

Aggregation state of amphiphilic cationic tetraphenylporphyrin derivatives in aqueous microheterogeneous systems

M. A. Gradova,^{a*} K. A. Zhdanova,^b N. A. Bragina,^b A. V. Lobanov,^a and M. Ya. Mel'nikov^c

^aN. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences,
4 ul. Kosygina, 119991 Moscow, Russian Federation.

Fax: +7 (495) 938 2484. E-mail: m.a.gradova@gmail.com

^bM. V. Lomonosov Moscow State University of Fine Chemical Technologies,
86 prosp. Vernadskogo, 119571 Moscow, Russian Federation.

Fax: +7 (495) 434 8711. E-mail: n.bragina@mail.ru

^cM. V. Lomonosov Moscow State University,
1/3 Leninskie Gory, 119991 Moscow, Russian Federation.

Fax: +7 (495) 932 8846. E-mail: melnikov46@mail.ru

The conditions for the formation of ionic associates and *J*- and *H*-aggregates from amphiphilic cationic tetraphenylporphyrin derivatives in aqueous solutions of anionic surfactants and polyelectrolytes in a wide range of concentrations and pH are considered. The aggregation behaviors of tetraphenylporphyrin and its cationic derivatives in aqueous microheterogeneous systems are compared illustrating the influence of the porphyrin molecular structure on its ability to form supramolecular aggregates of a certain structure. The mechanism of the promoting action of premicellar solutions of anionic surfactants on the formation of *J*-aggregates of the diprotonated form of cationic porphyrins was proposed. The acid-base properties of a number of new synthetic *meso*-aryl-substituted porphyrins in aqueous microheterogeneous systems were characterized.

Key words: tetraphenylporphyrin, cationic porphyrins, *J*-aggregates, porphyrin–surfactant complexes, premicellar aggregates, anionic surfactants, polyelectrolytes, microheterogeneous systems.

Nowadays, the study of self-assembly of supramolecular structures and supermolecular aggregates from porphyrins makes it possible to model complicated biological processes involving these structures and also to design novel diagnostic agents and drugs and promising nanomaterials with controlled properties, whose variety is provided by a comparatively easy structural modification of porphyrin molecules.¹ The photophysical properties and catalytic and biological activity of porphyrins substantially depend on the parameters of the medium affecting the acid-base and aggregation behavior of chromophores that necessarily should be taken into account when developing porphyrin-based supramolecular assemblies based on the porphyrins.² Thus, it is of great significance to establish the relationships between the porphyrin molecular structure and its ability to self-assemble into the ordered supramolecular aggregates in various media.

Most of the porphyrins are capable of self-assembling into two main types of homoaggregates: *H*-aggregates with the low-ordered columnar arrangement of the chromophores formed due to the hydrophobic interaction of the macrocycles in polar solvents and *J*-aggregates with the

highly ordered arrangement of the chromophores in molecular ensembles of the ladder type stabilized by ionic and stacking interchromophore interactions and having valuable optical properties.³ Ionized forms of porphyrins are also characterized by the formation of heteroaggregates⁴ and ionic associates of different types involving both low-molecular-weight substances and polyelectrolytes^{5–7} (PE) and surfactants^{8,9} with an opposite sign of the charge.

Taking into account the promising use of the tetraphenylporphyrin derivatives as photosensitizers in photodynamic therapy of malignant neoplasms^{10,11} and in the photoinactivation of biological objects,¹² it is necessary to study in detail the aggregation state of potential photosensitizers in aqueous microheterogeneous systems imitating the intracellular medium. In this case, it is necessary to take into account the form in which the chromophore exists in solution, since this form predetermines the type of intermolecular interactions under specific conditions of the medium.

