

# A Large Entropic Term Due to Water Rearrangement is Concomitant with the Photoproduction of Anionic Free-Base Porphyrin Triplet States in Aqueous Solutions<sup>†</sup>

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Received 2 September 2006; accepted 1 November 2006; published online 9 November 2006; DOI: 10.1562/2006-09-02-RA-1026

## ABSTRACT

The enthalpy change,  $\Delta_T H$ , and volume change,  $\Delta_T V$ , associated with triplet state formation upon excitation of free-base meso-tetra-(4-sulfonatophenyl)porphyrin, TSPP<sup>4-</sup>, its Zn derivative, ZnTSPP<sup>4-</sup>, and meso-tetra-(4-carboxyphenyl)porphyrin, TCPP<sup>4-</sup>, were obtained in aqueous solutions by the application of laser-induced optoacoustics spectroscopy in the presence of phosphate salts of various monovalent cations (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup> and Cs<sup>+</sup>). A linear correlation was found between  $\Delta_T H$  and  $\Delta_T V$  at different phosphate concentrations for the free-base porphyrins. The intercepts ( $132 \pm 8 \text{ kJ mol}^{-1}$  for TSPP<sup>4-</sup> and  $164 \pm 23 \text{ kJ mol}^{-1}$  for TCPP<sup>4-</sup>) of these plots correspond to the respective value of the triplet energy content obtained from phosphorescence at 77 K (140 and 149 kJ mol<sup>-1</sup>). This suggests that  $\Delta_T G$  for the triplet state formation is independent of the medium and an enthalpy–entropy compensation is responsible for the much smaller and salt-dependent  $\Delta_T H$  values obtained at room temperature. The Gibbs energy for triplet state formation of the free-base porphyrins at room temperature is thus mainly determined by the entropic term due to solvent rearrangement. The  $\Delta_T H$  values for <sup>3</sup>ZnTSPP<sup>4-</sup> at different buffer concentrations and different cations are all between 130 and 150 kJ mol<sup>-1</sup>, close to the triplet energy obtained from phosphorescence ( $E_T = 155 \text{ kJ mol}^{-1}$ ). The solvent structure and the nature of the counterion have a negligible influence on the <sup>3</sup>ZnTSPP<sup>4-</sup> formation due to the blockage of the electron pairs on the central N atoms. Thus, the small  $\Delta_T V$  value should be due to intrinsic bond changes upon <sup>3</sup>ZnTSPP<sup>4-</sup> formation and no correlation between  $\Delta_T H$  and  $\Delta_T V$  should be expected in this case. The enthalpy change determines the Gibbs energy for <sup>3</sup>ZnTSPP<sup>4-</sup> formation at room temperature.

## INTRODUCTION

The entropy change for the triplet-triplet energy transfer process has been analyzed in several fundamental papers (1–3). An important conclusion has been that the entropy change for the triplet energy transfer reaction in organic solvents is associated with intrinsic geometric changes in the molecular

species. For example, entropy changes determined by direct measurement of the temperature dependence of the equilibrium constant between triplet energy donors and acceptors by nanosecond laser flash photolysis were attributed to intrinsic changes in the flexible partner in the triplet-triplet energy transfer process (2). Thus, when a nonrigid energy acceptor, such as 4-methylbiphenyl, or donor, such as 4-methylbenzophenone, goes from the ground to the triplet state or from the triplet to the ground state, respectively, the so-called rigid partner, *e.g.*, 10,10-dimethylanthrone as energy donor and 9,9-fluorene as energy acceptor, was assumed to have a negligible entropic change, and conclusions were drawn about the conformational changes in the flexible partner giving rise to the entropic changes (2). It is, however, difficult to measure directly the entropy change upon triplet state formation, without resorting to an energy or electron transfer equilibrium process.

In a series of laser-induced optoacoustic spectroscopy (LIOAS) studies with water-soluble chromophores we have shown that, in systems in which the strong water–chromophore interactions determine the enthalpy and structural volume changes produced during the photoinduced reaction, there is a compensation between those changes when measured in aqueous solutions with different structurally perturbing cations and/or anions (depending on the systems). The variations observed in the thermodynamic parameters upon variations in the solvent properties were attributed to changes in specific chromophore–medium (water or even protein environment) interactions (4,5). When the Gibbs energy is the same irrespective of the medium-perturbing factor, this enthalpy–structural volume change compensation affords the entropy change for the particular reaction analyzed, *e.g.*, for the formation of free radicals in the case of the quenching of the metal-to-ligand charge transfer state of Ru(bby)<sub>3</sub><sup>2+</sup> by the methyl viologen dication (6) as well as for the quenching of flavin triplet states by amines and amino acids, again in the presence of various monovalent cations (7,8).

We have recently analyzed by LIOAS the triplet state formation upon excitation of meso-tetrakis-(4-sulfonatophenyl)porphyrin, TSPP<sup>4-</sup>, in the presence of various monovalent cations (9). We found a linear correlation between the enthalpy change ( $\Delta_T H$ ) and the volume change ( $\Delta_T V$ ) for the triplet state formation, with an intercept (*i.e.*,  $\Delta_T V = 0$ ) similar to the triplet energy determined by phosphorescence at 77 K. The interpretation of these data is that in fluid media the entropic

<sup>†</sup>This paper is part of a symposium-in-print dedicated to Professor Eduardo A. Lissi on the occasion of his 70th birthday.

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term for triplet state formation is large and has an important contribution from the solvent (water) reorganization, in turn dependent on the water structure (9).

The large value of  $\Delta_T V$  associated with the triplet state formation of free-base water-soluble porphyrins (a contraction of *ca* 8 cm<sup>3</sup> mol<sup>-1</sup>) (10) together with the small value of  $\Delta_T V$  for the same process in the Zn(II) porphyrin chelates, in protonated porphyrins, in porphyrin dimers, and in free-base nonwater-soluble porphyrin in micelles (11) were the basis for the postulation that the hydrogen bridges between water and the nitrogen atoms of the porphyrin ring and the changes in those interactions with water upon formation of the triplet state are responsible for the changes in the solvation sphere that give rise to the large  $\Delta_T V$  values. When those interactions are blocked such as in the protonated porphyrins, in the dimers, and in the Zn(II) derivatives, or when the nonwater-soluble porphyrin tetraphenylporphyrin is in a nonaqueous medium (micelles), the values of  $\Delta_T V$  are very small (11).

