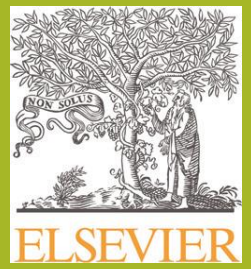
Studying the Interaction Between Trinuclear Ruthenium Complexes and Human Serum Albumin by Means of Fluorescence Quenching

By: Faezeh Naseri



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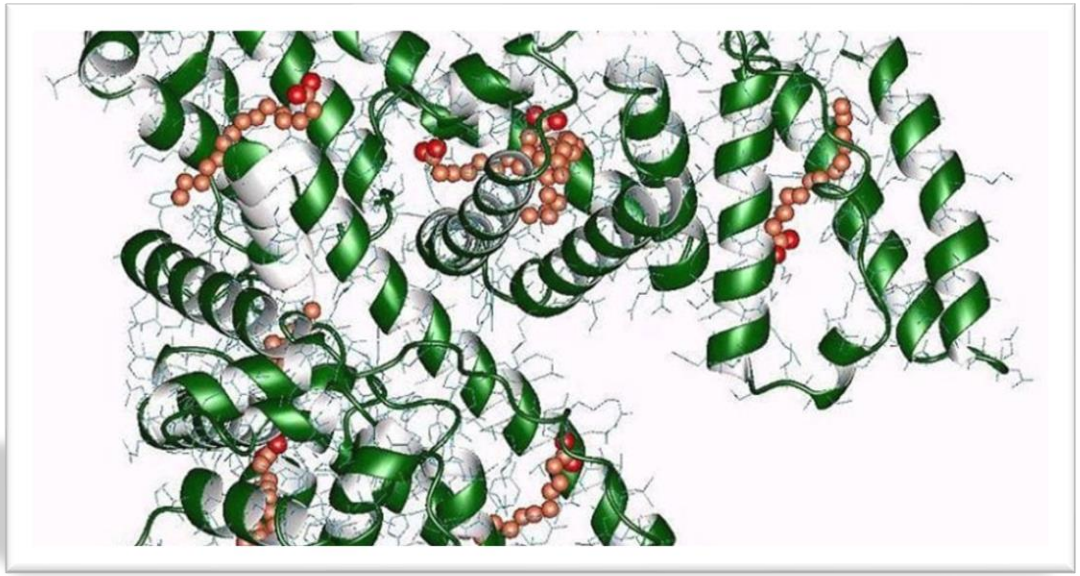
Introduction

Human serum albumin (HSA) is produced in the liver and is the most abundant protein present in blood plasma.



HSA

This protein acts in several physiological processes, including the regulation of **osmotic pressure, transmission, distribution and metabolism of several ligands,** and it is responsible for **regulating blood PH.**

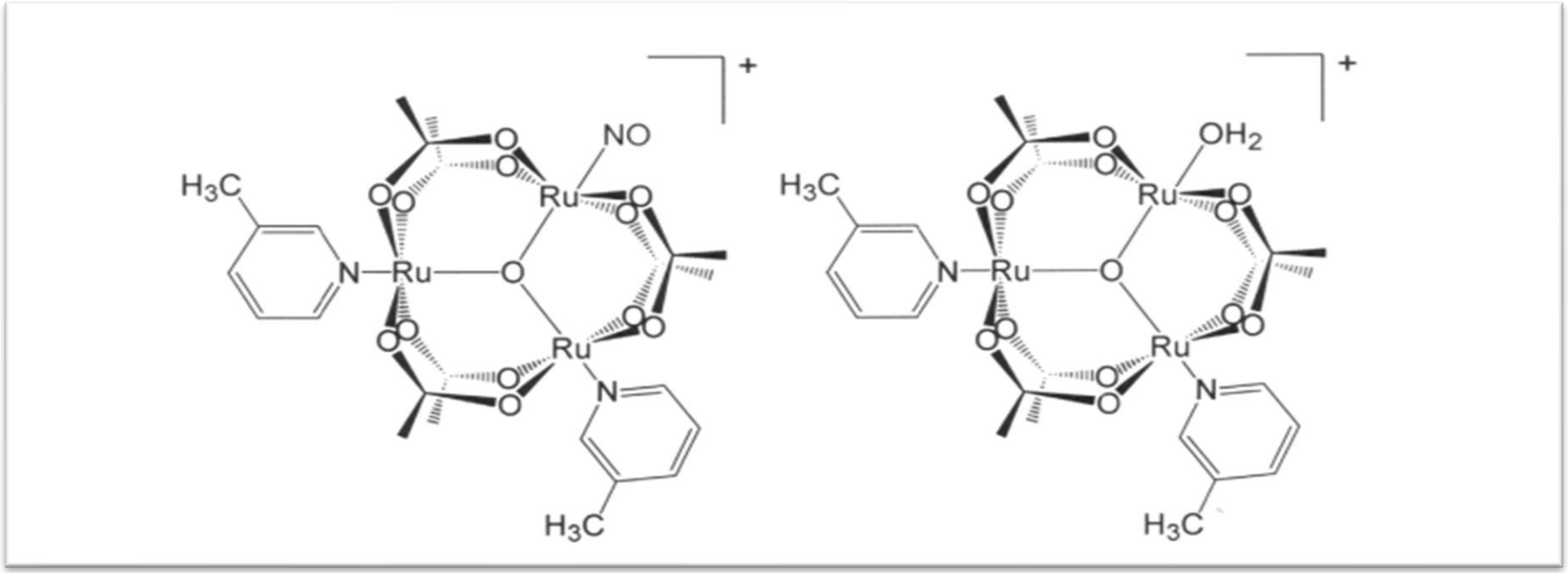
Binding affinity to HSA is highly related to the **distribution, free concentration** and **metabolism of ligands or drugs,** therefore it is of great importance to study these interactions.

