

# Spectroscopic Investigation of Procaine Interaction with Human Serum Albumin

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## Abstract

The interaction of Human Serum Albumin (HSA) with local anaesthetic, procaine hydrochloride is an important study from the viewpoint of pharmaceutical sciences to clarify the structure, function, and properties of HSA-drug complexes.

The investigation has been carried through UV-absorption, Fluorescence and FTIR spectroscopy. The secondary structure of the protein and the binding mechanisms of the drug have been studied using Fourier self-deconvolution techniques on the obtained IR spectra.

Analysis of UV-absorbance and fluorescence spectra of procaine-HSA complexes showed a weak binding ability in quenching the intrinsic fluorescence of HSA by combinations of static and dynamic quenching procedures.

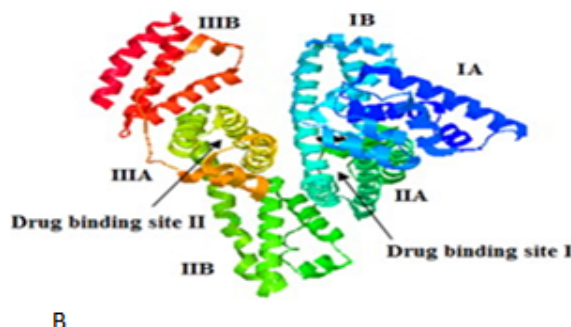
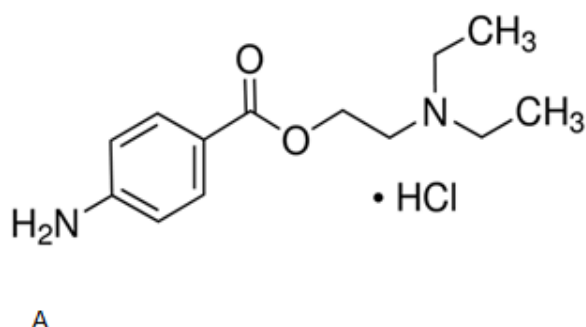
The binding constant (k) is calculated by graphical analysis and found to be in the range of  $(1.115-1.156) \times 10^3 \text{ M}^{-1}$  at 293 K. Spectral analysis of HSA-procaine compound has

revealed a relative decrease in the intensity of the absorption band of  $\alpha$  helix relative to that of  $\beta$ -sheets. This change in intensity is mainly due to the formation of H-bonding in procaine-HSA complex.

**Keywords:** Procaine; HSA; Binding constant; Protein secondary structure; FT-IR spectroscopy

## Introduction

Procaine hydrochloride, 2-(diethyl amino) ethyl p-amino benzoate has the molecular formula  $\text{C}_{13}\text{H}_{21}\text{N}_2\text{O}_2 \cdot \text{HCl}$ , and its chemical structure is shown in **Figure 1** [1]. It is a local anesthetic drug applied as an injection during surgery or other medical and dental procedures. It functions as a sodium channel blocker to ease the pain and it is known to be a short acting intravenous drug with adverse side effects such as cardiac and neurological toxicity [2]. Other studies on procaine have shown it to increase dopamine and serotonin in the brains of animals [3].



**Figure 1:** (A) Is the chemical structure of procaine (B) is the chemical structure of HSA.

Human serum albumin (HSA) is composed of three (196-383) and III (residues 384-585) and each of which is divided into two subdomains (A and B) and able to bind to a large

number of ligands at two main binding sites, identified as site I and site II which are shown in **Figure 1B** [4-6]. Site I is dominated by strong hydrophobic interaction with most neutral, bulky, heterocyclic compounds, while site II is dominated by dipole-dipole, van der Waals, and/or hydrogen-bonding interactions - with many aromatic carboxylic acids [7].

HSA is a single monomeric protein formed by 585 amino acids with 0.6 mM concentration in the blood plasma [8]. HSA has a dominant role in transporting and arranging various compounds such as fatty acids, hormones, tryptophan, steroids, metal ions, therapeutic agents and large number of drugs. HSA is considered as the major soluble protein components of the circulatory system, contributing to colloidal oncotic blood its pressure, binding and transferring poorly soluble drugs in water [9]. A weak drug binding has a short lifetime which is insufficient to provide therapeutic effect, whereas a strong binding reduces the free fraction of drugs with undesirable side effects because of its slow metabolism and excretion [10-13].

Evaluation of mechanisms of interaction of local anaesthetics can provide important information about their storage, transportation, evacuation in the blood makes it possible to predict their pharmacokinetic and pharmacodynamics properties such as the degree and duration of anesthetizing action, the rate of metabolism and/or utilization in peripheral tissues [14-16].

