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Spectral Studies on the Interaction of Sanguinarine Chloride with Cytidine

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In this work, the interaction of sanguinarine chloride (SAN) with cytidine at physiological pH 7.2 was investigated with the help of UV-visible, fluorescence, ^{1}H NMR spectral measurements and thermodynamic calculations. It was found that the interaction between sanguinarine chloride and cytinde results in hypochromism and red shift of the sanguinarine chloride E absorption band (274 nm) and one sanguinarine chloride molecule can bind with two cytidine molecules with the binding constant in the order of 10^7, indicating that the binding of sanguinarine chloride with cytidine is not only exothermic but entropy-driven with $\Delta_rH_m^\Theta$ = - 4.87 kJ mol^{-1}, $\Delta_rS_m^\Theta$ = 0.13 kJ mol^{-1} K^{-1} and $\Delta_rG_m^\Theta$ = - 44.45 kJ mol^{-1} at 298.15 K.

Key Words: Sanguinarine chloride, Cytidine, Spectroscopy, Interaction.

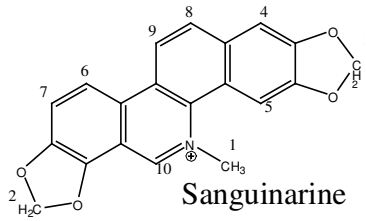
INTRODUCTION

This paper is a continuation of the systematic investigations on interaction of alkaloids with nucleic acids[1-4], because alkaloids have been known to play important roles in medicinal chemistry due to their extensive biological activities. As one of the important alkaloid, sanguinarine chloride (Fig. 1a) presents potent anticancer[5], apoptosis[6], antiinflammatory[7], antimicrobial[8] and antioxidant[9] activities. Despite the arguments that its interactions with DNA may interfere with DNA systhesis, the anticancer mechanisms of sanguinarine chloride is still not clear. In contrast to the large number of studies on the interaction mechanism of sanguinarine chloride with DNA, the studies on the interaction of sanguinarine chloride with the base of nucleosides are scanty[10-15].

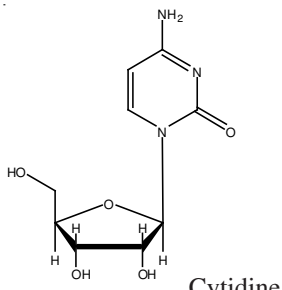
In this paper, the interaction of sanguinarine chloride with cytidine (Fig. 1b), which is one of four bases in the basic materials of DNA, was closely examined by using UV-VIS and fluorescence spectroscopies.

EXPERIMENTAL

Sanguinarine chloride (≥ 99 %) was kindly supplied by Yongfeng Boyuan Industry Co. Ltd., Jiangxi Province, PRC. sanguinarine chloride solution was prepared prior to be used and kept in dark to prevent light. Cytidine with a purity of ≥ 99 % was purchased from Sigma chemical Co. (USA). Sanguinarine chloride and cytidine were used without further purification. Their binding experiments were performed in PBS buffer (0.02 mol/L $Na_2HPO_4{\cdot}12H_2O$, 0.02 mol/L $NaH_2PO_4{\cdot}2H_2O$ and pH = 7.20 ± 0.05). Three-distilled water and analytical grade reagents were used in this work.



(a)



(b)

Fig. 1. Chemical structures of sanguinarine and cytidine

UV-VIS spectra were recorded using a Varian CARY 1E UV-VIS spectrometer with a resolution of 0.2 nm at room temperature in the region of 250-400 nm. Fluorescence spectra were acquired using an F-4500 fluorescence spectrophotometer employing a 500 W Hg-Xe high pressure lamp. The fluorescence

lifetime measurements were performed on an Edinburgh FLS920 spectrometer in matched an nF900 light source and an R928-PA detector with special spectral processor software. ^{1}H NMR spectra were acquired using a Bruker ARX-400 nuclear magnetic resonance spectrometer and D_2O was used as a NMR solvent. All temperatures are controlled using a PolyScience water baths with uncertainty of ± 0.02 K.