Ruthenium

Since the discovery of the use of ruthenium compounds as metallodrugs, many studies are being developed to synthesize new compounds that may be used in the treatment of various diseases, including cancer.

The development of metallodrugs allows the replacement of currently used drugs, since the coordination to a metal center may increase the activity of a given drug, as well as reduce its side effects.

$[Ru_3O(CH_3COO)_6(L)_3]PF_6$



[Ru3O(CH3COO)6(3-pic)2(NO)]PF6

[Ru3O(CH3COO)6(3-pic)2(H2O)]PF6

Material and methods

- HSA was purchased from Sigma-Aldrich.
- The average molecular weight value of 66,500 g/mol was used in the preparation of protein solutions.
- Prior to each experiment, all solutions were freshly prepared in phosphate buffer (pH 7.4).
- Deionized water was used in the preparation of buffer solution.
- Stock solutions (2.31×10^{3} M) were prepared by dissolving an adequate mass of each complex in acetonitrile.

Kinetic experiments

❑ The absorption spectra were recorded in an Agilent 8453 spectrophotometer in the 190–1100 nm region, using a quartz cell with 1.0 cm optical path.

❑ A solution of the complex [Ru3O(CH3COO)6(3-pic)2(NO)]PF6 (2.31 $\times 10^3$ M) was prepared in acetonitrile and an aliquot of this solution was added to a buffer solution containing albumin (1 $\times 10^6$ M) in order to provide a concentration of 5.37 $\times 10^6$ M .

❑ The solution was incubated at 303 K in the presence of ambient light and the absorption spectra were recorded as a function of time.

The kinetic constant and the half life time

$$[A] = [A_0]e^{-kT}$$

$$t_{1/2} = \ln 2/k$$

Fluorescence spectroscopy

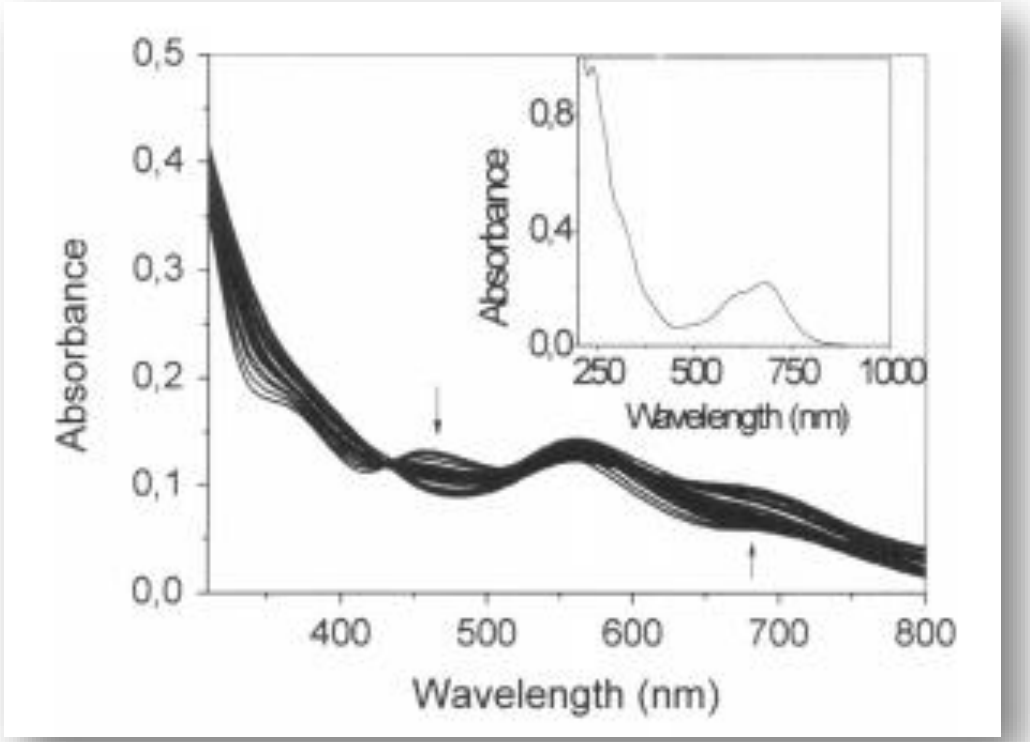
- Fluorescence spectra of the solution of HSA in the absence and presence of the complexes (0–18.5× 10^6 M) were recorded in a Shimadzu fluorescence spectrophotometer model RF-5301PC,using a quartz cell with 1.0 cm optical path.
- During a typical fluorescence measurement, 3.0 mL of HSA solution (1.0 × 10^6 M) was firstly added to a 1.0 cm quartz cell and the fluorescence spectrum was recorded.
- the complex solution aliquots were gradually added to the cell using a micropipette and the solution was incubated in the presence of ambient light for 5 min and for 120 min.

