From 8-hydroxy-5-sulfoquinoline to new related fluorogenic ligands for complexation of aluminium(III) and gallium(III)

Franck Launay,†[a] Valérie Alain,[a] Émilie Destandau,[a] Nathalie Ramos,[a] Élisabeth Bardez,*[a,b] Paul Baret[c] and Jean-Louis Pierre[c]

[a] *Laboratoire de Photophysique et Photochimie Supramoléculaires et Macromoléculaires (CNRS UMR 8531), École Normale Supérieure de Cachan, 61 avenue du Président Wilson, 94235 Cachan cedex, France*

[b] *Laboratoire de Chimie Générale, Conservatoire National des Arts et Métiers (CNRS UMR 8531), 292 rue Saint-Martin, 75003 Paris, France. E-mail: bardez@cnam.fr*

[c] *Laboratoire d'Etudes Dynamiques et Structurales de la Sélectivité (LEDSS, CNRS UMR 5616), Université Joseph Fourier, B. P. 53, 38041 Grenoble cedex 9, France. E-mail: Paul.Baret@ujf-grenoble.fr*

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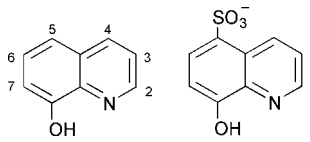
The hexadentate tripodal ligand O-TRENSOX (already known as a siderophore), incorporating three 8-hydroxy-5-sulfoquinoline (8-HQS) subunits, was investigated as a potential fluorogenic ligand of Al(III) and Ga(III). For the sake of comparison, every chelation study was also carried out with n-BUSOX, a ligand similar to one arm of O-TRENSOX. Chelations were studied at the optimal pH for fluorescence emission: pH = 4 for Al(III) and pH = 2 for Ga(III). An outstanding 'tripod' effect is exhibited by the values of the stability constants: with O-TRENSOX, $\log \beta_{111} = 24.8$ for Al(III) and 33.7 for Ga(III), whereas with n-BUSOX, $\log \beta_{110} = 8.6$ for Al(III) and 11.6 for Ga(III) at 25 °C. O-TRENSOX is nearly as efficient for Ga(III) chelation as for Fe(III). When increasing the [metal]/[ligand] ratio, fluorescence emission rose until either 1 : 1 chelation with n-BUSOX or 3 : 1 chelation with O-TRENSOX was achieved. Then, the resulting fluorescence intensity levelled off. The fluorescence emission intensity from n-BUSOX chelates was observed to be tenfold larger than that from O-TRENSOX chelates, suggesting that a self-quenching process occurs within the latter complexes. In terms of selectivity, ions such as Zn(II) or Cd(II), known to form strongly fluorescent complexes with 8-HQS, are not chelated at pH = 2 by n-BUSOX and O-TRENSOX. Thus, they are not potential interferences for Ga(III) determination, whereas Fe(III) strongly interferes, quenching the fluorescence. Conversely, although less stable at pH = 4, the chelates of Zn(II) and Cd(II) are possible interferences for Al(III) determination because of their strong fluorescence emission.

Chelation of metal cations by 8-hydroxyquinoline (oxine, 8-HQ), 8-hydroxy-5-sulfoquinoline (8-HQS) and their derivatives has long been extensively used in analytical chemistry.[1] 8-HQ is soluble in most organic media, and its neutral chelates can be solubilized in chlorinated solvents, for example chloroform.[1,2] This property makes 8-HQ and many of its substituted derivatives good agents for metal extraction from aqueous phases. In 8-HQS, the presence of the sulfonate group allows solvation of chelates in aqueous media, which enlarges the range of possible applications.



8-HQ 8-HQS

Furthermore, both 8-HQ and 8-HQS are fluorogenic ligands of most metal ions, except transition-metal ions. The fluorometric determination of metal ions constitutes then an important potential application for these chelating agents. For this purpose, 8-HQS is generally preferred to 8-HQ,[3] in particular because of its use in chromatographic applications when separations are conducted with purely aqueous solvents.[4] Fluorescence of oxinates was also shown to be enhanced in micellar media or in vesicles, which allows improvements in analytical determinations of a wide variety of metals.[5–7]

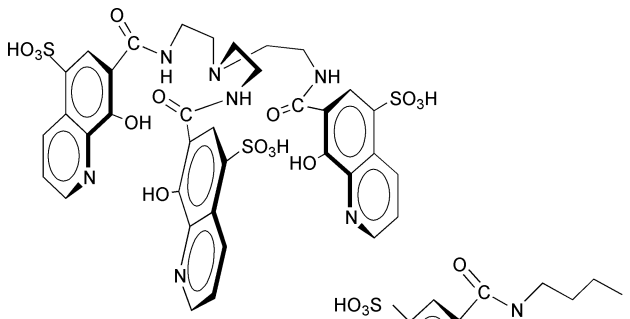
Interpretation of the fluorogenic character of these ligands, that is, the marked increase in their fluorescence emission triggered by complexation, is not straightforward. The very weak fluorescence of the free ligands was first tentatively explained by a simple molecular orbital picture,[8] which was not accounted for by the measurements of fluorescence and phosphorescence quantum efficiencies at 80 K.[9] That is why this phenomenon was revisited by one of us[10,11] and shown to be related to the excited-state amphoterous behavior of hydroxyquinolines, already reported in the 60s and 70s:[12–14] on electronic excitation, the hydroxyl group and the pyridinic nitrogen atom of these compounds become very strongly acidic and basic, respectively, and excited-state tautomerization occurs, as a result of coupled proton transfer and intramolecular charge transfer. The excited-state tautomers of both 8-HQ and 8-HQS (and of their derivatives) turn out to be very weakly fluorescent. In the case of 8-HQ, which is more soluble in organic media than in water, photoinduced tautomerization was shown to occur in any solvent, even apolar ones, according to intramolecular mechanisms within either internally H-bonded molecules or dimers.[10,11] On the con-

† Present address: Laboratoire Systèmes Inerfaciaux à l'Échelle Nanométrique (CNRS ESA 7069), Université Paris 6, Tour 55, 4 place Jussieu, 75252 Paris cedex 05, France.

trary, when complexation of metal cations takes place, involving the prototropic functions, fluorescence is no longer quenched, provided that the metal ions are not transition-metal ions.[15] The fluorescence intensity depends on the nature of the complexed metal ion[4] and on the solvent or the micro-environment.[5–7,16] In particular, elements of the IIIA group, that is aluminium, gallium and indium, were observed to lead to very large fluorescence enhancements on complexation.[4]

Unfortunately, a large number of metal ions can be complexed by 8-HQ or 8-HQS, but not alkaline ions.[1,3–5] The resulting lack of selectivity must consequently be stressed. Therefore, many attempts have been made to improve the selectivity, especially through substitutions at positions 2, 5 or 7 of the quinoline nucleus, but the obtained ligands remained in most cases able to complex competitively several metal ions.[1] Moreover, 8-HQ or 8-HQS and their substituted derivatives are only bidentate, whereas tri- or hexadentate ligands are expected to give higher thermodynamic stability. Concerning aluminium(III), gallium(III) and indium(III), it was in fact shown that hexadentate tripodal ligands combine large stability constants, such as those obtained with macrocycle-based ligands, and satisfactory kinetics, as observed with flexible linear ligands.[17]

In this paper, the hexadentate tripodal ligand O-TRENSOX is studied as a potential fluorogenic complexing agent of aluminium(III) and gallium(III) in aqueous solutions. O-TRENSOX consists of three 8-HQS (SOX for sulfoxine) subunits connected to a tris(2-aminoethyl)amine (TREN) framework *via* amide linkages. It was recently proposed by some of us for iron complexation in aqueous solutions.[18] The selectivity of O-TRENSOX towards Fe(III) was attested to by the large stability constant of the 1 : 1 chelate. However, the iron chelates are nonfluorescent, consistent with the lack of fluorescence exhibited by transition-metal oxinates (see above), whereas aluminium and gallium chelates are fluorescent.



It is worth noting that an interesting application of good fluorogenic ligands of aluminium is the fluorometric determination of Al(III), for example, in tap water or sodas, or in biological fluids. Quantitative aluminium determination is, in fact, of real concern to the scientific community because of the neurotoxicity of aluminium, and of its possible role in Alzheimer's disease.[19,20] Aluminium compounds are in widespread use in many areas: in water treatment (aluminium salts are used as clarifying agents), in food additives (for example, anticaking agents), in medicines (for instance, antiacids),[20a] *etc.* Aluminium itself is widely used in light alloys for cans. Concerning gallium, new chelators may be useful for developing radiopharmaceuticals based on ^{67}Ga and ^{68}Ga.[20a,21,22]

In the present work, complexation reactions were also carried out with a ligand whose structure is close to that of one arm of O-TRENSOX: 1-*n*-butyl-5-sulfo-8-hydroxyquinoline-7-carboxamide (n-BUSOX), for the sake of comparison with O-TRENSOX. Moreover, some experiments were also carried out with the original 8-HQS ligand. These results, in addition to comparisons with the literature data, have allowed us to elucidate the relationships between these three ligands.

Experimental

Materials

Analytical reagent grade aluminium perchlorate $Al(ClO_4)_3 \cdot 9H_2O$ (Aldrich, 98%) and anhydrous gallium chloride $GaCl_3$ (Aldrich, 99.999%) were used to prepare the aluminium and gallium stock solutions. Unfortunately, as far as we are aware, solid gallium perchlorate $Ga(ClO_4)_3$ is no longer commercially available. Nevertheless, thanks to a small quantity of solid $Ga(ClO_4)_3$ remaining at our disposal, we found no significant differences between $GaCl_3$ and $Ga(ClO_4)_3$ for complexation of Ga(III), in terms of the stability constants of the complexes and fluorescence emission. Zinc perchlorate [$Zn(ClO_4)_2 \cdot 6H_2O$, purity 99%] and cadmium perchlorate [$Cd(ClO_4)_2 \cdot 6H_2O$] were from Alfa Aesar, whereas iron(III) perchlorate, $Fe(ClO_4)_3 \cdot xH_2O$, was from Aldrich.

