

## Kinetics of the Formation of Metalloporphyrins and the Catalytic Effect of Lead Ions and Hydrogen Ions

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The reaction mechanism of Lead ions catalyzing complexation reactions between TIPP and metal ions was investigated by researching the kinetics of the formation of metalloporphyrins by UV/Vis-spectra, and verified by exploring the formation of metalloporphyrins catalyzed by acetic acid. Kinetics studies suggested that the fluctuations of reaction rate indicated the formation of metalloporphyrin was step-wise, including the pre-equilibrium step (the coordination of the pyrroline nitrogens to  $M^{n+}$ ) and the rate-controlling step (the deprotonation of the pyrrole proton). In the pre-equalization step, a sitting-atop (SAT) structure formed first with the complexation between larger radius of  $Pb^{2+}$  and TIPP, changed the activation, then  $Pb^{2+}$  left with the smaller radius of metal ions attacking from the back of the porphyrin ring center. In the rate-controlling step, two pyrrole protons dissociated to restore a stable structure. This was verified by adding acetic acid at different reaction times.

**Key Words :** 5,10,15,20-Tetrakis(4-isopropylphenyl)porphyrin (TIPP), Metalloporphyrin, Catalysis, Kinetics, UV/Vis-spectra

### Introduction

Many metalloporphyrins and analogues, such as hemoglobin,<sup>1</sup> myoglobin,<sup>2</sup> and chlorophyll,<sup>3</sup> play significant roles *in vivo*. The metalloporphyrin formation reaction is also one of the most important processes in biological systems, and the importance has been pointed out in relation to the biosynthesis of heme.<sup>4-8</sup> In 1960, Fleisher and Wang proposed that an intermediate should exist in the metalation process,<sup>9</sup> and this intermediate was called a sitting-atop (SAT) complex, in which two pyrroline nitrogens coordinate to the metal ion and two protons on the pyrrole nitrogens remain.<sup>10</sup> Recently, the SAT complex of 5,10,15,20-tetraphenylporphyrin was successfully detected during the reaction with the Cu(II) ion in acetonitrile.<sup>11,12</sup> The metalloporphyrin formation between metal ions ( $M^{n+}$ ) and a general porphyrin ( $H_2P$ ) certainly proceeds by multiple steps. At least two steps, the coordination of the pyrroline nitrogens to  $M^{n+}$  and the deprotonation of the pyrrole proton, should be involved in the overall process of the reaction.<sup>11</sup>

To elucidate the metalation mechanism, kinetic observation for each step is needed. The study of the movable mechanism of porphyrin complexes in organisms and plants benefits from learning the formation and conditions of metalloporphyrins.

### Experimental

**Instrument.** UV absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Elemental analyses were performed on a VARIO elemental analyzer (Germany). <sup>1</sup>H NMR spectra were obtained on a

Bruker Avance DRX 300 MHz nuclear magnetic resonance spectrometer (Fällanden, Switzerland). All the experiments were carried out at  $20 \pm 1$  °C.

**Materials.** The 5,10,15,20-tetrakis(4-isopropylphenyl) porphyrin (TIPP) was synthesized by using microwave. All reagents (including vanadium(IV)oxy acetylacetonate, cobalt(II) acetate, copper(II) acetate, zinc(II) acetate, nickelous(II) acetate, lead(II) acetate, acetic acid, DMF and  $CH_2Cl_2$ ) were analytical grade.

**Kinetic Measurements.** Absorption spectra were recorded on a UV-vis spectrophotometer (UV-2450). The absorbance changes for the reaction between the VO(II) ion and  $H_2TIPP$  were monitored at 422 nm. The solvent that used for the kinetics was the mixture of  $CH_3OH$  and  $CH_2Cl_2$  ( $CH_3OH : CH_2Cl_2 = 1:9$ ). All the experiments were carried out at  $20 \pm 1$  °C.

