รายงานวิจัยฉบับสมบูรณ์

โครงการ การสังเคราะห์ การศึกษาโครงสร้าง และการนำไปใช้ของ polyaza tris-ferrocene dinuclear cryptate

โดย ดร. เอกสิทธิ์ สมสุข
ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

30 มิถุนายน 2547

สัญญาเลขที่ TRG4580067

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

Abstract

Project Code: TRG4580067

Project Title: Synthesis, Structure, and Application of Polyaza Tris-ferrocene Dinuclear Cryptates.

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Project Period: July 2002-June 2004

In this project entitled "synthesis, structure, and application of polyaza tris-ferrocene dinuclear cryptates have been proposed for the study. Unfortunately, 1,1'-dibromoferrocene, a starting compound for the synthesis of the proposed compound, is unstable. The project has been modified by using commercial diamino compounds. An acridine-based spacer supramolecule was successfully synthesized and characterized with ^{1}H, ^{13}C, and 2D-NMR spectroscopy. Attempting to get a single crystal of this compound is in progress.

บทคัดย่อ

ในโครงการนี้ในชื่อ การสังเคราะห์ โครงสร้างและการนำไปใช้ ของ polyaza tris-ferrocene dinuclear cryptates ได้นำเสนอในโครงการวิจัยต่อ สกว.นั้น ได้พบอุปสรรคในการทดลองเพื่อเตรียมสารตั้งต้นคือ 1,1'-dibromoferrocene นั้นไม่เสถียรที่อุณหภูมิห้อง โครงการได้เปลี่ยนไปใช้สารที่ซื้อได้และปรากฏว่าการสังเคราะห์ acridine-based spacer supramolecule นั้นได้ประสบความสำเร็จและได้พิสูจน์โครงสร้างด้วย ในขณะนี้อยู่ระหว่างการตกผลึกเพื่อให้ได้โครงสร้าง 3 มิติต่อไป

Background

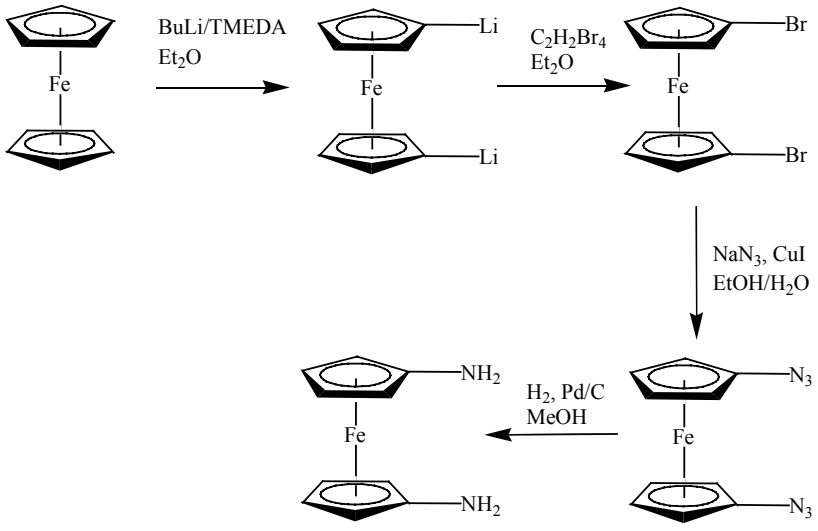
The activation of small molecules, such as H_2, O_2 and N_2, is currently of interest in both academia and industry. It is a multi-electron process that requires extra force to be exerted. In contrast to electron transfer systems in nature such as photosynthesis in plants, electrons are transferred very efficiently. Knowledge obtained from the investigation would be useful for the development of both clean energy generators such as fuel cells and a catalytic transformation of ammonia that is a precursor for explosive materials and fertilizers.

Objectives

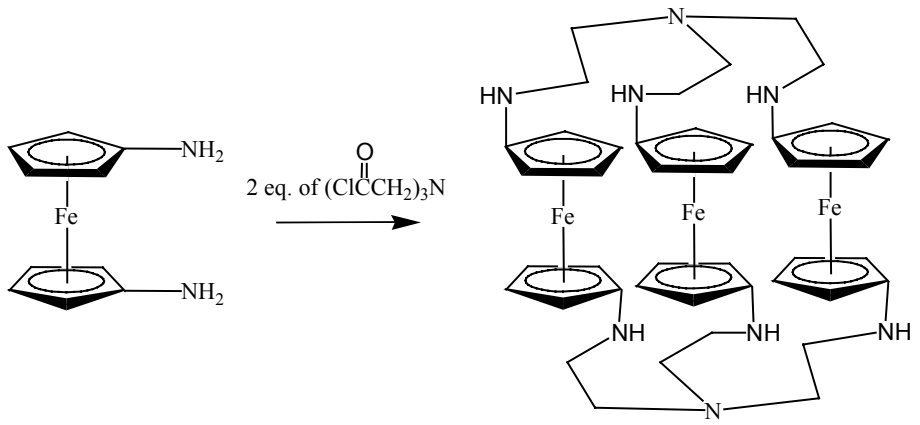
To synthesize polyaza tris-ferrocene cryptate complexes which potentially display a characteristic for the activation of small molecules.

The proposed experiments

The synthesis of the dinuclear cryptates could be carried out as summarized in Scheme 1-3. Starting from ferrocene, 1,1'-diaminoferrocene can be obtained by the Arnold's method (Scheme 1). Subsequent treatment with two equivalents of N $(CH_2COCl)_3$ will furnish polyaza tris-ferrocene cryptand(Scheme 2).

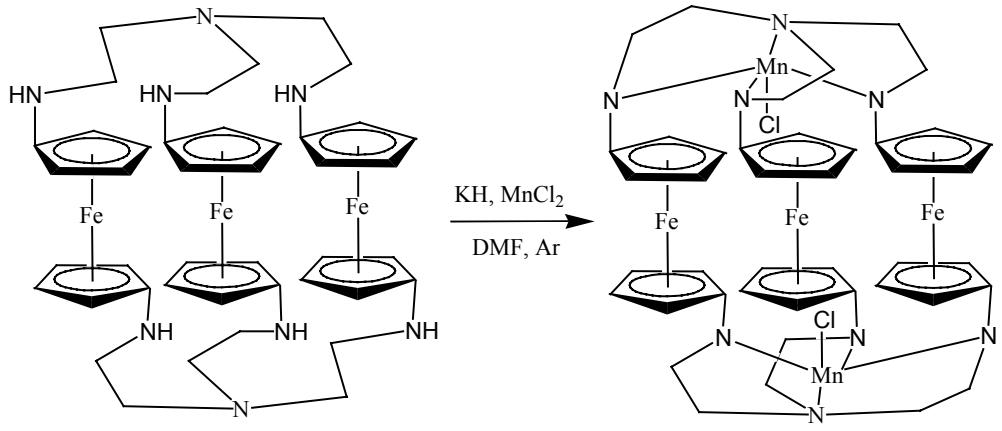


Scheme 1. Synthesis of 1,1'-diaminoferrocene.



Scheme 2. Synthesis of polyaza trisferrocene cryptand.

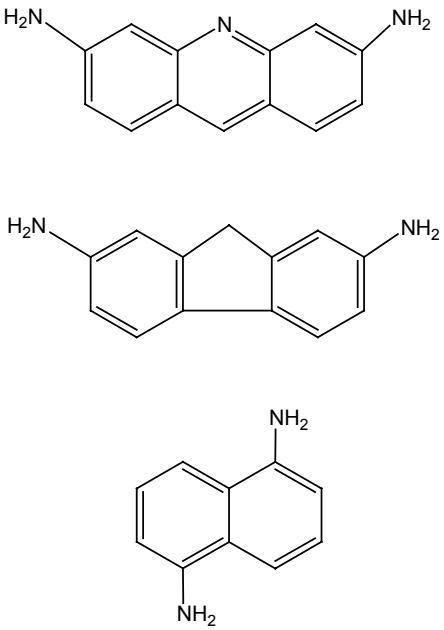
Finally manganese found in photosynthetic systems will be incorporated into the cavity (Scheme 3). Borovik and coworkers showed that dioxygen could be activated by a Mn(II) complex yielding a monomeric Mn(III) complex with a terminally bonded hydroxo ligand.



Scheme 3. The incorporation of Mn into the cavity of the cryptand.

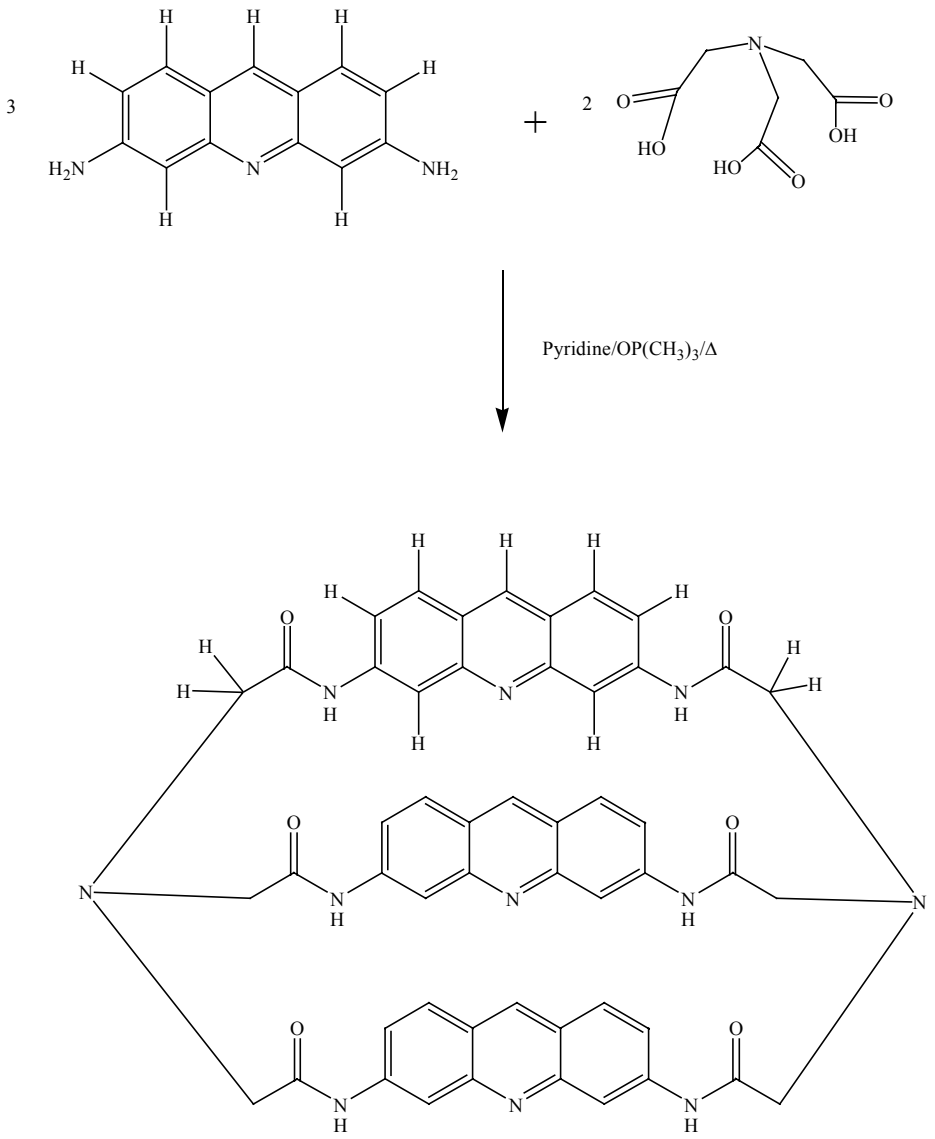
The actual results

Unfornately, 1,1'-dibromoferrocene is unstable at room temperature in Thailand. Attempting to synthesize this compound has not been successful and the yield is not great (< 5%). The plan has changed to look at new spacers which contain aromatic rings such as diamino compounds in Scheme 4. These diamino compounds are commercially available.



