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Hyperporphyrin effects extended into a J-aggregate supramolecular structure in water

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SUMMARY

Materials and methods.

General. ^{1}H-NMR spectra were recorded at room temperature on a Varian Mercury 400-MHz FT NMR spectrometer and chemical shifts are referenced to the d_6-DMSO signal (^{1}H δ 2.50 ppm downfield) relative to $(CH_3)_4Si$ at 0 ppm. Data are recorded as follows: chemical shift, multiplicity (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, (br) broad signal, relative intensities, and identification. Coupling constants are given in Hz. UV-vis spectra were recorded on a double-beam Cary 500-scan spectrophotometer (Varian); cuvettes (quartz QS Suprasil, Hellma) ranging in thickness from 1 to 0.1 cm were used for measuring the absorption spectra. Infrared (IR) spectra were recorded in a Nicolet 6700 FT IR (Thermo Scientific) in ATR mode. Mass spectra were obtained with a LC/MSD-TOF (Agilent Technologies). pH measurements were performed on a CRISON Micro pH 2000 pH-meter (Crison 52-04 glass electrode) at room temperature. The pH-meter was calibrated prior to each measurement with buffers at pH=7.00 and 4.00 (Metrohm). Thin layer and flash column chromatography were performed on Merk SiO_2 plates (0.2 mm thickness) with fluorescence indicator (F_{254}), Florisil® (60-100 mesh, Fluka), silica gel 60A (35-70 μm, 230-240 mesh). All reagents were of commercial grade quality and distilled and dried following standard organic laboratory procedures before they were used. Water of Millipore Q quality (18.2 MW.cm, obtained from Milli-Q1 Ultrapure Water Purification Systems, Millipore, Billerica, MA) was used throughout the sample purification procedures and for the preparation of all analytical porphyrin solutions.

HPLC analysis. The HPLC analysis were performed on a Shimadzu high-performance liquid chromatograph equipped with two LC-10AS pumps, a Shimadzu CBM controller, an analytical precolumn Resolve C18 (Waters), and a Nucleosil 120-5C18 analytical column, using an elution gradient consisting of a mixture of methanol and tetrabutylammonium phosphate buffer (3 mmol L^{-1}; pH=7.0) (1:1 v:v) to pure methanol over a period of 30 minutes at a flow rate of 0.6 mL min^{-1} (~2700 - 1050 psi). All solvents were HPLC grade and were carefully degassed prior to use. At time 0 the sample was injected. The elution profile was monitored at λ = 414 nm (UV-Vis detector SPD-6AV).

Spectrophotometric titrations of the porphyrins. The pK_a values of the porphyrin were determined at room temperature by monitoring the absorbance changes at a fixed wavelength (typically at an absorption maximum of one of the species involved in the acid-base equilibrium) of micromolar solutions of the substance, at different pH values, which were obtained by micropipette addition over the porphyrins of consecutive relatively small aliquots of freshly prepared acid solutions in water in the UV-Vis cell. All titrations were experimentally reproducible when repeated several times, preparing in each case new fresh solutions of all the reagents. The porphyrin solutions in water were carefully degassed by gentle bubbling a nitrogen gas stream prior to the spectrophotometric titrations. The apparent pK_a values were then obtained from the Henderson-Hasselbach equation by graphic interpolation using the following expression: pK = pH + log ([acid]/[base]) where the ratio of acid to base in each solution was calculated, at a given wavelength, as: $[acid]/[base] = (Abs_{base} - Abs)/(Abs - Abs_{acid})$. All the spectra used in the pK_a determinations showed neat isosbestic points. The experimental numerical values are presented in the following pages.

Peak Force Microscopy (PFM) Images were acquired in a Multimode Atomic Force Microscope attached to a Bruker® Nanoscope V electronics unit. The imaging mode used for these experiments

was Scan Assist Tapping Mode: in this operation mode the scanning probe performs individual force curves on the sample surface while maintaining a constant vertical force. The vertical force was set at 10 pN – 20 pN that is a suitable threshold in order to ensure that the sample deformation due to the contact of scanning probe with the sample is kept at a minimum value. The scanning probe used in this study was a Bruker® SLN-10 triangular probe made of silicon nitride, whose spring constant is 0.35 $N \cdot nm^{-1}$.

