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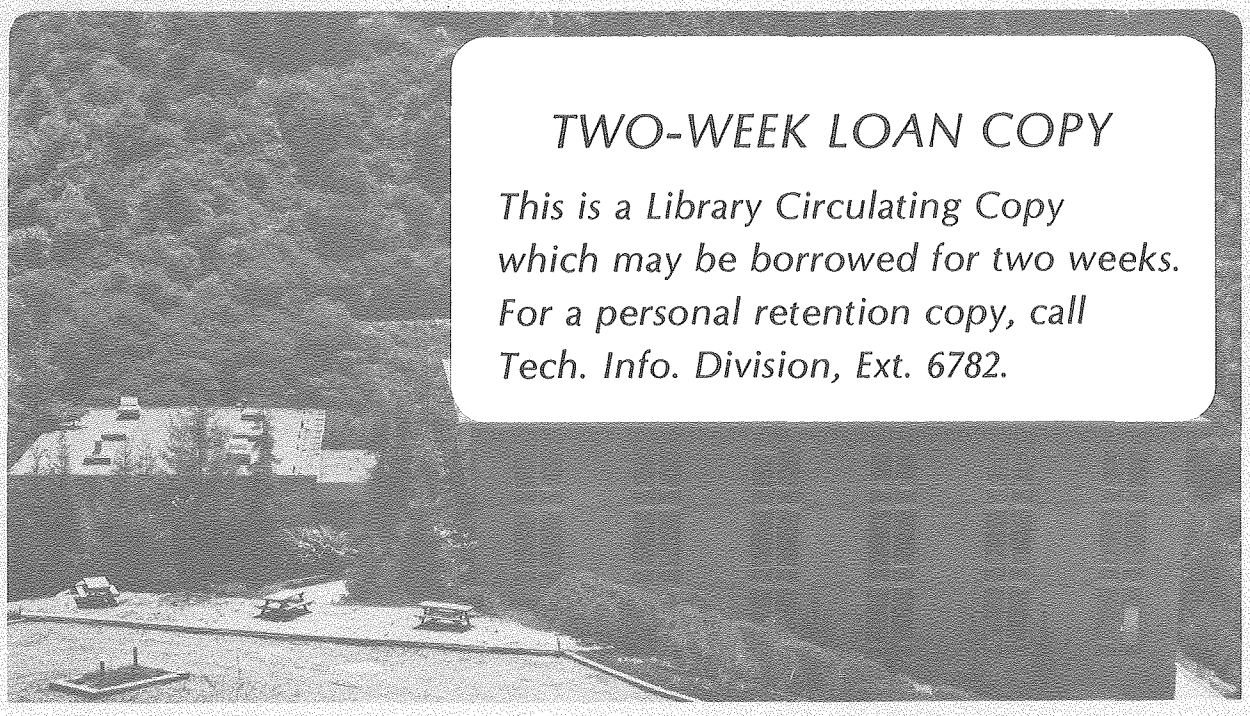

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The Synthesis, Thermodynamic Behavior, and Biological Properties of Metal-Ion-Specific Sequestering Agents for Iron and the Actinides

By

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Abstract

Enterobactin, a microbial iron-transport compound which is a hexadentate ligand via its three catechol groups, has been studied by potentiometric, spectrophotometric and electrochemical techniques. The proton-dependent stability constant for the ferric enterobactin complex has been determined spectrophotometrically by competition vs EDTA. At physiological pH, enterobactin is by far the most powerful iron chelating agent yet characterized. In a biomimetic approach to the design and synthesis of ferric-ion-specific sequestering agents that could prove to be new therapeutic agents for iron mobilization in man, several structural analogs of enterobactin have been chosen as target compounds. Because of the large number of chemical and biological similarities of plutonium(IV) with iron(III) a biomimetic approach has also been taken in the synthesis of a new class of actinide-specific sequestering agents which incorporate four catechol groups. In general both the iron and actinide sequestering agents are derivatives of 2,3-dihydroxybenzoic acid or the 5-sulfo-2,3-dihydroxybenzoic acid derivatives. Both MECAM [1,3,5-N,N',N"-tris(2,3-dihydroxybenzoyl)triaminomethylbenzene] and 3,4-LICAMS [1,5,10-N,N',N"-tris(5-sulfo-2,3-dihydroxybenzoyl)triazadecane] have been found to remove iron from the human iron transport protein transferrin. In fact, all of the synthetic tricatecholate ligands rapidly remove iron from human transferrin – in marked contrast to

the trihydroxamate ligands such as desferrioxamine B (the most commonly used drug for iron removal in man). The details of the kinetics of transferrin iron removal by 3,4-LICAMS have been investigated. In addition, the mode of coordination to ferric ion of enterobactin and analogous compounds has been investigated and the change in this coordination as a function of protonation of the metal-ligand complex characterized. Protonation of these compounds occurs through one-proton steps to give a salicylate mode of bonding in which the carbonyl oxygen of the 2,3-dihydroxybenzoyl group coordinates to the metal. The solution thermodynamics of the complexes formed between the tetra-catechol sequestering agents and thorium(IV) have been investigated in a preliminary fashion and these sequestering agents have undergone animal tests for their efficacy in decorporation of plutonium. Two of these compounds have been found to be far superior at low concentration to any of the sequestering agents presently used in actinide contamination treatment.

Introduction

Certain types of anemia require regular transfusions of whole blood, since the victims of these diseases cannot correctly manufacture their own. One such disorder, β-thalassemia, is described in more detail elsewhere in this publication.[1] Because the body lacks any mechanism for excreting significant amounts of serum iron, the iron contained in transfused blood (∿ 250 mg per pint) can accumulate to lethal levels. At this time the only treatment for Cooley's anemia is continual transfusion and so some way must be found to efficiently remove this excess iron. Thus there is an obvious need for a drug which can selectively bind iron *in vivo* and facilitate its excretion.

Dr. Anderson has described the current use of desferrioxamine B for clinical iron removal in the treatment of β-thalassemia.[1] Desferrioxamine B belongs to the class of compounds called siderophores,[2] which were discussed by Professor Neilands.[3] Siderophores are produced by microorganisms for the purpose of binding exogenous ferric ion and facilitating its transport across the cell membrane. These compounds generally utilize either hydroxamic acid or catechol groups to bind ferric ion and form very stable, high spin, octahedral complexes. Desferrioxamine B is one of the hydroxamate type siderophores (Figure 1). It is a linear molecule consisting of alternating units of succinic acid and 1,5-diaminopentane, which combine to give three

hydroxamic acid groups. Although the effectiveness of desferrioxamine therapy has been improved, there appear to be fundamental limitations to its potential for iron removal which are probably inherent to hydroxamates in general.

This led to our interest in the second major type of siderophores, the catechols. The best known member of this class of compounds is enterobactin, a cyclic triester of 2,3-dihydroxybenzoylserine, shown in Figure 2. Although there were indications that enterobactin formed very stable ferric complexes,[4,5] the formation constant of ferric enterobactin had never been determined. Therefore, our first priority was to determine the iron affinity of enterobactin.

Solution Equilibria of Ferric Enterobactin

The potentiometric titration curve of ferric enterobactin, shown in Figure 3, has a sharp inflection after the addition of six equivalents of base. Such a break indicates that the six phenolic oxygens from the three dihydroxybenzoyl groups are displaced by ferric ion in the ferric enterobactin complex. This interpretation is further supported by the absorbance maximum at 490 nm (ε 5600), which is very similar to simple tris(catecholato)iron(III) complexes.[6,7] The very low pH at which complexation of enterobactin occurs, with virtually complete complex formation by pH 6, is a strong indication of a very stable complex. However, the titration is prematurely terminated at pH 3.8 by the precipitation of a purple

neutral iron complex (whose composition and structure will be discussed later) which makes it impossible to determine the stability constant of ferric enterobactin from potentiometric data alone.

Instead, the stability constant of enterobactin has been determined spectrophotometrically by competition with EDTA, as described by the equation:

$$Fe(ent)^{3-} + EDTA^{4-} + 6H^{+} \overset{K_x}{\rightleftharpoons} Fe(EDTA)^{-} + H_6ent \qquad (1)$$

It is necessary to take advantage of the strong pH dependence of Eq. 1. At neutral or basic pH, this equilibrium lies completely on the side of ferric enterobactin. At pH 5, however, a measurable distribution of ferric ion is obtained with less than a tenfold excess of EDTA. The intense charge transfer band of ferric enterobactin provides a convenient way of determining the concentration of $Fe(ent)^{3-}$, and the remaining concentrations are obtained from mass balance considerations. Using the literature value for the formation constant of ferric EDTA,[8] one can calculate a value of the proton-dependent equilibrium constant

$$K_6^* = \frac{[Fe(ent)^{3-}][H^+]^6}{[Fe^{3+}][H_6ent]} = 10^{-9.7(2)} \qquad (2)$$

In order to convert K_6^* into the conventional, i.e. proton independent, formation constant, it is necessary to know the six ligand protonation constants of enterobactin.

Unfortunately, the ester groups of enterobactin are extremely susceptible to base-catalyzed hydrolysis, which precludes independent measurement of ligand pKa's. However, we have measured the protonation constants of the bidentate ligand 2,3-dihydroxy-N,N-dimethylbenzamide as log K_1^H = 12.1 and log K_2^H = 8.4. By using these values as estimates for the enterobactin protonation constants, we have estimated the overall formation constant of ferric enterobactin to be

$$K_{ML} = \frac{[Fe(ent)^{3-}]}{[Fe^{3+}][ent^{6-}]} \geq 10^{52} \qquad (3)$$

This is the largest formation constant ever reported for a ferric complex and indicates the exceptional stability of ferric enterobactin.

