

สำนักหอสมุดกลาง พระจอมเกล้าลาดกระบัง

รายงานการวิจัย

อัตราการเกิดปฏิกิริยาระหว่างเหล็กและกลุ่มสารไพริดอกซาลไพริดีลไฮดราโซน
IRON CHELATORS OF PYRIDOXAL PYRIDYL HYDRAZONE

ผู้วิจัย รองศาสตราจารย์ ดร. ประยงค์ ควงดี

ได้รับทุนสนับสนุนงานวิจัยจากเงินงบประมาณแผ่นดิน
ประจำปีงบประมาณ 2549

คณะวิทยาศาสตร์
สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหาร ลาดกระบัง

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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์อื่นใด
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ACKNOWLEDGEMENTS

This study was supported by a grant from National Research Council of Thailand.



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HYDRAZONE

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ประจำปีงบประมาณ 2549 จำนวนเงิน 300,000 บาท

ระยะเวลาทำการวิจัย 1 ปี ตั้งแต่ 1 กันยายน 2548 ถึง 1 ตุลาคม 2549

หน่วยงานและผู้ดำเนินการวิจัย รองศาสตราจารย์ ดร. ประยงค์ ดวงดี คณะวิทยาศาสตร์
สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง โทรศัพท์ 027373000 ต่อ 6246

บทคัดย่อ

จากข้อมูลการวัดค่าคงที่การเกิดปฏิกิริยา (pK) และเปอร์เซ็นต์การกระจายของสารประกอบ (Complex species distribution) ที่เกิดขึ้น ปฏิกิริยาเคมีระหว่างเหล็ก (Fe(II), Fe(III)) และสารไพริดีออกซาลไฮดราโซน (ligand) ก่อให้เกิดกลุ่มสารประกอบ (Complex species) เกิดขึ้นทั้งหมด 4 ชนิดในช่วง pH 3.5-8.5 ดังนี้ (i) 1:1-Fe(II):ligand (1), (ii) 1:1-FeOH:ligand (2), (iii) 1:1-Fe(III):ligand (3), and (iv) 1:2-Fe(III):ligand (4) สารไพริดีออกซาลไฮดราโซนใช้ในการทำปฏิกิริยามีทั้งหมด 3 ตัวดังนี้ (a) pyridoxal 2-pyridyl hydrazone (PPH), (b) 1-[N-methylpyridoxylidenium]-2-[2'-pyridyl] hydrazine iodide (MPH) และ (c) 1-[N-ethoxy carbonyl methyl pyridoxylidenium]-2-[2'-pyridyl] hydrazine bromide (EPH) การศึกษาปฏิกิริยาเคมีระหว่างเหล็ก (Fe(II), Fe(III)) และกลุ่มสารไพริดีออกซาลไฮดราโซนในครั้งนี้ใช้เทคนิคการวัดโดยวิธี Potentiometry และ Spectrophotometry ร่วมกัน

สารไพริดีออกซาลไฮดราโซนทั้งหมด 3 ตัวนี้สามารถทำปฏิกิริยากับเหล็กได้เป็นอย่างดีให้ค่าคงที่การเกิดปฏิกิริยา (pK) สำหรับสารประกอบที่ (1) ให้ค่า 7.42 (PPH), 6.04 (MPH) และ 5.78 (EPH) สารประกอบที่ (3) ให้ค่า 7.84 (PPH), 6.10 (MPH), และ 5.80 (EPH) สารประกอบที่ (4) ให้ค่า 12.74 (PPH), 12.30 (MPH), และ 11.76 (EPH) เมื่อเปรียบเทียบกับสารไพริดีออกซาลไฮดราโซนอีกตัว pyridoxal isonicotinoyl hydrazone (PIH) ให้ค่า [1:1-Fe(II):ligand=8.73, 1:1-Fe(III):ligand=8.03] แสดงให้เห็นว่าสารไพริ

ด็อกซาลไฮดรอกโซนเมื่อทำปฏิกิริยากับโลหะ กิ่งข้างของวงแหวนไพริดีนออกซาล ได้แก่
กลุ่ม CH₃, N-Me และ CH₂OH จะไม่มีผลต่อการเกิดปฏิกิริยาแต่อย่างใด

นอกจากนั้นจากข้อมูลยังแสดงให้เห็นว่า สารประกอบที่ (3) จะเปลี่ยนไปเป็น
สารประกอบที่ (4) [Fe(II):ligand(3)→1:2-Fe(II):ligand(4)] ที่ pH>7 สารประกอบที่ (1)
จะไม่เป็นเช่นนั้นไม่มีแนวโน้มที่จะเกิดปฏิกิริยาแบบ 1:1-Fe(II):ligand→1:2-Fe(II):ligand

ในแง่ชีวเคมี ค่าคงที่การเกิดปฏิกิริยา (*pK*) ที่วัดได้ไม่มีความสัมพันธ์ใดๆกับ
ค่าตัวเลข การตั้งหลักจากร่างกาย ซึ่งทดลองกับหนู หรือค่า IC₅₀ (inhibitory concentration
50%) ที่แสดงความสามารถต้านเชื้อมาลาเรียของสารประกอบกลุ่มนี้