- The wavelength 280 nm was used for sample excitation (tryptophan excitation).
- The fluorescence spectrophotometer was set up with a slit width of 5 nm. The fluorescence emission spectra were measured at 298,303, and 308 K.

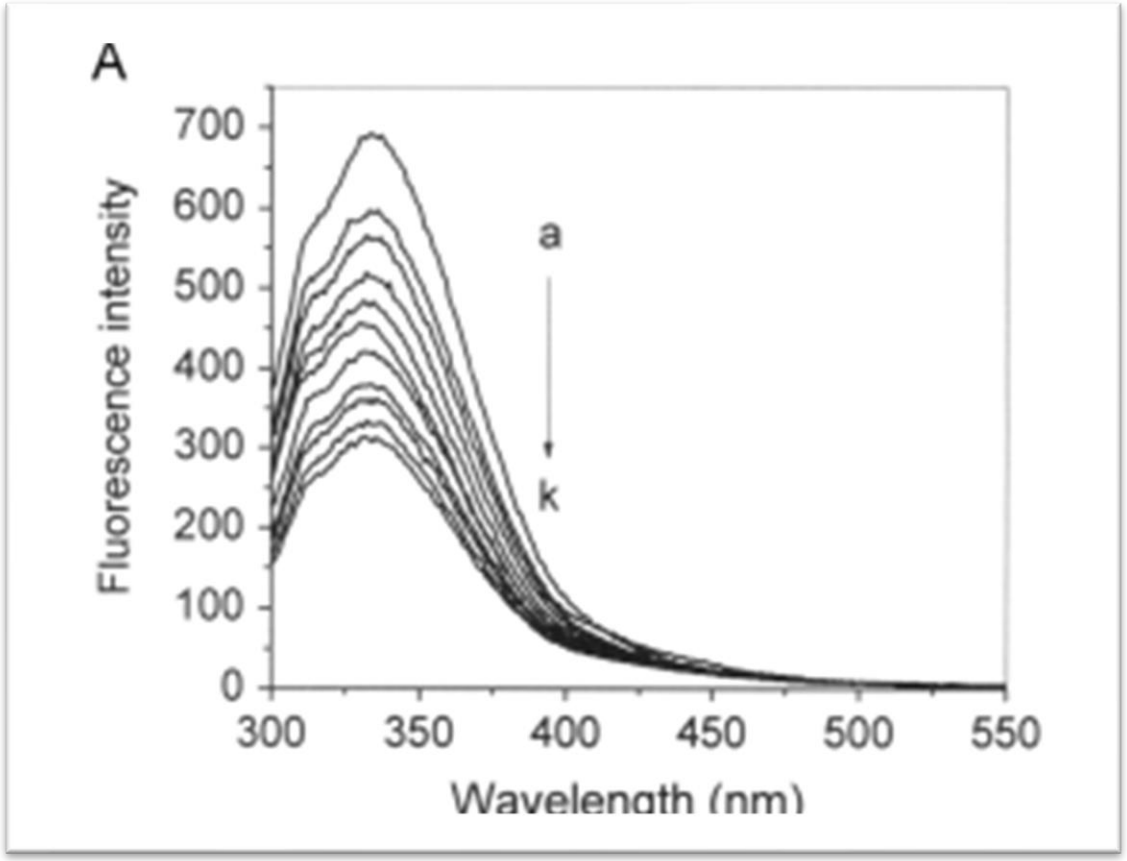
$$F_{corr} = F_{obs} e^{(A_{ex} + A_{em})/2}$$