Scheme 4 diamino compounds in the study

The reaction between diaminoacridine and nitriloacetic acetic acid was carried out as shown in Scheme 5. The product was purified by crystallization in chloroform and ether. The ^{1}H-NMR, ^{13}C-NMR, ^{13}C-DEPT, and HETCOR spectra of the product are shown in the Figure 1-4. ESI-MS showed the molecular ion at 902.1 ($M+H^{+}$). Attempting to crystallize the product has not been successful yet. The next step will be applied this methodology to synthesize this kind of cryptands with different diamino compounds.



Scheme 5 Synthesis of new acridine cryptand.

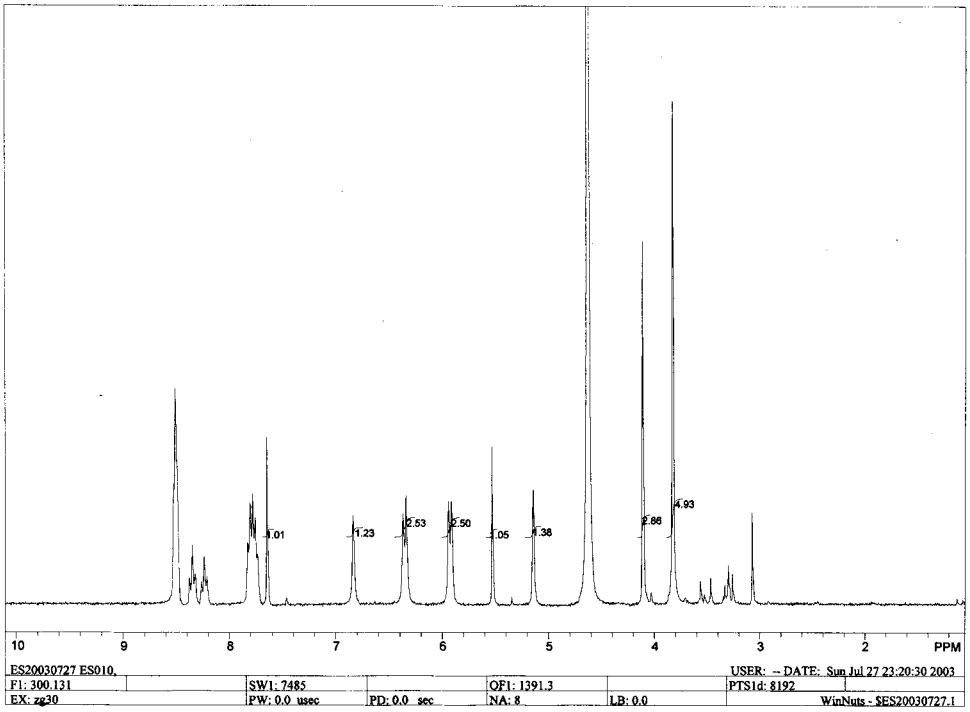


Figure 1 300-MHz ^{1}H NMR spectrum of the crude product.

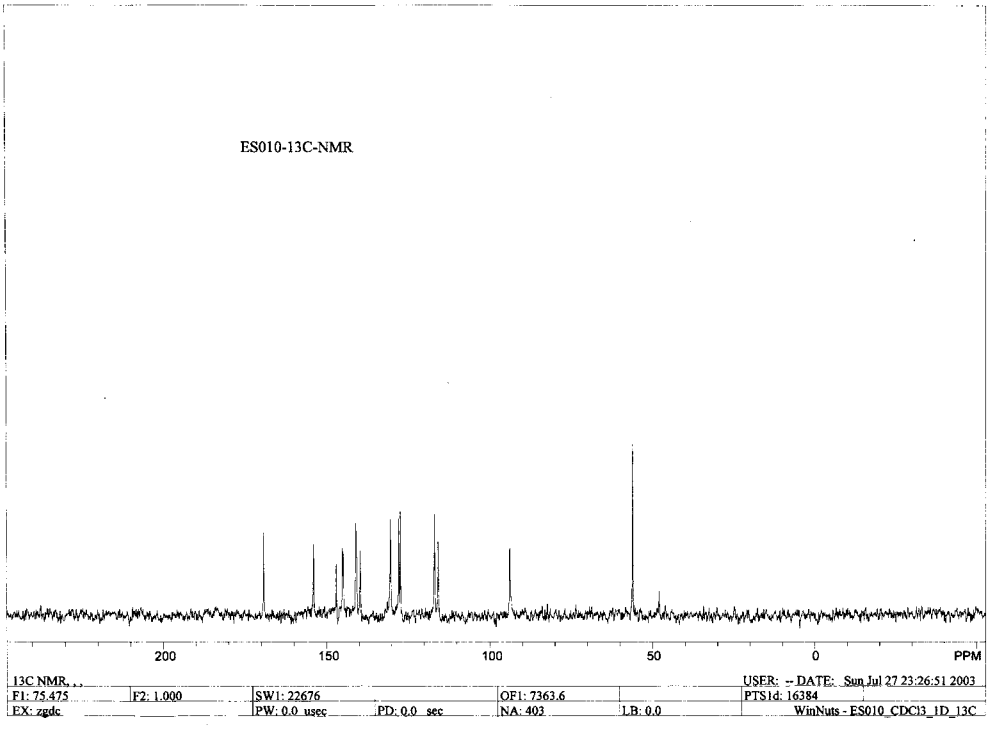


Figure 2 75-MHz ^{13}C NMR spectrum of the crude product.

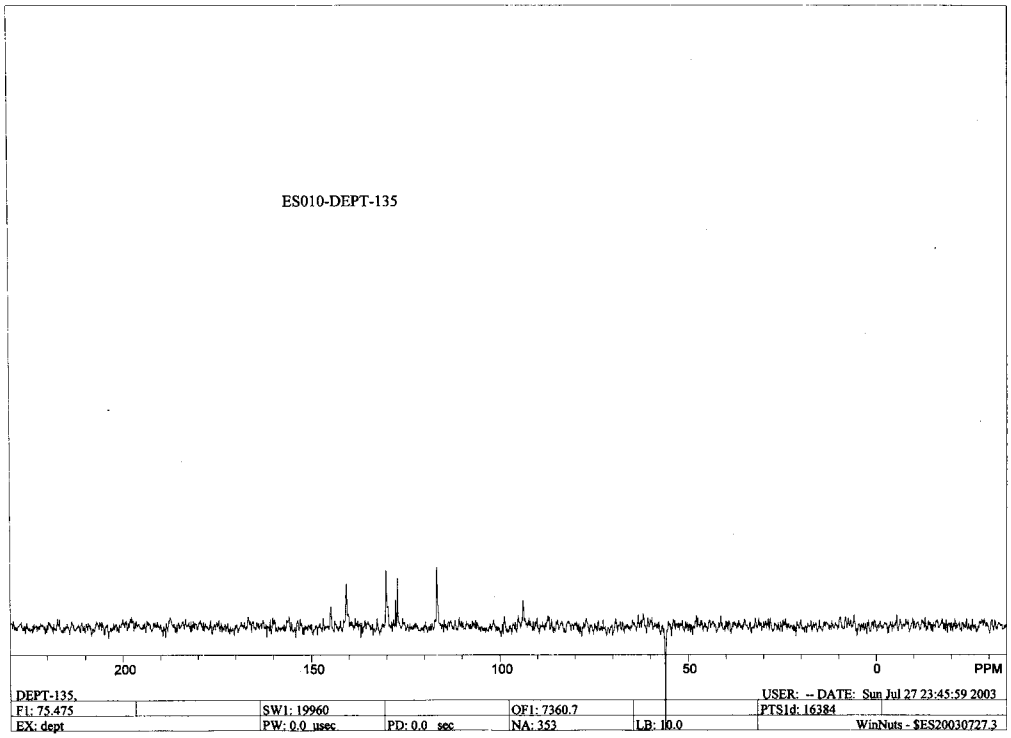


Figure 3 75-MHz ^{13}C-DEPT-135 spectrum of the crude product.

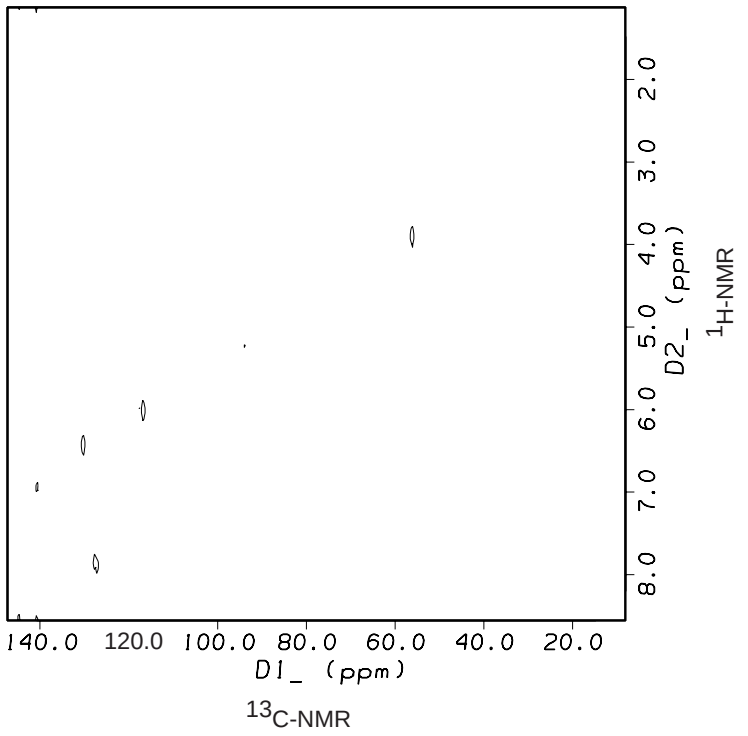


Figure 4 HETCOR spectrum of the crude product.

In addition to this project, our research group has carried out some small projects which could be published on international journals. The manuscripts are shown in the appendix.

The first manuscript is entitled to "A Facile One-Pot Synthesis of Acridine-Based Spacer Supramolecular Molecules". Please see the appendix A.

The second manucript is entitled to "A magnesium(II) complex of *cis*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylate".

The third manuscript is entitled to "Investigation of Iron-Saccharide Interactions in Complexes by Continuous-Flow Dissolution". which could be submitted to Stach-Starke or Journal of Inorganic Biochemistry. Please see the appendix B. The project has been presented at BioThailand 2003 at Pattaya and the Science and Technology Congress of Thailand at Khonkaen.

Output from this project

1. The manuscript entitled "crystal structure of magnesium(II) complex of *cis*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylate" has been submitted to Z Kristallographic-New Crystal Structure since May 5, 2004. Please see the appendix B.
2. The manuscript entitled "Investigation of Iron-Saccharide Interactions in Complexes by Continuous-Flow Dissolution" was submitted to Journal of Inorganic Biochemistry in March 2004 and was rejected in April 2004. Please see in the appendix D for reviewer's comments.
3. The manuscript entitled "Investigation of Iron-Saccharide Interactions in Complexes by Continuous-Flow Dissolution" was submitted to Biomacromolecules in April 2004 and was rejected in June 2004. Please see in the appendix E for reviewer's comments.
4. The manuscript entitled "Investigation of Iron-Saccharide Interactions in Complexes by Continuous-Flow Dissolution" was submitted to Journal of Pharmaceutical and Biomedical Analysis in June 2004 and was rejected two weeks later. Please see in the appendix F for reviewer's comments.

