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pH Dependence of Circularly Polarized Emission and Total Emission from Europium(III)/L-Malic Acid and Europium(III)/L-Malic Acid/Terbium(III) Complexes in H_2O and D_2O Solutions

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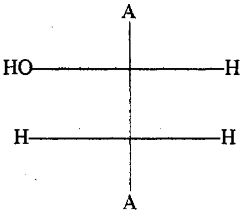
The circularly polarized emission (CPE) and total emission (TE) spectra are reported for Eu^{3+}/L-malic acid and Eu^{3+}/L-malic acid/Tb^{3+} complexes in H_2O and D_2O solutions under various pH conditions. Variations in CPE and TE intensities and band splittings as well as in CPE sign patterns as functions of pH are correlated with lanthanide ion/ligand binding characteristics and with structural changes within lanthanide ion/malic acid complexes. In the Eu^{3+}/L-malic acid system, the emission bands associated with the $^5D_0 \rightarrow {}^7F_0$, $^7F_1$, and $^7F_2$ Eu^{3+} transitions are measured. In the mixed system Eu^{3+}/L-malic acid/Tb^{3+}, the Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ emission as well as the Eu^{3+} $^5D_0 \rightarrow {}^7F_0$, $^7F_1$, and $^7F_2$ emissions are measured. The relative intensities of the Tb^{3+} and Eu^{3+} emissions in these mixed systems are followed as a function of Eu^{3+}:Tb^{3+} concentration ratios, solution pH, and solvent (H_2O or D_2O), and are related to energy transfer between Tb^{3+} ions and Eu^{3+} ions. Significant enhancement of TE and CPE intensities is found in going from H_2O to D_2O solvent. Furthermore, rather substantial qualitative and quantitative alterations are observed in the TE and CPE spectra across the pH range 1–13, indicative of significant changes in lanthanide ion/ligand binding and in the structural characteristics of the complex species formed.

I. Introduction

The interaction of lanthanide metal ions with carboxylic acids has been of considerable interest for many years.[1] Stepwise stability constants have been determined for many lanthanide complexes in solution,[1,2] but little progress has been made in unraveling the complicated structural features of the lanthanide/carboxylic acid complexes in aqueous solution under various pH conditions. This subject is of particular interest at present due to the widespread use of lanthanide ions as structure probes of metal binding sites in biological macromolecules such as proteins and nucleic acids.[3] Given the high propensity of trivalent lanthanide ions for binding to oxygen donor atoms (vs., for example, nitrogen or sulfur atoms), it is believed that the lanthanide binding sites in most protein or polypeptide systems include oxygen-containing donor groups such as –OH and –COOH. For this reason, lanthanide complexes with amino- or hydroxy-substituted carboxylic acids are expected to provide good model systems for eventually studying lanthanide ion–protein interactions.

Considerable use has been made of lanthanide ions as NMR probes in structure studies, and the absorption spectra (including circular dichroism, magnetic circular dichroism, and total unpolarized absorption) associated with lanthanide ion f–f transitions have also been employed to probe the structural features of lanthanide binding sites in a variety of molecular systems (simple and complex).[3] Our own studies in this area have focused on the use of lanthanide ion emission spectra to probe structure and structural changes at lanthanide binding sites.[4-8] Of special interest is the chiroptical probe, circularly polarized emission (CPE), which is expected to be particularly sensitive to very subtle structural features and structural changes in the ligand environment about the lanthanide reporter ion. CPE from lanthanide ions bound to asymmetric ligands or at asymmetric sites of biomolecular systems has also been reported and studied by Steinberg and co-workers at the Weizmann Institute.[9]

In the present study we examine the pH dependence of both CPE and total emission (TE) observed for Eu^{3+}/L-malic acid (Eu^{3+}/L-Mal) in H_2O and D_2O and for the "mixed" system Eu^{3+}/L-Mal/Tb^{3+} in H_2O and D_2O. Malic acid is a potentially terdentate ligand with two carboxylate groups and one (α-substituted) hydroxy group.