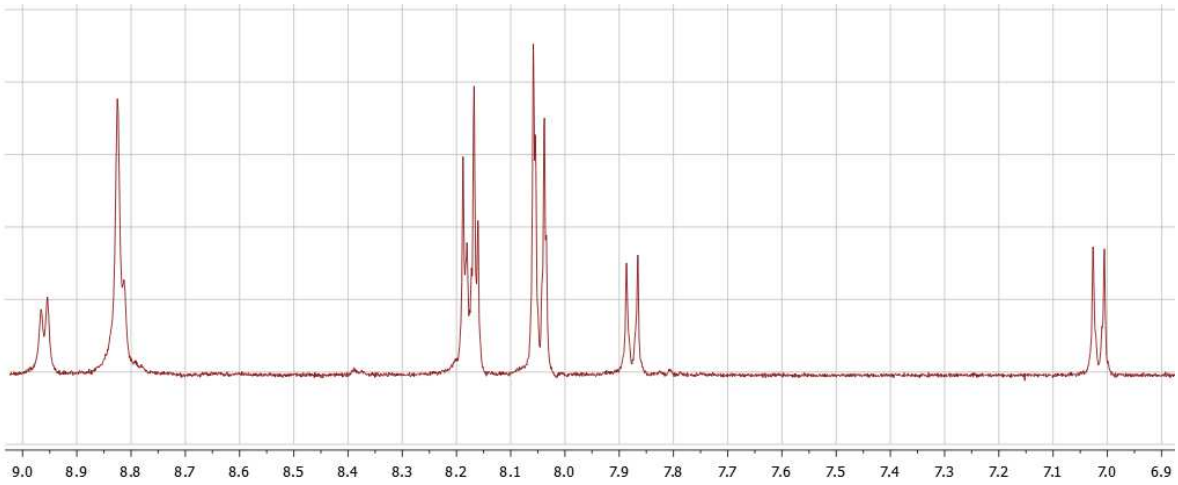
Solution deposition procedure for PFM imaging: The reported images correspond to the observation of different samples deposited under the same experimental conditions and were imaged in several points of the surfaces in order to assess the homogeneity and type of the deposited particles. The sample was deposited as follows: One drop (~10 µl) of the nanoparticle solution was brought into contact with a freshly cleaved highly ordered pyrolytic graphite (HOPG) surface during 40 seconds and the solution was then blotted off with the tip of a sheet of filter paper. Finally the substrate surface was dried by blowing with a nitrogen gas stream. In order to infer the thickness of the two kinds of aggregates a statistic evaluation was performed by measuring the height of a large number of particles through section analysis. This experimental procedure allows the deposition of a representative sample of the solution particles in suspension on the HOPG surface.

Synthesis and purification of the trisodium salt of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin ($TPPS_3NH_2$)

The title porphyrin was obtained from direct sulfonation of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (ref. 1) with hot sulfuric acid following a procedure adapted from refs. 2,3.

5-(4-aminophenyl)-5,10,15-triphenylporphyrin (150 mg, 0.238 mmol) was poured into 3 mL of ice-cold sulfuric acid, the suspension was sonicated, and the resulting homogeneous solution was then heated under magnetic stirring to 100 ºC for 6 h. The dark green solution was further stirred for 4 more days at room temperature and poured into 200 mL of cold water to form a precipitate. The dark green suspension was centrifuged at 6000 rpm for 15 min, the acidic supernatant was decanted and the precipitate was dissolved in 5 mL of water and neutralized with sodium carbonate. Inorganic salts in the resulting red solution were removed by medium pressure reverse phase column chromatography using MCI GEL CHP20P (Diaion®, Supelco), in which the porphyrin is slightly retained thus allowing the elimination of inorganic salts using water as the eluent; increasing gradients of methanol (from 0% to 50%) conveniently eluted the porphyrin which was concentrated in the rotary-evaporator and lyophilized to give a 89 yield (198 mg, 0.211 mmol) of the free base porphyrin trisodium salt as a red solid. HPLC analysis of the obtained product (page 5) showed the presence of considerable amounts of a regioisomer in which a sulfonato group was located at the *meta* position of the phenyl ring (ref. 4). Samples of enough analytical quality for the purpose of this work were obtained after further tedious chromatographic enrichment using gradient elution (from 100% H_2O to 100% CH_3OH) by means of C-18 (polygoprep 100-50 Macherey-Nagel) medium pressure column chromatography. The purest fractions were finally concentrated under reduced pressure and lyophilized (page 5).