In the present study we extend the studies on the influence of different phosphate salts and different concentrations on the triplet state formation of free-base tetracarboxylated porphyrin, TCPP<sup>4-</sup>, of free-base tetrasulfonated porphyrin, TSPP<sup>4-</sup>, and of its Zn(II) derivative, ZnTSPP<sup>4-</sup> and obtain interesting conclusions regarding the entropy changes upon triplet state formation.

## MATERIALS AND METHODS

*Meso*-tetrakis-(4-sulfonatophenyl)porphyrin, TSPP<sup>4-</sup>, free base, its Zn(II) chelate, ZnTSPP<sup>4-</sup>, and *meso*-tetrakis-(4-carboxyphenyl)porphyrin, TCPP<sup>4-</sup>, were obtained from Porphyrin Products. The calorimetric reference, Bromocresol Purple (BCP, Fluka, standard grade), lithium dihydrogen phosphate, LiH<sub>2</sub>PO<sub>4</sub> (Aldrich, 99%), lithium hydroxide, LiOH (Sigma, 99%), sodium dihydrogen phosphate, NaH<sub>2</sub>PO<sub>4</sub> (Merck, 99%), disodium hydrogen phosphate, Na<sub>2</sub>HPO<sub>4</sub> (Fluka, 99%), potassium dihydrogen phosphate, KH<sub>2</sub>PO<sub>4</sub> (Merck, 99%), dipotassium hydrogen phosphate, K<sub>2</sub>HPO<sub>4</sub> (Merck, 99%), cesium dihydrogen phosphate/dicesium hydrogen phosphate, CsH<sub>2</sub>PO<sub>4</sub>/Cs<sub>2</sub>HPO<sub>4</sub> (Aldrich, 99%), ammonium dihydrogen phosphate, NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> (Fluka, 99%), diammonium hydrogen phosphate, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (Fluka, 99%) were used as received. Water was deionized and purified by a Millipore-Milli-Q System equipment.

The buffers were prepared as already published (7–9). Solutions of TSPP<sup>4-</sup> and ZnTSPP<sup>4-</sup> were prepared by dissolving the necessary amount of chromophore in the different buffer solutions to give a final concentration of 20 × 10<sup>-6</sup> M with an absorbance of approximately 0.1 at the excitation wavelength, whereas for TCPP<sup>4-</sup> the final concentrations were 4 × 10<sup>-7</sup> M with an absorbance approximately 0.025 at the excitation wavelength and 4.2 × 10<sup>-6</sup> M for some fluorescence measurements. The precise absorbance depended on the experiments. The solutions were purged with argon during 20 min before the measurements.

Absorption spectra were recorded using a Shimadzu UV-2401 PC spectrophotometer.

Steady-state fluorescence measurements were performed using a Cary Eclipse (Varian) spectrofluorometer delivering noncorrected spectra. The measurements were performed in a quartz cell (1 cm pathlength) at room temperature. Fluorescence spectra were registered between 600 and 800 nm exciting at different wavelengths with 5 nm excitation and emission slits, 750 V PMT voltage, 120 nm min<sup>-1</sup> scan rate and 1 nm data interval. To avoid inner filter effects, the absorption of the solutions at the excitation wavelength was kept < 0.10.

Transient absorption measurements were carried out with the equipment previously described (9,12). Laser excitation at 425, 518 and 580 nm for the transient absorption and the LIOAS experiments was from a Nd:YAG-pumped Beta Barium Borate Optical Parametric Oscillator (OPO-C-355; Laser Technik Vertriebs GmbH, Ertes-

tadt-Friesheim, Germany), as previously described (13). Excitation pulses at 568 nm were from a dye laser (SL4000 G; Spectron Laser System, Rugby, UK) with rhodamine-6 G, pumped by the 11-ns pulses from a Nd:YAG laser (SL802; Spectron Laser System), whereas at 532 nm the second harmonic was used directly. Incident laser energies were varied with a neutral density wedge and measured with a pyroelectric energy meter (RJP732 head connected to an Rm-6600A Universal Radiometer; Laser Probe, Inc.). The experiments were carried out in the linear regime of amplitude *vs* laser fluence, up to 200 μJ/pulse. The analyzing beam from a pulsed 150 W Xe arc was focused into the 1 cm optical-pathlength sample cuvette, perpendicular to the excitation, after passing through a monochromator, focused again into a second monochromator slit and detected by a Hamamatsu R3896 photomultiplier wired with a five dynode chain for rapid detection. The output of the photomultiplier was fed through a 50 Ω load resistor into a TDS 520A Tektronix transient recorder. The signals were taken by and elaborated with a home-made program in a Compaq Tru64 Unix workstation. 49 signals were averaged for each decay. A personal computer with Origin program was used for further treatment of the averaged transient absorbance data.

The LIOAS setup has been described in several papers (11,13–16). Excitation was performed using the laser described above. The cuvette was in a temperature-controlled (±0.02°C) holder FLASH 100 (Quantum Northwest, Spokane, WA) equipped with a V103-RM ultrasonic transducer. The signal was fed into a 5662 preamplifier (Panametrics, Inc., Waltham, MA). One hundred shots were averaged for each waveform of the sample and calorimetric reference each time under the same conditions. Absorbances at  $\lambda_{exc}$  for the calorimetric reference and the samples were matched within 2% and were *ca* 0.10 or 0.025 depending on the substrate (the exact value depending on the sample). The laser beam was shaped by a 0.5 mm-width slit in front of the cuvette (13).