**Syntheses.** To a stirred solution of  $CH_2Cl_2$  (30 mL) and DMF (5 mL) was added TIPP (0.78 g, 0.1 mmol), two drops of acetic acid ( $10^{-3}$  mol/L,  $CH_2Cl_2$  as the solvent) and acetate (0.5 mmol). The reaction mixture was heated to reflux and the reaction was tracked by TLC, then allowed to cool at room temperature, and the reaction mixture was back extracted several times with an amount of distilled water, then concentrated in vacuo, obtaining purple crystals of the crude metal porphyrin. The crude was applied to a silica gel column and eluted with dichloromethane, then concentrated in vacuo, obtaining bright red purple crystals. The results are shown in Table 1.

### Results and Discussion

#### The Formation of Metalporphyrin Catalyzed by $Pb^{2+}$ .

**Table 1.** The results of 5,10,15,20-tetrakis(4-isopropylphenyl) porphyrin and its metalloporphyrins

Compounds	Reaction Time (h)	Yield (%)	Elemental Analysis (%)			UV-Vis ( $\lambda_{\max}$ , CH <sub>2</sub> Cl <sub>2</sub> ) <sup>c</sup>	<sup>1</sup> H NMR $\delta$ H (300 MHz, CDCl <sub>3</sub> )
			C <sup>b</sup>	H <sup>b</sup>	N <sup>b</sup>		
H <sub>2</sub> TIPP	2	25.28	85.88 (85.89)	6.87 (6.95)	7.14 (7.15)	419.5(5.45) 516(4.30) 551(4.25) 591(4.15) 648(4.14)	-2.78 (bs, 2H, NH), 1.52 (d, 24H, CH <sub>3</sub> ), 3.24 (Sep, 4H, CH), 7.57 (d, 8H, m-Ph2H), 8.12 (d, 8H, O-Ph2H), 8.84 (s, 8H, $\beta$ CH)
CoTIPP	1	95.58	79.88 (80.07)	6.24 (6.24)	6.62 (6.67)	412(5.39) 529(4.46)	Null <sup>d</sup>
NiTIPP	2	92.17	80.03 (80.10)	6.43 (6.24)	6.58 (6.67)	418(5.14) 529(4.91)	1.50 (d, 24H, CH <sub>3</sub> ), 3.22 (Sep, 4H, CH), 7.53 (d, 8H, m-Ph2H), 7.93 (d, 8H, O-Ph2H), 8.76 (s, 8H, $\beta$ CH)
CuTIPP	1	96.24	79.67 (79.64)	6.28 (6.21)	6.56 (6.63)	417(5.72) 540(4.63)	Null <sup>d</sup>
ZnTIPP	1	96.77	79.55 (79.46)	6.23 (6.19)	6.71 (6.62)	420.5(5.52) 548.5(4.42) 587(4.26)	1.55 (d, 24H, CH <sub>3</sub> ), 3.27 (Sep, 4H, CH), 7.59 (d, 8H, m-Ph2H), 8.13 (d, 8H, O-Ph2H), 8.98 (s, 8H, $\beta$ CH)
PbTIPP <sup>a</sup>	3	85.29	68.15 (68.06)	5.36 (5.30)	5.49 (5.67)	420(4.91) 466.5(5.28) 517(4.16) 551(4.19) 612(4.23) 661(4.36)	1.53 (d, 24H, CH <sub>3</sub> ), 3.25 (Sep, 4H, CH), 7.56 (d, 8H, m-Ph2H), 8.14 (d, 8H, O-Ph2H), 8.95 (s, 8H, $\beta$ CH)
VOTIPP <sup>a</sup>	2	91.36	79.42 (79.32)	6.21 (6.18)	6.55 (6.61)	425(5.77) 548(4.76) 585(4.56)	1.47 (d, 24H, CH <sub>3</sub> ), 3.18 (Sep, 4H, CH), 7.50 (d, 8H, m-Ph2H), 7.90 (d, 8H, O-Ph2H), 8.75 (s, 8H, $\beta$ CH)