RESULTS AND DISCUSSION

In order to discuss the interaction of sanguinarine chloride with cytidine, the concentration of sanguinarine chloride was fixed at 20.0 μmol/L and the concentrations of cytidine were designed at 0, 40, 80, 120, 160, 200, 240, 280, 320, 360 and 400 μmol/L. All experiments were carried out at (298.15, 308.15, 318.15 and 328.15 K) kept at a constant temperature for using CS 501 thermostated bath with a Beckmann thermometer purchased from Huanghua Meter Factory (Hebei Province, China) with a precision of ± 0.02 K and inspected using an accurate thermometer purchased from Fuqiang Meter Factory (Hebei province, China) with the precision of ± 0.02 K. A base line correction was made for the UV spectra recorded in the PBS solutions containing cytidine. The absorption spectra of sanguinarine chloride at 298.15 K are shown in Fig. 2. E absorption band at 274 nm and K absorption band at 327 nm are due to the aromatic chromophore (π-π*) of sanguinarine chloride. A conspicuous hypochromism on E band of sanguinarine chloride at 274 nm was found with the increasing cytidine concentration (0-400 μmol/L). Meanwhile, the absorption spectra show a decrease of peak intensity about 32 % and a small red shift about 11 nm at E absorption band of sanguinarine chloride. In contrast, minor but a little noticeable peak shift and hypochromism can be defined for the K absorption from Fig. 2. The hypochromicity and bathochromicity of E absorption band are due to the effective interaction of sanguinarine chloride with cytidine.

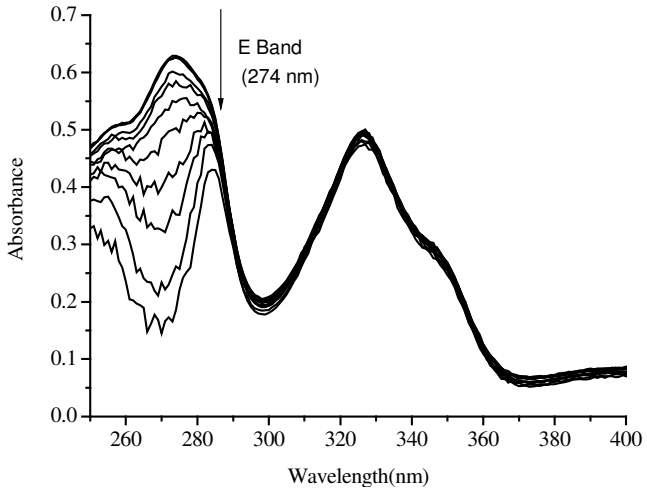


Fig. 2. Absorption spectral changes of sanguinarine chloride (20.0 μM) with inceasing cytidine concnetration (up to down: 0-400 μmol/L)

Meanwhile, the concentration of sanguinarine chloride was fixed at 5.0 μmol/L and the concentrations of cytidine were designed at 0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375 and 400 μmol/L for emission spectral measurements. All experiments were carried out at (298.15, 308.15, 318.15 and 328.15) K kept at a constant temperature for using CS 501 thermostated bath with a Beckmann thermometer. A strong emission spectrum of sanguinarine chloride at 419 nm in the range of 350-520 nm was observed at an excited wavelength of 274 nm. The emission spectra of sanguinarine chloride decreased with increasing of cytidine concentration at 298.15 K (Fig. 3). Meanwhile, a fluorescence quenching of sanguinarine chloride was observed, but not altering the emission maximum and shape of the peaks. These results show that there are the binding of sanguinarine chloride with cytidine. The fluorescence intensity tends to be constant at a high concentration of cytidine, which shows the binding of sanguinarine chloride to cytidine reached saturation.

