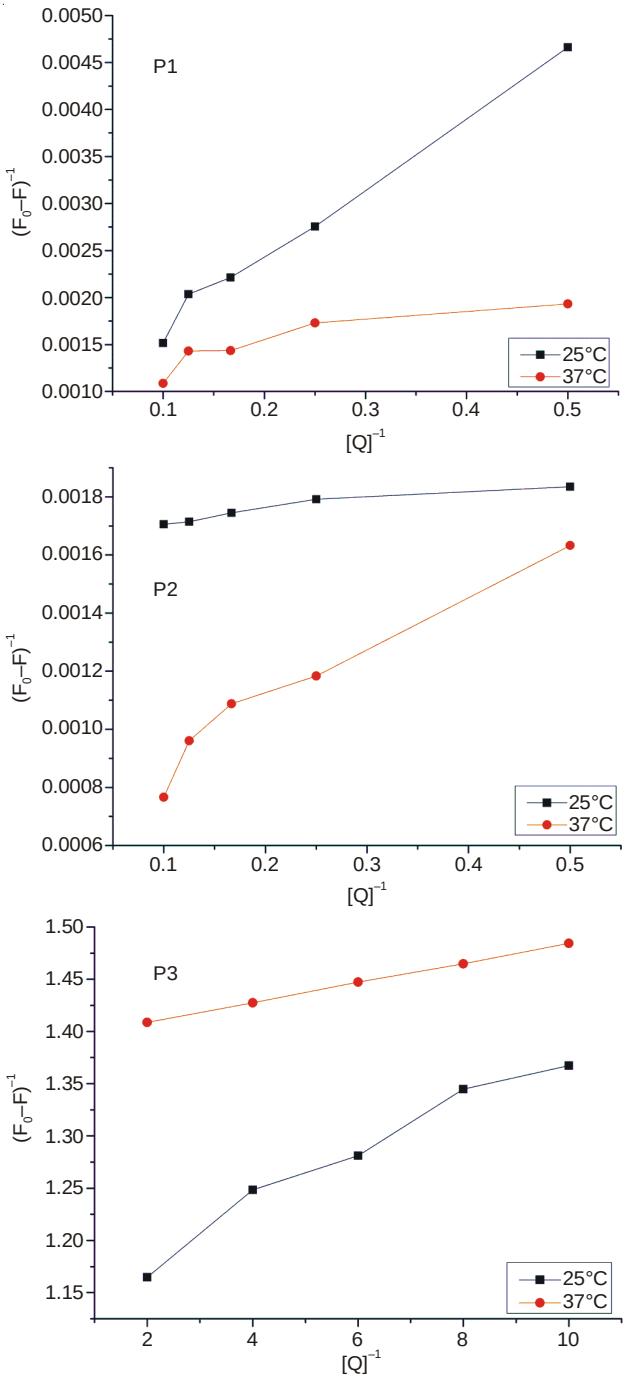



Fig. 6. Lineweaver-Burk plots of amino acid derivatives of aspirin at 25 and 37 °C

three kinds of derivatives and the role of human serum albumin, the experimental results are shown in Table-2.

TABLE-2
THERMODYNAMIC PARAMETERS OF
DRUG-HUMAN SERUM ALBUMIN BINDING PROCESS

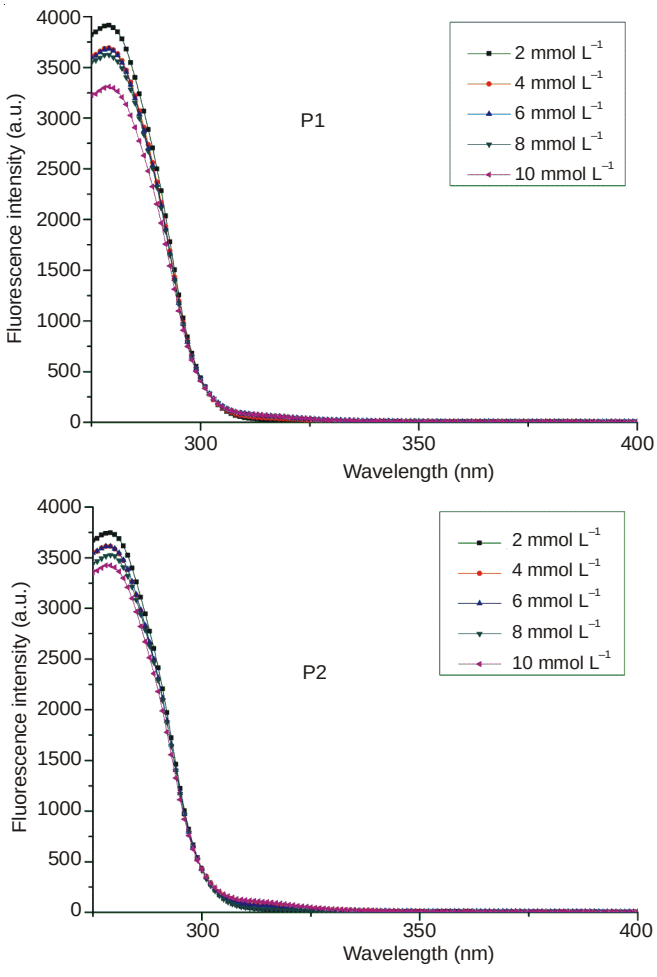
| Pharmaceutical | Temp. (°C) | ΔH (kJ mol$^{-1}$) | ΔG (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|
| P1 | 25 | 52.17 | -19.56 | -240.70 |
| | 37 | 52.17 | -22.46 | -240.07 |
| P2 | 25 | 112.88 | -17.57 | -43.78 |
| | 37 | 112.88 | -22.82 | -43.74 |
| P3 | 25 | -20.83 | -20.29 | 1.81 |
| | 37 | -20.83 | -20.27 | 1.81 |