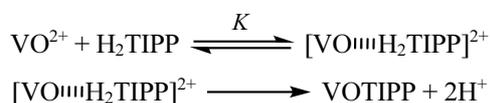
<sup>a</sup>Have not been reported.<sup>13</sup> <sup>b</sup>Theoretical values are given in parentheses. <sup>c</sup>The values of log<sub>10</sub>( $\epsilon$ ) are given in parentheses ( $\epsilon$  represents the molar absorbance). <sup>d</sup>The <sup>1</sup>H NMR spectras cannot be obtained due to paramagnetic or other factors.<sup>14</sup>

It was found that Pb<sup>2+</sup> can catalyze the formation of VO(II), Co(II), Ni(II), Cu(II), Zn(II) and other metalloporphyrins. In VO(II) and TIPP, for example, the reaction was detected in a large excess (100 times greater than TIPP) concentration of VO<sup>2+</sup>, which is considered to be the first order reaction. The changing absorbance of the system was measured in the characteristic wavelength (422 nm). The apparent rate constant ( $k_{\text{obs}}$ ) were fitted by using Guggenheim method.

$$\ln(A_t - A_{t+\Delta t}) = -k_{\text{obs}}t + C$$

$A_t$  and  $A_{t+\Delta t}$  in formula are the absorbance of  $t$  and  $t + \Delta t$ , respectively.  $\Delta t$  usually takes 3-4 times of the half-life time of the reaction. The fitting correlation coefficient was greater than 0.99 (Fig. 1). There was a linear relationship between  $k_{\text{obs}}$  and the concentration of Pb<sup>2+</sup> when the concentration of Pb<sup>2+</sup> was small (at least in five experimental points). When the concentration of Pb<sup>2+</sup> was large, the  $k_{\text{obs}}$  grew slowly with increasing concentration of Pb<sup>2+</sup>, tending to remain unchanged, and the following reaction mechanism was proposed according to the experimental results.<sup>15</sup>

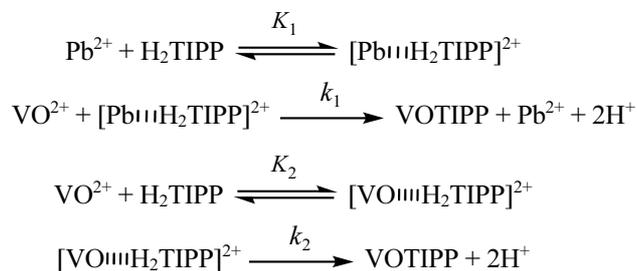
The formation mechanism(a) of VOTIPP as follows:



The  $k_{\text{obs}}$  of this mechanism can be expressed as:

$$k_{\text{obs}} = \frac{k \cdot K[\text{VO}^{2+}]}{1 + K[\text{VO}^{2+}]} \quad (1)$$

The reaction mechanism(b) of VOTIPP with the presence of Pb<sup>2+</sup> as follows:

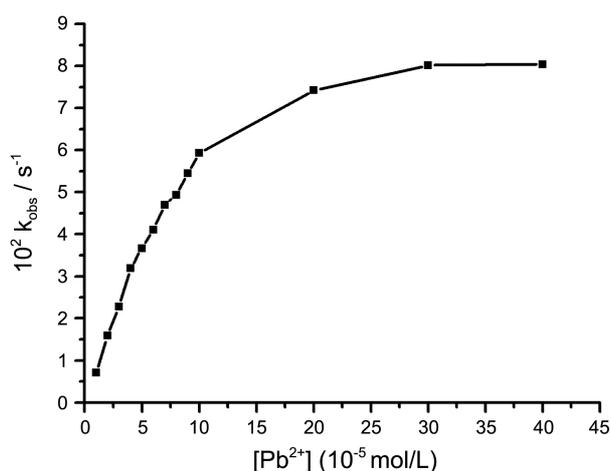


The  $k_{\text{obs}}$  of this mechanism can be expressed as:

$$k_{\text{obs}} = \frac{(k_1 \cdot K_1[\text{Pb}^{2+}] + k_2 \cdot K_2)[\text{VO}^{2+}]}{1 + K_1[\text{Pb}^{2+}] + K_2[\text{VO}^{2+}]} \quad (2)$$

$K$ ,  $K_1$  and  $K_2$  are the equilibrium constants of the pre-balancing steps;  $k$ ,  $k_1$  and  $k_2$  are the rate constants of the rate-controlling steps.

