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Intermolecular interactions between natural humic substances and tricyclic antidepressants

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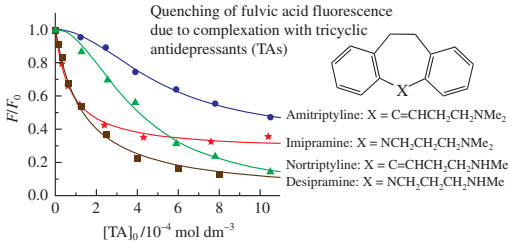
Intermolecular interactions between humic substances (HSs) and the tricyclic antidepressants (TAs) amitriptyline, nortriptyline, imipramine and desipramine were detected for the first time by fluorescence spectroscopy. Two simplest models assuming coordination of one or two of TA molecules at the binding centers of HSs were proposed to explain the photodegradation of TAs caused by sunlight photolysis of humic substances.



Humic substances (HSs) are naturally occurring photoactive components of surface waters.[1] These compounds generate active intermediates (triplet states, hydrated electrons and reactive oxygen species) under solar irradiation, which can react with dissolved organic pollutants initiating their degradation and mineralization.[1–4]

Tricyclic antidepressants (TAs) are used for the treatment of depression, anxiety, eating disorders, panic or phobic disorders and chronic pain syndromes. These compounds cannot be completely removed by common wastewater treatment, so they have been detected extensively in soils and surface waters.[5–7] Accumulation of TAs in the aquatic environment leads to detrimental effects on aquatic organisms due to a chronic exposure.[8]

It was found[9] that both amitriptyline and nortriptyline undergo photodegradation in the presence of fulvic acid. The photodegradation mechanism was proposed including electron transfer between triplet state of the fulvic acid and TAs similar to other amine drugs.[10,11] However, it is unclear whether electron transfer takes place on collision of both reagents (dynamical quenching) or in a complex between HS and TAs (static quenching). The latter occurred in the fulvic acid-assisted photodegradation of the amine drug propranolol.[11]

The aim of this work was to study the interaction of dibenzocycloheptadiene-type (amitriptyline and nortriptyline) and dibenzazepine-type (imipramine and desipramine) TAs with several HSs by optical spectroscopy and fluorescence techniques. Attention was focused on the mechanism of HS fluorescence quenching by TAs and a relationship between the structures of TAs and quenching ability.[†]

Figure 1 shows the absorption spectra of the test HSs and TAs. Only the HSs exhibited absorption at 365 nm, and none of the TAs demonstrated absorption at wavelengths longer than 330 nm. The excitation of HS alone led to fluorescence in a range of 400–750 nm with maxima at 480–540 nm and fluorescence quantum yields of 1.5–4% at neutral pH.[12] The kinetics of HS fluorescence is described by a set of at least three exponents

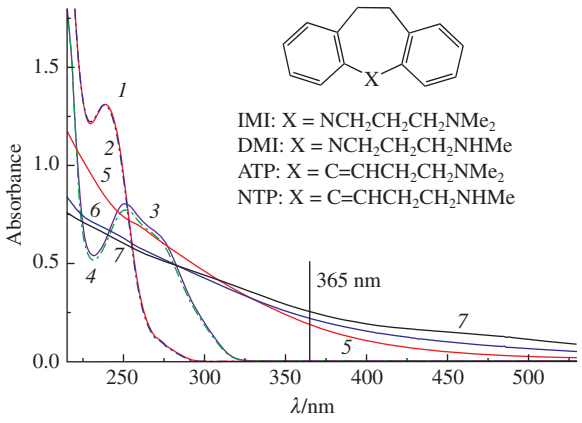


Figure 1 Absorption spectra of (*1*) ATP, (*2*) NTP, (*3*) IMI, (*4*) DMI, (*5*) NL, (*6*) HA, and (*7*) CS. The concentration of TAs was 10^{-4} mol dm^{-3}, and the concentrations of NL, HA and CS were 30, 20 and 20 mg dm^{-3}, respectively.

