nucleophilic reactivity of OH^- versus Cl^-.

The previously reported[1] rate of oxygen atom exchange between OCl^- and H_2O is not correct. We show that the rate is relatively rapid even in Cl^--free solutions with high OH^- concentrations. Our ^{17}O studies indicate that the exchange rate constant is greater than 5×10^{-3} s^{-1} and is less than 5×10^{2} s^{-1}. On the basis of a previous correlation[7] for rate constants of nucleophiles with HOCl, we predict a rate constant equal to approximately 4.5×10^{4} M^{-1} s^{-1} for the transfer of Cl^+ from HOCl to OH^-. This would correspond to an exchange rate constant of 2.7×10^{-2} s^{-1} for OCl^- and H_2O (a $t_{1/2}$ value of 25 s) that is independent of OH^- and Cl^- concentrations.

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Kinetic and Equilibrium Studies of the Complexation of Aqueous Iron(III) by Daunomycin, Quinizarin, and Quinizarin-2-sulfonate

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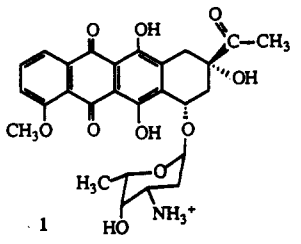
The equilibria and kinetics for the reaction of solvated iron(III) with daunomycin, quinizarin, and quinizarin-2-sulfonate have been studied in 0.01 and 0.06 M H^+, at 25 °C in 0.50 M $NaClO_4/HClO_4$ in water and 42.8% by volume methanol for quinizarin. The ligands (QzH_2) all have the 1,4-dihydroxyanthraquinone function, which is used to complex iron(III) to give $(H_2O)_4Fe^{III}(QzH)$ and $((H_2O)_4Fe^{III})_2(Qz)$ complexes from the analysis of the spectrophotometric equilibrium data with $[Fe(III) \gg [QzH_2]$. Stopped-flow kinetic studies indicate that the reaction is biphasic and this is attributed to successive formation of $(H_2O)_4Fe^{III}(QzH)$ and $((H_2O)_4Fe^{III})_2(Qz)$. The major reaction pathway for the two stages involves hydrolyzed iron(III) ($(H_2O)_5FeOH^{2+}$) and QzH_2 or $(H_2O)_4Fe^{III}(QzH)$, but the reaction of the bis(μ-hydroxo)iron(III) dimer with QzH_2 and $(H_2O)_4Fe^{III}(QzH)$ makes a significant contribution. Daunomycin is generally about 20 times slower to react than the other ligands. The rate constants for the various stages and paths are compared and discussed.

Introduction

This study was undertaken to investigate the kinetics of complexation of aqueous iron(III) by daunomycin (daunorubicine) (**1**). Daunomycin and the structurally similar adriamycin



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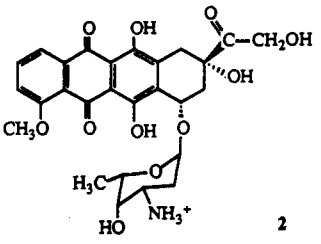

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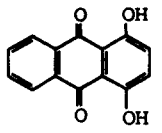

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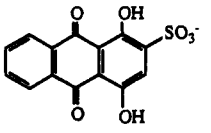

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(doxorubicin) (**2**) show outstanding anticancer potency although they have quite different ranges of application[1] and adriamycin has about twice the cardiotoxicity of daunomycin.[2,3] Zweier and co-workers[4-6] have observed that adriamycin is complexed and oxidized by aqueous iron(III) while daunomycin forms a complex with iron(III) but is not oxidized. Zweier suggested that the oxidation involves the ketol sidechain of adriamycin and that the toxicity is realted to radical products of the oxidation, while Gianni et al.[7] isolated the major oxidation products.

In order to provide kinetic background information, the functionally related quinizarin (1,4-dihydroxy-anthraquinone) (**3**) and quinizarin-2-sulfonate (**4**) also have been studied. Daunomycin has several potential sites for coordination of iron, but the hydroxyquinone function would be the expected site, and the behavior of daunomycin should parallel that of the simpler hydroxyquinones if this is true. Previous experience[8,9] has shown that a knowledge of the equilibrium constants for complex formation is very useful for the kinetic interpretation of such systems. Kiraly and Martin[10] reported the pK_a's and equilibrium constants for the 1:1 iron(III) complexes of quinizarin (in 50% ethanol) and daunomycin. The quinizarin-2-sulfonate ($QzSH_2^-$) system was studied spectrophotometrically by Thomson and Atkinson,[11] who concluded that there are $FeQzSH^+$ and $Fe_4(QzSH)_3^{6+}$ complexes, but the conclusions were critized by Budesinsky[12] because only one acidity was used. Preliminary spectrophotometric and kinetic studies indicated that these systems may not have been fully characterized, and new multiwavelength spectrophotometric equilibrium measurements are reported here.

Biochemical studies have concentrated on the tris(daunomycin) and tris(adriamycin) complexes of iron(III). The main question is whether the iron(III) complexes are strong enough to persist in the biological system at adventitious iron(III) concentrations (~ 2 μM) and in competition with transferrin ($K = 10^{31}$), but this work has largely ignored the oxidation of adriamycin. May et al.[13] studied adriamycin complexation potentiometrically and spectrophotometrically and reported $\beta_3 = 10^{33.4}$ M^{-3}. Beraldo et al.[14] critically discussed some of the work of Kiraly and Martin and May et al. and differences in the ligand pK_a values. Martin[15]

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has reviewed the results and suggests that the pK_a's of Beraldo et al.[14] are in error. Beraldo et al.[16] reported potentiometric results that gave $\beta_3 = 10^{28.4}$ M^{-3} for adriamycin and daunomycin. Neither May et al.[13] nor Beraldo et al.[16] reported the oxidation of adriamycin, nor did they correct for hydrolysis of iron(III). Gelvan and Sumuni[17] concluded that the tris(adriamycin) complex is not a colloidal aggregate, as suggested by Beraldo et al.,[16] and that it has an effective formation constant of $10^{16.2}$ M^{-3} in competition with iron(III) hydrolysis at pH 7.4. They agree with Kessel[18] that the complex is too weak to be the adriamycin carrier in serum where it must compete with transferin, although it might form in the intercellular environment. On the other hand, Loevstad[19] has reported that adriamycin takes up some iron from iron-saturated transferrin. Hannun et al.[20] found that the tris(adriamycin)iron(III) complex is a potent inhibitor of protein kinase *c*. In the present study, the formation constant of the mono(daunomycin)iron(III) and higher formation constants of other iron(III) complexes are used to estimate a value for β_3 for the tris(daunomycin) complex.