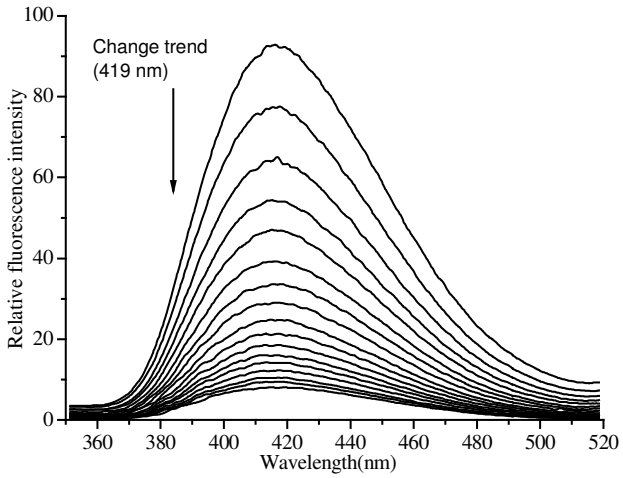


Fig. 3. Emission spectral changes of sanguinarine chloride (5.0 μmol/L) with increasing cytidine concentration (up to down: 0-400 μmol/L)

Chemical shift of sanguinarine chloride binding to cytidine is shown in Table-1.

The chemical shifts of hydrogen in -CH_3 (sites 1 and 10) appear at δ = 4.394 ppm in the ^{1}H NMR spectrum of sanguinarine chloride (Table-1). However, with increasing cytidine, the chemical shift of hydrogen atoms in -CH_3 (sites 1 and 10) shifts to 4.688 ppm. The shift is due to the interaction of nitrogen atom in sanguinarine chloride bonding with cytidine decreases shielding effect of hydroxyl hydrogen atoms of -CH_3 (site 1) and -CH= (site 10) in sanguinarine chloride, so that the signal of chemical shift towards lower magnetic field. Especially, the chemical shifts are obvious when cytidine was added in the sanguinarine chloride solution, which indicates the interaction of sanguinarine chloride with cytidine is strong.

TABLE-1
CHEMICAL SHIFT OF SANGUINARINE CHLORIDE BINDING TO CYTIDINE

Species	1	2	3	4	5	6	7	8	9	10
S (δ/ppm)	4.394	6.123	6.351	6.864	7.348	7.454	7.556	7.661	9.23	4.394
S/C (δ/ppm)	4.688	6.116	6.344	6.917	7.415	7.503	7.575	7.746	9.276	4.688
S/C (Δδ/ppm)	+0.294	-0.007	-0.007	+0.053	+0.067	+0.049	+0.019	+0.085	+0.046	+0.294

The quenching data of sanguinarine chloride by cytidine were fitted to the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{sv}[Q]^n = 1 + k_q \cdot \tau_0 [Q]^n \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of cytidine, respectively. [Q] denotes the concentration of cytidine and K_{sv}, the Stern-Volmer quenching constant, is the product between the rate constant for quenching (k_q) and the lifetime of sanguinarine chloride in the absence of quencher (τ_0). The Stern-Volmer quenching plot of sanguinarine chloride with the increasing concentration of cytidine is shown in Fig. 4.

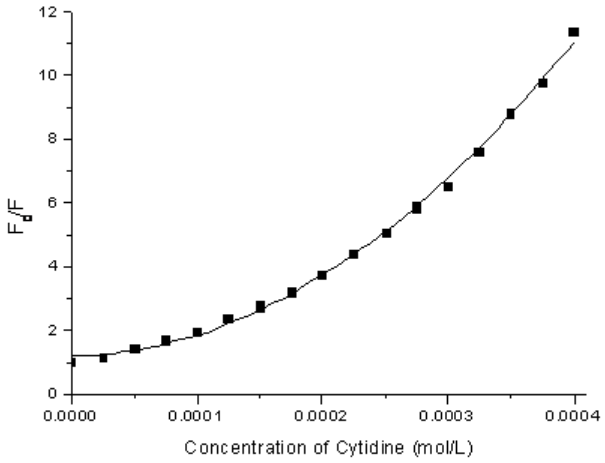


Fig. 4. Stern-Volmer quenching plot of sanguinarine chloride with the increasing concentration of cytidine in pH = 7.2 buffer at 298.15 K

The Stern-Volmer plot is nonlinear and approximate quadratic. One possibility is the existence of both dynamic and static quenching mechanisms. Because the measurement of the fluorescence lifetime is more definitive method to distinguish dynamic or static quenching[16,17], the fluorescence lifetimes of sanguinarine chloride in the absence and presence of cytidine are determined. According to the data in Table-2, the fluorescence lifetime ratios of sanguinarine chloride in the absence and presence of cytidine are near to 1, which shows that the quenching is static.