HSA contains a single intrinsic tryptophan residue (Trp 214) in domain IIA and its fluorescence is highly sensitive to the ligands bounded nearby [17-21]. Therefore, it is often used as a probe to investigate the binding properties of drugs with HSA.

Qualitative analysis of chemical binding to HSA can be detected by fluorescence spectra. The excitation wavelength, the emission wavelength, and the fluorescence intensity are the main three parameters used to study the synthetic information of the samples. In addition, the curve spectra can also provide important information. If there is any shift in the excitation or emission wavelength of the spectra, it could be an indication of HSA conformational changes [22].

Fluorescence quenching is formed by either dynamic or static quenching. Dynamic quenching is produced by intermolecular collision between a quencher and fluorescent molecules at an excited state, at which effective collision and the quenching constant should increase with increasing temperature. Usually dynamic quenching does not affect the structure and bioactivity of protein. Static quenching is caused by the intramolecular interaction of quenchers with fluorescent molecules at a ground state, which forms a new complex with changed structures. In static quenching, the constancy of new complexes decreases with the increase in temperature, since high temperatures contribute to molecular diffusion and the detachment of weakly bound complexes. Accordingly, dynamic quenching and static quenching can be distinguished by the impact of temperature change [23].

A considerable amount of scientific research has proven a strong connection between band positions of the IR spectra and changes to the secondary structure of the studied proteins. The energy associated with the observed absorption bands in several proteins are linked to certain molecular vibrations in the

secondary structures, such as  $\alpha$  helix or  $\beta$  -sheet structures [23-25]. The vital functions of each protein are dependent on the three dimensional structures of its components of peptides and polypeptide.

There are nine IR bands, named amide A, amide B and amides (I -VII), in the order of decreasing frequency [23,24]. Amide I band ( $1600 -1700$ )  $\text{cm}^{-1}$  with its large intensity, is the most useful for the analysis of the secondary structure of proteins. This band involves mainly C=O stretching vibration [26,27]. The amide II band ( $1480 -1600$ )  $\text{cm}^{-1}$  and amide III band ( $1220-1320$ )  $\text{cm}^{-1}$  contribute greatly to studies despite of the complex nature of their compositions and can be used for secondary structure prediction [28-30]. The spectra in the region from about ( $1300-500$ )  $\text{cm}^{-1}$  arise due to complicated combinations of (C-C, C-O, C-N) stretching and bending vibration. This range, referred to as the fingerprint region, is important because each compound produces its unique pattern of peaks.

The aim of the present work is to study the interaction between procaine and HSA. The importance of the study comes from the fact that procaine shows a short duration of action and adverse side effects. It is thus highly important for pharmaceutical sciences to clarify the structure, function, and properties of HSA- procaine complexes. Therefore, procaine's storage and transportation by proteins in the blood plasma becomes an important issue which requires investigations of the interaction mechanisms and determining the binding constant between procaine and HSA. In order to attain these objectives, UV-Vis absorption spectroscopy, fluorescence spectroscopy and FTIR spectroscopy were employed to carry out detailed investigation of procaine-HSA association.

## Material and Methods

### Materials

HSA (fatty acid free) and Procaine hydrochloride in powder form, were purchased from Sigma Aldrich chemical company and were used without any further purifications.

### Preparation of stock solutions

HSA was dissolved in phosphate buffered saline (80 mg/ml or 1.2 mM). The concentration of HSA in the buffer solution was calculated using its listed molecular weight of 66.5 kDa. Procaine hydrochloride, with the molecular weight of (272.77 g/mol) [28], was dissolved in double distilled water to prepare the following concentration (0.6, 0.8, 1.0, 1.2, 2.0, 2.4, and 4.80) mM. In the final step, each drug solution was added to an equal volume of the protein solution to attain the desired drug concentrations of (0.3, 0.4, 0.5, 0.6, 1.0, 1.2 and 2.4) mM. The solutions of procaine and HSA were incubated for 2 h at 20°C [31] before any further preparations for spectroscopic measurements.

### UV-Vis absorption spectra

The absorption spectra were obtained by the use of a Nano Drop ND-100 spectrophotometer. The absorption spectra were recorded for 5 - 10  $\mu\text{L}$  liquid samples of free HSA (40 mg/ml or

0.6 mM) and for its complexes with procaine solutions with the following concentrations (0.3, 0.4, 0.5, 0.6, 1.0, 1.2, and 2.4) mM. Repeated measurements were taken for all the samples and no significant differences were noticed. The UV-absorption spectra of procaine-HSA complex are obtained at the wavelength of 280 nm.