Because of the very weak acidity of the phenolic oxygens of enterobactin, the full impact of log K_{ML} would be realized only above pH 12. What we are really interested in is the ligand's ability to sequester ferric ion at physiological pH. We also need some method of comparing the relative effectivenss of various ligands which can take into account changes in ligand protonation constants, the hydrolysis of some ferric complexes, and the formation of polynuclear species in some systems. Thus we have chosen to compare ligands by calculating the equilibrium concentration of free hexaaquoiron(III) in a pH 7.4 solution which is 1 μM in iron and 10 μM in ligand. The results are expressed as pM values

(pM = - log $[Fe(H_2O)_6^{3+}]$), with a larger pM indicative of a more stable complex under the prescribed conditions. This gives a direct measurement of the relative iron binding affinity, since in a competition between two ligands under the conditions stated, the ligand with larger pM will dominate. Table I lists pM values of all the siderophores for which formation constants are known. [Note that the pM value for enterobactin is determined directly from the observed equilibrium constant K_6^* (Eq. 2) and not from the estimated K_{ML} (Eq. 3).] Although the trihydroxamic acid siderophores such as ferrioxamine B and ferrichrome form very stable complexes, it is clear that enterobactin not only has a much larger formation constant, but is also many orders of magnitude more effective at sequestering ferric ion at physiological pH. Although enterobactin itself is not suitable for chelation therapy (due in part to the facile hydrolysis of its ester linkages at physiological pH) it does represent a uniquely promising model on which to base the design of new synthetic ferric ion sequestering agents.

Synthetic Analogues of Enterobactin

With these results in mind, we have prepared and evaluated a number of catecholate type ligands. The structural formulas for these ligands are shown in Figure 4. Like enterobactin, all these ligands can bind ferric ion via six phenolic oxygens from three catechol groups. Unlike enterobactin, they are also hydrolytically stable over normal pH ranges.

The sulfonation of these types of ligands is designed to serve a number of purposes. It stabilizes the catechol groups against oxidation to the corresponding quinone, and also increases the otherwise very low water solubility of these ligands. In addition, sulfonation substantially lowers the ligand protonation constants. Because of the very weak acidity of the catechol ligating groups, competition from hydrogen ion is a significant interference to metal complexation, even at physiological pH. By decreasing the affinity of the ligating groups for hydrogen ion, it is possible to enhance metal complexation at neutral pH.

The formation constants of the ferric complexes of these synthetic catecholate ligands have been determined spectrophotometrically by competition with EDTA, as described above for enterobactin. The first three (most acidic) ligand protonation constants have been determined by potentiometric titration of the free ligand. The second, more basic, set of protonation constants are too large to be determined readily potentiometrically. Thus the proton-dependent stability constant is expressed as

$$K_3^* = \frac{[FeL][H]^3}{[Fe][H_3L]} \qquad (4)$$

Such constants are valid over the pH range in which the final three phenolic protons are essentially undissociated, i.e. up to $\sim$ 10.5. As with enterobactin, one can estimate the

higher ligand protonation constants based on values reported for simple catechols and then convert K_3^* into K_{ML} (K_{ML} = [FeL]/([Fe][L])), which is written in terms of the fully deprotonated form of the ligand. Values of log K_3^* and log K_{ML} are listed in Table II.

It is much easier to make direct comparisons between these catecholate compounds and other classes of ligands such as the hydroxamates in terms of pM values rather than log K_{ML}. Therefore, the K_3^*'s have been used to calculate pM values under the same conditions prescribed above for the siderophores: pH 7.4, 1 μM total Fe^{3+}, and 10 μM total ligand. These pM values are also listed in Table II.

The pM value of 3,4-LICAMS, 3,4,3-LICAMS, MECAM, and MECAMS are all exceptionally high, ranging from 28.5 to 31.0. Although not as powerful as enterobactin, these ligands are clearly superior to the usual amino acid type ligands such as diethylenetriaminepentaacetic acid and to the hydroxamate based siderophores. In particular, desferrioxamine B has a pM value of 26.6, so that the ligands described above are up to 10,000 times more effective at sequestering Fe(III) at pH 7.4 than is desferrioxamine B.

The CYCAM type ligands (Figure 4) are significantly less effective than their linear analogues, such as 3,4-LICAMS, or the other platform type ligand, MECAMS. It appears that the combination of having the two ends of an aliphatic amine linked to form a cyclic group and having the amide nitrogens

actually contained within this ten-membered ring leads to rather severe strain when the molecule is configured to fully encapsulate a ferric ion. In MECAM and enterobactin, the amide nitrogens are appended to, rather than contained within, the central rings. This additional flexibility appears to be necessary for an effective ligand. In addition, the triester ring of enterobactin is flexible, as opposed to the rigidly planar benzene ring of MECAMS. This extra conformational freedom is probably a contributing factor to the enhanced stability of enterobactin over MECAM.

The Mode of Coordination of Enterobactin and Analogous Tricatechols

At high pH, the tricatecholate ligands bind iron via the six phenolic oxygens, just as observed for the tris complexes of simple catechols such as 2,3-dihydroxybenzamide, Tiron, or catechol itself. In the MECAM system, this red $[Fe(MECAM)]^{3-}$ complex has an absorbance maximum at 492 nm with $\varepsilon = 4700\ M^{-1}\ cm^{-1}$. As the pH is lowered, the λ_{max} shifts to longer wavelengths and an isosbestic point is formed at 542 mn, as shown in Figure 5. Such data may be analyzed using the equation

$$\varepsilon_{obsd} = \frac{1}{K_{MH_nL}} \frac{(\varepsilon_{ML} - \varepsilon_{obsd})}{[H]^n} + \varepsilon_{MH_nL} \qquad (5)$$

in which ε_{obsd} is the absorbance observed at a given pH divided by the analytical iron concentration, and ε_{ML} and

ε_{MH_nL} are the molar extinction coefficients of the $[Fe(MECAM)]^{3-}$ and $[Fe(H_nMECAM)]^{(3-n)^-}$ species. The exponent $\underline{n}$ is the stoichiometric coefficient of hydrogen ion in the reaction:

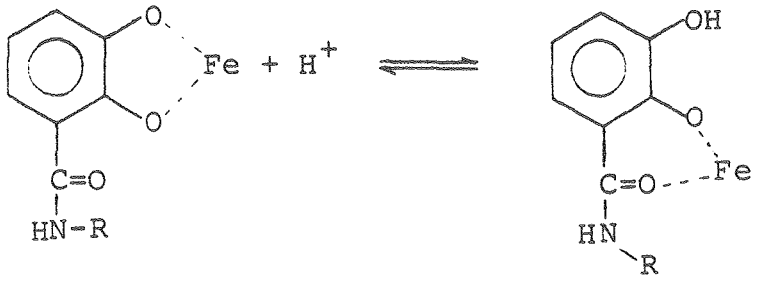
$$Fe(MECAM)^{3-} + nH^+ \rightleftharpoons^{K_{MH_nL}} Fe(H_nMECAM)^{(3-n)^-} \quad (6)$$

$$K_{MH_nL} = \frac{[Fe(H_nMECAM)]}{[Fe(MECAM)][H]^n} \quad (7)$$

Linear plots of ε_{obsd} vs $(\varepsilon_{ML} - \varepsilon_{obsd})/[H]^n$ are obtained only for $\underline{n} = 1$, which establishes the equilibrium of Eq. 6 is a one-proton reaction. The slope of this plot gives log K_{MHL} = 7.08(5). As the pH is lowered further, a second one-proton reaction occurs (with an isosbestic point at 588 nm) for which log K_{MH_2L} = 5.6(1). Below pH 4.8, a dark purple complex precipitates, which is the neutral $Fe(H_3MECAMS)$ complex. Thus ferric MECAM reacts in a series of 3 one-proton steps.

The solid-state IR spectra of free H_6MECAM, $K_3Fe(MECAM)$, and $Fe(H_3MECAM)$ are shown in Figure 6. There is an amide carbonyl band for the free H_6MECAM ligand at 1635 cm^{-1} which shifts to 1620 cm^{-1} upon formation of the red $K_3Fe(MECAM)$ complex. In the spectrum of the neutral, triply-protonated complex, $Fe(H_3MECAM)$, the carbonyl band is absent, and has apparently moved underneath the phenyl ring modes at 1580 and 1540 cm^{-1}. Such a shift is characteristic of a metal-bound carbonyl, and indicates a shift in the mode of bonding from a "catecholate-type" in which coordination is by the two phenolic oxygens to a "salicylate type" in which

coordination is by one phenol and the carbonyl oxygen, as shown below.



Spectrophotometric data indicate the presence of analogous, sequential one-proton reactions for the ferric complexes of enterobactin and all the synthetic tricatecholate ligands except TRIMCAMS (Figure 4), in which the amide carbonyl groups have been relocated α to the central benzene ring, and are not a substituent of the catechol rings as in MECAMS. Thus the carbonyl is no longer available to form a six-membered chelate ring with the ortho phenolic oxygen. The protonation reaction of TRIMCAMS thus provides a powerful test of the catecholate-salicylate mode of bonding equilibrium that we propose.

The visible spectra of ferric TRIMCAMS as a function of pH are shown in Figure 7. There is a single, sharp isosbestic point at 540 nm which remains throughout the addition of two equiv. of hydrogen ion to $[Fe(TRIMCAMS)]^{6-}$. A plot of Eq. 6 is linear for $n = 2$, with

$$K^{2}_{MH_2L} = \frac{[Fe(H_2TRIMCAMS)^{4-}]}{[Fe(TRIMCAMS)^{6-}][H^+]^2} = 10^{13.7} \qquad (9)$$

These results thus support the model proposed above in which one-proton reactions are due to a shift from a catecholate to a salicylate mode of bonding.