5. The manuscript entitled “Investigation of Iron-Saccharide Interactions in Complexes by Continuous-Flow Dissolution” has been resubmitted to Journal of Pharmaceutical and Biomedical Analysis in July 2004.
6. The research on iron-saccharide interactions has been presented at BioThailand 2003 at Pattaya and the Science and Technology Congress of Thailand 2003 at Khonkaen.
7. The research on iron-saccharide interactions has been implemented in two teaching classes, SCCH109 and SCCH448, at Mahidol University. Please see the appendix G.

Appendix

Appendix A

A Facile One-Pot Synthesis of Acridine-Based Spacer Supramolecular Molecules

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The design and synthesis of host molecules capable of strong complexation of biomolecules is an important area of molecular recognition. Anion receptors which are capable of mimic biological activities have played important roles in a number of areas including biochemistry, environment, and chemistry.

The objective of this research was to design and synthesize new macrocyclic cryptands which two cations and a large anion could be trapped inside the cavity in order to investigate their interactions. The synthesis involved linking two tripodal units with spacers. Acridine has been studied in the intercalation with nucleic acids. Lehn and coworkers had synthesized an amine cryptand in which three fluorogenic acridine units was capped by tris(2-aminoethyl)amine (TREN) (**1** in Figure 1). The preparation of **1** was carried out via Schiff base reaction with 2 steps. Recently, amide cryptands have been paid much attention because they exhibit the less pH-dependence and more solubility in organic media than polyaza systems. Moreover, amide cryptands have been predicted to be a good receptor due to strong dipole and induced dipole interactions.

We present here a simple one-pot synthesis of a new amide cryptand with acridine as spacers (**2**). The designed ligand was expected to capable of being incorporated with two metal ions to form coordinations at two tripodal units. Based on the molecular

modeling, the distance of two metal ions were about 9 Å in which a large guest anion should be able to form interactions with two metal ions with the stabilization from nitrogen atoms from acridine units. Cryptand synthesis is generally carried out in a dilution condition via acyl chloride or Schiff base. One-pot synthesis of cryptands is usually rare with a few examples. Bhattajaree reported the synthesis of an amide cryptand via the reaction between and ester and diamino compounds. Attempting to synthesize **2** with general methods starting with nitriloacetyl chloride or nitrilomethyl acetate and diaminoacridine hemisulfate had been failed. However, the designed acridine amide cryptand was successfully achieved by overnight refluxing the suspension of diaminoacridine hemisulfate and nitriloacetic acid in pyridine with a small amount of trimethylphosphite. The moderate yield was obtained with 20 %. The product was characterized with ^{1}H-NMR, ^{13}C-NMR, DEPT, and ESI-MS which were consistent with the proposed structure of **2** (Figure 2).

Conformation analysis of **2-6H**$^{+}$ were performed using UFF and VALBOND forcefield on Cerius^{2}. The minimized structure were shown in Figure 3. It should be noted that central nitrogen atoms of acridine units oriented towards the cavity could be utilized for anion complexation.

Protonated-**2** (**2-6H**$^{+}$) showed excellent fluorescent enhancements which could be used as a fluorogenic sensor of anions. The fluorescence spectra of **2-6H**$^{+}$ with anions were shown in the supplementary material. The fluorescence of **2-6H**$^{+}$ was not small as **1-6H**$^{+}$ probably due to the less flexibility of **2-6H**$^{+}$.

2 usually changes the color from yellow to red as a solution of HCl is added. The coordination chemistry of **2** has been investigated by treating it with $Fe(ClO_4)_3$ in MeOH resulting a dark red solution. The reaction of **2** and $Cu(ClO_4)_2$ was carried out first, then NaN_3 was added, resulting a dark brown solution.

In conclusion, we have developed a simple synthetic method of an acridine amide cryptands. The details of the work on the interactions of **2** and anions and metal complexation are now in progress.

Appendix B

Crystal Structure of Hexaaquamagnesium (II) bis (*cis*-9,10-dihydro-9,10-ethanoanthracene-11,12-carboxylic carboxylate) dihydrate

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In the title compound, hexaaquamagnesium bis(*cis*-9,10-dihydro-9,10-ethanoanthracene-11,12-carboxylic carboxylate) dihydrate $[Mg(H_2O)_6][(C_{18}H_{12}O_4)_2]\cdot 2H_2O$ (**I**) is a carboxylate complex of an anthracene adduct. Magnesium ion binds two carboxylate ions in an outer-sphere coordination mode via water molecules. The interaction between the metal, two carboxylate and two carboxylic groups was attached by a network of hydrogen bonds.

Comment

The role of magnesium in biological systems has been explored in very much detail (Cowan, 1998; Silva, 2001). There are many examples of the binding of magnesium to oxygen-containing ligands such as carboxylates, hydroxyls, and water. In nature, magnesium binds nucleic acids via water molecules while it can bind to proteins directly (Black, 1994). The role of water molecules in the inner coordination to

magnesium cations in enzymes was investigated by *ab initio* molecular orbital calculation (Katz, 1998). The interactions between magnesium-bound water molecules and carboxylate groups can facilitate the catalytic activity of enzymes by changing the pK_a of the water molecule and controlling the orientation of required functional groups. A detailed knowledge of the coordination of magnesium could provide a guideline for drug designs of certain metalloproteins. Here we report the crystal structure of the title compound (**I**). To our knowledge, no crystal structures of metal ion complexes of the carboxylate of the anthracene adduct have been reported.

The crystal structure of (**I**), with the atom-numbering scheme, is shown in Fig. 1. The bond lengths of CX—OX and CX and OY are X and Y, respectively while the bond length of CX-OX and CX-OY are approximately equal indicating that Mg(II) cation is attached to two carboxylate groups. In addition to the electrostatic interactions, the crystal structure of **I** is stabilized by a network of hydrogen bonds and van der Waal interactions. Intermolecular hydrogen bonds were formed between the carboxylic and the adjacent carboxylate groups. The stacked aromatic rings are found between the unit cell along the b axis with a distance of 4.132 Å indicating the π-π interactions. Mg(II) cation is bounded with six water molecules at the inner coordination and two water molecules at outer coordination. The bond lengths between the magnesium cation and oxygen atoms of inner water molecules are in the range of 2.04-2.12 Å. The outer water molecules are 4.317(4) and 7.925(4) Å apart from the Mg ion. The distances between Mg and each oxygen atom of the carboxylic and the carboxylate group (O2, O3, O13, and O18) are 7.514(4), 9.925(2), 9.795(2), 7.664(5) Å, respectively. The Mg atom is closer to the carboxylate group with C4 (8.103(1) Å) than the carboxylic group with C8 (9.895(2) Å). The carboxylic carboxylate group is twisted with the dihedral angle of $21.2(2)^o$ while the aromatic rings are bent inclining to each other with the dihedral angle of X. The complexes are oriented in the alternative interactions between the electrostatic interactions of Mg(II) and carboxylate groups and the van der Waal interactions of aromatic moieties. This results to the orientation of the unit cell of (**I**) consisting of alternative layers between hydrophobic (aromatic moiety of anthracene adducts) and hydrophilic groups (Mg(II) cation).

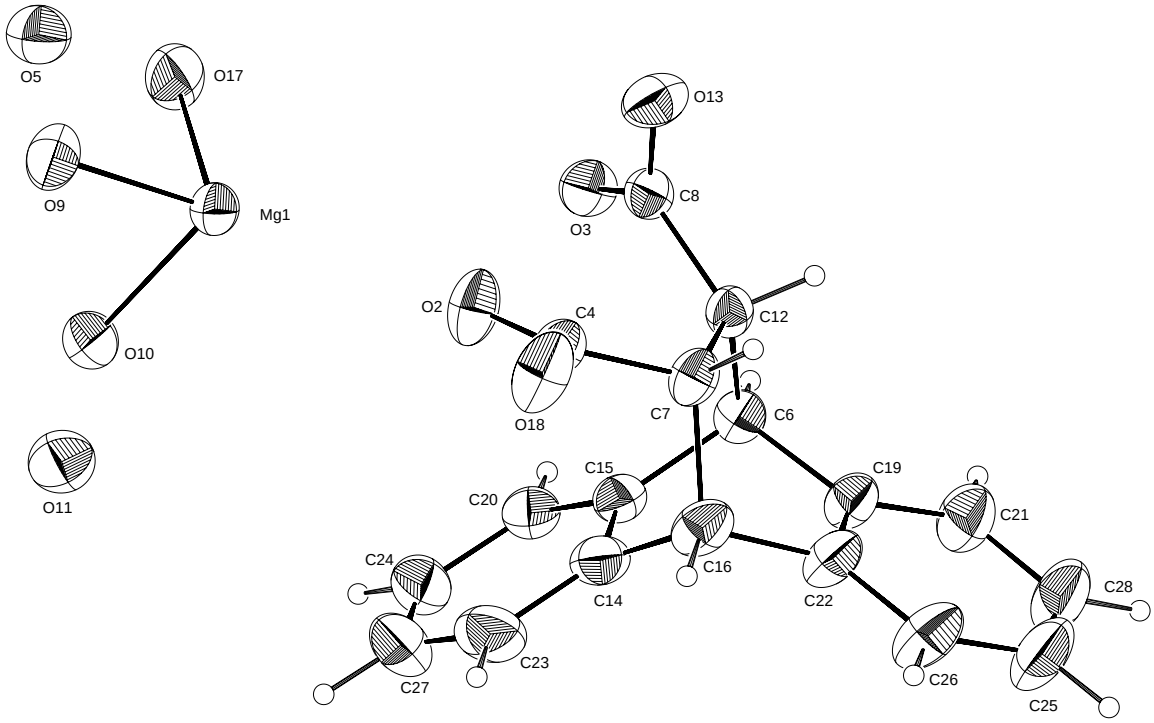
O5
O17
O9
Mg1
O10
O11
O13
C8
O3
O2
C4
C12
O18
C7
C6
C15
C19
C20
C21
C24
C16
C28
C14
C22
C23
C26
C27
C25

