Макрогетероциклы
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DOI: 10.6060/mhc120986g

Determination of Stability of Molecular Complexes of Zinc(II) *meso*–Tetraphenylporphyrin with Heterocyclic *N*–Oxide and Pyridine by Different Methods

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Stability constants of molecular complexes of zinc(II) meso-tetraphenylporphyrin with pyridine and picoline N-oxide were determined from ^{1}H NMR titration data. Stability constants were matched with data, obtained from calorimetry of titration and spectrophotometric titration. It was found that the constants differ significantly depending on the method of determination.

Keywords: *meso*-Tetraphenylporphyrin, calorimetry of titration, molecular complexes, *N*-oxides.

Определение устойчивости молекулярных комплексов *мезо*–тетрафенилпорфирина цинка с пиридином и его *N*–оксидами различными методами

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В работе определены константы устойчивости молекулярных комплексов цинк(II) тетрафенилпорфирина с гетероциклическими N-оксидами в хлороформе по данным ^{1}H ЯМР титрования. Полученные результаты сопоставлены с данными, определенными на основании калориметрического и спектрофотометрического титрования.

Ключевые слова: *мезо*-Тетрафенилпорфирин, калориметрическое титрование, молекулярные комплексы, *N*-оксиды.

Introduction

Biochemical activity of heterocyclic *N*-oxides is directly connected with the processes of complexation with metalloporphyrins in living organisms.[1-6] Catalytic oxi-dation of nitrogen-containing heterocycles to *N*-oxides by metalloporphyrins leads to a very high yield of the desired product (90 %),[7] therefore, it is likely that one of the stages of metabolism of nitrogen-containing heterocyclic compounds in living organisms is the oxidation with metalloporphyrins.

Heterocyclic *N*-oxides can cause apoptosis in human cells K562.[8] Main stage is coordination of *N*-oxides

on chromoproteins of cells (cytochrome *b*, *c*1, P450).[9] Therefore, identification of the basic laws that determine the stability of the complexes of metalloporphyrins with heterocyclic *N*-oxides, it is certainly an actual task.

The stability of molecular complexes are traditionally determined by the spectrophotometric method and the calorimetric titration.

However, each of these methods has highs and lows: the use of the first method can be followed by photochemical reactions, and the second is not a direct method and does not always correlate well with the thermal effects of multiple equilibrium processes (the formation of n–ν-, n–π-, π–π-complexes, *etc*.) that take place in solution. NMR spectroscopy is a direct method, which allows to obtain data with minimal external influence on the system under study. Therefore, in this paper, we try to estimate the possibility of determination the stability constants of complexes of zinc(II) *meso*-tetraphenylporphyrin (ZnTPP) with pyridine and 4-methylpyridine *N*-oxides in chloroform by ^{1}H NMR spectroscopy. Selecting objects of study was caused by different orientation of the ligands in the complexes with metalloporphyrins. In accordance with the X-Ray data of this complexes, pyridines are perpendicular to the plane of the metalloporphyrins and plane of *N*-oxides at an angle of 30 degrees.[10] This difference can significantly change the heat effect of the coordination process due to interactions of π–π-type.

Experimental

^{1}H NMR analysis was carried out on Bruker 200 at 25 °C. 2000 scans were performed for each sample.

For ^{1}H NMR study it was prepared 12 solutions by dilution method with constant concentration of zinc(II) *meso*-tetraphenylporphyrin and excess of ligand from 0 to 20 in accordance with Table 1.

Table 1. The concentration ratio of ZnTPP:L.