A = carboxyl group

L-malic acid

The absorption and circular dichroism (CD) spectra of Pr^{3+}/L-Mal in aqueous solution were previously reported as a function of pH by Katzin,[10] and we have previously reported the CPE and TE spectra of Tb^{3+}/L-Mal in aqueous solution under various pH conditions.[6] Although the absorption and CD data suggest that rather substantial alterations occur in the Ln^{3+}/carboxylic acid complex with very small changes in pH, the very weak absorption and CD intensities associated with the diagnostic lanthanide ion f–f transition make these spectroscopic techniques only marginally useful except when lanthanide ion concentrations are relatively high. Emission spectra provide significantly greater sensitivity.

Our previous TE and CPE studies of Tb^{3+}/L-Mal in aqueous solution indicated substantial structural modification as a function of pH. The interpretation of the data in the case of Tb^{3+} complexes is made difficult, however, because the most intense (and diagnostically most useful) emission bands originate with transitions between free ion levels of $J = 4$ ($^5D_4$ initial state) and $J = 5$ ($^7F_5$ final state). These bands are split into many components in the presence of the low-symmetry crystal field provided by the L-Mal ligands and solvent molecules. In the case of Eu^{3+}/L-Mal, the most intense emissions are observed for the transitions of $^5D_0 \rightarrow {}^7F_0$, $^7F_1$, and $^7F_2$ free-ion parentage, and crystal field induced band splittings are somewhat less complicated. In fact, splitting of the $^5D_0 \rightarrow {}^7F_0$ emission may be taken as direct evidence of either ion-pair formation (as, for example, in polynuclear species) or the presence of more than one type of Eu^{3+} complex in solution.

Previous studies[6] of the "mixed" Eu^{3+}/L-Mal/Tb^{3+} system in aqueous solution suggested that significant Tb^{3+} to Eu^{3+} energy transfer occurs and that the efficiency of the transfer increases as solution pH is raised (approaching 100% in the alkaline pH >8 region). It was hypothesized that this energy transfer occurred between a Eu^{3+} ion and a Tb^{3+} ion held in close juxtaposition *either* by their simultaneous binding at two different sites on a single ligand molecule *or* by their being linked through a hydroxy ion or solvent molecule bridge. We examine this Tb^{3+} to Eu^{3+} energy transfer phenomenon in greater detail in the present study. If in fact the solvent molecules or anions (in the alkaline pH region) play a prominent role in mediating the Tb^{3+} to Eu^{3+} energy transfer process, then one might expect the efficiency of the transfer to be sensitive to the replacement of H$_2$O by D$_2$O. Quantum efficiencies of lanthanide ion emission in D$_2$O are known to be somewhat greater than in H$_2$O.[11-13] Although the reason for this is not known with certainty, it is believed to be related to the differences in vibrational frequencies of D$_2$O and H$_2$O vibrational modes and the role of these vibrational modes in promoting radiationless processes (lanthanide ion to solvent energy transfer and/or intersystem crossings and internal conversions within the lanthanide ion or lanthanide complex).

II. Experimental Section

TbCl$_3$·6H$_2$O and EuCl$_3$·6H$_2$O were prepared by dissolving the 99.9% oxides (Alfa Inorganics) in 12 M HCl, heating almost to dryness, and removing the rest of the free water in a vacuum desiccator. pH adjustment was accomplished using HCl and NaOH or DCl and NaOD, depending upon whether the solvent was H$_2$O or D$_2$O. L-Malic acid was obtained from Aldrich. Solutions were made by dissolving the hydrated rare earth chloride and L-malic acid with H$_2$O or D$_2$O directly in the fluorescence cuvette.

Most of the emission results reported in this paper were obtained on aqueous solutions of Eu^{3+}/L-Mal and Tb^{3+}/L-Mal in which the metal ion:ligand concentration ratios were 1:5. These solutions (with either H$_2$O or D$_2$O as solvent) were 0.25 M in L-malic acid and 0.05 M in lanthanide ion (Eu^{3+} or Tb^{3+}). Solutions of Eu^{3+}/L-Mal/Tb^{3+} were studied at three different Eu^{3+}:Tb^{3+} concentration ratios, but the ratio of L-malic acid concentration to total lanthanide ion concentration was maintained at 5:1. The Eu^{3+}:Tb^{3+} concentration ratios used were 0.5:1, 1.0:1, and 2.0:1.