The LIOAS signal analysis procedure has been widely described in several publications and reviewed (11,14–16). The pressure evolution upon excitation of the sample is often assumed to be a sum of mono-exponential functions, but only one exponential was enough to fit the signals with the systems studied in this work. The convolution of the sample decay function and the reference signal (obtained by exciting the reference compound in a solution with the same buffer concentration as the sample) yields the fractional amplitudes ( $\varphi_i$ ) and the lifetimes ( $\tau_i$ ) of the transients (Sound Analysis 3000; Quantum Northwest, Inc., Spokane, WA). At a given temperature and for each resolved *i*-th step the fractional amplitude  $\varphi_i$  is the sum of the fraction of absorbed energy released as heat and a term containing the molar structural volume change ( $\Delta_i V$ ), according to Eq. (1) (14,17),

$$\varphi_i = \alpha_i + \frac{\Phi_i \Delta_i V}{E_\lambda} \left( \frac{c_p \rho}{\beta} \right)_T \quad (1)$$

$$\text{with } \alpha_i = \frac{q_i}{E_\lambda}$$

where  $E_\lambda$  is the molar excitation energy,  $\Phi_i$  is the quantum yield of the respective process,  $q_i$  is the heat released in the step involved,  $\beta = (\partial V/\partial T)_P/V$  is the volume expansion coefficient,  $c_p$  is the specific heat capacity at constant pressure, and  $\rho$  is the mass density of the solvent, the three latter quantities at temperature  $T$  (several temperatures method, ST). The temperature where  $\beta = 0$ ,  $T_{\beta=0}$ , was the same as in neat water ( $T_{\beta=0} = 3.90^\circ\text{C}$ ) (14) up to 10 mM of all cation buffers. Above this concentration,  $T_{\beta=0}$  was lower in the presence of the various cations and different concentrations of them. The  $T_{\beta=0}$  values were between 3.10 and 3.60°C. The values of  $(c_p \rho/\beta)_T$  (Table 2) were determined by comparing the LIOAS signals of the reference (BCP) in neat water with those in the desired solution at each temperature following the procedure described by Gensch *et al.* (14).

The two-temperatures (TT) method in LIOAS (14) was applied to determine the values of the enthalpy and the structural volume change in some cases. The calorimetric reference and the sample solution (both in the same buffer), at matched absorbances at  $\lambda_{exc}$ , were measured at  $T_{\beta>0} = 6^\circ\text{C}$ . At  $T_{\beta=0}$  the sample signal observed should only be due to structural volume change ( $\Delta_{str} V$ ). Upon deconvolution

of the signal for the sample at  $T_{\beta=0}$  and at  $T_{\beta>0}$  with those for the reference at 6°C, the values of the enthalpy and structural volume change are derived, under the assumption that the isothermal compressibility is the same at both temperatures. This is a very good assumption for aqueous solutions (14). The volume change and the fraction of heat released were calculated with Eqs. (2) and (3).

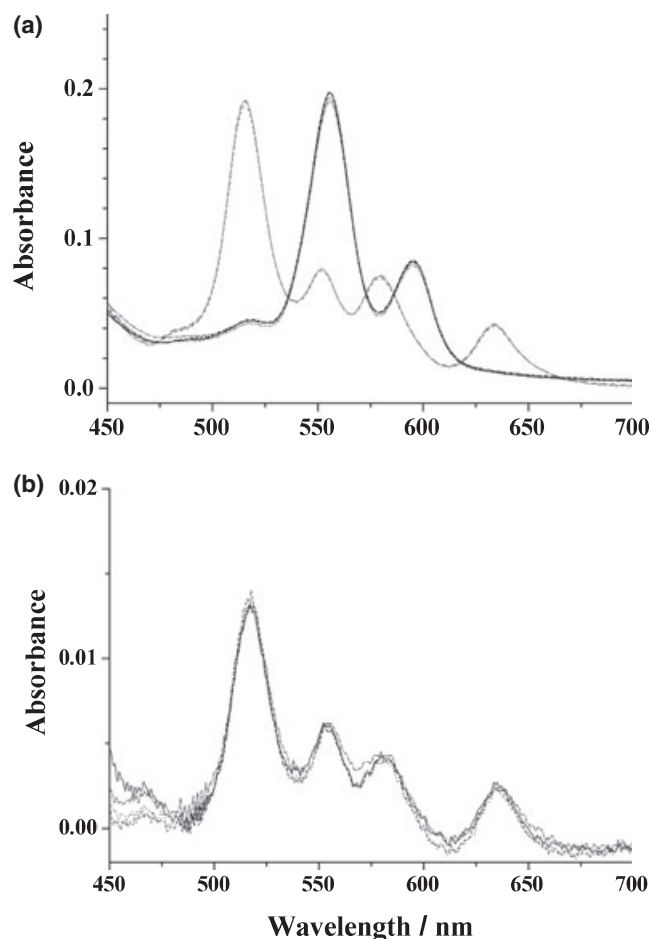
$$\Delta V_i = \varphi_{i(T_{\beta=0})} \left( \frac{\beta}{c_p \rho} \right)_{T_{\beta>0}} E_{\lambda} \quad (2)$$

$$\alpha_i = \varphi_{i(T_{\beta>0})} - \varphi_{i(T_{\beta=0})} \quad (3)$$

## RESULTS

### Absorption spectra

The absorption spectra of the porphyrins in the presence of the various cations were all identical (Fig. 1), and were the same before and after laser exposure.



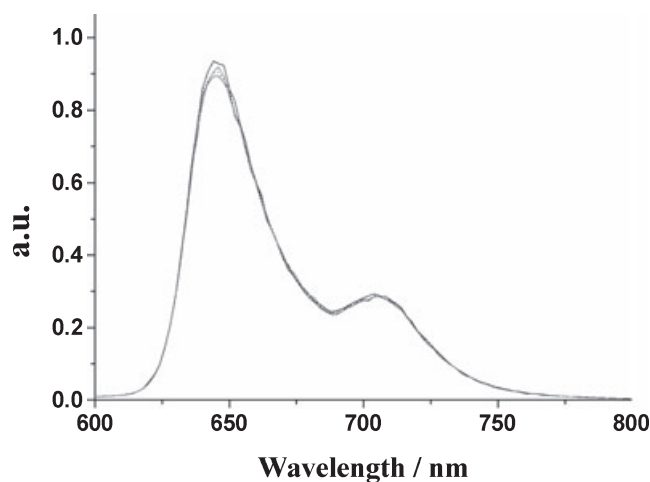
**Figure 1.** Absorption spectra of (a) TSPP<sup>4-</sup> in phosphate buffer of 9 × 10<sup>-3</sup> M (—) Cs<sup>+</sup>, (- - -) Li<sup>+</sup>, (.....) 18 × 10<sup>-3</sup> M Cs<sup>+</sup> and of ZnTSPP<sup>4-</sup> in phosphate buffer of 75 × 10<sup>-3</sup> M (—) NH<sub>4</sub><sup>+</sup>, (---) Na<sup>+</sup>, (.....) Li<sup>+</sup>, 15 × 10<sup>-3</sup> M (- · - · -) Cs<sup>+</sup>, and (- - - - -) K<sup>+</sup> and (b) 4 × 10<sup>-7</sup> M TCPP<sup>4-</sup> in the presence of 0, 15, 30 and 75 × 10<sup>-3</sup> M Cs<sup>+</sup> as phosphate buffer (the traces essentially overlap within the error of the measurements).