All aqueous solutions were prepared with Millipore filtered water (conductivity $< 10^{-6}\ \Omega^{-1}\ cm^{-1}$ at 25 °C). The metal-ion stock solutions were prepared in dilute perchloric acid solutions [at pH = 3.5–4 for Al(III) and at pH = 2 for Ga(III)]. Redistilled perchloric acid from Aldrich containing 69.5% $HClO_4$ (purity 99.999%) was diluted to prepare every acidic solution. Buffers used in neutral media (pH ≈ 5–9), either to determine the best pH range for observing the fluorescence of the metal chelates or for the pK_a determination of n-BUSOX, were prepared from MES, HEPES, HEPBS and CHES (GOOD® buffers purchased from Sigma). Basic solutions were prepared from dilution of sodium hydroxide (Aldrich, 99.998%).

8-Hydroxy-5-sulfoquinoline (Aldrich) was twice recrystallized from hot water. Elemental analysis showed that 8-HQS · $2H_2O$ was obtained. O-TRENSOX was synthesized according to the procedure described earlier.[18a,23] Analysis showed that crystallization involved 3 H_2O molecules.

Synthesis of n-BUOX and n-BUSOX

1-*n*-Butyl-8-hydroxyquinoline-7-carboxamide (n-BUOX). A mixture of 8-hydroxyquinoline-7-carboxylic acid (2 g, 10.6 mmol), carbonyldiimidazole (CDI, 1.88 g, 11.6 mmol) in dry THF (180 mL) was refluxed for 1 h under argon. A solution of *n*-butylamine (1.68 g, 23 mmol) in THF (10 mL) was added and the mixture refluxed overnight. After evaporation of THF, the crude product was taken up into chloroform and the solution treated successively with ammonium chloride and brine, then dried on magnesium sulfate. Chloroform was evaporated and treatment with diethyl ether–pentane (1 : 1 v/v) gave the amide as a beige powder (1.805 g, yield: 70%), pure enough for the following step of the synthesis. ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 0.97 (t, 3H, CH_3); 1.45 (sext, 2H, CH_2); 1.66 (p, 2H, CH_2); 3.55 (q, 2H, CH_2); 7.32 (dd, 1H, CH); 7.49 (dd, 1H, CH); 7.9 (br s, 1H, NH); 8.13 (dd, 1H, CH); 8.83 (dd, 1H, CH); 9.32 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 13.8 (CH_3); 20.16 (CH_2); 31.6 (CH_2); 39.6 (CH_2); 113.7 (C); 117.4 (CH); 123.1 (CH); 126.9 (CH); 129.9 (C); 135.9 (CH); 138.6 (C); 148.4 (CH); 152.6 (C); 166.1 (C=O). MS-DCI: $[M + H]^+ = 245$. Anal. calc. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; O, 13.10; found: C, 68.81; H, 6.70; N, 11.62%.

1-*n*-Butyl-5-sulfo-8-hydroxyquinoline-7-carboxamide (acidic form) (n-BUSOX). At 0 °C under argon, n-BUOX (1.4 g, 5.74 mmol) was dissolved in 20% oleum (fuming sulphuric acid, 25 mL). The mixture was stirred overnight and then poured onto

ice. Excess sulfuric acid was neutralized at 0 °C with 10 M NaOH, giving a yellowish precipitate. This solid was dissolved in refluxing methanol and the hot solution was filtered in order to eliminate residual sodium sulfate. Evaporation, then addition of diethyl ether gave a precipitate, which was recrystallized in water. The product was dried under vacuum (1.85 g, yield: 53%). ^{1}H NMR (DMSO-d_6, 300 MHz, δ): 0.89 (t, 3H, CH_3); 1.35 (sext, 2H, CH_2); 1.56 (p, 2H, CH_2); 3.35 (m, 2H, CH_2); 8.13 (dd, 1H, CH); 8.58 (s, 1H, CH); 9.11 (dd, 1H, CH); 9.56 (br s, 1H, NH); 9.62 (dd, 1H, CH); 13.30 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6, 100 MHz, δ): 13.8 (CH_3); 19.7 (CH_2); 30.8 (CH_2); 39.0 (CH_2); 111.6 (C); 124.1 (CH); 124.3 (CH); 127.6 (C); 132.4 (C); 134.1 (C); 143.1 (CH); 146.2 (CH); 153.3 (C); 168.5 (C=O). FAB MS: $[M + H]^+ = 325$; $[M - CH_3(CH_2)_3NH)]^+ = 252$. Anal. calc. for $C_{14}H_{16}N_2O_5S \cdot 0.5H_2O$: C, 50.44; H, 5.14; N, 8.40; S, 9.62; O, 26.40; found: C, 50.63; H, 5.00; N, 8.50; S, 9.78%. UV-vis (H_2O, pH = 11.4) λ_{max}/nm (log ε): 269 (4.48); 342 (3.96).

N-Methylated derivative of n-BUOX. Methylation of the pyridinic nitrogen atom of n-BUOX was obtained by addition of a large excess of methyl iodide (2 mL, 32 mmol) to a solution of n-BUOX (49 mg, 0.2 mmol) in acetone. The mixture was refluxed for 40 h. The iodide compound was then transformed into the betaine by treating its aqueous solution with potassium carbonate, followed by extraction with chloroform. The solvent was evaporated and the N-methyl derivative was obtained as a red compound in good yield (88%). ^{1}H NMR ($CDCl_3$, 200 MHz, δ): 0.94 (t, 3H, CH_3); 1.43 (sext, 2H, CH_2); 1.65 (quint, 2H, CH_2); 3.48 (q, 2H, CH_2); 5.00 (s, 3H, N^+–CH_3); 6.65 (d, 1H, CH); 7.37 (dd, 1H, CH); 8.06 (d, 1H, CH); 8.35 (d, 1H, CH); 8.37 (d, 1H, CH). Anal. calc. for $C_{15}H_{18}N_2O_2 \cdot 1.13H_2O$: C, 64.64; H, 7.33; N, 10.05; found: C, 64.64; H, 7.27; N, 9.68%.

Methods

The pK_a values of n-BUSOX were determined spectrophotometrically at 25 °C. n-BUSOX concentration was 4.7×10^{-5} mol L^{-1} and the spectra (not shown) were recorded from pH = 1 to 12.5. They showed isosbestic points at 275 and 383 nm (between pH = 1 and 4.5) and at 307 and 387 nm (between pH = 4.5 and 12.5). The analysis of the spectra by the Schwarzenbach method[24] gave pK_a values of 2.8 ± 0.1 and 6.8 ± 0.1, for the pyridinium function and the hydroxyl group, respectively.

The equilibrium constants for the formation of the aluminium and gallium chelates were determined by a spectrophotometric titration method at the optimum pH value for the fluorescence emission of the chelate [*i.e.*, pH ≈ 2 for Ga(III) and pH ≈ 4 for Al(III), see below]. The absorption spectra were recorded after progressive additions of the metal ion to a solution of ligand at a given concentration C_0. The added solutions of the metal ion contained the ligand at the same concentration C_0, such that the ligand concentration remained unchanged upon metal addition. The concentration C_0 was 1.82×10^{-5} mol L^{-1} for O-TRENSOX. For n-BUSOX, two concentrations were used: 1.93×10^{-5} and 4.95×10^{-5} mol L^{-1}. Complexation usually occurred within 5 min for Ga(III) and within 10 to 15 min for Al(III), which was checked by monitoring for up to 30 min the fluorescence intensity at the maximum emission wavelength of the chelate. Thus, after each metal addition, the solution was equilibrated for the required time. Aqueous solutions of perchloric acid were used to operate at pH = 2 or 4, rather than buffers, because of possible competitive complexation by the buffer components. Similarly, the ionic strength was not fixed by an external salt, because of possible fluorescence quenching effects by the electrolyte ions. Thus, the ionic strength, mainly due to perchloric acid, was 10^{-2} and about 10^{-4} mol L^{-1} for gallium and aluminium complexation, respectively. The solutions were maintained at 25 °C. Regarding the complexations of Zn(II) and Cd(II), they were carried out at a total ligand concentration $C_0 = 1.82 \times 10^{-5}$ mol L^{-1} for O-TRENSOX and $C_0 = 1.93 \times 10^{-5}$ mol L^{-1} for n-BUSOX.

The UV-visible absorption spectra were recorded either with a Uvikon-940 KONTRON spectrophotometer or with a CARY 5E (Varian). For each titration, 30 to 35 spectra corresponding to progressive additions of metal ion were recorded. The spectrophotometric data were globally analyzed by the program SPECFIT to determine the stoichiometries and the stability constants of the chelates. In the case of n-BUSOX titration, at least 14 to 18 spectra were recorded below R = [metal]/[ligand] = 1, in order to improve the quality of the analysis in the range R = 0–1 and the accuracy of the stability constants of the possible 1 : 3 and 1 : 2 chelates.

Corrected fluorescence spectra were obtained from either a SLM 8000 C spectrofluorometer or a SPEX 1681 Fluorolog. Excitation wavelengths were either 340 or 350 nm, for complexations with n-BUSOX, and 390 nm, for complexations with O-TRENSOX. When spectral evolution was to be characterized, the spectra were normalized to the same absorbance at the excitation wavelength. The fluorescence quantum yields of the free ligands or chelates were measured from solutions where the concentration of every ligand or chelate was $\approx 2 \times 10^{-5}$ mol L^{-1}, using quinine sulfate dihydrate (NIST 936a) in 0.5 mol L^{-1} H_2SO_4 ($\Phi_0 = 0.544$) as the standard.[25]

Results and discussion

Deprotonation constants

8-HQS is a bifunctional compound whose pK_a values slightly depend on ionic strength; moreover, its reported pK_a values may differ somewhat according to the method used for the determination (*i.e.*, potentiometry or spectrophotometry). Reasonable pK_a values for ionic strengths between 0 and 0.1 mol L^{-1} can be obtained by working out the average of the pK_a values collected in ref. 26 from Martell's constants tables,[27] which gives: pK_a (=NH$^+$–/=N–) = 4.00 ± 0.15 and pK_a (–OH/–O$^-$) = 8.5 ± 0.2 (Table 1).