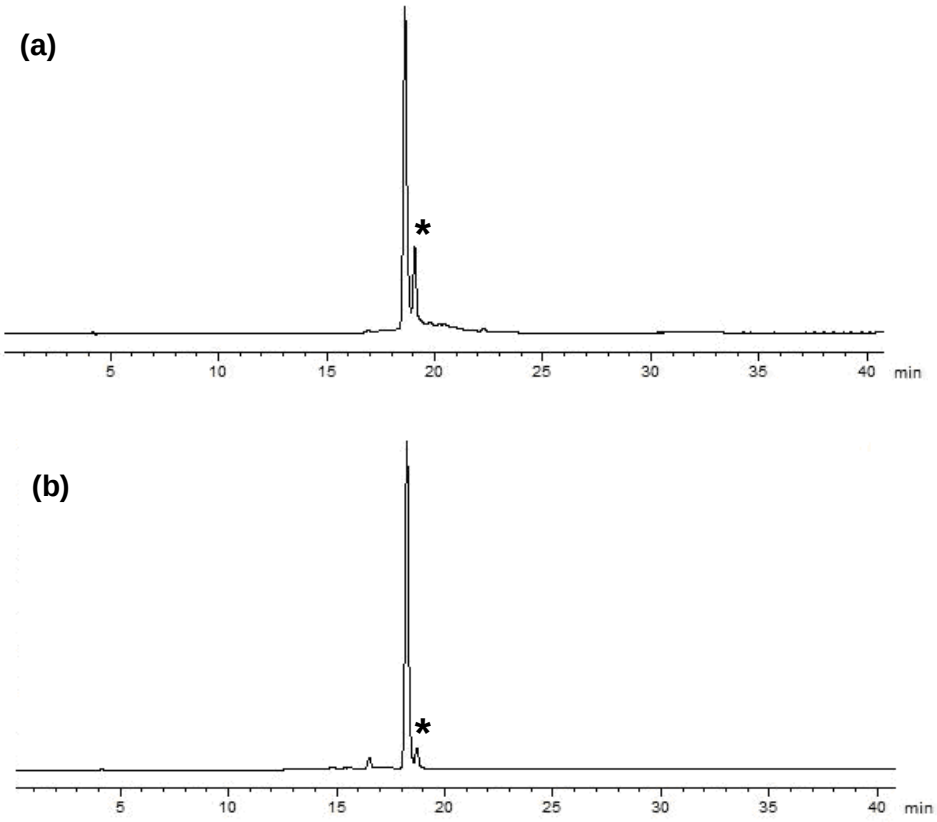
EM [ES(-), CH_3OH/H_2O 1:1, m/z]: 288.7 $[M\text{-}3Na^+]^{3-}$, 433.6 $[M\text{-}3Na^+\text{+}1H^+]^{2-}$, 444.5 $[M\text{-}2Na^+]^{2-}$, 868.1 $[M\text{-}3Na^+\text{+}2H^+]^-$; IR ($\upsilon_{max}$): 3370, 1599, 1176, 1121, 1035, 1010, 991, 964, 853, 794, 734, 629 cm^{-1}; UV-Vis (H_2O, N_2 purged) λ_{max}, nm (ε): 415 (320.000), 519 (13.400), 560 (9.100), 579 (7.900) and 639 (4.100). ^{1}H-NMR (400 MHz, DMSO-d_6, rt): 8.96 (d, 2H, *J* = 4.4, β-pyrrole); 8.82 (s, 6H, β-pyrrole); 8.17 (d, 6H, *J* = 8.0, 4-sulfonatophenyls); 8,05 (d, 6H, *J* = 8,0, 4-sulfonatophenyls); 7,87 (d, 2H, *J* = 8,0, 4-aminophenyl); 7,01 (d, 2H, *J* = 8.0, 4-aminophenyl); 5,59 (s, 2H, aniline-NH_2); −2,86 (br s, 2H, NH-pyrrole).



Aromatic region of the ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of the title compound.