Abstract

The formation constants and species distribution over pH range 3.5–8.5, herein presented, for four different iron complexes, (i) 1:1-Fe(II):ligand(1), (ii) 1:1-FeOH:ligand(2), (iii) 1:1-Fe(III):ligand(3), and (iv) 1:2-Fe(III):ligand(4), arising from interaction of aqueous Fe(II) and Fe(III) ions with three biologically active chelators, (a) pyridoxal 2-pyridyl hydrazone (PPH), (b) 1-[N-methylpyridoxylidinium]-2-[2'-pyridyl] hydrazine iodide (MPH) and (c) 1-[N-ethoxy carbonyl methyl pyridoxylidinium]-2-[2'-pyridyl] hydrazine bromide (EPH), has been determined by glass electrode potentiometry. All three ligands bind effectively to iron(II) and iron(III), giving *pK* values of (1) ranging from 7.42 (PPH), 6.04 (MPH), to 5.78 (EPH), and of (3) 7.84 (PPH), 6.10 (MPH), and 5.80 (EPH), and *pK* values of (4) 12.74 (PPH), 12.30 (MPH), and 11.76 (EPH), comparable to pyridoxal isonicotinoyl hydrazone (PIH) [1:1-Fe(II):ligand=8.73, 1:1-Fe(III):ligand=8.03] indicating that metal binding essentially is not appreciably influenced by the CH₃, N-Me, and CH₂OH side chains, on the pyridoxal ring, whereas species of group (3) easily invert into (4) at pH>7. Group (1) do not lend themselves to similar 1:1-Fe(II):ligand→1:2-Fe(II):ligand inversion, at higher pH. The rates of mobilization of labeled iron from hypertransfused rats, and the IC₅₀ (inhibitory concentration 50%) values of the iron dependent antimalarial activities of the ligands could not be correlated with ligand affinities for iron.

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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
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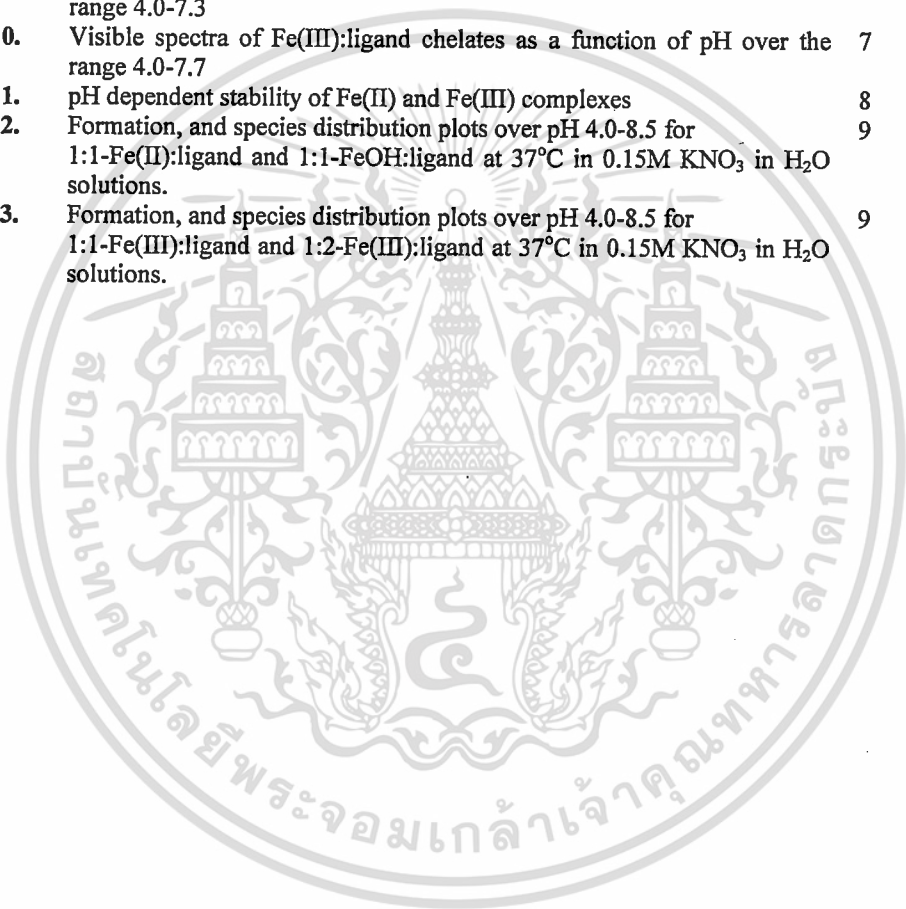
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List of Abbreviations

| | |
|------------|--|
| PPH | pyridoxal 2-pyridyl hydrazone |
| MPH | 1-[N-methylpyridoxylidenium]-2-[2'-pyridyl] hydrazine iodide |
| EPH | 1-[N-ethoxy carbonyl methyl pyridoxylidenium]-2-[2'-pyridyl] hydrazine bromide |
| PIH | pyridoxal isonicotinoyl hydrazone |



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INTRODUCTION

An important goal in chelation therapy of certain hemoglobinopathies such as Cooley's anemia (β thalassemia)¹ has been the development of lipophilic chelating agents of high affinity for iron, capable of removal of toxic surpluses of iron (iron overload) from the body². Toxic accumulation of iron in certain blood disorders frequently arises from repeated blood transfusions needed to overcome the hemoglobinopathy situation. The only effective iron chelators in current clinical use is desferrioxamine B^{3,4} (desferal, DFO), a costly microbial trishydroxamic acid siderophore, requiring sub-coetaneous administration due to its (i) rapid degradation in the serum, and (ii) poor intestinal absorption. Because of these disadvantages, considerable effort has been expended in search for alternatives for this drug. Over the last decade, new classes of synthetic pyridoxal-containing ligands have been developed as potential substitutes for DFO endowed with oral activity, transmembrane mobility, and low cost.⁵⁻⁸

The acyl-hydrazone from pyridoxal and isonicotinic acid hydrazide is pyridoxal isonicotinoyl hydrazone (PIH) (Fig. 1), an orally effective chelators of moderate affinity for iron (II) and iron (III). Its potential has been examined and substantiated in a variety of biological testings.^{8,9}