Among many synthetic porphyrins, water-soluble anionic tetraphenylporphyrin derivatives are most studied in respect of the aggregation behavior, and they easily form

J-aggregates in an acidic medium,¹³ including that in the presence of polycations^{14,15} and cationic surfactants in the premicellar concentration range.¹⁶ Unlike them, the formation of *J*-aggregates in an acidic medium by the cationic tetraphenylporphyrin derivatives is much more difficult because of a strong electrostatic repulsion of the peripheral substituents,¹⁷ but these derivatives are also capable of forming stable ionic associates.¹⁸

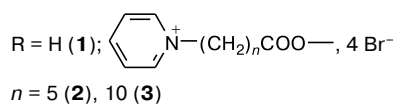
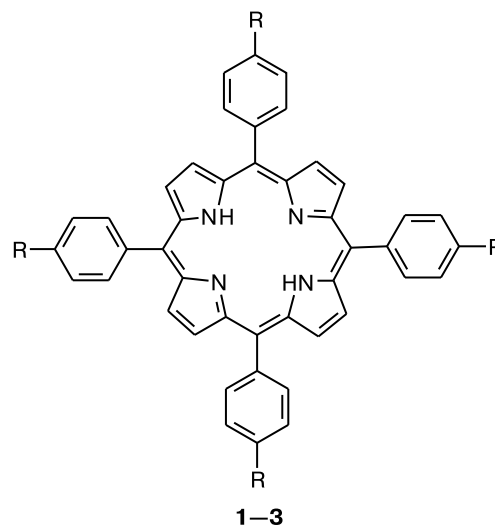
The aggregation behavior of the tetraphenylporphyrin hydrophobic derivatives in aqueous solutions is poorly studied. The studies were mainly devoted to the solubilization of the monomeric form of the dye in micellar solutions of the surfactant and prevention of aggregation of the *H* type accompanied by the complete fluorescence quenching and a sharp decrease in the extinction of the chromophore.^{19,20} For example, for water-insoluble tetraphenylporphyrin, the conditions of *J*-aggregate formation are limited to the interfacial self-assembly at the interface²¹ or in mixed solvents,²² whereas *J*-aggregated forms were not obtained earlier for the amphiphilic cationic derivatives of this compound described below.

Therefore, we aimed at studying the acid-base equilibrium and aggregation behavior of the tetraphenylporphyrin cationic derivatives in aqueous solutions and microheterogeneous systems. In this work, we also compared the obtained results with the earlier data on unsubstituted tetraphenylporphyrin.

Experimental

Unsubstituted 5,10,15,20-tetraphenylporphyrin (**1**) was synthesized at the Ivanovo State University of Chemical Technology (Ivanovo, Russia) according to a standard procedure and used without additional purification. Amphiphilic cationic porphyrins, viz., 5,10,15,20-tetrakis[4-(6-pyridiniohexanoyl)oxyphenyl]porphyrin tetrabromide (**2**) and 5,10,15,20-tetrakis[4-(11-pyridinioundecanoyl)oxyphenyl]porphyrin tetrabromide (**3**), being esters of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin and saturated monocarboxylic acids with the terminal pyridinium group, were synthesized and purified at the Chair of Chemistry and Technology of Biologically Active Compounds named after N. A. Preobrazhensky of the M. V. Lomonosov Moscow State University of Fine Chemical Technologies according to the previously described procedure.²³

Porphyrin stock solutions ($5 \cdot 10^{-5}$ mol L⁻¹) were prepared by the dissolution of an accurately weighed sample in the required amount of acetone (high-purity grade) or DMF (high-purity grade). The following commercial compounds were used for the preparation of series of solutions of surfactants and polyelectrolytes with a concentration of 2%: sodium dodecyl sulfate (SDS, Scharlau, Germany), sodium bis(2-ethylhexyl) sulfosuccinate (AOT, Acros Organics, Belgium), polyethylene glycol 1000 (PEG, Merck, Germany), poly-*N*-vinylpyrrolidone (PVP, Sigma, USA), bovine serum albumin (BSA, Serva, Germany), sodium polyphosphate (SPP, Fluka, Switzerland), and bidistilled water. The acidity of the medium in the studied solutions was controlled by the addition of a necessary volume of H₂SO₄ or NaOH with a concentration of 0.1 mol-equiv L⁻¹.