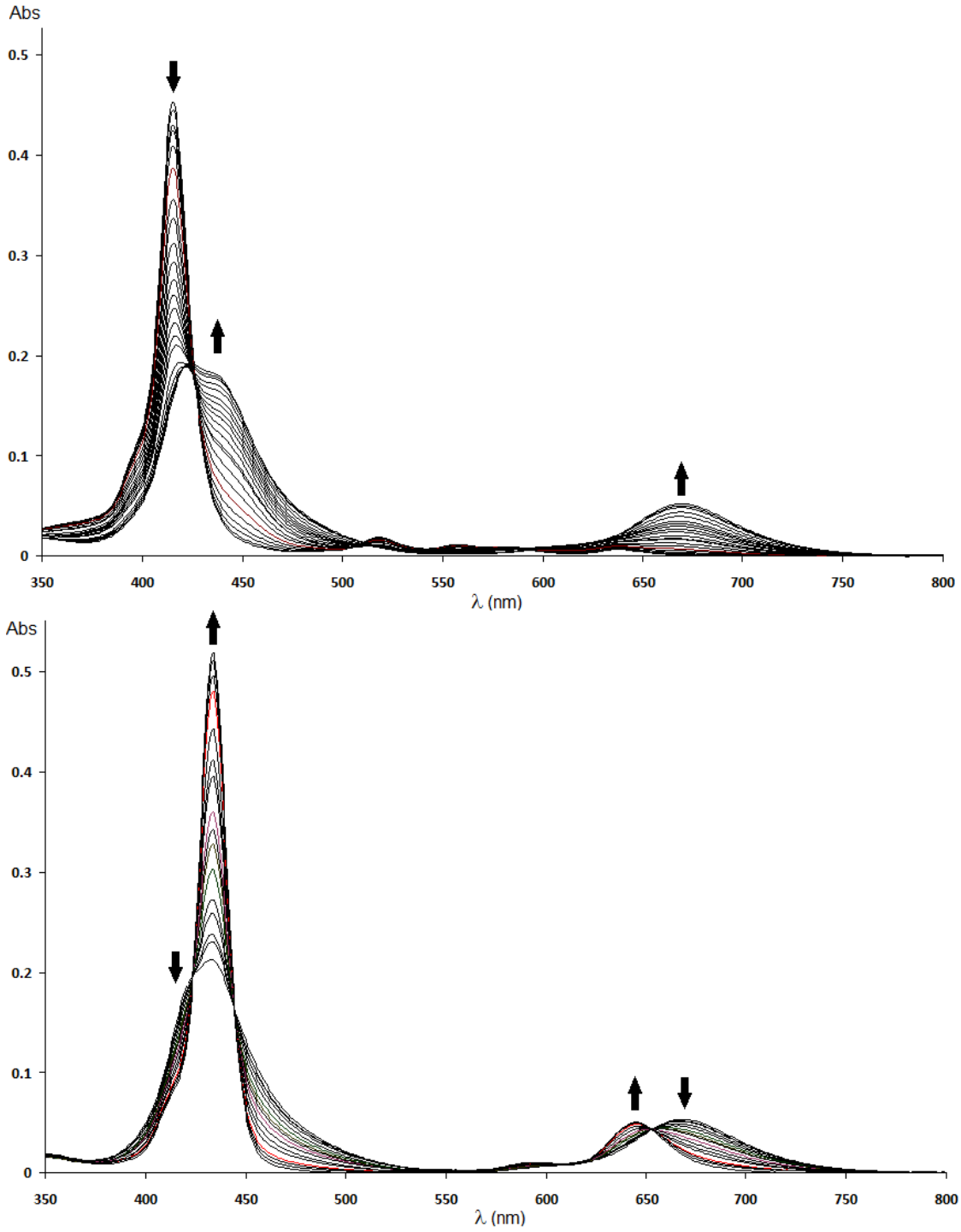
HPLC analysis of

5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin ($TPPS_3NH_2$) samples



Reverse-phase analytical HPLC chromatograms of **(a)** a mixture of $TPPS_3NH_2$ (18.4 min) and its regioisomer (18.9 min) in which one sulfonato group is located at the *meta* position of the phenyl ring, as obtained from the direct sulfonation of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin in hot sulfuric acid (*****impurity, up to 15 % of the total amount of porphyrin; the nature of this compound was reported in ref. 4); and (**b**) the sample of $TPPS_3NH_2$ used in this work obtained after tedious successive chromatographic enrichment using gradient elution (from H_2O to CH_3OH) in C-18 medium pressure column chromatography (*****impurity < 2 % of the total amount of porphyrin). Under the experimental conditions of sulfonation reported in the previous page, partially sulfonated porphyrins bearing only two sulfonato groups were not detected in any case.

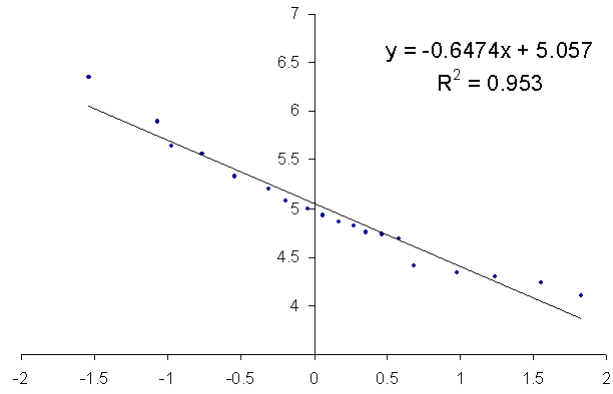
Spectrophotometic titration of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin ($TPPS_3NH_2$) with H_2SO_4 in water.



Spectrophotometric titration (cell path 1 cm) of a 1.40 10^{-6} M aqueous solution of $TPPS_3NH_2$ with an aqueous solution of sulfuric acid (see the quantitative details on the next page). The titration shows two well differentiated processes. Top: titration from the free-base porphyrin to its diprotonated form (inner core nitrogen atoms) leading to a splitting of the Soret band in the diacidic species (see the main text); Bottom: protonation of the anilinic nitrogen atom leading to the disappearance of the hyperporphyrinc effects (see the main text).

Numerical values used to calculate the pK_a values.
$[TPPS_3\text{-}NH_2] = 1.40\ 10^{-6}$ M

	Abs (a)	**[H_2SO_4] M** (b)	**pH** (c)	**log[H_4P^{2+}/H_2P]**
0	0.45			--
1	0.44	2.12 e-07	6.35	-1.54
2	0.43	6.34 e-07	5.90	-1.07
3	0.42	1.13 e-06	5.65	-0.97
4	0.41	1.39 e-06	5.56	-0.76
5	0.39	2.33 e-06	5.33	-0.54
6	0.35	3.18 e-06	5.20	-0.31
7	0.34	4.16 e-06	5.08	-0.19
8	0.31	4.99 e-06	5.00	-0.04
9	0.29	5.91 e-06	4.93	0.06
10	0.27	6.81 e-06	4.87	0.17
11	0.26	7.65 e-06	4.82	0.27
12	0.24	8.67 e-06	4.76	0.36
13	0.23	0.91 e-05	4.74	0.47
14	0.22	1.03 e-05	4.69	0.58
15	0.21	1.95 e-05	4.41	0.68
16	0.18	2.25 e-05	4.35	0.98
17	0.17	2.53 e-05	4.30	1.24
18	0.16	2.93 e-05	4.24	1.55
19	0.16	3.91 e-05	4.11	1.83
20	0.15			--