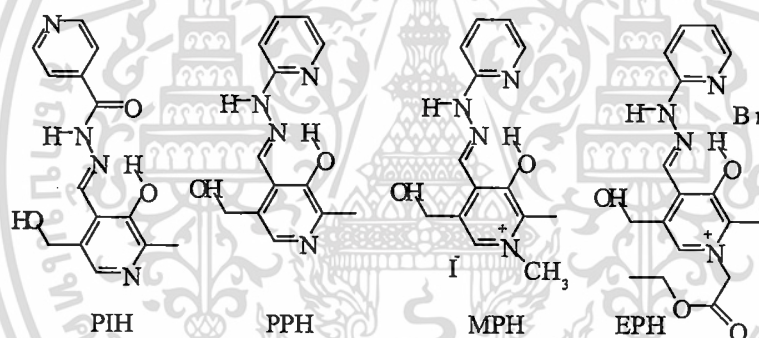


Fig. 1 The structures of PIH, PPH, MPH and EPH

Recently we have shown that aryl-hydrazone of pyridoxal such as pyridoxal 2-pyridyl hydrazone (PPH)^{10a}, 1-[N-methyl-pyridoxylidonium]-2-[2'-pyridyl] hydrazine iodide (MPH)^{10b}, and 1-[N-ethoxy-carbonylmethyl-pyridoxylidonium]-2-[2'-pyridyl] hydrazine bromide (EPH) (Fig.1) are more effective than PIH in assays using hypertransfused rats^{9a}. Structure-activity-relationship (SAR) study has shown that the iron mobilizing capability in vivo increases in the ordering: PIH < PPH < MPH < DFO < EPH^{9c}.

Complex formation between iron(II) and iron(III), and PIH, has been investigated extensively by spectroscopic and physicochemical methods¹¹⁻¹⁴. In the solid state, PIH exists as a neutral cyclic dimer {preferred motif R²(16)¹⁵} of its trans keto-form (Fig.2). Interestingly, PIH on complexation with Fe²⁺ ions undergoes prototautomeric change, yielding crystalline {(PIH)₂Fe(III) x 0.5 SO₄} complex, in which, the ligand exists as an zwitter-ionic cyclic dimer {preferred motif R²(26)} of its enolic-form (Fig.3)^{11b}. The observed facile Fe(II) → Fe(III) + e⁻ conversion with concomitant stabilization of the ligand enolic form arises most likely from "metal-coordination effect", as encountered with other ligands such as oxalates, EDTA, phosphates, etc.¹⁶

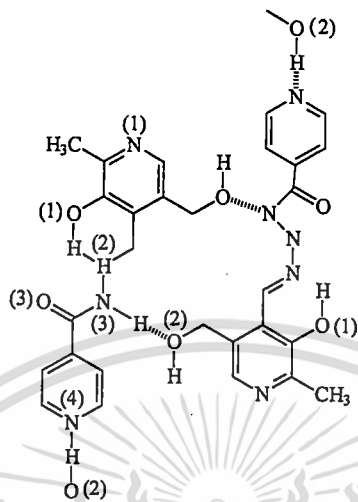


Fig. 2 16-Membered ring dimer of PIH

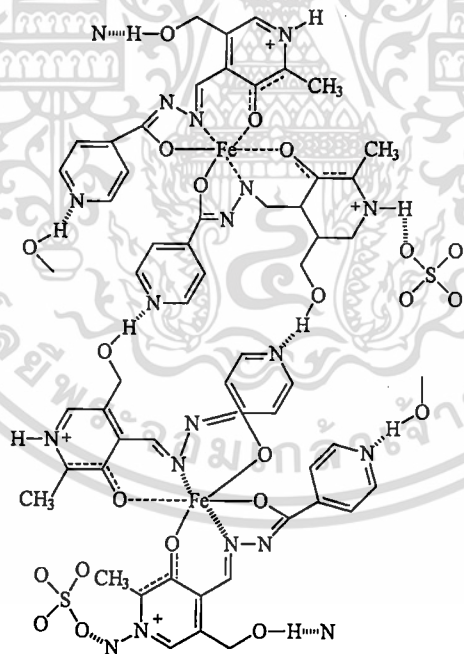


Fig. 3 26-Membered ring dimer of $(PIH)_2Fe(III) 0.5 SO_4$

Of particular interest is the generation and trapping, under anaerobic conditions, of Fe(III)-chelate radical of structure $\{[MPH]Fe(II)\}^+ X^- \rightarrow \{[MPH]Fe(III)\}^\bullet X^-$ (Fig.4), which is capable of quick killing of the drug-resistant specie of *Plasmodium falciparum*.¹⁹

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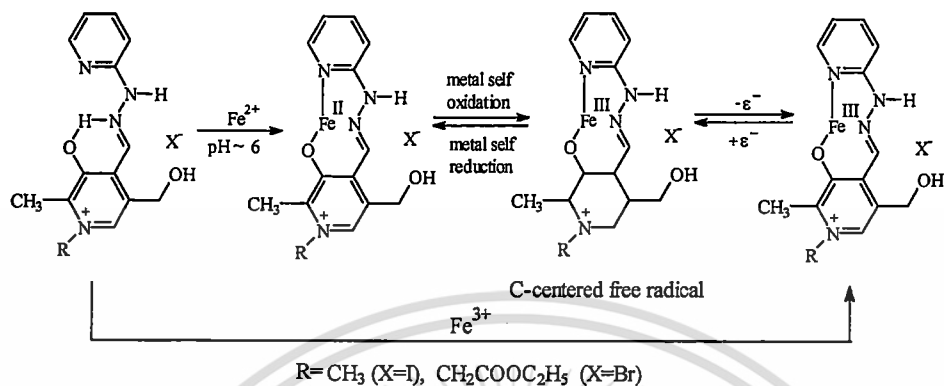