In n-BUSOX, the pK_a values determined in this work are pK_a (=NH$^+$–/=N–) = 2.8 ± 0.1 and pK_a (–OH/–O$^-$) = 6.8 ± 0.1 (see Experimental and Table 1). The fully protonated species, denoted (nBS)H_2, and the monoprotonated form denoted (nBS)H$^-$ are both further involved in chelation equilibria. The global charges take into account the negative

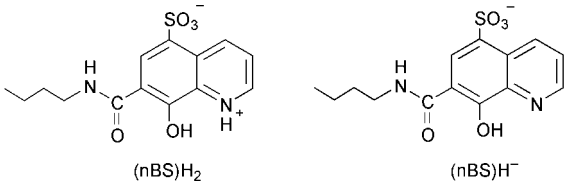


Table 1 Deprotonation constants of the ligands (25 °C)

	pK_a		
	8-HQS[a]	n-BUSOX[b]	O-TRENSOX[c]
=NH$^+$–/=N–[d]	4.0	2.8	1.8 2.5 2.9
–NH$^+$/–N[e]	—	—	5.8_6
–OH/–O$^-$	8.5	6.8	7.2 7.9 8.1

[a] Average values from ref. 26. [b] This work. Accuracy: ±0.1 pK_a units. [c] G. Serratrice, personal communication. [d] Heterocyclic nitrogen atom. [e] Tertiary amine group of O-TRENSOX.

charge of the sulfonate group, the sulfonic acid group being deprotonated between pH ≈ 0 and pH ≈ 1. The pK_a values are lower than those of 8-HQS, because of the electron-withdrawing character of the carbonyl group, which increases the mobility of the leaving protons.

A similar effect is observed in O-TRENSOX, which possesses seven acido-basic functions (Table 1).[18b] The fully protonated form will be further denoted $(OTR)H_7^{+}$, and the species obtained by consecutive deprotonations of the pyridinium groups, which can be involved in complexation reactions at either pH ≈ 2 or 4, will be denoted $(OTR)H_6$, $(OTR)H_5^{-}$, $(OTR)H_4^{2-}$ (the global charges again take into account the charges of the three sulfonate groups). Below pH = 5, the tertiary nitrogen atom is protonated and cannot participate in coordination of metal ions.

The "hard" cations Al^{3+} and Ga^{3+} strongly polarize the solvated water molecules, giving rise to hydrolysis. The corresponding overall equilibrium constants β[28,29] are given in Table 2. For both cations, the stability of the soluble tetrahydroxometallate $M(OH)_4^{-}$ forms must be emphasized. In an aqueous solution where the total concentration of Al(III) or Ga(III) is 1.00×10^{-6} mol L^{-1}, formation of $Al(OH)_4^{-}$ occurs above pH = 7 (at ionic strength $I = 0.1$ mol L^{-1}),[28] and that of $Ga(OH)_4^{-}$ above pH = 4.2 (calculated at $I = 0$ from β values in ref. 29).

Optimal pH range for observation of the fluorescence emission from the aluminium and gallium chelates

Each ligand possesses then several acido-basic forms, and each metal ion several hydrolytic species. Consequently, according to the pH of the medium, various equilibria can underlie the complexation process whose efficiency may depend on acidity. In fact, hydronium ions compete with the metal ions for binding to the coordination sites of the ligand, and hydroxyl ions compete with the ligand to coordinate the metal ions.

With fluorogenic ligands, a simple way of investigating and depicting the influence of pH on the efficiency of complexation is to study the variation of the fluorescence emitted by chelates as a function of pH. Moreover, for analytical purposes, it is necessary to know the optimum pH for complex detection. This study was then carried out prior to any other.

Chelates of both Al(III) and Ga(III) with O-TRENSOX, n-BUSOX and 8-HQS, respectively, were formed for ratios R = [metal]/ligand] of *ca.* 23 for O-TRENSOX (*ca.* 7.5 per 8-HQS subunit) and *ca.* 7.5 for n-BUSOX and 8-HQS. Metal ions were in excess, because it was observed that the fluorescence signal was improved in such conditions (see below). Fluorescence spectra were recorded on excitation at 340 nm from pH = 1 to ≈7, either in diluted perchloric acid or in GOOD® buffers, thus minimizing competitive complexation of Al(III) and Ga(III) by the non-fluorogenic components of the buffer itself[30] (complexation of aluminium was indeed observed with acetate and phosphate buffers, see Experimental). The spectra were then normalized such as to correspond to the same absorbance at the excitation wavelength.

Whatever the conditions, the emission spectra showed only one wide band. The fluorescence signals of the free ligands in the same conditions were very low (see below). The consequence is a high sensitivity of the fluorescence emission of the ligands to complexation of most possible metal ion impurities, in the absence of aluminium or gallium. Because some metals form highly fluorescent chelates with 8-HQS, the presence of such metal impurities even at concentrations as low as 0.1% can multiply the emission of the "free ligand" by a factor of 2 or more. Consequently, we did not evaluate the fluorescence enhancement on chelation by the ratio of the fluorescence emissions of chelates *vs.* ligands (at constant wavelength), but rather by the intensity of the sole fluorescence emission of chelates at their maximum emission wavelength (*ca.* 480 nm for aluminium chelates and *ca.* 500 nm for gallium chelates, see below).

Bell-shaped variations of the fluorescence emission of chelates as a function of pH were observed (not shown). For the three ligands, the maxima were around pH = 4 for Al(III) and pH = 2 for Ga(III), consistent with previous results for chelation of Al(III) and Ga(III) by 8-HQS.[31] Because metal ions were introduced in excess, hydroxide precipitation occurred when the pH was increased above pH ≈ 5–6 for Al(III) and pH ≈ 3 for Ga(III). Consequently, the present study was carried out only at the optimum pH values obtained above, because many of the experiments reported here required the [metal]/[ligand] ratio to vary from a large excess of ligand to a large excess of metal. Solutions were unbuffered and pH was monitored by addition of perchloric acid (see Experimental).

Stability constants of aluminium complexes

Spectrophotometric titrations of n-BUSOX by Al(III) solutions were carried out at a total ligand concentration of either $C_0 = 1.93 \times 10^{-5}$ mol L^{-1} (pH = 4.02 ± 0.08) or $C_0 = 4.95 \times 10^{-5}$ mol L^{-1} (pH = 3.70 ± 0.07). For these pH values, more than 90% of the ligand is in the $(nBS)H^{-}$ form (see pK_a values in Table 1). The ratio R = [Al(III)]/[ligand] was varied from 0 to 100. The spectra obtained with the largest C_0 concentration are displayed in Fig. 1, for $R \leqslant 2.5$. During complexation, the band of the free ligand located at 420 nm (very likely due to the tautomeric form of the hydroxyquinoline moiety)[10] disappeared, and the band at 279 nm shifted to 267 nm. No additional band specific to the chelate(s) appeared at long wavelengths. Most of the changes occurred between $R = 0$ and $R = 1$, and the isosbestic points appearing at 236,

Table 2 Equilibrium constants for hydroxo Al(III) and Ga(III) complexes (25 °C)

	Al(III)[a]	Ga(III)[b]
log β_1 [c]	8.21[d]	11.4[e]
log β_2 [d]	19.0[f]	22.1[e]
log β_3 [g]	27.0[h]	31.7[e]
log β_4 [i]	31.4[h]	39.4[e]
log K_{sp} [j]	−33.5	−35.7

$K_{sp} = [M^{3+}][OH^{-}]^3$. [a] From ref. 28. [b] From ref. 29.
[c] $\beta_1 = \dfrac{[MOH^{2+}]}{[M^{3+}][OH^{-}]}$. [d] $\beta_2 = \dfrac{[M(OH)_2{}^{+}]}{[M^{3+}][OH^{-}]^2}$.
[e] $I = 0$ M. [f] Ionic strength $I = 0.6$ M. [g] $\beta_3 = \dfrac{[M(OH)_3]}{[M^{3+}][OH^{-}]^3}$.
[h] $I = 0.1$ M. [i] $\beta_4 = \dfrac{[M(OH)_4{}^{-}]}{[M^{3+}][OH^{-}]^4}$. [j] Solubility product:

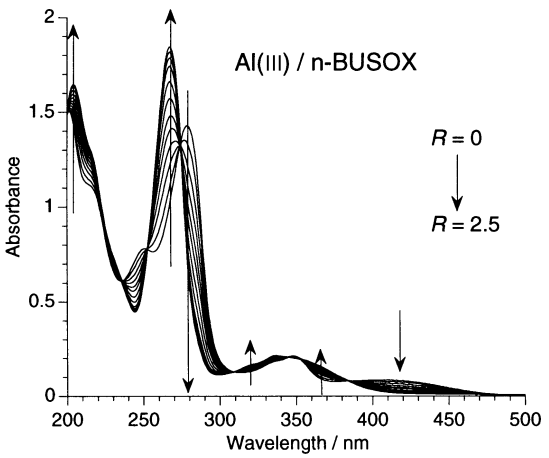


Fig. 1 Absorption spectra recorded upon complexation, at pH ≈ 3.7, of Al(III) by n-BUSOX as a function of R = [Al(III)]/[n-BUSOX] in the range $R = 0$ to 2.5. Ligand concentration: $C_0 = 4.95 \times 10^{-5}$ mol L^{-1}.

252, 274, 309, 344, 353 and 385 nm at low R values became a little blurred when R increased (not shown), which means that different stoichiometries of chelates may exist. Beyond $R = 2.5$, only slight changes were observed, mainly a continuous increase of the band at 267 nm, which levelled off for $R \geqslant 55$.

As for oxinates, three complexes of aluminium with the bidentate ligand n-BUSOX are expected to be formed competitively: $Al(nBS)_3^{3-}$, $Al(nBS)_2^{-}$ and $Al(nBS)^{+}$, with different predominance ranges when the ratio [Al(III)]/[ligand] is increased (Scheme 1).

Considering that most of the aluminium is in the Al^{3+} form (see hydrolysis constants in Table 2), the main equilibria involved in complexation are:

$$3(nBS)H^- + Al^{3+} \rightleftharpoons Al(nBS)_3^{3-} + 3H^+$$

$$2(nBS)H^- + Al^{3+} \rightleftharpoons Al(nBS)_2^{-} + 2H^+$$

$$(nBS)H^- + Al^{3+} \rightleftharpoons Al(nBS)^{+} + H^+$$

However, the best fit of the spectral data by global analysis (using the SPECFIT program) either for the whole range of R values, or for $R \leqslant 2.5$, was obtained from a model where only the two 1 : 2 and 1 : 1 chelates are formed, whatever the C_0 value. In each case, the reconstruction, from the calculated equilibrium constants, of the absorption spectra of the free ligand and of the separate chelates was consistent with the experimental results, and constituted a criterion for the acceptability of the fit.