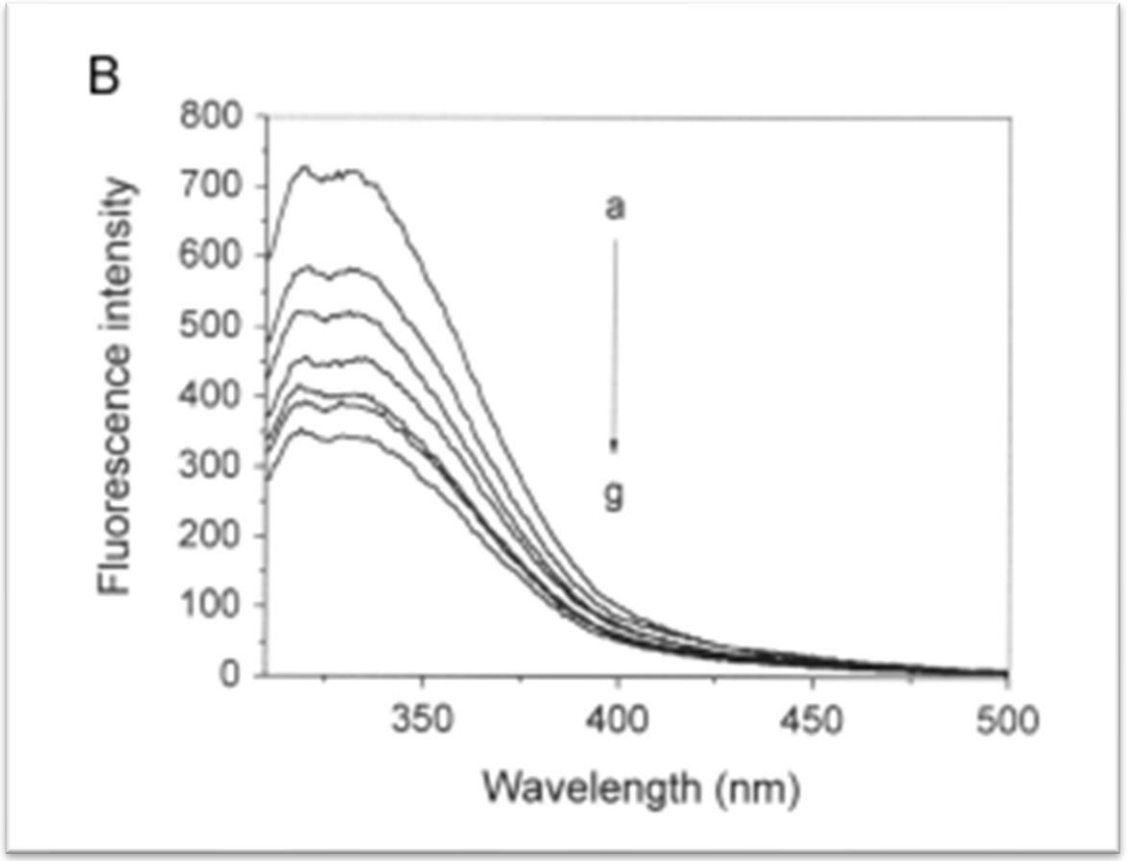
Results and discussion

Fluorescence-quenching of HSA by $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ and $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$