Removal of Iron from Human Transferrin

Most normal body iron is contained either in hemoglobin or in the storage proteins ferritin and hemosiderin. Certainly for the heme proteins it is unlikely that chelating agents will be able to remove significant amounts of iron — and the iron in the storage proteins is also quite inaccessible. However, the high-spin Fe(III) in the iron transport protein transferrin is relatively labile, and apotransferrin is able to obtain iron from ferritin. Thus if we could remove and excrete transferrin iron, we may then allow the apotransferrin to mobilize stored iron. The key factor is obviously the ability to remove ferric ion from transferrin. This is even more of a kinetic problem than a thermodynamic one. Thus, while the hydroxamates such as desferrioxamine B are thermodynamically capable of removing iron from transferrin, kinetically they are able to do so at a useful rate only in the presence of other ligands.

The pM values of most of the catecholate ligands are well above that of transferrin, indicating that iron removal is thermodynamically favored. The question remains, however,

as to the rate of this exchange reaction. Therefore we have investigated the kinetics of iron removal from transferrin by these types of catecholate ligands. The addition of 3,4-LICAMS to diferric transferrin results in the series of spectra shown in Figure 8. The absorbance maximum shifts smoothly from the 470 nm λ_{max} of diferric transferrin (ε = 2500/Fe) to the 495 nm λ_{max} of ferric 3,4-LICAMS (ε = 5500). With excess 3,4-LICAMS, plots of $\ell n[(A - A_\infty)/(A_o - A_\infty)]$ vs time are linear over three half-lives (Figure 9).

Previous results have indicated that iron removal from transferrin might involve the formation of a ternary iron-transferrin-ligand intermediate, which dissociates into FeL and apotransferrin.[12,13] Such a scheme is outlined in Eq. 10.

$$FeTr + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} FeTrL \xrightarrow{k_2} FeL + Tr \qquad (10)$$

Such a mechanism predicts a hyperbolic relationship between k_{obsd} and the concentration of the competing ligand, and Figure 10 shows that such a relationship does pertain. One can express k_{obsd} as

$$k_{obsd} = \frac{k_2[L]K_{eq}}{2.3 + 2.3\ K_{eq}[L]} \qquad (11)$$

where $K_{eq} = k_1/k_{-1}$. The data for 3,4-LICAMS were refined by a nonlinear least-squares fit of k_{obs} vs [L] to give values

k_2 = 0.066(4) min^{-1} and K_{eq} = 4.1(6) x 10^2 ℓ mol^{-1}. Similar results are obtained with MECAM and enterobactin as competing ligands, although the low solubility of these ligands limits the ratio of ligand:transferrin. Table III lists the percentage of iron removed in 30 min at a specified ligand concentration. While desferrioxamine B can remove less than 5% of the iron at a 100:1 excess of desferrioxamine, 3,4-LICAMS removes 50% of transferrin iron at only a 40:1 ratio. Thus the catecholate type ligands represent an effective combination of both a high affinity for ferric ion coupled with the ability to remove iron from transferrin at a reasonable rate.

Specific Sequestering Agents for Actinide(IV) Ions

In addition to the problems posed by acute and chronic iron-overload poisoning, we are also interested in the problems posed by trans-uranium actinide contamination. There are a number of similarities in the coordination chemistry of Fe(III) and Pu(IV). Indeed, the great biological hazard of plutonium is because, once in the body, it is associated with the iron binding protein's transferrin and ferritin and is deposited essentially irreversibly in iron storage sites. Using enterobactin as a model, we can predict that catechol-based ligands should form very stable plutonium complexes – especially when incorporated in large multidentate ligands.

In order to satisfy the preferred higher coordination number of the actinides, we have synthesized several potentially octadentate, tetracatecholate compounds with the structures

shown in Figure 11. Of these, we have studied the solution chemistry of 3,4,3-LICAMS with Th(IV) as a model for Pu(IV). The potentiometric equilibrium curve of a 1:1 ratio of Th(IV): 3,4,3-LICAMS is shown in Figure 12. Complexation is complete by ∿ pH 8, which indicates fairly strong complexation. However, the sharp inflection at 7.75 equivalents of base per Th(IV) shows that the thorium species formed under these conditions is polynuclear, with at least three and possibly more thorium ions per molecule. Polynuclear complexes of thorium are quite common with ligands having fewer than eight donor groups.[14-17] In particular, Th(IV) forms a ligand-bridged dimeric complex with tiron[15] (1,2-dihydroxy-3,5-disulfobenzene), even in the presence of a large excess of ligand.

The coordination of all four catechol groups of 3,4,3-LICAMS to Th(IV), displacing the eight catechol protons, would result in a break in the titration curve at 8 equiv. of base, compared to the observed break at 7.75 equiv. The titration of fewer than the expected number of protons, plus the polymeric nature of the Th(IV):3,4,3-LICAMS complex both suggest that all four dihydroxybenzoyl groups are not coordinating, thus leaving coordination sites available for hydroxyl bridging groups.

Unfortunately, the complexity of the Th(IV):3,4,3-LICAMS system precludes the calculation of formation constants. However, the relative sequestering abilities of 3,4,3-LICAMS and DTPA have been investigated by direct competition between

these two ligands using difference ultraviolet spectoscopy to measure the fraction of thorium bound to 3,4,3-LICAMS. At pH 6.5, the Th(IV) is bound predominantly to DTPA. The fraction of Th(IV) bound to 3,4,3-LICAMS then increases rapidly with increasing pH, leveling off around pH 7.5 with $\sim$ 80% of the thorium associated with 3,4,3-LICAMS. Thus at physiological pH, the 3,4,3-LICAMS complex is slightly favored thermodynamically over Th(IV)-DTPA.

Several linear and cyclic tetracatecholates have been evaluated in vivo for their ability to enhance the excretion of Pu(IV) from mice.[18] Between 20-30 μmole/Kg of the ligand was administered 1 hr after the injection of plutonium citrate. The mice were sacrificed after 24 hr and the distribution of Pu(IV) in the tissues and excreta were measured. The percentages of plutonium excreted are listed in Table IV. About 60-65% of the Pu(IV) is excreted with 3,4,3- and 4,4,4-LICAMS, which is roughly comparable to the effectiveness of DTPA at this dose level. The length of the middle bridging alkane group in the LICAMS series appears to be critical, since the presence of a propyl group at this position reduces the amount of Pu(IV) excreted to $\sim$ 40%. The cyclic catecholates are less effective than the linear compounds. The unsulfonated ligand 3,3,3,3-CYCAM binds plutonium and restricts its deposition in the liver and skeleton, which is where 80% of injected plutonium is deposited in the control. However, the 3,3,3,3-CYCAM complex

apparently dissociates in the kidneys, so the Pu(IV) is simply concentrated in this organ.

Although 3,4,3-LICAMS and DTPA removed roughly equal amounts of plutonium under the conditions described above, 3,4,3-LICAMS offers several advantages over DTPA. The catecholate ligands do not form strong complexes with most divalent metal ions and thus the depletion of essential metals such as Zn, Mn, Cu and Co, (which is a severe problem in DTPA therapy) is greatly reduced. In addition, dose-response studies show that the 3,4,3-LICAMS dose can be reduced by a factor of 100 (to 0.3 μmoles/Kg) and still effect ∿ 50% excretion of Pu(IV). In contrast, DTPA therapy at this level is virtually ineffective at this dose level.[19] Finally, 3,4,3-LICAMS is substantially more effective than DTPA for removing Pu which has been deposited in the skeleton. The properties and chemistry of these compounds are described in greater detail in a monograph resulting from another symposium of the 1979 Washington ACS meeting.[20]

Summary

The results described above show that these new catecholate compounds do indeed form exceptionally stable complexes with very hard Lewis acids such as Fe^{3+}, Th^{4+} and Pu^{4+}. The sulfonation of these ligands increases their water solubility, stabilizes the catechol groups against air oxidation, and enhances their ability to sequester metal

ions at neutral and slightly acidic pH. The iron complexes are among the most stable ever characterized, and these types of ligands are thermodynamically able to compete with transferrin for serum iron. In contrast to the hydroxamates, our kinetic studies have shown that the catechol-based ligands are in fact capable of removing substantial amounts of iron from transferrin within a few minutes.

With Th(IV), the tetracatecholates form complex polynuclear species. Competition studies in vitro indicate these complexes are slightly more stable than the monomeric Th:DTPA complex at pH 7.4. In vivo studies on plutonium decorporation from mice show that 3,4,3-LICAMS is as effective as DTPA at fairly high doses. However, dose-response curves show that the 3,4,3-LICAMS retains 80% of its effectiveness at very low concentrations, whereas the DTPA activity drops to essentially zero under the same conditions.

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Table I. Relative ferric ion complexing abilities at neutral pH for human transferin and several microbial iron sequestering agents.

Ligand[a]	pM[b]	log K_{ML}	Ref.
Enterobactin	35.5	52	6
Desferrioxamine E	27.7	32.5	9
Desferrioxamine B	26.6	30.6	9
N-acetyl-desferrioxamine B	26.5	30.8	9
Desferrichrysin	25.8	30.0	9
Desferrichrome	25.2	29.1	9
Aerobactin	23.3	23.1	10
Rhodotorulic acid	21.9	- [c]	11
Human transferrin	23.6	-	6

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[a]The structural formulas of several of the siderophores are shown in Figures 1 and 2, for the other siderophore structures see Ref. 2. The "des" prefix explicitly denotes the iron free siderophore ligand.

[b]Calculated for pH 7.4, 10 μM ligand, and 1 μM Fe^{3+}.

[c]Exists in solution solely as an Fe_2L_3 dimer under these conditions.

Table II. Relative ferric ion complexing abilities at neutral pH for synthetic tricatechol sequestering agents.