№	Volume (ml)			Ratio
	$CDCl_3$	*N*-oxide in $CDCl_3$ 13.3 mM	ZnTPP in $CDCl_3$ 1 mM	ZnTPP:L
	1.00	1.00	0.0	0:1
	0.57	0.03	0.4	1:1
	0.54	0.06	0.4	1:2
	0.51	0.09	0.4	1:3
	0.48	0.12	0.4	1:4
	0.45	0.15	0.4	1:5
	0.42	0.18	0.4	1:6
	0.39	0.21	0.4	1:7
	0.36	0.24	0.4	1:8
	0.30	0.30	0.4	1:10
	0.15	0.45	0.4	1:15
	0.0	0.60	0.4	1:20

In accordance with the work of the authors,[9] the stability constants from the NMR data for the reaction:

$$ZnTPP + L \leftrightarrow ZnTPP \cdot L$$

should be calculated by the Equation:

$$K = \frac{[ZnTPP \cdot L]}{[ZnTPP] \cdot [L]} = \frac{\alpha}{(1-\alpha)\cdot([L]_0 - \alpha[ZnTPP]_0)} \quad (1),$$

where

$$\alpha = \frac{(\delta_i - \delta_0)}{(\delta_{max} - \delta_0)} \quad (2),$$

δ – peak signal position of protons 1-8 of ZnTPP.

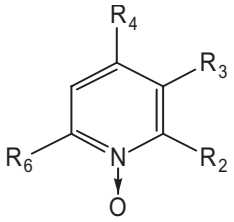
Thus, substituting (2) in Equation (1), we obtain an equation with two unknowns (*K* and δ_{max}), which is minimized in the program MathCad to find the global minimum of a function $S_{ad} = f(K, \delta_{max})$ with physically reasonable values of K and δ_{max}.

Spectrophotometric measurements were performed on a spectrophotometer UNICO 2800. For the calculation of the stability constants by the method of spectrophotometric titration we used approaches described earlier:[12] saturation curve method, methods of isomolecular series, dilution method, Brown and Drago methods.

The technique of calorimetric studies, the description and characteristics of an automatic differential titration calorimeter, as well as methods for calculating the thermodynamic characteristics of complexation are given in [13].

Zinc(II) *meso*-tetraphenylporphyrin (ZnTPP) 97 % was purified by column chromatography on Al_2O_3 (Brockmann II). Benzene was used as an eluent. To remove the solvent molecules from ZnTPP, it was dried to constant weight in vacuum.

Heterocyclic *N*-oxides (Figure 1) were synthesized and purified by techniques.[14-16] Pyridine, after drying over KOH was fractionally distilled. Chloroform-*d* was obtained from commercial suppliers and was of spectroscopic purity and was used as received, chloroform was purified by technique.[17]



$R_{2,3,6}$=H, R_4=NO_2 – *N*-oxide 4-nitropyridine (4-NPyO);
$R_{2,6}$=H, R_3=CH_3, R_4=NO_2 – *N*-oxide 3-methyl-4-nitropyridine (3-MeNPyO);
$R_{3,6}$=H, R_2=CH_3, R_4=NO_2 – *N*-oxide 2-methyl-4-nitropyridine (2-MeNPyO);
$R_{2,6}$=CH_3, R_4=NO_2 – *N*-oxide 2,6-dimethyl-4-nitropyridine (2,6-Me_2NPyO);
$R_{3,6}$=H, R_4=CH_3 – *N*-oxide 4-methylpyridine (4-MePyO);
$R_{2,3,6}$=H, R_4=$CH_2CH_2C_6H_4$-*p*OCH_3 – *N*-oxide 4-(4'-methoxystyryl) pyridine (4-MeOStPyO);
$R_{2,3,6}$=H, R_4=$CH_2CH_2C_6H_4$-*p*N$(CH_3)_2$ – *N*-oxide 4-(4'-dimethylaminostyryl)pyridine (4-DPyO);

Figure 1. *N*-oxides.