Additional emission measurements were carried out on Eu^{3+}/L-Mal solutions in which the metal ion:ligand concentration ratios were 1:1 and 1:3. For the 1:1 solutions, pH could not be raised above 2.5 without precipitation occurring and for the 1:3 solutions precipitation appeared around pH 8.5. Emission measurements were also made on aqueous solutions of Gd^{3+}/L-Mal/Tb^{3+} in which the Gd^{3+}:L-Mal:Tb^{3+} concentration ratio was 1:10:1.

pH measurements of each solution were taken on an Orion Model 701 digital pH meter using a standard glass electrode.

All emission spectra were measured at 180 °C ("head on") using apparatus constructed in this laboratory.[6] TE and CPE were measured at room temperature, with a spectral band-pass of 2 nm. Both TE and CPE are reported in arbitrary intensity units. We denote TE intensity by I ($I_L + I_R$) and CPE intensity by ΔI ($I_L - I_R$), where I_L and I_R are, respectively, the observed intensities of left and right circularly polarized emission. Although I and ΔI were measured in relative intensity units, absolute values of the ratio $\Delta I/I$ ($=g_{em}$ or emission dissymmetry factor)[6] were measured.

Except where noted in subsequent presentation and discussion of results, all emission spectra were recorded using an excitation wavelength of 365 nm. At this excitation wavelength both Tb^{3+} and Eu^{3+} are excited and will emit; however, the Tb^{3+} emission is considerably stronger than the Eu^{3+} emission. Several experiments were conducted in which Tb^{3+} was *selectively* excited at 488 nm. Eu^{3+} exhibits no emission under 488-nm excitation.

All emission spectra were obtained on solutions which were optically dilute and were measured in spectral regions where "inner filter" effects were determined to be negligible.

III. Results

A. Eu^{3+}/L-Mal (1:5). The TE and CPE of 1:5 Eu^{3+}/L-Mal solutions were followed as a function of pH in both D$_2$O and H$_2$O. No difference in either TE or CPE line shape was observed in comparing the D$_2$O and H$_2$O spectra, but the emission intensity was much greater in D$_2$O than in H$_2$O. Both the TE and CPE were observed to be very dependent on pH. Representative TE spectra taken at selected pH values are shown in Figure 1 and the corresponding CPE traces are shown in Figure 2.

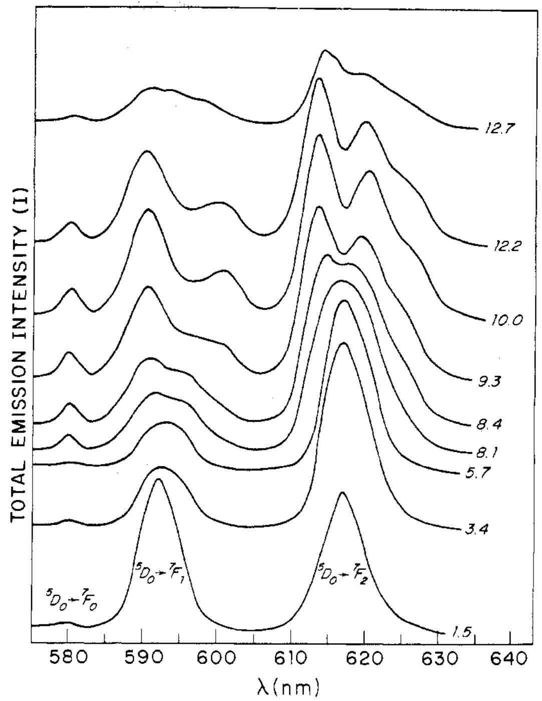


Figure 1. Total emission (TE) spectra of 1:5 Eu^{3+}/L-Mal D$_2$O solutions in the region of the Eu^{3+} $^5D_0 \to {}^7F_0$, $^7F_1$, and $^7F_2$ transitions for various values of solution pH. The intensity units are arbitrary, but all spectra are drawn to the same scale.