## Fluorescence

The following concentrations of procaine-HSA complex (0.3, 0.4, 0.5, 0.6, 1.0, 1.2, and 2.4) mM were prepared for the fluorescence study. The fluorescence measurements were performed by a Nano Drop ND-3300 Fluorospectrometer at 25°C. The excitation source comes from one of three solid-state light emitting diodes (LED's) with excitation source options include: UV LED with maximum excitation 365 nm, Blue LED with excitation 470 nm, and white LED from 500 to 650 nm excitation. A 2048-element CCD array detector covering 400-750 nm, is connected by an optical fibre to the optical measurement surface. The excitation is performed at the wavelength of 360 nm and the maximum emission wavelength is at 440 nm.

## FTIR spectroscopic measurements

The FTIR spectra were measured by a Bruker IFS 66/S spectrophotometer equipped with a liquid nitrogen-cooled MCT detector and a KBr beam splitter. The spectrometer was continuously purged with dry air during the measurements. Samples in the form of thin films are prepared after 2 h of incubation of HSA with procaine solution at room temperature, 30 µl of the complex sample were placed on a certain area on a silicon window plate and left to dry in a closed chamber at room temperature. The dehydrated films were formed on one side of a silicon window with different concentrations of procaine keeping the same protein content in all samples. The obtained absorption spectra were kept in the range of (400-4000) cm<sup>-1</sup>. Each spectrum was obtained by taking the average of 60 scans to enhance the signal to noise ratio, and the spectral resolution was at 4 cm<sup>-1</sup>. The aperture setting remained at 8 mm during all measurements, because it gave the best signal to noise ratio.

Baseline correction, normalization and peak areas calculations were done for all the obtained spectra by using OPUS software. The peak positions were determined using the second derivative of the spectra and the use of Fourier self-deconvolution technique. The infrared spectra of HSA and the procaine-HSA complex were recorded for the region of (1200-1700) cm<sup>-1</sup>. The FTIR spectrum of free HSA was acquired by subtracting the absorption spectrum of the buffer solution from the spectrum of the protein solution. For the net interaction effect, the difference spectra {(HSA-procaine)-(HSA)} were generated using the featureless region of the spectra at (1800-2200 cm<sup>-1</sup>) as an internal standard [27]. The accuracy of this subtraction method is tested using several control samples with the same protein and drug concentrations, which resulted in a flat base line formation as it should.

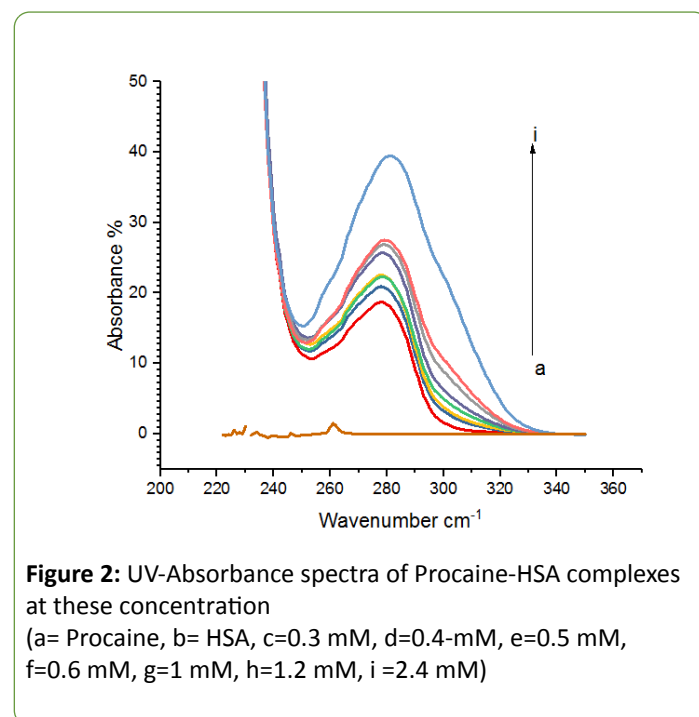
The obtained spectral differences were used here, to investigate the nature of the drug-HSA interaction. Silicon windows (NICODOM Ltd) were used as spectroscopic cell

windows, 30µl of each sample of HSA-procaine at the following concentrations of (0.15, 0.3, 0.6, 0.7, 0.9) mM was spread on silicon window using spin coater to obtain equal thickness of each sample, and then incubator was used to evaporate the solvent in order to obtain transparent thin film on the silicon window. All solutions were prepared at the same time at room temperature and were stored at the same closed chamber.

## Results

### UV-Vis absorption spectroscopy

UV-Vis absorption spectroscopy is one of the most common and effective experimental techniques used in calculating binding constants for several drug-protein complexes [32-34]. A complex has formed by the interaction of procaine with HSA as shown from the absorption spectra in **Figure 2**. The intensity of UV absorption of HSA increased with increasing concentrations of procaine indicating formation of procaine-HSA complex. A small red shift of the absorption peak at 280 nm is noticed with the increase of procaine concentration most likely due to the complex formation of procaine with HSA.