The formation equilibrium constants calculated by the program (at constant pH) are expressed by:

$$K_{11} = \frac{[Al(nBS)^+]}{[(nBS)H^-][Al^{3+}]}$$

$$K_{12} = \frac{[Al(nBS)_2^-]}{[(nBS)H^-]^2[Al^{3+}]}$$

The values of the overall stability constants β_{mlh} of the equilibria

$$mM + lL + hH \rightleftharpoons M_mL_lH_h$$

defined as:

$$\beta_{mlh} = \frac{[M_mL_lH_h]}{[M]^m[L]^l[H]^h}$$

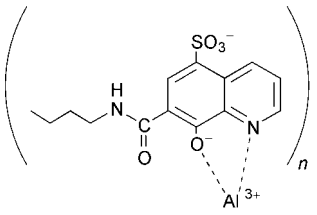
can be obtained from

$$pK_a(\text{–OH/–O}^-) = pK_1 = \frac{[(nBS)^{2-}][H^+]}{[(nBS)H^-]}$$

and from the equilibrium constants K_{1l} (*i.e.*, K_{11} and K_{12}). In the present case,

$$\log \beta_{1l0} = \log K_{1l} + lpK_1 - lpH$$

Thus $\log \beta_{110} = 8.4 \pm 0.3$ and $\log \beta_{120} = 16.2 \pm 0.6$. Unfortunately, the ligand concentrations for spectrophotometric titrations used here (1.93×10^{-5} or 4.95×10^{-5} mol L^{-1}) were too low for significant formation of the 1 : 3 chelate, and the β_{130} value could not be obtained from these experiments. Larger ligand concentrations are not suitable for fluorescence requirements; because we wanted to characterize chelations in possible conditions for fluorometric measurements, no further experiments were carried out such as to obtain β_{130}.



n = 1, 2 or 3
global charge: 3–2n

Scheme 1

Fig. 2 shows the distribution curves of the species (expressed as the ratio of their concentration C *vs.* C_0), in the case where $C_0 = 4.95 \times 10^{-5}$ mol L^{-1}, as a function of R. The maximum concentration of the 1 : 2 chelate corresponds to $R = 0.64$ and to about 45% of n-BUSOX engaged in this chelate, whereas 27.5% is already involved in the 1 : 1 chelate. Besides, it is worth noting that the stability constants are not large enough for aluminium to be quantitatively chelated at $R = 1$, even at the largest ligand concentration used here. In fact, examination of the distribution curves in Fig. 2 shows that for $R = 1$, the concentrations of the 1 : 2 and 1 : 1 chelates are 0.21 C_0 and 0.42 C_0, respectively, which corresponds to only 63% of aluminium chelated. Dilution is, of course, unfavorable to chelation, and at the lowest ligand concentration, $C_0 = 1.93 \times 10^{-5}$ mol L^{-1}, only 54% of aluminium was found to be complexed for $R = 1$.

The log β values obtained for the complexation of Al^{3+} by n-BUSOX are 0.8 to 1.4 units smaller than those reported for chelation by the ligand 8-HQS[26,32] (Table 3), thus indicating a slight decrease of oxinate stability when 8-HQS is a subunit of n-BUSOX. This observation likely results from the electron-withdrawing effect of the carbonyl group in n-BUSOX, which weakens the electron density on the complexing groups of the 8-HQS entity.

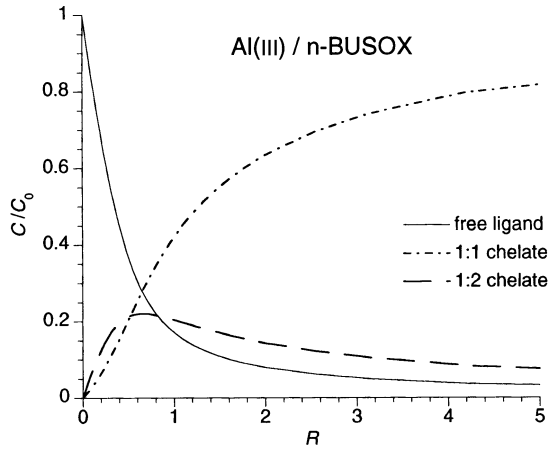


Fig. 2 Distribution curves of free n-BUSOX, in the range $R = 0$ to 5, and of its 1 : 2 and 1 : 1 aluminium chelates as a function of R = [Al(III)]/[n-BUSOX], calculated by the SPECFIT program from the spectral data. Ligand concentration: $C_0 = 4.95 \times 10^{-5}$ mol L^{-1}, pH ≈ 3.7.

Table 3 Overall stability constants for Al(III) and Ga(III) complexation by 8-HQS, n-BUSOX and O-TRENSOX (25 °C)

	Al(III)	Ga(III)
8-HQS	$\log \beta_{110} = 9.24 \pm 0.14$[a] $\log \beta_{120} = 17.61 \pm 0.24$[a] $\log \beta_{130} = 24.7 \pm 0.35$[a]	$\log \beta_{110} = 11.97$[b] $\log \beta_{120} = 23.44$[b] $\log \beta_{130} = 33.4$[b]
n-BUSOX	$\log \beta_{110} = 8.4 \pm 0.3$[c] $\log \beta_{120} = 16.2 \pm 0.6$[c]	$\log \beta_{110} = 11.6 \pm 0.43$[d] $\log \beta_{120} = 22.7 \pm 0.7$[d] $\log \beta_{130} = 32.7 \pm 1.1$[d]
O-TRENSOX	$\log \beta_{111} = 24.8 \pm 0.8$[c] $\log \beta_{211} = 30.4 \pm 1.0$[c] $\log \beta_{311} = 34.3 \pm 1.0$[c]	$\log \beta_{111} = 33.7 \pm 1.2$[d] $\log \beta_{211} = 40.5 \pm 1.3$[d] $\log \beta_{311} = 44.7 \pm 1.4$[d]

[a] From ref. 26 and 32 ($I = 0.1$ M, KNO_3). [b] From ref. 32 and 34 ($I = 0.1$ M, NaCl). [c] $I = 10^{-4}$ M. [d] $I = 10^{-2}$ M (see Experimental).

Let us now turn our attention to complexation of aluminium by the hexadentate ligand O-TRENSOX. Experiments were carried out at pH = 3.8 ± 0.1, the total ligand concentration was $C_0 = 1.82 \times 10^{-5}$ mol L^{-1}, and the ratio R = [Al(III)]/[ligand] was varied from 0 to 230. Fig. 3 shows the evolution of the absorption spectra in the range R = 0–12, which looks very similar to that obtained with n-BUSOX (Fig. 1). For $R > 12$, the spectral changes are minimal, indeed. Three ranges of R values can be distinguished, corresponding to three successive systems of isosbestic points whose wavelengths are given in parentheses: R = 0 to 1.04 (240, 251, 273, 306, 334, 357 and 392 nm), R = 1.04 to 3.02 (264, 280, 296 and 348 nm) and R = 3.02 to 230 (266, 278, 353 and 401 nm). This shows that three consecutive steps can be considered.

At pH = 3.8, O-TRENSOX is mostly in the $(OTR)H_4^{2-}$ form (see acidity constants in Table 1). When R is increased, the Al^{3+} ions may successively and/or competitively enter the tripod. Complexations can then be expressed by the following main global equilibria:

$$(OTR)H_4^{2-} + Al^{3+} \rightleftharpoons Al(OTR)H^{2-} + 3\,H^+$$

$$(OTR)H_4^{2-} + 2\,Al^{3+} \rightleftharpoons Al_2(OTR)H^{+} + 3\,H^+$$

$$(OTR)H_4^{2-} + 3\,Al^{3+} \rightleftharpoons Al_3(OTR)H^{4+} + 3\,H^+$$

The assumption that, at large R values, three Al^{3+} can be bound to one molecule of ligand, each arm of the tripod chelating one Al^{3+}, is buttressed by the lack of rigidity of the tripodal structure, all the more pronounced in that the keystone tertiary nitrogen atom is protonated and not involved in metal bonding, as already mentioned.

The best fit of the spectral data was obtained from the above model including the three chelates. The equilibrium constants calculated by the program (at constant pH) are expressed as follows:

$$K_{11} = \frac{[Al(OTR)H^{2-}]}{[(OTR)H_4^{2-}][Al^{3+}]}$$

$$K_{21} = \frac{[Al_2(OTR)H^{+}]}{[(OTR)H_4^{2-}][Al^{3+}]^2}$$

$$K_{31} = \frac{[Al_3(OTR)H^{4+}]}{[(OTR)H_4^{2-}][Al^{3+}]^3}$$

If the acidity constants of the –OH groups and of the tertiary amine nitrogen atom of O-TRENSOX (given in Table 1) are denoted pK_1, pK_2, pK_3 and pK_4, respectively, the logarithm of the overall complexation constant for m aluminium ions bound to the tripod is:

$$\log \beta_{m11} = \log K_{m1} - 3\mathrm{pH} + \sum_{i=1}^{4} pK_i$$

The best fits of the spectral data led to the following values: $\log \beta_{111} = 24.8 \pm 0.8$, $\log \beta_{211} = 30.4 \pm 1.0$, $\log \beta_{311} = 34.3 \pm 1.0$ (reported in Table 3).

The distribution curves of the species are displayed in Fig. 4. They show that the concentration of the 1 : 1 chelate is maximum when $R = 1$ and equal to *ca.* 0.73 C_0. Nevertheless, the 2 : 1 chelate is already present, and its concentration is 0.135 C_0. Aluminium, engaged within both complexes, is thus fully chelated.

Regarding the formation of the 1 : 1 chelate, the "tripod effect", that is the cooperative effect between the three arms of the ligand, is demonstrated by the enhancement of the stability constant by a factor of $10^{16.4}$ when O-TRENSOX is used instead of n-BUSOX. The large β_{111} constant for Al(III) complexation by O-TRENSOX is comparable to those obtained with other tripodal ligands, for example tripodal aminophenolate ligands studied by Caravan and Orvig,[17] whose stability constants are either $\approx 10^{22}$, or $\approx 10^{29}$, depending on whether the 1 : 1 chelates involve no proton or one proton.

A last comment must be made concerning the 3 : 1 chelate, which is formed for $R \geqslant 1.5$ (Fig. 4). Here the three arms of the ligand O-TRENSOX can move away from each other to accommodate three aluminium ions within the tripod. When observing the distribution curves, one can see that the rise in 3 : 1 chelate concentration is nevertheless rather moderate when R is increased. Large R values must be reached for the 3 : 1 chelate to prevail. For $R = 70$, only 90% of O-TRENSOX is involved in the 3 : 1 chelate, and for the largest aluminium excess used here, $R = 230$, it is 97% (distribution curves are not shown at large R values). This observation will be important when interpretating the evolution of fluorescence during metal ion additions (see below).