The aggregation behavior of porphyrin **1** and its derivatives was studied in solutions obtained by the injection of 200 μL of a stock solution of porphyrin in acetone into an aqueous solution of the surfactant or polyelectrolyte with a specific concentration and beforehand established pH followed by monitoring the absorption spectra or light scattering intensity of the system and recording fluorescence spectra. The content of the organic solvent in all studied solutions did not exceed 5%.

The absorption spectra of the solutions were recorded on a HACH DR 4000V spectrophotometer (Hach, USA) in the wavelength range 350–800 nm with an increment of 1 nm in a quartz cell (*l* = 10 mm) at ~20 °C. Fluorescence spectra were obtained on a Shimadzu RF-5000 spectrofluorimeter (Japan) under similar conditions using the excitation wavelength corresponding to the absorption maximum of a certain form of porphyrin at the Soret band. The particle size was approximately estimated on a Photocor Complex dynamic light scattering spectrometer (Russia) with a laser source (power 15 mW, wavelength 632.8 nm) at an angle of 90°. The data obtained were processed using the Origin 6.1 program, and the 3D visualization of the molecular structures was performed with the HyperChem 7.0 program package.

Results and Discussion

The spectral parameters and photophysical properties of amphiphilic cationic porphyrins in organic solvents are similar to those for unsubstituted tetraphenylporphyrin and are predominantly determined by the electron density distribution in the conjugated system of the macrocycle poorly affected by the peripheral long-chain substituents. However, the presence of four large pyridylacetyl fragments significantly affects the aggregation behavior of tetraphenylporphyrin derivatives in aqueous and aqueous–organic media.

By analogy to tetraphenylporphyrin, synthetic cationic porphyrins are amphoteric compounds and form a series of ionized forms depending on the acidity of the medium.

Doubly-charged ions of the chromophore are involved in the protolytic equilibrium, because the addition or detachment of the second proton in the macrocycle core occurs nearly simultaneously with the addition or detachment of the first proton.²⁴ The diprotonated forms of compounds **1**, **2**, and **3** are characterized by a higher symmetry (D_{4h}) than that for the neutral ligand (D_{2h}) and the coplanar arrangement of the *meso*-aryl group in the macrocycle plane, resulting in the bathochromic shift of the absorption maximum in the range of the Soret band from 414 to 438 nm and a decrease in the number of *Q* absorption bands from four to two (Fig. 1, *a*).

The diprotonated form of compounds **2** and **3** is characterized by fluorescence at 670 nm, but its intensity is decreased over the neutral form of the chromophore due to partial aggregation in an acidic medium. In an alkaline medium, compound **2** represents the strongly aggregated form of the chromophore with a low extinction and no fluorescence (see Fig. 1, *b*).

In some cases, the presence of polyelectrolytes has a significant influence on the aggregation state of the ion-

ized forms of the chromophores due to the formation of ionic porphyrin–PE associates, which provide the neutralization of charges on the peripheral substituents and spatial bringing together of ion-bound chromophores at a distance sufficient for the manifestation of van der Waals forces and stacking interactions between p-electron systems of the adjacent macrocycles. The full compensation of charges results in the formation of stacked *H*-aggregates with the maximum overlap of the aromatic systems involving no ionic interactions, which are characterized by the hypsochromic shift of the Soret band by 10 nm with its simultaneous broadening and decreasing extinction. In neutral aqueous solutions of SPP, which is inorganic polyanion, both cationic porphyrins **2** and **3** are completely aggregated according to the *H* type, whereas in acidic and alkaline media preventing the formation of ionic aggregates, the presence of SPP exerts no effect on the form of the chromophore (Fig. 2).