Ligand[a]	pM[b]	log K_3^*	log K_{ML} (est.)
3,4,3-LICAMS	31.1	8.51(3)	43
MECAMS	29.4	6.6(1)	41
MECAM	29.1	9.5(3)	46
3,4-LICAMS	28.5	6.40(9)	41
TRIMCAMS	25.1	4.4(1)	41
3,4,3-CYCAMS	24.9	3.4(1)	38
3,4,3-CYCAM	23.0	1.5(1)	38

[a]For the structural formulas of these compounds see Figure 4.

[b]As defined in Table I.

Table III. Relative kinetics of iron removal from human transferrin by several iron sequestering agents.

Ligand	[L]/[Tr][a]	% Fe Removed[b]
3,4-LICAMS	40	50
3,4,3-LICAMS	100	60
Desferrioxamine B	100	5
EDTA	2500	37
MECAM	1	13
Enterobactin	1	6
3,4-LICAMS	1	6
EDTA	1	0

[a]Ratio of ligand to transferrrin conentration; [L] = ligand, [Tr] = diferric transferrin.

[b]After 30 minutes.

Table IV. Relative efficacy of Pu removal from mice by several tetracatechol ligands

Ligand[a]	% Pu Excreted (24 hrs)
3,4,3-LICAMS (i.v.)	64.6
4,4,4-LICAMS (i.v.)	59.3
3,3,3-LICAMS (i.v.)	44.1
4,3,4-LICAMS (i.v.)	43.4
2,3,2-LICAMS (i.p.)	37.0
2,3,3,3-CYCAMS (i.v.)	38.6
3,3,3,3-CYCAMS (i.v.)	35.3
3,3,3,3-CYCAM (i.m.)[b]	11.1
$CaNa_3DTPA$ (i.p.)	63.2
Desferrioxamine B (i.p.)	47.8
Control	6.1

[a]See Figure 11 for structural diagrams.

[b]Note that this is the unsulfonated derivative.

Figure Captions

Figure 1. Structural formulas of representative types of hydroxamate siderophores.

Figure 2. Structural formula of the tricatecholate siderophore enterobactin.

Figure 3. Potentiometric equilibrium curve of a 1:1 solution of ferric enterobactin. T = 25°C, μ = 0.10 M (KNO_3), a = moles of base per mole of iron.

Figure 4. Structural formulas and acronyms of synthetic tricatecholate ligands.

Figure 5. Visible spectra of ferric MECAM, as a function of pH, from pH 6.5 to 7.5. $[Fe(MECAM)^{3-}]$ = 2×10^{-4}, μ = 0.10 (KNO_3), T = 25°C.

Figure 6. Infrared spectra of KBr pellets of MECAM (-·-·-·), [Fe(MECAM)$^{3-}$] (------), and Fe(H_3MECAM) (———).

Figure 7. Visible spectra of ferric TRIMCAMS as a function of pH from pH 6 to 10.

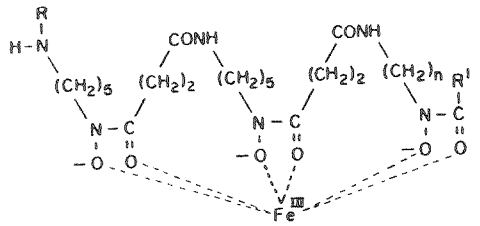
Figure 8. Spectral changes accompanying iron removal from transferrin. The bottom curve represents the unreacted transferrin and the top curve the final product, ferric 3,4-LICAMS.

Figure 9. Iron removal from ∿ 0.2 mM diferric transferrin by various concentrations of 3,4-LICAMS.

Figure 10. Plot of the observed rate constant for iron removal from transferrin (∿ 0.4 mM) vs the concentration of 3,4-LICAMS. The points represent the experimental data, the line is calculated from the derived rate constants.

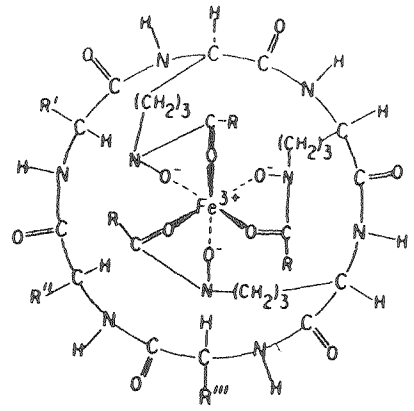
Figure 11. Structural formulas and acronyms for synthetic tetracatecholate ligands.

Figure 12. Potentiometric equilibrium curve of a 1:1 ratio of Th(IV) 3,4,3-LICAMS. [Th] = [LICAMS] = 2×10^{-3} M, T = 25°C, μ = 0.10 (KNO_3).



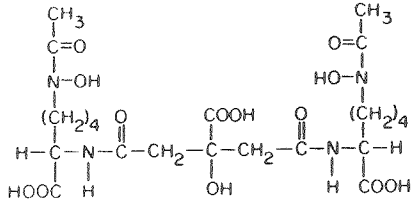
FERRIOXAMINE B

(R = H, R′ = CH_3)

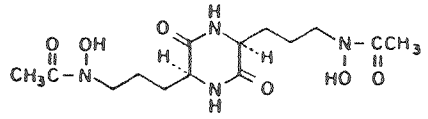


FERRICHROME

(R = CH_3, R′ = R″ = R‴ = H)