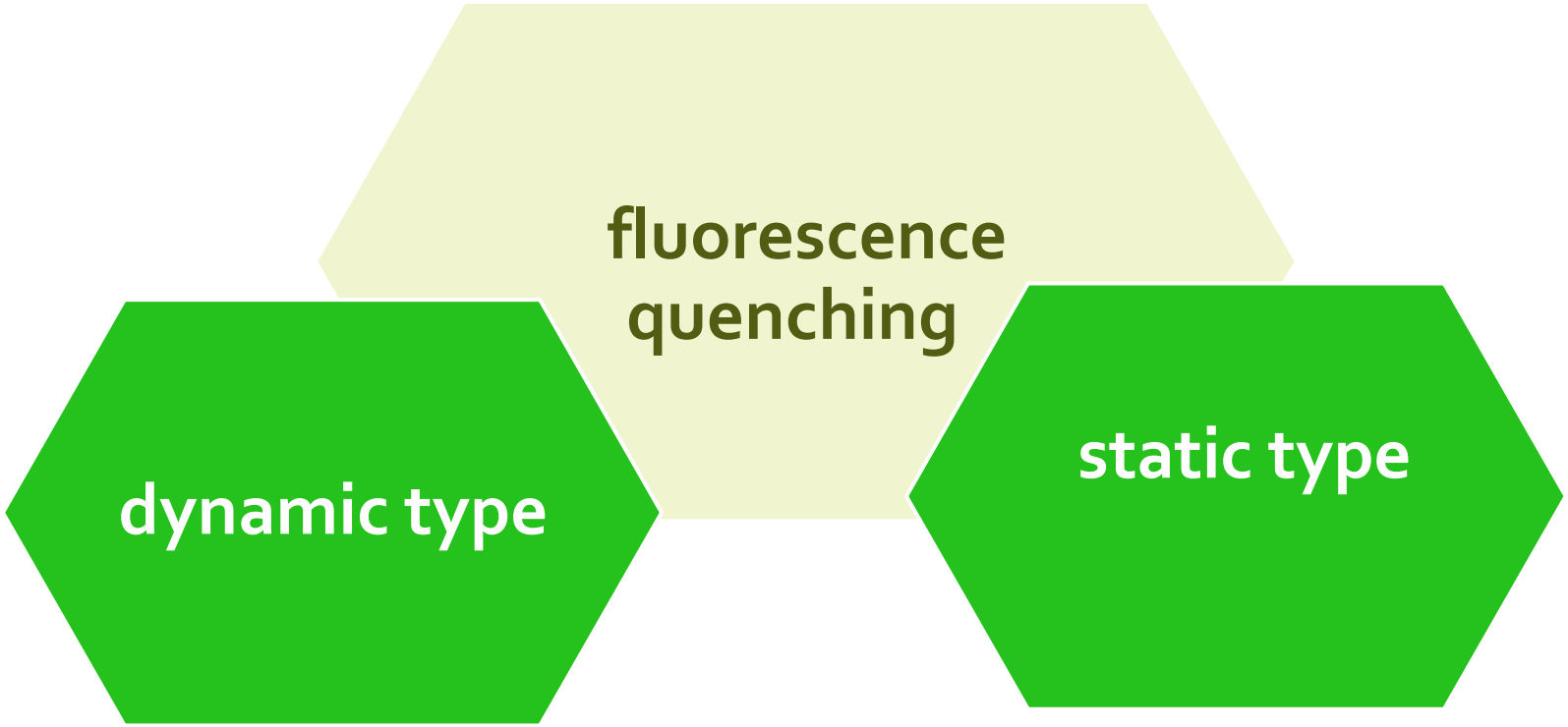


Fluorescence spectra of HSA in presence of [Ru3O(CH3COO)6(3-pic)2(NO)]PF6





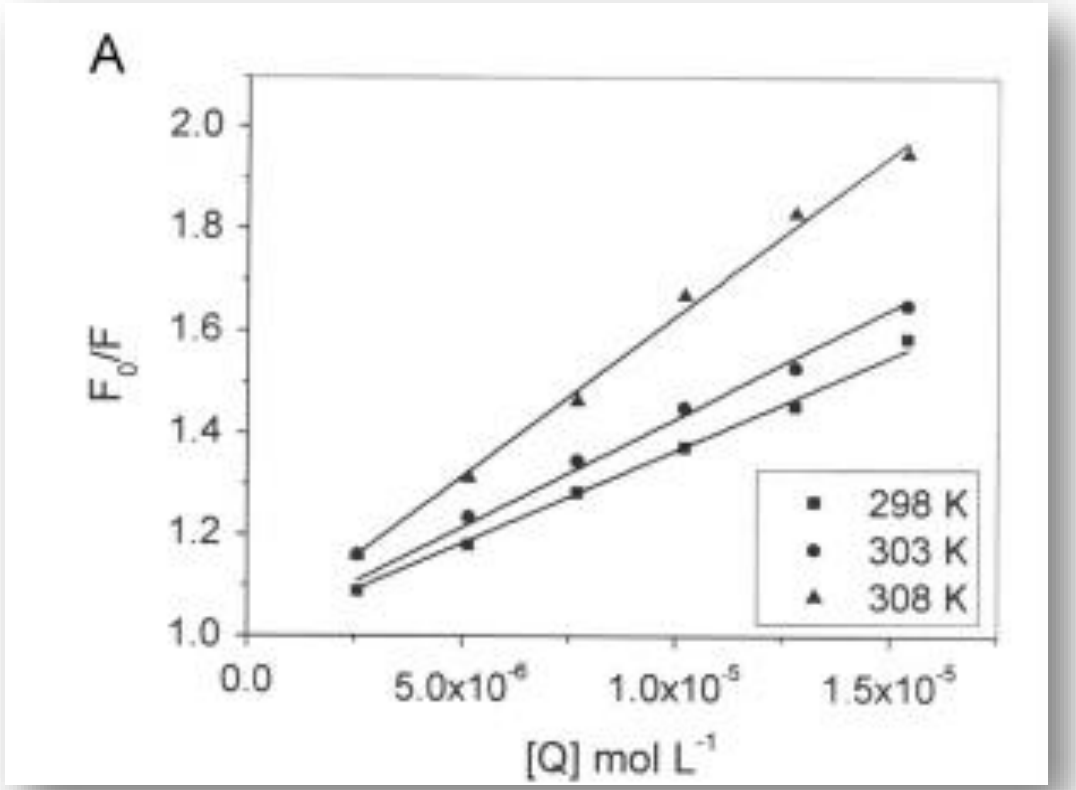
(A) for 5 min incubation; concentrations of quencher: 0; 2.57; 5.15; 7.70; 10.2; 12.8;15.4; 17.9; 20.5; 23.1 × 10^6 M , and (B) for 120 min incubation; concentrations of quencher 0; 3.08; 6.16; 9.24; 12.3; 15.4; 18.5 × 10^6 M. Both experiments were carried out at 303 K, λexc¼280 nm.