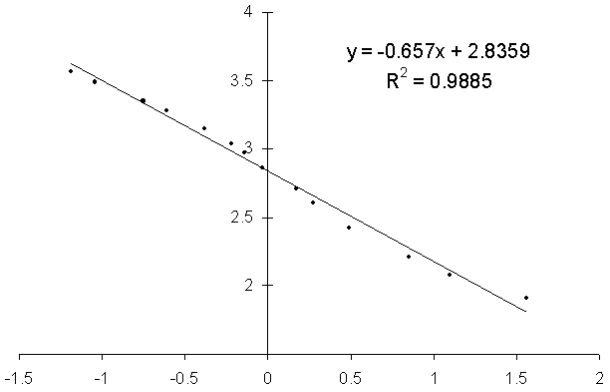
This unusually bad correlation for the inner-core protonation of the porphyrin ring has been attributed to the stabilization of the mono-protonated species by the peripheral amino group (ref. 5) leading to two separate pK_a values for the two-proton process.

(a) λ = 415 nm.

(b) The concentration in each solution has been corrected taking into account the amount of acid neutralized by the porphyrin (from the experimental $[H_4P^{2+}/H_2P]$ value and assuming a two proton acid-basic process). Uncorrected values, though, do not lead to meaningful differences in the linear correlation of the data points.

(c) Calculated from the concentration of sulfuric acid.

	Abs (a)	**[H_2SO_4] M** (b)	**pH** (c)	**log[H_5P^{3+}/H_4P^{2+}]**
21	0.21			--
22	0.23	1.38 e-04	3.57	-1.19
23	0.24	1.69 e-04	3.49	-1.04
24	0.26	2.23 e-04	3.35	-0.75
25	0.27	2.75 e-04	3.28	-0.61
26	0.30	3.79 e-04	3.15	-0.38
27	0.33	4.88 e-04	3.04	-0.22
28	0.34	5.87 e-04	2.97	-0.14
29	0.36	7.57 e-04	2.86	-0.03
30	0.39	1.12 e-03	2.71	0.17
31	0.41	1.44 e-03	2.61	0.27
32	0.44	2.28 e-03	2.42	0.49
33	0.48	3.97 e-03	2.21	0.85
34	0.49	5.61 e-03	2.08	1.10
35	0.51	8.89 e-03	1.91	1.56
36	0.52			--

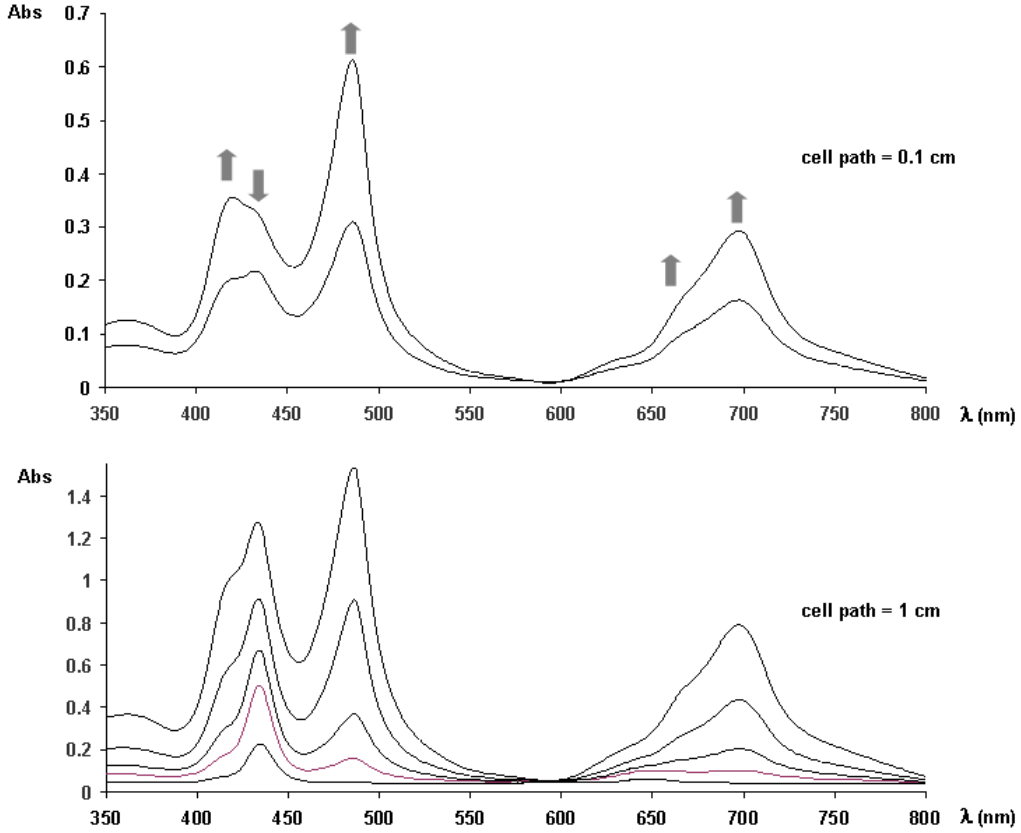


(a) λ = 433 nm.

