

Physico-chemical properties of β -diketone phosphorus-containing dendrimers

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Protolytic, absorbance and fluorescence properties of β -diketone phosphorus-containing dendrimers based on cyclotriphosphazene core were studied. Dendrimer solutions in acetone are characterized by intense absorbance band at ≈ 340 nm ($\epsilon_{340} \approx 8.5 \cdot 10^4 \text{L/mol}\cdot\text{cm}$) and fluorescence band with maximum at 440 nm. Position of these maxima does not change in various solvents, unlike the bands of monomer β -diketone. It was found that dendrimer aggregation is accompanied by appearance of a second absorbance band $\epsilon_{400} \approx 4.5 \cdot 10^3 \text{L/mol}\cdot\text{cm}$, by red shift of emission spectra $\Delta\lambda \approx 10$ nm and also by decrease in surface tension of acetone solution. Ability of dendrimer aggregates to solubilize organic substrates was observed with the fluorescent indicator acridine.

Introduction

Dendrimers [1] are branched cascade macromolecules, which have globular structure with multivalent central nucleus, intermediate branches and terminal functional groups on the surface. Cavities of the nanometric size can also be distinguished between the branches of dendrimer molecules [1-5]. The structural

the “host-guest” mechanisms provides a prospective of their application as nanocarriers for transmembrane transfer of pharmaceutical substances [8,9]. In addition, dendrimers have been used as luminescence nanomarkers for detection of cancer [10,11].

Researchers have paid particular attention to the study and development of physical and

easy functionalization of the surface of these molecules and for elaboration of new specific reagents for selective extraction of metal ions, high sensitive fluorescence sondes [6] and effective catalytic nanosystems [7]. Ability of dendrimers to interact with organic substances by

new classes of phosphorus-based dendrimers with cyclotriphosphazene core [12], likely due to the success of these dendrimers in such fields as nanomedicine, biology and chemical engineering [13]. β -Diketones on the other hand are well known by their own fluorescence and high ability

for complexation with metal ions [14,15]. Therefore, functionalization of dendrimer surface by β -diketone groups constitutes as rational way for developing new nanomarkers and nanoreactors based on them. Present study focuses on protolytic, optical and aggregation properties of β -diketone phosphorus-containing dendrimers based on cyclotriphosphazene core, which have been first synthesized by authors [16].

Experimental part

The physico-chemical properties of phosphorus-containing dendrimers were studied using as example the terminal β -diketone (Md), monomer branch (Bd), and dendrimers of first (Gd1) and fourth (Gd4) generations. The structures of these substances are shown on Fig. 1. Starting solutions of substances were prepared by dissolving their weighted portions in organic solvents (acetone, acetonitrile, DMSO, toluene and chloroform). All solvents were spectroscopic grade by «Merck».



Figure 1. Structures of terminal β -diketone (Md), monomer branch (Bd), dendrimers of first (Gd1) and fourth generation (Gd4)

The acidity of the solutions was controlled by a pH meter (pH-340) with a glass electrode ESL-43-07 (Belarus). The formal dissociation constants of Bd and Md (pK^f) were determined in acetone solution due to limited

solubility of these compounds in water. The aliquot parts of the compounds solutions which contained a twofold excess of hydrochloric acid were titrated with standardized base solution at constant temperature (23°C). Then, a threefold excess of sodium hydroxide was added to the same portion of Md and Bd solutions and the obtained mixtures were titrated with hydrochloric acid. The content of the analyzed compounds in the solution was tested by the "Put-Found" method. Based on obtained pH-metric curves (an example is shown in supporting information S1) the pK^f values were calculated using the Hyperquad-2000 program.

Absorption spectra were measured using a spectrophotometer UV/Vis 2800 (Unico, USA). The absorbance of Gd4 acetone solutions was registered in cuvettes with $l = 1$ cm in the range 200-800 nm using acetone as the reference solution. The interaction of dendrimers with organic substrates was studied using the example of fluorescent indicator acridine (Acr). Working solution of Acr was prepared by dissolving its exact mass in bidistilled water (pH= 3). The changes in the absorption spectra of Acr after adding of Gd4 were studied in water-acetone solution ($\omega_{\text{water}}:\omega_{\text{acetone}} = 20:80$, pH = 3).

The excitation and emission spectra of Gd4, Bd and Md in different solvents were measured with a LS55 spectrofluorimeter (Perkin-Elmer, UK) in the optimal conditions at maximum wavelengths for each investigated system. The spectra were recorded in the range

between 200 and 800 nm by taking away the background of solvent. The data collection frequency was 0.5 nm. The thickness of the cuvette was 1 cm, excitation and emission slit width were 10 and 20 nm, respectively. The fluorescence maxima agreed within 0.5–1 nm in the independent parallel measurements.