fluorescence quenching
dynamic type
static type

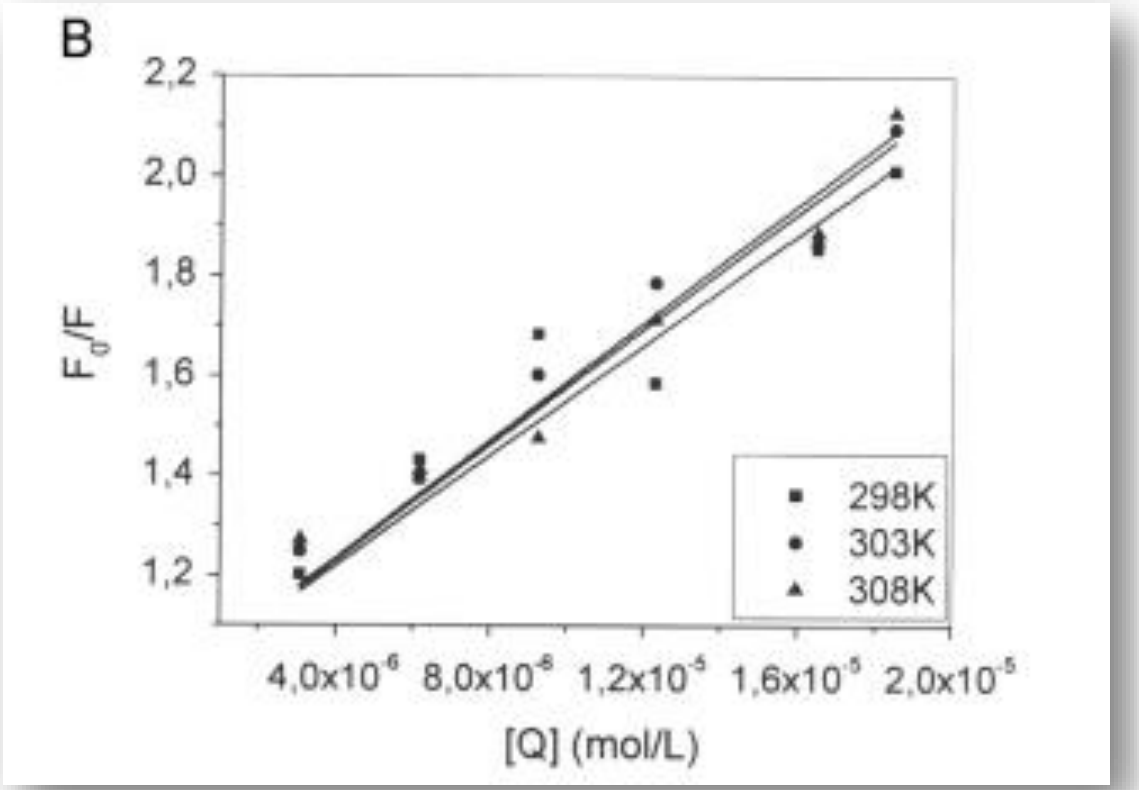
Stern–Volmer equation

$$F_0/F = K_{SV}[Q] + 1 = \tau_0 k_q [Q] + 1$$

Stern–Volmer plots



HAS-$[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$



HSA-$[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$

Stern–Volmer quenching constants for HSA-[Ru3O(CH3COO)6(3-pic)2(NO)]PF6 system and HSA-[Ru3O(CH3COO)6(3-pic)2(H2O)]PF6 system at different temperatures

HSA-$[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$				HSA-$[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$		
T (K)	K_{sv} (10^4 M^{-1})	k_q (10^{12} M^{-1} s^{-1})	R	K_{sv} (10^4 M^{-1})	k_q (10^{12} M^{-1} s^{-1})	R
298	3.67 ± 0.0538	3.67 ± 0.0538	0.999	4.67 ± 0.3381	4.67 ± 0.3381	0.996
303	4.29 ± 0.1094	4.29 ± 0.1094	0.996	5.18 ± 0.2335	5.18 ± 0.2335	0.998
308	6.30 ± 0.0895	6.30 ± 0.0895	0.998	5.31 ± 0.2272	5.31 ± 0.2272	0.998