**Figure 2:** UV-Absorbance spectra of Procaine-HSA complexes at these concentration (a= Procaine, b= HSA, c=0.3 mM, d=0.4-mM, e=0.5 mM, f=0.6 mM, g=1 mM, h=1.2 mM, i=2.4 mM)



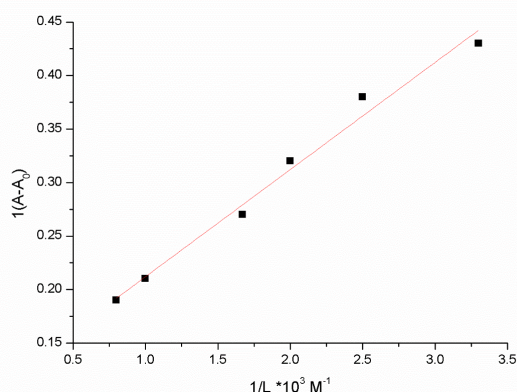
$$K = \frac{[\text{Procaine:HSA}]}{[\text{procaine}][\text{HSA}]} \quad (2)$$

The absorption data were treated using linear reciprocal plots based on the following equation [35].

$$\frac{1}{A - A_0} = \frac{1}{A_\infty - A_0} + \frac{1}{[A_\infty - A_0]K} \cdot \frac{1}{L} \quad (3)$$

Where  $A_0$  corresponds to the initial absorption of protein at 280 nm in the absence of ligand,  $A_\infty$  is the final absorption of the ligated protein, and  $A$  is the recorded absorption at different procaine concentrations ( $L$ ). The double reciprocal plot of  $1/(A - A_0)$  versus  $1/L$  is linear (**Figure 3**) and the binding constant ( $K$ )

can be calculated from the ratio of the intercept to the slope, which is found to be  $1.115 \times 10^3 \text{ M}^{-1}$ . This binding constant value shows a relatively weak procaine-HSA interaction in comparison to other drug-HSA complexes with binding constants in the range of  $10^5$  and  $10^6 \text{ M}^{-1}$  [35-37]. The reason for the weak formation can be attributed to the presence of mainly hydrogen-bonding interaction between protein donor atoms and the procaine polar groups or an indirect drug-protein interaction through water molecules [38].



**Figure 3:** The plot of  $1/(A-A_0)$  vs.  $1/L$  for HSA with different concentration of procaine

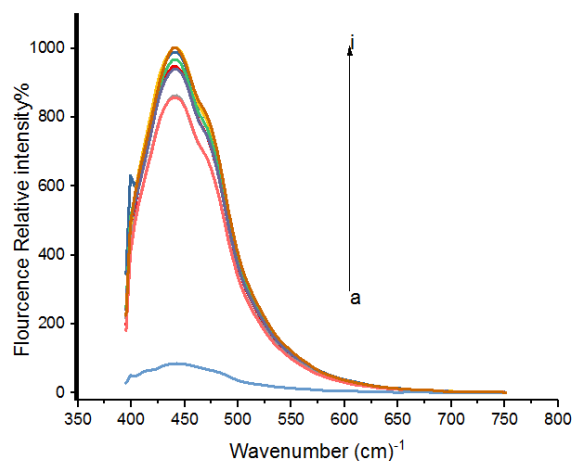
### Fluorescence Spectroscopy

Fluorescence quenching refers to any mechanism which reduces the fluorescence intensity of a fluorophore. Fluorescence quenching of HSA results from the tryptophan, tyrosine, and phenylalanine residues. The intrinsic fluorescence of HSA is almost due to the contribution of tryptophan alone, because phenylalanine has very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is ionized or near an amino group, a carboxyl group, or a tryptophan residue [22]. However, in fluorescence spectra studies on protein-drug interactions, the fluorescence intensity of proteins are usually sensitive to interference by ligands or newly produced complexes which exhibit significant fluorescence at or near the chosen excitation or emission wavelengths [39]. The ligand-binding process during protein-ligand complex is mainly governed by four types of weak, noncovalent forces including hydrogen bond, van der Waals force, electrostatic, and hydrophobic interactions [40].

When HSA is excited at 280 nm, it emits strong intrinsic fluorescence at 440 nm which is shown in **Figure 4**. This phenomenon is due to Trp residue located at the 214th position of the chain and the Try residues in HAS [41,42]. Further, the fluorescence intensity of HSA decreases due to the increase of procaine concentration, while the peak position shows little or no change at all.