Fig. 4 Carbon-centered Fe(III)-chelate free radical of PPH, MPH, EPH

Studies have shown that depending on pH the pyridoxal-based chelators exist in aqueous medium as distinctly different species^{20,21}. Each of these can react with iron ions differently to yield iron-complexes, which differ from each other in composition, stability, transmembrane transport, and oxidation-reduction potentials. All these arguments, underscores the importance of complex formation between the chelating agents PPH, MPH, and EPH, and Fe²⁺ and Fe³⁺ ions. In this paper we report the results of solution studies of the formation constants between the three structurally related ligands, PPH, MPH, EPH, and the Fe²⁺, and Fe³⁺ ions. The formation constant between PIH and Fe(II) has been reported earlier^{12,13}, and more spectroscopic and physicochemical analyses were performed mainly on PIH, indicating that depending on pH, the ligand reacts with iron(III) to form two distinctly different complexes, (i) the corresponding 1:1-Fe(III):ligand^{12a}, and (ii) the respective 1:2-Fe(III):ligand chelates.^{11a12b}

The ultraviolet and the visible spectra of PPH, MPH, and EPH, both in presence and in absence of Fe²⁺, and Fe³⁺ ions, are presented in Figs. 7-10. The formation, and species distribution plots of iron complexes with Fe²⁺, and Fe³⁺ ions, as a function of pH are depicted respectively in Figs. 12 and 13.

MATERIALS AND METHODS

Chelating agents. All reagents were of the highest purity commercially available and were used without further purification. PPH mp 288°C (free base)^{1a}, MPH mp 235-6°C (free base)^{1b}, EHP mp 204°C, UV (MeOH, c. 5.10⁻⁶M) : λ_{\max} 651 nm (log ϵ 1.55), and PIH, mp 245°C (monohydrochloride), were prepared according to literature and recrystallized from methanol.

Stock solutions. Nitric acid and sodium hydroxide solutions were obtained from "Merck" (Titrisol). The concentrations of these solutions were checked regularly by acid-base titration in the course of electrode calibration²³. Stock solutions of 0.2 mm of the ligands were freshly prepared before each titration by dissolving the respective ligands in 0.15 M KNO₃ (Merck Analytical grade). Double-distilled water was used throughout the experiment. Stock solutions of 0.15 M Fe(NO₃)₃ were standardized by reduction with SnCl₂ followed by titration with standard KMnO₄. Solutions of Fe(NO₃)₂ were prepared by allowing equimolar solutions of FeSO₄ and Ba(NO₃)₂ in freshly distilled water to mix under positive pressure of oxygen-free nitrogen. The supernatant was decanted and filtered under N₂ before being added to HNO₃, to yield a final Fe(II) concentration of approx. 0.15 M. The exact iron(II) concentration was determined by titration with standard KMnO₄.

Spectrophotometric Measurements. The optical absorbance spectra of the ligand and metal complex species were measured with Kontron Uvikon 810 fast scan spectrophotometer and matched quartz cells of light path length of 1.000 ± 0.001 cm. Ionic strength was maintained at 0.15 M with reagent grade KNO₃.

Formation Constants. The Fe(II) complexes are more soluble than the corresponding Fe(III) species and the formation constants were determined at 10⁻⁴ M iron(II) concentration by potentiometric titration at 37°C under pure N₂ over the pH range 3.5-8.5, and great care had been taken to exclude oxygen. The less soluble Fe(III) complexes were studied similarly at 10⁻⁵ M concentrations. Potentiometric measurements were carried out in a capped titration vessel, fitted with an Ingold No. 405-M5 micro glass electrode. Electrode potentials were measured by use of a purpose-built automatic titrator of CG 822 Schott pH meter, with a 5 ml piston burette. The titrations were performed under inert atmosphere by flowing nitrogen to the titration vessel. The entire apparatus was kept in a thermostat at 37 ± 0.5°C. All measurements were made at an ionic strength of 0.15 M (KNO₃). The electrode calibrations were made by acid-base titration²³ under identical above conditions.

Calculations. The intercept and the slope from electrode equation followed from analysis of calibration data using the least-square programs in Microsoft office. The SUPERQUAD computer program²² was also used in the search of the complexation model.

RESULTS AND DISCUSSION

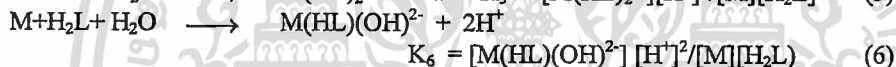
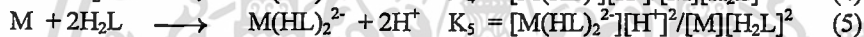
The protonation constants of PPH, MPH, EPH, and PIH, have been reported elsewhere^{1d} to be characterized by pK_a values of 2.4 – 10 in the uncomplexed ligands. The stepwise formation constants of the Fe(II) and Fe(III) chelate produced here are assembled in Tables 3 and 4. Structural formulae of the ligands included in this study are delineated in Fig.1. The protonation species of PPH (1a-1d), and of MPH, and EPH (2a-2d) are represented in Fig. 5-6.

It has been noted that PIH and PPH can be characterized by four protonation constants corresponding to the formation of H_4L^{2+} , H_3L^+ , H_2L , and HL^- species (1a-1d, Fig. 5). The ligands based on pyridoxal betaine, MPH and EPH, have been shown to be characterized by only three protonation constants corresponding to H_3L^+ , H_2L , and HL^- (2a- c, Fig. 6). Because of the large number of ionisable protons on the ligands, iron-ligand species are potentially complicated and it is necessary to specify the equilibria and the stoichiometry of the various complexes.

Each of the synthetic ligands, protonation and deprotonation species would correspond to respected cases described in these mechanisms.



For the iron complex species will be described as following.



Where H_2L is free ligand, H^+ is proton, OH^- is a hydroxyl group, and M is either Fe(II) or Fe(III). $M(HL)^-$ is mono-iron complex, $M(HL)_2^{2-}$ is 1:2-Fe(III):ligand complex and $M(HL)(OH)^{2-}$ is a hydroxyl containing 1:1-Fe(II)(OH):ligand complex.

