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Study on the interaction characteristics of dexamethasone sodium phosphate with bovine serum albumin by spectroscopic technique

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The interaction of dexamethasone sodium phosphate (DEX-P) with bovine serum albumin (BSA) was studied by fluorescence quenching in combination with UV-Vis spectroscopic method under near physiological conditions. The fluorescence quenching rate constants and binding constants for BSA–DEX-P system were determined at different temperatures. The fluorescence quenching of BSA by DEX-P is due to static quenching and energy transfer. The results of thermodynamic parameters, ΔH (-161.0 kJ /mol), ΔS (-468.0 J/mol K) and ΔG (-21.54~-16.86 kJ/mol), indicated that van der Waals interaction and hydrogen bonding played a major role for DEX-P–BSA association. The competitive experiments demonstrated that the primary binding site of DEX-P on BSA was located at site III in sub-domain III_A of BSA. The distance between BSA and DEX-P is estimated to be 1.23 nm based on the Förster resonance energy transfer theory. The binding constant (K_a) of BSA–DEX-P at 298 K was 2.239×10^4

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L/mol. Circular dichroism spectra, synchronous fluorescence and three-dimensional fluorescence studies showed that the presence of DEX-P could change the conformation of BSA during the binding process.

KEYWORDS: Fluorescence; Bovine serum albumin; Dexamethasone sodium phosphate; Interaction characteristics

1. Introduction

Serum albumin is one of the most abundant proteins in circulatory system of a wide variety of organisms and one of the most extensively studied proteins at all. The albumins make a significant contribution to colloid osmotic blood pressure and aid in the transport, distribution and metabolism of many endogenous and exogenous ligands. Protein–drug binding greatly influences absorption, distribution, metabolism and excretion properties of typical drugs[1]. Protein binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in distribution, excretion and therapeutic effectiveness. Dexamethasone (DEX) is a glucocorticoid and belongs to a group of medicines called corticosteroids. Dexamethasone sodium phosphate (DEX-P) is a water-soluble prodrug that is administered parenterally. Under physiological conditions, DEX-P exists principally as a di-anion. Its aqueous solubility and charge render DEX-P a much better candidate for transdermal iontophoretic delivery than the parent molecule (DEX)[2]. DEX-P is rapidly absorbed after oral administration and up to 65% of a dose is excreted in the

urine in 24 h. The plasma concentration of DEX-P was found to be highest (3 μg/L) at 4 h, declining rapidly to about 0.5 μg/L at 24 h [3]. Thereby, it is important and necessary to study the interaction of DEX-P with serum albumins at molecular level.

Several methods have been applied to drug–protein binding studies among them equilibrium dialysis (ED) is considered as a reference method, However, ED suffers from many drawbacks such as low throughput, nonspecific adsorption to dialysis membrane, volume shifts, the Donnan effect, and it additionally requires sensitive analytical method for the determination of free drug concentration[4]. Capillary electrophoresis in the frontal analysis mode (CE/FA) is a promising technique for assessment of drug–protein interaction. This method offers certain advantages over the traditional ones such as simplicity, low sample requirements and consumption, short analysis times, high separation efficiencies, and high sample throughput[5]. Fluorescence spectroscopy is an effective tool for the investigation of conformational changes of protein under physiological conditions because of its accuracy, sensitivity, rapidity and convenience [6]. It can reveal the accessibility of drugs to albumin's fluorophores, which can help us to understand the binding mechanisms of albumin–drug and to provide information on the structural features for determining the therapeutic effectiveness of the drug. However, one of the major problems associated with measurement of fluorescent organic matter in natural samples is the

inner-filter effect (IFE), sometimes referred to as self-absorption [7]. The overall human plasma protein binding of DEX has been investigated by ED and the results suggested that the binding was linear and existed primary to albumin with little or no binding to corticosteroid binding globulin [8]. The HSA-DEX interaction was also evaluated using the capillary electrophoresis/frontal analysis (CE/FA) [9]. A study was designed to examine the interaction of DEX-P with BSA and HSA under simulated physiological conditions in terms of buffer composition and protein concentration. The binding parameters obtained from the CE/FA experiment were compared to the ones calculated [10]. In another study, the interactions of DEX with low concentrations of BSA and HSA were examined by a fluorescence quenching and Fourier transformation infrared spectroscopy, and both the binding and thermodynamic parameters were calculated, but not correction of the inner-filter effect[11].

Figure 1. Chemical structure of dexamethasone sodium phosphate

In this work, DEX-P was selected as model drug (Figure 1), because it is a much better candidate for transdermal iontophoretic delivery than the parent molecule (DEX) [2]. To provide important insight into the interaction of the physiologically important protein BSA with drugs, this study examined, for the first time, the interaction between BSA and DEX-P under near physiological conditions by the fluorescence quenching in combination with UV-Vis spectroscopic method.