The intrinsic fluorescence of HSA is highly sensitive to any slight changes in the local environment of HSA, and becomes obviously weakened by the following factors such as protein

conformational transition, bio-molecule binding, and denaturation [43].



**Figure 4:** Fluorescence emission spectra of HSA in the presence of various concentration of procaine (a=free procaine 4.8 mM, b=2.4 mM, c=1.2 mM, d=1.0 mM, e=0.6 mM, f=0.5 mM, g=0.4 mM, h= 0.3 mM, i=free HSA)

The mechanisms of fluorescence quenching are either dynamic quenching or static quenching. Dynamic and static quenching are caused by different means, namely diffusion for dynamic quenching and ground state complex formation for static quenching [38]. In addition, it is known that higher temperatures increase diffusion coefficients leading to an increase in dynamic quenching constants. In contrast, the rise of temperature can decrease the stability of a complex which leads to a decrease in the static quenching constant.

In order to confirm the nature of the quenching mechanism, the fluorescence quenching can be analyzed by the Stern-Volmer equation [44]

$$\frac{F_0}{F} = 1 + K_{q0}[L] = 1 + K_{sv}[L] \quad (4)$$

Where  $F$  and  $F_0$  are the fluorescence intensities with and without quencher,  $k_q$  is the quenching rate constant of the biomolecule,  $K_{sv}$  is the Stern Volmer quenching constant,  $[L]$  is the concentration of procaine, and  $\tau_0$  is the average life time of the molecule without quencher  $10^{-8}$  s.

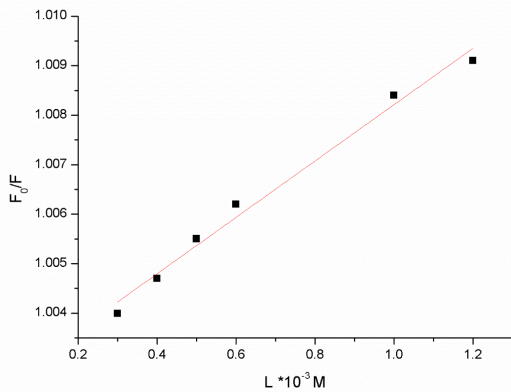
The value of  $K_{sv}$  ( $0.00566 \times 10^3 \text{ Mol}^{-1}$ ) is equal to the slope of a best fit straight-line analysis as shown in **Figure 5**. The quenching rate constant  $K_q$ , can be calculated using the fluorescence life time of  $10^{-8}$  s for HSA [45]. The obtained value for  $K$  is  $5.66 \times 10^8 \text{ Mol}^{-1} \text{ s}^{-1}$ , which is two orders of magnitude smaller than the maximum scatter collision quenching constant for various quenchers with biopolymer  $2 \times 10^{10} \text{ Mol}^{-1} \text{ s}^{-1}$  [46]. This indicates that procaine has low binding affinity with HSA, and the binding is weak and can be easily reversed. Other low binding constants within similar range of  $10^3 \text{ Mol}^{-1}$  have also been reported [47]. Usually, low binding to HSA results in a shorter life time or poorer distribution of drugs in the plasma,



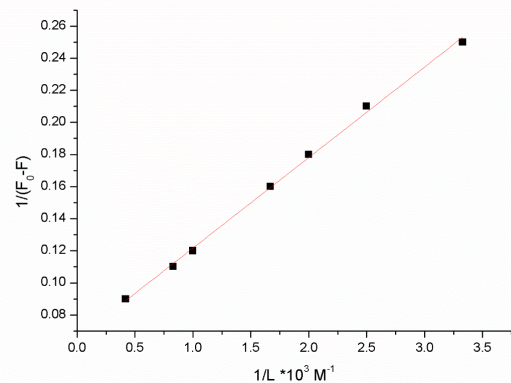
whereas the strong binding reduces the levels of free plasma [48]. Therefore, the binding is weak and can be attributed to combinations of static and dynamic quenching, Lineweaver-Burk equation, is used [49,50]

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{1}{KLF_0} \quad (5)$$

Where K is the static quenching constant with the unit of  $L \text{ mol}^{-1}$ , which describes the binding efficiency of micro molecules to biological macromolecules at ground state [51]. The value of K can be determined from the slope and the intercept in **Figure 6**. The value of K is  $1.156 \times 10^3 \text{ Mol}^{-1}$ , which agrees well with the value obtained earlier by UV spectroscopy. The weak quenching constant in this case has led to a lower value of binding constant between the drug and HSA due to an effective hydrogen bonding between procaine and HSA. The interaction of procaine molecules with HSA is initiated by electrostatic forces, but subsequently the hydrophobic interactions play the major role in procaine-HSA interactions. The low value of the binding constant showed that procaine quenched the intrinsic fluorescence of HSA through dynamic quenching mechanism. Furthermore, hydrophobic interaction played a major role in the binding process. Using fluorescence and UV absorption spectroscopy showed that procaine induces protein structural changes. The red shift in **Figure 2**, and the good linearity in the Stern-Volmer equation imply the role of static quenching by procaine [52].