Stability constants of gallium complexes

Studies of gallium chelation by n-BUSOX and O-TRENSOX were carried out at pH = 2.00 ± 0.05. The main difference with the previous experiments was that the predominant forms of the ligands were either $(nBS)H_2$ or $(OTR)H_6$ (see acidity constants in Table 1). Progressive complexations were again followed by recording the absorption spectra as a function of the ratio R = [Ga(III)]/[ligand]. The spectra underwent similar changes as seen for Al(III) chelation when R was increased; in particular, isosbestic points occurred at nearly

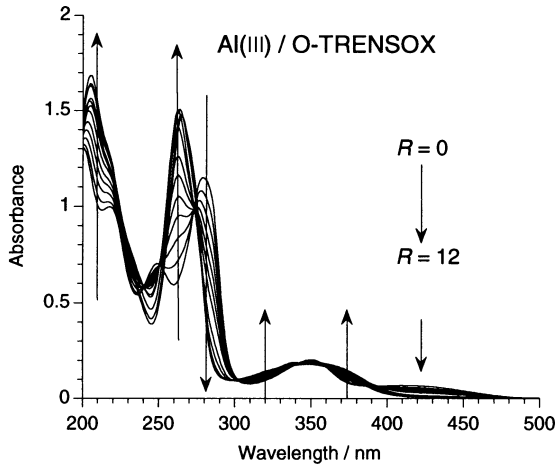


Fig. 3 Absorption spectra recorded upon complexation, at pH ≈ 3.8, of Al(III) by O-TRENSOX as a function of R = [Al(III)]/[O-TRENSOX] in the range R = 0 to 12. Ligand concentration: $C_0 = 1.82 \times 10^{-5}$ mol L^{-1}.

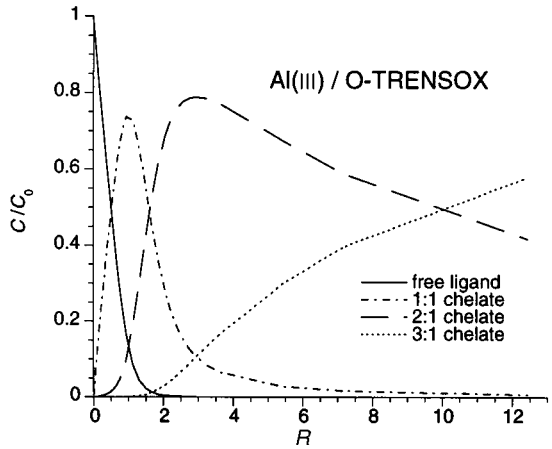
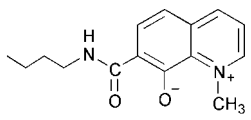


Fig. 4 Distribution curves of free O-TRENSOX, and of its 1 : 1, 2 : 1 and 3 : 1 aluminium chelates as a function of R = [Al(III)]/[O-TRENSOX] in the range R = 0 to 12, calculated by the SPECFIT program from the spectral data. Ligand concentration: $C_0 = 1.82 \times 10^{-5}$ mol L^{-1}, pH ≈ 3.8.

the same wavelengths as reported above. Yet, with most of the nitrogen atoms of the hydroxyquinoline subunits being protonated at pH = 2, the spectra of the free ligands $(nBS)H_2$ and $(OTR)H_6$ are quite different from those of $(nBS)H^-$ and $(OTR)H_4^{2-}$, and look much more like those of the chelates than the spectra recorded at pH ≈ 4. The spectral evolutions throughout gallium additions to either n-BUSOX or O-TRENSOX solutions (see Fig. 5) were consequently less pronounced than for Al(III) complexation (*cf.* Fig. 1 and 3). During n-BUSOX titration, the main changes happened for $R \leqslant 2.5$ [Fig. 5(a)]. Beyond $R = 2.5$, evolutions were very slight and the spectra levelled off for $R \geqslant 55$. Concerning O-TRENSOX titration, no spectral evolution was noticeable beyond $R = 3.5$ [Fig. 5(b)].

In the case of n-BUSOX, the possibility of a salicylate-type complexation, involving the carbonyl and hydroxyl groups, must be discussed because such a complexation mode was observed at pH < 2 for ferric ions.[18b,33] However, we have just mentioned that there were no essential differences between the spectra of gallium and aluminium chelates, the latter involving quinolinate-type complexation, whereas a large spectral change between the two modes of chelation was reported in the case of Fe^{3+} ions.[18b] Moreover, the N-methylated derivative of n-BUOX, synthesized on that occasion (see Experimental) because it can only provide a salicylate-type complexation, did not show any real tendency for gallium chelation.

Consequently, only a quinolinate-type complexation was considered. However, three complexes could be shown to be



N-Methylated derivative of n-BUOX

competitively formed on increasing R: $Ga(nBS)_3^{3-}$, $Ga(nBS)_2^-$ and $Ga(nBS)^+$. The analysis of the spectral data by SPECFIT gave the formation constants K_{1l} (K_{11}, K_{12} and K_{13}) expressed by:

$$K_{11} = \frac{[Ga(nBS)^+]}{[(nBS)H_2][Ga^{3+}]}$$

$$K_{12} = \frac{[Ga(nBS)_2^-]}{[(nBS)H_2]^2[Ga^{3+}]}$$

$$K_{13} = \frac{[Ga(nBS)_3^{3-}]}{[(nBS)H_2]^3[Ga^{3+}]}$$

The logarithms of the overall stability constants were again calculated from the acidity constants [pK_1 and $pK_2 = pK_a$ (=NH^+–/=N–)] and the K_{1l} constants, according to:

$$\log \beta_{1l0} = \log K_{1l} + l(pK_1 + pK_2) - 2l\text{pH}.$$

The obtained values were $\log \beta_{110} = 11.6 \pm 0.4$, $\log \beta_{120} = 22.7 \pm 0.7$, $\log \beta_{130} = 32.7 \pm 1.1$. The distribution curves of the chelates are shown in Fig. 6(a). They show that the maximum concentration of the 1 : 3 chelate (0.085 C_0) occurs

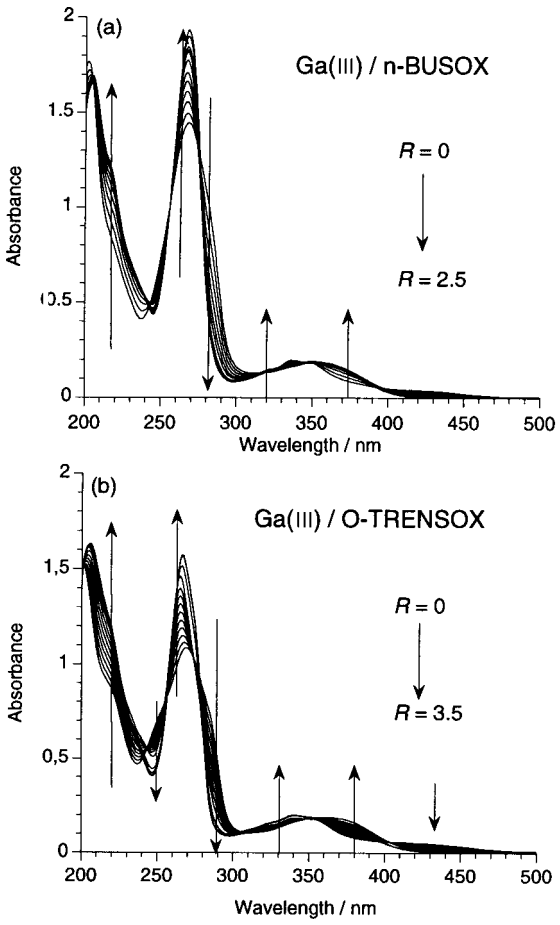


Fig. 5 Absorption spectra recorded upon complexation at pH = 2 of Ga(III): (a) by n-BUSOX as a function of R = [Ga(III)]/[n-BUSOX] in the range $R = 0$ to 2.5. Ligand concentration: $C_0 = 4.95 \times 10^{-5}$ mol L^{-1}; (b) by O-TRENSOX as a function of R = [Ga(III)]/[O-TRENSOX] in the range $R = 0$ to 3.5. Ligand concentration: $C_0 = 1.82 \times 10^{-5}$ mol L^{-1}.

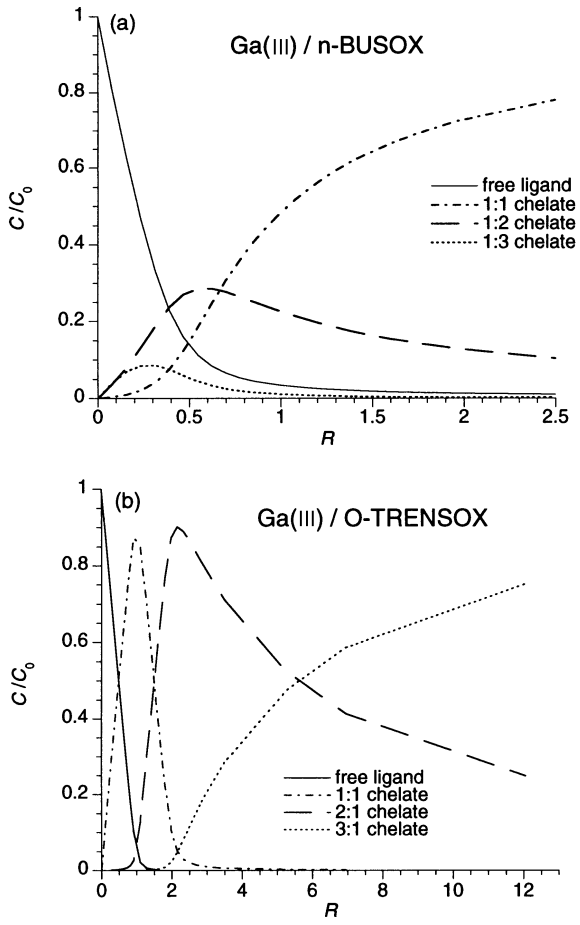


Fig. 6 Distribution curves, calculated by the SPECFIT program from the spectral data, at pH = 2, of: (a) the free ligand and the 1 : 3, 1 : 2 and 1 : 1 chelates of Ga(III) and n-BUSOX as a function of R = [Ga(III)]/[n-BUSOX] in the range $R = 0$ to 2.5. Ligand concentration: $C_0 = 4.95 \times 10^{-5}$ mol L^{-1}; (b) the free ligand and the 1 : 1, 2 : 1 and 3 : 1 chelates of Ga(III) and O-TRENSOX as a function of R = [Ga(III)]/[O-TRENSOX] in the range $R = 0$ to 12. Ligand concentration: $C_0 = 1.82 \times 10^{-5}$ mol L^{-1}.

at $R \approx 0.3$, whereas the maximum concentration of the 1 : 2 chelate (0.285 C_0) is observed at $R \approx 0.55$. For $R = 1$, the three chelates 1 : 1, 1 : 2 and 1 : 3 are present at concentrations of 0.48 C_0, 0.225 C_0 and 0.01 C_0, respectively. Thus, 71.5% of Ga(III) is chelated. It is worth noting that carrying out the titration with a ligand solution diluted by a factor of 2.5 (*i.e.*, $C_0 = 1.93 \times 10^{-5}$ mol L^{-1}) did not permit fitting of the data with the three-chelate model, but rather with a model consisting only of $Ga(nBS)_2^-$ and $Ga(nBS)^+$. The values of the formation constants K_{11} and K_{12} obtained from the analysis were consistently equal (within the experimental errors) to those obtained at higher ligand concentration, when the third chelate is formed.