2. Experimental

2.1. Drugs and reagents

Commercially available bovine serum albumin (BSA, catalog no. A-7030, purity: 98%, M: 68000. BSA stock solution (1.0×10^{-4} M) was prepared by dissolving an appropriate amount of BSA with 0.1 M Tris–HCl (pH 7.4) buffer solution, and kept in the dark at 4 ˚C. BSA working solutions were prepared by diluting the stock solution with water. DEX-P (purity: 98.7%) was purchased from the Jinyue Pharmacy Factory (Tianjin, China). A stock solution (1.0×10^{-3} M) of DEX-P was prepared in water, and stored in refrigerator at –4 ºC. Tris–HCl buffer (pH 7.40) consists of Tris (0.1 M) and HCl (0.1 M), and NaCl solution (0.5 M) was used to maintain the ion strength. All chemicals were of analytical reagent grade or better. Purified water was prepared by an XGJ-30 highly pure water machine (Yongcheng purification Science & Technology Co. Ltd., Beijing, China).

2.2. Equipment

All fluorescence measurements were performed on an F-7000 Fluorescence spectrophotometer (Hitachi, Japan) which was equipped with a 1 cm quartz cell and thermostat bath. The spectrum data points were collected from 280 to 500 nm. The widths of the excitation and the emission slit were both set at 5 nm. Fluorescence measurements were carried out at room temperatures.

Circular dichroism (CD) spectra were obtained on a MOS-450/SFM300 circular dichroism spectrometer (Bio-Logic Com. Germany). The absorption spectra were performed on an TU-1900 double light Spectrophotometer (Beijing TAYASAF Science & Technology Co., Ltd, China) using a 1 cm quartz cell in the wavelength range of 200–500 nm. All pH measurements were performed with a pHS-3C pH meter (Shanghai, China).

2.3. Determination of fluorescence intensity

Five 10-mL clean and dried test tubes were taken, and 2 mL of 0.5 M NaCl, 2.0 mL Tris–HCl buffer (pH 7.40), 0.25 mL of 4.0×10^{-5} M BSA, and different volumes (2.0–4.0 mL) of DEX-P standard solution of 1.936×10^{-4} M were added in each test tube, and diluted to 10 mL with water. The concentration of BSA was 1.0×10^{-6} M, and that of DEX-P was 9.5, 19, 28.5, 38.0, and 47.5×10^{-6} M. Sixth test-tube containing only BSA solution at pH 7.4 was marked as ‘‘control’’, and seventh test-tube containing only 9.5×10^{-6} M DEX-P was used for the comparison. After mixing the solutions, these were allowed to stand for 15 min for maximum binding of DEX-P to BSA. The fluorescence intensity after the correction of inner-filter effect was calculated by the equation [12]: $F_{cor}=F_{obs}\exp(\frac{1}{2}A_{ex}+\frac{1}{2}A_{em})$, where F_{obs} is fluorescence intensity measured before the correction of inner-filter effect, A_{ex} and A_{em} are absorbance of the test solution at excitation and emission wavelengths, respectively. The corrected fluorescence intensity was used for studying on the interaction of DEX-P and BSA. After corrected inner-filter

effect, fluorescence intensity (F_0) in the absence of quencher DEX-P and the fluorescence intensity (F) in the presence of quencher DEX-P were measured at a wavelength of λex 280 nm and λem 340 nm under temperature of 298, 303 and 308 K for estimating the interaction between DEX-P and BSA.

3. Results and discussion

3.1. Fluorescence quenching mechanism

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in fluorescence quenching of excited state fluorophores. These include molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Figure 2 shows the fluorescence spectra of BSA in the absence and presence of DEX-P after corrected inner-filter effect.

Figure 2. Fluorescence spectra of BSA in the absence or presence of DEX-P at λex 280 nm and 298K after corrected inner-filter effect and absorption spectra a→f: BSA,1.0×10^{-6} M; DEX-P ($\times10^{-6}$ M), 0, 9.5, 19, 28.5, 38.0 and 47.5; For inner-filter effect, their absorbance at λex 280 nm were 0.018, 0.048., 0.070, o.103, 0.130 and 0.159, and absorbance at λem 340 nm were 0.008, 0.009, 0.008, 0.011, 0.011 and 0.012, respectively; g: DEX-P, 9.5×10^{-6} M; 0.1 M NaCl, 0.02 M Tris–HCl buffer (pH 7.40)

No fluorescence of DEX-P was observed. The fluorescence spectra of BSA show a broad band with maximum at ~340 nm. It is observed that both fluorescence intensity decreases and absorbance of BSA increases with increasing concentration of DEX-P. A maximum fluorescence emission of BSA underwent spectral shift from 340 to 345 nm, and a maximum

absorption of BSA underwent spectral shift slightly. It is suggested that BSA and DEX-P formed a complex, and an energy transfer between DEX-P and BSA occurred.

The fluorescence quenching data are analyzed by the Stern–Volmer equation [13]:

$$F_0/F = 1+k_q\tau_0[Q] = 1+k_{sv}[Q] \qquad (1)$$

where F_0 and F are the fluorescence intensity in the absence and presence of quencher, respectively. k_q is the quenching rate constant, τ_0 is the fluorescence life time of biopolymer BSA ($\tau_0=10^{-8}$ s)[14], k_{SV} and $[Q]$ are the Stern–Volmer quenching constant and concentration of quencher, respectively.

In this work, the Stern–Volmer plots of F_0/F vs concentration of DEX-P were obtained (Fig.3). The values estimated are given in Table 1.