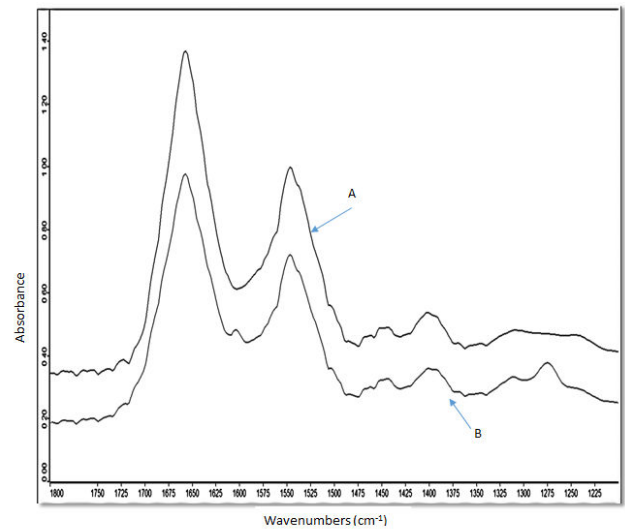
**Figure 5:** The Stern-Volmer plot for procaine -HSA complexes



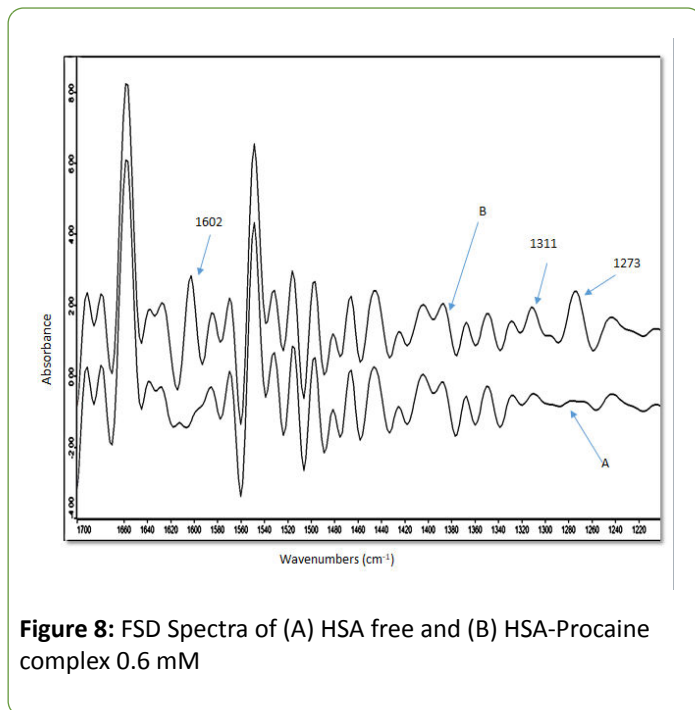
**Figure 6:** The plot of  $1/(F_0 - F)$  vs.  $1/[L(10)-3]$

### FTIR Spectroscopy

The absorption spectra of HSA free and HSA-procaine complex are presented in **Figure 7** showing the major amide bands (I, II, III) and the fingerprint region. All major peaks have been identified by taking the second derivative of the spectra and by applying Fourier self-deconvolutions (FSD) to the spectra as shown in **Figure 8**.



**Figure 7:** Spectra of HSA free (A) and Procaine-HSA complex 0.9 mM (B)



vibration modes [53]. As for amide III ( $1220=1320$ )  $\text{cm}^{-1}$ , the absorption bands are caused by in-phase combination of the N-H bending and the C-N stretching vibration with some contributions from the C-O in-plane bending and the C-C stretching vibration [53,54].

The component bands of amide I were attributed according to the well-established assignment criterion [55,56]. The bands range ( $1615-1637$ )  $\text{cm}^{-1}$  and ( $1680-1700$ )  $\text{cm}^{-1}$  are generally assigned to  $\beta$ -sheets, ( $1638-1648$ )  $\text{cm}^{-1}$  are assigned to random coil, ( $1649-1660$ )  $\text{cm}^{-1}$  to  $\alpha$ -helix and ( $1660-1680$ )  $\text{cm}^{-1}$  to  $\beta$ -turn structure. The amide II components are assigned in the following order ( $1488-1504$ ) and ( $1585-1600$ )  $\text{cm}^{-1}$  to  $\beta$ -sheets, ( $1504-1527$ )  $\text{cm}^{-1}$  to random coil, ( $1527-1560$ )  $\text{cm}^{-1}$  to  $\alpha$ -helix and ( $1564-1585$ )  $\text{cm}^{-1}$  to turn structure [57]. The component bands of Amide III have been assigned as follows:  $\alpha$ -helix ( $1330-1290$ )  $\text{cm}^{-1}$ ,  $\beta$ -turn ( $1290-1270$ )  $\text{cm}^{-1}$ , random coil ( $1270-1250$ )  $\text{cm}^{-1}$  and  $\beta$ -sheets ( $1250-1220$ )  $\text{cm}^{-1}$  [56].