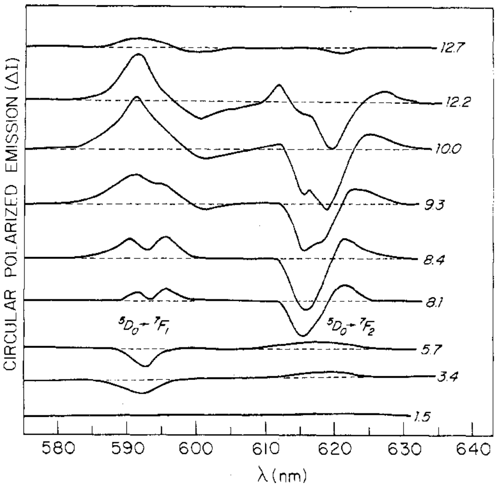


Figure 2. Circularly polarized emission (CPE) spectra of 1:5 Eu^{3+}/L-Mal D$_2$O solutions in the region of the Eu^{3+} $^5D_0 \to {}^7F_0$, $^7F_1$, and $^7F_2$ transitions at various values of solution pH. All spectra are drawn to the same scale.

The rather substantial changes observed in both the TE and CPE spectra (shown in Figures 1 and 2) as pH is varied between 1.5 and 12.7 clearly demonstrate that rather profound alterations take place in the immediate environment of the Eu^{3+} ions. At very low pH (below 2), neither of the malic acid carboxyl groups is ionized and no CPE is observed. Still in the acid region (5 > pH > 2), a readily observable negative CPE band associated with the $^5D_0 \to {}^7F_1$ (0–1) transition appears, but only very weak (and positive) CPE is observed in the region of the $^5D_0 \to {}^7F_2$ (0–2) transition. The TE spectra show some sign of broadening when pH is raised above 2 (but is kept below 5). In approximately neutral solution (pH

5.0–7.5), the intensity of the 0–1 CPE diminishes while there is still only very weak CPE observed for the 0–2 transition. The TE intensity of the $^5D_0 \rightarrow {}^7F_0$ (0–0) transition remains very weak relative to that of the 0–1 and 0–2 transitions over the entire pH range 2.0–7.5. The CPE of the 0–0 transition is vanishingly small over this pH range.

In the midalkaline range (pH 7.5–8.0) fairly strong CPE appears in the 0–2 band, and weak positive CPE is also seen in the 0–1 band. The intensity of the 0–0 total emission also increases dramatically relative to the 0–1 and 0–2 emissions, although CPE in the 0–0 band remains unobservable. At around pH 8.5, the CPE of both the 0–1 and 0–2 transitions begins to increase significantly and the associated TE bands exhibit some splitting. This splitting becomes more pronounced at pH ~9.0. In this pH region the 0–1 TE band is clearly split into three components with maxima appearing at 590, 596, and 600 nm, while the 0–2 TE band also splits into three components at 613, 619, and 624 nm. The 0–1 and 0–2 TE grows steadily more intense as the pH is raised above 9.0, indicating substantial changes in the ligand environment or in metal/ligand binding.

In the region of pH 9.5–12.5, a new TE and CPE pattern is evident. Only two components are found in the 0–1 emission; both the TE and CPE due to the 596-nm component (seen at pH ~9.0) have disappeared. Three components remain in the 0–2 TE, but the peaks at 613 and 619 nm become better resolved (due to sharpening within the band profile). Finally, at pH values greater than 12.5, the 0–1 and 0–2 TE intensities diminish greatly and the CPE is entirely quenched. This presumably accompanies complete hydrolysis of the complex at these high pH values just prior to precipitation of europium hydroxide.

B. Tb^{3+}/L-Mal (1:5). TE and CPE data for 1:5 Tb^{3+}/L-Mal in H_2O at various pH values have been reported previously,[6] so it is not necessary to dwell on these results here. In the present study, data were obtained for Tb^{3+}/L-Mal in D_2O, but the TE and CPE spectra were similar to those obtained in H_2O solution except for a large enhancement of Tb^{3+} emission intensity by D_2O.