Figure 3. Stern-Volmer plots of F_0/F vs concentration of DEX-P under different temperatures after corrected inner-filter effect
F_0/F data are average with relative standard deviation of 6.9, 8.5 and 8.9% at 298, 303 and 308 K, respectively.

Table 1. Quenching parameter of BSA–DEX-P at different temperatures

The variation of F_0/F against DEX-P concentration Q fits in the equation of y = m x + c with correlation coefficient (R) greater than 0.99. Obviously, the quenching parameters are the lack of temperature dependence, and the rate constants k_q at temperature ranged from 298 to 308 K greater than the maximum scatter collision quenching constants of various quenchers with the biomolecule (2.0×10^{10} L/mol s)[15], which suggests that

the quenching is not initiated by dynamic quenching but by static quenching resulted from the formation of a complex.

3.2. Binding constant and binding site number

The binding of DEX-P with BSA to form complex in the ground state is further understood on the basis of available binding site number and binding constant of the complex formation process. For static quenching, the following equation was used to calculate the binding constant and binding sites [16,17]:

$$\log[(F_0 - F)/F] = \log K_a + n \log[Q] \qquad (2)$$

where K_a and n are the binding constant and binding site number, respectively. The plots of log $[(F_0\text{-}F)/F]$ vs log $[Q]$ are linear. Binding constant (K_a) and the binding site number (n) could be calculated from the intercept and slope, as shown in Table 2.

Table 2. Regression equation, correlation coefficient (R), binding constant K_a and the number of binding site n between DEX-P and BSA at different temperatures

It was noticed that the binding constant values decreased with increase in temperature due to reduction of the stability of DEX-P–BSA complexes. The binding constants of DEX-P and BSA in the temperature range of 298–308 K ranged from 2.239×10^4 to 6.918×10^2 L/mol. The average value of DEX-P and BSA was 8.18×10^3 L/mol. It is of the same order of magnitude as the binding constant (7.87×10^3 L/mol) obtained for DEX-P–BSA using CE/FA and that (2.22×10^3 L/mol) reported using ED for DEX-P–BSA [10]. Otherwise, the binding constants of DEX-P–BSA and DEX–HAS/BSA are

near[9,11], showing DEX-P and DEX having similar bioactivity.

3.3. Interaction forces between DEX-P with BSA

The interaction forces between drug and biomolecules include hydrogen bonds, van der Waals forces, electrostatic and hydrophobic interactions[18]. The temperature dependence of the interaction of DEX-P with BSA was investigated at 298, 303 and 308 K. The thermodynamic parameters can be evaluated from the Van't Hoff equation:

$$\mathrm{Ln}\ K_a = -\Delta H/\mathrm{RT} + \Delta S/\mathrm{R} \qquad (3)$$

where K_a is the binding constant at corresponding temperature T, and R is the gas constant. The enthalpy change (ΔH) and entropy change (ΔS) can be obtained from the slope and the ordinates at the origin of the Van't Hoff plot, respectively (Fig.4).

The free energy change, ΔG is determined from the following relationship

$$\Delta G = \Delta H - T\Delta S \qquad (4)$$

The values of ΔG, ΔS and ΔH are calculated and summarized in Table 3.

Figure 4. Van't Hoff plot for the interaction of DEX-P with BSA in 0.02 M Tris–HCl buffer (pH 7.40)–0.1 M NaCl solution at 298, 303 and 308 K and pH 7.40.

Table 3. Thermodynamic parameters of the interaction between DEX-P and BSA at different temperatures

The negative values of free energy (ΔG) supports the assertion that the binding process is spontaneous. The negative enthalpy (ΔH) and entropy (ΔS) values of the interaction of DEX-P and BSA indicate that the binding is

mainly enthalpy-driven and the entropy is unfavorable for it. This is similar with the interaction of DEX and BSA [11]. So Van der Waals interaction and hydrogen bond play major roles in the binding process [19].

3.4. Binding site of DEX-P on BSA

BSA has two tryptophan residues, Trp-212 and Trp-134. Similar to HSA, Trp-212 is located in subdomain IIA whereas Trp-134 is localized in the sub-domain IA [20]. On the basis of the probe-displacement method, there are at least three relatively-high specific drug-binding sites on the BSA molecules. These sites, commonly called warfarin, ibuprofen and digoxin-binding sites, are also denoted as Site I, Site II, and Site III, respectively[21,22]. To further determine the binding site of DEX-P, the competitive experiments were carried out at a temperature of 298 K using warfarin, ibuprofen and digoxin as a Site I-, Site II- and Site III-specific probe, respectively. The concentration ratio of BSA and probe was 1:1 (2×10^{-6} M: 2×10^{-6} M). The plots of log $[(F_0-F)/F]$ vs log $[C_{DEX\text{-}P}]$ in the absence and presence of site specific probe were prepared. The values of binding constant (K_a) and the binding site number (n) were calculated from the intercept and slope based on the Eq. (2). Binding constant and the binding site number were obtained.

Table 4. Binding constant K_a and binding site number n between DEX-P and BSA in the presence of site-specific probe

The data in Table 4 show that the binding constants for DEX-P–BSA system were 2.239×10^4 L/mol, while in the presence of site specific probe the binding constants decreased obviously. In the digoxin probe case, the binding constants decreased to 3.412×10^3 L/mol. It is shown the competition of digoxin with DEX-P at a same site. The competitive experiments suggested that the primary binding site of DEX-P on BSA was located at site III in sub-domain III_A of BSA.