### Fluorescence measurements

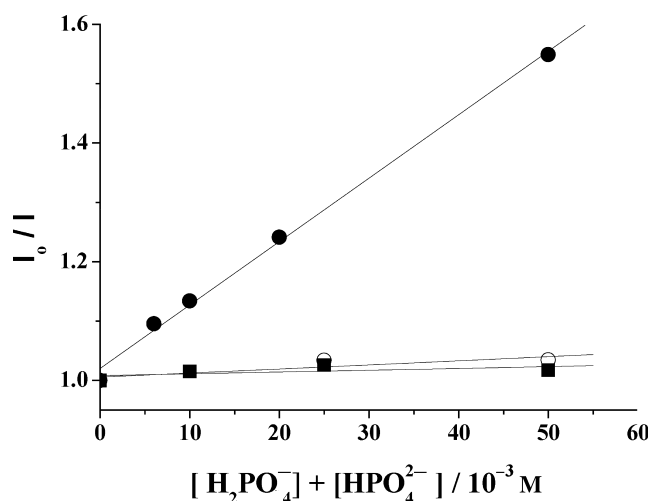
In view of the higher aggregation tendency of TCPP<sup>4-</sup> than TSPP<sup>4-</sup>, fluorescence measurements were carried out with the former in order to find the maximum concentration at which aggregates can be neglected.

The fluorescence emission maxima of TCPP<sup>4-</sup> in aqueous solution are at 646 and 702 nm. In all cases, the fluorescence spectrum (normalized relative to the maximum emission value for comparative purposes) remained unchanged irrespective of the excitation wavelength.

Emission spectra of 4 × 10<sup>-7</sup> M TCPP<sup>4-</sup> were recorded in the presence of different concentrations of Li<sup>+</sup> and Cs<sup>+</sup> phosphate salts (typically 0, 10, 25 and 50 × 10<sup>-3</sup> M, corresponding, respectively, to 0, 15, 37.5 and 75 × 10<sup>-3</sup> M cation concentrations). In all cases, the emission spectra remained almost the same (Fig. 2) and negligible quenching is observed (Fig. 3).



**Figure 2.** Noncorrected fluorescence spectra ( $\lambda_{\text{exc}} = 425$  nm) of a 4 × 10<sup>-7</sup> M aqueous solution of TCPP<sup>4-</sup> with different concentration of Li<sup>+</sup> as phosphate: (—) 0 M, (.....) 15 × 10<sup>-3</sup> M, (- · - · -) 37.5 × 10<sup>-3</sup> M and (- - - - -) 75 × 10<sup>-3</sup> M.



**Figure 3.** Stern-Volmer plot for the quenching of the integrated fluorescence intensity from TCPP<sup>4-</sup> by phosphate salts of (●) Cs<sup>+</sup>, [TCPP<sup>4-</sup>] = 4.2 × 10<sup>-6</sup> M,  $\lambda_{\text{exc}}$  532 nm, (○) Cs<sup>+</sup> and (■) Li<sup>+</sup> for [TCPP<sup>4-</sup>] = 4 × 10<sup>-7</sup> M,  $\lambda_{\text{exc}}$  425 nm.

Absorption and fluorescence spectroscopic studies indicate that at higher concentration ( $4.2 \times 10^{-6}$  M) TCPP $^{4-}$  is highly aggregated at the same pH (6.9 in buffered solutions). A significant decrease in the emission as a function of the concentration of phosphate salt is then observed (Fig. 3, ●). These results are in good agreement with the UV–visible spectra showing aggregation of TCPP $^{4-}$  as the concentration of the phosphate salts is increased (results not shown). Thus, the fluorescence quenching is probably caused by salt-mediated aggregation of TCPP $^{4-}$ .

### Transient absorption measurements

The triplet absorbance decays of the three porphyrins in the presence of various concentration of phosphate buffer are shown in Fig. 4a–c for TSPP $^{4-}$ , ZnTSPP $^{4-}$  and TCPP $^{4-}$ , respectively. For the purpose of comparison, the experiments were carried out with solutions of the same absorbance at the excitation wavelength and under experimental conditions where no aggregation occurs. The decay traces in the various salts for each porphyrin overlap each other demonstrating that the values of  $\Phi_T$  do not change in the presence of different cations. Different time scales are shown to illustrate that the various salts at various concentrations affect neither the triplet yield nor the triplet decay.

### LIOAS

The several-temperatures (ST) method in LIOAS was in general used for the elaboration of the data upon excitation of TSPP $^{4-}$ , ZnTSPP $^{4-}$  and TCPP $^{4-}$ , whereas in some experiments with TSPP $^{4-}$ , the two-temperatures (TT) method was used. Only one exponential term was needed to fit the data for the convolution of the signals for the sample and reference (both in the same buffer) at each temperature.

Good linear correlations were found when  $\varphi$  was plotted as a function of the ratio of thermoelastic parameters (Eq. [1]) with the ST method (examples are shown in Fig. 5, data in Table 1). The data obtained with the TT method (Eqs. [2] and [3]) are also in Table 1.