[†] Amitriptyline hydrochloride (ATP, 98%, Sigma), nortriptyline hydrochloride (NTP, 98%, Sigma), imipramine hydrochloride (IMI, ⩾99%, Sigma), desipramine hydrochloride (DMI, ⩾98%, Sigma), fulvic acid from Henan ChangSheng Corporation (CS), Nordic Lake fulvic acid (NL, IHSS reference 1R105F) and humic acid sodium salt (HA, Aldrich, CAS number 68131-04-4) were used without additional purification. The pH was controlled by an ANION-4100 ion meter (Infraspak-Analit, Russia) with an ESK-10614 combined electrode. Analytical grade NaOH or $HClO_4$ were used for pH adjustment. Deionized water was used for preparation of solutions.

The UV and fluorescence spectra were recorded using an Agilent 8453 spectrophotometer and a FLSP920 spectrofluorimeter (Edinburg Instruments), respectively. An Xe900 ozone free xenon lamp (λ_{ex} = 260, 280 and 365 nm) and an EPL-375 diode laser (Edinburg Instruments, λ_{ex} = 375 nm; pulse duration, 75 ps) were used as excitation sources, respectively.

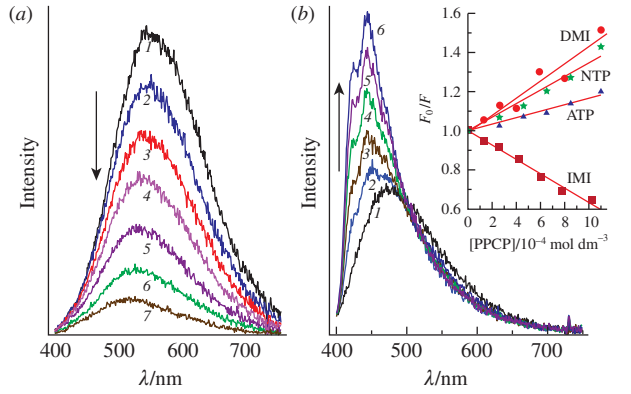


Figure 2 (*a*) Fluorescence spectra of CS in the presence of (*1*) 0, (*2*) 3.3, (*3*) 6.5, (*4*) 13, (*5*) 26, (*6*) 40 and (*7*) 80×10^{-5} mol dm^{-3} DMI; [CS] = = 20 mg dm^{-3}, pH 6.7, λ_{ex} = 365 nm. (*b*) Fluorescence spectra of NL in the presence of (*1*) 0, (*2*) 26, (*3*) 42, (*4*) 60, (*5*) 78 and (*6*) 102×10^{-5} mol dm^{-3} IMI; [NL] = 30 mg dm^{-3}, pH 6.9, λ_{ex} = 365 nm. Insert: dependence of the ratio F_0/F upon the concentration of TAs (points) and best linear fits by equation (1) (lines).

(Figure S1, Table S1, Online Supplementary Materials) due to the presence of a large number of chromophores with different lifetimes of excited states in the HSs. The average lifetimes of HS fluorescence $\tau_{av} = \Sigma\tau_i A_i$ were 1.2–2.1 ns (τ_i and A_i are the characteristic lifetimes and relative amplitudes of fluorescence signals, respectively). Among TAs, only IMI and DMI demonstrated fluorescence with a maximum at 400 nm (Figure S2, Online Supplementary Materials) upon excitation (λ_{ex} = 260 nm for NTP and ATP and 280 nm for DMI and IMI) in agreement with published data.[13]

Absorption spectroscopy did not reveal interactions between HSs and TAs up to a 10^{-3} mol dm^{-3} drug concentration. However, the fluorescence intensity of HSs in the presence of TAs changed (Figure 2 and S3). In all cases, there is a blue shift of the HS emission maximum with a simultaneous change in the integral fluorescence intensity F of HSs [Figure 2(*b*)]. The ratio F_0/F was a linear function of TA concentrations, which allowed us to determine Stern–Volmer parameters (K_{SV}) and the rate constants of dynamic quenching (k_q) by equation (1).