(b) The concentrations of sulfuric acid are uncorrected (the acid concentration is at least two orders of magnitude higher than the porphyrin concentration).

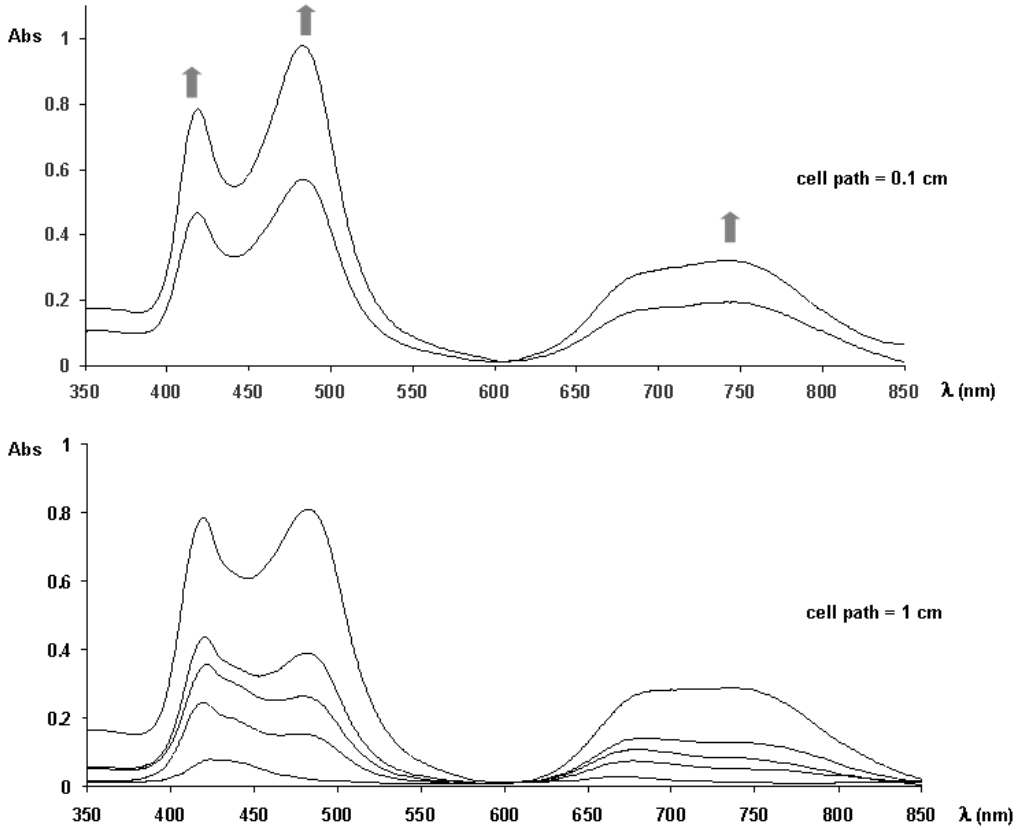
(c) Calculated from the concentration of sulfuric acid.

Aggregation of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin ($TPPS_3NH_2$) in aqueos HCl 0.1 M