C. Eu^{3+}/L-Mal/Tb^{3+} (365-nm Excitation). Three Eu^{3+}:Tb^{3+} concentration ratios were chosen (0.5, 1, 2) and TE and CPE data were taken at a variety of pH values in H_2O and D_2O for all three solutions. The intensity and splitting patterns observed for spectra obtained in D_2O and H_2O for a particular Eu^{3+}:Tb^{3+} ratio were the same (although the usual intensity enhancement in D_2O was observed), but spectra obtained for different ratios differed. With 365-nm excitation, where both Eu^{3+} and Tb^{3+} are excited the predominant emission was from the Eu^{3+} ion, although Tb^{3+} emission could be measured and was not completely quenched. While the CPE of the Eu^{3+} 0–2 transition was not changed from that obtained without Tb^{3+} present, the CPE of the Eu^{3+} 0–1 transition was entirely quenched. The Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ (4–5) emission was monitored and the CPE observed within this band was substantially the same in qualitative features (sign and intensity patterns) as that observed for Eu^{3+}-free Tb^{3+}/L-Mal solutions, except for a drastic reduction in net intensity.

At very low pH (less than 1.5), the TE spectra are simply described as superpositions of Eu^{3+} and Tb^{3+} spectra. The Tb^{3+} emission dominates, since it is inherently a much stronger emitter than Eu^{3+}. No CPE is seen for either ion at low pH. In D_2O, when the pH is raised to 2.5, the Tb^{3+} emission becomes very weak but the Eu^{3+} emission is easily seen (one is able visually to observe the emitted light change from green to red). In H_2O, however, little change in Tb^{3+} emission intensity is seen as the pH is raised to ~2.5. As one goes to higher Eu^{3+}:Tb^{3+} ratios, the intensity of Tb^{3+} emission decreases.

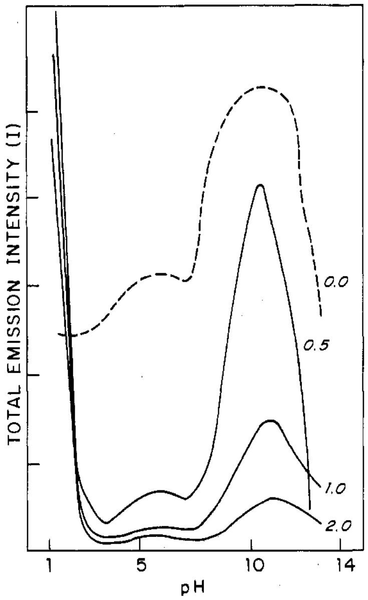


Figure 3. Intensity of the Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ transition in D_2O solution for Eu^{3+}/L-Mal/Tb^{3+} as a function of pH. The various Eu^{3+}:Tb^{3+} concentration ratios used are indicated to the right of each trace. Solid curves are plotted on an $I \times 15$ scale.

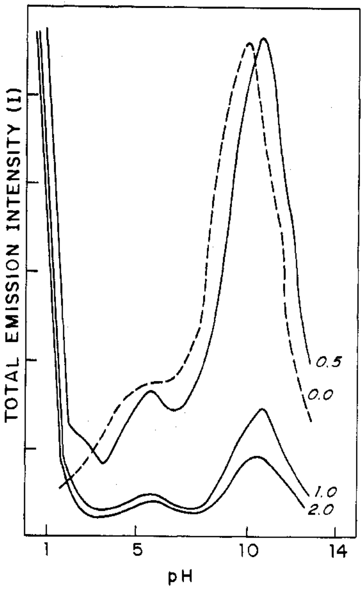


Figure 4. Intensity of the Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ transition in H_2O solution for Eu^{3+}/L-Mal/Tb^{3+} as a function of pH. The various Eu^{3+}:Tb^{3+} concentration ratios used are indicated to the right of each trace. Solid curves are plotted on an $I \times 15$ scale.

The intensity of the 4–5 Tb^{3+}/L-Mal emission in D_2O is shown in Figure 3, and the H_2O results are shown in Figure 4. It is clear that the positions of the maxima found in the alkaline regions of the Tb^{3+} emission intensity vs. pH plots correspond to the same pH values that result in strong CPE, as discussed in the previous section.