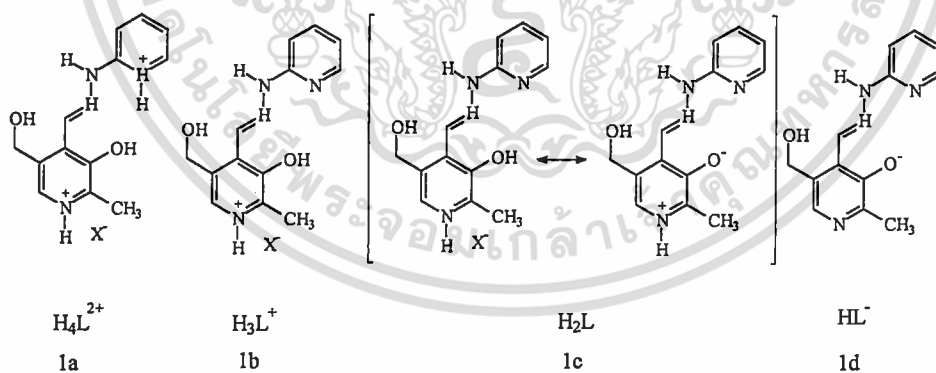


Fig. 5 Protonation Species of PPH

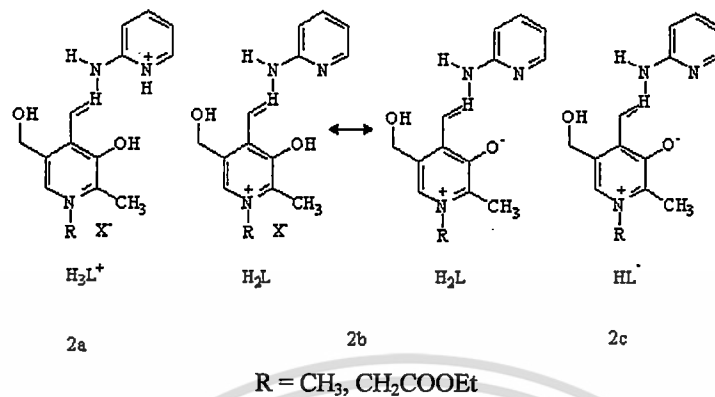


Fig. 6 Protonation Species of MPH and EPH

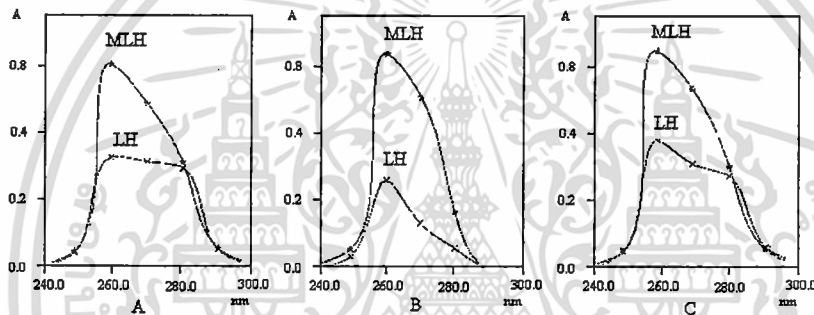


Fig. 7 UV spectra of 1:1-Fe(II):ligand

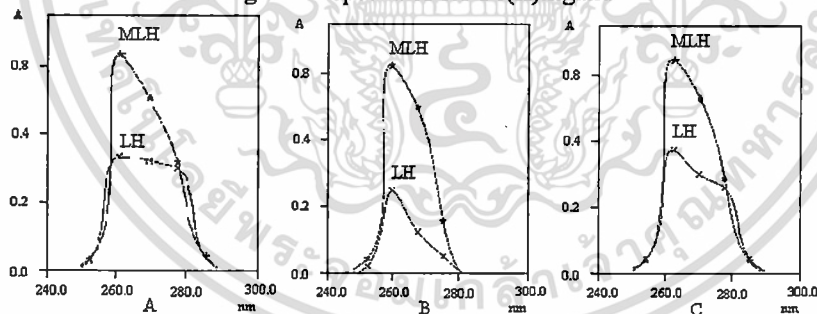


Fig. 8 UV spectra of 1:1-Fe(III):ligand

The UV spectra of ligands in the presence, and in the absence of iron salts at pH 3.5 (Figs. 7 and 8) clearly indicates the involvement of the pyridoxal-phenolate oxygen site in the iron binding process. The UV band at 260 nm is attributed to pyridoxal phenolate anion absorption²⁴. Most convincingly, the binding of the metal to the ligand is noted at pH > 3.5 (see, Figs. 12 and 13), giving rise to visible absorption bands at 400 and 475 nm (see Figs. 9 and 10, and Tables 1 and 2). In the range of physiological pH (6.8-7.6), the iron-chelates exhibit new visible bands in the 470-480 nm range of the spectrum. On increasing the pH beyond 6.8-7.7, the respective chelates become sparingly soluble in water, rendering the chelates solutions unsuitable for further spectroscopic study.

สำนักหอสมุดกลาง พระจอมเกล้าลาดกระบัง

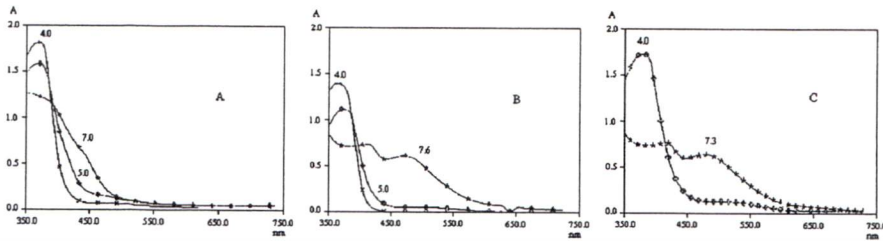


Fig. 9 Visible spectra of Fe(II):ligand chelates as a function of pH over the range 4.0-7.3
A=PPH, B=MPH, C=EPH

