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QUENCHING MECHANISM OF HUMAN SERUM ALBUMIN FLUORESCENCE BY GANGLERON

K. R. GRIGORYAN[*], A. G. GHAZARYAN[**]

Chair of Physical and Colloid Chemistry YSU, Armenia

Interaction between Gangleron (spasmolytic and anaesthetizing drug) and human serum albumin (HSA) was studied using UV/vis and fluorescence spectroscopy methods. The quenching mechanism of HSA fluorescence was discussed based on interaction studies carried out at different temperatures (298, 303 and 309 *K*). Stern-Volmer constant (K_{SV}), quenching rate constant (k_q) and activation energy of bimolecular quenching (E_a) were evaluated. UV/vis absorption spectra were used to confirm the quenching mechanism.

***Keywords*:** human serum albumin, Gangleron, fluorescence spectroscopy, UV/vis spectroscopy, dynamic quenching.

Introduction. Gangleron (*Gangleronum*) is an effective synthetic spasmolytic and anaesthetizing drug. It may be used for central nervous system as a tool in various stages of pathological conditions involving smooth muscle spasm and to prevent strokes [1, 2]. Distribution and metabolism of biologically active compounds such as metabolits, drugs and other organic compounds in the body are correlated with their affinities towards serum albumin [3]. Human serum albumin (HSA) the most important and abundant constituent of blood plasma, extensively and reversible binds and transports drugs to the targets. The mechanism of drug-protein interaction can affect the biological activity (efficiency and rate of delivery) of the drug. Thus, it is important to study the interaction between Gangleron and HSA to control the pharmacological response of the drug.

The methods of fluorescence spectroscopy combined with UV/vis and other spectroscopic methods have been widely used for monitoring molecular interactions involving proteins [4–7]. In this paper the interaction between HSA and Gangleron was studied using fluorescence and UV/vis spectroscopy methods.

Materials and Methods. HSA fatty acids free (< 0.005%) and was purchased from "Sigma Chemical Co" (USA). Concentration of HSA (0.4 *mg*/*ml*) was determined by spectrophotometric method at λ =280 *nm* and ε =36 600 $M^{-1}cm^{-1}$ [8]. 1.5% solution of Gangleron in 2 *ml* ampoules (Armenia) was used. UV/vis spectra were recorded on spectrophotometer Specord 50PC (Germany). A 1 *cm* quartz cell was used for measurements. Varian spectrofluorimeter was used to measure the

[*] E-mail: kara@ysu.am [**] E-mail: armghazaryan@ysu.am

fluorescence spectra. Fluorescence emission spectra were recorded at three different temperatures (298, 303 and 309 *K*) in the range of λ=310–450 *nm*. An excitation wavelength of 280 *nm* was chosen. The widths of the excitation and emission slits were set to 5 *nm*. The temperature of sample was kept by recycling water during the experiment. To construct the graphs Origin 8.0 software was used.

Results and Discussion. The intrinsic fluorescence emission of Trp 214 of HSA was observed at 340 *nm* on excitation at 280 *nm*. Gangleron has fluorescence emission at 260 *nm*. To obtain the proper fluorescence intensity values, fluorescence data must be corrected for the inner filter effect caused by attenuation of the excitation beam and emission signal because of absorption by quencher and fluorophore. These absorption events lead to artificial decrease in the fluorescence intensities; this effect is corrected using the equation

$$F_{corr} = F_{obs} \cdot 10^{\frac{OD_{ex}+OD_{em}}{2}}, \quad (1)$$

where F_{corr} and F_{obs} are the corrected and observed fluorescence intensities, respectively [9]; OD_{ex} is the absorbance value at the excitation wavelength and OD_{em} is the absorbance value at the emission wavelength. The observed spectra (curves 1–4) display a single broad band centered at around 340 *nm* (Fig. 1).

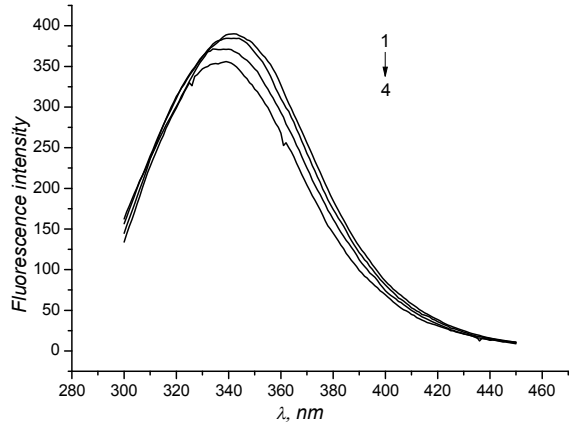

Fig. 1. Fluorescence spectra of HSA at the presence of Gangleron. [HSA] = 0.4 *mg*/*ml*: 1 – [Gang] = 0; 2 – [Gang] = $9.4 \cdot 10^{-6}$ *M*; 3 – [Gang] = $1.87 \cdot 10^{-5}$ *M*; 4 – [Gang] = $2.8 \cdot 10^{-5}$ *M*.