Table 3 allows us to compare these values with the stability constants of the chelates of 8-HQS and Ga(III),[32,34] and to again see a slightly weakened stability when the 8-HQS unit is included in n-BUSOX as the ligand, in line with the previous observation for Al(III) complexation.

Concerning O-TRENSOX titration, the analysis showed that the three chelates $Ga(OTR)H^{2-}$, $Ga_2(OTR)H^+$ and $Ga_3(OTR)H^{4+}$ were unambiguously formed and gave the corresponding formation constants K_{11}, K_{21} and K_{31}; calculation of the overall stability constants was again carried out, using the sum of all the O-TRENSOX acidity constants, pK_i, except for the smallest (see Table 1), according to:

$$\log \beta_{m11} = \log K_{m1} - 5\mathrm{pH} + \sum_{i=1}^{6} pK_i$$

The following values were obtained: $\log \beta_{111} = 33.7 \pm 1.2$, $\log \beta_{211} = 40.5 \pm 1.3$, $\log \beta_{311} = 44.7 \pm 1.4$ (reported in Table 3). The "tripod effect" relative to the formation of 1 : 1 chelates is now represented by an increase by a factor of $10^{22.1}$ when using O-TRENSOX for gallium complexation, rather than n-BUSOX. Concerning the $\log \beta_{111}$ value, it is only one unit larger than the value of $\log \beta_{130}$ obtained with n-BUSOX, within experimental errors. This may be related to the different arrangement of the three asymmetric bidentate 8-HQS subunits around the metal ion in the 1 : 3 chelate with n-BUSOX (*mer* isomer) and in the 1 : 1 chelate with O-TRENSOX (*fac* isomer).[35]

Comparison between the stability of similar chelates (*i.e.*, with the same ligand and the same stoichiometry) of aluminium and gallium, shows the larger stability of gallium complexes over aluminium chelates, already reported for 8-HQS[32] or other tripods.[17] Besides, once again, the stability constant β_{111} of the gallium 1 : 1 chelate formed with O-TRENSOX is comparable to that reported for other tripodal ligands.[17]

It must also be mentioned that $\log \beta_{111}$ is only 2.8 units below the stability constant of the 1 : 1 chelate between Fe(III) and O-TRENSOX ($\log \beta_{111} = 36.5 \pm 0.1$).[18b] In fact, Ga(III) and Fe(III) cations are structurally quite similar: ionic radii are 0.645 Å for Fe(III) and 0.620 Å for Ga(III); moreover, their hydrolysis constants and their rate constants for exchange of a water molecule from the first coordination shell are close to each other.[35]

The distribution curves of the species when complexation of gallium was carried out by O-TRENSOX are displayed in Fig. 6(b). They give rise to similar comments as for aluminium chelation; however, the improved stability of the gallium complexes, compared to the aluminium ones, underlies the distribution of every species and is exhibited, for instance, by the larger maximum concentrations of Ga(III) chelates compared to Al(III) chelates (*cf.* Fig. 4). The maximum concentration of the 1 : 1 chelate (0.87 C_0) is obtained for $R = 1$, the remaining ligand being shared between the 2 : 1 chelate (0.065 C_0) and the uncomplexed form (0.065 C_0), whereas the maximum concentration of the 2 : 1 chelate (0.89 C_0) is obtained for R = 2.2. Although the total concentration of ligand ($C_0 = 1.82 \times 10^{-5}$ mol L^{-1}) is small, complexation is very effective. Above $R = 3.5$, there is no 1 : 1 chelate present and the slow conversion of the 2 : 1 into the 3 : 1 chelate induces no appreciable changes in the spectra.

Fluorescence emission from the aluminium and gallium chelates

The fluorescence spectra of the chelates were recorded in the same ranges of [metal]/[ligand] ratios as those used for the spectrophotometric titrations. Thus, when large excesses of metal were added, the predominant species were either the 1 : 1 chelate in the case of n-BUSOX, or the 3 : 1 chelate in the case of O-TRENSOX, with opening up of the tripod. In these chelates, the 1 : 1 stoichiometry for metal ion and complexing 8-HQS subunits was attained. It is important to keep this in mind for the discussion below.

Figs. 7(a) and (b) display the spectral evolutions during Al(III) and Ga(III) chelations by n-BUSOX. The increases in fluorescence intensity at the maximum emission wavelengths are shown in Fig. 7(c) as a function of metal ion addition, for both cations. Replacing n-BUSOX by O-TRENSOX modified neither the shape of the spectra, nor the wavelength emission range of the chelates; the evolution of the fluorescence intensity was rather similar to that in Fig. 7, but the level of emitted fluorescence was approximatively ten times lower. Fig. 7 shows that the fluorescence emission levelled off above $R \approx 55$ for both Al(III) and Ga(III) complexation by n-BUSOX. With O-TRENSOX as the ligand, the maximum fluorescene emission was reached for $R = 3.5$ for Ga(III) chelation, whereas $R \approx 230$ was required for Al(III) chelation, consistent with the evolution of the absorption spectra (see above). No significant spectral shifts occurred upon progressive addition of metal ions. The wavelengths of the emission maxima are given in Table 4; they are ≈480 nm for the aluminium chelates, and ≈500 nm for the gallium chelates, independent of the ligand, n-BUSOX or O-TRENSOX, as already reported above for optimal pH determination. Table 4 also indicates the emission maxima for oxinates formed with 8-HQS, which shows the hypsochromic shifts (≈20–30 nm) of chelate emis-

Table 4 Fluorescence maximum wavelength (λ_{max}), quantum yield (Φ_F), molar absorption coefficient [$\varepsilon(\lambda_{max})$] and the value of the product $\varepsilon(\lambda_{max}) \cdot \Phi_F$, for Al(III) and Ga(III) 1 : 1 chelates of 8-HQS and n-BUSOX and 3 : 1 chelates of O-TRENSOX, formed at large R values [that is 1 : 1 chelates between 8-HQS, or 8-HQS sub-units, and Al(III) or Ga(III)]

	Al(III)			Ga(III)		
pH	4			2		
Ligand	8-HQS	n-BUSOX	O-TRENSOX	8-HQS	n-BUSOX	O-TRENSOX
Fluo. λ_{max}/nm[a]	510	483	479	522	500	495
Φ_F	0.105 ± 0.005	0.36 ± 0.02	0.039 ± 0.002	0.048 ± 0.002	0.22 ± 0.01	0.019 ± 0.001
Abs. λ_{max}/nm	356	337	340	361	337	340
$\varepsilon(\lambda_{max})$[b]	3220	3900	10 600	3060	3900	10 450
$\varepsilon(\lambda_{max}) \cdot \Phi_F$	340	1400	415	150	860	200

[a] ±2 nm (flat maxima). [b] Accuracy: ±5%.

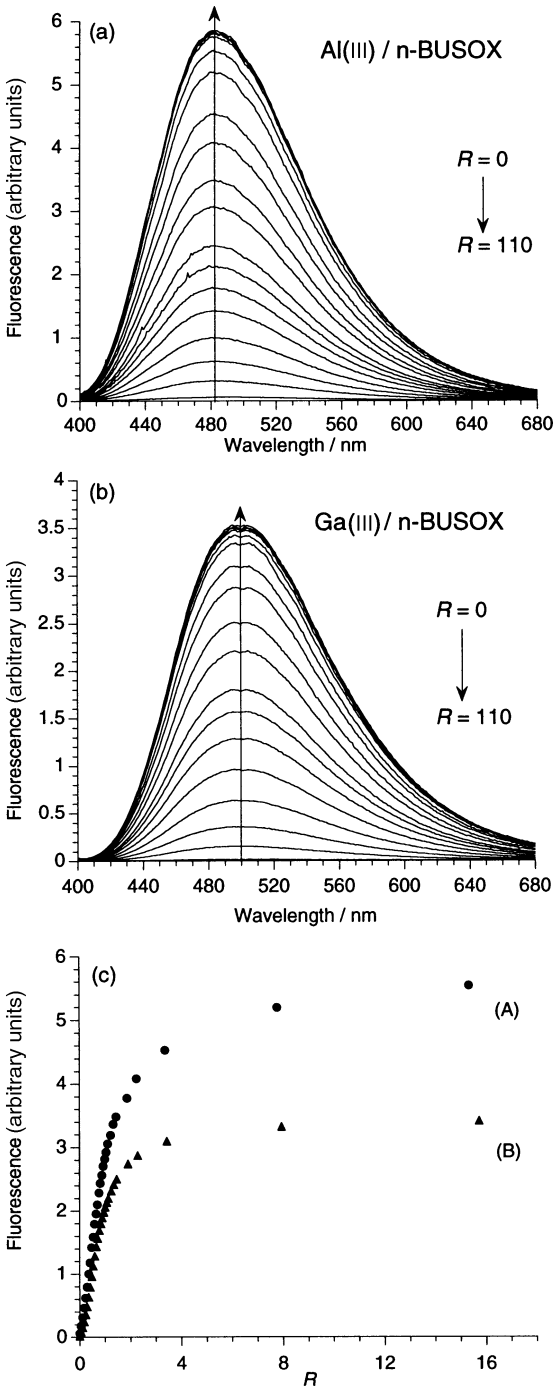


Fig. 7 Corrected fluorescence spectra recorded upon complexation by n-BUSOX of: (a) Al(III) at pH = 4; (b) Ga(III) at pH = 2, as a function of R = [metal]/[n-BUSOX] in the range $R = 0$ to 110. From $R = 55$ to 110, the spectra are superimposed. Ligand concentration: $C_0 = 1.93 \times 10^{-5}$ mol L^{-1}, $\lambda_{exc} = 350$ nm. (c) Increase in the fluorescence intensity, measured at the maximum emission wavelength λ_{fluo}, as a function of R: (A) Al(III) chelation, $\lambda_{fluo} = 483$ nm; (B) Ga(III) chelation, $\lambda_{fluo} = 500$ nm.

sions when the 8-HQS units are involved in n-BUSOX and O-TRENSOX. These shifts are a typical consequence of the electron-withdrawing character of the carbonyl group on the hydroxyquinoline ring.