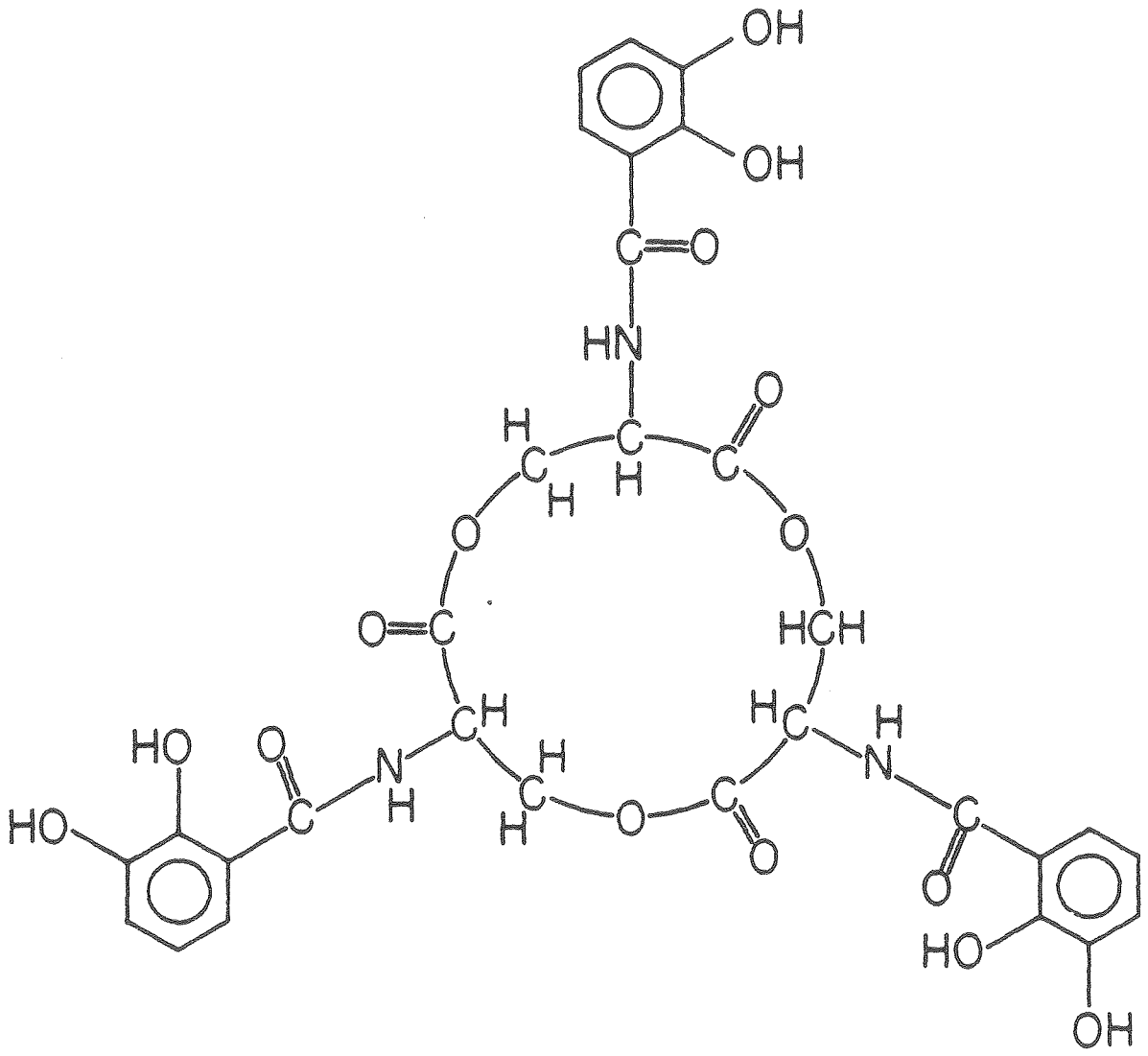
AEROBACTIN



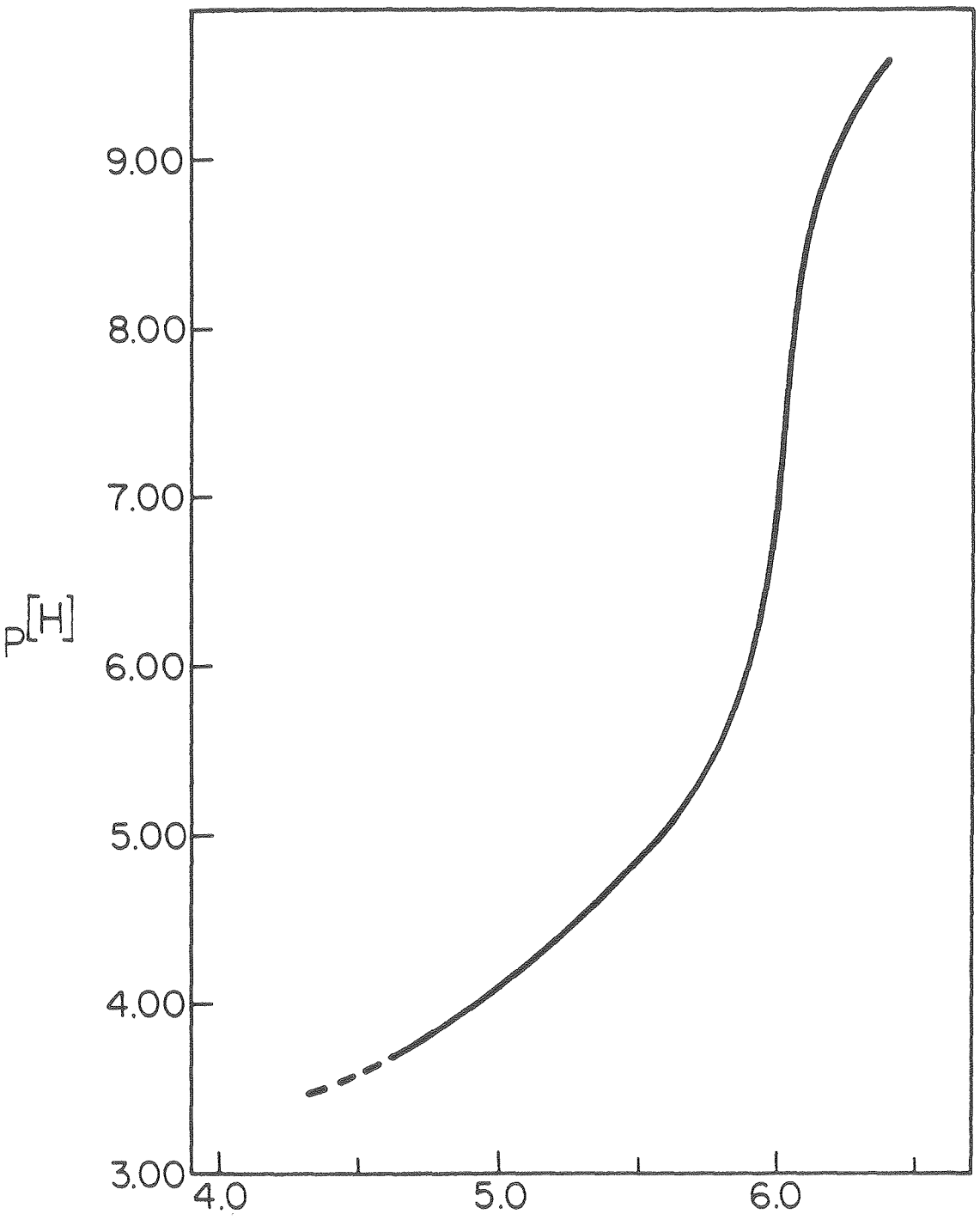
RHODOTORULIC ACID

XBL 7910-12513

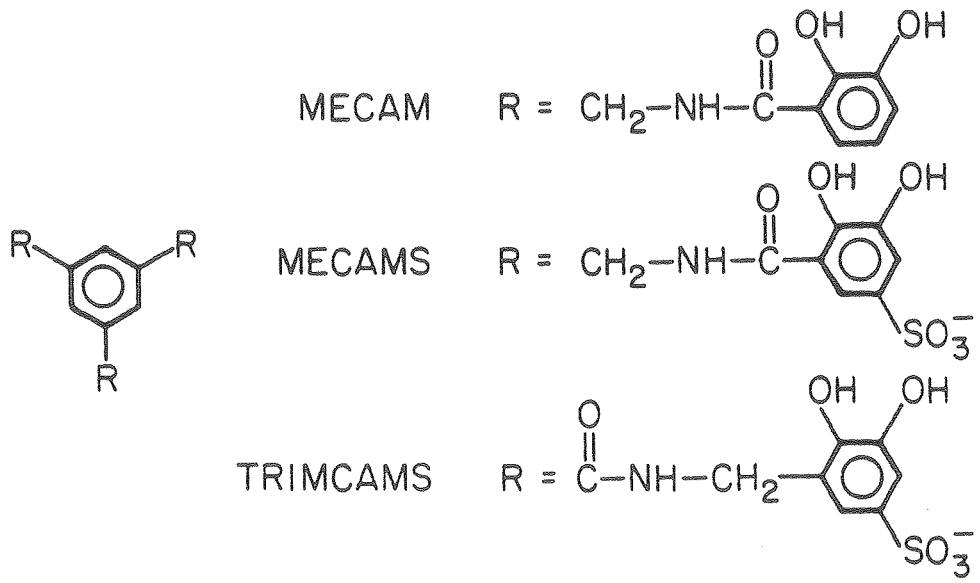
ENTEROBACTIN

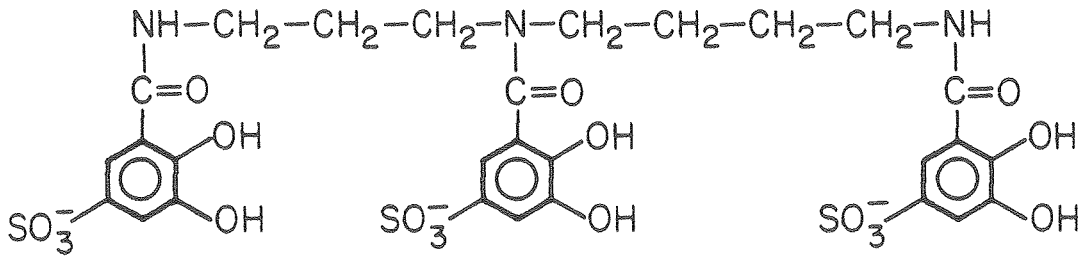


XBL 7610-4903

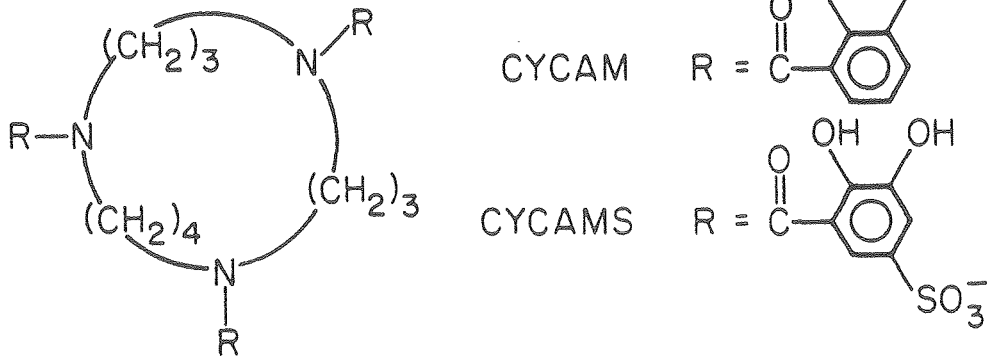

9.00
8.00
7.00
6.00
5.00
4.00
3.00
p[H]
4.0
5.0
6.0
a (MOLES BASE PER MOLE OF METAL)