Results and Discussion

Consider the data on the example of 4-DPyO (Figure 2, Table 2). The largest shift in the strong field reveals the signals of protons 2, 6 and 2', 6' of *N*-oxide, *i.e.* reaction center of complexation of *N*-oxide with ZnTPP is oxygen

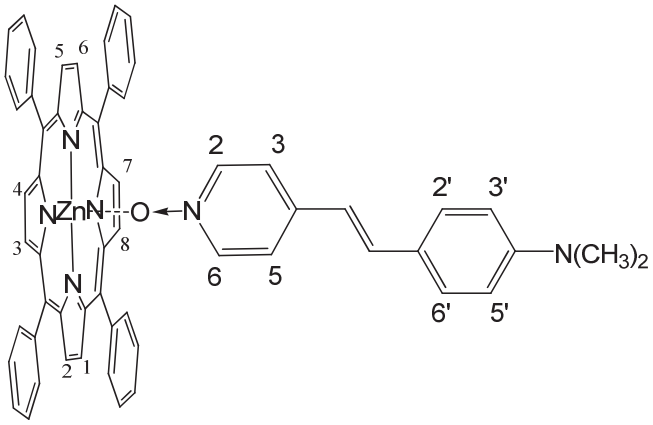


Figure 2. Schematic structure of the molecular complex ZnTPP·4-DPyO.

atom of NO-group.

Proton signals of 1-8H in ZnTPP undergo the maximum shift in the strong field ($\Delta\delta_{1\text{-}8H}$ = 0.065 ppm), whereas the signals of protons in the phenyl residues are not displaced. Thus, we can say with confidence that the coordination of *N*-oxide is realized on the central atom of the metal porphyrin is in NO-group (Figure 2).

Similarly, the experimental data of ^{1}H NMR spectroscopy of molecular complexes in all of these heterocyclic *N*-oxides with ZnTPP were analyzed. It was found that in all cases, coordination of *N*-oxides at the central metal ion in ZnTPP takes by NO-group of *N*-oxide. Stability constants of molecular complexes were calculated from spectral data (Table 3).

Stability constants of molecular complexes of heterocyclic *N*-oxides with ZnTPP were calculated by the chemical shifts of ZnTPP 1-8H protons, because its resonance is much more intensive, and therefore is easy to detected. Titration curve of 4-DPyO·ZnTPP is presented in Figure 3. Stability constants of molecular complexes within the error limits are consistent with constants calculated from calorimetric titration data (Table 3).

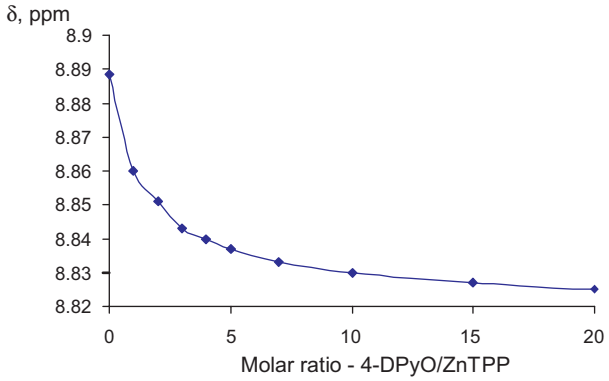


Figure 3. Titration curve of zinc *meso*-tetraphenylporphyrin by *N*-oxide 4-(4'-dimethilaminostiryl)pyridine in chloroform.

Table 2. Changing the proton signals of 4-DPyO and ZnTPP under complexation.

Proton	$\delta_{N\text{-oxide}}$, ppm	$\delta_{complex}$, ppm	Δδ
4-DPyO			
$H_{2,6}$	8.10 d	6.86 d	1.24
$H_{3,5}$	7.40 d	7.4 d	No change
$H_{2',6'}$	7.29 d	~7.1	0.19
CH=	7.09 d	7.08 d	No change
=CH	6.74 d	~6.75	
$H_{3',5'}$	6.71 d	6.7d	
$N(Me)_2$	3.04 s	3.02 s	
ZnTPP			
m- H	8.20-8.23	8.16-8.23	No change
o-,*p*- H	7.74-7.77 m	7.68-7.74 m	
1,8 - H	8.94 s	8.875 s	0.065

However, it should be noted that the solution is unstable because it can provide a very wide range of values of the constants which satisfactorily describe the experimental data. Variability of solutions is related with a small number of experimental points to be processed. Expansion of the experimental series is not possible for technical reasons because duration of a single experiment leads to evaporation of the solvent and the concentration of the reagents changes. In summary, we conclude that the method of ^{1}H NMR spectroscopy allows to determine the centers responsible for complex formation, and to estimate the stability constants of molecular complexation.