Fig. 1

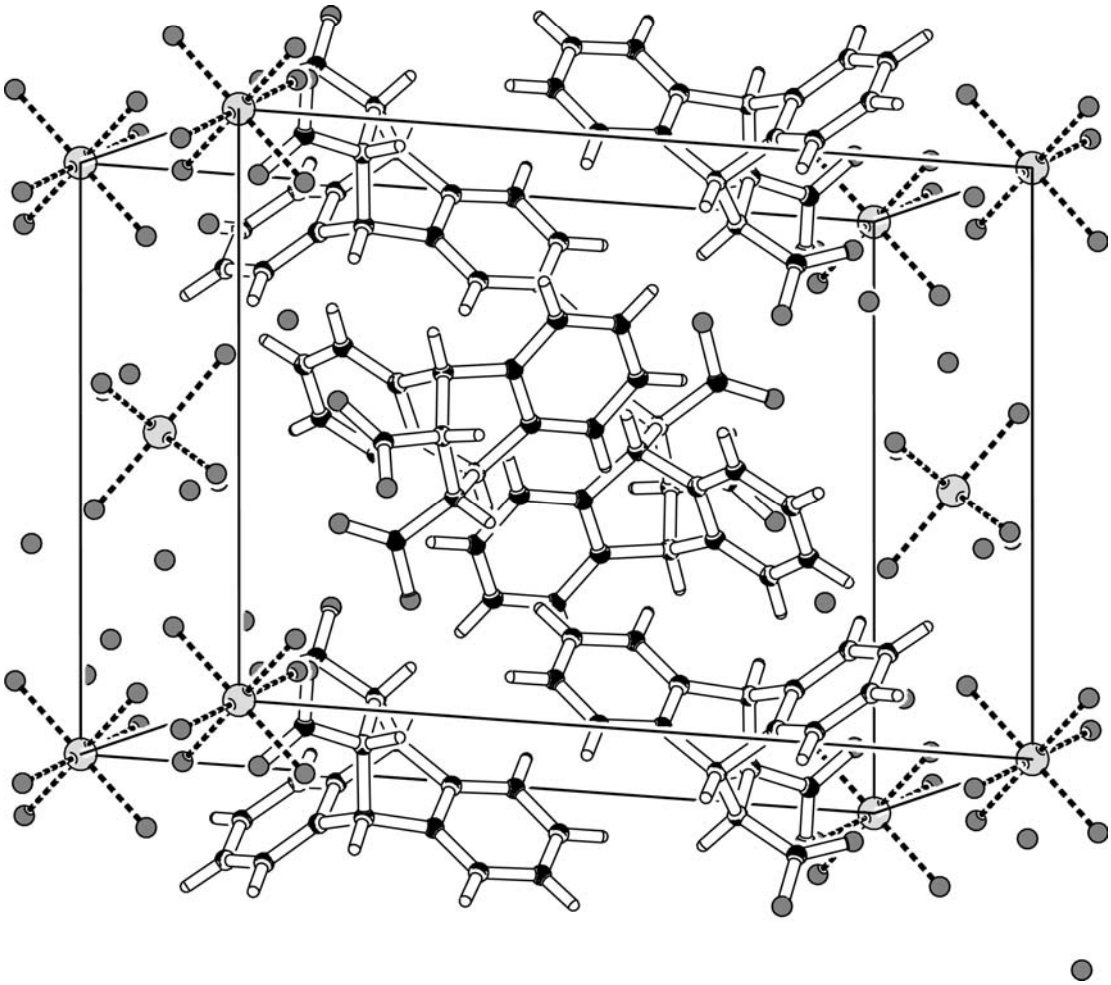

Fig.2

Experimental

The dicarboxylic acid was synthesized by a cycloaddition reaction of maleic anhydride with anthracene and then hydrolyzed with NaOH solution (Bachmann, 1948). The title compound was crystallized by slow evaporization from the aqueous solution prepared by the reaction of equimolar amounts of the dicarboxylic acid and $Mg(NO_3)_2$.

Crystal data

$C_{18}H_{28}MgO_{12}$ $M_r = 460.7181$

Monoclinic $P2_1/c$

a = 18.3330 (2)Å

b = 12.1670 (3)Å

c = 8.5210(5)Å

α = 90.00°

β = 96.0920 (9)°

γ = 90.00°

V = 1889.94 (12)Å^3

Z = 4

D_x = 1.353 Mg m^{-3}

Density measured by: not measured

fine-focus sealed tube

Mo *K*α radiation

λ = 0.71073

Cell parameters from 4369

θ = 1.018—27.485 °

μ = 0.128 mm^{-1}

T = 293(2) K

Cube

colorless

Data collection

KappaCCD

CCD

Absorption correction: none

4653 measured reflections

4454 independent reflections

2822 observed reflections

Criterion: >2sigma(I)

θ_{max} = 28.26 °

h = -23 $\rightarrow$23

k = -15 $\rightarrow$0

l = 0 $\rightarrow$11

Refinement

Refinement on F^2

full matrix least squares refinement

R(all) = 0.0768

R(gt) = 0.0583

wR(ref) = 0.1629

wR(gt)= 0.1484

S(ref) = 1.257

4444 reflections

250 parameters

0 restraints

Only coordinates of H atoms refined

Calculated weights calc

Δ/σ_{max} = 0.001

$\Delta\rho_{max}$ = 0.483eÅ^3

$\Delta\rho_{min}$ = -0.357eÅ^3

Extinction correction: none

Atomic scattering factors from International Tables Vol C Tables 4.2.6.8 and 6.1.1.4

Data collection: KappaCCD; cell refinement: HKL Scalepack (Otwinowski & Minor, 1997); data reduction: Denzo and Scalepak (Otwinowski & Minor, 1997); program(s) used to solve structure: *SIR*97(Cascarano, 1996); program(s) used to refine structure: *SHELXL*-97 (Sheldrick, 1997)

Table 1. Selected interatomic distances and angles *(*Å*, °)*

Mg1—O9	2.070(2)
Mg1—O10	2.120 (2)
Mg1—O17	2.035 (2)
O2—C4	1.215 (3)
O3—C8	1.253 (3)
O13—C8	1.263 (3)
O18—C4	1.314 (3)

O9—Mg1—O10	85.48(7)
O9—Mg1—O10	95.52(7)
O9—Mg1—O17	91.92(7)
O9—Mg1—O17	88.08(7)
O10—Mg1—O17	88.69(7)
O10—Mg1—O17	91.31(7)

O2—C4—O18	122.5(1)
O3—C8—O13	122.9(2)
O2—C4—C7	124.1(1)
O18—C4—C7	113.3(5)
O3—C8—C12	121.1(5)
O13—C8—C12	115.8(3)

The authors would like to thank the Postgraduate Education and Research Program in Chemistry (PERCH) and the Thailand Research Fund (TRF) for partially financial supports.

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Appendix C

Investigation of iron(III)-saccharide interactions in complexes by continuous-flow dissolution

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ABSTRACT

This paper is aimed to study the nature of iron(III)-saccharide interactions in complexes and to look for new iron-containing drugs for supplementing organisms. Iron (III)-saccharide complexes were synthesized by reacting $FeCl_3$ and saccharides (rice starch, sucrose, and dextran) in basic solutions and their characteristics were compared with synthetic iron-oxide. Both crystalline and amorphous iron(III) complexes were obtained and identified by X-ray diffraction (XRD). Amorphous samples were chosen to

investigate the interactions between iron(III) and saccharides by continuous-flow dissolution experiments. In acidic solution, the rate of iron release from amorphous iron complexes increased with the following order: iron-rice starch ≈ iron-oxide << iron-sucrose ≈ iron-dextran. The colloidal structure of iron-rice starch was proposed to describe its similar dissolution profile with that of iron-oxide.

KEYWORDS: iron deficiency, iron-saccharide, iron-rice starch, continuous-flow, dissolution.

1. Introduction

Iron is essential for most living organisms because it is required for many metabolic processes including oxygen transport, drug metabolism, steroid synthesis, DNA synthesis, ATP production and electron transport[1]. However, the concentration of iron within cells must be well controlled due to the cytotoxicity of excess iron. Iron is incorporated into organs and tissues in two major forms: heme and non-heme iron. Most of our dietary intake of iron is in the form of non-heme iron(III) ion. Some components in the diet such as phosphates, phytate, carbonate, and oxalate greatly decrease the bioavailability of iron because precipitates with iron are formed. The use of iron in biological systems is associated with two problems: low solubility of free metal ions and generation of toxic oxidants. Iron absorption can be enhanced by complexing iron with sugars[2-10]. Complexation of iron with saccharides and polyols has been extensively studied because saccharides are able to stabilize iron(III) oxides to maintain relatively high concentration of iron in a soluble form at physiological conditions.

Iron deficiency is one of the most severe nutritional problems worldwide[11]. Iron-saccharide complexes, such as iron-dextran, iron-sucrose, iron-gluconate, and iron-polysaccharide, have been used for the treatment of iron-deficiency anemia[12, 13]. Oral iron formulations are usually preferred whenever iron replacement therapy is needed. However, some patients who may not be tolerated with oral iron supplements are usually treated with parenteral iron. Iron(II) sulfate has long been accepted for the effective oral treatment of iron deficiency although side effects have been reported. Polysaccharide iron complex (PIC), synthesized by the reaction of $FeCl_3$ and dextrose, is widely used for the oral treatment due to its effectiveness, safe, and free from side effects. Intravenous iron dextran has been used for the treatment in several groups of patients especially in the

U.S. However, severe side effects, including sarcoma, sterile abscess formulation, tissue necrosis and death have been reported and are associated with the repeated injection of iron-dextran[14]. In contrast, clinical use of iron-sucrose in Europe for 50 years has been shown that it is safe for the intravenous treatment of iron-deficiency anemia[15-17]. Hence, it is valuable to carefully investigate how iron interacts with saccharides for the design of new iron-containing drugs.

Although the interactions between metals and sugars or polysaccharides have been studied extensively[2-10, 18-31], how metals, especially iron(III), interact with polysaccharides is not well understood. The interesting question, whether coordination bonds between iron(III) and the alcoholic hydroxyl group of saccharides are formed, or iron-oxide particles are "packed" in polysaccharides, still remains unsolved[32]. Sucrose, dextran and rice starch were chosen for study due to their importance in medicine and nutrition. Iron-rice starch, iron-sucrose, iron-dextran complexes and iron-oxide were prepared using the previously reported methods[25, 33]. The interactions between iron(III) and saccharides were investigated by kinetic dissolution experiments. Crystallinity of complexes was identified by X-ray diffraction (XRD). Only the amorphous samples were chosen for dissolution experiments using a continuous-flow dissolution system[34-38] in acidic solutions of pH 1, which is similar to the pH of gastric juice in the human stomach. Then various analytical tools such as thermal gravimetric analysis (TGA), differential thermal analysis (DTA), electron paramagnetic resonance (EPR), and ^{13}C-cross polarization/magic-angle spinning NMR (^{13}C-CP/MAS NMR) were employed to extract the chemical structures of complexes especially iron-rice starch in order to describe the different characteristic profiles in the dissolution experiments.

1. Experimental

1.1. Materials

Sucrose and dextran (MW 15,000-20,000) were purchased from Fluka (Switzerland). Rice starch was purchased at a local market in Bangkok, Thailand. Iron (III) chloride of purity greater than 98% was obtained from Merck (Germany). Deionized water was used in preparation of reagents.

1.2. Preparation of iron-containing samples

1.2.1. Iron-oxide (1)

$FeCl_3$ (3.6 g) was dissolved in deionized water (400 mL). Then 5 M NaOH was added dropwise with stirring to obtain a pH of 9. Red-brown precipitates were observed instantly. The suspension was incubated at 90°C (incubation temperature) for 2 hours with gentle stirring. The precipitates were centrifuged and decanted and then washed with deionized water.

1.2.2. Iron-rice starch complex (2), iron-sucrose complex (3) and sodium-rice starch

Aqueous starch suspension or sucrose solution (saccharide 5 g in 50 mL of water) was heated at 90°C for 1 hour with continuous agitation. Then 10 mL of 5 M NaOH was added to the solution. The basic saccharide solution was added slowly to the ferric chloride solution (3.6 g of $FeCl_3$ in 400 mL of water). Subsequently, 5 M NaOH was added dropwise to obtain a pH of 9. The suspensions were then incubated for 2 hours and the precipitates were centrifuged and washed as in the above procedure.

Sodium-rice starch was prepared with the similar method without adding $FeCl_3$.

1.2.3. Iron-dextran complex (4)

Dextran solution (5 g in 50 mL of water) was heated at 90°C for 1 hour with continuous agitation. Then 10 mL of 5 M NaOH was added to the solution. The basic solution was added slowly to the ferric chloride solution. Subsequently, 5 M NaOH was added dropwise to obtain a pH of 9. The suspensions were incubated for 2 hours before 150 mL of 2-propanol was added and then the precipitates were centrifuged and washed as in the above procedure.

All iron-containing samples were oven-dried at 50°C and ground in an agate mortar prior to chemical analysis.