3.5. Energy transfer from BSA to DEX-P

Fluorescence resonance energy transfer is an important technique for investigating a variety of biological phenomena including energy transfer processes[23]. Here the donor and acceptor are BSA and DEX-P, respectively. It was observed that there is spectral overlap between fluorescence emission of BSA and absorption spectra of DEX-P in the wavelength range of 285–450 nm, as shown in Fig. 5.

Figure 5. Spectral overlap of DEX-P absorption with BSA fluorescence in 0.02 M Tris–HCl buffer (pH 7.40) –0.1 M NaCl solution at 298 K
(a) absorption spectra of DEX-P; (b) fluorescence spectra of BSA-DEX-P; (c) fluorescence spectra of BSA; DEX-P, 1.0×10^{-6}M; BSA, 1.0×10^{-6}M

The fluorescence emission of BSA–DEX-P solution at an excitation wavelength of 280 nm is from BSA only since DEX-P is a non-fluorescence drug molecule. However, at this wavelength DEX-P has weak absorption. It suggested the possibility of fluorescence resonance energy transfer from BSA to DEX-P molecules in solution.

The region of integral overlap is used to calculate the critical energy transfer distance (R_0) between BSA (donor) and DEX-P (acceptor) according to Foster's non-radioactive energy transfer theory using Förster's equation[16,24]. Based on this theory, the efficiency (E) of energy transfer between donor (BSA) and acceptor (DEX-P) can be calculated by Equation (3):

$$E = R_0^6/(R_0^6 + r^6) \qquad (3)$$

Where, r is the binding distance between donor and acceptor, and R_0 is the critical binding distance. When the efficiency (E) of energy transfer is 50%, which can be calculated by Equation (4):

$$R_0^6 = 8.8\times10^{-25} k^2 n^{-4} \Phi_D J \qquad (4)$$

Where, the k^2 is the spatial orientation factor of the dipole, n is the refractive index of medium, Φ_D is the quantum yield of the donor in the absence of acceptor and J is the overlap integral of the emission spectrum of the donor and the absorption spectrum of the acceptor. The J can be calculated by Equation:

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda / \sum F(\lambda)\Delta\lambda \qquad (5)$$

Where, $F(\lambda)$ is the fluorescence intensity of the fluorescent donor of wavelength, λ, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength, λ. In the present case, k^2, n and Φ_D are 2/3, 1.336 and 0.118, respectively [25].

The efficiency (E) of energy transfer can be determined by Equation:

$$E = 1 - F/F_0 \quad (6)$$

Where, F_0 and F are the fluorescence intensities of BSA solutions in the absence and presence of DEX-P, respectively.

From the overlapping we found R_0 = 0.79 nm from Eq. (4) using k^2 = 2/3, n = 1.336 and Φ_D =0.118 (tryptophan residue) for the aqueous solution of BSA. J could be calculated from Eq. (5) and the corresponding result was 7.278×10^{-18} cm^3/mol. E calculated from Eq. (6) was 4.452%. At the same time, the binding distance (r) between BSA and DEX-P was obtained to be 1.23 nm by Eq. (3). The donor-to-acceptor distance, r<8 nm, indicated that the energy transfer from BSA to DEX-P occurs with high possibility[26]. It also suggested that the bindings of DEX-P to BSA molecules were formed through energy transfer, which quenched the fluorescence of BSA molecules, indicating the presence of static quenching interaction between BSA and DEX-P.

3.6. Change of BSA conformation

3.6.1. Circular dichroism studies

Circular dichroism is a sensitive technique to monitor conformational changes in protein structure[28]. CD spectra of BSA and BSA-DEX-P are shown in Fig.6.

Figure 6. CD spectra of BSA and BSA−DEX-P in 0.02 M Tris–HCl buffer (pH 7.40)–0.1 M NaCl solution at 298 K
(a) BSA, 1.0×10^{-6}M; (b) BSA/DEX-P, 1.0×10^{-6}M/1.0×10^{-6}M

In BSA spectrum, there are negative peaks in the ultraviolet region, one at 209 nm and the other at 222 nm, which are characteristic of the α-helical structure of a protein. Trynda-Lemiesz et al. explained that both of the negative peaks between 208-209 and 222-223 nm contribute to the transfer for the peptide bond of the α-helix[27]. In the presence of DEX-P, the intensity of both the negative peaks increased slightly, proving the change of the α-helical structure of BSA due to the formed complex of BSA and DEX-P. The CD spectra observed for BSA in the presence of DEX-P are similar in shape, indicating that the structure of BSA is also predominantly α-helical[28].

3.6.2. Synchronous fluorescence spectral change of BSA

Synchronous fluorescence is a kind of simple and sensitive method to measure the fluorescence quenching. It can provide the information of polarity change around the chromophore micro-environment. Δλ, representing the difference between excitation and emission wavelengths, is an important operating parameter. When Δλ is 15 nm, synchronous fluorescence is characteristic of tyrosine residue, while when Δλ is 60 nm, it provided the characteristic information of tryptophan residues[29]. The synchronous fluorescence spectra of tyrosine residue and tryptophan residues in BSA with addition of DEX-P were observed, as shown in Fig. 7.

