

OPTICAL STUDIES OF STABILITY AND AGGREGATION STATE OF PORPHYRINS IN ACID AQUEOUS SOLUTION*

I. Šimkienė^a, A. Kinduryš^a, J.G. Babonas^{a,b}, V. Snitka^c, R. Rodaitė-Riševičienė^c, and J. Beinoras^b

^a Semiconductor Physics Institute, A. Goštauto 11, LT-01108 Vilnius, Lithuania

^b Vilnius Gediminas Technical University, Saulėtekio 11, LT-10223 Vilnius, Lithuania

^c Kaunas University of Technology, Studentų 65, LT-51369 Kaunas, Lithuania

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Stability and aggregation state of aqueous solution of *meso*-tetra (4-sulfonatophenyl) porphine were studied by measuring the absorption spectra in UV–VIS range. Long-duration temporal variations of the optical features were examined and interpreted as being induced by the change of the forms of porphyrin. The observed effects were explained by the variation of acidity of the aqueous solution induced by the change of the composition of solution and due to the possible influence of photodegradation. It was also concluded that *J*-aggregates are most favourably adsorbed on the solid substrates from aqueous solution of porphyrin.

Keywords: spectroscopy, porphyrin, aggregation

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1. Introduction

The porphyrins and their solutions were widely investigated mainly as therapeutic drugs, targeting agents, and photosensitizers in photodynamic therapy [1]. Various solutions of porphyrins were studied taking into account the characteristics of biological media.

Recently, organic compounds have also attracted attention as perspective materials for molecular electronics (see, e.g., [2] and references therein). In addition, it is well known [3] that due to spontaneous self-aggregation, large molecular assemblies form in the aqueous/organic solutions of porphyrins. In the solid state the resulting structure with large channels can act as a host for solvent molecules and can be used as microporous molecular material for sensors and chemical separation. From this point of view, the studies of time stability and deposition regularities of porphyrins from aqueous solutions, which are the most promising for technical applications, are of a great interest.

The formation of supramolecular organizations was indicated and investigated by several optical methods like absorption, circular dichroism, fluorescence, and resonance light scattering. As a result of experimen-

tal investigations, characteristic bands of various porphyrin formations were revealed [3–10].

The aggregation kinetics of porphyrins was also extensively studied. It was found that *J*-aggregate formation of a water-soluble dilute ($1.5 \cdot 10^{-5}$ M) porphyrin in acid aqueous media saturated at several hours [8]. The large-scale aggregates of protonated porphyrin dimers were formed in water-organic solutions in a time period exceeding 68 h at porphyrin concentration $1.5 \cdot 10^{-5}$ M [9], and a green precipitate was noticed after several days [10]. The formation of homoassociations of diprotonated porphyrins in acid solutions was indicated [11] by measuring the signal of circular dichroism which increased strongly during the first few days and asymptotically varied after 2–3 months. It should be noted that in the porphyrin system the delay or lag period of up to 1 h was found [3].

The task of the present work was twofold. On the one hand, long-duration temporal changes were noticed and investigated in pure aqueous solution of *meso*-tetra(4-sulfonatophenyl) porphine (TPPS₄). On the other hand, the investigated aqueous solution contained porphyrin in various aggregation states. Therefore, it was possible to examine the change of aggregation state during several experimental runs under conditions similar to those, which could be used in

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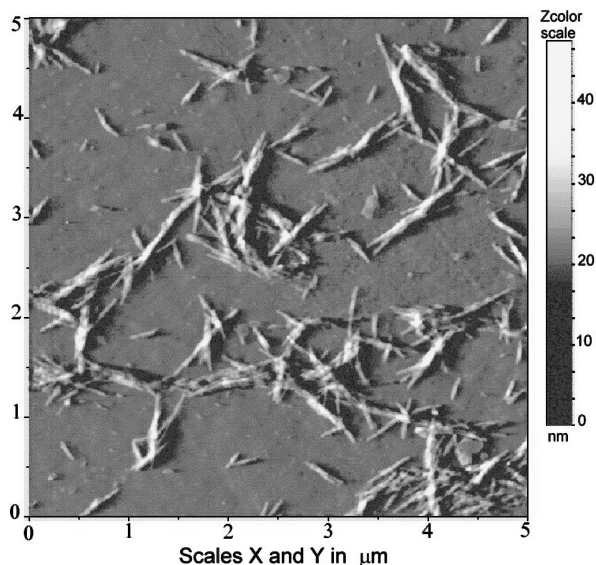


Fig. 1. AFM image of TPPS₄ deposited on mica from aqueous solution 10 days after preparation. The image was obtained in a contact mode.

fabrication of electronic devices at deposition of liquid precursors on substrates.