1.3. Characterization

1.3.1. X-Ray Diffraction

A D8 Advance Bruker analytical X-ray system was operated at the CuKα wavelength of 32.297 nm, 40 mA, and 40 keV. The spectra over the range of 20-70^{o} 2θ were recorded at a scan rate of 0.020 2θ/s and a step time of 5 s.

1.3.2. Thermal analysis

Thermal Gravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) were performed with the TA instruments-SDT-2960 simultaneous analyzer. Samples were placed in an alumina cup and heated from 30^{o}C to 450 ^{o}C at a heating rate of 5 ^{o}C min^{-1} in a nitrogen atmosphere. A purge gas was a flowing dry nitrogen at a rate of 100 mL min^{-1}.

1.3.3. Electron Paramagnetic Resonance (EPR)

The spectra were recorded for amorphous samples in the X-band region (9.2 GHz, λ = 3.2 cm) at room temperature. Mn(II) complex was used as the standard for the g-value.

1.3.4. ^{13}C-Cross Polarization/Magic-Angle Spinning NMR spectroscopy (^{13}C-CP/MAS NMR)

The ^{13}C-CP/MAS NMR spectra of sodium-rice starch and sample **2** were acquired using the high power cross-polarization sequence at ambient temperature at 125 MHz on a Bruker DRX-500 spectrometer. The sweep width of spectra was 50,000 Hz and the recycle time was 1 s. The numbers of scans were 5,600 and 70,000 for sodium-rice starch and sample **2**, respectively. Chemical shifts were determined by using adamantane as a reference.

2.4 A continuous-flow dissolution setup

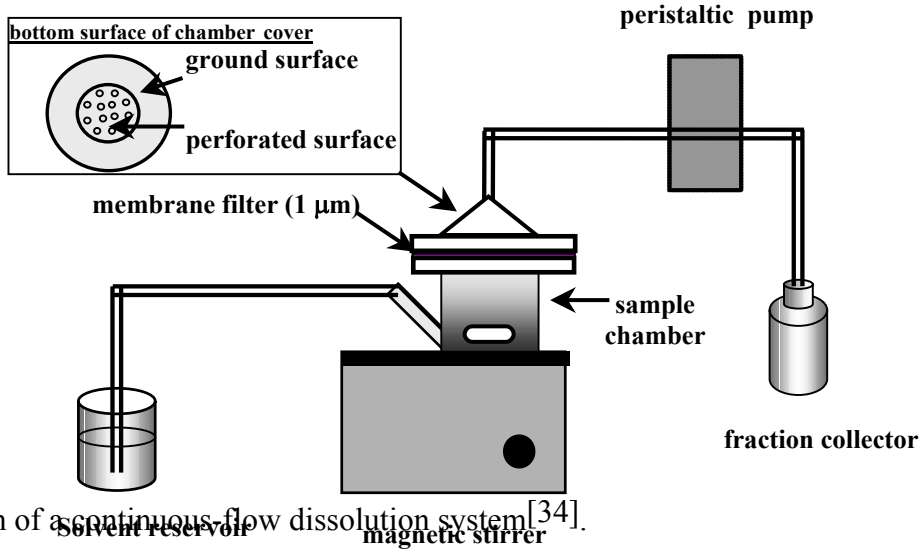


Figure 1. Diagram of a continuous-flow dissolution system[34].

A dissolution chamber and its cover were constructed from borosilicate glass to have a capacity of approximately 10 mL. The outlet of the chamber was furnished with a glass microfiber filter membrane to allow dissolved matter to flow through. Leachate was pumped through the chamber using a peristaltic pump at approximately 4 mL min^{-1}, using a tygon tubing of 2 mm inner diameter.

2.4 Kinetic dissolution procedure

A weighed sample, i.e. iron-oxide or iron-saccharide complexes, was transferred to a dissolution chamber. The 0.5 M hydrochloric acid solution was flowed

through to dissolve the solid sample. The solutions from the dissolution chamber were collected in small plastic bottles at 3 – 10 mL volume intervals. The solutions were then diluted with deionized water and subjected to flame atomic absorption spectrometric determination.

3. Results and discussion

3.1 Complex preparation and XRD patterns

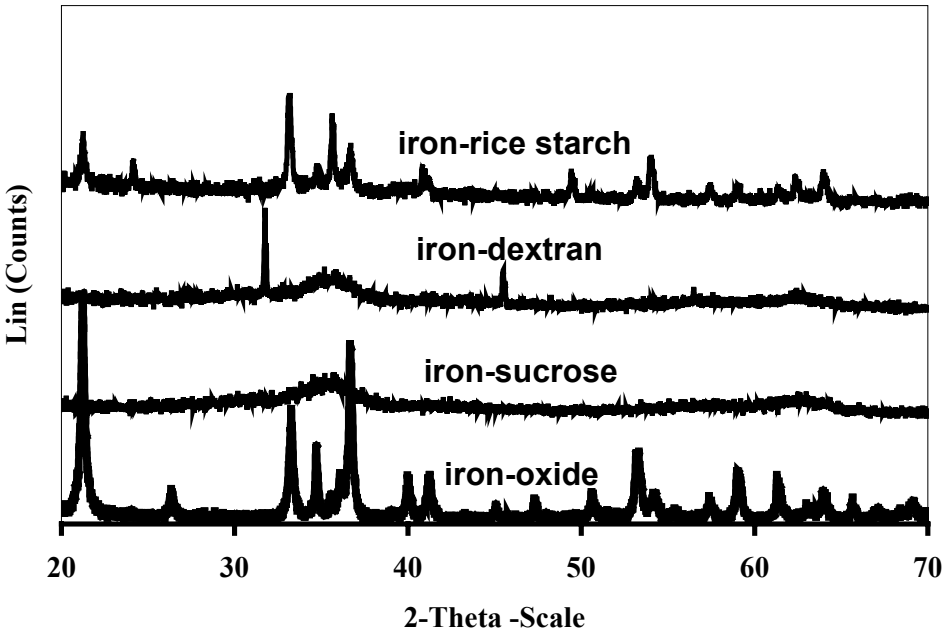


Figure 2. XRD patterns of samples **1-4** (preparation condition: pH 11, incubation temperature 70 ^{o}C, 2 days).

The addition of iron(III) to basic saccharide solutions resulted in suspensions of iron complexes. XRD patterns in Figure 2 clearly showed that the synthetic samples of iron-rice starch and iron-oxide were crystalline which were hematite and goethite[39], respectively while iron-dextran and iron-sucrose were amorphous at this preparation condition (pH 11, incubation temperature at 70 ^{o}C for 2 days). Crystalline samples were not suitable for the dissolution experiments due to nonreproducible results. Therefore, the condition to prepare the amorphous phase of complexes was optimized. The conditions of iron(III)-rice starch preparation at various pH, incubation time, and incubation temperature were carried out. It was found that at pH 11, incubation temperature at 50, 70, and 90 ^{o}C for 2 days, all experiments gave crystalline samples

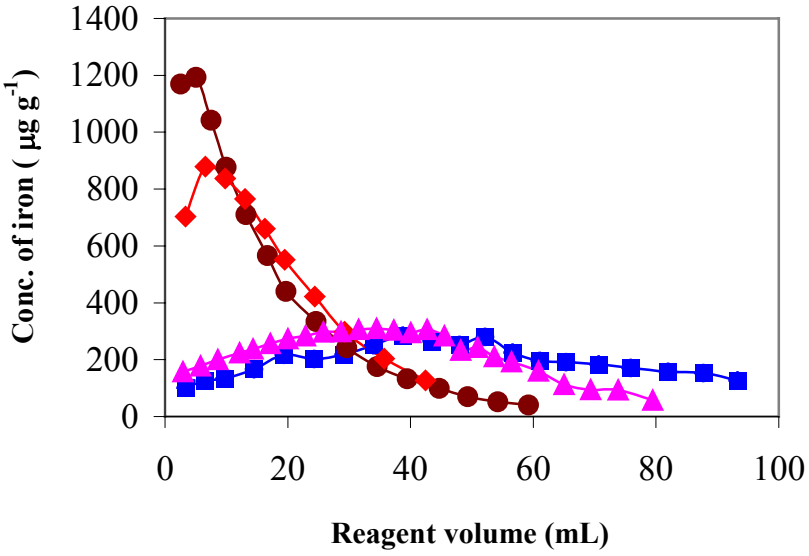
possibly with the hematite phase. At various pH of 5, 7, 9, and 11 and an incubation temperature of 90 ^{o}C, crystalline products were obtained for pH 5, and 11 and amorphous products were obtained for pH 7, and 9. Conditions such as pH, incubation temperature, and incubation time must be well controlled in the preparation of iron-containing drugs. Subtle change in the complex preparation can lead to different complex structures and properties.

3.2 Dissolution study of iron(III)-saccharide complexes using a continuous-flow dissolution system

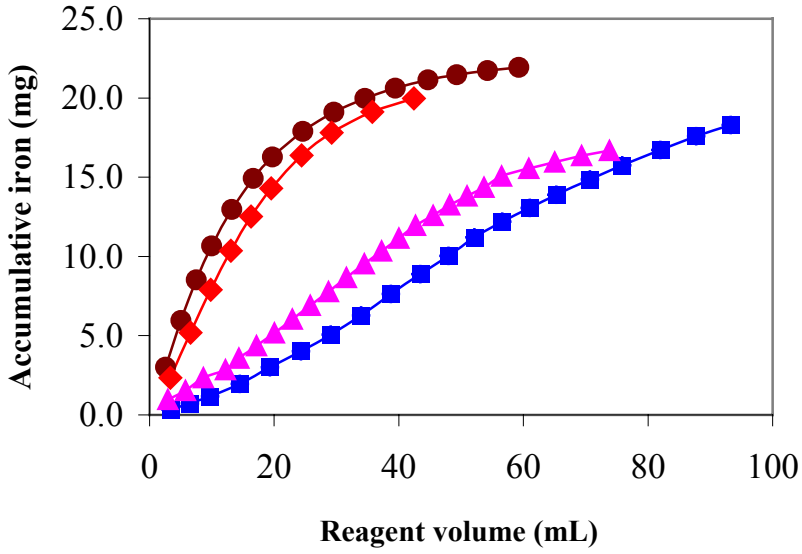
The dissolution or releasing experiments of iron-oxide or ferritin in hydrochloric solution or under reductive conditions have been studied extensively in batch experiments[40-44]. The batch experiment performs dissolution or releasing of metals in an equilibrium condition. In this study, continuous-flow dissolution experiments were carried out to gradually dissolve iron(III) complexes with a fresh reagent and iron concentrations in the leachate collected in fractions were determined. Thus, the exhaustive dissolution can be performed. A graphical plot of concentration of iron and dissolution volume provides a dissolution profile which offers the kinetic information about the release of iron from the sample into solution.

The dissolution of ferric ion in an acidic solution was studied using the dissolution profile obtained from the continuous-flow system. The 0.5 M hydrochloric acid was used to dissolve iron-containing samples. The leachate was collected in fractions for the determination of iron concentration by flame atomic absorption spectrometric method. The patterns (narrow or broad) of dissolution profiles was used to indicate the interactions betweens iron(III) and matrices. The dissolution characteristics on the dissolution profiles (Figure 3) were divided into 2 groups: the first group is iron-dextran and iron-sucrose and the second group is iron-oxide and iron-rice starch complexes. The iron-dextran and iron-sucrose were dissolved in 0.5 M HCl readily after the addition of acid and then the concentration of dissolved iron decreased exponentially. The iron-oxide and iron-starch complex were gradually dissolved after the addition of acid. The dissolution patterns of iron-oxide and iron-rice starch complex were similar. The plots of accumulative concentration of iron in the leachate were also shown in Figure 3(b). The approximate rate of iron release from amorphous iron complexes increased

with the following order: iron-rice starch ≈ iron-oxide << iron-sucrose ≈ iron-dextran. From Table 1, the % Fe dissolved by various concentrations of HCl increased with the order: iron-rice starch < iron-oxide < iron-sucrose < iron-dextran. The slower release of iron-rice starch complex compared to iron-sucrose and iron dextran was described as a consequence of the different structures of the iron(III) complexes. However, the rate of release of iron-rice starch complex (**2**) was slightly slower than that of iron-oxide. The argument that the saccharide complexes were overloaded with iron-oxide was ruled out otherwise the rate of release of iron-sucrose should be approximately the same as iron-oxide.