XBL 793-8732





3,4–LICAMS



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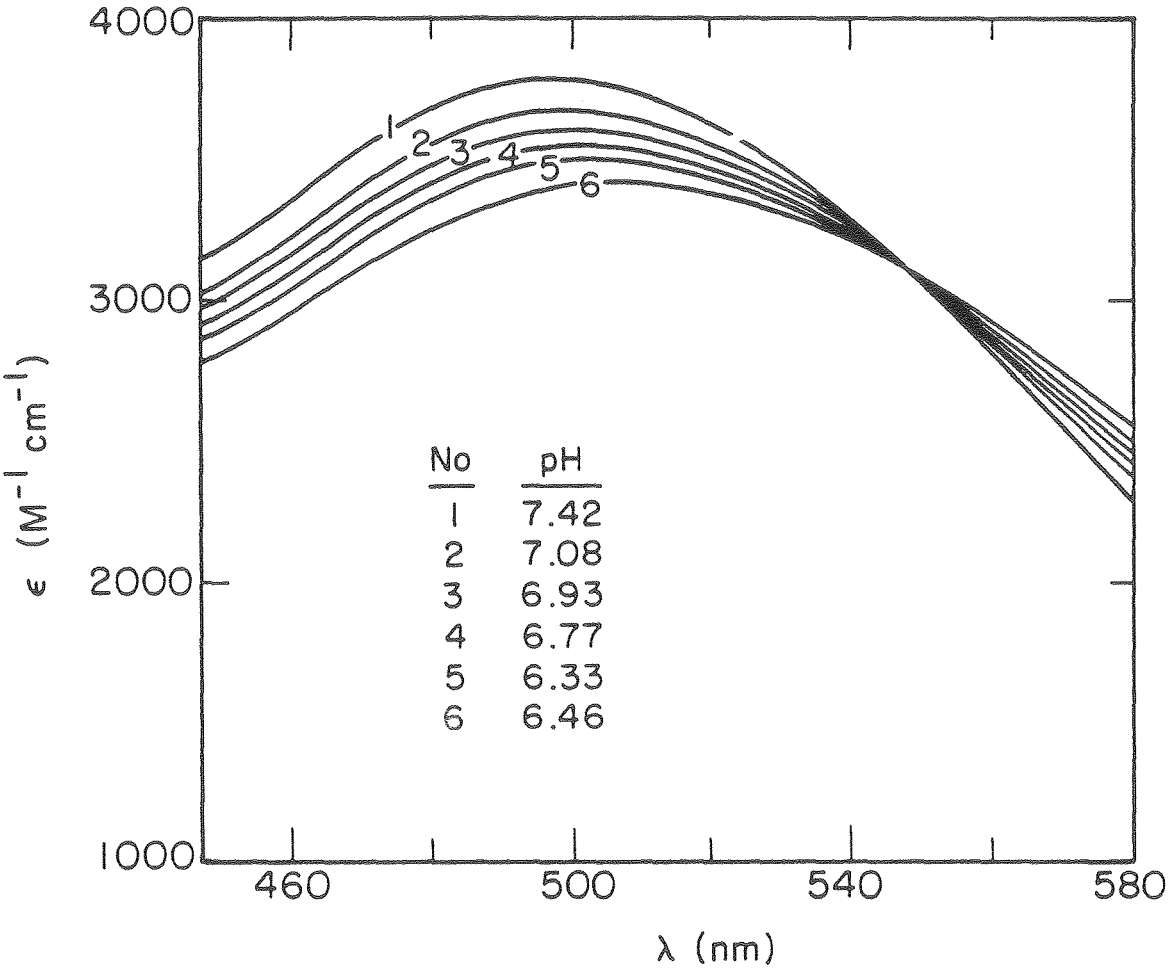
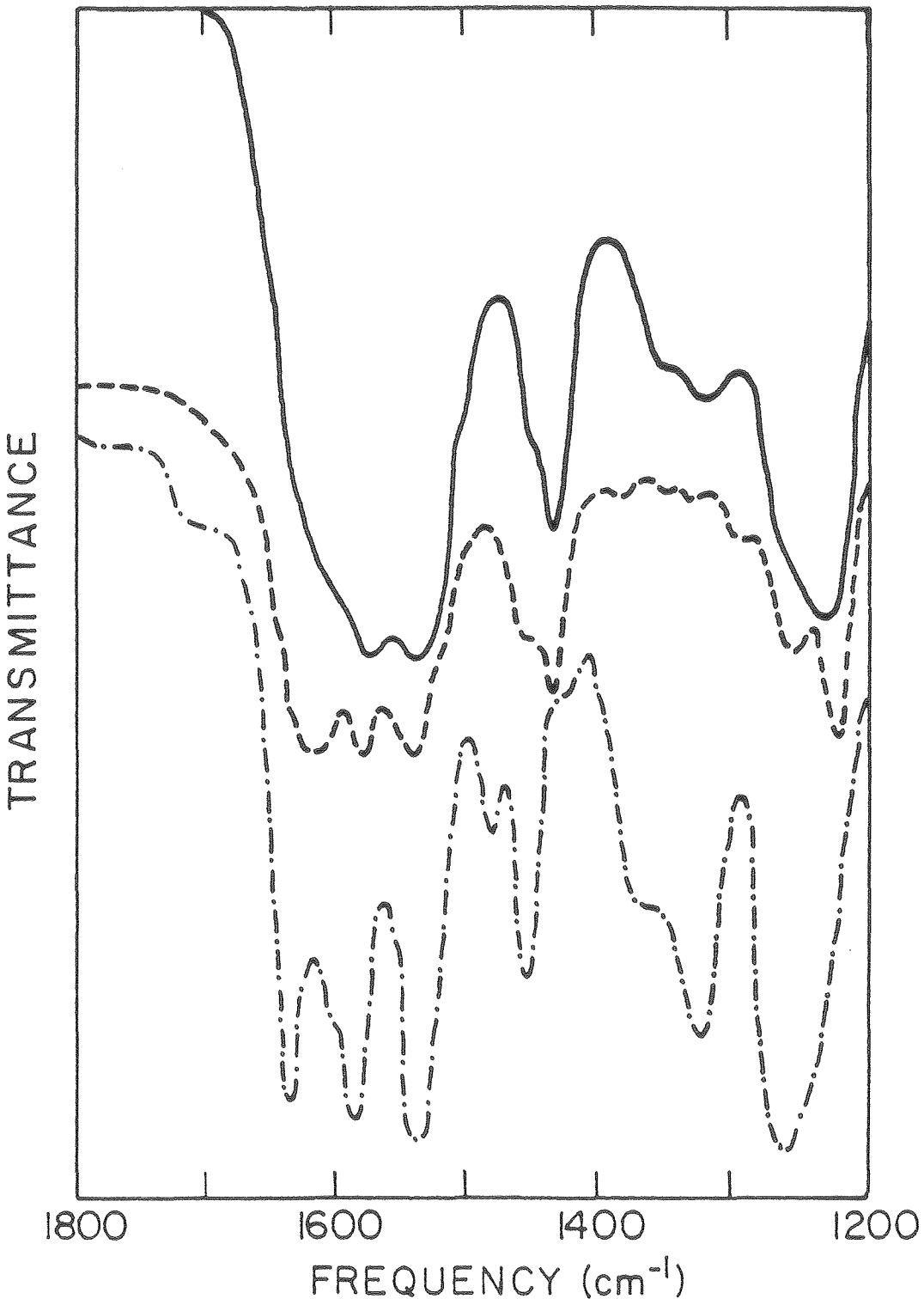

4000
3000
2000
1000
460
500
540
580
ε (M⁻¹ cm⁻¹)
λ (nm)
1
2
3
4
5
6
No pH
1 7.42
2 7.08
3 6.93
4 6.77
5 6.33
6 6.46

XBL 793-8735



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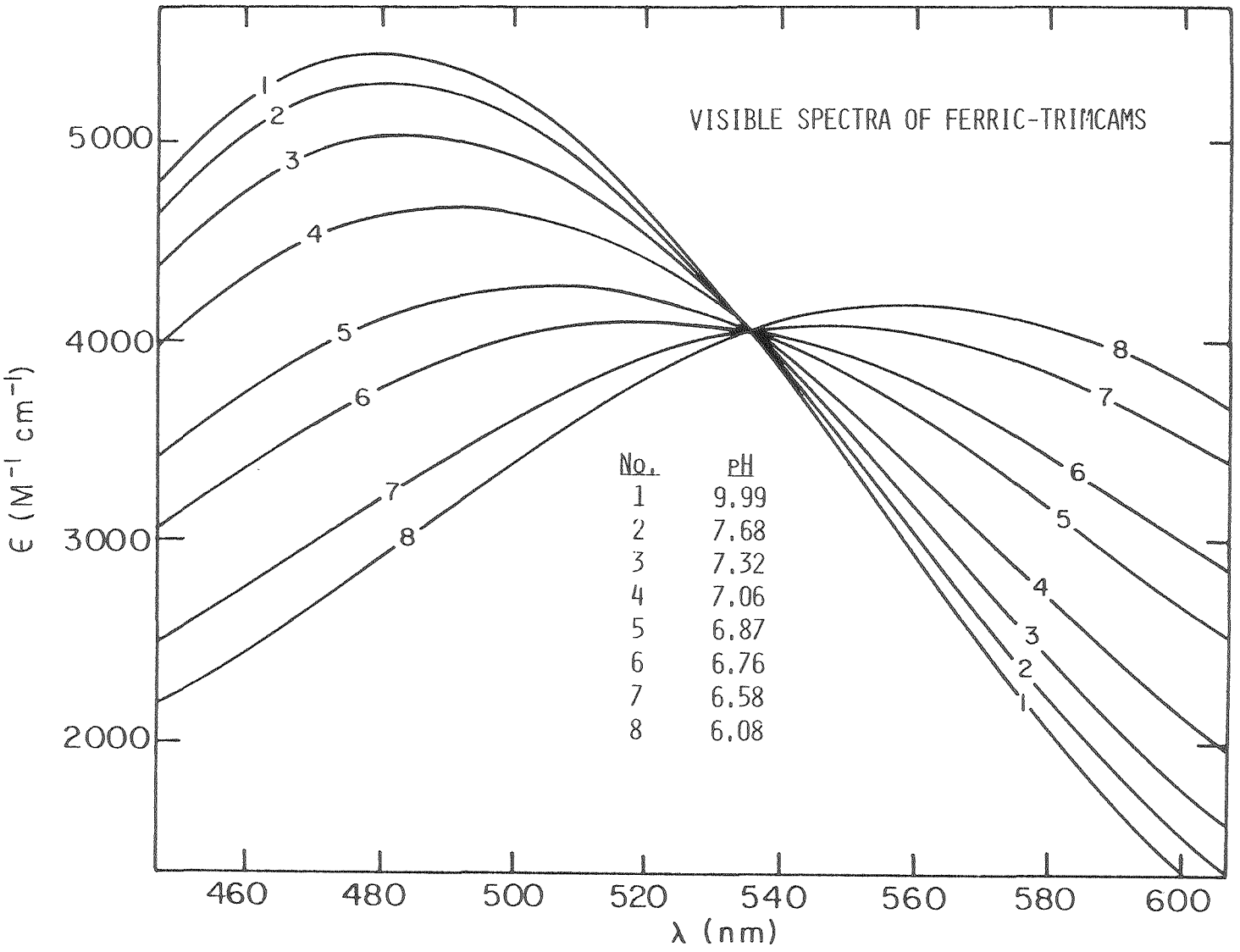