2. Experimental

The samples studied were prepared from *meso*-tetra(4-sulfonatophenyl) porphine dihydrochloride (Frontier Scientific, Inc., Logan), which had been dissolved in a purified water. The acid solution of TPPS₄ with pH value equal to 2.5 was obtained. The solution was stored in dark at room temperature for 10 days, the time assumed to be sufficiently long to complete the aggregation process. However, successive long-duration changes were still observed in the optical absorption of aqueous solution of porphyrin. The TPPS concentration in aqueous solution was evaluated spectroscopically and the results will be discussed in the next section.

Figure 1 shows the AFM micrograph of initial aqueous solution of TPPS deposited on mica substrate 10 days after the preparation. As it is seen, the stick-like structures of 0.1–1.0 μm in length, 50–100 nm in width, and 20–25 nm in height have been formed.

Experimental series of 6 runs of optical measurements started 12 days after preparation of aqueous solution and was completed in the next 12 days. Each experimental run was carried out using the cuvette with a newly refilled aqueous solution. The optical spectra were measured by means of a spectrophotometer SPECORD UV–VIS using quartz cuvettes of 0.1–2.0 mm in length. The absorption coefficient of the

samples of solid solutions was calculated neglecting the scattering effects. The position and halfwidth of separated and deconvoluted overlapping bands were determined with accuracy of $\pm 2 - 3$ nm assuming the Lorentzian-type lineshape. In the fitting procedure the band characteristics and background were treated as adjustable parameters. It should be noted that the concentration values determined below were just the approximate values for the amounts of porphyrin of a particular form in a mixture of various forms.

3. Results and discussion

The long-duration temporal changes of the spectra are illustrated by Fig. 2. The examples of the convolution of the spectra in the vicinity of Soret and some *Q*-bands are presented in Figs. 3 and 4, respectively. The fine structure of the absorption spectra shows that porphyrins of various forms have been present in the initial aqueous solution (Fig. 2(a)). The locations of strong absorption bands observed at 437, 590, and 643 nm (Figs. 3 and 4) were in agreement with those for monomer of diprotonated porphyrin [11]. It is known that in aqueous diluted ($\sim 1 \cdot 10^{-6}$ M [6]) acidic (pH < 4) solutions porphyrin in a dianion form (H₄TPPS₄)²⁻ is monomeric with Soret band at 432 nm [12], 434 nm [4, 5], or 437 nm [13], depending on the pH values and concentration of aqueous solution. The *Q*-bands *Q*(1, 0) and *Q*(0, 0) corresponding to *D*_{4h} symmetry are located at 594 nm and 644 nm [4, 5] (or 642 nm [12]), respectively. The relative intensity and lineshape of the *Q*-bands (Figs. 2(a), 4(a)) was similar to those observed in [12] for monomeric dianionic H₄²⁺TPPS₄⁴⁻ in aqueous solution of concentration $5 \cdot 10^{-5}$ M, pH 4.5. In the investigated samples the concentration of porphyrin in diprotonated form $\sim 1.5 \cdot 10^{-4}$ M was estimated spectroscopically using the molar absorption coefficient values for developed B ($4.42 \cdot 10^5$ cm⁻¹/M for 434 nm) and *Q*-bands ($4.60 \cdot 10^4$ cm⁻¹/M for 644 nm) [4].

The porphyrin molecule in diacid form has a positive charge that interacts with sulphonatophenyl anionic groups of other molecules and tends to aggregate. Monomers of (H₄TPPS₄)²⁻ assemble to form the *J*-aggregates of diprotonated porphyrin due to edge-to-edge interaction [14] exhibiting an intense Soret band B_J at 490 nm [3]. At high porphyrin concentrations ($\sim 1 \cdot 10^{-4}$ M [6]) another band B_H at 420 nm [6, 7] was observed which has been assigned to the manifestation of *H*-type face-to-face aggregates. In this