The influence of polyelectrolytes on the aggregation equilibrium of compound **2** is especially pronounced in a neutral medium, where most of the ionic groups of the polymer remain charged. The binding of the monomeric form of the chromophore with the substrate is accompanied by an increase in the extinction, a decrease in the peak half-width at the Soret band, and an increase in the fluorescence intensity, whereas the formation of homo- and heteroaggregates of the dye results in an opposite effect (Table 1).

The partially ionized forms of the porphyrins due to the electrostatic repulsion during aggregation tend to shift the monomers relative to each other retaining the coplanar orientation of dipole moments of electron transitions. This results in the conjugation and coherent propagation of excitation over the delocalized system of chromophores in the composition of the *J*-aggregate responsible for the appearance of an exciton band in the absorption spectra, which is strongly shifted to the red range relative to the

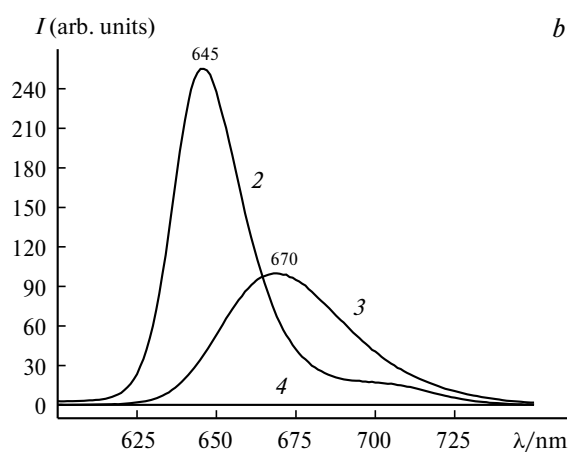
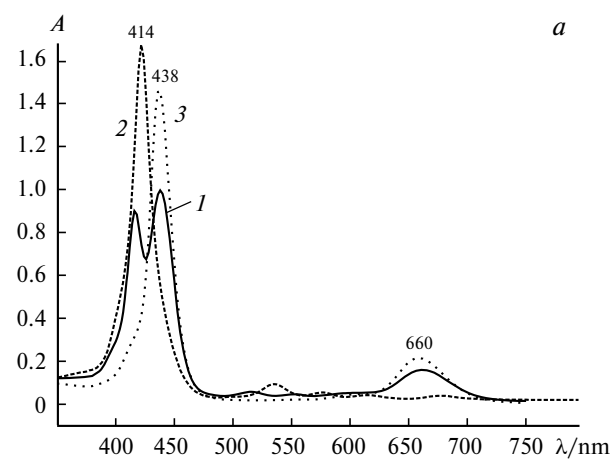


Fig. 1. Absorption (*a*) and fluorescence (*b*) spectra of the monomeric form of compound **2** at pH 3.5 (*1*), 6.5 (*2*), 1.5 (*3*), and 11.5 (*4*).

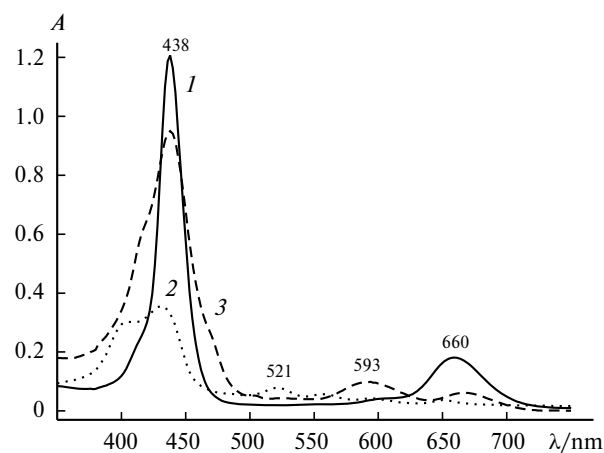


Fig. 2. Absorption spectra of compound **2** in a solution of SPP at pH 1.5 (*1*), 6.5 (*2*), and 11.5 (*3*).