Results and Discussion

Because of the low solubility of quinizarin in water, this ligand was studied in 42.8% by volume methanol (mole fraction 0.25) in water at 0.50 M ionic strength ($NaClO_4/HClO_4$). This solvent was chosen because it provided adequate solubility and because the hydrolysis constant of $Fe(OH_2)_6^{3+}$ (eq 1) has been determined[21] as $K_m = 8.7 \times 10^{-3}$ M in this medium. The other two systems were studied in aqueous 0.50 M $NaClO_4/HClO_4$, where $K_m = 1.9 \times 10^{-3}$ M.[22]

$$Fe(OH_2)_6^{3+} \overset{K_m}{\rightleftharpoons} Fe(OH_2)_5(OH)^{2+} + H^+ \qquad (1)$$

The bis(μ-hydroxo)iron(III) dimer (eq 2) is also a species of potential importance under the conditions of our study. The

$$2Fe(OH_2)_6^{3+} \overset{K_D}{\rightleftharpoons} ((H_2O)_4Fe(OH))_2^{4+} + 2H^+ \qquad (2)$$

formation of this dimer has been studied[23] in ethanol–water mixtures, and the results give a value of $K_D = 3.7 \times 10^{-3}$ M (eq 2) in 60% by volume ethanol and 0.50 M $NaClO_4/HClO_4$ at 25 °C. In this medium, the value of K_m (7.3×10^{-3} M) is close to that in our methanol–water mixture, and the value of K_D in ethanol/water has been assumed for the latter medium. In aqueous 0.50 M $NaClO_4/HClO_4$, $K_D = 1.9 \times 10^{-3}$ M.[22] Although the individual hydrolyzed aquairon(III) species constitute less than 10% of the total iron, they can be kinetically important, and their formation contributes to the total acidity of the solutions. This effect has been included in the present analysis.

Spectrophotometric Equilibrium Measurements. In aqueous acid, the ligands studied here all show absorbance maxima in the 460–500-nm region of the visible spectrum. When iron(III) perchlorate is added to these solutions, a new peak appears in the 610–630-nm region. This observation is consistent with all the previous studies.[4,10,11] As the iron(III) concentration is increased, the ligand peak intensity changes very little and an isosbestic point is observed for [iron(III)]/[ligand] < 20 at ~485 nm for quinizarin and quinizarin-2-sulfonate and at 412 nm for daunomycin. Further addition of iron(III) causes a general increase in absorbance throughout the visible region and loss of the isosbestic behavior. There also is an increase in absorbance in the 300–400-nm region, which is associated with complex formation but also is caused by absorption of $Fe(OH_2)_6^{3+}$ and its hydrolysis products. Because of the latter complication, our quantitative observations have focused on the 600–700-nm region. Over periods of several hours, the iron(III)–quinizarin solutions show some fading of absorbance around 600 nm due to the formation of iron(II) (based on the characteristic red color with 1,10-phenanthroline). This redox process has not been studied, and the equilibrium measurements here refer to the solutions within a few minutes after mixing.

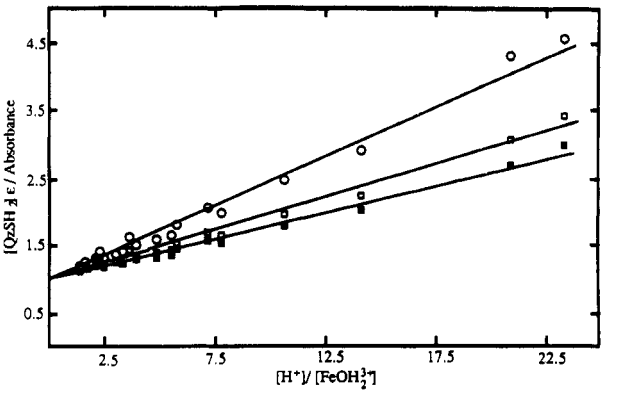

Figure 1. Spectrophotometric results for the determination of the formation constant in the aquairon(III)–quinizarin-3-sulfonate system at 670 (○), 630 (□) and 610 nm (■). Lines are best fits to a one-species model and should have the same slope if the model is valid. The data shown represents one-third of the total data set, and some points near the intercept have been omitted for clarity.

In order to determine the chemical species present at equilibrium, the absorbance was determined for solutions of each of the ligands with various concentrations of iron(III) ($[Fe(III)]_{tot} \gg$ [ligand]) and H^+. In the preliminary analysis, a single complex was assumed with a formation constant K_{f1} defined by eq 3, where

$$Fe(OH_2)_6^{3+} + QzH_2^n \overset{K_{f1}}{\rightleftharpoons} (H_2O)_4Fe(QzH)^{(2+n)+} + H^+ \qquad (3)$$

QzH_2^n represents the protonated form of the ligands **1**, **3**, or **4**, with $n = +1$, 0, and −1, respectively.