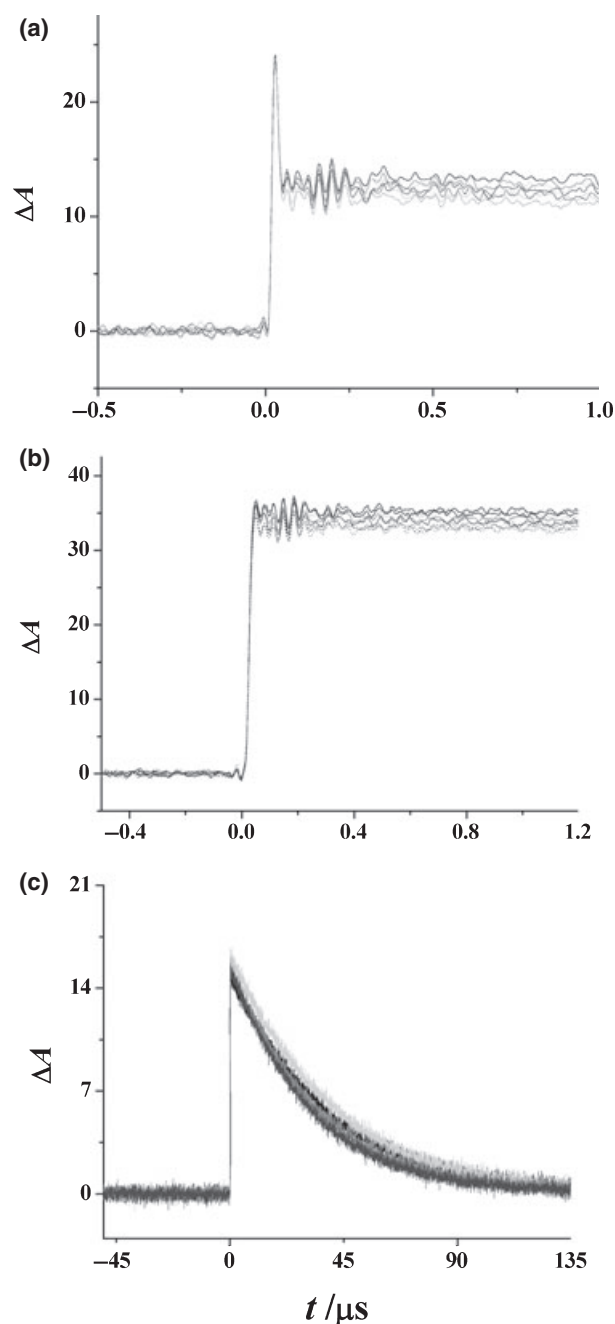
From simple energy balance considerations, the fraction of heat released during the production of triplet state,  $\alpha$ , is related to the triplet enthalpy content,  $\Delta_T H$ , according to Eq. (4),

$$\Delta_T H = \frac{E_\lambda - \alpha E_\lambda - E_F \Phi_F}{\Phi_T} \quad (4)$$

where  $E_F$  is the average energy of the fluorescence emission,  $\Phi_F$  is the fluorescence quantum yield and  $E_\lambda$  is the molar excitation energy. The values used were  $E_F = 174$  kJ mol $^{-1}$  (10),  $\Phi_F = 0.058$  (10),  $\Phi_T = 0.78$  (18–20) for TSPP $^{4-}$ ,  $E_F = 198$  kJ mol $^{-1}$  (18),  $\Phi_F = 0.043$  (18),  $\Phi_T = 0.84$  (18) for ZnTSPP $^{4-}$ , and  $E_F = 183$  kJ mol $^{-1}$  (18),  $\Phi_F = 0.03$  (the value in ethanol was used [21]),  $\Phi_T = 0.76$  in phosphate buffer (22) identical for all cations and concentrations for TCPP $^{4-}$  (as shown in Fig. 4).

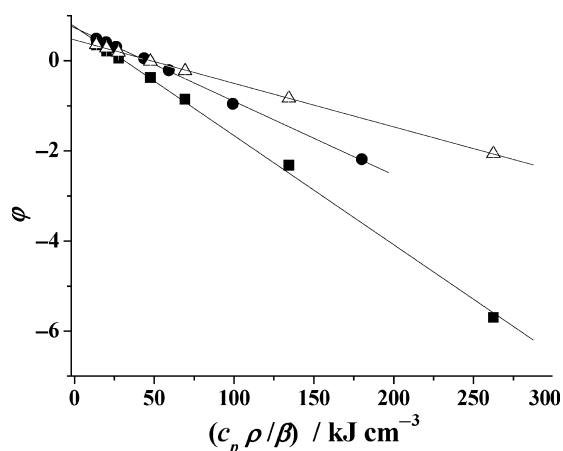
### DISCUSSION

The exponential term sufficient for the fitting of the LIOAS signals and associated with a short time constant



**Figure 4.** Triplet absorption decay of (a) TSPP $^{4-}$ ,  $\lambda_{\text{exc}} = 532$  nm,  $\lambda_{\text{obs}} = 460$  nm, (b) ZnTSPP $^{4-}$ ,  $\lambda_{\text{exc}} = 560$  nm,  $\lambda_{\text{obs}} = 470$  nm and (c) TCPP $^{4-}$ ,  $\lambda_{\text{exc}} = 425$  nm,  $\lambda_{\text{obs}} = 400$  nm. The traces overlap for solutions in the presence of phosphate buffer in (a) of Li $^+$  9, 18 and  $90 \times 10^{-3}$  M, as well as Cs $^+$  9, 37.5 and  $37.5 \times 10^{-3}$  M + 5% ethanol, in (b) of  $75 \times 10^{-3}$  M Cs $^+$ , K $^+$ , NH $_4^+$ , Li $^+$ , and  $15 \times 10^{-3}$  M Cs $^+$ , K $^+$ , NH $_4^+$ , Na $^+$  and Li $^+$  in (c) of  $75 \times 10^{-3}$  M Cs $^+$ , Na $^+$ , Li $^+$  and K $^+$ , [TCPP $^{4-}$ ] =  $4 \times 10^{-7}$  M. The spike in (a) is caused by fluorescence.

( $\tau < 10$  ns) was assigned to the production of the respective triplet state. The decay of the various triplet states analyzed under all conditions takes place in a time longer (several  $\mu$ s) than the integration time of the experiment in the Ar-purged solutions.



**Figure 5.** Relationship between  $\phi$  and  $(c_p \rho / \beta)_T$  according to Eq. (1) in phosphate buffer with different cations and concentrations for  $\text{TSPP}^{4-}$  in (●)  $37.5 \times 10^{-3} \text{ M Cs}^+$ , (■)  $9 \times 10^{-3} \text{ M Li}^+$  and (Δ)  $\text{ZnTSPP}^{4-}$  in  $15 \times 10^{-3} \text{ M K}^+$ ; pH = 6.9  $\pm$  0.1.

### $\text{TSPP}^{4-}$

$\text{TSPP}^{4-}$  and its Zn(II) derivative do not form any aggregate at neutral pH up to about  $10^{-3} \text{ M}$  (11,18,23), whereas  $\text{TCPP}^{4-}$  produces aggregates under some conditions even at pH = 7.50 (24) (*vide infra*).