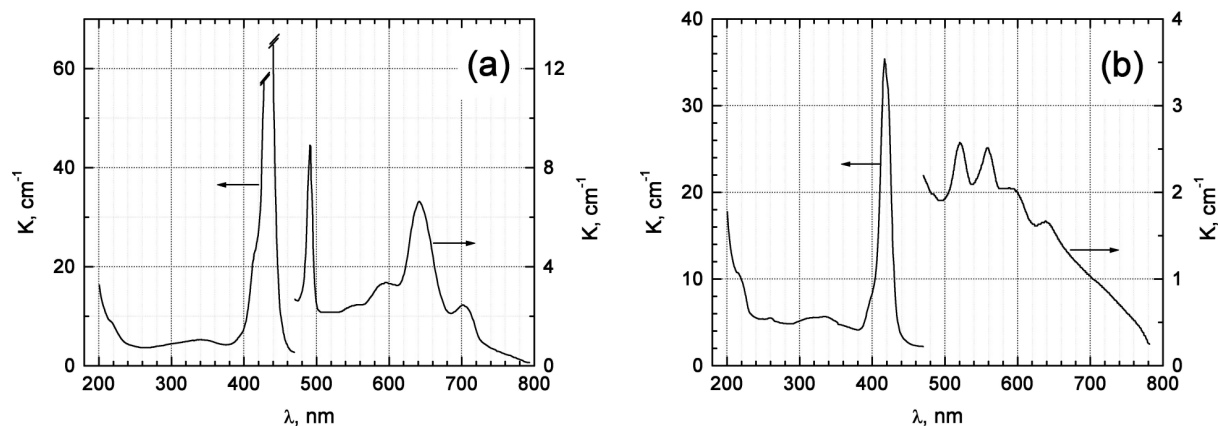


Fig. 2. Spectra of aqueous solution of TPPS₄ at (a) 290 h (1st run) and (b) 550 h (6th run) after preparation.