In all cases, a least-squares analysis of the absorbance–concentration data according to the predictions of eq 3 gave values of K_{f1} which varied systematically with the observation wavelength. Some representative results for the iron(III)–quinizarin-2-sulfonate ($QzSH_2^-$) system are shown in Figure 1. For the experimental conditions of [iron(III)] $\gg$ $[QzSH_2^-]_{tot}$, the model from eq 1 predicts that the variation of absorbance with reagent concentrations should be given by eq 4, where l (cm) is the cell path

$$\frac{\text{absorbance}}{l[QzSH_2]_{tot}} = \frac{\epsilon_1 K_{f1}[FeOH_2^{3+}]}{[H^+] + K_{f1}[FeOH_2^{3+}]} \qquad (4)$$

length, ϵ_1 (M^{-1} cm^{-1}) is the molar absorptivity of the product complex (the only absorbing species at the observation wavelengths), and $FeOH_2^{3+}$ represents $Fe(OH_2)_6^{3+}$ calculated from the total iron(III) and appropriate hydrolysis constants. This equation can be rearranged to eq 5, which predicts that the plots

$$\frac{l\epsilon_1[QzSH_2]_{tot}}{\text{absorbance}} = \left(\frac{1}{K_{f1}}\right)\left(\frac{[H^+]}{[FeOH_2^{3+}]}\right) + 1 \qquad (5)$$

in Figure 1 should be linear with an intercept of unity and the same slope for different wavelengths. Clearly the latter condition is not satisfied. Fits of the absorbance–concentration data to the single species model (eq 3) give wavelength-dependent values for K_{f1} of 7.6 ± 0.5, 11.3 ± 0.6 and 13.7 ± 0.7 at 670, 630, and 610 nm, respectively, as indicated by the different slopes of the lines in Figure 1.

The quinizarin system shows similar behavior with values of K_{f1} of 56 ± 4, 74 ± 5, and 78 ± 5 at 670, 630, and 610 nm,

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Table I. Summary of the Spectrophotometric Results for Iron(III) Complex Formation in Aqueous 0.50 M $NaClO_4/HClO_4$ at 25 °C

	ligand		
	quinizarin[a]	quinizarin-2-sulfonate	daunomycin
K_{f1}	45.7 ± 4.6	19.5 ± 2.3	23.1 ± 2.8
K_{f2}	36.3 ± 1.7	8.2 ± 0.6	2.0 ± 1.2
$10^{-3}\epsilon_1(610)$[b]	5.35	0.688	11.4
$10^{-3}\epsilon_2(610)$[b]	5.09	1.32	14.5
$10^{-3}\epsilon_1(630)$[b]	5.04	0.540	10.9
$10^{-3}\epsilon_2(630)$[b]	4.96	1.11	14.9
$10^{1-3}\epsilon_1(670)$[b]	3.34	0.242	7.69
$10^{1-3}\epsilon_2(670)$[b]	4.24	0.930	14.9
$10^{-3}\epsilon_{ligand}$ (λ_{max})	7.63 (472)	8.80 (462)	10.6 (480)[c,d]

[a] In 42.8% by volume of methanol in water. [b] Molar absorptivity in M^{-1} cm^{-1} and wavelength in nm; ϵ_1 is for the monoiron complex and ϵ_2 is for the diiron complex. [c] At 25 °C in 0.01 M $HClO_4$ and 0.50 M $NaClO_4$. [d] A value of 11.4 × 10^3 M^{-1} cm^{-1} at 470 nm in water has been reported: Gabbay, E. F.; Grier, D.; Fingerle, R. E.; Reimer, R.; Levey, R.; Pearce, S. W.; Wilson, W. D. *Biochemistry* **1976**, *15*, 2062.

respectively, for the one species model. The iron(III)–daunomycin system gives values for K_{f1} of 7.13, 10.4, and 11.2 at 670, 630, and 610 nm respectively. If the self-association of daunomycin is taken into account with a dimerization constant[10] of 1 × 10^4 M^{-1}, then the K_{f1} values are 13.7 ± 0.5, 18.8 ± 0.6 and 20.0 ± 0.6, respectively. The variation of K_{f1} with the observation wavelength indicates that eq 3 is not an adequate description of the system, although the fits at any given wavelength were satisfactory, as indicated by the standard errors. This conclusion is consistent with the observed loss of isosbestic behavior at higher iron(III) concentrations.

Since eq 3 will not explain the observations, the model was expanded to include the diiron complex as described in eq 6. The

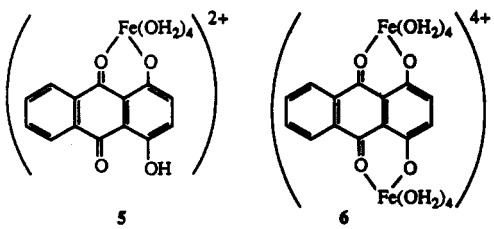
$$(H_2O)_4Fe(QzH)^{(2+z)+} + Fe(OH_2)_6^{3+} \overset{K_{f2}}{\rightleftharpoons} (H_2O)_4Fe(Qz)Fe(OH_2)_4^{(4+z)+} + H^+ \quad (6)$$

choice of this model was suggested in part by the kinetic observations discussed below. The combination of eqs 3 and 6 predicts that the absorbance should be described by eq 7, where ϵ_2 is the

$$\frac{\text{absorbance}}{l[\text{ligand}]_{tot}} = \frac{\epsilon_1 K_{f1}[H^+][FeOH_2^{3+}] + \epsilon_2 K_{f1}K_{f2}[FeOH_2^{3+}]^2}{[H^+]^2 + K_{f1}[H^+][FeOH_2^{3+}] + K_{f1}K_{f2}[FeOH_2^{3+}]^2} \quad (7)$$

molar absorptivity of the diiron(III) complex, and the iron(III) and H^+ concentrations are corrected as described for eq 4. Least-squares fits with the two species model (eq 7) for the data at all wavelengths give a good fit for all the ligands. The results are summarized in Table I.

The general spectrophotometric similarity of all of these systems is consistent with complexation involving the hydroxy and quinone oxygen functions in daunomycin. The most probable structures for the two complexes for quinizarin are shown by **5** and **6**, with



5 **6**

analogous structures for the other two ligands. A similar diiron(III)–quinizarin complex has been structurally characterized by Maroney et al.[24] for the Fe^{III}(salen) system.