TABLE-2
FLUORESCENCE EMISSION LIFETIMES OF SANGUINARINE CHLORIDE (4.89 μM) IN THE ABSENCE AND PRESENCE OF CYTIDINE (χ^2 IS EMISSION MAXIMA)

[Cytidine] (μM)	0	25	50
τ (ns)	3.17 ± 0.01	3.17 ± 0.01	3.18 ± 0.01
χ^2	1.16	1.08	1.08

A binding formation of sanguinarine chloride with cytidine is nonfluorescent. Besides, the processes can be distinguished by examining of the absorption spectrum of fluorophore. The collisional quenching only affects the excited states of the fluorophores, thus no changes in the absorption spectrum are predicted. In contrast, the ground state binding formation will frequently result in perturbation of the absorption spectrum of fluorophore[16,17]. The fluorescence excitation spectra of fluorophore is treated as the same as the absorption spectra of the fluorophore. The varieties of the excitation spectra of sanguinarine chloride (4.89 μM) caused by gradually increasing the concentration of cytidine at 298.15 K are shown in Fig. 5.

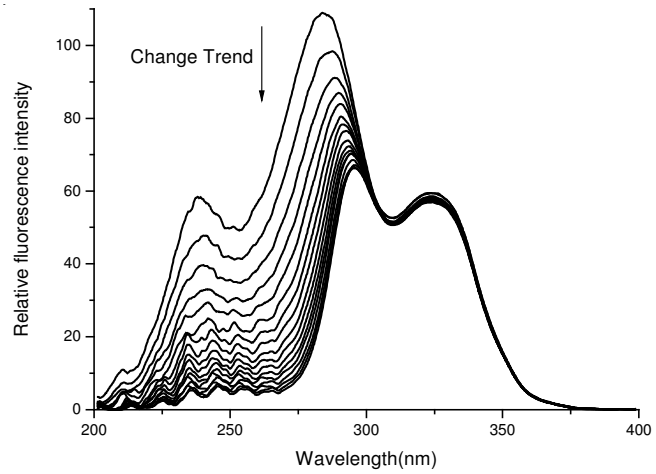


Fig. 5. Excitation spectra of sanguinarine chloride (4.89 μM) with increasing cytidine concentrations in pH = 7.2 buffer of (up to down: 0-400 μM)

The fluorescence intensities of sanguinarine chloride decrease with the increasing concentration of cytidine, which indicates that binding of sanguinarine chloride with cytidine is nonfluorescent. This phenomenon also proves that fluorescence quenching is static.

The binding constants of sanguinarine chloride with cytidine are calculated by the reported methods[16,17]. In static quenching,

$$\frac{[M]_0}{[M]} = \frac{F_0}{F} \quad (2)$$

where $[M_0]$ denotes the total concentration of sanguinarine chloride and [M] denotes the concentration of free sanguinarine chloride.

According to previous methods[16,17], the following formula can be obtained:

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

where [Q] denotes the concentration of free quencher. A linear plot of F_0/F to $[Q]^2$ indicates that one sanguinarine chloride can bind two cytidine (Fig. 6). By linear analysis from the data in Fig. 6, a binding constant K is calculated out as 6.14×10^7 at 298.15 K. The binding parameters of sanguinarine chloride-cytidine are shown in Table-2 at various temperatures.

