Journal of Chromatography, 482 (1989) 351–359
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 831

LANTHANIDE LUMINESCENCE QUENCHING AS A DETECTION METHOD IN ION CHROMATOGRAPHY

CHROMATE IN SURFACE AND DRINKING WATER

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SUMMARY

Dynamic quenching of Eu(III) and Tb(III) luminescence by inorganic anions as a detection method in ion chromatography was investigated. To obtain a high luminescence intensity, lanthanide(III) complexes are formed with ligands which make indirect excitation of the ions possible. Only a few anions (*e.g.*, nitrite, chromate) induce efficient dynamic luminescence quenching. Chromate is an efficient quencher of Tb–acac luminescence. Samples of tap water and surface water, spiked with chromate, were injected into a high-performance liquid chromatographic system with post-column addition of the luminescent complex. In this way, a detection limit of $1.1 \cdot 10^{-7}$ *M* (13 ppb) of chromate could be obtained.

INTRODUCTION

In addition to conductivity detection, spectroscopic methods have been introduced in ion chromatography, the best known being absorption and fluorescence detection applied in both direct and indirect modes[1]. The indirect mode is based on the displacement of chromophoric or fluorophoric eluent anions by the analyte. In our laboratory we have developed another indirect spectroscopic detection technique, *viz.*, phosphorescence detection, which is not related to displacement effects. In this method, the dynamic quenching of a phosphorescence signal is induced by the analyte. The decrease in signal monitored is independent of the concentration of the phosphorescent compound. This is an important advantage over indirect methods based on displacement effects, where low concentrations of analyte can only be observed if low concentrations of the chromophoric or fluorophoric eluent anions are used. Applications of indirect detection by dynamic quenching of phosphorescence have been described, with biacetyl[2–5] and, to a minor extent, brominated naphthalenes as phosphorophores, present as a solute in the eluent[3,4,6]. The phosphorescence could only be observed if oxygen was removed from the high-performance

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0021-9673/89/$03.50

liquid chromatographic (HPLC) system, which was achieved by purging nitrogen through the eluent vessel and using stainless-steel capillaries in the experimental set-up.

In addition to the compounds mentioned, which display a long-lived phosphorescence in liquid solutions, there are also inorganic compounds that show long-lived luminescence in liquid solutions, *e.g.*, the lanthanide ions Eu(III) and Tb(III). An important advantage of this type of luminescence is that the intensities and associated lifetimes are hardly or not quenched by oxygen[7]. Preliminary results have led to the conclusion that a number of inorganic anions cause efficient dynamic quenching of lanthanide luminescence[8]. However, excitation of the lanthanide(III) ions is not effective because of their low absorptivities. This is a serious hindrance for its applicability in ion chromatography: the noise on a weak luminescence signal is relatively high, which implies that the attainable detection limits based on a signal decrease are unfavourable.

The purpose of this study was to improve the excitation of Eu(III) and Tb(III) by making use of complexation and indirect excitation via a ligand with high absorptivity. Further, dynamic quenching of these lanthanide complexes by inorganic anions was studied with emphasis on the compatibility of this principle with HPLC conditions. The relevance of the method was shown by investigating its applicability to the determination of chromate in surface and drinking-water samples. Many epidemiological studies have indicated that chromium(VI) is carcinogenic in humans[9]. The maximum allowable concentration in drinking water established in 1963 by the World Health Organization is 50 ppb, so that a method of analysis should give a detection limit down to about 10 ppb.

EXPERIMENTAL

Chemicals

The lanthanide salts $EuCl_3 \cdot 6H_2O$ (99.9%) and $TbCl_3 \cdot 6H_2O$ (99.9%) and 2-thenoyltrifluoroacetone were purchased from Aldrich (Milwaukee, WI, U.S.A.). Acetylacetone (>99%) was from Merck (Hohenbrunn, F.R.G.), Trizma base (reagent grade) from Sigma (St. Louis, MO, U.S.A.), tetrabutylammonium bromide from Kodak (Rochester, NY, U.S.A.) and K_2CrO_4 (99.5%) from Merck (Darmstadt, F.R.G.). Acetonitrile (Baker analyzed HPLC reagent), ethanol (Baker analyzed reagent), methanol (Baker analyzed HPLC reagent), sodium nitrite (Baker laboratory grade), $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ (Baker analyzed reagent) were obtained from Baker (Deventer, The Netherlands). Deionized water was distilled twice before use.

Instrumentation

Batch experiments were carried out with a Perkin-Elmer (Beaconsfield, U.K.) MPF 44 fluorescence spectrometer, supplied with a continuous XBO 150-W xenon lamp and two Hamamatsu type R777-01-HA photomultipliers.

The HPLC sysem consisted of a Gilson (Villers le Bel, France) 302 HPLC pump equipped with a Gilson 802c manometric module, a Valco six-port injection valve, a stainless-steel column (250 × 3.1 mm I.D. for nitrite separations, 150 × 3.1 mm I.D. for chromate separations) packed with 5-μm LiChrosorb RP-18 (Merck, Darmstadt,

F.R.G.), a Kratos (Ramsay, NY, U.S.A.) URS 051 post-column unit (pump and mixing device) and a Perkin-Elmer LS-2 filter fluorimeter. The Perkin Elmer LS-2 luminescence detector, containing a xenon discharge lamp pulsed at line frequency, was employed in the time-resolved phosphorescence mode with a delay time of 0.1 ms and a gating time of 2.0 ms. As the excitation filter a UG 11 filter (which has maximum transmission between 300 and 350 nm) was used; maximum emission wavelengths of the Eu(III) and Tb(III) complexes were 614 and 545 nm, respectively.