$$F_0/F = 1 + K_{SV}[TA] = 1 + k_q t_{av}[TA] \quad (1)$$

The calculated values of k_q (1.4×10^{11}–4.0×10^{12} dm^3 mol^{-1} s^{-1}, Table S2) were higher than a diffusion-controlled rate constant in water ($k_{diff} \approx 6\times10^9$ dm^3 mol^{-1} s^{-1}) by one or two orders of magnitude, but this is not physically relevant. An increase in the parameter F in the presence of TAs [Figure 2(*b*)] also allowed us to exclude the process of dynamical quenching, which could lead only to a decrease in F. A reasonable explanation of the observed phenomena is the formation of HS–TA complexes in aqueous solutions leading to a modification of the emission spectra of HSs. The absence of changes from the absorption spectra upon complexation indicates that the HS–TA complexes were mainly formed by weak (van der Waals and dispersion) interactions close to those observed in the host–guest chemistry of cyclodextrins (CDs) and similar macromolecules.[14,15]

As HSs are complex macromolecules with an irregular quantity of different binding sites, the compositions and stability constants of complexes between HSs and TAs are difficult to determine. In the first approximation, we assumed that each HS molecule contains a number of independent effective binding centers (which can contain several binding sites) with identical binding strength to coordinate a TA molecule. As the absorption coefficient at an excitation wavelength (365 nm) remained unchanged upon complexation, the amount of absorbed light was constant with varying TA concentration. Assuming that the initial concentration of TAs is much greater than the concentration of HS binding centers, we obtained equation (2).

$$F/F_0 = \frac{1 + (\phi_{HS-TA}/\phi_{HS})K_{st}[TA]_0}{1 + K_{st}[TA]_0}, \quad (2)$$

where ϕ_{HS} and ϕ_{HS-TA} are the quantum yields of fluorescence of HS and HS–TA complex, respectively; $[TA]_0$ is the initial concentration of a selected TA, and K_{st} is the apparent stability constant of a 1 : 1 complex. Figures 3 and S4 present the best fits (solid lines) of the experimental values of F/F_0 using equation (2) with parameters listed in Table 1. The obtained values of K_{st} (10^2–10^4 dm^3 mol^{-1}) are not so high as the typical values of K_{st} (10^3–10^5 dm^3 mol^{-1}) for complexes between aromatic molecules and CDs.[14] This is indirect evidence that weak van der Waals and dispersion interactions are the main driving force of complexation between TAs and HSs.

The simplest model of 1 : 1 complexes cannot adequately account for experimental results for HA–IMI and NL–IMI complexes. We modified our model by assuming two types of binding centers with different stability constants, however it did not explain an almost parabolic increase in the F/F_0 ratio in the HA–IMI and NL–IMI complexes (Figure 3 and 4S, Online Supplementary Materials). Therefore, we took into account the addition of one more TA

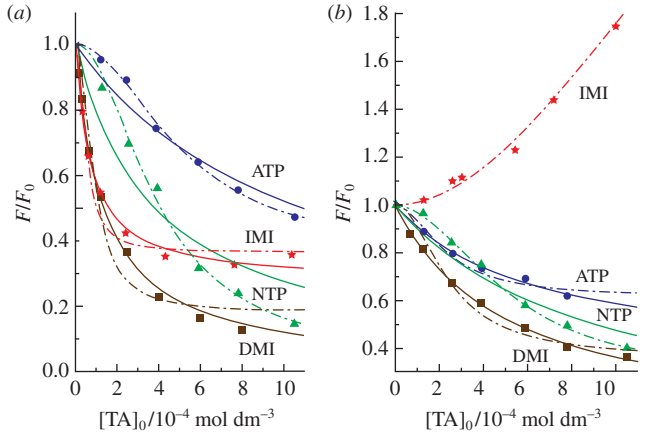


Figure 3 Dependence of the ratio F/F_0 upon the concentration of TAs (points) for (*a*) CS and (*b*) HA. Solid curves show the best fits using equation (2) with parameters listed in Table 1, and dotted curves show the best fits using equation (3) with parameters listed in Table 2.