$\Delta S > 0$ indicating the binding force between the quencher and human serum albumin are hydrophobic and electrostatic force; $\Delta S < 0$ indicating the binding forces are hydrogen bonds and van der Waals forces; if $\Delta H > 0$ and $\Delta S > 0$, the binding forces are hydrophobic, if $\Delta H \approx 0$ and $\Delta S > 0$, the binding forces are electrostatic force, if $\Delta H < 0$ and $\Delta S < 0$, the binding force is hydrogen bonds and van der Waals forces. The interaction between aspirin and its derivatives (P1, P2, P3) are respectively van der Waals forces and van der Waals forces, van der Waals forces and van der Waals forces and electrostatic force.

Add quencher into human serum albumin and keep the concentration of human serum albumin constant, make the difference between $E_m$ and $E_x$ $\Delta\lambda$ = 60 nm to obtain the Synchronous fluorescence spectra of human serum albumin (Fig. 7). Human serum albumin exhibited a strong fluorescence emission with a peak at 339 nm on excitation at 282 nm which is mainly due to its single tryptophan residue, while remarkable blue shifts at the maximum wavelength were observed, which suggested the fall of polarity and the increase of hydrophobicity around tryptophan residue. It is indicated that the quencher could bind to the human serum albumin.

## Conclusion

The addition of sample solution to human serum albumin solution resulted in a fluorescence quenching. The quenching intensity were different by sample and concentration and showed below. Human serum albumin exhibited a strong fluorescence



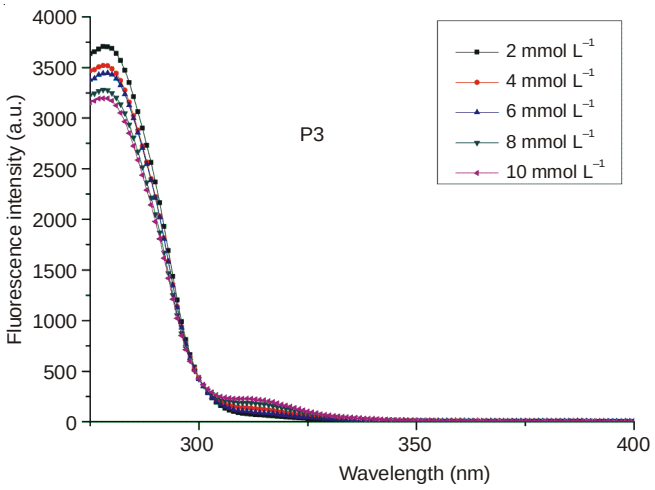



Fig. 7. Synchronous fluorescence spectra of human serum albumin

emission with a peak at 339 nm which was mainly due to its tryptophan residue, while this complex had no instrinsic fluorescence at around the wavelength. The fluorescence quenching between aspirin, P1 and human serum albumin was static, however, the fluorescence quenching between P2, P3 and human serum albumin was dynamic. The interaction between aspirin and its derivatives (P1, P2, P3) were respectively hydrophobic, electrostatic force, van der Waals forces and van der Waals forces. The synchronous fluorescence spectra showed that human serum albumin exhibited a strong fluorescence emission with a peak at 339 nm on excitation at 282 nm which was mainly due to its single tryptophan residue, while remarkable blue shifts at the maximum wavelength were observed, which suggested the fall of polarity and the increase of hydrophobicity around tryptophan residue. It is indicated that the quencher could bind to human serum albumin.

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