Changes in the dendrimer structure in the presence of acids and oxidizing additives were controlled by ^{31}P -NMR and keto–enol tautomerism was studied by ^1H -NMR using NMR spectrometer (Bruker AV 300). Surface tension (σ) of aqueous acetone solution of Gd4 was measured by capillary raising method according to literature [17].

Results and discussion

Keto–enol tautomerism. The percentage of keto and enol forms in the molecular structure of phosphorous-containing dendrimer with terminal β -diketones groups of the first generation Gd1 and its terminal β -diketone Md was calculated in different solvents, such as acetone and chloroform on the basis of obtained NMR spectra, Table 1. It was established that in the polar acetone solution the content of keto form in diketone fragment of molecule of dendrimer is higher than in less polar chloroform.

diketone groups of the first generation Gd1 and its terminal β -diketone Md

Studied molecule	Solvent	Percentage of keto form, %	Percentage of enol form, %
Gd1	acetone	7.4	92.6
	chloroform	4.3	95.7
Md	acetone	7.8	92.2
	chloroform	4.8	95.2

Protolytic properties. Using the example of monomer branch Bd it was shown that protonation of dendrimer molecule occurs on the azomethine nitrogen atom at pH 7.5 (pK_1^f), and protolytic dissociation of enol groups of dendrimer molecules in acetone solution occurs at pH higher than 9.5 (pK_2^f). Experimentally obtained value of formal dissociation constant of enol group of Md and pure acetone is equal to 9.1 and 10.2, respectively.

On the basis of data obtained the distribution diagram of protolytic forms of Bd was calculated, Fig. 2.

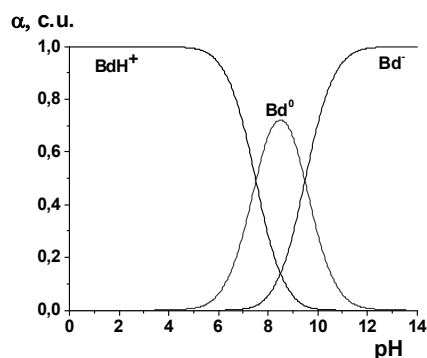
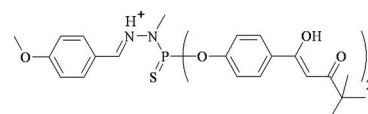


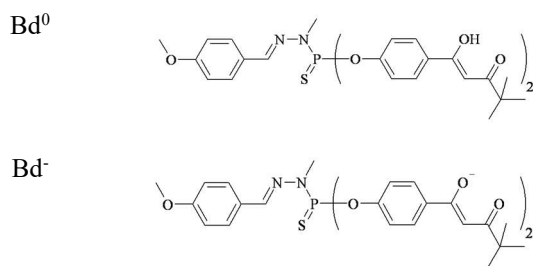
Figure 2. Distribution diagram of protolytic forms of Bd.

Table 1.

Content of keto and enol forms in the molecular structure of phosphorous-containing dendrimer with terminal β -

where
BdH⁺





Absorption and fluorescence characteristics.

Excitation and emission spectra of Gd1 in different solvents are characterized by intense bands at 360 nm and 440 nm, respectively. Position of these maxima does not change in various solvents (Fig. 3) and is almost independent on dendrimer generation (look at S2).

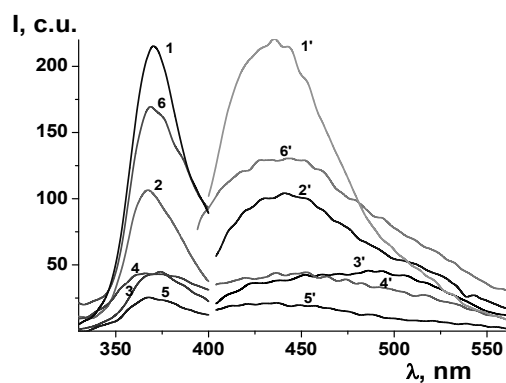


Figure 3. Excitation (1-5) and emission (1'-4') spectra of Gd1 in DMSO (1, 1'), acetone (2, 2'), toluene (3, 3'), acetonitrile (4, 4') chloroform (5, 5') and in water-acetone mixture (20:80) (6, 6'). $C_{Gd1} = 10^{-5}M$.

The emission intensity rises as basic properties of solvent increases (donor number for acetonitrile is equal to 14.1, acetone – 17.0, DMSO – 28.9), Fig. 2. This can be caused by increase of molecular electron density after abstraction of diketone hydrogen atom by a solvent. The same solvatochromic effect was observed in the spectra of monomer branch of dendrimer Bd (look at S3). Specifically, no correlation was found between emission spectra intensity and dielectric constants or dipolar

moment of the solvents (look at S4). Studied dendrimers are characterized by highest solubility in acetone and their intensity of fluorescence spectra is also high in this solvent. Therefore, acetone was chosen as solvent for further investigations.