(24) Maroney, M. J.; Day, R. O.; Psyris, T.; Fleury, L. M.; Whitehead, J. P. *Inorg. Chem.* **1989**, *28*, 173.

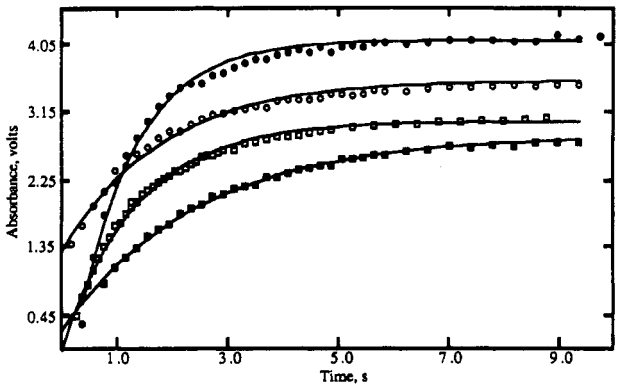
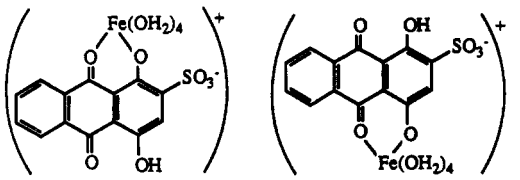


Figure 2. Variation of absorbance with time for the reaction of iron(III) and 3.9 × 10^{-5} M quinizarin in 42.8% methanol, 0.50 M ionic strength: 4.36 × 10^{-3} M iron(III), and 1.36 × 10^{-2} M $HClO_4$ observed at 610 nm (●), k = 0.98 s^{-1}, and 670 nm (○), k = 0.575 s^{-1}; 1.31 × 10^{-2} M iron(III), 8.00 × 10^{-2} M $HClO_4$ observed at 610 nm (□), k = 0.752 s^{-1}, and 670 nm (■), k = 0.402 s^{-1}. The curves are best fits to a first-order rate law, and k is the apparent first-order rate constant.

The values of K_{f1} and K_{f2} are more similar to each other for quinizarin than for the other two ligands as might be expected because the two complexing sites are equivalent in quinizarin. With quinizarin-2-sulfonate, the $-SO_3^-$ group makes the two sites nonequivalent, and there is the possibility of forming two monoiron(III) isomers (**7** and **8**). Analogous isomers are possible



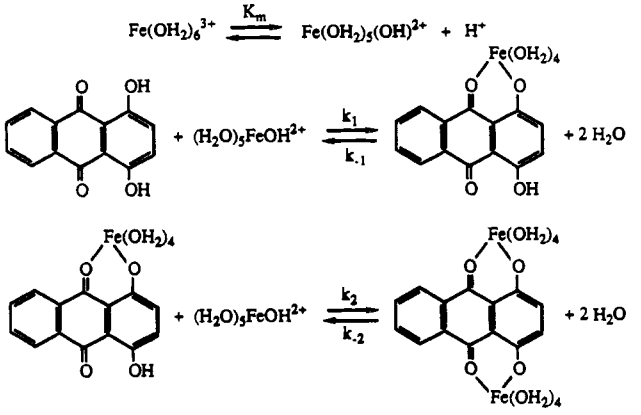
7 **8**

with daunomycin. Our data give the sum of the formation constants for these species (K_{f17} + K_{f18}). It might be argued that the added negative charge favors complexation adjacent to the $-SO_3^-$ substituent or that steric effects disfavor this site. There do not seem to be simple analogous systems which can be used to assess the probable effect of the sulfonate group. In daunomycin, the steric effect would seem to favor initial complexation remote from the amino–sugar substituent, and this is consistent with the larger difference between K_{f1} and K_{f2} in this system.

The results of Kiraly and Martin[10] give a value of K_{f1} = 10 for quinizarin (based on H^+ activity, at ~20 °C, in 50% ethanol, 0.15 M ionic strength, salt unspecified). If the salt is KNO_3, often used in Martin's laboratory, then one can estimate, from the solvent effect on Cl^- complexing,[20,22] that about 50% of the iron(III) is present as the nitrato complex. Their higher acidity (pH 0.46 and 1.02) and single wavelength (560 nm) would limit the possibility of determining K_{f2} for iron(III), and these factors, in addition to differences in conditions, may account for the discrepancy in the results. Kiraly and Martin found K_{f1} = 12.6 for daunomycin (at ~20 °C in water, 0.15 M ionic strength, pH 0.7, corrected for daunomycin dimerization) which is in better agreement with our value but is subject to the same limitations. Thomson and Atkinson[11] obtained K_{f1} = 30 for quinizarin-2-sulfonate and $\epsilon_1 \approx 500$ M^{-1} cm^{-1} at 600 nm (25 °C, 0.1 M $HClO_4$), both in reasonable agreement with our value of 19 and 688 M^{-1} cm^{-1} at 610 nm. The difference in this case may be due to the assignment of the second complex as $Fe_4(QzSH)_3$ by Thomson and Atkinson.

Stopped-Flow Studies. The time dependence of the absorbance in the 600–700-nm region has been used to study the kinetics of complex formation under conditions similar to those of the equilibrium studies ([Fe(III)] ≫ [ligand]). For all of these ligands, the absorbance change does not strictly obey a simple exponential first-order rate law. In addition, the rate of the absorbance change is larger when observed at 610 nm than when

Scheme I



observed at 670 nm. Some typical results for quinizarin are shown in Figure 2, where the curves represent the best fit to a first-order law. These small diagrams do not fully expose the nonexponential behavior, but the curves are persistently below the data near the middle of the change and above the data in the 70–90% region of the reaction. The wavelength dependence of the apparent rate is quite clear from the results in Figure 2.