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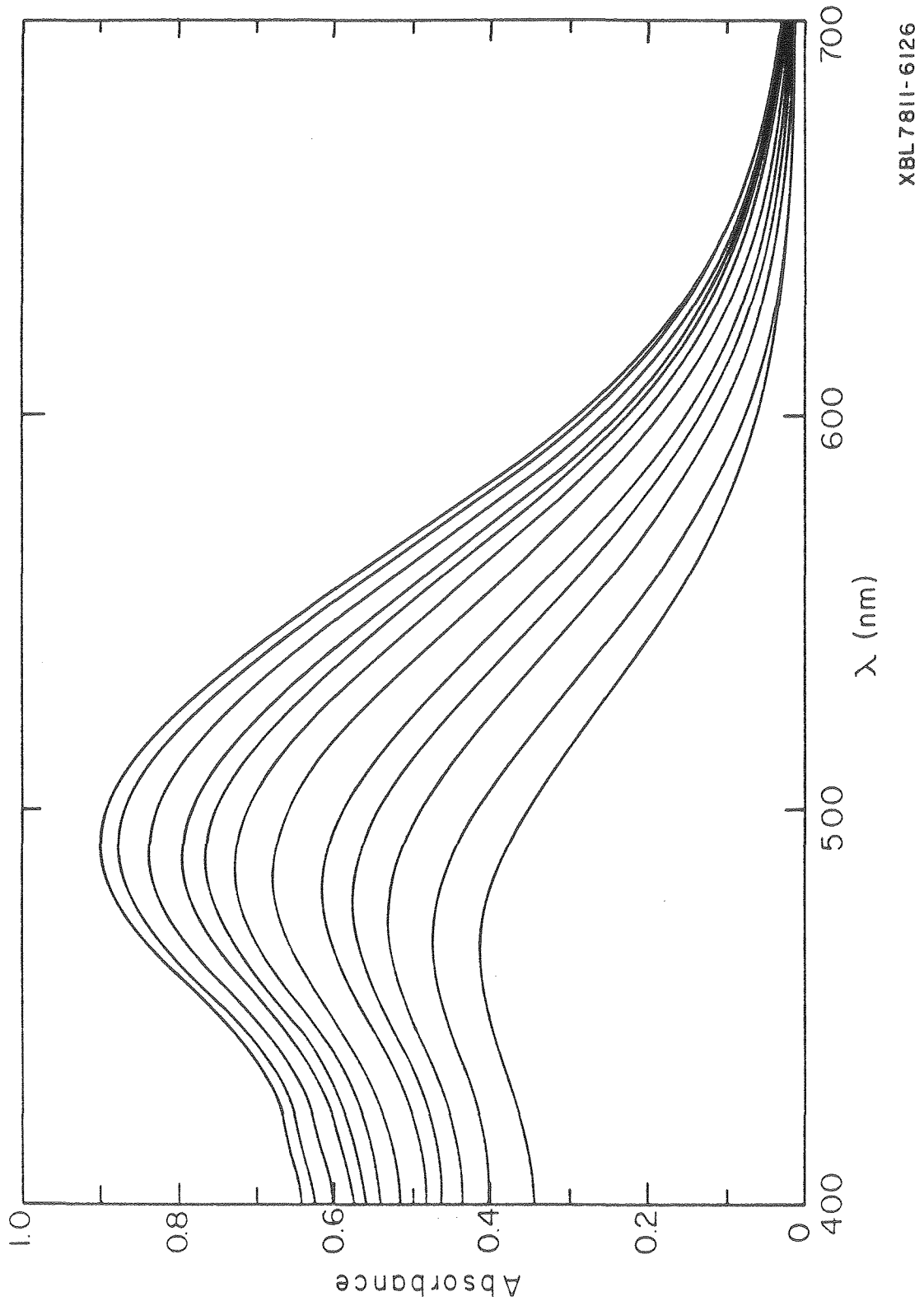
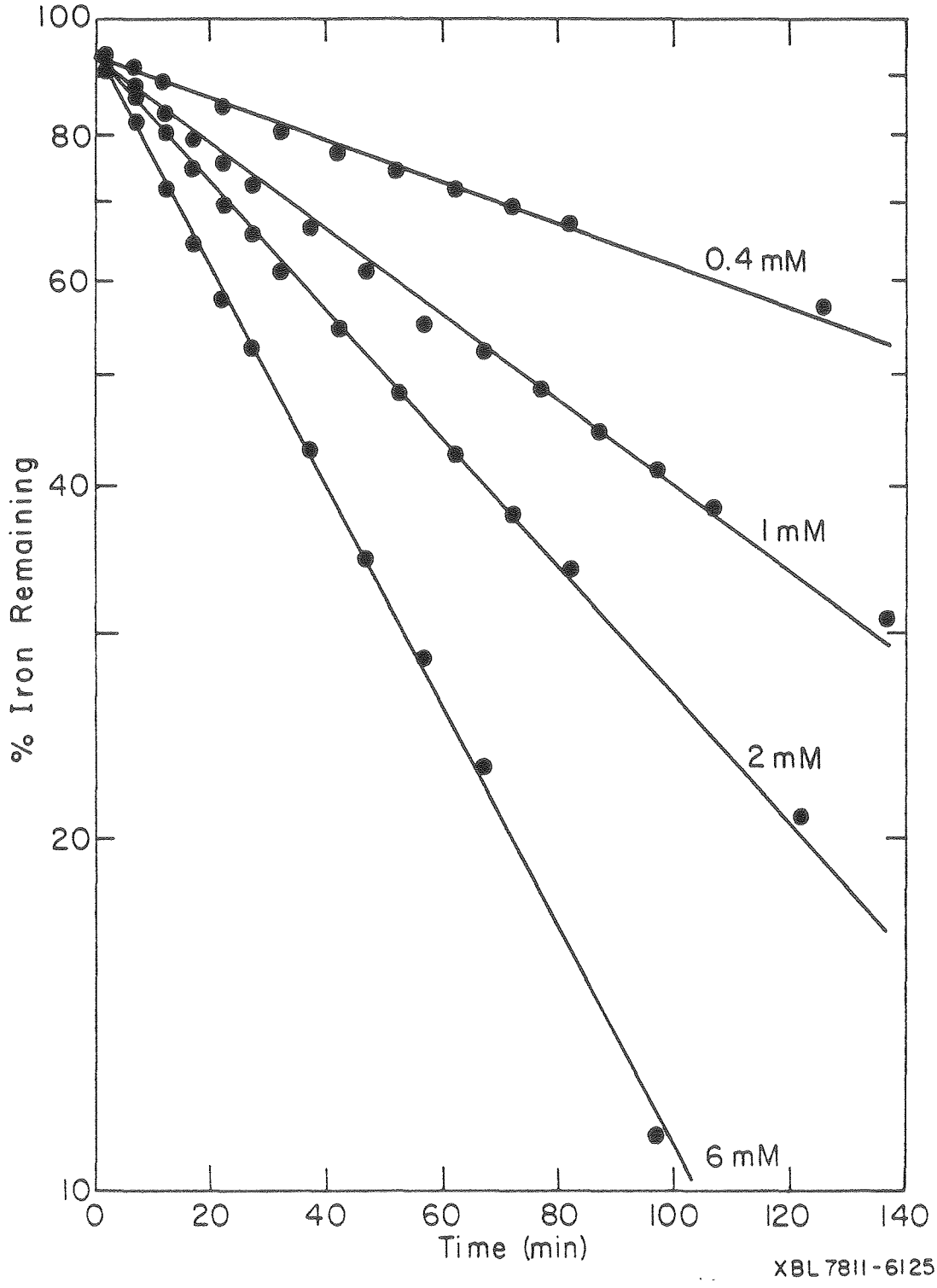
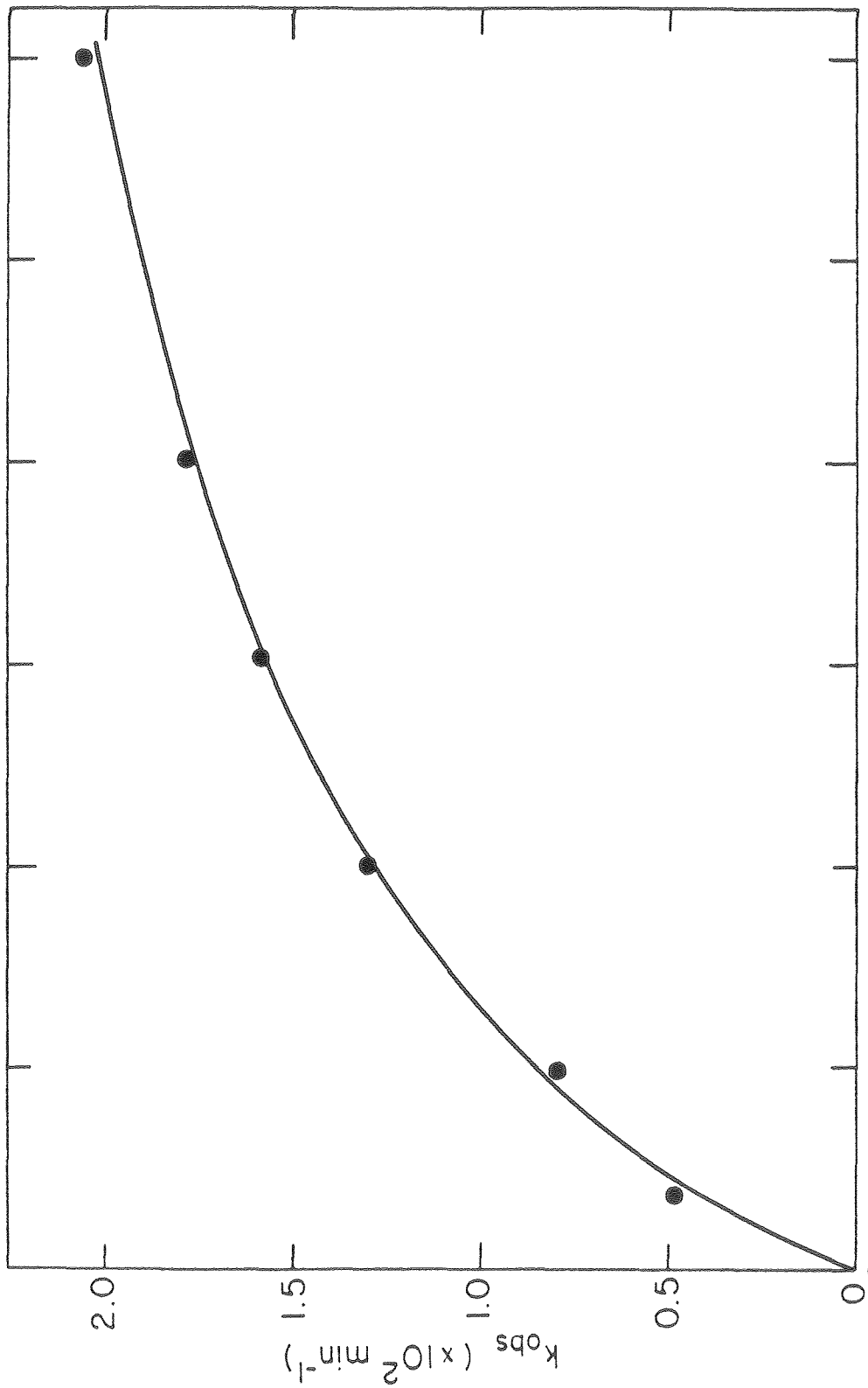
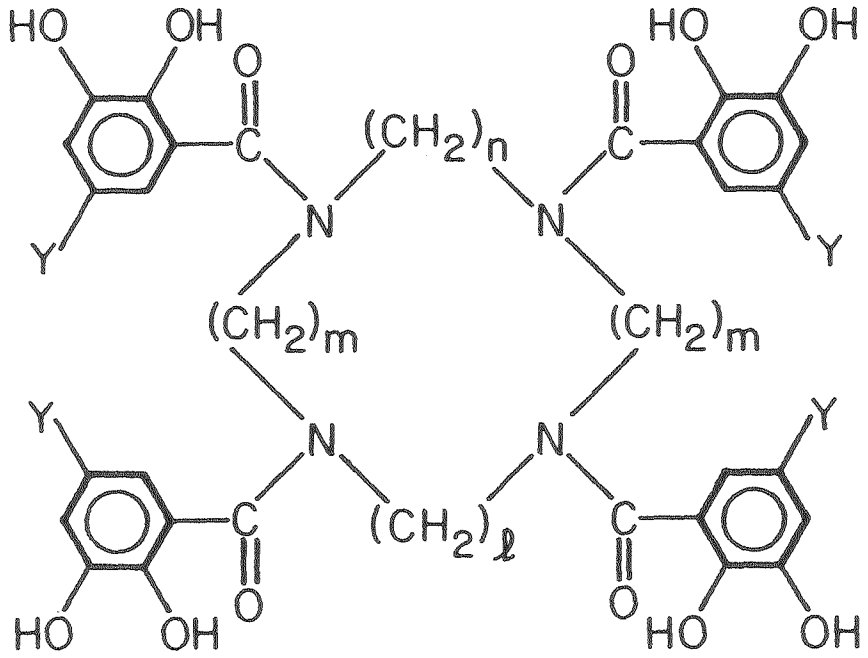