For  $[\text{Pb}^{2+}] = 0$ , the mechanism(b) reduced to mechanism(a)

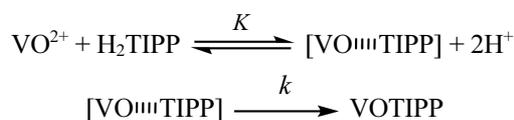


**Figure 1.** The relationship between  $k_{\text{obs}}$  and the concentration of  $\text{Pb}^{2+}$  in the formation of VOTIPP at room temperature.

and formula(2) reduced to formula(1). As far as formula(2) is concerned, it can be changed to the following equation when the concentration of TIPP was maintained at  $2.45 \times 10^{-6}$  mol/L and the concentration of  $\text{Pb}^{2+}$  was low.

$$k_{\text{obs}} = \frac{k_1 \cdot K_1 [\text{Pb}^{2+}] [\text{VO}^{2+}]}{1 + K_2 [\text{VO}^{2+}]} + \frac{k_2 \cdot K_2 [\text{VO}^{2+}]}{1 + K_2 [\text{VO}^{2+}]} \quad (3)$$

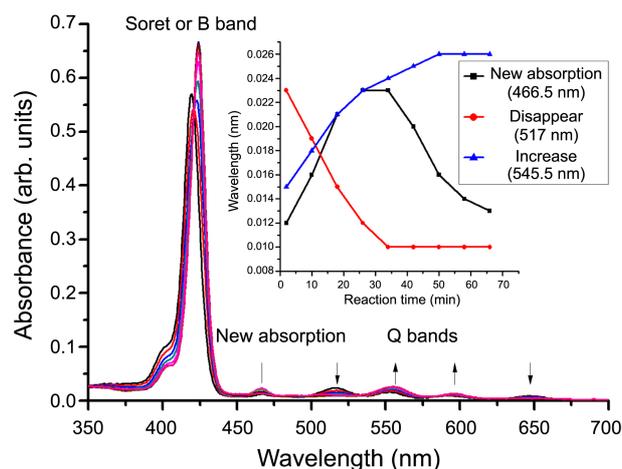
There was a linear relationship between  $k_{\text{obs}}$  and  $\text{Pb}^{2+}$ , at this point, which is consistent with the experimental results. There was no association between  $k_{\text{obs}}$  and  $\text{Pb}^{2+}$  from formula(2). Furthermore, the  $k_{\text{obs}}$  should be a constant when the concentration of  $\text{Pb}^{2+}$  is high, and  $K_1 * [\text{Pb}^{2+}] \gg K_2 * [\text{VO}^{2+}]$ . It was consistent with the trend of the experimental curve, thus proving the mechanism was reasonable. Experimentally it was assumed that protons dissociated in the rate-controlling step; its rationality can be proved by reductio ad absurdum. Assuming that protons dissociated in the pre-equilibrium step, then the reaction mechanism should be written as follows:



The rate equation of this mechanism can be expressed as:

$$-\frac{d[\text{H}_2\text{TIPP}]}{dt} = \frac{k \cdot K [\text{VO}^{2+}] [\text{H}_2\text{TIPP}]}{[\text{H}^+]^2 + K [\text{VO}^{2+}]} \quad (4)$$

For the approximate conditions in this experiment, formula(1) represented that the  $k_{\text{obs}}$  was a constant for a particular reaction; the reaction may be considered as the pseudo first-order reaction. However, with the on-going reaction,  $-d[\text{H}_2\text{TIPP}]/dt$  was not a constant according to formula(4) due to increasing  $\text{H}^+$ . There were some discrepancies compared to pseudo first-order reactions, which did not match the experimental results. It was found that the reaction could be considered as a first-order reaction during the first five half-lives. It could be identified that protons