The changes in water structure, due to different phosphate salts and concentrations, produce changes in the enthalpy and

volume changes in a wide range of *ca*  $50 \text{ kJ mol}^{-1}$  for  $\Delta_T H$  and of  $4 \text{ cm}^3 \text{ mol}^{-1}$  for  $\Delta_T V$ . A good linear correlation was obtained between  $\Delta_T H$  and  $\Delta_T V$  with a slope of  $13 \pm 2 \text{ kJ cm}^{-3}$  and an intercept of  $132 \pm 8 \text{ kJ mol}^{-1}$  (Fig. 6).

The buffer concentration and the nature of the cations (Table 1, Fig. 6) produce changes in the  $\Delta_T H$  and  $\Delta_T V$  values for  ${}^3\text{TSPP}^{4-}$  formation, although they do not affect the formation quantum yield (Fig. 4). The intercept in Fig. 6 agrees, within experimental error, with the value for the triplet energy obtained from phosphorescence data at 77 K ( $E_T = 140 \text{ kJ mol}^{-1}$ ) (18). This suggests that  $\Delta_T G$  for the triplet state formation is the same, independently of the measuring conditions, and an enthalpy–entropy compensation is responsible for the much smaller  $\Delta_T H$  values obtained at room temperature and in the presence of various salts.

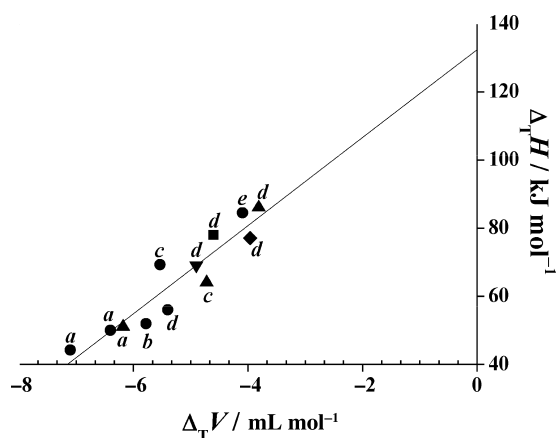
The role of the salt concentration in the energetic level and also in the structural volume change for  ${}^3\text{TSPP}^{4-}$  formation is documented by the values for  ${}^3\text{TSPP}^{4-}$  formation previously published in  $10^{-2} \text{ M}$  Tris buffer,  $\Delta_T V = -8.8 \text{ cm}^3 \text{ mol}^{-1}$  (10,11), whereas  $\Delta_T V$  varies between  $-3$  and  $-7 \text{ cm}^3 \text{ mol}^{-1}$  in phosphate buffer, depending on the salt concentration (see Table 1).

Different salt concentrations produce changes in the substrate environment structure, as shown by the variation in the  $(c_p \rho / \beta)$  values with temperature (Table 2). At  $6 \times 10^{-3} \text{ M}$  phosphate the  $(c_p \rho / \beta)$  values are the same as in neat water, whereas at higher salt concentration  $(c_p \rho / \beta)$  changes with the cation. Therefore, at high salt concentration a correlation

**Table 1.** Fraction of heat released,  $\alpha$ , structural volume change,  $\Delta_T V$ , and enthalpy change,  $\Delta_T H$ , for triplet state formation of porphyrins in the presence of different concentrations of various cations ( $\text{M}^+$ ) in phosphate buffers.\*

$[\text{M}^+] / \times 10^{-3} \text{ M}$	Method	$\alpha \pm 0.05$	$\text{cm}^{-3} \Phi_T \Delta_T V / \pm 0.07$	$\text{kJ mol}^{-1} q / \pm 10$	$\text{cm}^3 \text{ mol}^{-1} \Delta_T V / \pm 0.5$	$\text{kJ mol}^{-1} \Delta_T H / \pm 15$
$\text{TSPP}^{4-}$						
$\text{Li}^+ 9^\dagger$	TT	0.80	-5.54	180	-7.1	44
$\text{Li}^+ 9^\ddagger$	ST	0.76	-4.99	157	-6.4	50
$\text{Cs}^+ 9^\ddagger$	ST	0.75	-4.82	156	-6.2	51
$\text{Li}^+ 18^\dagger$	TT	0.78	-4.51	174	-5.8	52
$\text{Li}^+ 37.5^\dagger$	TT	0.72	-4.31	161	-5.5	69
$\text{Li}^+ 90^\dagger$	TT	0.66	-3.20	149	-4.1	84
$\text{Cs}^+ 37.5^\dagger$	ST	0.74	-3.68	166	-4.7	64
$\text{ZnTSPP}^{4-\S}$						
$\text{Li}^+ 15$	ST	0.50	-2.30	105	-2.7	132
$\text{Na}^+ 15$	ST	0.45	-1.93	94	-2.3	146
$\text{K}^+ 15$	ST	0.46	-2.03	98	-2.4	141
$\text{NH}_4^+ 15$	ST	0.43	-1.96	90	-2.3	150
$\text{Cs}^+ 15$	ST	0.51	-2.21	108	-2.6	129
$\text{Li}^+ 75$	ST	0.52	-2.27	110	-2.7	127
$\text{Na}^+ 75$	ST	0.50	-2.53	105	-3.0	133
$\text{K}^+ 75$	ST	0.51	-2.31	107	-2.7	130
$\text{NH}_4^+ 75$	ST	0.51	-2.52	106	-3.0	131
$\text{Cs}^+ 75$	ST	0.52	-2.44	109	-2.9	128
$\text{TCPP}^{4-\parallel}$						
$\text{Li}^+ 9$	ST	0.89	-4.5	208	-5.9	22
$\text{Na}^+ 9$	ST	0.90	-4.4	208	-5.8	22
$\text{Cs}^+ 9$	ST	0.96	-4.7	223	-6.2	11
$\text{Li}^+ 75$	ST	0.85	-3.8	198	-5.0	45
$\text{Na}^+ 75$	ST	0.81	-3.4	188	-4.5	57
$\text{K}^+ 75$	ST	0.84	-3.3	194	-4.3	49
$\text{NH}_4^+ 75$	ST	0.78	-3.5	180	-4.6	68
$\text{Cs}^+ 75$	ST	0.82	-3.2	190	-4.2	53

\*pH = 6.9  $\pm$  0.1, the errors are standard deviations of the data from several measurements.  $^\dagger \lambda_{\text{exc}} = 532 \text{ nm}$ .  $^\ddagger \lambda_{\text{exc}} = 580 \text{ nm}$ .  $^\S \lambda_{\text{exc}} = 568 \text{ nm}$ .  $^\parallel \lambda_{\text{exc}} = 517 \text{ nm}$ ,  $[\text{TCPP}^{4-}] = 4 \times 10^{-7} \text{ M}$ .