Table 1 Apparent stability constants of 1 : 1 complexes between HSs and TAs (K_{st}) and ratios of quantum yields of HS fluorescence without TAs and in the complex with TAs (ϕ_{HS-TA}/ϕ_{HS}) calculated using equation (2).

Humic substance	Tricyclic antidepressant							
	IMI		DMI		ATP		NTP	
	K_{st}/dm^3 mol^{-1}	ϕ_{HS-TA}/ϕ_{HS}	K_{st}/dm^3 mol^{-1}	ϕ_{HS-TA}/ϕ_{HS}	K_{st}/dm^3 mol^{-1}	ϕ_{HS-TA}/ϕ_{HS}	K_{st}/dm^3 mol^{-1}	ϕ_{HS-TA}/ϕ_{HS}
CS	13600	0.27	7400	0.000	920	0.00	2600	0.00
HA	–	–	1950	0.038	1640	0.38	1100	0.00
NL	–	–	420	0.000	160	0.00	330	0.00

Table 2 Apparent stability constants of 1 : 2 complexes between HSs and TAs (K_{st}) and ratios of quantum yields of HS fluorescence without TAs and in the complex with TAs ($\phi_{HS–TA}/\phi_{HS}$) calculated using equation (3).

Humic substance	Tricyclic antidepressant							
	IMI		DMI		ATP		NTP	
	K_{st}/dm^6 mol^{-2}	$\phi_{HS–TA}/\phi_{HS}$	K_{st}/dm^6 mol^{-2}	$\phi_{HS–TA}/\phi_{HS}$	K_{st}/dm^6 mol^{-2}	$\phi_{HS–TA}/\phi_{HS}$	K_{st}/dm^6 mol^{-2}	$\phi_{HS–TA}/\phi_{HS}$
CS	3.4×10^8	0.37	1.2×10^8	0.18	3.9×10^6	0.36	6.5×10^6	0.035
HA	3.0×10^5	4.30	1.6×10^7	0.36	1.7×10^7	0.61	3.6×10^6	0.260
NL	1.4×10^6	1.90	3.4×10^6	0.61	1.2×10^6	0.72	1.5×10^6	0.550

molecule with the formation of a 1 : 2 complex with two TA molecules bound at the same effective binding center of HS molecule and obtained equation (3).

$$F/F_0 = \frac{1 + (\phi_{HS-TA}/\phi_{HS})K_{st}[TA]_0^2}{1 + K_{st}[TA]_0^2} \quad (3)$$

Figure 3 shows the best fits (dotted lines) of experimental values of F/F_0 using equation (3) with parameters listed in Table 2. An analysis of information in Tables 1 and 2 led us to the following preliminary conclusions. (i) Interaction of ATP, NTP and IMI with HSs is better described by a 1 : 2 complexation model; a 1 : 1 model is preferable for DMI in the test range of TA concentrations. (ii) The apparent stability constants of 1 : 1 complexes are small (10^2–10^4 dm^3 mol^{-1}) to give indirect evidence that weak van der Waals and dispersion interactions are the main driving force of complexation between TAs and HSs. (iii) The values of $\phi_{HS–TA}$ are lower than those of ϕ_{HS} with the exception of HA–IMI and NL–IMI complexes. (iv) Dibenzazepine-type TAs (IMI, DMI) form relatively more stable complexes with HSs in comparison with those of dibenzocycloheptadiene-type TAs (ATP, NTP).

These preliminary conclusions are important for the explanation of the observed photodegradation of TAs in the presence of HSs.[9] Further steady state and laser flash photolysis experiments are required for the determination of the absolute efficiency of TA degradation in the presence of HSs and for the detection of the quenching of HS triplet states by TAs.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.07.024.

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