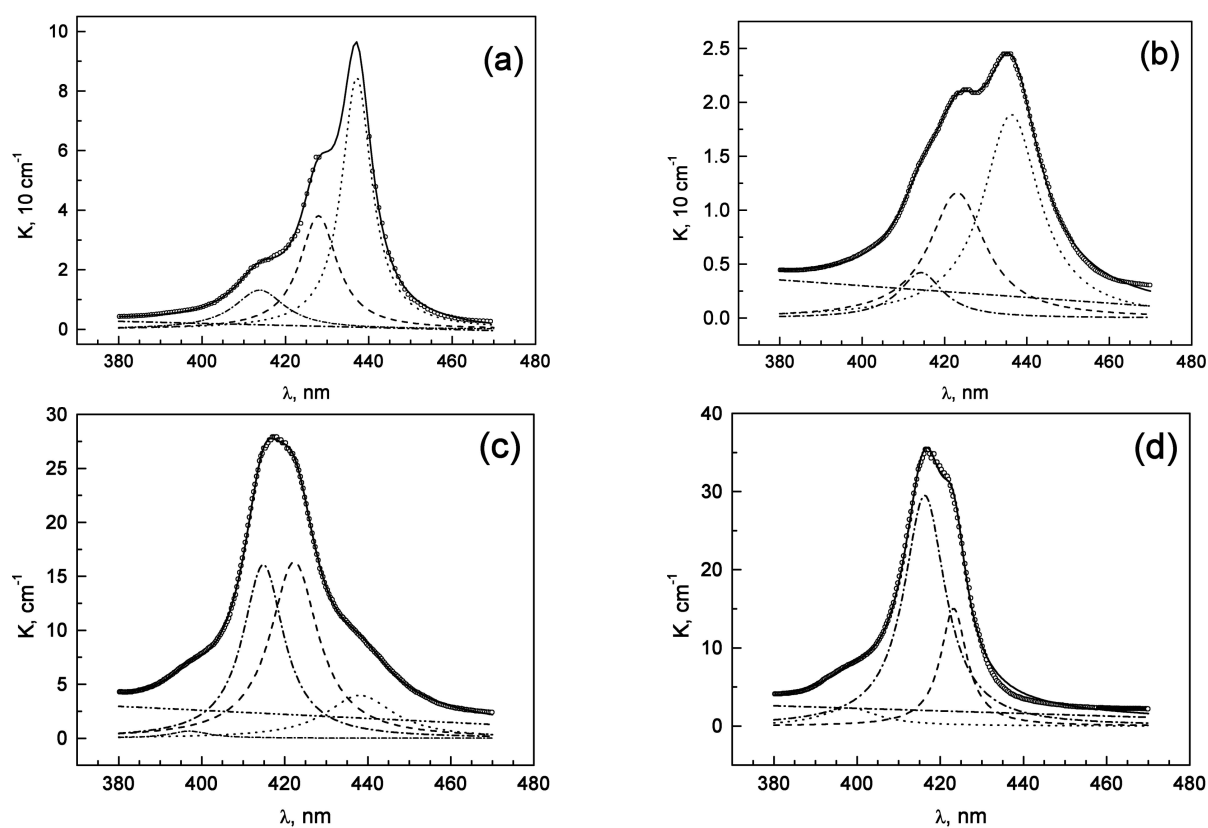


Fig. 3. Experimental data (points) and deconvolution of Soret band (curves) in the spectra of aqueous solution of porphyrins after (a) 290 h (1st run), (b) 360 h (2nd run), (c) 430 h (4th run), and (d) 550 h (6th run).

case three bands at 625, 668, and 707 nm were indicated [6]. The bands at 490 and 706 nm are characteristic for the aggregated monomeric dianionic species [12] observed in acidic aqueous solution and develop at increasing the acidity and ionic strength as well as the concentration of $(H_4TPPS_4)^{2-}$ [15]. As seen from Figs. 2(a) and 4(a), the sharp band at 490 nm and the separated band at 705 nm were well resolved and together with the observed though masked B_H and Q -bands indicated the presence of J -aggregates in the

initial solution. It should be noted that coexistence of monomers $(H_4TPPS_4)^{2-}$ and J -aggregates has been observed previously [16] and most favourably has occurred at moderate temperatures of 37.5 °C [17]. The self-aggregation model based on spectroscopic data was confirmed by the X-ray scattering studies [7].

In the next 12-day period, the changes in the optical spectra were still significant (Fig. 2(b)). In particular, the relative intensity inside the complex Soret band (Fig. 3) was redistributed over the com-