Binding parameters

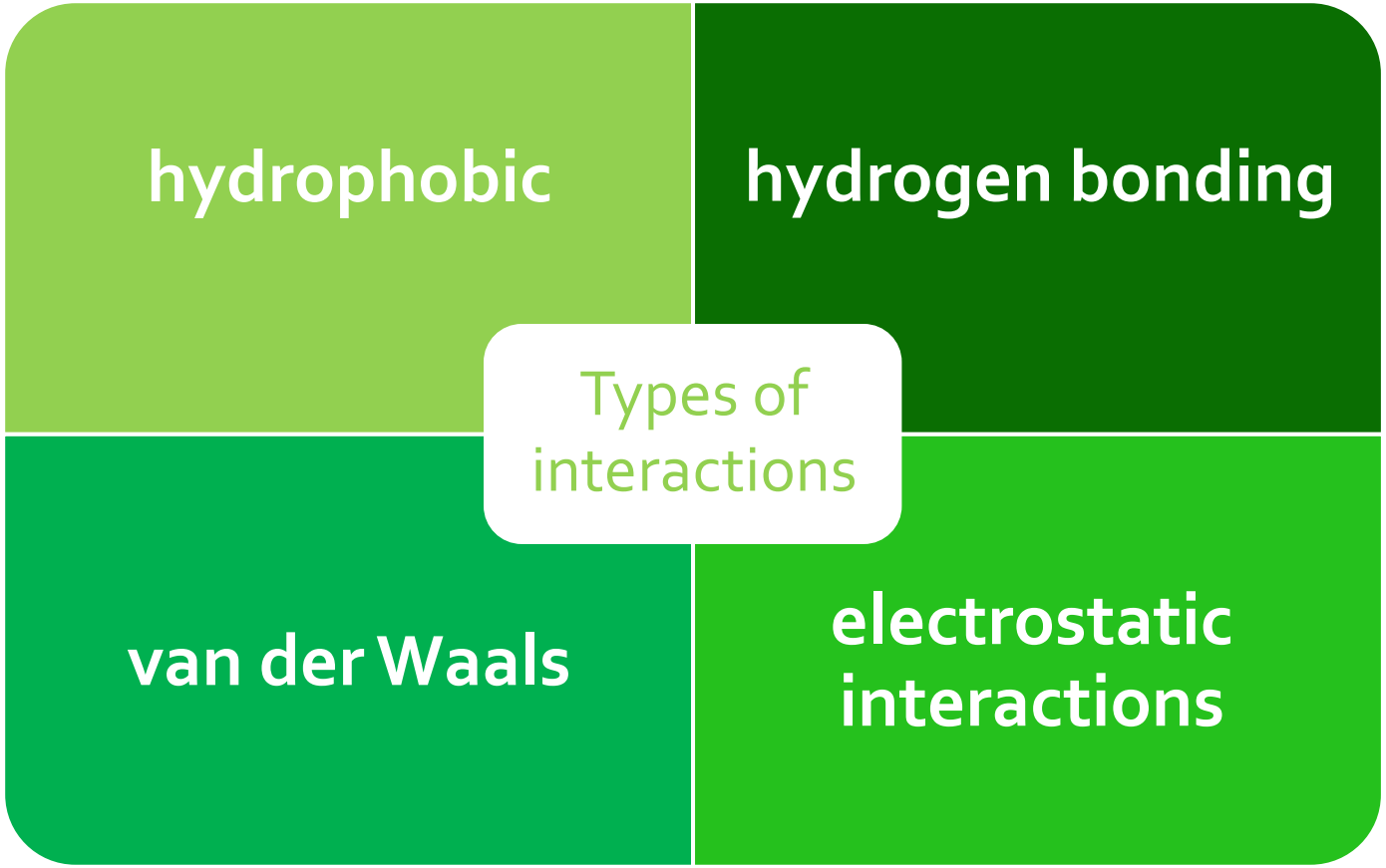
$$\log(F_0 - F)/F = \log K_b + n \log[Q]$$

	HSA-$[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$			HSA-$[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$		
T (K)	K_b (10^3 M^{-1})	n	R	K_b (10^3 M^{-1})	n	R
298	10.48 ± 0.085	0.95	0.999	12.88 ± 0.0327	0.86	0.995
303	15.55 ± 0.135	0.91	0.997	8.71 ± 0.0721	0.82	0.996
308	178.44 ± 0.137	1.08	0.997	4.78 ± 0.0443	0.77	0.991

The binding constant K, and the number of binding sites n of the HSA-[Ru3O(CH3COO)6(3-pic)2(NO)]PF6 and

HSA-[Ru3O(CH3COO)6(3-pic)2(H2O)]PF6 system at different temperatures.