The TE data for H_2O relative to those observed for D_2O at pH values around 10 are very dependent upon the Eu^{3+}:Tb^{3+} ratio. For low Eu^{3+}:Tb^{3+} ratios, significant $^5D_4 \rightarrow {}^2F_3$ Tb^{3+} emission is seen along with strong $^5D_0 \rightarrow {}^7F_1$ Eu^{3+} emission in H_2O, while the Tb^{3+} $^5D_4 \rightarrow {}^7F_3$ emission is somewhat reduced compared to that of the Eu^{3+} $^5D_0 \rightarrow {}^7F_1$ emission in D_2O. That is, in the alkaline pH region Tb^{3+} to Eu^{3+} energy transfer would appear to be more efficient in D_2O than in H_2O, possibly implicating solvent molecules or anions in the energy-transfer process. A comparison of the TE spectra

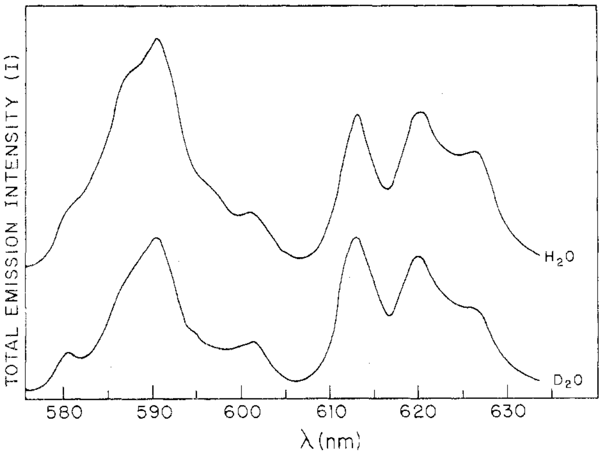


Figure 5. Total emission spectra of Eu^{3+}/L-Mal/Tb^{3+} solutions at pH 10.0 and a Eu^{3+}:Tb^{3+} concentration ratio of 0.5 in both D_2O and H_2O.

obtained in D_2O and in H_2O at pH 10 for a Eu^{3+}:Tb^{3+} ratio of 0.5 is shown in Figure 5.

D. Eu^{3+}/L-Mal/Tb^{3+} (488-nm Excitation). At 488-nm excitation only the Tb^{3+} ions are excited. Emission spectra on both 0.06 M:0.30 M Tb^{3+}/L-Mal solutions and 0.06 M:0.30 M:0.06 M Eu^{3+}/L-Mal/Tb^{3+} solutions were measured in the pH 7.5–9.0 region. Tb^{3+} emission from the mixed Eu^{3+}/L-Mal/Tb^{3+} system was found to be about 15 times weaker in intensity than that observed for the Tb^{3+}/L-Mal system. Emission was observed in the 0–0, 0–1, and 0–2 Eu^{3+} transitions for the mixed system, again indicating energy transfer from Tb^{3+} to Eu^{3+} in solution. Although the total emission intensity of the Tb^{3+} 4–5 transition was reduced in going from the Tb^{3+}/L-Mal system to the Eu^{3+}/L-Mal/Tb^{3+} system, the g_{em} ($\Delta I/I$) values associated with this transition remain essentially unchanged.

E. Gd^{3+}/L-Mal/Tb^{3+} (365-nm Excitation). To determine if Tb^{3+} emission intensity would be reduced in the presence of an ion like Gd^{3+} which *cannot* quench Tb^{3+} emission via an energy-transfer mechanism but which *may* displace Tb^{3+} ions from ligand coordination sites, we measured and compared the emission spectra of Tb^{3+}/L-Mal and Gd^{3+}/L-Mal/Tb^{3+} in H_2O solution at pH values of 2.0, 4.0, 6.5, 8.5, and 11.5. No quenching of the Tb^{3+} emission was observed in the presence of equimolar amounts of Gd^{3+}, and the g_{em} ($\Delta I/I$) values associated with the 4–5 Tb^{3+} transition remained unchanged. At 365-nm excitation, only the Tb^{3+} ions in the Gd^{3+}/L-Mal/Tb^{3+} system are excited.

F. Eu^{3+}/L-Mal (1:1 and 1:3). The pH of the 1:1 Eu^{3+}/L-Mal solutions could not be raised above 2.5 without precipitation occurring. At pH values less than 2.5, Eu^{3+} TE was observed but no CPE was found. The pH of the 1:3 Eu^{3+}/L-Mal solutions could be varied over the range ~1.0–8.5. Over this pH range, the TE and CPE spectra were found to be similar to those observed for the 1:5 Eu^{3+}/L-Mal solutions in the vicinity of the Eu^{3+} 0–0, 0–1, and 0–2 transitions.