Complete thermodynamic characteristics of the interaction of zinc *meso*-tetraphenylporphyrin with *N*-oxides, has been obtained by isothermal titration calorimetry method (Table 3). All of the investigated *N*-oxides formed with ZnTPP complexes with composition 1:1, what was determined by the inflection point of the integral titration curve, as well as

Table 3. Thermodynamic characteristics of ZnTPP with heterocyclic *N*-oxides in chloroform.

N-oxide	K_c, l·mol^{-1}	ΔH^0, kJ·mol^{-1}	ΔS^0, J·(mol·K)$^{-1}$	K_c^*, l·mol^{-1}
4-NPyO	5623 ± 401	-0.80±0.06	69±21	5381
3-MeNPyO	7861 ± 432	-1.64±0.18	69±21	7865
2-MeNPyO	6606 ± 537	-1.01±0.08	70±21	5985
2,6-Me_2NPyO	6693 ± 280	-0.95±0.40	70±20	5622
4-MePyO	8167 ± 449	-3.17±0.31	64±18	7412
4-MeOStPyO	4352 ± 213	-4.41±0.22	55±18	4035
4-DPyO	5608 ± 308	-4.97±0.10	55±18	5632

* The constants were determined by the method of 1H NMR titration. The error is 20-30 %.

a satisfactory description of the experimental calorimetric curve by the theoretical function with a dispersion of adequacy less than 10^{-3}.

The constants are determined by the method of ^{1}H NMR titration. The error is 20-30 %

Coordination of *N*-oxides in the metalloporphyrin is exothermic, but the process is controlled by entropy, which indicates the importance of solvation interactions of the initial reagents and the resulting molecular complex with solvent molecules. It should also be noted that in view of errors, the calculated values of stability constants are consistent with results obtained by NMR spectroscopy. Calorimetric titration method allowed to calculate the stability constants with an error of 8 %, while the error in determining the constants by NMR titrations ranges from 20 to 30 % due to the inability to determine δ_{max} because at comparable concentrations, it is impossible to achieve 100 % degree of complex formation, and the addition of a large excess of one component, the other proton signals could not be identified. Thus, δ_{max} can be obtained only by calculation, which greatly enhances the confidence interval of the stability constants of molecular complexes. Therefore, the stability constants of complexes of *N*-oxides with ZnTPP were also obtained by spectrophotometric titration.

In this work were evaluated stability constants of complexes of ZnTPP with pyridine *N*-oxide and 4-methylpyridine by spectrophotometric titration using the widely used approaches:[18] by the saturation curve (Figure 4), the method of isomolar series, the method of dilution, Brown's and Drago's methods. Moreover, the spectral data were analyzed in the regions 564, 604 and 566, 608 nm for the complexes of ZnTPP·4MePyO and ZnTPP·Py, respectively. The results are shown in Table 4. In general, the stability constants of the complex ZnTPP·Py calculated by various methods, within the error limits are the same (K = 3876 ± 374 l·mol^{-1}), which is consistent with published data.[19] In contrast to the stability constants of the complex ZnTPP·4-MePyO, the differences are so significant (from 800 to 15000 l·mol^{-1}), which makes the problematic choice of the mean value (Table 4). Additional studies by thin layer chromatography (eluent - chloroform, stationary phase - a plate "Sorbifil" Al_2O_3) of solutions ZnTPP and its complex with 4-MePyO, kept at room