(a) Dissolution profile



(b) Accumulative iron plot

Figure 3. Dissolution profile (a) and accumulative amount plot (b) obtained from dissolution experiments of iron-oxide (▲), iron-sucrose (◆), iron-dextran (●) and iron-rice starch (■) using 0.5 M hydrochloric acid.

Table 1. Dissolution data of iron from iron-containing samples **1-4**.

Iron-containing samples	%Fe dissolved in various concentrations of HCl (M)				
	0.05 M	0.1 M	0.3 M	0.5 M	0.7 M
Iron-oxide (**1**)	n.d.	4.9	24	60	60
Iron-rice starch (**2**)	n.d	4.7	27	44	45
Iron-sucrose (**3**)	14	29	65	78	n.d.
Iron-dextran (**4**)	37	66	83	82	n.d.

n.d. = not determined.

3.3 Structures of iron(III)-rice starch complex

Iron(III)-rice starch was chosen to investigate in more details because of its potential therapeutical use while iron-sucrose and iron dextran complexes have been well-known in the medical applications[14-17] and literature[31]. The similar characteristics of dissolution patterns of samples **1** and **2** led us to ask a question whether the sample **2** was the iron-oxide adsorbed on the surface of starch. It has been known that the alcoholic hydroxyl group of polysaccharides is deprotonated in alkaline medium and coordinated to iron(III). However, the question about how iron(III) interacts with polysaccharides is still unanswered. Two models have been proposed to explain the structures of iron(III)-polysaccharide complexes[32]. The first assumption is that iron (III) is bound through the binding sites of the saccharide moieties and forms spatially separated iron(III) centers along the backbone of polysaccharides (site binding model). The second assumes that iron(III) forms FeOOH precipitate, which is covered by the polysaccharide and inhibits their aggregation of the subcolloidal FeOOH particles (colloidal model). In our study, the dissolution experiment, TGA, DTA, EPR, and ^{13}C CP/MAS solid state NMR were used to investigate the interactions between iron(III) and saccharides especially rice starch.

TGA and DTA data of saccharides and samples **1-4** under nitrogen atmosphere are summarized in Table 2. This showed that there was some amounts of water (10-15 %) which could be located in the matrix or in the coordination sphere of the iron(III) centers. The TGA and DTA of samples **1** and **2** were totally different indicating that sample **2** was not a mixture of saccharide complexes with the excessive iron-oxide sorbed

on them. The DTA of sucrose reflected by an endothermic peak centered at 189 ^{o}C possibly associated with the phase transition of sucrose. Based on the DTA thermograms of samples **1-4,** the thermal effect was exothermic except for iron-sucrose. It was related to the formation of new iron-oxide phases or the loss of coordination water or the redox reaction or metal-catalyzed oxidation[45].

Table 2. EPR, TGA, and DTA data of saccharides and iron-containing samples.

Samples	EPR g-value	TGA	DTA
Iron-oxide (**1**)	1.9417	loss 14 % (50-120 ^{o}C) loss 5 % (30-400 ^{o}C)	endo (55 ^{o}C) slightly exo (283 ^{o}C)
Rice starch	-	loss 10 % (50-120 ^{o}C) decompose (287 ^{o}C)	endo (50 ^{o}C) endo (274, 296 ^{o}C)
Iron-rice starch (**2**)	1.9547	loss 10 % (50-130 ^{o}C) loss 34 % (130-375 ^{o}C)	endo (50 ^{o}C) exo (266 ^{o}C)
Sucrose	-	decompose (213 ^{o}C)	endo (189 ^{o}C)(phase tr.) endo (222 ^{o}C) endo (283 ^{o}C)
Iron-sucrose (**3**)	1.9479	loss 11 % (50-120 ^{o}C) loss 18 % (120-430 ^{o}C)	endo (70 ^{o}C) endo (186 ^{o}C)
Dextran	-	loss 10 % (50-120 ^{o}C) decompose (230 ^{o}C)	endo (50 ^{o}C) endo (297 ^{o}C)
Iron-dextran (**4**)	1.9417	loss 10 % (50-120 ^{o}C) loss 25% (220-400 ^{o}C)	endo (70 ^{o}C) exo (270 ^{o}C)

The X-band EPR spectra of **1-4** in the solid state exhibited one broad band at $g \approx 1.9$. The g-values of **1-4** are approximately equal to 1.94 indicating the similar environment of iron(III) centers. It was assigned to a signal arising from strong interactions between the paramagnetic high-spin iron(III) centers in the polynuclear species[4, 46]. However, there was no signal of narrow band at $g \approx 4.3$ indicating that

there was no isolated high spin iron(III) with a rhombic symmetry in the complexes or the EPR linewidth was too broad. EPR data showed the similar g-values of both complexes indicating that the iron(III) cores of iron-oxide and iron-rice starch were rather similar and starch could stabilize the iron-rice starch complex to release iron slower.

NMR provides the complementary information of saccharide complex structures. The ^{13}C-CP/MAS NMR spectra of sodium-rice starch and iron-rice starch sample **2** were shown in Figure 4. Based on previous studies[47], the distinctive peaks of the ^{13}C-CP/MAS NMR spectra at highest and lowest field were assigned to the C-6 and C-1, respectively. Typically, the increase in the local short-range order narrows the ^{13}C resonance dramatically. The broadening resonances may be associated with the long-range order in the local site and probably the interaction between antiferromagnetic iron (III) centers and ^{13}C atoms of glucose units. Clearly the C-6 resonance was shifted to higher field indicating that there were more interactions between iron(III) centers and O-6 units of glucose than other oxygen atoms. The slightly change in the NMR spectrum of iron-rice starch complex was explained with the long-range interactions between iron(III) and starch. This led us to propose that the synthetic iron-rice starch sample **4** should be in the form of iron-oxide stabilized by rice starch. The possible structure of iron-rice starch is that iron-oxide is packed with water inside the helix structure of amylose in which the hydroxyl group of C-6 units of glucose pointing towards the helix center. Our results were in agreement with the structure of iron(III)-chitosan system[32], and polyvinyl alcohol (PVA) and polyacrylic acid (PAA) systems solubilizing iron-containing aggregates[48].

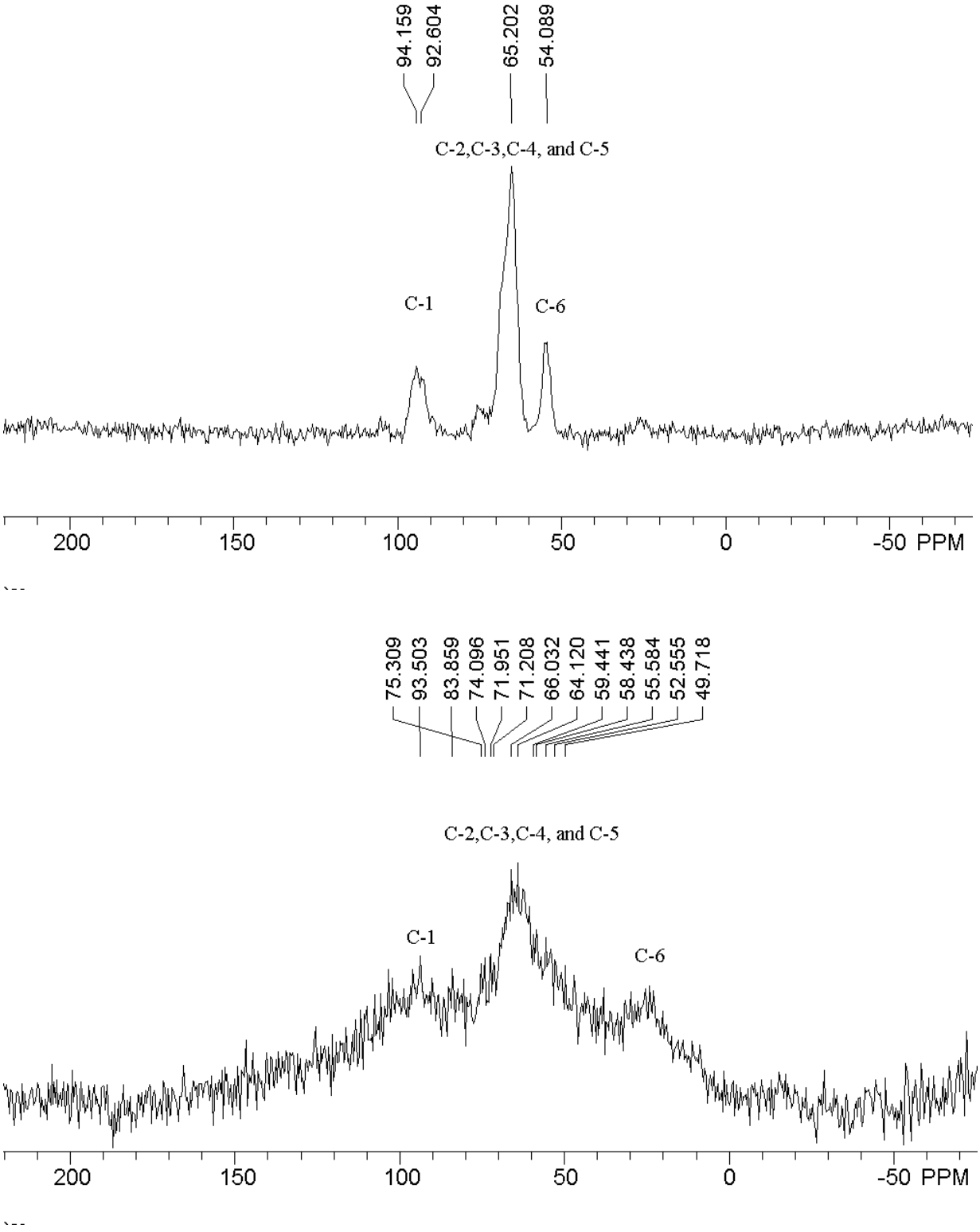


Figure 4. ^{13}C-CP/MAS NMR spectra of sodium-rice starch and iron-rice starch complex (**2),** respectively.

Conclusions

We have shown the use of the continuous-flow dissolution system with other techniques for shedding light on the interactions between iron(III) and saccharides in complexes. The synthetic iron-rice starch complex was proposed to adopt a colloidal structure although the site-binding model was not ruled out. Furthermore, the continuous-flow dissolution system could be further applied for the therapeutical use to check the releasing of iron from iron-containing drugs and in search of new iron-supplements.

ACKNOWLEDGMENT. The authors would like to thank Dr. Tienthong Thongpanchang for useful discussions. This research was supported by the Thailand Research Fund (TRG4580067), the Postgraduate Education and Research Program in Chemistry (PERCH), and the Faculty of Science, Mahidol University.