The fluorescence spectra of sanguinarine chloride-cytidine binding are performed at 298.15, 308.15, 318.15 and 328.15 K. Thermodynamic parameters were estimated by the analysis of ln K *versus* 1/T plot (van't Hoff plot) obtained by the experimental data at the above temperatures. The gradient of this straight line of ln K *versus* 1/T is equal to -ΔH/R, the values of ΔH, ΔG and ΔS are calculated from the following relationships:

$$\Delta G = RT \ln K = \Delta H - T\Delta S \quad (4)$$

The van't Hoff plot for the binding of sanguinarine chloride to cytidine is depicted in Fig. 7. The values of the

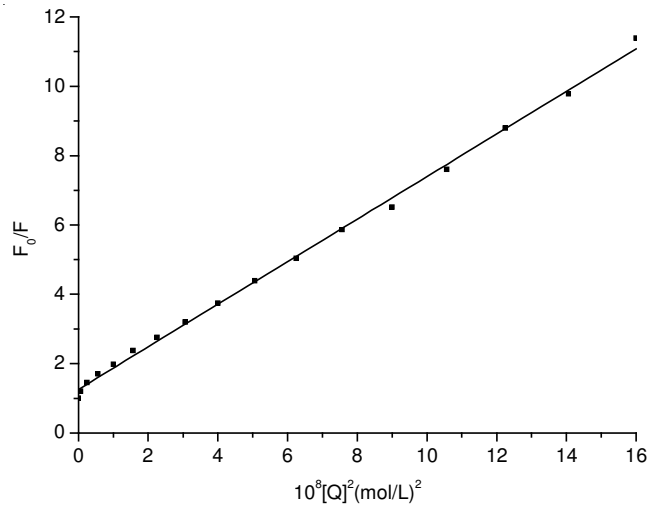


Fig. 6. A plot between F_0/F and concentration of free quencher

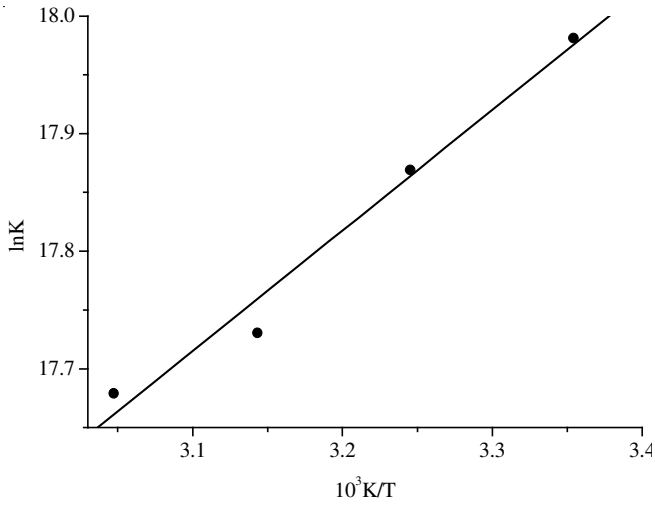


Fig. 7. Van't Hoff plot for the binding of sanguinarine chloride to cytidine

thermodynamic parameters are shown in Table-3. The negative ΔG and positive ΔS show that the interaction of sanguinarine chloride with cytidine is entropy-driven. The negative ΔH demonstrates the interaction is an exothermic reaction.

TABLE-3
THERMODYNAMIC PARAMETERS FOR SANGUINARINE CHLORIDE-CYTIDINE COMPLEXATION OBTAINED FROM FLUORESCENCE MEASUREMENTS IN pH = 7.2 BUFFER

T (K)	$K \times 10^7$	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol K)
298.15	6.14	-44.45	-4.87	0.13
308.15	6.00	-45.88	-4.87	0.13
318.15	5.57	-47.18	-4.87	0.13
328.15	5.15	-48.45	-4.87	0.13

Conclusion

The binding of sanguinarine chloride to cytidine results in hypochromism and bathochromism in absorption spectra and in emission spectra. These spectra features strongly support the interactions of one sanguinarine chloride molecule with two cytidine molecule. sanguinarine chloride binds to cytidine with a binding constant of 6.14×10^7 at 298.15 K. In addition, the thermodynamic data for the sanguinarine chloride binding to cytidine were also calculated and derived from experimental measurements, with the results of $\Delta_r H_m^\Theta$ = -4.87 kJ/mol, $\Delta_r G_m^\Theta$ = -44.45 kJ/mol and $\Delta_r S_m^\Theta$ = 0.13 kJ/mol K at 298.15 K. These results show that the binding of sanguinarine chloride to cytidine is not only exothermic but entropy-driven.

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