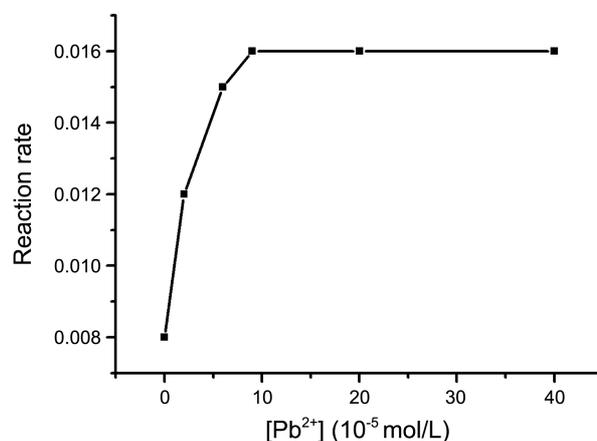


**Figure 2.** The change of absorption at 466.5, 517 and 545.5 nm in the formation of VOTIPP catalyzed by  $\text{Pb}^{2+}$  at room temperature.

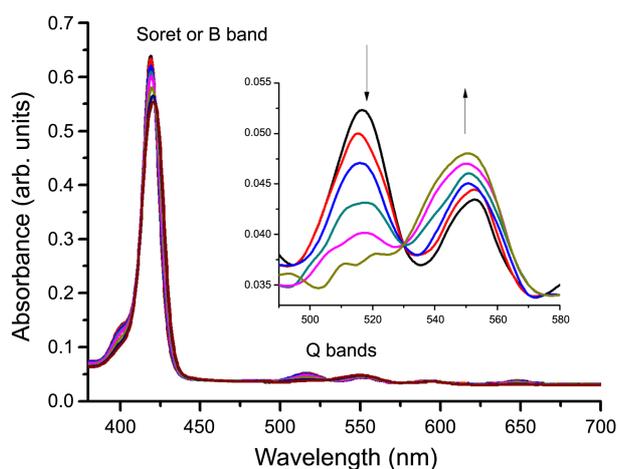
dissociated in the rate-controlling step.

New absorption appears at 466.5 nm when the concentration of  $\text{Pb}^{2+}$  reaches  $10^{-3}$  mol/L (Fig. 2), increasing with the decrease of absorbance at 517 nm and reaching the maximum when the absorption at 517 nm completely disappeared, then fading away until it disappeared. What's more, the increasing of the absorption at 545.5 nm proved that it was still accompanied by the generation of VOTIPP during the disappearance of absorbance at 466.5 nm. As is known, the disappearance of the absorbance at 517 nm and the increasing at 545.5 nm represent the generation of metalloporphyrin. However, the absorption at 466.5 nm heralded that a certain intermediate appeared and destroyed the plane of the porphyrin ring. This intermediate was consumed continuously by the attack of  $\text{VO}^{2+}$  ions. The acceleration of  $\text{Pb}^{2+}$  may be attributed to this intermediate.

The relationship between the formation rate (in 422nm) of metal porphyrin and  $\text{Pb}^{2+}$  is shown in Figure 3. The reaction rate was  $0.008 \text{ min}^{-1}$  without  $\text{Pb}^{2+}$ ; however, the reaction rate dramatically accelerated with the increasing of  $[\text{Pb}^{2+}]$ . The reaction rate was maintained at  $0.016 \text{ min}^{-1}$  when  $[\text{Pb}^{2+}]$  exceeded  $10^{-4}$  mol/L. It proved that  $\text{Pb}^{2+}$  played a catalytic



**Figure 3.** The relationship between reaction rate and the concentration of  $\text{Pb}^{2+}$  in the formation of VOTIPP at room temperature.



**Figure 4.** The UV-vis spectrum of the formation of VOTIPP in acetic acid.

role in the reaction process, the rate upgraded nearly two-fold.