Thermodynamic parameters and binding modes



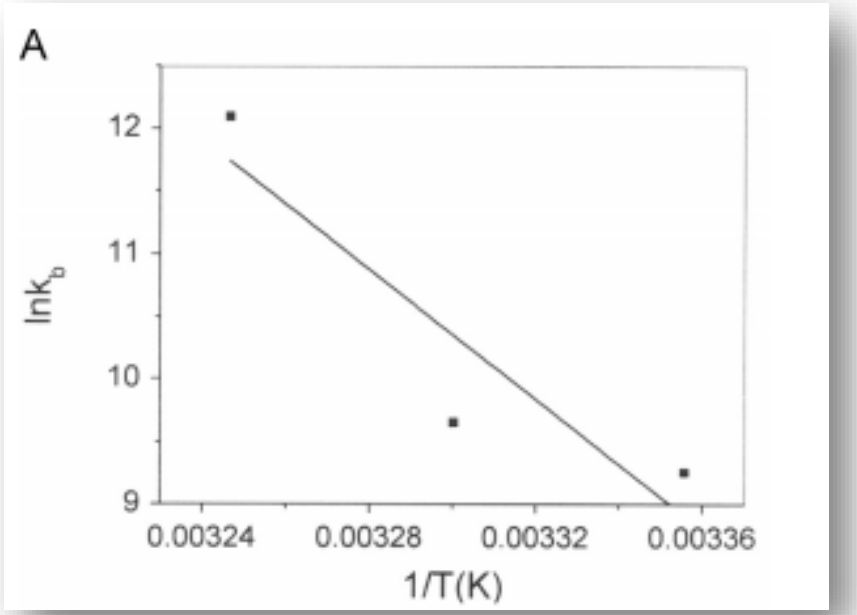
$$\ln K_b = -\Delta H/RT + \Delta S/R$$

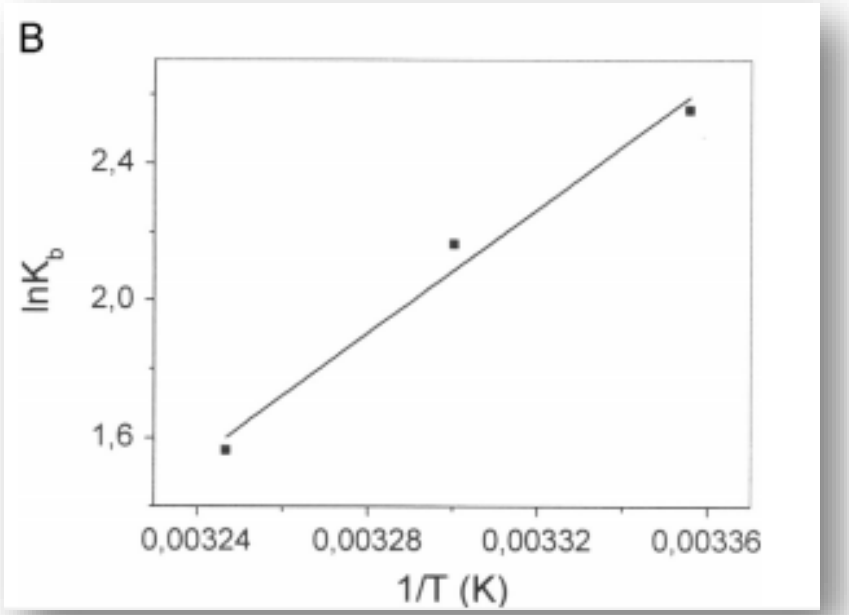
$$\Delta G = -RT \ln K_b$$

. Negative ΔH and ΔS values indicate the presence of hydrogen bonds and/or van der Waals forces

. Negative ΔH and positive ΔS values suggests the presence of electrostatic interactions

. Positive ΔH and ΔS values indicate the presence of hydrophobic interaction





	HSA-[$Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)$]$PF_6$			HSA-[$Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)$]$PF_6$		
T (K)	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
298			−22.94			−6.34
303	215	796	−24.32	−75.5	−231	−5.44
308			−30.96			−3.99

Conclusions

In this study we investigated the interaction between HSA and the $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ and $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$ complexes, by using fluorescence spectroscopy and the Stern–Volmer model. For both species studied, the fluorescence quenching was observed with increasing concentration of complexes, the reaction ratios with HSA are suggested to be 1:1 HSA: complex and the processes are spontaneous ($\Delta G<0$). It was observed that both dynamic and static quenching mechanisms are present and, despite the K_{sv} values increase with increasing temperature, our data suggests an important contribution of static quenching.

Complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ displays interaction with HSA by hydrophobic forces, likely because of the lipophilicity of the nitrosyl ligand, while complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$ is involved in the formation of hydrogen bonds with HSA, through its aquo ligand. It is worth mentioning that the results described in this study will be important to help define the distribution and transport of these candidates to metallodrugs in blood plasma. The high K_b value observed for the nitrosyl complex at 308 K suggests that this compound can be efficiently stored and transported in the body by HSA.

THANK YOU

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