IV. Discussion

The TE and CPE spectra described in section III (Results) clearly indicate that the ligand environment about the lanthanide ions and possibly the coordination geometry of the Ln^{3+}/L-Mal complexes are sensitive to solution pH. It is also apparent that the TE spectra are somewhat less sensitive probes of these structural features than are CPE spectra. The splittings within the various emission bands observed in the Eu^{3+} TE and CPE spectra may be attributed to one or both of the following: (a) crystal field splitting of the ^7F_J multiplet levels due to the low symmetry of the ligand environment; (b) the presence of nonequivalent Eu^{3+} ions in the solution leading to multiple emissions from the same Eu^{3+} transition. The latter explanation would require several "kinds" of Eu^{3+}/L-Mal complex species to be present in solution or would require the formation of polynuclear species which have nonequivalent metal sites.

It is expected that if more than one Eu^{3+} complex is present in solution or in the solid state, a splitting of the $^5D_0 \rightarrow {}^7F_0$ band at 580 nm should occur.[8,14] However, this band cannot be split by a low-symmetry ligand field. The TE spectra of several Eu^{3+}/L-Mal solutions were examined at the highest resolution possible with our instrument (spectral band-pass of 1 Å) and no splitting within the 0–0 band was observed. We conclude that the splittings observed in the TE and CPE bands of the 0–1 and 0–2 Eu^{3+} transitions are due solely to crystal field splittings induced by the low symmetry of the ligand environment.

On the basis of the spectroscopic data reported here it is not possible to reach definite conclusions concerning the detailed structural features of the complex species formed in aqueous solution by Eu^{3+}/L-Mal or by Eu^{3+}/L-Mal/Tb^{3+}. The structural chemistry of lanthanide ion complexes in aqueous solution is exceedingly complex and the data obtained with just one or two kinds of structural probes (such as TE and CPE) are not sufficient to provide detailed or conclusive characterizations of structure. However, it is possible and, we believe, useful to offer *qualitative* interpretations of the spectroscopic data in terms of structural characteristics and structural changes (which accompany variations in solution conditions such as pH and solvent type). While these interpretations cannot be subjected to the test of conclusive evidence, given the data reported here, they do provide useful working hypotheses for further structural studies on lanthanide ion complexes in solution. A detailed interpretation of the CPE data in terms of specific structural characteristics cannot be accomplished until the mechanism whereby the f–f transitions of chiral lanthanide complexes acquire optical activity is elucidated and a reliable theoretical model for f–f optical activity is developed. In the following discussion, explanations and interpretations of the effects of pH changes on the TE and CPE spectra of Eu^{3+}/L-Mal in solution are offered. In most cases these interpretations are based on qualitative structural arguments.

The relatively weak, negative CPE observed in the 0–1 emission band of Eu^{3+}/L-Mal at pH 2.0–5.0 is attributable to unidentate coordination of a singly deprotonated malic acid ligand to Eu^{3+}. L-Malic acid has a pK_1 of 3.41[1] and it is likely, therefore, that it can bind to Eu^{3+} only through a single deprotonated carboxyl group over the pH range 2–5. Since unidentate binding will provide only a very weak dissymmetric environment for the Eu^{3+} ion, one may expect that the CPE intensity will be weak, as is experimentally observed. As shown by the data displayed in Figures 3 and 4, energy transfer between Tb^{3+} and Eu^{3+} ions in mixed Eu^{3+}/L-Mal/Tb^{3+} systems is effective over the pH range ~3–5.

The second carboxylic acid proton of malic acid is ionized above pH 5 (pK_2 = 5.11).[1] The malic acid may now bind two different lanthanide ions (unidentate) or may bind to one ion in a bidentate fashion (chelate formation). The CPE data indicate a continuing unidentate malic acid coordination up to pH 7.5, since no significant change in CPE band shape is observed. A bidentate coordination should result in a significant change in CPE band shape since the dissymmetry provided by bidentate coordination will, in general, be considerably different (larger) than that provided by unidentate coordination.[15] The magnitude of the 0–1 CPE diminishes indicating that a structure change is taking place but that bidentate Eu^{3+}/L-Mal coordination does not occur in this pH region. The Tb^{3+} to Eu^{3+} energy transfer is still quite efficient in this region, although there is a slight decrease in efficiency at pH ~6. Martin and co-workers[16] have noted in pH titrations of lanthanide ion/malic acid solutions that the ionization of the second carboxylic acid proton is complete at a pH of 6.5. Thus, the spectroscopic changes occurring in the pH region 5.0–7.5 appear to be related to malic acid binding to *two* lanthanide ions (unidentate), as opposed to the binding of just one ion (bidentate). The very weak chirality (as evidenced by weak CPE intensities) argues against the two carboxylic acid groups of malic acid binding to one lanthanide ion through chelate formation.