These experiments showed that metal ions complex with porphyrin first, then two pyrrole protons dissociate during the formation of porphyrin in a rate-controlling step catalyzed by  $Pb^{2+}$ . Furthermore, if it is assumed that the dissociation of two pyrrole protons occurred in the pre-equilibration step, then the  $[H^+]$  will definitely affect the pre-equalization step. To this end, exploring the influence between  $H^+$  and the complexation was necessary.

#### The Formation of Metalporphyrin Catalyzed by $H^+$ :

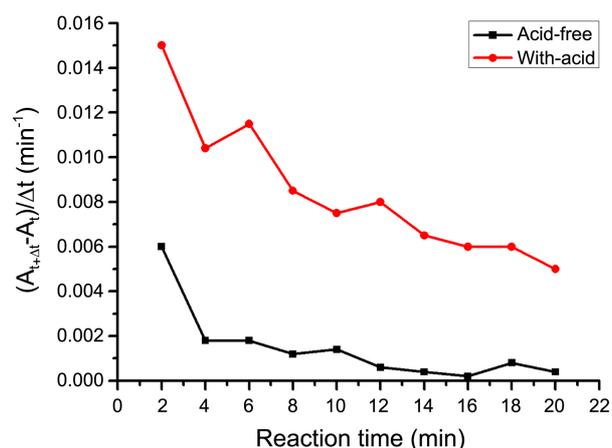
The formation kinetics of VOTIPP were explored in different ratios of acid by UV-vis. The rates of complexation reactions were very slow for Vanadyl ions and Lead ions; few changes of the UV absorption could be observed several hours later. The preparation of those metalloporphyrins takes several days refluxing in DMF; However, the reaction requires only 2 h by adding acetic acid. At the rate of TIPP:  $H^+ = 1:10$ , for example, the UV absorption peaks changed rapidly with the participation of acetic acid, decreasing at 517 nm, rising at 545.5 nm (Fig. 4).

The catalysis of acid was expressed by altering the structure of the porphyrin ring. The extent of change could be observed through the UV absorption of the same concentration of porphyrin. The changing absorbance of the system was measured in the characteristic wavelength (420 nm). Then the reaction rate could be calculated through the Bill-Lambert law. The relationship between the rate of the change of absorbance and reaction time was shown in Figure 5.

$$\nu_{(A)} = \frac{A_{(t+\Delta t)} - A_t}{\Delta t} = \frac{d_A}{d_t} = \epsilon b \frac{d_c}{d_t} = \epsilon b \nu_{(t)} = k \nu_{(t)}$$

$A_t$  and  $A_{t+\Delta t}$  in the formula are the absorbance of  $t$  and  $t + \Delta t$ , respectively.  $\Delta t$  usually takes 3-4 times of the half-life time of the reaction.  $\nu_{(A)}$  is the rate of the change of absorbance,  $\epsilon$  is the molar absorption coefficient,  $b$  is the width of the cuvette,  $k$  is the rate constant, and  $\nu_{(t)}$  is the formation rate.

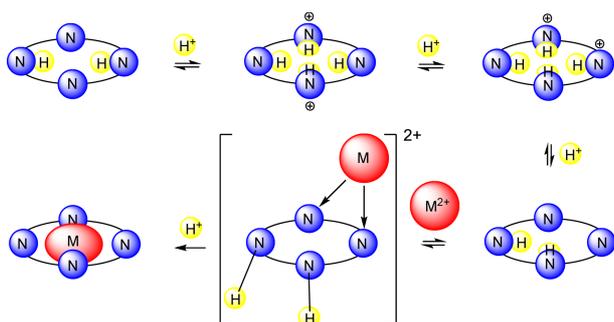
Different concentrations of  $H^+$  on the complexation bet-