The simplest explanation for the wavelength dependence of the apparent rate constant is that two processes are being observed. This also is consistent with the persistent deviations from a simple exponential dependence of the absorbance change. For quinizarin and quinizarin-2-sulfonate, these deviations generally are not large enough to allow two rate constants to be determined from observations at one wavelength. In order to determine the two rate constants needed to describe the system, parallel runs under identical concentration conditions at 610 and 670 nm have been fitted simultaneously to a two-exponential equation[25] to provide wavelength independent first-order rate constants, γ_1 and γ_2. The absorbance–time curves for the reaction of daunomycin at 610 nm were clearly not simple exponential curves, and the single wavelength data were fitted to a biphasic model[25] to determine γ_1 and γ_2.

The kinetic observations and the equilibrium study would be consistent with the reaction sequence shown in Scheme I, where charges are omitted to preserve generality. This scheme has been simplified in anticipation of the detailed analysis described below. No evidence has been found for a contribution from the reaction of $Fe(OH_2)_6^{3+}$ with QzH_2, and rate constant arguments presented later indicate that $Fe(OH_2)_6^{3+} + QzH^-$ is not contributing because of the small K_a of all the ligands, although it is the kinetic equivalent of the pathway shown in Scheme I.

The model in Scheme I predicts that γ_1 and γ_2 should be given by eq 8, where $k'_1 = k_1K_m[FeOH_2^{3+}]/[H^+]$, $k'_2 = k_2K_m$-

$$\gamma_{1,2} = \{(k'_1 + k'_2 + k_{-1} + k_{-2}) \pm [(k'_1 + k'_2 + k_{-1} + k_{-2})^2 - 4(k'_1k'_2 + k'_1k_2 + k_{-1}k_{-2})]^{1/2}\}/2 \quad (8)$$

$[FeOH_2^{3+}]/[H^+]$ since $[FeOH^{2+}] = K_m[FeOH_2^{3+}]/[H^+]$ and $[FeOH_2^{3+}]$ and $[H^+]$ are the concentrations of $Fe(OH_2)_6^{3+}$ and H^+ after correction for hydrolysis and dimerization. If the reverse rate constants are substituted by $k_{-1} = k_1K_m/K_{f1}$ and $k_{-2} = k_2K_m/K_{f2}$, then eq 8 predicts that the sum of the apparent pseudo-first-order rate constants will be given by eq 9.

$$\gamma_1 + \gamma_2 = (k_1K_m + k_2K_m)\frac{[FeOH_2^{3+}]}{[H^+]} + \left(\frac{k_1K_m}{K_{f1}} + \frac{k_2K_m}{K_{f2}}\right) \quad (9)$$

The plots in Figure 3 show that $\gamma_1 + \gamma_2$ appears to have the dependence on $[FeOH_2^{3+}]/[H^+]$ predicted by eq 9. If the K_{fi} are taken as knowns, then the data can be fitted to eq 9 with two parameters, k_1 and k_2, and the best-fit lines for this model are

(25) The data were fitted to the equation $A_t = A_\infty + A_1e^{-\gamma_1 t} + A_2e^{-\gamma_2 t}$ with A_∞, A_1, A_2, γ_1, and γ_2 as parameters.

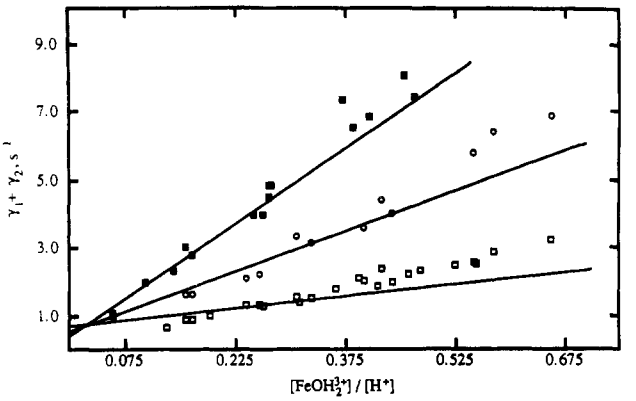


Figure 3. Variation of the sum of the experimental rate constants with $FeOH_2^{3+}$ and H^+ concentrations as predicted by eq 9: quinizarin in 42.8% methanol (×1.5) (■); quinizarin-2-sulfonate in water (○); daunomycin in water (×10) (□). All the data are obtained at 25 °C and 0.50 M ionic strength controlled with $NaClO_4/HClO_4$.

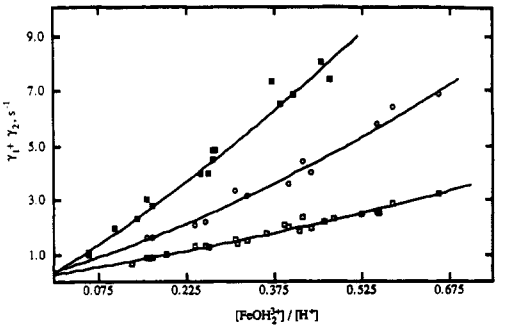
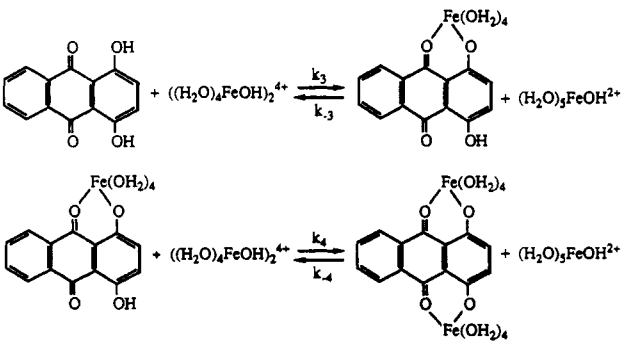


Figure 4. Variation of the sum of the experimental rate constants with $FeOH_2^{3+}$ and H^+ concentrations as predicted by eq 10: quinizarin in 42.8% methanol (×1.5) (■); quinizarin-2-sulfonate in water (○); daunomycin in water (×10) (□). All the data are obtained at 25 °C and 0.50 M ionic strength controlled with $NaClO_4/HClO_4$.