The peak positions of amide I bands in HSA spectrum stayed about the same as shown in **Table 1**. The only noticeable change is the disappearance of the small peak at  $1612$   $\text{cm}^{-1}$  and the rise of a new peak at  $1602$   $\text{cm}^{-1}$  with higher concentration of procaine. The amide II bands did not show any major changes in their peak positions with the different concentrations of HSA-Procaine complexes. As for amide III, the following peaks ( $1310$ ,  $1294$ ,  $1243$ , and  $1224$ )  $\text{cm}^{-1}$  maintain their positions, while the two weak bands at  $1277$  and  $1243$   $\text{cm}^{-1}$  have been overshadowed by a new peak at  $1273$   $\text{cm}^{-1}$ . The two new peaks at  $1602$  and  $1273$   $\text{cm}^{-1}$  are due to C=C (aromatic) stretching mode and C-O and C-N stretching mode of procaine, respectively [58-60]. Their dominant effect appears to be due to the presence of unbound procaine molecules within the HSA-procaine complex which is related to the weak binding constant between procaine and HSA.

**Table 1:** Absorption peak positions in Wave numbers ( $\text{cm}^{-1}$ ) for Procaine-HSA at different concentrations.

Bands	Range ( $\text{cm}^{-1}$ )	Free HSA	concentrations					
			0	0.15 mM	0.3 mM	0.6 mM	0.7 mM	0.9 mM
<b>Amide I</b>	1700-1600	----	----	1603	1603	1603	1603	
		1613	1612	--	--	--	--	
		$\beta$ -sheets	1627	1628	1626	1626	1626	1627
		Random	1640	1641	1640	1640	1641	1641
		$\alpha$ -helix	1657	1658	1658	1657	1659	1659
<b>Amide II</b>	1600-1480	1679	1678	1678	1679	1679	1679	
		$\beta$ -sheets	1694	1694	1694	1694	1694	1694
		$\beta$ -sheets	1498	1498	1498	1498	1498	1498
	$\beta$ -sheets	1515	1516	1515	1515	1516	1516	
	Random	1532	1532	1532	1532	1532	1532	
	$\alpha$ -helix	1549	1549	1549	1549	1549	1549	

	Turn	1569	1569	1569	1569	1569	1569
	$\beta$ -sheets	1585	1585	1585	1584	1584	1584
<b>Amide III</b>	1320-1220						
	$\beta$ -sheets	1224	1224	1224	1224	1224	1224
	$\beta$ -sheets	1244	1244	1244	1244	1244	1244
	Random	1267	1267				
				1273	1273	1273	1273
	Random	1277	1277				
	Turns	1295	1295	1295	1295	1295	1295
	$\alpha$ -helix	1309	1309	1309	1310	1310	1310

The relative intensities of all component bands of amide I, amide II, and amide III were calculated and listed in **Table 2**. The following bands showed major increase in their intensities with increasing procaine concentration in the complex (1603, 1309, and 1273)  $\text{cm}^{-1}$ , while these bands (1627, 1548)  $\text{cm}^{-1}$  showed very small increase in their relative intensities. On the other

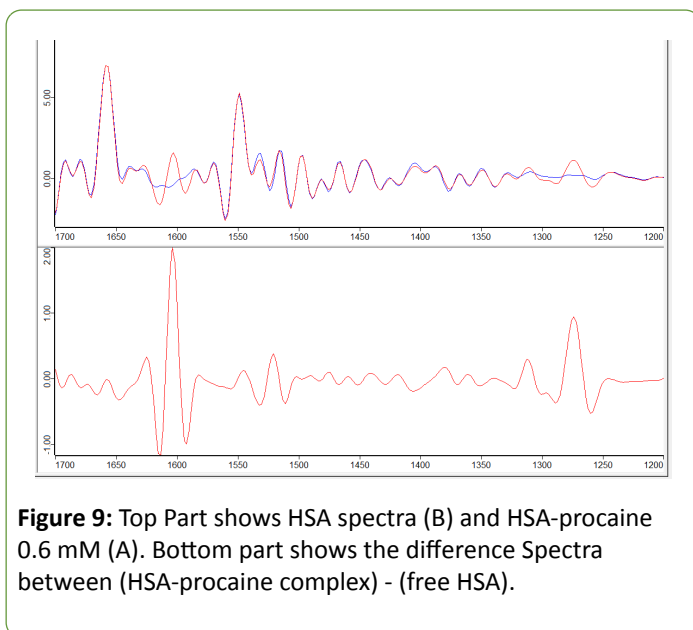
hand, the following bands (1657, 1531, and 1294)  $\text{cm}^{-1}$  show little or no decrease in their intensities. The bands at (1613, 1277, and 1266)  $\text{cm}^{-1}$  disappeared due to overlapping by neighboring bands and the rest of the bands showed no change in their intensities.