Figure 7. Synchronous fluorescence spectra of BSA with different amount of DEX-P in 0.02 M Tris–HCl buffer (pH 7.40)–0.1 M NaCl solution at 298 K

(A) Δλ= 15 nm, and (B) Δλ= 60 nm; T=298K; a→f: BSA,1.0×10^{-6}M; DEX-P(10^{-6} M), 0, 9.5, 19, 28.5, 38.0, and 47.5

When the drug DEX-P was gradually added, the peak wavelength of tyrosine residues (Δλ=15 nm) did not change, and the fluorescence intensity decreased regularly. Otherwise, the peak of tryptophan residues (Δλ=60 nm) in BSA underwent spectral shift, and the fluorescence intensity and the pitch of quenching for Δλ = 60 nm was much higher than those for Δλ = 15 nm. Such result means DEX-P is closer to tryptophan residues than tyrosine residues, namely binding sites mainly are focused on tryptophan moiety.

3.6.3. Three-dimensional fluorescence spectral change of BSA

The three-dimensional fluorescence spectrum is another powerful method for studying conformation change of BSA. In this work, the three-dimensional fluorescence spectra and the contour spectra of BSA and BSA–DEX-P systems were observed, as shown in Fig. 8, and the characteristic parameters are summarized in Table 5.

Figure 8. Three-dimensional fluorescence spectra of (A) and contour spectra (B) of BSA and BSA−DEX-P systems in 0.02 M Tris–HCl buffer (pH 7.40)–0.1 M NaCl solution at 298 K
A-1 and B-1: BSA, 1×10^{-6}M; A-2 and B-2: BSA−DEX-P, 1×10^{-6}M/4×10^{-5}M,

Table 5. Three-dimensional fluorescence spectra characteristic parameters of the BSA and BSA–DEX-P systems

From Fig. 8, peak 1 (λex/λem =225/340 nm) reveals the spectral characteristic of tryptophan and tyrosine residues. After the addition of DEX-P, the fluorescence intensity of BSA decreased from 174.2 to 42.69. This suggests a less polar environment of both residues and almost all the

hydrophobic amino acid residues of BSA were buried in the hydrophobic pocket. Less polar environment means that the binding position between DEX-P and BSA located within this hydrophobic pocket, the addition of DEX-P changed the polarity of this hydrophobic microenvironment and the conformation of BSA[30]. In Fig. 8, peak 2 (λex/λem =275/340 nm) reveals the fluorescence spectra behavior of polypeptide backbone structures, which is caused by the transition of π-π* of BSA's characteristic polypeptide backbone structure C=O [31].

After the addition of DEX-P, the fluorescence intensity of BSA decreased from 170.9 to 87.36, and from the contour spectra in Fig.8, after addition of DEX-P in BSA, fingerprint lines of contour spectra changed to be sparse markedly. These revealed that the microenvironment and conformation of BSA were changed in the binding reaction. The interaction between DEX-P and BSA induced the unfolding of the polypeptides chains of BSA and conformational change of BSA.

4. Conclusions

The results showed that dexamethasone sodium phosphate can bind to bovine serum albumin by hydrogen bonding and Van der Waals forces. The primary binding for dexamethasone sodium phosphate was located at site III in subdomain IIIA of bovine serum albumin. The result demonstrated that the presence of dexamethasone sodium phosphate induced some micro environmental and conformational changes of bovine serum albumin

molecules. Circular dichroism spectra, synchronous fluorescence and three-dimensional fluorescence studies showed that the presence of dexamethasone sodium phosphate could change the conformation of bovine serum albumin during the binding process. The results are of great importance in pharmacy, pharmacology and biochemistry, and are expected to provide important insight into the interactions of the physiologically important protein bovine serum albumin with drugs.

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Table 1

Quenching parameter of BSA–DEX-P at different temperatures

T (K)	Regression equation	R	k_{sv}(L/mol)	k_q(L/mol s)
298	F_0/F=0.991+0.768×10^4 [Q]	0.9971	7.68×10^3	7.68×10^{11}
303	F_0/F=1.011+0.725×10^4 [Q]	0.9900	7.25×10^3	7.25×10^{11}
308	F_0/F=1.031+0.705×10^4C [Q]	0.9935	7.05×10^3	7.05×10^{11}

Table 2

Regression equation, correlation coefficient (R), binding constant K_a and the number of binding site n between DEX-P and BSA at different temperatures

T (K)	Regression equation	R	K_a(L/mol)*	n
298	$\log[(F_0-F)/F]=4.350+1.114 \log [Q]$	0.9963	$(2.239\pm0.155)\times10^4$	1.11
303	$\log[(F_0-F)/F]=3.160+0.8468 \log [Q]$	0.9918	$(1.445\pm0.123)\times10^3$	0.85
308	$\log[(F_0-F)/F]=2.840+0.7669 \log [Q]$	0.9915	$(6.918\pm0.615)\times10^2$	077

* (average ±Standard Deviation), n=3

Table 3

Thermodynamic parameters of the interaction between DEX-P and BSA at different temperatures