**Figure 6.** Relationship between the enthalpy change,  $\Delta_T H$ , and the structural volume change,  $\Delta_T V$ , for  ${}^3\text{TSPP}^{4-}$  formation, as derived from LIOAS data. (●)  $\text{Li}^+$ , (▼)  $\text{Na}^+$ , (■)  $\text{NH}_4^+$ , (◆)  $\text{K}^+$  and (▲)  $\text{Cs}^+$  at various concentrations:  $a = 9 \times 10^{-3} \text{ M}$ ,  $b = 18 \times 10^{-3} \text{ M}$ ,  $c = 37.5 \times 10^{-3} \text{ M}$ ,  $d = 75 \times 10^{-3} \text{ M}$  (9) and  $e = 90 \times 10^{-3} \text{ M}$ . The slope of the linear fitting is  $12 \pm 2 \text{ kJ cm}^{-3}$  and the intercept,  $132 \pm 8 \text{ kJ mol}^{-1}$ .

**Table 2.** Ratio of thermoelastic parameters at various temperatures in the presence of different phosphate salt concentrations.\*

Temp (°C)	$(c_p \rho / \beta)_T / \text{kJ mol}^{-1}$				
	$\text{H}_2\text{O}^\dagger$	$\text{Li}^+$ $9 \times 10^{-3} \text{ M}$	$\text{Li}^+$ $18 \times 10^{-3} \text{ M}$	$\text{Li}^+$ $75 \times 10^{-3} \text{ M}$	$\text{Cs}^+$ $75 \times 10^{-3} \text{ M}$
30	13.710	13.71	13.71	13.43	13.41
20	20.174	20.17	20.17	18.91	19.04
15	27.701	27.70	27.70	25.30	25.71
10	47.609	47.61	45.73	45.45	40.49
8	69.397	69.40	—	52.27	53.73
6	134.342	134.34	118.42	92.71	80.61
5	262.453	262.45	246.31	145.09	125.12

\*Measurements carried out at  $\text{pH} = 6.9 \pm 0.1$  according to the procedure previously described (14). †Literature values (25).

(albeit small) could be observed with the nature of cations, and it is not surprising that at  $6 \times 10^{-3} \text{ M}$  buffer the same values of  $\Delta_T V$  and  $\Delta_T H$  were observed for all cations. The differences in the thermodynamic values at higher salt concentration are significant. This is evident by the direct comparison of the LIOAS data in  $75 \times 10^{-3} \text{ M}$   $\text{Li}^+$  and  $\text{Cs}^+$ .

The increase in ionic strength produces modifications in the water structure reflected in the  $\Delta_T H$  and  $\Delta_T V$  values. This effect allowed us to extend the range of the  $\Delta_T H$  vs  $\Delta_T V$  relationship in order to distinguish between experimental errors and real changes, as shown in Fig. 6. The results are in good agreement with the interpretation previously given (9). Thus, again in this case, the enthalpy–entropy compensation is an experimental finding outside the experimental error.

This type of compensation has been observed in several systems in which breaking and reforming of hydrogen bonds are implied such as micellization (26), redox reactions with blue copper proteins (27), inter- and intramolecular electron transfer reactions (4,6) and decay of the red-shifted transient in a sensory rhodopsin (5).

The value of  $T \Delta_T S$  in  $6 \times 10^{-3} \text{ M}$  lithium phosphate is close to  $80 \text{ kJ mol}^{-1}$ , larger than  $\Delta_T H$ . The value of  $T \Delta_T S$  was calculated taking into account that  $\Delta_T G$  is constant in this series and equal to  $E_T = 140 \text{ kJ mol}^{-1}$  from phosphorescence data (18), whereas  $\Delta_T H$  was taken from Table 1. Therefore, the entropic term at room temperature should be the most important factor determining the efficiency of the reaction in this case.

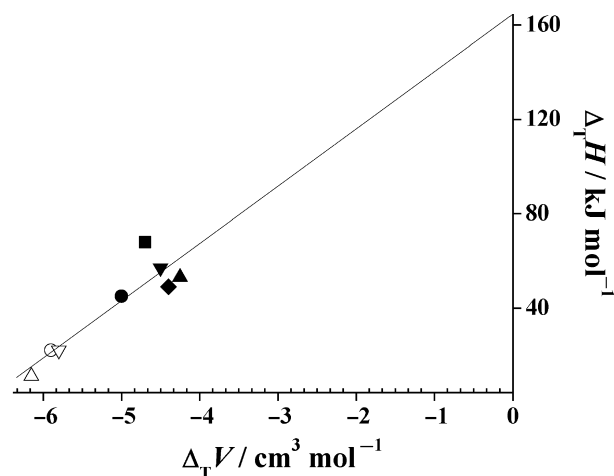
### TCPP $^{4-}$

The fluorescence experiments (Fig. 3) show that the formation of aggregates depends on both porphyrin and salt concentration. Aggregation is negligible at  $[\text{TCPP}^{4-}] = 4 \times 10^{-7} \text{ M}$  up to  $0.05 \text{ M}$  phosphate salt. The same quantum yield for triplet formation is observed under these conditions (Fig. 4).

In spite of the fact that no changes in  $\Delta_T V$  and  $\Delta_T H$  are observed for the various cations at each buffer concentration, two sets of values are obtained at two buffer concentrations (Fig. 7).