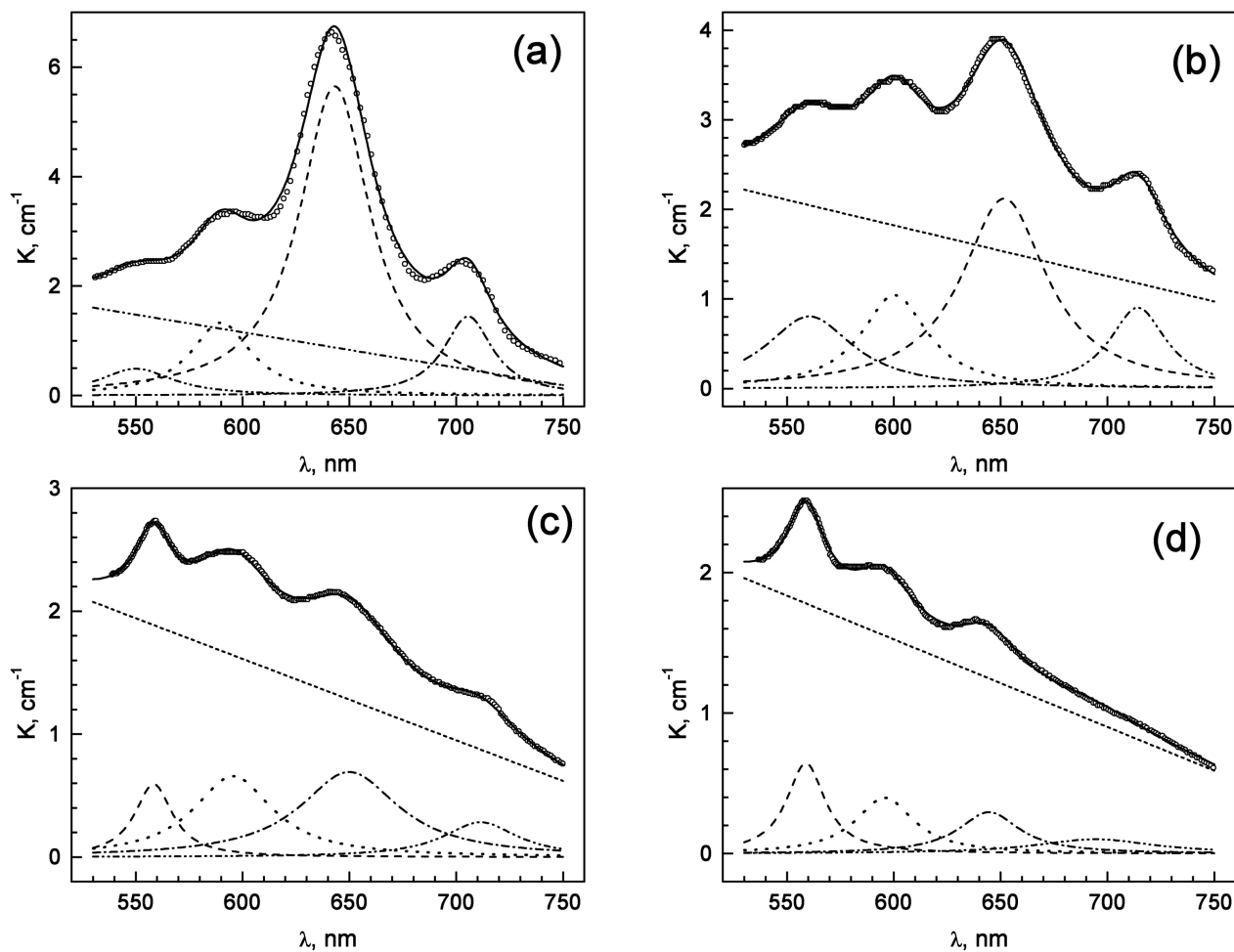


Fig. 4. Experimental data (points) and deconvolution of Q -bands (curves) in the spectra of aqueous solution of porphyrins after (a) 290 h (1st run), (b) 360 h (2nd run), (c) 430 h (4th run), and (d) 550 h (6th run).

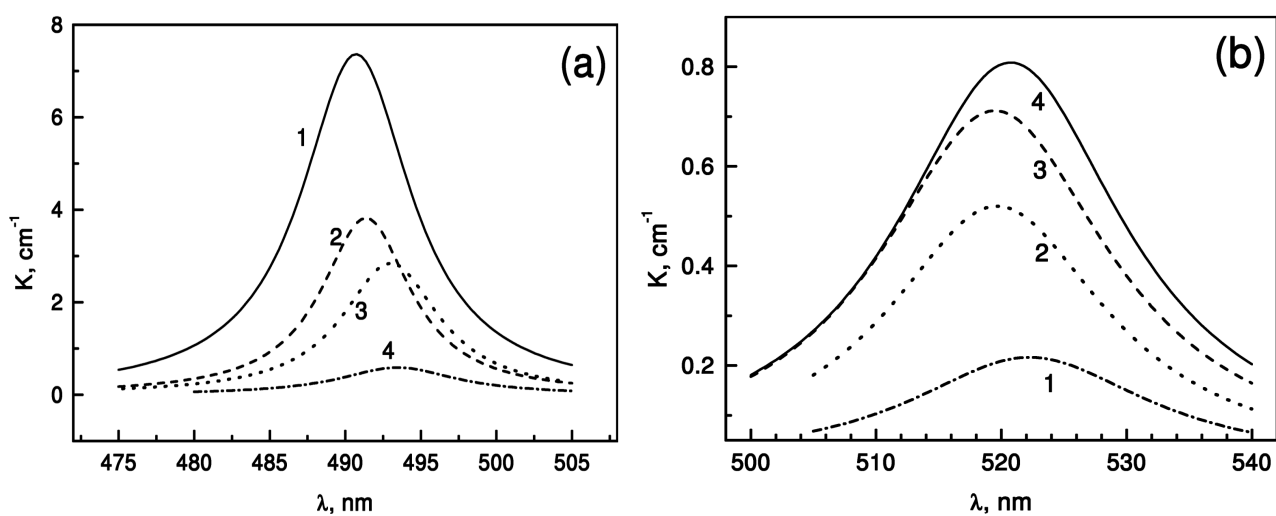


Fig. 5. Absorption spectra in the vicinity of (a) 490 nm and (b) 520 nm bands measured at various time after preparation of aqueous solutions in different experimental runs. (a) 290 h (1st run, 1); 360 h (2nd run, 2), 390 h (3rd run, 3), 430 h (4th run, 4); (b) 390 h (3rd run, 1), 430 h (4th run, 2), 460 h (5th run, 3), 550 h (6th run, 4).

ponents. As followed from decomposed spectra in the region of Soret bands (Fig. 3), the main band at ~ 437 nm decreased and disappeared in the next experimental runs. The changes in the intensity of the band at 422 nm were probably related to those of 414 nm band. The relative intensity of the band at ~ 414 nm increased and it became dominant in the last run in the series (Figs. 2(b), 3). Simultaneously, the changes in the region of Q -bands occurred: the longest-wavelength band at ~ 706 nm disappeared and the bands at approximately 520, 560, 590, and 650 nm were observed. In addition, the intensity of the band at 490 nm decreased in the next experimental runs whereas that of the 520 nm band increased (Fig. 5). The shift of the bands presented in Fig. 5 should be also noted.