The pH range 7.5–9 appears to be a region of structural transition. The TE bands split, and new CPE develops in the 0–1 and 0–2 transitions. Throughout this region, the efficiency of energy transfer between Tb^{3+} and Eu^{3+} decreases, as illustrated by the growth in Tb^{3+} ion emission (see Figures 3 and 4). The symmetry about the lanthanide ion must be quite low, since the 0–1 band of Eu^{3+} splits into three components. The situation in the 0–2 band is less clear. That the ligand environment has become less symmetric than at lower pH values is suggested by the sudden increase in intensity of the 0–0 band upon reaching pH 7.5. The rather severe selection rules which govern the intensity of this band apparently are relaxed owing to the presence of low-symmetry ligand fields or the formation of ion pairs (such as in polynuclear coordination species).

In the pH region 9.5–12.0, a coordination symmetry different from that in the pH region 7.5–9.0 is indicated by the disappearance of one component of the 0–1 TE band and its associated CPE. It is possible that in this pH range coordination to Eu^{3+} occurs through three malic acid donor groups (two carboxyl and one hydroxyl). That is, the malic acid becomes terdentate in its binding to Eu^{3+}. Terdentate binding vs. bidentate or unidentate binding should provide the largest dissymmetry in the ligand field of the Eu^{3+} ion. In this case the asymmetric sites within the malic acid ligands should be rigidly fixed relative to the Eu^{3+} ion and the ligand fields will present a definite and strong chirality (or dissymmetry) to the chromophoric electrons of the metal ion. In fact, the *net* CPE intensities associated with the 0–1 and 0–2 Eu^{2+} transitions *do* increase over the pH range 9.0–10.5 (see Figure 2 for representative data) and the splitting patterns are altered. Support for this type of malic acid coordination (terdentate) in the pH range 9.0–12.0 is also provided by the energy-transfer data. The Tb^{3+} to Eu^{3+} energy-transfer efficiency is very low in this pH region. Eu^{3+} ions tightly bound to malic acid ligands through three donor groups (terdentate) would be somewhat better protected from direct contacts with excited Tb^{3+} ions (and vice versa) than would Eu^{3+} ions less tightly bound through unidentate and bidentate complex formation with malic acid. That is, if the Ln^{3+} ions (Tb^{3+} and Eu^{3+}) are each bound to malic acid ligands through terdentate coordination rather than through bidentate or unidentate coordination, it is likely that they will be relatively inaccessible for direct energy-transfer contacts. This assumes, of course, that the energy-transfer process depends on a short-range interaction mechanism (such as quadrupole–quadrupole coupling),[17] and that the terdentate coordinated malic acid ligands will, in effect, "insulate" the bound ions from direct contacts with other ions.

Finally, above pH 12.5 complete hydrolysis of the complex takes place. The CPE drops quickly to zero and the TE decreases to values expected if no malic acid was coordinated. The TE also broadens and loses structure, indicative of a significant alteration in the coordination geometry or structure of the emitting ion. Eventually, precipitation of the lanthanide hydroxide takes place.

The CPE and TE data reported in this study provide a probe for following the binding characteristics and structural changes of the Eu^{3+}/L-Mal system as a function of solution pH. The information obtained is qualitative in the sense that the *detailed* nature of ion/ligand binding and of the complex structure cannot be deduced directly from the spectroscopic observables. It is clear that the structural chemistry of Ln^{3+}/carboxylic acid systems in aqueous solution is quite complex and that emission studies such as the one reported here will not yield *definitive* structural characterizations. However, the CPE technique does provide a very sensitive probe or diagnostic tool for following structural changes in the coordination environment of the emitting ion and, in some cases, the CPE observables can be related to specific structural features of the complex species.

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References and Notes

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