Table 1. Parameters of the Soret band for compound **2** in the presence of PE

PE	I		II		III	
	λ_{\max}/nm	$\Delta\nu_{1/2}/\text{cm}^{-1}$	λ_{\max}/nm	$\Delta\nu_{1/2}/\text{cm}^{-1}$	λ_{\max}/nm	$\Delta\nu_{1/2}/\text{cm}^{-1}$
H ₂ O	438	1038	414	1051	438	2099
PVP	440	1142	422	840	448	2489
PEG	436	989	—	—	446	2533
BSA	438	1246	422	840	—	—
SPP	440	1186	—*	—	442	2324

Note. The measurements were carried out at pH 1–2 (I), 5–6 (II), and 11–12 (III).

* *H*-Aggregation occurs.

Soret band of the diprotonated monomer and for the bathochromic shift of the *Q* band by the value inversely proportional to the interchromophore distance in the *J*-aggregate.

In the case of porphyrin **1**, we obtained *J*-aggregated forms in acidified aqueous solutions of anionic surfactants in the premicellar concentration range. This effect can be explained by the presence of premicellar aggregates (in particular, stable dimers) of the anionic surfactant and premicellar surfactant aggregates of a small size²⁵ capable of forming ionic associates with the cationic form of the dye, thus providing the necessary orientation for the coherent interaction of the *p*-electron systems of the chromophores. This mechanism is proposed as an explanation of the known promoting effect of premicellar solutions of ionogenic surfactants on the formation of *J*-aggregates of the water-insoluble tetraphenylporphyrin derivatives.²⁶

In the case of the cationic amphiphilic tetraphenylporphyrin derivatives, a similar approach allowed us to obtain for the first time *J*-aggregates of compounds **2** and **3** and to compare the results with the data for porphyrin **1**. A comparison of the absorption spectra of cationic derivatives **2** and **3** with the spectra of porphyrin **1** in an aqueous solution of SDS with a concentration of $5 \cdot 10^{-4}$ mol L⁻¹ indicates that the structures of *J*-aggregates are similar, but compounds **2** and **3** with their bulky substituents appear to have a reduced packing density and coherent length, which results in a significant broadening of the *Q* band and the superposition of the absorption maxima of the monomer and aggregate in the range of the Soret band (Fig. 3, *a*). For both cationic derivatives **2** and **3**, the concentration range and optimum of the formation of *J*-aggregates in a premicellar aqueous solution of SDS are similar to those for unsubstituted porphyrin **1** (see Fig. 3, *b*). However, the aggregation rate is substantially decreased because of the lower diffusional mobility of the cationic derivatives and steric hindrances for monomer packing.

A similar effect of surfactant-induced self-assembly of *J*-aggregates from compounds **2** and **3** is also observed in a premicellar aqueous solution of AOT, which is an anionic surfactant and retains the ionized form in an acidic

medium. The aggregated form of the chromophore with absorption maxima at 475 (Soret band) and 690 nm (*Q* band) begins to predominate in solution with time (Fig. 4, *a*). The exterior of the solution also changes from transparent to opalescent, indicating the presence of dis-