The observed spectral changes can be correlated to the spectral properties of porphyrins determined in aqueous solutions of different acidity [12, 18]. The spectral features shown in Fig. 2(b) can be ascribed to the formation of free-base species in the aqueous solutions with high pH values. The spectra are similar to those observed in [16] for TPPS₄ in aqueous solution with pH 5.15 and 6.9. In basic solutions with pH values 6.3 [8], 7.4 [15], 9.0 [7], and 10.0 [4] the free-base porphyrin is formed with characteristic intense Soret band at 414 nm. Due to a lower symmetry D_{2h} , four weak Q -bands were noticed at 516, 552, 580, and 634 nm attributed to Q_4 , Q_3 , Q_2 , and Q_1 , respectively [7]. As it is known [6], the aggregation in aqueous solutions leads to an increase in the width of Soret band but no new bands have been noticed. Therefore, according to the changes in the optical features of the investigated samples, the contribution of free-base porphyrin with D_{2h} symmetry became dominant after 6 experimental runs performed in 12 days. The concentration of free-base TPPS₄ estimated from the molar absorption coefficient of 411 nm [4] and 413 nm [19] bands using the reference data ($5.33 \cdot 10^5$ cm⁻¹/M and $5.1 \cdot 10^5$ cm⁻¹/M, respectively) for porphyrin in aqueous solutions with corresponding pH values 10.0 and 6.0 was $\sim 0.7 \cdot 10^{-4}$ M. The data allows one to assume that the acidity of investigated aqueous solution of porphyrin decreased in this solution (Fig. 2(b)).

It is worthwhile to note that a mixture of (H₄TPPS₄)²⁻ and free-base (H₂TPPS₄)⁴⁻ was observed [7] at pH 4.0 due to the close proximity to the pK_a value of 4.52. The aggregation of free-base porphyrin in acidic media was indicated both in homogeneous solution and in the presence of surfactant [14].

The absorption bands in the UV spectral region have not changed significantly during the experimental in-

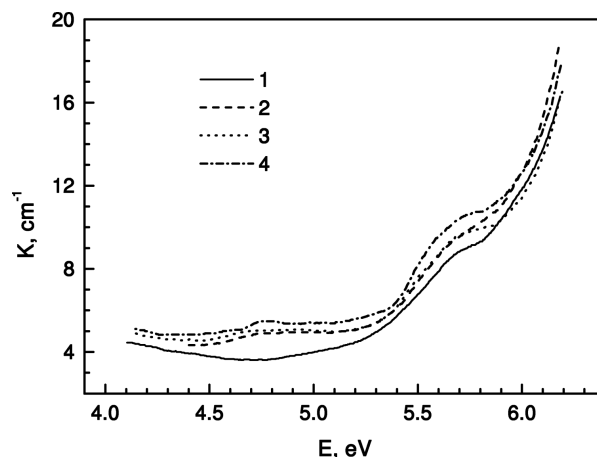


Fig. 6. Absorption spectra in UV region measured in different runs at various time after preparation of aqueous solution: 290 h (1st run, 1), 390 h (3rd run, 2), 460 h (5th run, 3), 550 h (6th run, 4).

vestigations though the bands at 260 nm (4.8 eV) and 220 nm (~ 5.6 eV) have slightly increased (Fig. 6).

The temporal changes of the optical spectra of investigated porphyrin solutions were not significant after 12 days of measurements. It should be noted that after one year the pH value of investigated sample, which experienced multiple experimental runs, was 7.9 and it differed from that (pH 2.5) of the control sample of aqueous solution.