**Table 2:** Relative intensity of absorption bands for Procaine-HSA at different concentrations.

Bands	Range ( $\text{cm}^{-1}$ )	Intensity %	Intensity %	Intensity %	Intensity %	Intensity %	Intensity %
<b>Amide I</b>	1700-1600	0	0.15 mM	0.3mM	0.6mM	0.7mM	0.9mM
<b><math>\beta</math>-sheets</b>	1606-1620	3	5				
<b><math>\beta</math>-sheets</b>	1620-1633	5	7	7	9	17	14
<b>Random</b>	1633-1647	8	8	9	9	10	8
<b><math>\alpha</math>-helix</b>	1649-1670	46	40	40	46	25	39
<b>Turns</b>	1672-1686	18	19	20	18	21	18
<b><math>\beta</math>-sheets</b>	1687-1700	20	21	24	18	26	21
<b>Amide II</b>	1600-1480						
<b><math>\beta</math>-sheets</b>	1487 1506	12	12	13	13	14	14
<b><math>\beta</math>-sheets</b>	1506-1523	14	13	13	14	11	13
<b>Random</b>	1523-1539	11	15	11	10	10	9
<b><math>\alpha</math>-helix</b>	1541- 1558	23	25	23	25	20	24
<b>Turns</b>	1560-1585	19	19	19	20	21	21
<b><math>\beta</math>-sheets</b>	1585-1600	21	16	21	18	24	19
<b>Amide III</b>	1320-1220						
<b><math>\beta</math>-sheets</b>	1222-1256	51	41	42	34	30	29

Random	1259-1287	15	21	30	40	43	45
Turns	1288-1302	5	6	5	5	5	5
$\alpha$ -helix	1302-1319	29	32	23	21	22	21

The difference in FSD spectra [(HSA+ procaine)-(HSA)] were obtained to investigate the intensity variations and the results are shown in **Figure 9**. The strong negative features at 1601 and 1277  $\text{cm}^{-1}$  were due to absorbance by free procaine. While the band at 1309  $\text{cm}^{-1}$  formed by double contributions from free procaine and procaine-HSA complex. The positive peaks shown at 1657 and 1554  $\text{cm}^{-1}$  represent the decrease in intensity of the  $\alpha$ -helix bands of the amide I and amide II respectively. This decrease is attributed to the unfolding of the protein in the presence of procaine as a result of the H-bonding formation with protein C=O and C-N groups [61]. As for any possible changes in the intensity of  $\alpha$ -helix in the amide III region, it could not be determined due to the overlapping by the procaine band at 1309  $\text{cm}^{-1}$ . In general these changes of the peak positions, intensities and peak shapes are considered to be overwhelming evidences of secondary structure changes have occurred as a result of procaine interaction with HSA.



**Figure 9:** Top Part shows HSA spectra (B) and HSA-procaine 0.6 mM (A). Bottom part shows the difference Spectra between (HSA-procaine complex) - (free HSA).

## Conclusions

In this paper, the interaction between procaine and HSA was investigated by fluorescence spectroscopy, UV-Vis absorption and FTIR spectroscopy. The results indicate that the fluorescence quenching mechanism of HSA by Procaine binding indicate contributions from both a static and dynamic quenching process. The binding reaction of procaine with HSA showed a binding constant is in the range of  $(1.115-1.156) \times 10^3 \text{ M}^{-1}$ . The experimental results pointed to contributions from different interaction mechanisms between procaine and HSA. It seems that, the complexation of procaine molecules with HSA is initiated by electrostatic forces, but subsequently the

hydrophobic interaction played a major role in this complexation.

The infrared spectroscopic results show that drug- protein binding occurs with the participation of the tryptophan residues and some conformation of the protein secondary structure. The variations in the relative intensities of the absorption bands showed a slight decrease in  $\alpha$ -helical contents and a minor increase in  $\beta$ -sheet contents.

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