Absorbance
1.0
0.8
0.6
0.4
0.2
0
400
500
600
700
λ (nm)
XBL 7811-6126


100
80
60
40
20
10
% Iron Remaining
0.4 mM
1 mM
2 mM
6 mM
0
20
40
60
80
100
120
140
Time (min)
XBL 7811-6125



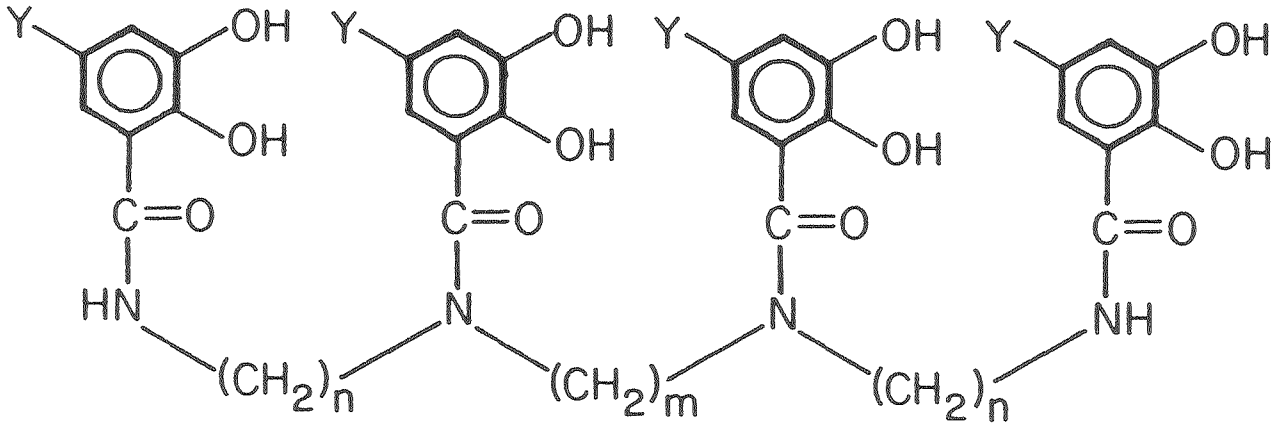
XBL 7811-6124



n = m = ℓ = 3; Y = H 3,3,3,3-CYCAM

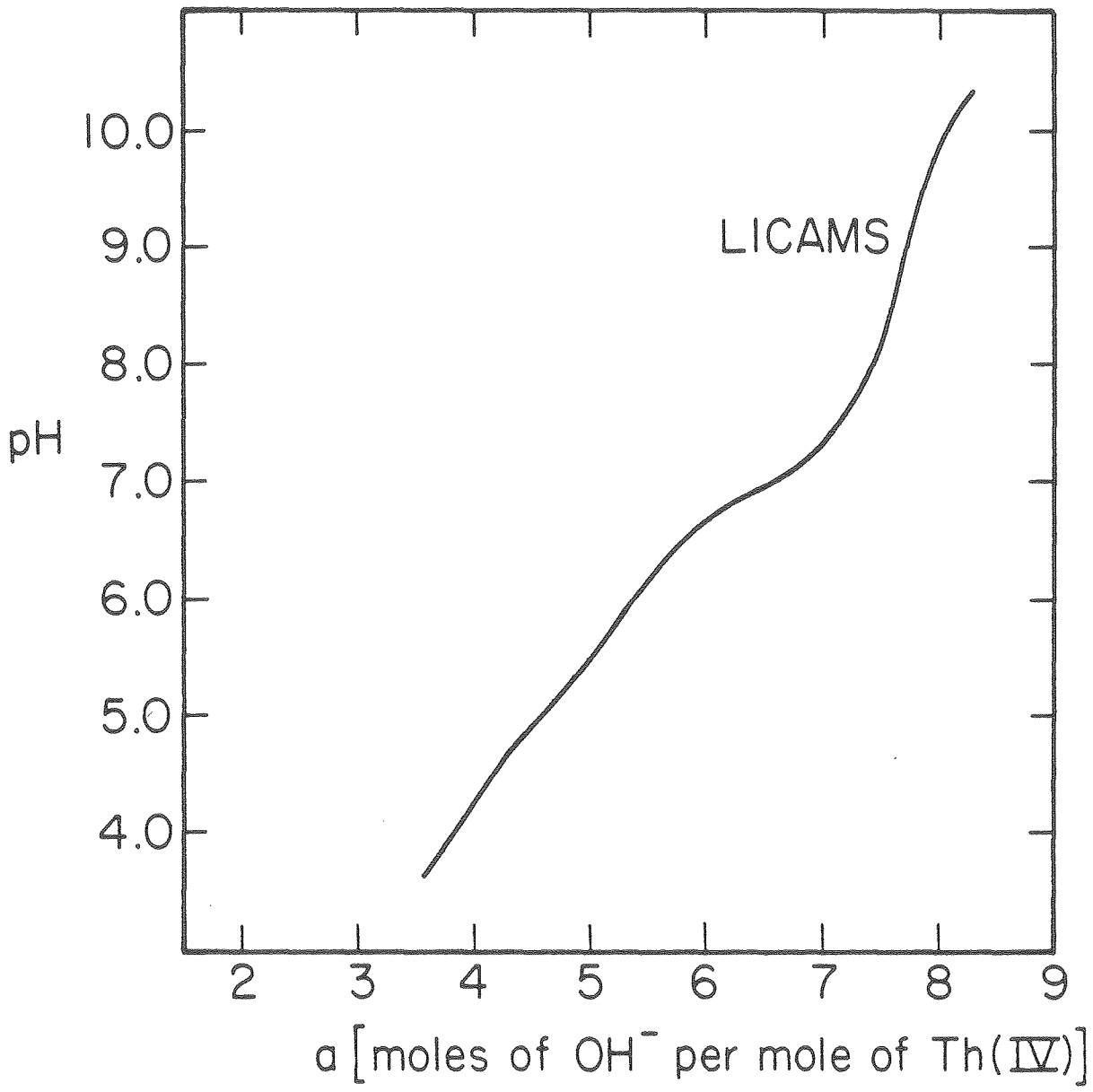
n = m = ℓ = 3; Y = SO_3Na 3,3,3,3-CYCAMS

XBL 7911-12704



n = m = 4; Y = H	4,4,4-CYCAM
n = m = 4; Y = SO_3Na	4,4,4-CYCAMS
n = 3; m = 4; Y = H	3,4,3-CYCAM
n = 3; m = 4; Y = SO_3NA	3,4,3-CYCAMS

XBL 7911-12703



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