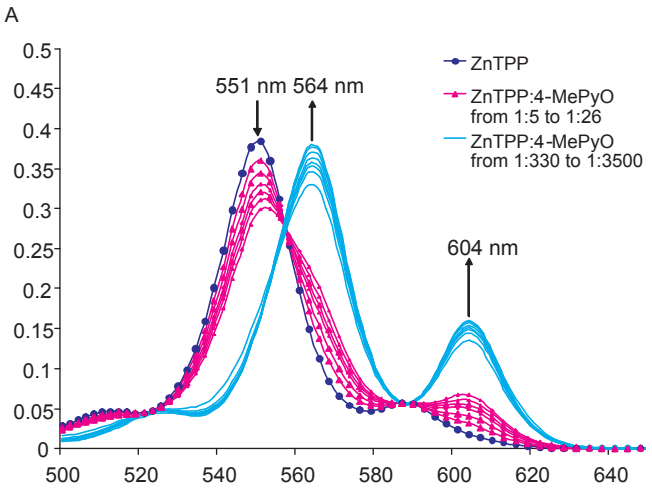


Figure 4. Absorption spectra of zinc *meso*-tetraphenylporphyrin in chloroform by the titration of *N*-oxide 4-methylpyridine.

Table 4. Stability constants of ZnTPP·4MePyO and ZnTPP·Py determined by different methods.[20]

K	The method and conditions
	ZnTPP·4MePyO
	The method of isomolar series
8357	By the fitted spectra, the absorption maximum of the complex is 564 nm
8537	By the fitted spectra, the absorption maximum of the complex is 604 nm
1630	By absorbance at 564 nm
7114	By absorbance at 604 nm
	By the saturation curve
4465	By absorbance at 564 nm
1983	By absorbance at 604 nm
5008	By the fitted spectra, the absorption maximum of the complex is 564 nm
1809	By the fitted spectra, the absorption maximum of the complex is 604 nm
	The method of dilution
6723	C_{ZnTPP} = 5.6·10^{-5} mol/l, dilution rate = 2
9152	C_{ZnTPP} = 1.95·10^{-5} mol/l, dilution rate = 2
14920	C_{ZnTPP} = 1.95·10^{-5} mol/l, dilution rate = 3
	Brown's method
1428	C_{ZnTPP} = 1.12·10^{-5} mol/l, 564 nm
1700	C_{ZnTPP} = 1.12·10^{-5} mol/l, 604 nm
5000	C_{ZnTPP} = 1.94·10^{-5} mol/l, 564 nm
3448	C_{ZnTPP} = 1.94·10^{-5} mol/l, 604 nm
	Drago's method
4000	Graphical solution at 564 nm, C_{ZnTPP} = 1.94·10^{-5} mol/l
3850	Graphical solution at 604 nm, C_{ZnTPP} = 1.94·10^{-5} mol/l
846	By the fitted spectra, the absorption maximum of the complex is 564 nm
1013	By the fitted spectra, the absorption maximum of the complex is 604 nm
1000	Least-squares method, 564 nm
900	Least-squares method, 604 nm
	ZnTPP·Py
	By the saturation curve
4111	By absorbance at 566 nm, C_{ZnTPP} = 5·10^{-5} mol/l
4124	By absorbance at 608 nm, C_{ZnTPP} = 5·10^{-5} mol/l
	Brown's method
4166	By absorbance at 566 nm, C_{ZnTPP} = 5·10^{-5} mol/l
4000	By absorbance at 608 nm, C_{ZnTPP} = 5·10^{-5} mol/l
	The method of dilution
23978	C_{ZnTPP} = 5·10^{-5} mol/l, dilution rate = 2, 566 nm
7785	C_{ZnTPP} = 5·10^{-5} mol/l, dilution rate = 2, 608 nm
	Drago's method
5600	Graphical solution at 566 nm, C_{ZnTPP} = 5·10^{-5} mol/l
5000	Graphical solution at 608 nm, C_{ZnTPP} = 5·10^{-5} mol/l
3716	By the fitted spectra, absorbance of ZnTPP at 551 nm
3500	Least-squares method, 566 nm
3500	Least-squares method, 608 nm

light for 10-15 min, and stored in the dark, showed that in the presence of 4-MePyO in the light ZnTPP is destroyed.

Conclusion

Thus, the method of NMR spectroscopy allows to establish the reaction centers, responsible for complexation, which is important in the investigation of ligands with two reaction centers. Also, the method allows to determine the values of stability constants, which are comparable with that determined by calorimetric titration.

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Received 11.09.2012
Accepted 19.11.2012