The variations in the fine structure of porphyrins in aqueous solutions can be explained by the influence of several factors. Firstly, the long-duration temporal changes can be related to the increase of the pH value. The acidity in subsequent experimental runs could have diminished due to the formation of volatile HCl and hypochlorous acid HOCl. Decomposition of HOCl leads to the formation of active molecular oxygen [20]. It should be also noted that $1.27 \mu\text{m}$ IR radiation, the intensity of which is close to that in sun light, causes excitation of oxygen molecules dissolved in the water saturated with air at normal atmospheric pressure [21]. The influence of oxygen can result in a destruction of double carbon bonds and opening of the pyrrole ring [1]. Secondly, the relative amount of porphyrins in different aggregation states can be changed in subsequent experimental runs because a certain part of porphyrin was presumably deposited on the cuvette walls and was removed by cleaning. As a result, the total porphyrin concentration was reduced. Thirdly, the relative changes in the band intensities as well as the decrease of total absorption indicated the decrease of porphyrin concentration which could be due to photodegradation [1, 18, 22]. A small though well observable increase of UV absorption (Fig. 6) indicated the influence of this

factor. It should be noted that after 12 days of measurements, the cuvette of porphyrin with $\sim 1 \text{ cm}^3$ volume experienced the $\sim 2 \cdot 10^4 \text{ lm}\cdot\text{s}$ ($\sim 30 \text{ J}$) dose of light irradiation by room illumination. This value is sufficiently high, though lower than illumination doses usually applied in photobleaching experiments.

On the basis of experimental observations it can be concluded that the use of pure water as a solvent leads to the formation of unstable aqueous solution of TPPS₄. In addition, it is reasonable to predicate that *J*-aggregates are firstly adsorbed on solid surfaces from the aqueous solution of porphyrins present in several aggregation forms.

Acknowledgements

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References

- [1] R. Rotomskis, S. Bagdonas, and G. Streckyte, Spectroscopic studies of photobleaching and photoproduct formation of porphyrins used in tumour therapy, *J. Photochem. Photobiol. B* **33**, 61–67 (1996); R. Rotomskis, G. Streckyte, S. Bagdonas, Phototransformations of sensitizers 1. Significance of the nature of the sensitizer in the photobleaching process and photoproduct formation in aqueous solution, *J. Photochem. Photobiol. B* **39**, 167–171 (1997); Phototransformations of sensitizers 2. Photoproducts formed in aqueous solutions of porphyrins, *J. Photochem. Photobiol. B* **39**, 172–175 (1997).
- [2] C.A. Richter, C.A. Hacker, L.J. Richter, and E.M. Vogel, Molecular devices formed by direct monolayer attachment to silicon, *Solid-State Electr.* **48**, 1747–1752 (2004).
- [3] R.F. Pasternack, C. Fleming, S. Herring, P.J. Collings, J. dePaula, G. DeCastro, and E.J. Gibbs, Aggregation kinetics of extended porphyrin and cyanine dye assemblies, *Biophys. J.* **79**, 550–560 (2000).
- [4] E.B. Fleischer, J.M. Palmer, T.S. Srivastava, and A. Chatterjee, Thermodynamic and kinetic properties of an iron–porphyrin system, *J. Amer. Chem. Soc.* **93**, 3162–3167 (1971).
- [5] R.F. Pasternack, P.R. Huber, P. Boyd, G. Engasser, L. Francesconi, E. Gibbs, P. Fasella, G.C. Venturo, and L. de Hinds, On the aggregation of *meso*-substituted water-soluble porphyrins, *J. Amer. Chem. Soc.* **94**, 4511–4517 (1972).
- [6] R. Rubires, J. Crusats, Z. El-Hachemi, T. Jaramillo, M. Lopez, E. Valls, J.-A. Farrera, and J.M. Ribo, Self-assembly in water of sodium salts of *meso*-sulfonatophenyl substituted porphyrins, *New J. Chem.* **23**, 189–198 (1999).
- [7] S.C.M. Gandini, E.L. Gelamo, R. Itri, and M. Tabak, Small angle X-ray scattering study of *meso*-tetrakis(4-sulfonatophenyl) porphyrin in aqueous solution: A self-aggregation model, *Biophys. J.* **85**, 1259–1268 (2003).
- [8] O. Ohno, Y. Kaizu, and H. Kobayashi, *J*-aggregate formation of a water-soluble porphyrin in acidic aqueous media, *J. Chem. Phys.* **99**, 4128–4139 (1993).
- [9] A.V. Udal'tsov, M. Tosaka, and G. Kaupp, Microscopy of large-scale porphyrin aggregates formed from protonated TPP dimmers in water-organic solutions, *J. Mol. Struct.* **660**, 15–23 (2003).
- [10] A.V. Udal'tsov, L.A. Kazarin, and A.A. Sweshnikov, Self-assembly of large-scale aggregates of porphyrin from its dimmers and their absorption and luminescence properties, *J. Mol. Struct.* **562**, 227–239 (2001).
- [11] R. Rubires, J.-A. Farrera, and J.M. Ribo, Stirring effects on the spontaneous formation of chirality in the homoassociation of diprotonated *meso*-tetraphenylsulfonato porphyrins, *Chem. Eur. J.* **7**, 436–446 (2001).
- [12] D.L. Akins, S. Özçelik, H.-R. Zhu, and C. Guo, Fluorescence decay kinetics and structure of aggregated tetrakis(*p*-sulfonatophenyl)porphyrin, *J. Phys. Chem.* **100**, 14390–14396 (1996).
- [13] Y. Minna and L. Yaogun, Analysis of *meso*-tetrakis(4-sulfonatophenyl)porphyrin in aqueous solution by constant wavelength synchronous fluorescence spectroscopy, *Chem. Mag.* **5**, 65 (2003).
- [14] S.C.M. Gandini, V.I. Yushmanov, I.E. Borissevich, and M. Tabak, Interaction of the tetra(4-sulfonatophenyl)porphyrin with ionic surfactants: Aggregation and location of micelles, *Langmuir* **15**, 6233–6243 (1999).
- [15] C.Z. Huang, Y.F. Li, N. Li, K.A. Li, and S.Y. Tong, Spectral characteristics of the aggregation of α , β , γ , δ -tetrakis(*p*-sulfophenyl)porphyrin in the presence of proteins, *Bull. Chem. Soc. Jpn.* **71**, 1791–1797 (1998).
- [16] L. Kelbauskas, S. Bagdonas, W. Dietel, and R. Rotomskis, Excitation relaxation and structure of TPPS₄ *J*-aggregates, *J. Lumin.* **101**, 253–262 (2003).
- [17] K. Kemnitz and T. Sakaguchi, Water-soluble porphyrin monomer–dimer systems: Fluorescence dynamics and thermodynamic properties, *Chem. Phys. Lett.* **196**, 497 (1992).
- [18] S. Bagdonas and R. Rotomskis, Investigation of spectroscopic properties of *meso*-tetra(4-sulfonatophenyl)porphine (TPPS₄) in aqueous solutions of different acidity, *Lithuanian J. Phys.* **38**, 75–78 (1998).
- [19] C.Z. Huang, Y.F. Li, and P. Feng, Determination of proteins with α , β , γ , δ -tetrakis(4-