Concerning the enhancement of the fluorescence intensity when the metal ion is progressively added and complexed by the ligand, the increase is monotonous whatever the stoichiometries of the successive chelates. This may seem surprising at first sight. Nevertheless, this observation can be interpreted in light of the processes underlying the fluorescence behavior of hydroxyquinoline: emission is very weak in neutral media, but arises in concentrated acidic media, when the emitting species is the hydroxyquinolinium ion.[10] In fact, chelation by metal ions, acting similarly to protons, impairs the photoinduced charge transfer from the oxygen atom to the pyridinium ring that, in the free ligands, accompanies tautomerization and leads to fluorescence quenching. Therefore, chelation prompts fluorescence.[15] The larger the positive charge density of the metal ion, the stronger this effect, and the greater the fluorescence enhancement. Consequently, the trivalent aluminium or gallium ions are good candidates for off–on switching of fluorescence upon complexation, as already observed[4] and mentioned in the introduction. Moreover, one aluminium or gallium ion bound per 8-HQS subunit is more efficient than one ion simultaneously bound to two or three 8-HQS, which explains the monotonous fluorescence increase upon addition of metal ions until a 1 : 1 stoichiometry between the metal ions and complexing 8-HQS subunits is reached.

The fluorescence quantum yields Φ_F of the ultimate chelates, measured at large R values, indicate the maximum fluorescence emission that can be attained on complexation of the ligands. Quantum yields of aluminium and gallium 1 : 1 oxinates formed with 8-HQS itself were also determined, so as to demonstrate the actual consequences of including 8-HQS into the ligands n-BUSOX and O-TRENSOX. The results are reported in Table 4.

In every case, the fluorescence quantum yields of the free ligands were measured and found to be: for n-BUSOX, $\Phi_F^L = 1.3 \times 10^{-3}$ (pH = 2) and 1.5×10^{-3} (pH = 4); for O-TRENSOX, $\Phi_F^L = 3 \times 10^{-4}$ (pH = 2) and 8×10^{-4} (pH = 4). For 8-HQS, Φ_F^L was equal to 3×10^{-3} (pH = 4). Because of the difficulty of eliminating any metallic impurity leading to a fluorescent chelate (see above), we will not claim that these values are the absolute quantum yields of the "free ligands". Nevertheless, fluorescence enhancements upon complexation are really dramatic, and increase in the order O-TRENSOX < 8-HQS < n-BUSOX.

The disappointing fluorogenic character of O-TRENSOX appears clearly when observing that the Φ_F values of the 1 : 1 chelates formed with n-BUSOX are approximately tenfold larger than those of the corresponding 3 : 1 chelates formed with O-TRENSOX, whereas in both cases every 8-HQS unit is bound to one metal ion (Table 4). Consequently, when the chelated 8-HQS moieties are brought together into the tripod, there is an unfavorable "self-quenching" effect that is superimposed onto the fluorogenic character of O-TRENSOX. Therefore, the increase in fluorescence intensity when going from 1 : 3 to 1 : 1 chelates with n-BUSOX, and from 1 : 1 to 3 : 1 chelates with O-TRENSOX, may additionally result from the decrease in the self-quenching effect when the metal–hydroxyquinoline moieties move away from each other. Nevertheless, they always remain linked and in mutual interaction within the tripod, whereas they can get free from each other in the case of n-BUSOX. Thus, the rise in fluorescence intensity on metal chelation results from the complex interplay of two concomitant photophysical effects.

The self-quenching effect is consistent with the results of a previous study carried out on 8-HQS chelates. Indeed, the fluorescence lifetimes of Al(III), Ga(III) and In(III) chelates were shown to decrease when the ligand-to-metal ratio was increased.[36] Moreover, the increasing interaction between the neighboring chromophores, when going from 1 : 1 to 1 : 2 and 1 : 3 Al(III) complexes with 8-HQ, had been previously demonstrated by the polarization of the fluorescence.[37] Similar self-quenching effects were also reported for other multichromophoric chelating agents based on coumarins linked to crown ethers[38a] or on naphthalenic fluorophores bound to calixarenes.[38b] However, a tripodal ligand containing three dansyl chromophores was recently reported to exhibit no intramolecular interactions, which shows that

chromophores involved in tripodal structures are not necessarily interacting. In the absence of interaction, the molar absorption coefficient is three times larger than that of the single chromophore; moreover, lifetime and quantum yield are unchanged by insertion into the tripod.[39]

Two final additional comments are still to be made when reading Table 4: (i) the favorable effect of the electron-withdrawing carbonyl group on the fluorescence efficiency is shown by an improvement in chelate Φ_F by factors of 3 or 4 and (ii) the larger fluorescence quantum yields for the Al(III) chelates compared to the equivalent Ga(III) chelates are consistent with the mechanism of fluorescence triggering described above. In fact, the Ga(III) ion possesses a smaller charge density than Al(III) because its radius is larger, leading to a smaller efficiency in triggering the fluorescence of hydroxyquinolines.

What about the fluorogenic behaviour of the 8-HQS subunits, after insertion into n-BUSOX or O-TRENSOX?

A full picture of the ability of the 8-HQS moieties to give rise to a fluorescence emission on chelation needs to take into account both molar absorption coefficients and quantum yields of the formed chelates. In fact, in dilute solutions, the intensity of the fluorescence emitted from a fluorescent species is proportional to both fluorescence quantum yield Φ_F and absorbance. Comparison between 8-HQS sub-units in terms of fluorometric response to metal ion complexation requires then comparison of the product $\varepsilon(\lambda_{exc})\cdot\Phi_F$, where $\varepsilon(\lambda_{exc})$ represents the molar absorption coefficient at the excitation wavelength in similar conditions of stoichiometries between the metal ion and 8-HQS ligand.

Because only a global comparison is needed in the present case, we will content ourselves with calculating the product $\varepsilon(\lambda_{max})\cdot\Phi_F$, $\varepsilon(\lambda_{max})$ being the molar absorption coefficient at the maximum absorption wavelength of the lowest energy band of every chelate formed at large R values, that is when the 1 : 1 metal ion : 8-HQS stoichiometry is attained. Thus, Table 4 also shows: (i) $\varepsilon(\lambda_{max})$ determined for each chelate and (ii) the product $\varepsilon(\lambda_{max})\cdot\Phi_F$ calculated from the Φ_F values. Regarding the molar absorption coefficients, no difference can be seen (within the experimental errors) between the complexes formed by a given ligand and either Al(III) or Ga(III). For a given metal ion, the O-TRENSOX chelates, as expected, exhibit larger values of the molar absorption coefficient, because of the presence of three chromophores, compared to the bidentate ligands. If the chromophores in O-TRENSOX were independent, the absorption coefficients of the complexes would be three times larger than those of the n-BUSOX chelates. This is not the case, but the discrepancy between the measured values and this ideal situation is only 10%. Nevertheless, because of the large quantum yields of the n-BUSOX chelates, n-BUSOX (*i.e.*, its 8-HQS subunit) clearly behaves as the best fluorogenic chelator of Al(III) and Ga(III), as shown by the $\varepsilon(\lambda_{max})\cdot\Phi_F$ product.

What about the fluorometric determination of aluminium or gallium using n-BUSOX or O-TRENSOX?

Can we now assess the actual improvements when using n-BUSOX or O-TRENSOX rather than 8-HQS, keeping in mind that the aim in using such ligands is metal determination under conditions where metal, although present in small quantities, has to be fully and selectively recovered by chelation?

In the ground state, the "tripod" effect on the stability constants is outstanding, in particular for Ga(III), allowing traces of metal ion to be complexed by O-TRENSOX. At the levels of concentrations used in this study, aluminium and gallium were fully complexed for $R = 1$, no excess ligand was then required. Unfortunately, the stability constants of the complexes with the bidentate ligands 8-HQS and n-BUSOX (present at concentrations not exceeding *ca.* 10^{-5}–10^{-4} mol L^{-1} because of the requirements of fluorometric measurements) are then not sufficient to quantitatively bind the metal.

In short, O-TRENSOX shows a cooperative effect of its three arms in metal binding and, from the standpoint of stability, is much more interesting than 8-HQS and n-BUSOX. Conversely, O-TRENSOX exhibits a disappointing fluorogenic character compared with 8-HQS and particularly with n-BUSOX. Hence, both ligands, n-BUSOX and O-TRENSOX, represent an improvement *vs.* 8-HQS, each of them for different reasons. Unfortunately, these two ligands do not combine their qualities.

Concerning the selectivity towards Al(III), severe interferences were reported when using 8-HQS, either with Fe(III) leading to fluorescence quenching, or for instance with Zn(II) at pH = 6.[5,6] The Zn(II) ion was, in fact, observed to form strongly fluorescent chelates with 8-HQS at pH = 6 or 7, as was Cd(II), also belonging to the IIB group.[4] On the contrary, no interference study was previously carried out for Ga(III) complexation at pH values as low as 2. Consequently, in the present work, a few experiments were carried out concerning the chelation of Fe(III), Zn(II) and Cd(II) by either n-BUSOX or O-TRENSOX, at pH = 2 and 4, respectively, in order to get an idea of some possible interferences when these ligands are used as fluorogenic agents of Ga(III) or Al(III).

Chelation of iron by O-TRENSOX was observed to be very efficient, and addition of Fe(III) to a fluorescent solution of 1 : 1 aluminium chelate (at pH = 4) or 1 : 1 gallium chelate (at pH = 2) led to quantitative fluorescence quenching. Such a quenching effect can even be used for iron determination. Al(III) or Ga(III) fluorometric determination is then impaired in the presence of Fe(III), without appropriate masking agents.