T (K)	K_a (L/mol)	R	ΔH (kJ/mol)	ΔS (J/mol K)	$\triangle G$ (kJ /mol)
298	2.239×10^4	0.9963	-161.0	-468.0	-21.54
303	1.445×10^3	0.9918			-19.20
308	6.918×10^2	0.9915			-16.86

Table 4

Binding constant K_a and binding site number n between DEX-P and BSA in the presence of site-specific probe

System	K_a (L/mol)	n	R
DEX-P–BSA	2.239×10^4	1.11	0.9963
DEX-P–BSA–warfarin	1.227×10^4	1.05	0.9962
DEX-P–BSA–ibuprofen	8.710×10^3	1.03	0.9935
DEX-P–BSA–digoxin	3.412×10^3	0.92	0.9939

Table 5

Three-dimensional fluorescence spectra characteristic parameters of the BSA and BSA–DEX-P systems

Systems	Parameters	Peak 1	Peak 2
BSA	Peak position(λex/λem, nm)	225/340	275/340
	Relative intensity	174.2	170.9
	Stokes shift Δλ (nm)	115	65
BSA–DEX-P	Peak position(λex/λem, nm)	225/335	285/340
	Relative intensity	42.69	87.36
	Stokes shift Δλ (nm)	110	55

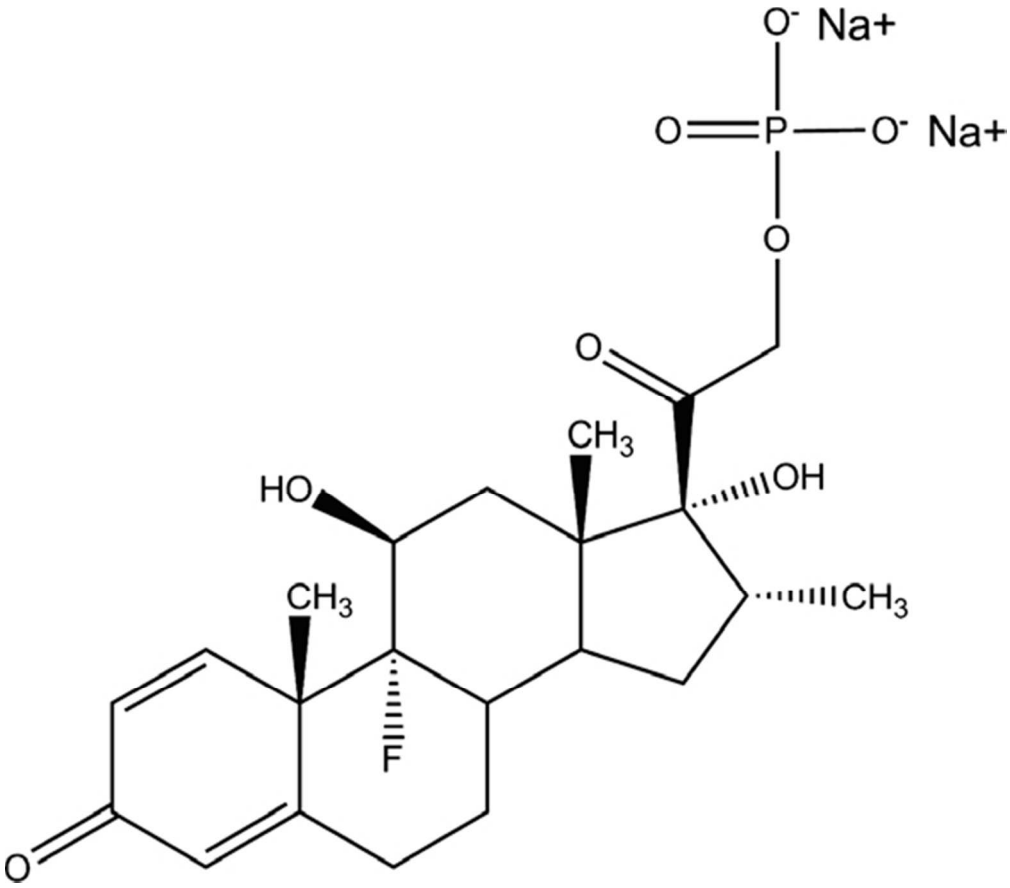

Figure 1
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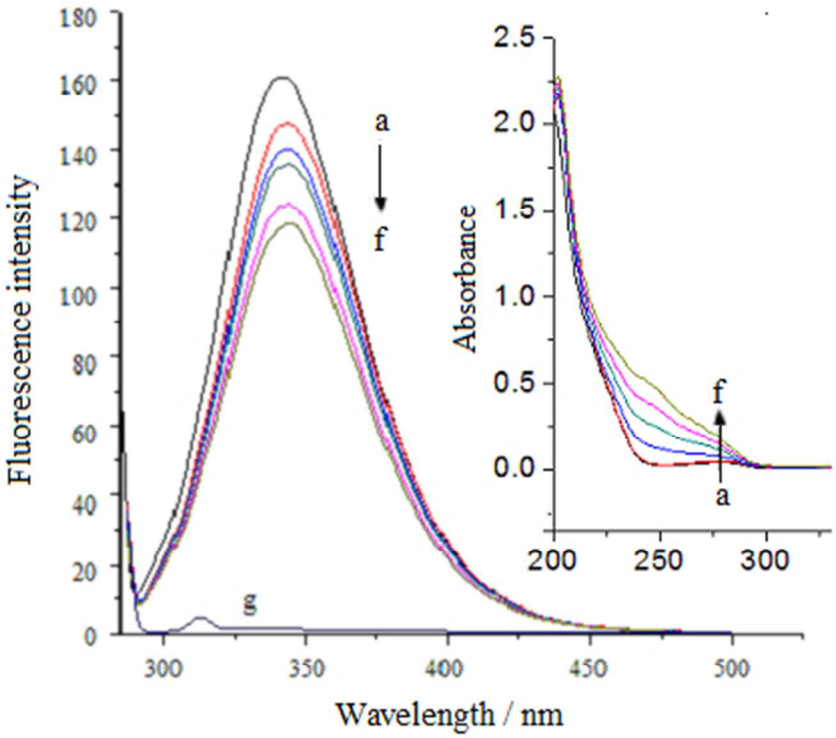