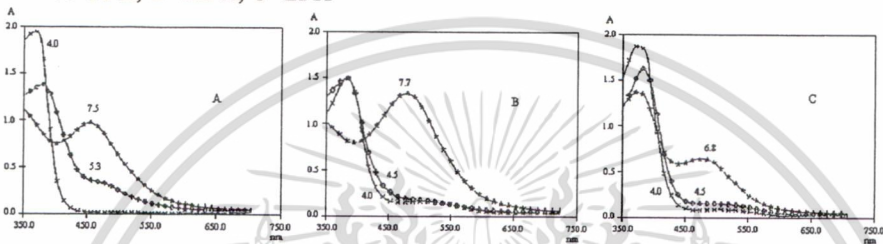


Fig. 10 Visible spectra of Fe(III):ligand chelates as a function of pH over the range 4.0-7.7
A=PPH, B=MPH, C=EPH

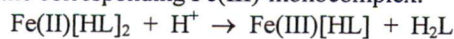
Table 1. Absorbance of Fe(II) : ligand chelates as a function of pH over the range 4.0-7.3
A=PPH, B=MPH, C=EPH

| | pH | λ_{max} (nm) | pH | λ_{max} (nm) | pH | λ_{max} (nm) |
|---|-----|-------------------------|-----|-------------------------|-----|-------------------------|
| A | 4.0 | 375 | 5.0 | 375 | 7.0 | 375 |
| B | 4.0 | 375 | 5.0 | 375 | 7.6 | 422, 472 |
| C | 4.0 | 388 | -- | -- | 7.3 | 420, 470 |

Table 2. Absorbance of Fe(III) : ligand chelates as a function of pH over the range 4.0-7.7
A=PPH, B=MPH, C=EPH

| | pH | λ_{max} (nm) | pH | λ_{max} (nm) | pH | λ_{max} (nm) |
|---|-----|-------------------------|-----|-------------------------|-----|-------------------------|
| A | 4.0 | 378 | 5.0 | 390 | 7.1 | 378, 475 |
| B | 4.0 | 392 | -- | -- | 7.7 | 480 |
| C | 4.0 | 395 | 5.0 | 400 | 6.8 | 385, 472 |

The titration measurements in this study included experiments of metal:ligand ratios, ranging from 1:1, 1:2, 1:3 through 1:8. The resulting data allowed the conclusion that the reaction of the ligands with Fe^{2+} ions gives rise to two distinctly different Fe(II) chelates, (i) the respective mono complex, $M(HL)^-$ and (ii) an hydroxyl containing complex, $M(HL)(OH)^{2-}$ in a ratio depending on the ligand structure. Unlike the association with Fe(II) ions, the reaction similarly of the ligands with Fe^{3+} ions leads, in addition to corresponding (iii) monocomplex $M(HL)^-$, also to (iv) the respective dicomplex, $M(HL)_2^{2-}$ in a ratio shown to be a function of pH (see Table 5). Thus, the mono Fe(II)-complex is quite stable over the pH range, 3.5-7.7, but, the corresponding Fe(III)-dicomplex is definitely unstable at pH below 5. Indeed, at $pH < 5$, the Fe(III)-dicomplex tends to loose one of its ligands to yield the corresponding Fe(III)-monocomplex.



The reaction appears to be reversible since on pH increase the Fe(III)-mono complex transforms fully into its Fe(III)-dicomplex species (see Fig. 11).

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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา 7- ละต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

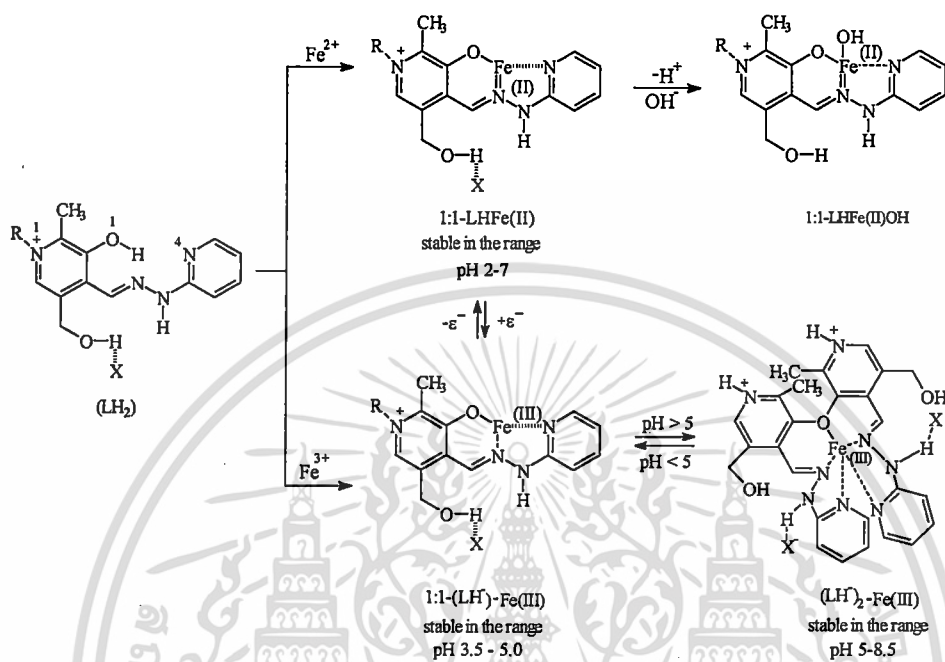


Fig. 11 pH dependent stability of Fe(II) and Fe(III) complexes

Table 3. Complex formation constant of 1:1-Fe(II):ligand and 1:1-Fe(II)(OH):ligand

| Ligand | 1:1-Fe(II):ligand pK ₄ | 1:1-Fe(II)(OH):ligand pK ₆ |
|-------------------|--------------------------------------|--|
| PPH | 7.42 ± 0.11 | 12.29 ± 0.32 |
| MPH | 6.04 ± 0.20 | 10.97 ± 0.22 |
| EPH | 5.78 ± 0.33 | 11.06 ± 0.70 |
| PIH ¹² | 8.73 ± 0.11 | - |