Scheme II



shown in Figure 3. It is apparent that the fit is moderately successful for quinizarin, but there are substantial systematic deviations for both quinizarin-2-sulfonate and daunomycin. The problem is that the data require a steeper slope (larger k_1 and/or k_2), but this also causes the intercept to increase and the best fits are a compromise between these two effects. Stated in another way, the kinetic data require much larger values of the K_{fi} to decrease the intercept and give a satisfactory fit. The K_{fi} values must increase about 2.5 times to give reasonable fits to this model. It seems more probable that our K_{fi} values might be too large because our equilibrium model is a minimal one and the inclusion of other species would reduce K_{f1} and K_{f2}.

The simplest explanation which we have found to account for the above inconsistency is the involvement of the bis(μ-hydroxo)iron(III) species in the complex formation reactions. This species is known to be present in solutions in the acidity and iron(III) concentration ranges used in this study. If the model is expanded by the addition of the reactions in Scheme II to those in Scheme I, then the values of γ_1 and γ_2 can be fitted to the

Table II. Results of Dual Wavelength Fits to a Two-Exponential Model for the Reaction of Iron(III) with Quinizarin in 42.8% by Volume Methanol at 25 °C in 0.50 M $NaClO_4/HClO_4$

$10^2[Fe(III)]_{tot}$, M	$10^2[H^+]$, M		γ_1, s^{-1}		γ_2, s^{-1}	
	init.	final[a]	obsd	calcd[b]	obsd	calcd[b]
0.436	1.36	1.52	1.62	1.30	0.40	0.42
0.872	1.36	1.66	2.40	2.20	0.82	0.77
1.31	1.36	1.79	3.68	3.00	1.20	1.10
2.18	2.26	2.79	3.98	4.05	1.37	1.41
0.436	3.00	3.10	0.93	0.90	0.37	0.27
1.31	3.00	3.28	2.20	2.18	0.97	0.77
1.31	3.00	3.28	2.47	2.18	0.75	0.77
2.18	3.00	3.45	3.20	3.34	1.35	1.24
1.73	5.16	5.41	1.80	2.02	0.85	0.70
0.436	6.45	6.50	0.495	0.58	0.16	0.16
0.436	6.45	6.50	0.57	0.58	0.15	0.16
1.31	6.45	6.61	1.47	1.37	0.39	0.45
2.18	6.45	6.71	1.83	2.13	0.80	0.74
3.43	6.45	6.85	3.38	3.17	0.97	1.17
4.28	6.45	6.94	3.40	3.92	1.54	1.48
1.31	8.00	8.13	1.20	1.17	0.34	0.37

[a] Initial $[H^+]$ corrected for H^+ from formation of $Fe(OH)^{2+}$ and $(FeOH)_2^{4+}$. [b] Calculated from least-squares fit to eq 10.

Table III. Results of Dual Wavelength Fits to a Two-Exponential Model for the Reaction of Iron(III) with Quinizarin-2-sulfonate at 25 °C in Aqueous 0.50 M $NaClO_4/HClO_4$

$10^2[Fe(III)]_{tot}$, M	$10^2[H^+]$, M		γ_1, s^{-1}		γ_2, s^{-1}	
	init.	final[a]	obsd	calcd[b]	obsd	calcd[b]
1.31	1.36	1.62	5.08	4.66	1.30	1.15
0.872	1.36	1.54	3.53	3.31	0.851	0.759
0.436	1.36	1.48	1.70	1.84	0.38	0.376
2.81	2.26	2.58	5.48	5.50	1.40	1.41
1.74	2.26	2.50	4.63	4.49	1.12	1.11
1.31	2.26	2.42	3.28	3.46	0.706	0.807
0.872	2.26	2.36	2.66	2.38	0.654	0.513
0.436	2.26	2.30	1.34	1.31	0.267	0.256
3.49	8.00	8.13	2.84	3.18	0.721	0.734
2.83	8.00	8.10	2.60	2.56	0.526	0.563
2.18	8.00	8.07	1.85	1.98	0.353	0.412
1.31	8.00	8.04	1.35	1.25	0.252	0.243

[a] Initial $[H^+]$ corrected for H^+ from formation of $Fe(OH)^{2+}$ and $(FeOH)_2^{4+}$. [b] Calculated from least-squares fit to eq 10.

predicted rate law with K_{f1} and K_{f2} fixed at the values from the equilibrium study.

The combination of Schemes I and II predicts that $\gamma_1 + \gamma_2$ is given by eq 10, where $FeOH_2$ and $(FeOH)_2$ represent $Fe(OH_2)_6^{3+}$

$$\gamma_1 + \gamma_2 = k_1K_m\frac{[FeOH_2]}{[H^+]} + k_3[(FeOH)_2] + k_2K_m\frac{[FeOH_2]}{[H^+]} + k_4[(FeOH)_2] + \frac{\left(k_1K_m + k_3K_D\frac{[FeOH_2]}{[H^+]}\right)}{K_{f1}} + \frac{\left(k_2K_m + k_4K_D\frac{[FeOH_2]}{[H^+]}\right)}{K_{f2}} \quad (10)$$

and $((H_2O)_4FeOH)_2^{4+}$ respectively. The results of least-squares analysis with this model are shown in Figure 4, and the observed and calculated values of γ_1 and γ_2 for the different systems are given in Tables II–IV. The kinetic parameters are summarized in Table V.

Summary and Conclusions

The value of K_{f1} for daunomycin can be combined with the pK_a[15] of ~10 to give the conventional formation constant $\beta_1 = [FeQzH]/[FeOH_2][QzH] = 10^{11.4}$ M. Because of the possible biological relevance, it is of interest to estimate β_3 for daunomycin from its β_1, β_2, and β_3 of reasonable models. The model ligands 8-hydroxyquinoline and its 5-sulfonate have pK_a's in the same range as daunomycin (9.6 and 8.4, respectively), the same charge type, and fairly similar structure at the coordination site. The models suggest[26] that $\beta_2 = 10^{12}\beta_1$ and $\beta_3 \approx 10^{10}\beta_2$, so that one can estimate that $\beta_3 \approx 10^{33}$ M^{-3} for daunomycin. It seems likely that β_3 for adriamycin would be of similar magnitude since the β_1 values are similar for the different ligands studied here.