**Figure 5.** The change of absorbance of the formation of VOTIPP at 422 nm in neutral and acidic conditions.

ween TIPP and  $VO^{2+}$  have similar influences and the changes of absorbance have similar trends. Accordingly, at the rate of 1:10 shown in Figure 5, for example, the reaction rate catalyzed by  $H^+$  was significantly faster than in neutral condition; the curves appeared twice the acceleration process catalyzed by  $H^+$ , while it appeared less obvious under neutral condition. The first acceleration ( $0.0115 \text{ arb.} \cdot \text{min}^{-1}$ ) appeared at 6 min catalyzed by  $H^+$ , and the reaction rate rapidly declined at the beginning of the formation. This might be attributed to the slow generation of the intermediate, the first segment of acceleration might be related to the catalytic of intermediate. Then the rate began to decline with the consumption of the intermediate. During the rate-controlling step, the second acceleration ( $0.008 \text{ arb.} \cdot \text{min}^{-1}$ ) appeared at 12 min catalyzed by  $H^+$ , the second segment of acceleration might be related to the dissociation of two pyrrole protons so that the porphyrin ring restored to stability. The kinetics catalyzed by  $H^+$  studies suggest that the formation of metalloporphyrin was step-wise. Otherwise there would not be two distinct acceleration periods. The reaction rate catalyzed by  $H^+$  was significantly faster than during neutral conditions. If the dissociation of protons occurred in pre-equalization step, the  $H^+$  will inhibit the forward movement of the balance in acidic condition, the reaction rate will be lower than neutral condition at least in the pre-equilibrium step, this was inconsistent with the experimental facts. So, the dissociation of protons occurred in the rate-controlling step, rather than the pre-equilibration step.

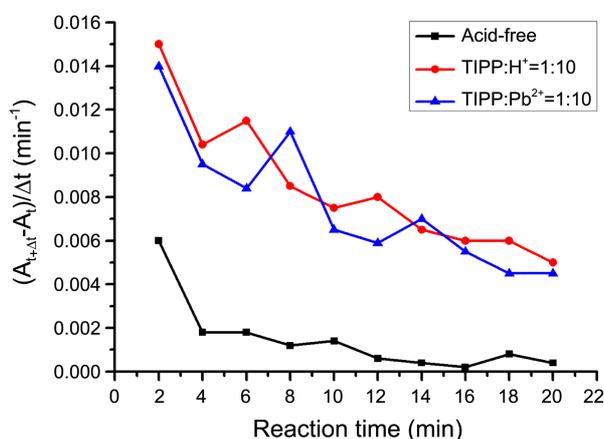
There was a significant period of acceleration 6 minutes later (Fig. 5), existing a certain relationship with hydrogen ions. This might be attributed to the porphyrin ring that accepted two protons, then the two hydrogen atoms in porphyrin ring shift from counterpoint to ortho due to conjugation. The isomer (hydrogens adjacent to free base) was raised by Rothmund.<sup>16</sup> The two nitrogens that have complexing ability transformed to ortho and became closer together (about 0.144 nm), making it easier to form the intermediate. Certainly, the presence of this isomer has been confirmed.<sup>17</sup>



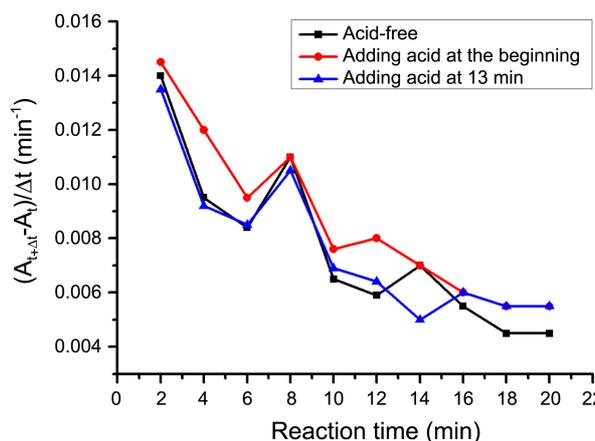
**Figure 6.** The possible process of the formation of metalloporphyrin catalyzed by  $H^+$ .