Figure 2
115x105mm (96 x 96 DPI)

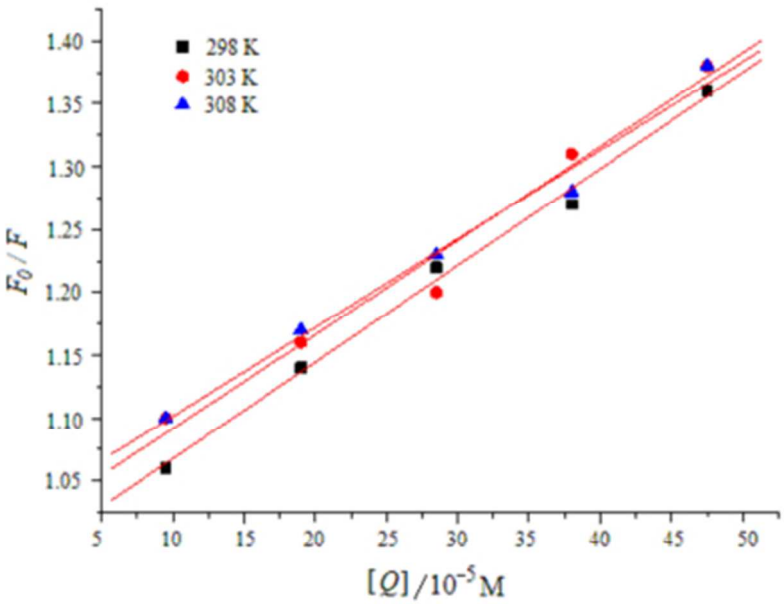

Figure 3
109x89mm (96 x 96 DPI)

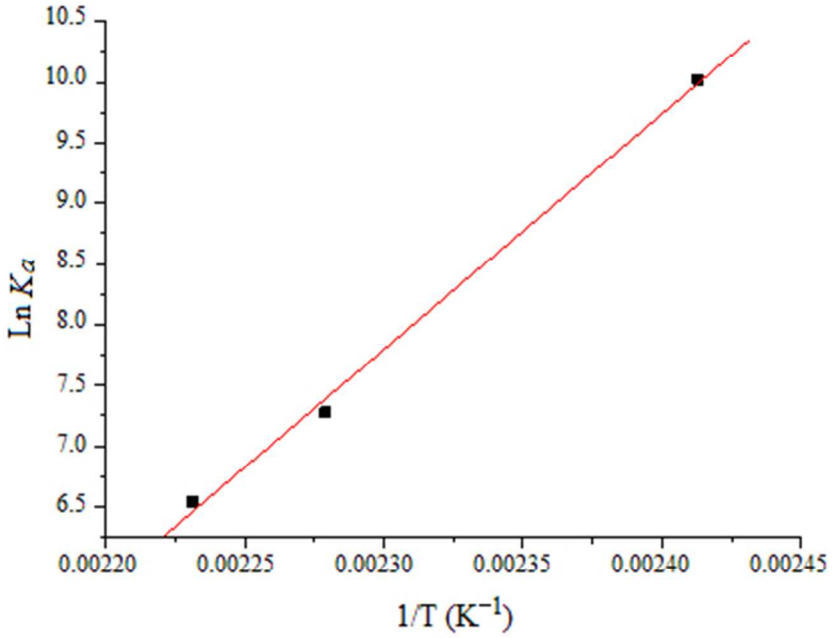

Figure 4
123x94mm (96 x 96 DPI)

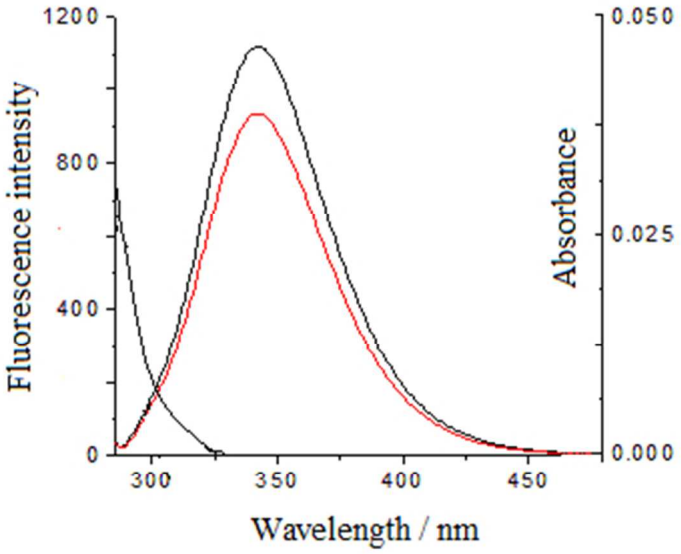

Figure 5
95x85mm (96 x 96 DPI)