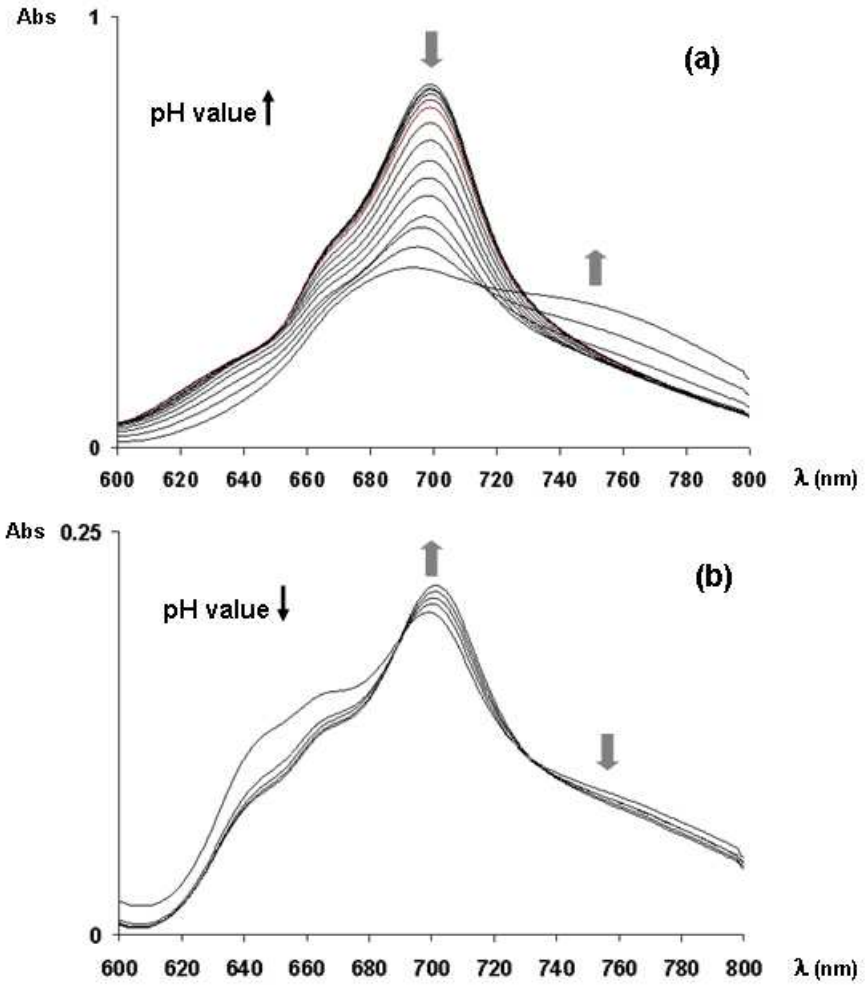
UV-Vis spectra of solutions of increasing concentration of $TPPS_3NH_2$ in aqueous HCl 0.1 M; (bottom): 7.88 10^{-7} M; 3.94 10^{-6} M; 7.88 10^{-6} M; 1.58 10^{-5} M; 3.15 10^{-5} M; (top): 5.91 10^{-5} M; 1.18 10^{-4} M. All spectra correspond to freshly prepared solutions obtained by the addition of an aliquot of an aqueous stock solution (~10^{-3} M) of the free-base porphyrin into the acidic water.

Aggregation of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin ($TPPS_3NH_2$) in AcOH/AcONa 0.1 M at pH=4



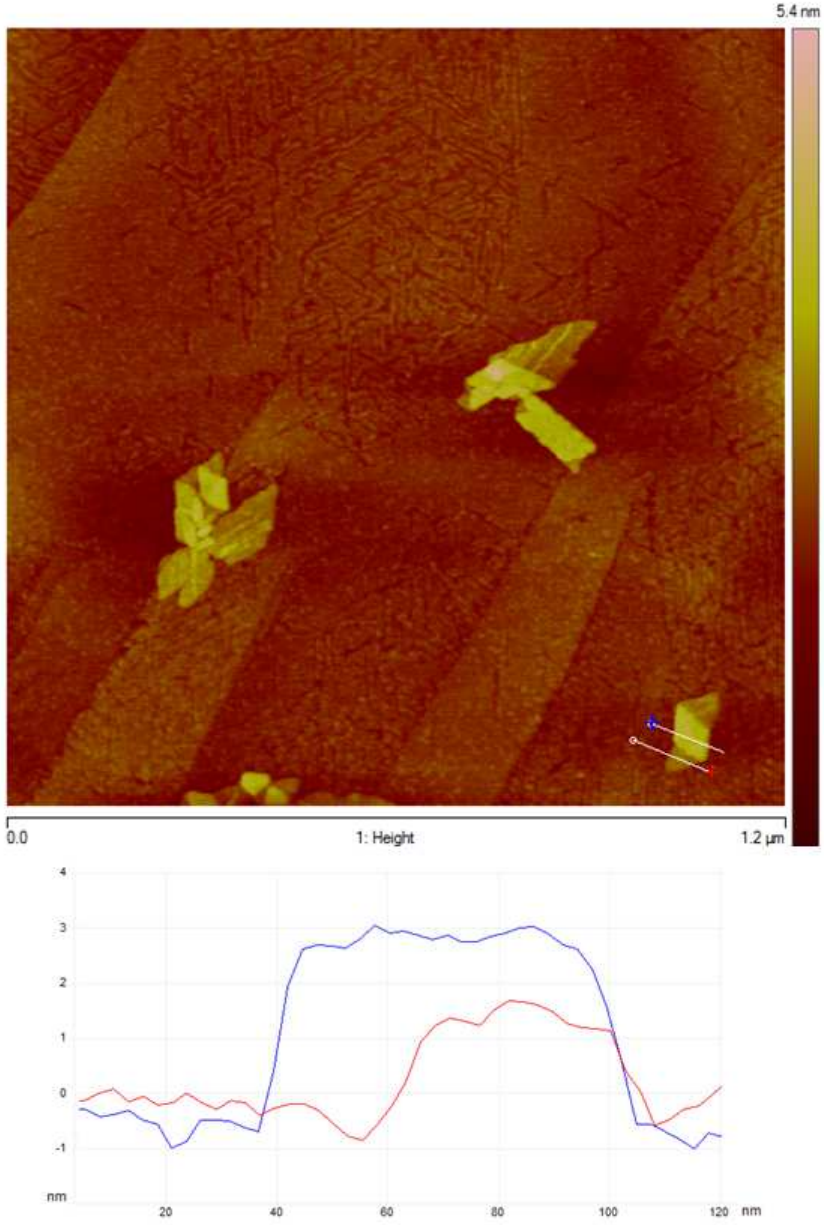
UV-Vis spectra of solutions of increasing concentration of $TPPS_3NH_2$ in buffered solution of AcOH/AcONa 0.1 M at pH=4.0; (bottom): $6.25\ 10^{-7}$ M; $3.13\ 10^{-6}$ M; $4.60\ 10^{-6}$ M; $6.25\ 10^{-6}$ M; $1.29\ 10^{-5}$ M; (top): $3.94\ 10^{-5}$ M; $7.09\ 10^{-5}$ M. All spectra correspond to freshly prepared solutions obtained by the addition of an aliquot of an aqueous stock solution (~10^{-3} M) of the free-base porphyrin into the acidic water.