The possible process of the formation of metalloporphyrin catalyzed by  $H^+$  is shown in Figure 6. It becomes easier to be complexed by those two nitrogens when metal ions are close to the porphyrin ring, then dissociating two protons, meanwhile, macrocyclic conjugated system restore to steady state. The formation of intermediates was slow and the dissociation of protons was fast. The dissociation of protons during the complexation reactions between TIPP and metal ions occurred in the rate-controlling step, rather than the pre-balance step. This reasonably explains the phenomenon of acceleration in complexation reaction kinetics. What's more, it provides the basis of the formation mechanism of metal porphyrins catalyzed by lead ions.

Different concentrations of  $H^+$  and  $Pb^{2+}$  on the complexation between TIPP and  $VO^{2+}$  have similar influence and the changes of absorbance at different conditions have similar trends. Accordingly, at the rate of 1:10, for example, shown in Figure 7, as can be seen, the reaction rate catalyzed by  $Pb^{2+}$  significantly faster than neutral condition, the curves appeared twice the acceleration process catalyzed by  $Pb^{2+}$ . The first acceleration ( $0.011 \text{ arb. min}^{-1}$ ) appeared at 8 min, and the reaction rate rapidly declined at the beginning of the formation. This might be attributed to the slow generation of the intermediate, the intermediates formed with  $Pb^{2+}$  were difficult to  $H^+$ , the first segment of acceleration might be related to the catalytic of intermediates. Then the rate began



**Figure 7.** The change of absorbance of the formation of VOTIPP at 422 nm in different conditions.



**Figure 8.** The influence of  $H^+$  in the formation of VOTIPP catalyzed by  $Pb^{2+}$ .

to decline with the consumption of the intermediate. During the rate-controlling step, the second acceleration ( $0.007 \text{ arb. min}^{-1}$ ) appeared at 14 min and might be related to the dissociation of Hydrogen ions so that the porphyrin ring restored to stability. The kinetics catalyzed by  $Pb^{2+}$  studies suggested that the formation of metalloporphyrin was step-wise. Otherwise there would not be two distinct acceleration periods.

Figure 8 shows the influence of  $H^+$  in the pre-equilibration step and rate-controlling step catalyzed by  $Pb^{2+}$  (at the rate of  $TIPP:Pb^{2+} = 1:10$ ). As can be seen from the curve, compared with neutral condition, the reaction rate of the pre-equilibration step does not slow down but improved when the acid was added at the beginning. The reaction rate also improved during the rate-controlling step and may be attributed to the catalytic effect of  $H^+$  and  $Pb^{2+}$ . The reaction rate was significantly faster than during neutral conditions when adding acid at the beginning. If the dissociation of protons occurred in the pre-equalization step, the  $H^+$  will inhibit the forward movement of the balance in acidic condition, the reaction rate will be lower than neutral condition at least in the pre-equilibrium step, this was inconsistent with the experimental facts. So, the dissociation of protons did not occur in the pre-equilibration step. However, the reaction rate of the rate-controlling step declined about  $0.002 \text{ arb. min}^{-1}$  when the acid was added at 13 min.  $H^+$  may inhibit the forward movement of the balanced system, and it most like the dissociation of hydrogen ions. This further validated the formation mechanism of metalloporphyrin catalyzed by  $Pb^{2+}$ .

## Conclusions

Lead ions can catalyze the complexation reactions between TIPP and  $VO^{2+}$ . It forms a tetragonal conical configuration without plane symmetry when substituted hydrogen atoms are on the nitrogen of the porphyrin ring. There was an existing great repulsive force in congested benzenes.  $VO^{2+}$  attack from the rear center of the porphyrin ring more easily; Hydrogen ions can catalyze the complexation reactions between TIPP and  $VO^{2+}$ . This may be attributed to the

porphyrin ring that accepts two protons, then the two hydrogen atoms in the porphyrin ring shift from counterpoint to ortho due to conjugation. More importantly, this isomer is more conducive to complexation reactions; Kinetic studies verified that the dissociation of protons during the complexation reactions between TIPP and metal ions occurred in the rate-controlling step, rather than in the pre-balance step.

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