Interesting, that for β -diketone Md the change of the solvent nature leads to a change not only in the spectra intensities, but also to the excitation and fluorescence maxima shifts, Fig. 4. Therefore, the solvatochromic effects of solvents on spectra of monomer β -diketone Md and dendritic molecules (Gd1, Bd) are different.

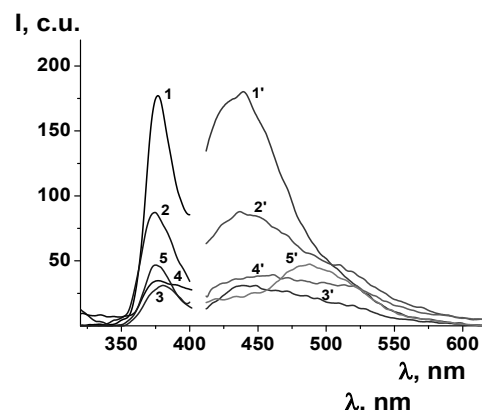


Figure 4. Excitation (1-5) and emission (1'-4') spectra of Md in DMSO (1, 1'), acetone (2, 2'), toluene (3, 3'), acetonitrile (4, 4') and chloroform (5, 5'). $C_{Md} = 10^{-3}M$.

Absorption characteristics of dendrimers were studied on Gd4. It was found that absorption spectra of acetone solution of Gd4 are characterized by an intense band at 340 nm, with molar absorption coefficient $\epsilon_{340} \approx 8.5 \cdot 10^4 L/mol \cdot cm$. The difference in the position of the maxima of the absorption and excitation bands can be caused by the high ability of dendrimers to self-absorption. It is noteworthy that at concentration of Gd4 more than 30 μM

(higher than critical concentration of aggregation - CCA) the appearance of a second band at 400 nm ($\epsilon_{400} \approx 4.5 \cdot 10^3 \text{ L/mol}\cdot\text{cm}$) was observed (look at S5). This effect can be explained by intermolecular interaction of dendrimers and formation of their aggregates.

Self-aggregation ability. Decrease in surface tension of aqueous-acetone solution (20:80) at concentration of Gd4 \geq CCA (in interval 20-50 μM , Fig. 5) proves the β -diketone phosphorus-containing dendrimer ability for self-aggregation. Such dendrimers are poorly soluble compounds, and in aqueous-acetone solution at $C_{\text{Gd4}} \approx 100 \mu\text{M}$ they precipitate.

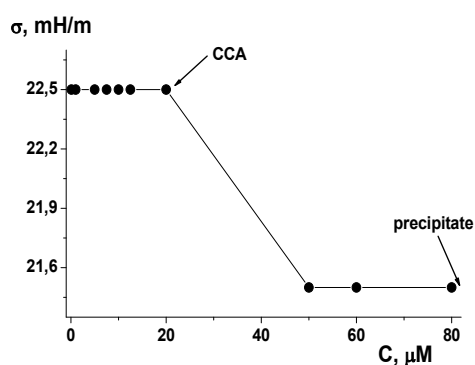


Figure 5. Dependence of the surface tension of Gd4 aqueous-acetone solution on its concentration. ($\omega_{\text{H}_2\text{O}}:\omega_{\text{CH}_3\text{CO}}$ = 20:80), $t = 26 \text{ }^\circ\text{C}$.

Acridine molecule was chosen as indicator of dendrimer molecule-organic substrate interaction due to its small size (effective size of acridine molecule is around 1.1 nm [18]), which allow Acr to penetrate into the cavities between the dendrimer branches. Acridine high fluorescence and absorbance intensities (ϵ_{440} in benzene solution $> 2 \cdot 10^4 \text{ L/mol}\cdot\text{cm}$ [19]) allows for use of highly sensitive spectrophotometric and fluorescent methods of

analysis to study the “host-guest” mechanism of interaction of dendrimers with organic substrates. Selected experimental conditions (pH = 3) excluded electrostatic attraction between acridine and dendrimer terminal groups. At such pH value acridine and dendrimer molecules are protonated and the interaction between these molecules can occur due to the formation of hydrogen bonds and hydrophobic effect.

The deviation of the absorption spectrum of the mixture of Acr and Gd4 from the additivity law proves the interaction of components in the system, Fig. 6a. Gradual addition of Gd4 to Acr aqueous-acetone solution (20:80) allowed us to establish two different mechanisms of interaction of dendrimer molecules and its aggregates with the organic molecules.