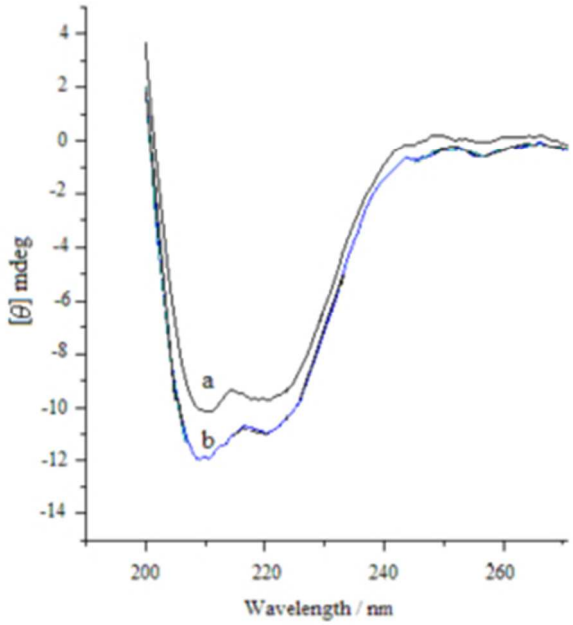

Figure 6
83x86mm (96 x 96 DPI)

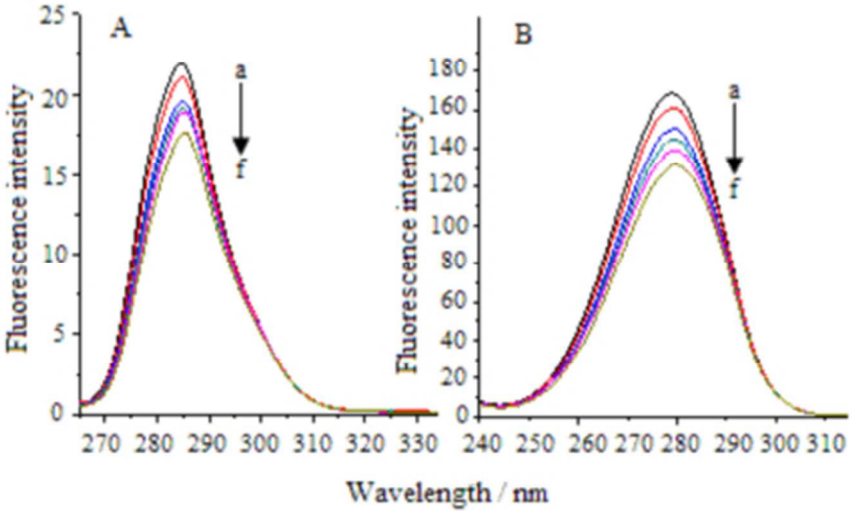

Figure 7
119x79mm (96 x 96 DPI)

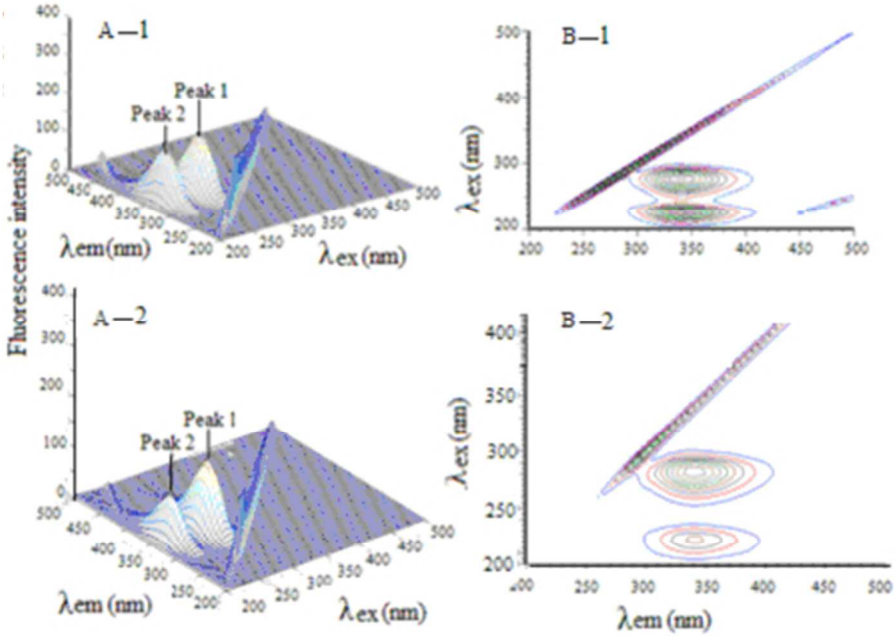

Figure 8
120x87mm (96 x 96 DPI)

Graphical abstract