sulfophenyl)porphine by measuring the enhanced resonance light scattering at the air/liquid interface, *Anal. Chim. Acta* **443**, 73–80 (2001).

[20] A.U. Khan and M. Kasha, Singlet molecular oxygen evolution upon simple acidification of aqueous hypochlorite: Application to studies on the deleterious

health effects of chlorinated drinking water, *Proc. Natl. Acad. Sci. USA* **91**, 12362–12364 (1994).

[21] A.A. Krasnovsky, N.N. Drozdova, A.V. Ivanov, and R.V. Ambartsumian, Activation of molecular oxygen by infrared laser radiation in pigment-free aerobic systems, *Biochemistry (Moscow)* **68**, 963–966 (2003).

PORFIRINŲ RŪGŠTINIAME VANDENS TIRPALE PASTOVUMO IR AGREGATINĖS BŪSENOS OPTINIAI TYRIMAI

I. Šimkienė^a, A. Kindurys^a, J.G. Babonas^{a,b}, V. Snitka^c, R. Rodaitė-Riševičienė^c, J. Beinoras^b

^a *Puslaidininkų fizikos institutas, Vilnius, Lietuva*

^b *Vilniaus Gedimino technikos universitetas, Vilnius, Lietuva*

^c *Kauno technologijos universitetas, Kaunas, Lietuva*

Santrauka

Matuojant sugerties spektrus UV ir regimajame spektriniuose ruožuose, tirti *mezo*-tetra(4-sulfonatofenil)porfirino tirpalų vandenyje pastovumas ir agregatinės formos. Nagrinėti ypatumų optiniuose spektruose ilgalaikiai kitimai, kurie aiškinami porfirinų for-

mos ir agregatinių būsenų pokyčiais. Pastebėti efektai buvo susieti su vandens tirpalų rūgštingumo kitimu, kurį sukelia tirpalo sudėties kitimas, taip pat su galima fotodegradacijos įtaka. Padaryta išvada, kad iš porfirino vandens tirpalų *J* agregatai greičiausiai įgeriami ant kietų padėklų.