It was found, that the dendrimer-acridine associates with highest optical density are formed in the system at ratio $C_{\text{Gd4}}:C_{\text{Acr}} = 1:96$, Fig. 6b. From our point of view, this effect can be explained by Acr molecule connection to each β -diketonate terminal group due to intermolecular hydrogen bonds formation. Further increase in the concentration of Gd4 promotes the decreasing of optical density of the solution probably due to reducing the number of attached molecules of Acr to dendrimer molecule.

a

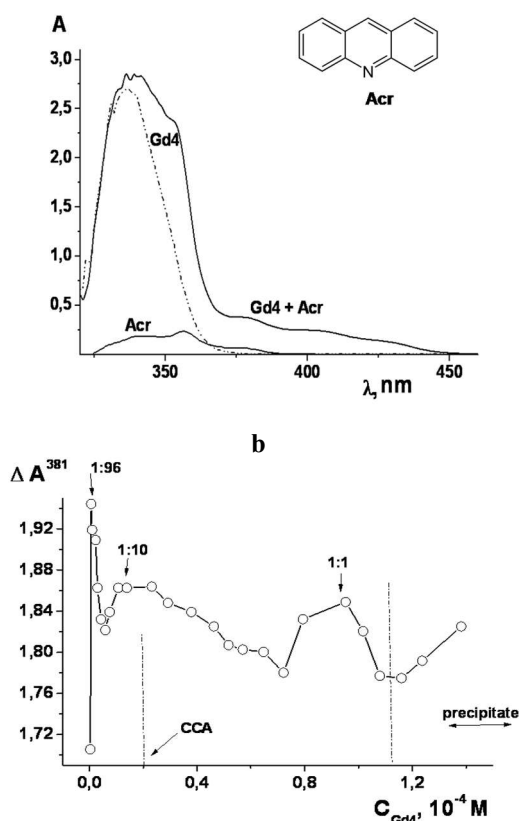


Figure 6. Changes of absorbance of acridine aqueous-acetone solution at the presence of Gd4. $C_{Acr} = 1 \cdot 10^{-4}$ M (a, b), $C_{Gd4} = 1 \cdot 10^{-5}$ M (a), pH=3. Reference solution – water-acetone mixture ($\omega_{H_2O}:\omega_{(CH_3)_2O} = 20:80$, pH = 3).

On the other hand, when the dendrimer concentration is greater than the CCA, the Acr is solubilized by Gd4 aggregates and the optical density of solutions gradually decreases. At equimolar ratios of the concentrations of acridine and dendrimer, a sharp change in the character of the absorption spectrum of the solution is observed, which is presumably due to the formation of the mixed associates $C_{Gd4}:C_{Acr} = 1:1$. With a further increase in the Gd4 concentration, a precipitate forms in the solution, Fig. 6b.

Observed changes of fluorescence of acridine molecule have also presented evidences of the ability of dendrimer aggregates to solubilize

the organic substrates. Thus, Fig. 7 shows that interaction between acridine molecules and dendrimers aggregates is accompanied by red shift of excitation band and blue shift of emission band relative to pure Acr spectra. The maxima of the absorption and fluorescence bands of Gd4-Acr mixture coincide with the maxima of the corresponding bands of the pure dendrimer. This effect can occur due to complete solubilization of Acr molecules by Gd4 aggregates.

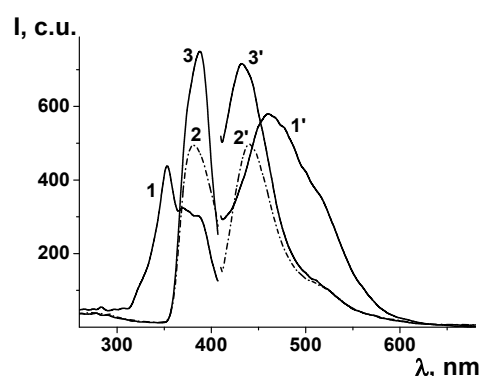


Figure 7. Excitation (1-3) and fluorescence (1'-3') spectra of acridine (1, 1'), Gd4 aggregates (2, 2') and their mixture (3, 3') in acetone. $C_{Gd4} = 5 \cdot 10^{-5}$ M (b); $C_{Acr} = 1,6 \cdot 10^{-5}$ M, $\lambda_{ex} = 380$ nm, $\lambda_{em} = 440$ nm.

Increase of fluorescence intensity of organic substrates at the presence of Gd4 aggregates proves the prospective of application of such dendrimers for elaboration of effective fluorescent markers.

Conclusions

The evaluated values of the acid-base dissociation constants and molar absorption coefficients of molecules of β -diketone phosphorus-containing dendrimers based on the cyclotriphosphazene core are found useful in

further studies of the coordination of metal ions to the functional groups of this class of dendrimers. The ability of such dendrimers for self-aggregation and solubilization of organic substrates can be used for development of new effective organized nanosystems for targeted drug delivery.

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