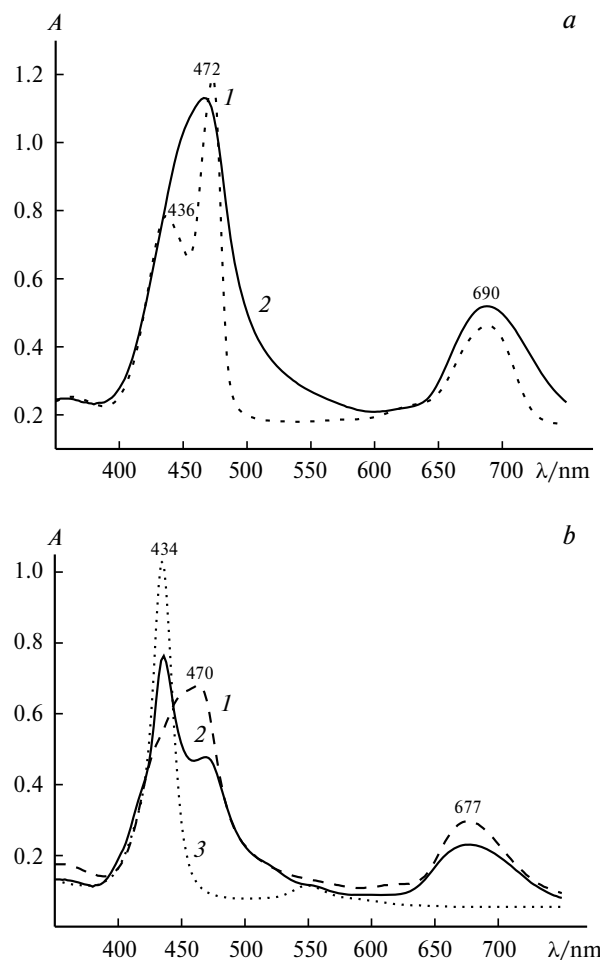


Fig. 3. (a) Absorption spectra of compounds **1** (*1*) and **2** (*2*) in an aqueous solution of SDS with a concentration of $5 \cdot 10^{-4}$ mol L⁻¹. (b) Dependence of the shape of the absorption spectra of compound **3** in an aqueous solution of SDS on the surfactant concentration (mol L⁻¹): $5 \cdot 10^{-4}$ (*1*), $1 \cdot 10^{-4}$ (*2*), and 0 (*3*).

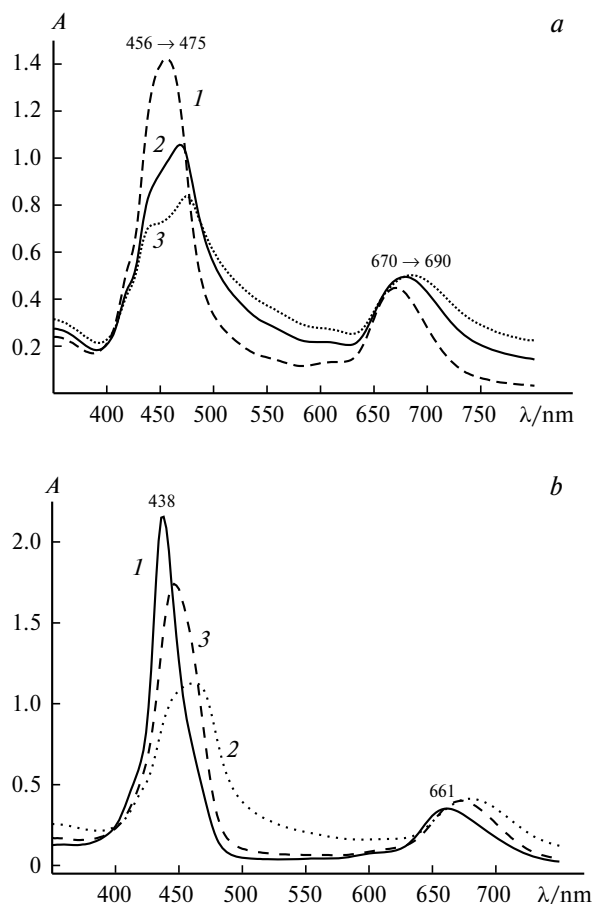


Fig. 4. (a) Changes in the absorption spectra of compound **2** in an aqueous solution of AOT with a concentration of 10^{-4} mol L $^{-1}$ with time: 0 (**1**), 5 (**2**), and 10 (**3**) min. (b) Dependences of the shape of the absorption spectra of compound **2** in an aqueous solution of AOT on the surfactant concentration (mol L $^{-1}$): $5 \cdot 10^{-5}$ (**1**), $1 \cdot 10^{-4}$ (**2**), and $1 \cdot 10^{-3}$ (**3**).