Table 4. Complex formation constant of 1:1-Fe(III):ligand and 1:2-Fe(III):ligand

| Ligand | 1:1-Fe(III):ligand pK ₄ | 1:2-Fe(III):ligand pK ₅ |
|-------------------|---------------------------------------|---------------------------------------|
| PPH | 7.84 ± 0.03 | 12.74 ± 0.52 |
| MPH | 6.10 ± 0.10 | 12.30 ± 0.32 |
| EPH | 5.80 ± 0.08 | 11.76 ± 0.44 |
| PIH ¹² | 8.03 ± 0.10 | - |

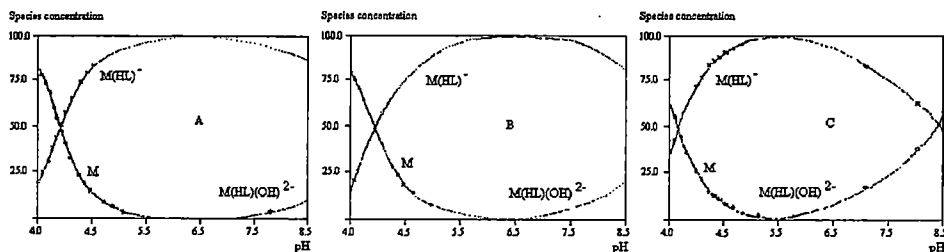


Fig. 12 Formation, and species distribution plots over pH 4.0-8.5 for 1:1-Fe(II):ligand and 1:1-Fe(OH):ligand at 37°C in 0.15M KNO₃ in H₂O solutions. A=PPH, B=MPH, C=EPH

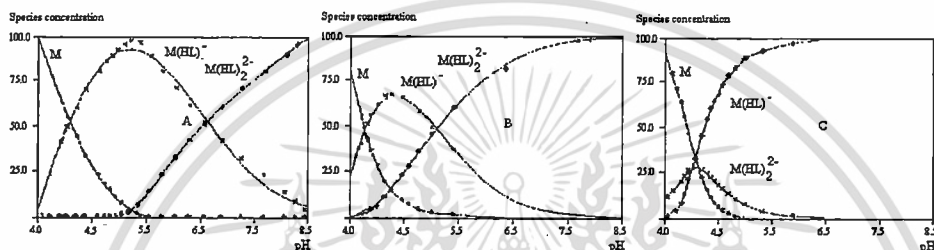


Fig. 13 Formation, and species distribution plots over pH 4.0-8.5 for 1:1-Fe(III):ligand and 1:2-Fe(III):ligand at 37°C in 0.15M KNO₃ in H₂O solutions. A=PPH, B=MPH, C=EPH

The formation and species distribution of the complexes between the ligand and the ions of Fe(II) and Fe(III), over the pH range 4.0-8.5 are presented in Figs. 12 and 13, respectively. It can be seen that depending on pH range, and the iron species, each ligand gives rise to two sets of iron complexes. One contains the species of 1:1-Fe(II):ligand (1); and that of hydroxo 1:1-Fe(OH):ligand complex (2). The second, comprise the forms of 1:1-Fe(III):ligand (3), and 1:2-Fe(III):ligand (4). In the 4.0-7.0 pH range, PPH gives rise to a single, relatively stable form of 1:1-Fe(II):ligand complex (1). As the pH increases, the latter (1) lends itself to hydrolysis to yield the hydroxo-complex 1:1-Fe(OH):ligand (2). The influence of pH-increase, on 1:1-Fe(III):PPH from follows a distinctly different reaction course, inducing the 1:1 → 1:2 conversion of (3) → (4). Unlike 1:1-Fe(II):ligand (1), the corresponding Fe(III) species (3), shows no tendency to hydrolysis. By the same token, on pH elevation the 1:1-Fe(II):ligand species could not be induced to 1:1-Fe(II):ligand → 1:2-Fe(II):ligand conversion, prior to (1) → (2) hydroxylation. These could be rationalized in terms of tendencies of the nitrogen of PPH, MPH, and EPH hydrazone bridge and pyridyl ring, to favor complex formation with Fe(II), while the single phenolate oxygen is assumed to form a stronger complex with Fe(III)^{17,28}. It is significant that unlike 1:1-PIH:Fe(II), which lends itself to easy Fe(II) → Fe(III) oxidation²⁹, following a modification in the redox potential of the metal ion in the complex¹⁹, the redox potential of the metal ion in 1:1-PPH:Fe(II), seems to be unaffected when the ligand becomes “softer”²⁷.

Table 5 Species distribution of Fe(II):ligand and Fe(III):ligand at physiological pH

| | Fe(II):ligand | | | Fe(III):ligand | | |
|-----|-----------------|-----------------------------------|----------------------------|------------------|---|----------------------------|
| | Fe(II)(HL) % | Fe(II)(OH)(HL) ²⁻ % | Free Fe ²⁺ % | Fe(III)(HL) % | Fe(III)(HL) ₂ ²⁻ % | Free Fe ²⁺ % |
| PPH | 95 | 5 | 0 | 50 | 50 | 0 |
| MPH | 87 | 13 | 0 | 15 | 85 | 0 |
| EPH | 80 | 20 | 0 | 0 | 100 | 0 |

Biological relevance. Interestingly, at physiological pH (7.2-7.4), PPH exists as a 50%:50% mixture of (3):(4), whereas the relevant values for MPH, and EPH, are 15%:85%, and 0%:100% respectively (see Table 5). It can be seen that the iron is completely chelated at physiological pH. It is conceivable to assume that enzymatic reduction of Fe(III)-dicomplex at physiological pH should yield the respective highly lipophilic and pH stable Fe(II)-monocomplex, capable of interacting with protoplasmic carriers with enhanced transmembrane mobility.