It can be clearly seen that the presence of Gangleron induces a decrease in fluorescence intensity with a shift to low wavelengths. To analyze the mechanism (static or dynamic) of fluorescence quenching the Stern-Volmer equation can be used [9]. Based on the calculated fluorescence data at three temperatures (298, 303 and 309 *K*), the plots F_0/F against $[Q]$ are constructed (Fig. 2):

$$F_0 / F = 1 + K_{SV}[Q] = 1 + k_q \tau_0 [Q], \quad (2)$$

where F_0 and F are the fluorescence intensities of HSA in the absence and presence of quencher respectively, $[Q]$ is the concentration of quencher and K_{SV} is the Stern-Volmer quenching constant, k_q is the quenching rate constant of biological macromolecule, τ_0 is the average lifetime of the molecule without any quencher and the fluorescence lifetime of the biopolymer is 10^{-8} *s* [10]. From the temperature

dependence of k_q using Arrhenius' equation (Eq. 3) the activation energy of bimolecular collision (E_a) has been determined. The values of K_{SV} and k_q at mentioned temperatures and E_a are listed in the Table. Linear form of Stern-Volmer dependence indicates the homogeneous quenching (dynamic or static) process in the system. It means the existence of one kind of fluorophores is equally accessible for the quencher. Dynamic and static quenching can be distinguished by their differing dependence on temperature and viscosity [9]. Dynamic quenching depends on diffusion. The bimolecular quenching constants are expected to increase with the increase of temperature. As a result of temperature increasing the diffusion coefficient is enlarging.

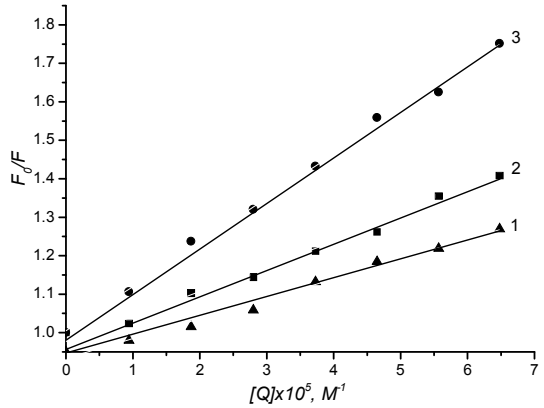

Fig. 2. Stern-Volmer dependence for HSA–Gangleron system. 1) $T = 298$ *K*; 2) 303 *K*; 3) 309 *K*.

The results showed (Fig. 2) that the values of K_{SV} increased with temperature increasing, which indicates that the quenching mechanism of HSA–Gangleron interaction was initiated by dynamic collision.

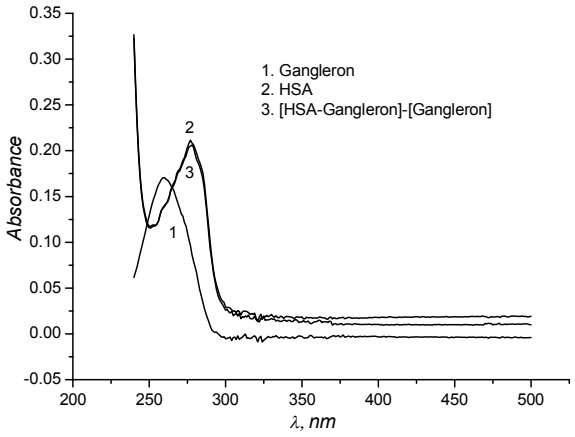

Fig. 3. UV/vis spectra of Gangleron (1), HSA (2) and HSA–Gangleron (3) system. [HSA] = 0.4 *mg*/*ml*, [Gang] = $9.4 \cdot 10^{-6}$ *M*.

UV/vis absorption spectroscopy method was used to confirm dynamic quenching mechanism. UV/vis spectra of HSA, Gangleron and HSA–Gangleron system are presented on Fig. 3. The absorption spectra of Gangleron are characterized by an absorption peak at 260 *nm* (curve 1). The absorption of HSA (curve 2) at 280 *nm* was almost unchanged after addition of Gangleron (curve 3), which confirms that the interaction between Gangleron and HSA is mainly a dynamic process.

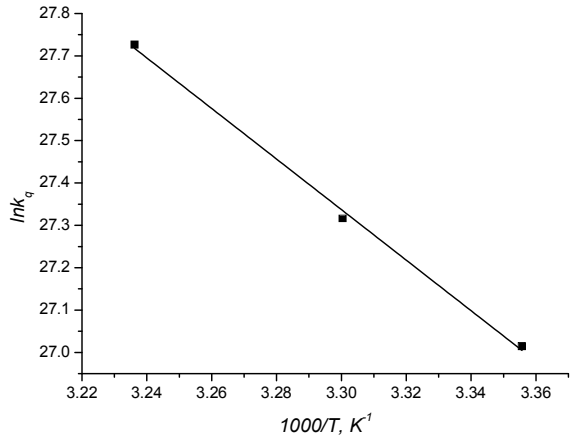

Fig. 4. Arrhenius' dependence for HSA–Gangleron system.

From temperature dependence of k_q the activation energy of bimolecular collision (E_a) has been determined due to Arrhenius' equation:

$$\ln k_q = -\frac{E_a}{RT} + \ln A, \tag{3}$$

where R is the Universal gas constant; A is the pre-exponential factor.

Stern-Volmer quenching constants and activation energy for the interaction of HSA and Gangleron

T, K	K_{SV}, $10^3 M^{-1}$	k_q, $10^{11} M^{-1} \cdot s^{-1}$	E_a, $kJmol^{-1}$
298	5.4	5.4	49.64
303	7.3	7.3	
309	11.0	11.0	

Conclusions. UV/vis and fluorescence spectroscopy methods were used to study the interaction between HSA and Gangleron. HSA fluorescence intensities were determined taking into account the inner filter effect detected in this system due to fluorescence properties of HSA and Gangleron. Dynamic quenching mechanism of HSA fluorescence was established based on the studies carried out at different temperatures (298, 303 and 309 *K*). This mechanism of quenching was confirmed by UV/vis absorption spectroscopy method. Stern-Volmer constant (K_{SV}), quenching rate constant (k_q) and activation energy of bimolecular quenching (E_a) were evaluated.

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