perse phase. This phenomenon is observed in a fairly narrow range of surfactant concentrations (10^{-4} – 10^{-3} mol L $^{-1}$) with an optimum value of 10^{-4} mol L $^{-1}$ for AOT and $5 \cdot 10^{-4}$ mol L $^{-1}$ for SDS, respectively. The typical spectrum of the monomeric diprotonated form of the chromophore without any signs of aggregation is observed beyond this concentration range (see Fig. 4, *b*).

As known, the addition of electrolytes to an aqueous solution of surfactants results in the shielding of the electrostatic repulsion of polar groups, a decrease in the micelle formation threshold, and the formation of pre-micellar surfactant aggregates in a pre-micellar solution.²⁷ The distinct ability of the studied anionic surfactants to promote the *J*-aggregation of diprotonated forms of porphyrins is manifested in this concentration range. Therefore, we estimated the relative sizes of aggregated particles in aqueous solutions of these surfactants at pH 1.5 before and after porphyrin addition. According to the dynamic light scattering in pre-micellar aqueous solutions of AOT with a concentration of 10^{-4} mol L $^{-1}$, individual particles with the hydrodynamic radius 50 ± 10 nm were observed after acidification with sulfuric acid. Upon the addition of porphyrin, a smooth growth of the intensity of light scattering and a parallel increase in the particle size from 100 nm to several μ were observed against the background of decreasing contribution of the finer surfactant aggregates to the light scattering intensity. The data obtained favor the proposed model of formation of hybrid ionic porphyrin–surfactant associates involving oligomeric pre-micellar aggregates of the surfactant in an acidified aqueous solution. The structure proposed for fragment of the *J*-aggregate based on compound **2** formed with the participation of the AOT dimer is shown in Fig. 5.

Thus, the synthetic amphiphilic cationic tetraphenylporphyrin derivatives in aqueous microheterogeneous sys-

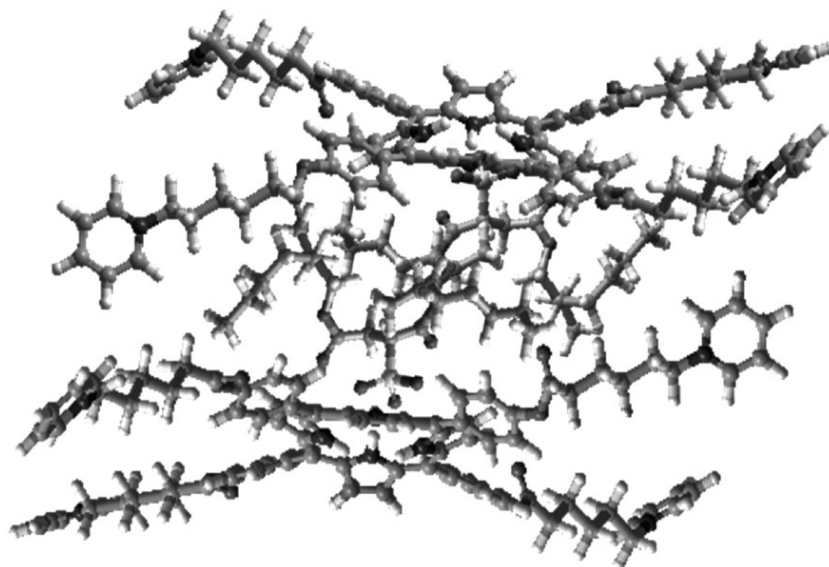


Fig. 5. Structure proposed for the *J*-aggregate of compound **2** involving the AOT dimer.

tems are capable of forming homoaggregates of the *J* and *H* types and mixed ionic associates in the presence of the oppositely charged polyelectrolytes and premicellar aggregates of anionic surfactants.

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