Acid-base reversibility between J-aggregates of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin ($TPPS_3NH_2$) in acidic medium at different pH values

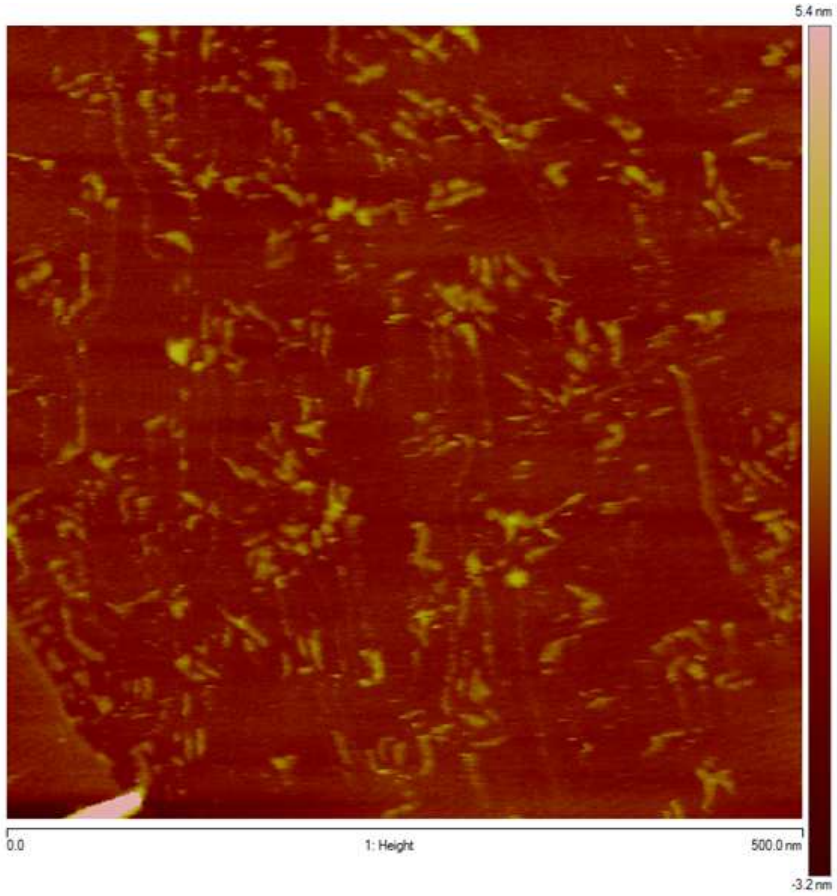


UV-Vis spectra obtained with **(a)** successive additions of small volumes of a NaOH 0.26 M solution into a 3.15 10^{-5} M solution of $TPPS_3NH_2$ in HCl 0.1 M. Further addition of base leads to the spectrum the monomeric free-base porphyrin; **(b)** successive additions of small volumes of an aqueous 2.25 M HCl solution into a 1.13 10^{-5} M solution of $TPPS_3NH_2$ in aqueous HCl 10^{-4} M; cell paths = 1 cm.

Atomic Force Microscopy images



PFM image (topographical map) of **$TPPS_3NH_2$** aggregates deposited on higly orientedd pyrolitic graphite (HOPG) from a 8.8 10^{-5} M aqueous solution of the porphyrin at pH=4.0 (HCl). The inset shows the height analysis of the section of the particle which points to the folding of a 2D monolayered structure into a bilayer.



PFM image (topographical map) of **$TPPS_3NH_2$** aggregates deposited on HOPG from a 4.0 10^{-5} M aqueous solution of the porphyrin at pH=1.3 (HCl). At this pH value the aggregates are composed of the triprotonated form and flocculate from the solution; the image evidences the lack of regular mono/bilayered structures such as those formed at higher pH values when the peripheral amino group is in its basic neutral form.

References

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