The metal:ligand association involves most favorably the neutral form of the ligand, H₂L. This is adequately verified on comparing plots of species distribution in percentage against pH of the ligands³⁰ reported in Part 4^{1d}, with plots of formation of corresponding 1:1-Fe(II):ligand versus pH demonstrated in Fig. 12. This indicates that the biologically most active chelators (EPH, MPH) sequester favorably Fe(II) ions at pH well below 7.4, whereas the maximal sequestration of the Fe(III) ions occurs near the physiological pH (see Fig. 13). This can, at least partially, explain the remarkable enhancement^{20,32} in antimalarial activity on going from PIH:Fe(III), to PPH:Fe(II), to MPH:Fe(II)-EPH:Fe(II), when the neutral forms of which pass from the blood media (pH ~ 7.2) to the paritized red blood cell and then to the triphozoite (a well defined stage in the intraerythrocytic life-cycle of *Plasmodium falciparum*³³, the pH of its food vacuole estimated to be approximately 5.2³⁴⁻³⁶). Significantly, the population of the neutral form, H₂L, of the weaker antimalarial (PPH, PIH), increases by only 0.3-fold as they approach the parasite food vacuole. This associates metal coordination with the acidity of the cell compartment³⁶ in which the metal-sequestration takes place³².

The affinities of PPH, MPH, EPH, and PIH for Fe(III) (Table 4) are comparable, indicating that the N-CH₃, and N-CH₂COOEt side chains on the pyridoxal ring have minor influence on metal binding. However, the rates of mobilization of labeled iron from ⁵⁹Fe-transferrin, and ⁵⁹Fe-haem-damaged red blood cells (HDRBC) in hypertransfused rats were shown to increase in the ordering, PIH < PPH < MPH < EPH < DFO^{9,10b}, indicating no simple correlation between the affinity for iron and the iron-mobilizing capacity of the chelators to excrete iron from the body. Indeed, in view of the complexity of the living cell, it is not likely to expect straightforward relationship between the affinity for iron and the ability of the chelators to mobilize the metal in vivo^{10b}. Affinity for iron is an important factor, but it is certainly one among many others. This subject is worthy of further bioinorganic chemical study.

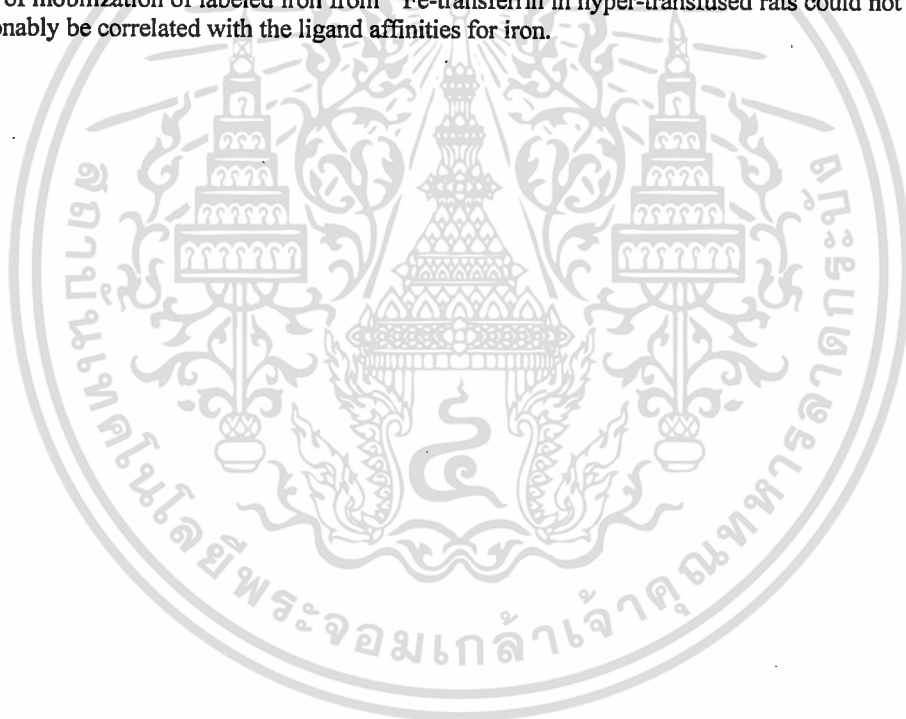
CONCLUSIONS

Potentiometric pH titration of PPH, MPH, and EPH with iron(II) and iron(III) ions, under pure nitrogen at 37°C in 0.15 M KNO₃, led to the detection of four distinctly different species for each ligand, 1:1-Fe(II):ligand (1), 1:1-Fe(II)OH:ligand (2), 1:1-Fe(III):ligand (3), and 1:2-Fe(III):ligand (4) with formation constants, pK values that are comparable with PIH.

The (1) species are steady below pH 7. Above pH 7, they tend to hydrolyze to produce (2). At physiological pH, the iron is completely chelated. The (1) is not sensitive to oxidation to produce, either (3), or (4).

The (3) species are not steady at pH above 6, around which they lend themselves to reversible inversion to yield (4), which is steady to hydrolysis. At physiological pH, the (3):(4) ratios vary respectively from 50:50, in PPH, to 15:35 in MPH, to 0:100 in EPH.

The affinities for iron(III) among the “softer”²⁷ ligand, PPH, MPH, EPH, and the “harder”²⁷ ones, PIH are quite comparable (Table 3), indicating that, neither the electron-withdrawing, N-CH₃, N-CH₂COOEt, nor the electron-donating, CH₃, and CH₂OH, side chains on the pyridoxal ring have noticeable influence on metal binding. Moreover, the rates of mobilization of labeled iron from ⁵⁹Fe-transferrin in hyper-transfused rats could not reasonably be correlated with the ligand affinities for iron.



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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา 14 จะต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้



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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้