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Appendix D

Date: Wed, 21 Apr 2004 21:01:34 +0100 (BST)
To: scess@mucc.mahidol.ac.th
To: jib@mail.chem.sc.edu
Subject: JIB, Editor's decision: reject, Somsook, scess_20040323/1, Preparation, charact...
From: jib@mail.chem.sc.edu

Dear Dr Somsook,

On 23-Mar-2004 you submitted a new paper
number JIB_scess_20040323/1 to Journal of Inorganic Biochemistry entitled:

'Preparation, characterization and investigation of the interaction of iron(III)-saccharide complexes by continuous-flow system and related techniques'

The Editorial reference number for this paper is: JIB 04-0315

We regret to inform you that your paper has not been accepted for publication.
Comments from the editor are:

April 21, 2004

Prof. Ekasith Somsook
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RE: Manuscript JIB 04-0315

Authors: Ekasith Somsook*, Duangduean Hinsin, Pasakorn Buakhrong, Rattapon Teanchai, Manat Pohmakotr, Juwadee Shiowatana

Title: “Preparation, characterization and investigation of the interaction of iron(III)-saccharide complexes by continuous-flow system and related techniques”

Dear Prof. Somsook:

Your manuscript has been reviewed by three experts in the subject matter of the paper. Copies of their opinions are included (please see below). Although one reviewer favors returning the manuscript for revision and re-evaluation, the other two argue strongly in

favor of declination. Thus, I regret to inform you that the consensus of the reviewers, with which I concur, is that the paper is not suitable for publication in the Journal.

While we are unable to publish your paper in the Journal, I hope that the reviews included below may be helpful to you. I am keeping a copy of your manuscript on file. Thank you for giving us the opportunity to review it.

Sincerely,

John H. Dawson
Editor-in-Chief, Journal of Inorganic Biochemistry
Carolina Distinguished Professor

----Reviewer #1----

Comments on "Preparation, characterization and investigation of the interaction of iron (III)-saccharide complexes by continuous-flow system and related techniwues" by E. Somsook et al.
Ref. No. JIB 04-0315

Althought the manuscript is rather diffuse, it contains sufficient amount of results for publishing in JIB. A great number of similar papers have already been published in this field. Perhaps this is the reason why there are an extensive number of references included, which are sometimes inadequate (the information is too old, see for example refs. 18, 19, 25, 66 and should be omitted). The main output, similarly to the previous papers, is that the alcoholic hydroxyl group of carbohydrates (even of polysaccharides) is deprotonated in alkaline medium, and co-ordinates to iron(III). This is not a novelty (see: B. Gyurcsik, L. Nagy, Coord. Chem. Rev., 203, 81-149, 2000.). The most interesting question, whether Werner-type complexes are formed with polysaccharides, or latter only “pack” the Fe-hydroxide particles, still remains unresolved. The best part of the manuscript is the continuous-flow work. I would suggest to expand on this in the future. As far as the appearance is co!
ncerned, the manuscript is very well presented.

Therefore, my final conclusion is that the manuscript could become suitable for publication after revision.

----Reviewer #2----

Manuscript Number: JIB 04-0315

Authors: Ekasith Somsook*, Duangduean Hinsin, Pasakorn Buakhrong, Rattapon Teanchai, Manat Pohmakotr, Juwadee Shiowatana

Title: “Preparation, characterization and investigation of the interaction of iron(III)-saccharide complexes by continuous-flow system and related techniques”

Recommendation: reject.

This manuscript describes complex formation of FeIII with some polysaccharides. A variety of techniques (NMR, EPR, XRD, TGA) where used to characterize the compounds. However, with regard to significance and novelty, the scientific outcome reported is poor. On page 17, the authors wrote that “the investigation of the microstructure of iron(III)-complexes and the interaction of iron(III) with other starch is in progress”.
I suggest to include this part in the manuscript, and to present a more significant paper.

----Reviewer #3----

THIS WAS SCANNED IN FROM A FAX

OPINION (JIB 04-0315)

Overall recommendation: Reject

General remarks:

According to the authors, two reasons induced their study:

-Contribution to the knowledge on the nature of metal-saccharide interactions, as stated on p. 4 (lines 9-11 from the top), is poorly understood.

-Looking for novel preparations for supplementing organisms with iron.

I am afraid that authors failed to meet the targets. The authors did not present any novel concept on the nature of interactions, which would result from their study. The preparations that they designed are poorly characterized.

On p. 4, the authors talk about side-effects of the therapy with Fe(III) - dextran complexes (by the way, which dextran?) and lack of such effects from the sucrose-Fe(III) complex. Such reality forms grim prospects for potential therapeutical use of starch-Fe (III) complexes. Formation of FeCl3 complexes with carbohydrates is controlled, among others, by equilibrium. Obviously, equilibrium is controlled by concentration, that is, by solubility of reagents. Because starch and products of its interaction with FeCl3 are poorly soluble, formation of the O-Fe valence bonds is likely. For instance, in the case of interaction of aqueous NaOH with starch, formation of the stable O-Na bond is postulated whereas alcoholates readily hydrolyze in aqueous solution. Under the conditions that the authors applied, formation of the O-Fe bonds could take place and it could be assisted by formation of some coordination compounds. Coordination could occur either intra- or inter-molecularly. Th!

is is also valid for the dextran-Fe(III) complexes. They are definitely more stable to proton and enzymes. In conclusion, if, indeed, authors look for novel preparations they should turn rather towards ferric (ferrous) complexes with lower oligosaccharides.

Procedures applied are not clear. Authors prepared the complex from a blend of 0.014 mole sucrose and 0.022 mole of FeCl3. I do not even ask why they operated with such either excess of the ferric salt if they assumed to coordinate one sucrose molecule by one Fe or its deficiency when two sucrose molecules would be coordinated by one Fe atom. However, initially, there was deficient NaOH in the reaction medium. Thus, the result anticipated by the authors could differ from that achieved. For instance, FeOCI could form and after coordination to starch, the chlorine atom could reside in the complex [see paper: K. Marusza and P. Tomasik, Starch/Staerke, 46, 13-17 (1994)]. Did authors check the reaction product for chlorine? How have the authors calculated proportions of reagents in case of dextran and starch? In order to compare the results, they should calculate the amount of polysaccharides in experiments in moles of glucose units. Because of the possibility of the intra- and !
inter-molecular coordination, the possibility of the formation of complexes with higher amount of Fe(III) should be limited. It is likely that the authors overloaded polysaccharide with iron compounds. The procedure applied by the authors could lead to mixtures of polysaccharide (saccharide) complexes with excessive iron oxide sorbed on them.

There is no information about how much iron was left in the mother liquor. It would be essential information for interpretation of the results of the iron extraction,

In Fig. 2, x-ray of original starch and NaOH treated starch should be quoted in order to be sure what is, actually, observed.

I am not sure whether Fig. 3 presents TGA and DTA or TG and DSC curves. Nothing is known about instrument used.

Fig. 5: The spectrum of the complex looks like it is, because of magnetic properties of the metal ion. Any precise reading of the positions of the peaks is impossible. Hence the conclusions drawn from that spectrum are meaningless.

Authors should support their discussion of dissolution of complexes and Fe(III) with microscopic images (SEM, AFM?) of particles subjected to extraction. There are no data providing evidence for either crystalline or amorphous character of the powders. Moreover, such classification of the powders seems to be insufficient because any aggregation of the powder particles can also influence the rate of extraction.

Presentation of the results of the iron extraction is misleading. It is not very important how much iron was extracted from the samples, but what percent of total iron in the samples was recovered. Extraction of iron from its complexes with polysaccharides can be controlled by acid-catalyzed hydrolysis of the polysaccharide matrix. It is likely that in their experiments, the authors extracted only that portion of iron, which, in the form of

iron oxide, was sorbed on the complex surface. I think, that the authors should estimate the rate of acid hydrolysis of polysaccharide prior and after complexation. It could shed light on the Fe(III)-polysaccharide interactions.

The abstract states that the complex with starch is colloidal. This statement did not receive any support.

Further remarks:

1. Title is misleading. I think that intentions of the authors would be followed by the title like: Iron(III) - saccharide interactions in complexes

2. Page 3, line 6 from the top. This statement is not generally true.

3. Page 5, p. 2.2.1. What comp1ex do the authors talk about?

4. Page 6, p. 2.3.1. Instruments should be properly described.

5, There are minor improvements needed in the text. Because my opinion about this paper is negative, I was not too specific in this point.

Yours sincerely,

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University of South Carolina, Columbia, SC 29208
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JIB website: http://www.elsevier.nl/locate/jinorgbio

Appendix E

Reviews for Somsook bm049754m

Reviewer #2

Manuscript Number: bm049754m
Manuscript Title: Investigation of iron(III)-saccharide interactions in complexes by continuous-flow system.
Corresponding Author: Somsook

Rate the overall importance of this paper to the field of Biomacromolecules

10 - High Importance / 1 - Low Importance: 3

Recommendation: Publication in any form would be premature at this time.

Additional Comments:

Authors have specified two targets. Their meeting would rationalize publication of this paper. They are: (i) contribution to poor knowledge of the nature of metal – saccharide interactions and (ii) designing novel saccharide – Fe(III) complexes – carriers of bioactive iron. Sucrose, dextran of M_w = 15 000 – 20 000, and rice starch have been selected as ligands.

Indeed, novel complexes were prepared and their selected properties thoroughly studied. The results look fairly promisingly and, seemingly, the complexes could pass to a biological screening. However, some elements of their structure were recognized but who knows what is their composition? How concentration of iron in Table 2 was estimated? There is no information in Experimental Part about reproducibility of the results, number of replications, and tests for purity of the complexes. Thus, one can say that the second target was met by halves.

Studies presented in this paper did not push forward our knowledge on the nature of metal – saccharide interactions [target (i)]. Conclusions well reflect it.

Interactions under study can, generally, involve formation of either Werner complexes (in case of sucrose ligand their inner coordination sphere could be precisely identified), salts of the L-O-Metal type, surface sorption complexes, and ion pairs. With starch, problem can additionally be complicated by the formation of inclusion and capillary complexes. There are reports in the literature, which fairly exhaustively discuss these problems. In this paper, apart from a speculative macrostructure, Authors propose no molecular structure.

It seems to me, that authors fall into a trap they prepared. They started from very scientific level and terminated this problem on the preparation of two amorphous physical blends of saccharides with an iron(III) compound. It can be deduced from g-factor in Table 1. Optionally there are hydrogen bonds of the following nature: glucose unit-O…H…O=Fe- Under acidic conditions, iron(III) is fairly readily available from

such preparations. Problem whether the iron compound is ferric oxide or ferric oxide hydroxide takes solution. TG analysis (Table 1) informs that all preparations contain approximately 10% water, and only iron oxide holds 14% water. Form of the presentation of thermogravimetric results makes discussion of the water status in the preparations impossible.

Language of this paper is awkward.

In order to make this paper publishable, the concept of presentation has to be substantially modified. In order to design report in acceptable language, authors should ask assistance of experienced, possibly native, English speaker.

As a consequence of proposed changes, revised paper should be submitted to a lower rank journal.

Reviewer #5

Manuscript Number: bm049754m
Manuscript Title: Investigation of iron(III)-saccharide interactions in complexes by continuous-flow system.
Corresponding Author: Somsook

Rate the overall importance of this paper to the field of Biomacromolecules

10 - High Importance / 1 - Low Importance: 4

Recommendation: Publish after minor revisions noted below.

Additional Comments: Somsook et al: "Investigation of iron(III)-saccharide interaction in complexes by continuous-flow system

This is a comprehensive study of "complexes" between iron and various other "compounds" utilising many modern analytical techniques and methods.