For Zn(II) and Cd(II), chelation experiments were carried out and followed as previously by recording the absorption and fluorescence spectra, either at pH ≈ 4 or at pH ≈ 2. Comparisons were simultaneously done (in similar experimental conditions) with Al(III) and Ga(III) chelations, at the respective pH values of 4 and 2.

At pH = 4, the evolution of the absorption spectra showed a decreasing stability of the chelates in the order Al(III) > Zn(II) > Cd(II). For example, Fig. 8 shows, for complexation by n-BUSOX at pH = 4, a close-up of the absorption band, which appears on chelation at ≈267 nm, at the expense of the free ligand band at ≈279 nm. The spectra

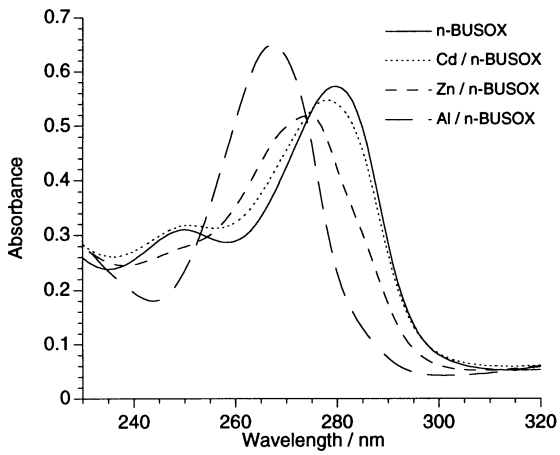


Fig. 8 Absorption bands in the 230–320 nm region of the spectrum of free n-BUSOX at pH = 4, and of the spectra obtained after additions of Cd(II), Zn(II) and Al(III) such that $R = 1$. Ligand concentrations $C_0 = 1.93 \times 10^{-5}$ mol L^{-1} or $C_0 = 1.82 \times 10^{-5}$ mol L^{-1}.

allow comparison of the spectral changes after additions of Cd(II), Zn(II) or Al(III) such that $R = 1$, confirming the order of stability given above. The stability constants were fully determined for chelation of Zn(II) by n-BUSOX. The obtained values: $\log \beta_{110} = 7.0 \pm 0.2$ and $\log \beta_{120} = 14.8 \pm 0.4$, are actually 1.4 units smaller than the corresponding constants for Al(III) chelation. Conversely, a strong fluorescence emission was observed with Zn(II) and Cd(II), in line with that reported for 8-HQS, making up for the lower chelate stabilities. Figs. 9(a) and (b) show the increase of the fluorescence intensity for Al(III), Zn(II) and Cd(II) complexes with n-BUSOX and O-TRENSOX, respectively, at pH = 4. The outstanding emission of zinc chelates prevents the determination of Al(III) in the presence of Zn(II) at pH = 4, and competitive effects were observed between Al(III) and Cd(II) complexations.

On the contrary, at pH = 2, only chelation of Ga(III) occurs significantly, and no real interfering signal is to be expected from the presence of Zn(II) and Cd(II), whatever the ligand, n-BUSOX [Fig. 9(c)] or O-TRENSOX [Fig. 9(d)]. Apart from Fe(III), the selectivity of n-BUSOX and O-TRENSOX towards Ga(III) is satisfactory at pH = 2.

Conclusions

O-TRENSOX, an excellent tripodal ligand of iron, incorporating three 8-hydroxy-5-sulfoquinoline (8-HQS) subunits, has been characterized as a fluorogenic ligand of aluminium and gallium, with the purpose of being used in metal determination. A new ligand, n-BUSOX, consisting of only one arm of O-TRENSOX, has been synthesized in order to be compared to O-TRENSOX and to bring out the specificity of the hexadentate tripod.

From the viewpoint of ground-state complexation and stability, it was possible to observe the 1 : 2 and 1 : 1 chelates of bidentate n-BUSOX as a function of the ratio R = [metal]/[ligand]. On the other hand, it was possible to form the 1 : 1, 2 : 1 and 3 : 1 complexes of O-TRENSOX. This ligand could thus accommodate three metal ions when metal was in large excess. The stability constants were measured and the large values obtained with O-TRENSOX confer to this compound a similar complexing ability as other tripodal ligands recently synthesized by Orvig's group.[17] Moreover, the stability constants were always improved when going from aluminium to gallium, chelation of gallium by O-TRENSOX being nearly as efficient as chelation of iron.[38b]

Regarding the fluorogenic properties, free O-TRENSOX was still less fluorescent than free 8-HQS and n-BUSOX. Fluorescence emission was triggered by complexation and the intensity was higher when aluminium was chelated rather than gallium. The mechanism of fluorescence enhancement is explained as resulting from the partial suppression of the photoinduced charge transfer within the quinoline nucleus upon excitation, because of the electrostatic influence of the bound cation. Therefore, Al^{3+}, whose charge density is larger than that of Ga^{3+}, is then the better candidate for fluorescence enhancement. Furthermore, the three chromophores of O-TRENSOX were shown to interact, which had two unfavorable effects on the expected properties of O-TRENSOX for ion detection: (i) the molar absorption coefficient was 10% less than three times that of n-BUSOX and (ii) above all, a strong self-quenching process occurred, lowering the quantum yield of chelates by a factor of ten, as compared with n-BUSOX. Thus, the latter ligand revealed itself a much better agent than O-TRENSOX for fluorometric determination of Al(III) and Ga(III), on the condition that the metal is fully

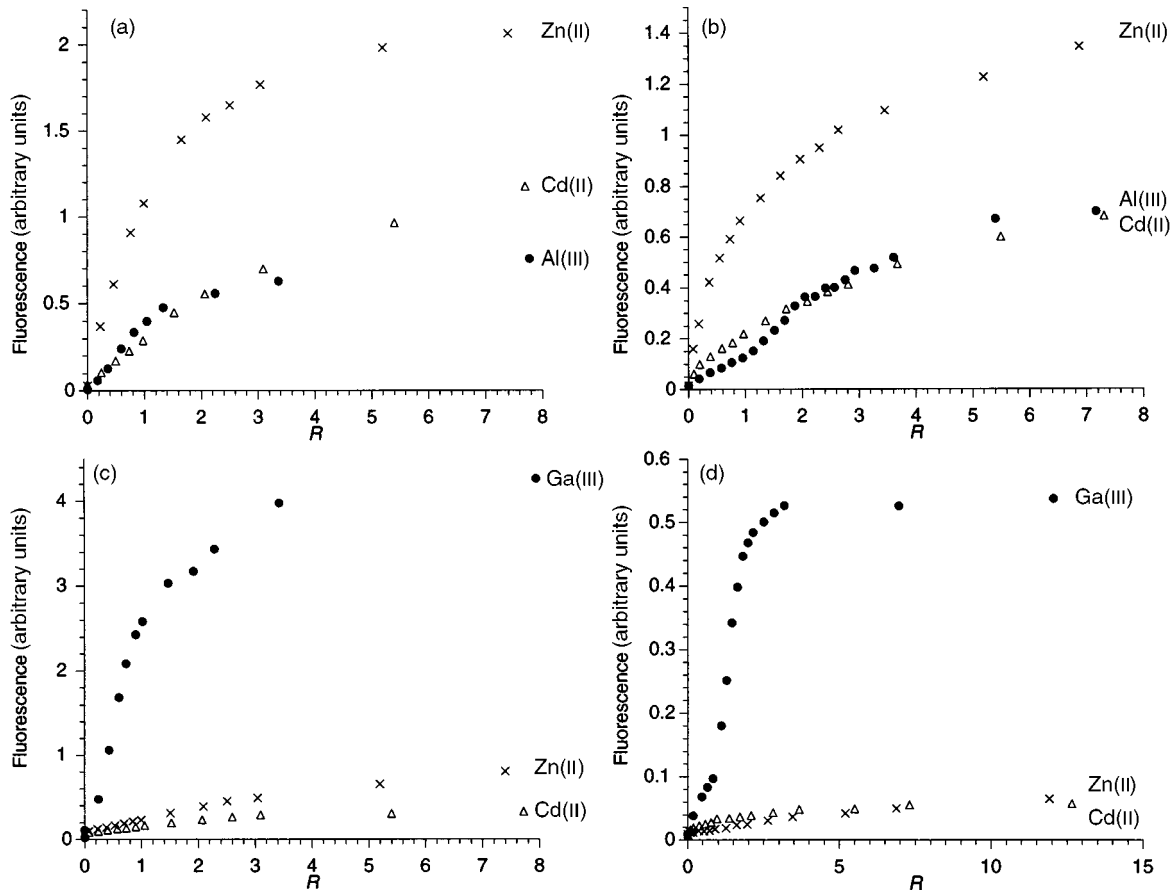


Fig. 9 Increase of the fluorescence intensity upon addition of the indicated metal ion as a function of R. (a) n-BUSOX at pH = 4, $\lambda_{exc} = 350$, $\lambda_{fluo} = 483$ nm. (b) O-TRENSOX at pH = 4, $\lambda_{exc} = 390$, $\lambda_{fluo} = 479$ nm, (c) n-BUSOX at pH = 2, $\lambda_{exc} = 350$, $\lambda_{fluo} = 500$ nm. (d) O-TRENSOX at pH = 2, $\lambda_{exc} = 390$, $\lambda_{fluo} = 495$ nm.

chelated. Compared to 8-HQS, n-BUSOX also exhibited much better performances for chelate fluorescence emission, which can be ascribed to the presence of the electron-withdrawing carbonyl group bound to position 7 of the nucleus; similarly to chelated metal ions, its role is to weaken the photoinduced charge transfer mentioned above that leads to fluorescence quenching.

Concerning the selectivity of n-BUSOX and O-TRENSOX, it was shown to be satisfactory for Ga(III) determination at pH = 2. At this pH, Zn(II) and Cd(II) do not interfere, but Fe(III) quenches the fluorescence signal. At pH = 4, for Al(III) determination, Zn(II) is strongly interfering and Cd(II) competes for the ligands.

In conclusion, both ligands O-TRENSOX and n-BUSOX are more interesting than their parent 8-HQS, for the different reasons already given. Gallium is more efficiently bound by every ligand, whereas aluminium gives the larger fluorescence emission. Because the global fluorescence signal obtained on chelation includes contributions in terms of stability and of fluorogenic effects, balanced results were observed in the present study where, as far as we are aware, the use of a tripodal fluorogenic ligand of aluminium and gallium is described for the first time.

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