Table IV. Results of Fits to a Two-Exponential Model for the Reaction of Iron(III) with Daunomycin at 25 °C in Aqueous 0.50 M $NaClO_4/HClO_4$

$10^2[Fe(III)]_{tot}$, M	$10^2[H^+]$, M		γ_1, s^{-1}		γ_2, s^{-1}	
	init.	final[a]	obsd	calcd[b]	obsd	calcd[b]
1.31	1.36	1.75	0.226	0.220	0.060	0.0546
0.872	1.37	1.61	0.185	0.159	0.050	0.0403
0.436	1.36	1.48	0.101	0.0907	0.0302	0.0255
2.18	2.26	2.74	0.261	0.256	0.0587	0.0640
1.74	2.26	2.63	0.206	0.212	0.0483	0.0529
1.31	2.26	2.53	0.157	0.165	0.0409	0.0420
0.872	2.26	2.43	0.115	0.116	0.0390	0.0309
0.436	2.26	2.33	0.0656	0.0654	0.0218	0.0206
2.18	3.00	3.39	0.203	0.214	0.0480	0.0537
1.74	3.00	3.30	0.176	0.175	0.0433	0.0443
1.31	3.00	3.21	0.137	0.136	0.0390	0.0352
0.436	3.00	3.06	0.0504	0.0538	0.0142	0.0185
2.83	4.05	4.86	0.199	0.203	0.0476	0.0511
2.18	4.50	4.76	0.146	0.158	0.0390	0.0404
1.31	4.50	4.64	0.0980	0.0992	0.0270	0.0273
3.49	6.50	6.82	0.187	0.183	0.0452	0.0464
2.83	6.50	6.74	0.161	0.149	0.0475	0.0383
2.18	6.50	6.67	0.109	0.116	0.0300	0.0312
1.31	6.50	6.59	0.0742	0.0734	0.0230	0.0222
3.49	8.00	8.25	0.159	0.152	0.0404	0.0391
2.83	8.00	8.19	0.119	0.124	0.0307	0.0327
2.18	8.00	8.13	0.101	0.0972	0.0283	0.0269
1.31	8.00	8.07	0.0636	0.0624	0.0220	0.0201

[a] Initial $[H^+]$ corrected for H^+ from formation of $Fe(OH)^{2+}$ and $(FeOH)_2^{4+}$. [b] Calculated from least-squares fit to eq 10.

Table V. Summary of Kinetic Results at 25 °C in 0.50 M $NaClO_4/HClO_4$

		10^{-2} × rate constant, M^{-1} s^{-1}		
reactant	k_i	quinizarin[a]	quinizarin-2-sulfonate	daunomycin
$FeOH^{2+}$	k_1	7.3 × 0.6	31.6 × 3.2	1.7 × 0.1
$(FeOH)_2^{4+}$	k_3	4.5 × 2.6	12.2 ± 4.2	0.39 ± 0.15
$FeOH^{2+}$	k_2	2.8 ± 0.2	4.8 ± 0.6	0.16 ± 0.01
$(FeOH)_2^{4+}$	k_4	2.3 ± 1.1	6.0 ± 0.9	0.18 ± 0.2

[a] Results in 42.8% by volume methanol in water.

In general, the kinetic results reveal that quinizarin (in 42.8% methanol) and quinizarin-2-sulfonate (in water) have similar reactivity, while daunomycin (in water) reacts about 20 times slower. This can be seen from Figures 2 and 3 when the scaling factors are taken into account.

The interpretation thus far for k_1 and k_2 has assumed that the reactants are $FeOH^{2+}$ and QzH_2, but the rate law does not distinguish this from $FeOH_2^{3+}$ and QzH^- as the reactants. In the latter case, the k_iK_m term is replaced by k_iK_a, where K_a is the acid dissociation constant of the ligand. Since K_a is $\leq 10^{-9}$ M for these ligands,[10,14,27] this leads to values of k_1 and k_2 in the range 10^8–10^9 M^{-1} s^{-1}. However, rate constants for substitution on $FeOH_2^{3+}$ are typically[8] in the range 10–10^3 M^{-1} s^{-1}, and much larger values would be unprecedented. This justifies our omission of the $FeOH_2^{3+}$ + QzH^- pathway in Scheme I. On the other hand, substitution reactions of $FeOH^{2+}$ have rate constants[8] in the range of 10^3 M^{-1} s^{-1}, and the values found here are normal.

The value of k_1 for quinizarin-2-sulfonate is almost identical to that of 3.1 × 10^3 M^{-1} s^{-1} for Tiron (1,2-dihydroxy-3,5-benzenesulfonate)[8] reacting with $FeOH^{2+}$. This similarity is

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reasonable because both ligands have a sulfonate group adjacent to the –OH reaction center of the ligand. The larger value of k_1 for the sulfonate compared to quinizarin indicates that initial complexation is preferred adjacent to the sulfonate group because of more favorable ion pairing in the precursor complex. This also is consistent with the rather similar values of k_2 for these two systems.

The reactivity of the bis(μ-hydroxo)iron(III) dimer is more difficult to categorize because of the few cases where it has been observed. The rate constants for the squarate anion,[28] Tiron,[8] and acetohydroxamic acid[29] are 4.5×10^5, 1.1×10^4, and 8.2×10^3 M^{-1} s^{-1}, respectively. For the systems studied here, the rate constants (k_3 and k_4) are all smaller than these values. It is not clear at present whether this highly variable reactivity reflects an associative mechanism or ligand steric and other structural features. It is noteworthy that the ratio k_3/k_4 is 2 for all the ligands studied here.