The range of changes in the thermodynamic parameters for  $\text{TCPP}^{4-}$  is smaller than for  $\text{TSPP}^{4-}$ . Again in this case, the intercept of this plot agrees, within experimental error, with the value obtained for the triplet energy from phosphorescence data at  $77 \text{ K}$  ( $E_T = 149 \text{ kJ mol}^{-1}$ ) (18). The buffer concentration strongly affects the enthalpy change for the triplet state formation, *i.e.* at  $6 \times 10^{-3} \text{ M}$  the  $\Delta_T H$  value is between 10 and  $20 \text{ kJ mol}^{-1}$ , whereas at  $50 \times 10^{-3} \text{ M}$   $\Delta_T H$  is between 40 and  $60 \text{ kJ mol}^{-1}$ . The triplet energy at  $77 \text{ K}$  obtained from phosphorescence is much larger than these  $\Delta_T H$  values. Therefore, the entropic contribution ( $T \Delta_T S$  is between 80 and  $140 \text{ kJ mol}^{-1}$ ) to the  $\Delta_T G$  value is even higher than in the case of  $\text{TSPP}^{4-}$  (*vide supra*).

$\text{TCPP}^{4-}$  is expected to have qualitatively the same behavior as  $\text{TSPP}^{4-}$  regarding the formation of hydrogen bonds with the water molecules. However, the smaller influence of the cations and buffer concentrations on the thermodynamic parameters should be due to the softer nature of the



**Figure 7.** Relationship between the enthalpy change,  $\Delta_T H$ , and the structural volume change,  $\Delta_T V$ , for  ${}^3\text{TCPP}^{4-}$  formation at  $[\text{TCPP}^{4-}] = 4 \times 10^{-7} \text{ M}$ . (○)  $\text{Li}^+$ , (x)  $\text{Na}^+$  and (8)  $\text{Cs}^+$  at  $[\text{M}^+] = 9 \times 10^{-3} \text{ M}$  and (◊)  $\text{NH}_4^+$ , (●)  $\text{Li}^+$ , (b)  $\text{Na}^+$ , (△)  $\text{K}^+$ , and (7)  $\text{Cs}^+$  at  $[\text{M}^+] = 75 \times 10^{-3} \text{ M}$ . The slope of the linear fitting is  $24 \pm 4 \text{ kJ cm}^{-3}$  and the intercept is  $164 \pm 23 \text{ kJ mol}^{-1}$ .

carboxylate groups than the respective sulfonate group in  $\text{TSPP}^{4-}$ . Therefore, the primary solvent sphere in the case of  $\text{TCPP}^{4-}$  might not be strong enough to extend the hydrogen bond network and a smaller amplification of the phenomena is observed.

### ZnTSPP<sup>4-</sup>

Within the experimental error, the  $\alpha$  values (Table 1) are the same under all conditions. As the quantum yield of  ${}^3\text{ZnTSPP}^{4-}$  formation is also the same under all conditions (Fig. 4), the  $\Delta_{\text{T}}H$  values should not vary for the different cations and concentrations. On the other hand, the volume changes,  $\Delta_{\text{T}}V$ , display a change between 2.3 and 3.0  $\text{cm}^3 \text{mol}^{-1}$ , a very small change to draw any conclusion in view of the error of the determinations (around 0.5  $\text{cm}^3 \text{mol}^{-1}$ ). The  $\Delta_{\text{T}}H$  values shown in Table 1 are within a small range between 130 and 150  $\text{kJ mol}^{-1}$ , all similar to the triplet energy obtained by phosphorescence at 77 K,  $E_{\text{T}} = 155 \text{ kJ mol}^{-1}$  (18). Therefore, the enthalpy change is the main factor in the Gibbs energy for  ${}^3\text{ZnTSPP}^{4-}$  formation.

The above observations indicate that the solvent structure and the nature of the counterion have no, or a negligible, influence on  ${}^3\text{ZnTSPP}^{4-}$  formation. In Zn(II) porphyrin the nitrogen atoms of the porphyrinic ring are blocked and are unable to make hydrogen bridges with water. Thus, the small  $\Delta_{\text{T}}V = -2 \text{ cm}^3 \text{mol}^{-1}$  should be due to intrinsic bond changes upon formation of  ${}^3\text{ZnTSPP}^{4-}$  and no correlation between  $\Delta_{\text{T}}H$  and  $\Delta_{\text{T}}V$  should be expected in this case.

### CONCLUSIONS

The thermodynamic parameters  $\Delta_{\text{T}}H$  and  $\Delta_{\text{T}}V$  for the triplet state formation of water-soluble free-base and Zn(II) derivative porphyrins could be obtained in aqueous solution by application of LIOAS using several and two-temperatures methods. The thermodynamic parameters for the free-base porphyrins are mainly determined by changes in strength and number of hydrogen bonds between nitrogen atoms from the porphyrinic ring and water that produce enthalpy and entropy changes. Thus, a linear correlation between  $\Delta_{\text{T}}H$  and  $\Delta_{\text{T}}V$  is clearly found for each free-base porphyrin, in various concentrations of phosphate salts of various monovalent cations. This compensation and the fact that the intercept of the respective plot is the value for the triplet energy determined by phosphorescence at low temperature where no entropic contribution is expected underline the concept that the entropic term, mainly determined by the water movement upon formation of the triplet state, cannot be neglected for triplet state formation in aqueous solution, for substrates that have a high interaction with water through hydrogen bonds. The entropic contribution to the Gibbs energy for the triplet state formation in the free-base porphyrins is very large at room temperature.

An alternative explanation for the contraction observed upon triplet state formation in the free-base porphyrins was suggested by one of the reviewers. The fast tautomerization in the central N atoms would produce hydrogen-bridged intermediate states that would be contracted with respect to the parent state (as observed). This is a possible explanation for an intrinsic contraction. However, should this be the whole effect, then the changes upon variation in salt concentration and

cation nature could not be explained. An interaction of the N atoms with the aqueous solution is required to explain the observed effects with both free-bases.

On the other hand, when the substrate does not form an extended hydrogen bond network with the solvent (as in the case of  $\text{ZnTSPP}^{4-}$ ), the enthalpy change is similar to the energy obtained from phosphorescence spectra at low temperature and the Gibbs energy is determined by the enthalpy change.

*Acknowledgements*—Gabriel O. Andrés was supported by a postdoctoral fellowship from the Alexander von Humboldt Foundation (Germany) and Franco M. Cabrerizo from the DAAD (German Office for Academic Exchange) and CONICET (Argentina). We thank Professor Wolfgang Lubitz for his support, and Heike Deinert, Dagmar Lenk and Gül Koc-Weier for their able technical assistance.

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