The use of the word "complex" can sometimes be confusing. The "iron-oxide complex" (page 4) might be OK if we agree that there is only one complex formed when iron and hydroxide ions interact. But the "iron-rice starch complex" - it can hardly be only one complex involved. This is also true for "the iron-dextran complex" (page 4). The "samples" ("solutions" would be a better word here) were prepared according to section 2.2 (page 4) and named 1, 2, 3 etc. and later inconsistently referred to in the text as, for instance, "complex 2" and "complex (2)". The text should be more stringent and the correct terms for the various solutions must be used.

There are many abbreviations in the text, for example TGA, DTA, XRD, EPR. These should be written by their full names the first time they appear in the text. NMR is an exception - it is too well-established as a technique to be misunderstood.

On page 6, flame AAS is mentioned as an analytical technique but the details about the analyte is missing (type of analyte, concentration range, wavelength).

Reviewer #10

Publish as is, no revisions necessary.

Rate of Originality: Fair (ranging from excellent to poor)

Rate of Technical Quality: Good (ranging from excellent to poor)

Clarity of Presentation: Good (ranging from excellent to poor)

Importance to Field: Good (ranging from excellent to poor)

Comments on "Investigation of iron(III)-saccharide interactions in complexes by continuous-flow system" by E. Somsook et al.
Ms Number BM049754M

Althought the manuscript is rather diffuse, it contains sufficient amount of results for publishing in Biomacromolecules. A great number of similar papers have already been published in this field. Perhaps this is the reason why there are an extensive number of references includeds. The main output, similarly to the previous papers citated here, is that the alcoholic hydroxyl group of carbohydrates (even of polysaccharides) is deprotonated in alkaline medium, and co-ordinates to iron(III). This is not a novelty (see: B. Gyurcsik, L. Nagy, Coord. Chem. Rev., **203**, 81-149, 2000.). The most interesting question, whether Werner-type complexes are formed with polysaccharides, or latter only "pack" the Fe-hydroxide particles, still remains unresolved. The best part of the manuscript is the continuous-flow work. I would suggest to expand on this in the future. As far as the appearance is concerned, the manuscript is very well presented.

Therefore, my final conclusion is that the manuscript is suitable for publication.

Reviewer #11

Investigation of iron (III)-saccharide interactions in complexes by continuous-flow system.
Authors: Somsook, et al.

This manuscript reports a study of iron (III) extraction from various iron (III)-saccharide complexes by using 0.5M HCl solution. Iron-sucrose, iron-dextran, iron-starch complex and iron oxide were selected for the extraction study. Among the four, iron complexes of sucrose and dextran were amorphous and iron was easily released; the other two were crystalline and slowly released. ^{13}C-CP/MAS NMR showed that C-6 of rice starch was shifted to higher field when complexed with iron, but no further study to define the structure of iron-rice starch complex. There were no structural analyses of iron-sucrose and iron-dextran complexes to differentiate the two from the rice starch complex. Thus, the study did not add new understanding of structures of iron-saccharide complexes. The

manuscript is aiming for drug-release study and is more suitable for pharmaceutical oriented journals instead of *Biomacromolecules*.

Table 2, How %Fe dissolved in different concentrations of HCl solutions was determined was not given in the Method.

Appendix F

Date: Sat, 26 Jun 2004 07:18:04 +0100 (BST)
To: scess@mucc.mahidol.ac.th
Subject: PBA, Editor's decision: reject, Somsook, scess_20040610/1, Investigation of iro...
From: jpba@ph.mukogawa-u.ac.jp

Dear Dr Somsook,

On 10-Jun-2004 you submitted a new paper
number PBA_scess_20040610/1 to Journal of Pharmaceutical and Biomedical Analysis entitled:

'Investigation of iron(III)-saccharide interactions in complexes by continuous-flow system'

We regret to inform you that your paper has not been accepted for publication.
Comments from the editor are:

Dear Dr. Somsook,

I am very sorry to inform you that your paper cannot be accepted, in its present form, for publication in the Journal of Pharmaceutical and Biomedical Analysis. The reports of the independent Reviewers are enclosed here.

However, if after careful consideration of the enclosed comments from the Reviewers you would care to resubmit your manuscript, it will be given further consideration. In case you decide to do so, it will be send to two Reviewers again.

Thank you for sending your paper to the Journal of Pharmaceutical and Biomedical Analysis for review. I look forward to your reply.

Best regards, and thank you for your co-operation,

Prof. Jun Haginaka
Editor

Reviewer I
This paper is focusing on the interaction of Fe(III) to carbohydrates by using several analytical methods. It is very interesting to study the interaction of metals to carbohydrates, however, the strategy of such a study should be well organized. For example, the authors have shown only the results of sucrose, rice starch and dextran as counter materials for complex formation with Fe(III). The systematic carbohydrate samples such as glucose, maltose, maltotriose, maltotetraose and oligosaccharides/polysaccharides which molecular weights were well defined, should be

examined to clarify the mechanism of the complex formation of Fe(III) with carbohydrates.

Furthermore, 13C NMR experiments are not suitable for samples containing paramagnetic metal such as Fe(III). Please consider 1H NMR experiments of the complexes under acidic conditions in which Fe(III) might be existing as an ion form.

Consequently, this reviewer thinks that the authors should show more convincible strategy and experimental data the complex formation of Fe(III) – carbohydrate for publication in this journal.

Reviewer II
This study presented physical characters of several kinds of complex such as ion-starch, ion-sucrose and iron-dextran, investigated by some analytical methods, though the title of this manuscript is ginvestigation of iron(III)-saccharide interactions in complex by continuously-flow system h.

At first this manuscript should be collected by native English speaker, because many sentences were understandable. In the worst case I may be writing this comment by my misunderstanding.

Author uses too many vague words such as can, may, would. Author should say more exact conclusion based on appropriate data of experiments.

Adding sugars to iron-supplement is suitable to increase the rate of iron assimilation. Hence investigation of interactions between ion and various carbohydrates is significant, and study of relation of structure to activity is important. However, the manuscript is generally ambiguous, and includes too many mistakes. I am afraid that the manuscript should not be acceptable.

Additional comments follow below.

2.2. Preparation of samples in 2. Experimental section
Author said complexes were made by known reaction in abstract, but no reference is in this section.

Page 4
Line 9, 16 gpH between 10 and 11 h
Why pH was not adjusted to exact value?
How did you measure pH value?

Page 5
Line 9 gTGA h, gDTA h and gTA h
Abbreviations should be shown.

Which compound (metal?) did you use as a reference for thermal analysis? Don fft need in this case?

Figure caption for figure 2 g c.incubation temperature 70 ‹C, 2 days)h
Is it OK? In experimental section g90 ‹C, 2 hh
Exact conditions should be shown in experimental section.

Page 8

1st line gAt an incubation temperature c..h is not clear.
Do you mean gVarious pH (5,7,9 and 11) were investigated at constant temperature (90 ‹C). Among used pH, c c.h ??

Line 9 gThis showed ccould ccenter. This water was probably c..in air.h is not clear.
These sentences is just prospect. If the water was from air, you should keep samples more careful!!

Figure 3 Symbols for iron-sucrose and iron-starch are same.

Title of Y axes for figure 3(a) gConcen. of ,,,h should be gConc. of c..h or gConcentration of c h

There are many other easy mistakes.

Editor fs Notes:
1. Reference to a journal publication should be as follows:

[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, J. Sci. Commun. 163 (2000) 51–59.

Reference to a book:

[2] W. Strunk Jr., E.B. White, The Elements of Style, third ed., Macmillan, New York, 1979.

Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing, Inc. New York, 1994, pp. 281–304.
Journal names should be abbreviated according to CAS (Chemical Abstracts Service): http://www.cas.org/

2. We don ft polish your English editorially. Please check your English by a native speaker.
3. On author fs names, the first name is first and the family name is last. Please check your names.

The editorial office has provided the comments as a file. Please click http://www.elsubmit.com/esubmit/pba/scess/20040610/1/Reject%28J04-101%29.doc to download.

Yours sincerely,

Dr. Jun Haginaka
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Appendix G

SCCH 448

Inorganic Chemistry

Laboratory 2 – Iron-saccharide complexes: When the chemical diversity meets the biological diversity.

Introduction

In human body, there are only 24 different essential elements. The greatest components, by mass, are oxygen (65%), carbon (18%), hydrogen (10%), and nitrogen (3%). The other elements, such as calcium, phosphorus, iron, and copper, are mineral and trace elements, which essential to the body's proper functioning. One vital trace element, **iron** (0.008%), will be presented here.

Iron is an essential element for all living organisms. In the human body, iron shows in all cell and has several vital functions, which are a carrier of oxygen to the tissues (hemoglobin-Hb), a facilitator of oxygen use and storage in the muscles (myoglobin), a transport medium for electrons within the cells (cytochrome), and an integral part of enzyme reactions in various tissues. When the body's iron supply is too low, it's called **iron deficiency**.

Iron status	Stored Iron#	Transport Iron*	Functional Iron~
Iron-deficiency anemia	Low	Low	Low
Iron-deficiency erythropoiesis	Low	Low	Normal
Iron depletion	Low	Normal	Normal
Normal	Normal	Normal	Normal
Iron overload	High	High	Normal

Stored Iron: Ferritin and Hemosiderin
* Transport Iron: Transferrin
~ Functional Iron: Hemoglobin, Myoglobin and Respiratory enzymes

Iron-deficiency anemia is one of the most common nutritional deficiencies and has many causes, as in infants and preschool children, Iron-deficiency anemia causes in developmental delays and behavioral disturbances. Adult men and post-menopausal women are not at risk of Iron-deficiency anemia.

Iron-saccharide complexes, such as iron-dextran, iron-sucrose, iron-gluconate, and iron-polysaccharide, have been used for the treatment of Iron-deficiency anemia.

Some is good for the oral treatment, like iron-dextran, but iron-sucrose is safe for the intravenous treatment.

Procedure

A. Preparation of Iron-saccharide complexes

Saccharides	Iron compounds
1. Tapioca flour	1. Iron (III) chloride
2. Sticky rice flour	2. Iron (III) nitrate
3. Rice flour	3. Iron (III) sulphate
4. Mung bean flour	
5. Corn flour	
6. Dextran	
7. Sucrose	

1. Iron-saccharide complexes

Aqueous starch suspension (saccharide 5 g in 50 ml of water) is heated at 90 °C for 1 h with continuous agitation. Then 10 ml of 5 M NaOH is added to the solution. The basic saccharide solution is added slowly to the iron (III) ion solution (3.6 g of iron (III) chloride in 400 ml of water). Subsequently, 5 M NaOH is added dropwise to obtain a pH between 10 and 11. The suspension is heated at 90 °C for 2 h with gentle stirring, and then centrifuge and decants, and wash precipitates with deionized water to remove NaCl

If precipitates have not occurred, the 150 ml of 2-propanol would be added prior to centrifugation.

2. Iron oxide complex

Iron (III) chloride (3.6 g) is dissolved in distilled water (400 ml), and then adds 5 M NaOH dropwise with stirring to obtain a final pH between 10 and 11. The precipitates are then heated and separated off same as iron-saccharide complexes

All samples are oven-dried at 50 °C and ground in an agate mortar prior to characterization.

B. Characterization of Iron-saccharide complexes

1. X-Ray Diffraction (XRD)
2. Thermogravimetry (TGA)

3. Differential Thermal Analysis (DTA)
4. Electron Paramagnetic Resonance (EPR)
5. Scanning Electron Microscope (SEM)
6. Mossbauer Spectroscopy

C. Molecular Modeling

In this part, you have to speculate what the structure of iron-starch would be. Draw on a paper. Do not think anything too complicated. Then you will attempt to make that structure on a molecular modeling software.