The rate constants for daunomycin are substantially smaller than the analogous rate constants for quinizarin or its sulfonate. Dimer formation of daunomycin has not been included in the kinetic analysis of daunomycin. About 50% of the daunomycin is dimerized at our typical concentration of 1×10^{-4} M, so that the rate constants would increase by a factor of ~2 at most if only the monomer was assumed to be reactive. Then, if $k_1 = 2(1.7 \times 10^2)$ for daunomycin it is similar to k_2 for quinizarin-2-sulfonate, and this may reflect the normal reactivity for $FeOH^{2+}$ at a neutral hydroxyquinone site in aqueous solution. The 10 times smaller value of k_2 for daunomycin can be attributed to a steric effect of the aminosugar substituent.

Experimental Section

Materials. Sodium quinizarin-2-sulfonate was prepared by the method of Marshall.[30] The product was recrystallized three times from hot water and charcoal to yield a golden brown-orange solid. The ^1H NMR spectrum (in DMSO at 300 MHz) gave the following chemical shifts (δ in ppm from internal TMS): 7.65 (3 H, singlet), 7.96 (6 and 7 H, seven-line multiplet), 8.25 (5 and 8 H, seven-line multiplet), 12.64 (OH, singlet), 13.28 (OH, singlet). The electronic spectrum in water has maxima at 278, 335, and 462 nm and a shoulder at 486 nm, consistent with the recent report of Mukherjee et al.[27]

Quinizarin (Eastman Chem. Co.) was used as supplied. In the aqueous 42.8% methanol solution used for this work, the electronic spectrum has maxima at 280, 326, and 472 nm, with molar absorptivities of 1.03×10^4, 3.06×10^3, and 7.63×10^3 M^{-1} cm^{-1}, respectively, and a shoulder at ~515 nm.

Daunomycin hydrochloride (daunorubicine) was used as obtained (Sigma Chemical Co). In aqueous 0.01 M $HClO_4$/0.50 M $NaClO_4$ the electronic spectrum has maxima at 288 and 480 nm, with molar absorptivities of 8.02×10^3 and 1.06×10^4 M^{-1} cm^{-1}, respectively, and a shoulder at ~494 nm.

Stock solutions of iron(III) perchlorate in 1.0 M perchloric acid were prepared from iron wire (99.9%, Baker and Adamson) and standarized for iron(III) and H^+ content as described previously.

Equilibrium Measurements. Solutions of ligand and iron(III) at various concentrations and acidities were mixed in volumetric flasks and diluted to volume, and the spectra were recorded between 250 and 700 nm within 2–5 min after mixing. The ligand concentration ranges were $(3.56–4.06) \times 10^{-5}$ M for quinizarin, $(1–2) \times 10^{-4}$ M for quinizarin-2-sulfonate and 1×10^{-4} M for daunomycin. The iron(III) concentrations were in the range 6×10^{-4} to 0.03 M and $[H^+]$ was between 0.01 and 0.06 M. The equilibrium constants were evaluated by least-squares analysis of the variation of absorbance with ligand, iron(III), and H^+ concentrations at 610, 630, and 670 nm.

Stopped-Flow Measurements. Solutions of iron(III) perchlorate at the desired concentration and acidity in 1.00 M $HClO_4/NaClO_4$ were mixed with aqueous solutions of ligand, except that both were in 42.8% methanol for quinizarin. The time–absorbance change (volts) data were collected on a transient recorder. The data was output on a digital voltmeter/printer and then analyzed by least-squares methods to give the model described in the text. Error limits given are 1 standard deviation.

The kinetics were studied between 1.36×10^{-2} and 8.00×10^{-2} M H^+ and 0.436×10^{-2} and 3.49×10^{-2} M iron(III) at ligand concentrations similar to those in the equilibrium study.

Instrumentation. Spectrophotometric measurements were done on a Hewlett-Packard 8451 diode array spectrophotometer equipped with a thermostated cylindrical cell holder.

The stopped-flow studies were done on a Tritech Dynamic Instruments system. The flow system is glass and Teflon, and the reservoir and drive syringes are immersed in a thermostated water bath with the temperature regulated to 25 °C by a YSI thermistor controller.

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Notes

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Kinetic and Thermodynamic Synergism of Chloride and Carbon Monoxide Binding to Bis(acetonitrile)bis((dimethylglyoximato)difluoroborato)iron(II)

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Introduction

Extensive investigations of CO binding to low spin FeN_4L_2 systems involving a tetradentate macrocyclic ligand (N_4) and a variety of neutral axial ligands (L) have been previously described.[1-4] Here we describe some remarkable effects of anions on the CO binding properties of the bis(acetonitrile) complex of bis((dimethylglyoximato)difluoroborato)iron(II) $Fe(dmgBF_2)_2(CH_3CN)_2$ (denoted as FeN_4A_2 hereafter) in acetonitrile solution.

Experimental Section

Materials. The complex FeN_4A_2 was prepared as described previously.[3] Tetraethylammonium cyanide and chloride and tetrabutylammonium thiocyanate and bromide (Aldrich) were used as received. Solvents and other reagents were of the highest purity available. Kinetic results were unaffected by drying the acetonitrile or on addition of small amounts (1%) of water.

Synthesis. $[Et_4N][FeN_4(CN)(CO)]$. Solid FeN_4A_2 (120 mg, 0.26 mmol) was added to 50 mL of CO-saturated tetrahydrofuran and stirred during dropwise addition of a CH_2Cl_2 solution of tetraethylammonium cyanide (40 mg). Concentration of the solution with CO purging resulted in a yellow precipitate, which was filtered off and dried in vacuo (yield 70 mg, 62%). IR (KBr): 2030 (ν_{CO}), 2200 (ν_{CN}) cm^{-1}.

Physical Measurements. Visible spectra were recorded using an Aminco DW-2a UV/vis spectrophotometer with temperature maintained at 25 ± 0.1 °C. Fast reactions were monitored using an Applied Photophysics flash photolysis apparatus and data processed as described pre-

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