For clean-up of surface water, disposable octadecyl extraction columns (Baker) were used.

RESULTS AND DISCUSSION

Indirect excitation of Eu(III) and Tb(III)

To achieve the efficient indirect excitation of lanthanide ions without the need for deoxygenation, in general three conditions should be fulfilled. First, a stable complex must be formed. Second, the ligand should have a high absorptivity, preferably at wavelengths higher than 280 nm, to guarantee a high output of the xenon excitation lamp. Third, the energy transfer to the lanthanide ion should be efficient compared with other energy decay processes of the ligand; here the energy of the lowest triplet electronic level of the ligand plays a crucial role. It is known that β-diketonates, $[RC(O)CR'C(O)R'']^-$, form stable complexes with lanthanide(III) ions[10]. Several β-diketonates have high triplet levels necessary for energy transfer to the resonance level of lanthanide ions. To obtain maximum luminescence intensities of Eu(III) and Tb(III), different β-diketonates should be chosen, as the energies of the resonance levels of Eu(III) and Tb(III) are different. 2-Thenoyltrifluoroacetate (ttac) and acetylacetonate (acac) have appropriate triplet energies for the indirect excitation of Eu(III) and Tb(III), respectively[11]. The indirect excitation of Tb(III) by acac is depicted schematically in Fig. 1 and characteristics of the Eu–ttac and Tb–acac complexes are given in Table I.

In the literature, generally well defined synthesized complexes of lanthanide ions and β-diketonates have been studied. In our study, we utilized mixed solutions of ligand and lanthanide(III) salt, as in buffered solutions these complexes are formed rapidly (the choice of the buffer is described below). As the stoichiometries of the complexes in water are not known, we prefer to write Eu–ttac and Tb–acac.

Solubility, influence of pH and temperature dependence

Eu–ttac. A solution of $1 \cdot 10^{-4}$ *M* Eu(III) and $3 \cdot 10^{-4}$ *M* ttac in buffer–ethanol (80:20, v/v) showed an irreproducible fluorescence signal, probably caused by the low solubility of ttac. With a mixture of buffer–ethanol (50:50, v/v) no solubility problems were observed, so this ratio was chosen for the experiments.

A maximum luminescence signal was observed at pH 7.0. At higher pH the luminescence intensity decreased, probably owing to hydrolysis of Eu(III). At lower pH protonation of the β-diketonate may interfere with the complexation of Eu(III), thus causing a lower signal. For these reasons, a Tris buffer of pH 7.0 was used.

The luminescence intensity of Eu–ttac in a solution of buffer–ethanol (50:50, v/v) was found to be strongly dependent on the temperature, which is in line with literature data on $Eu(ttac)_3$[13]. This was a serious problem with the experimental

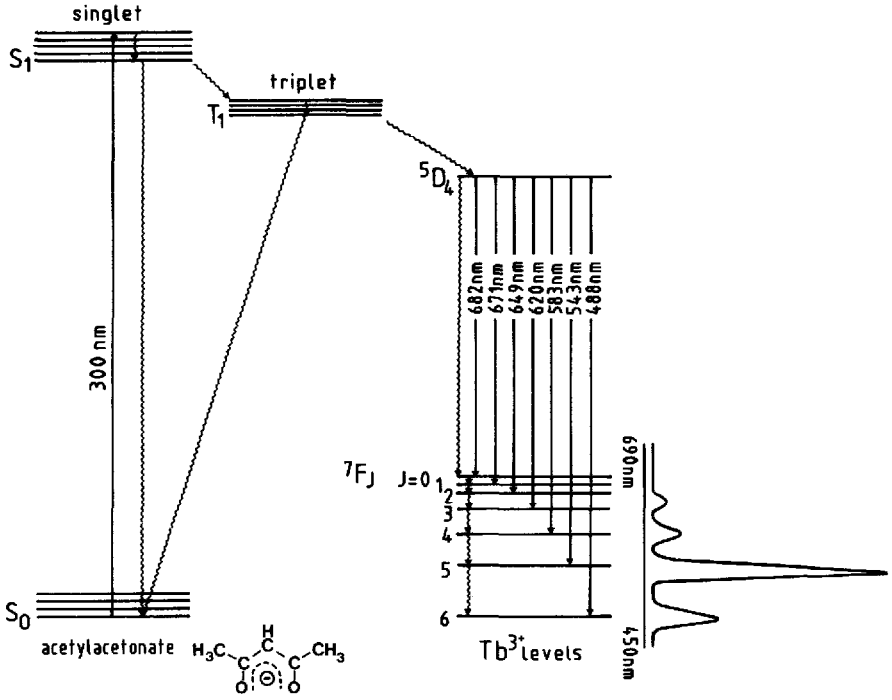

Fig. 1. Schematic representation of the indirect excitation of Tb(III) by acetylacetonate.

set-up that we used in flow experiments. The metal parts are not thermally isolated and the lamp produces so much heat that a rise in temperature of the inlet capillary connected to the flow cell of more than 10°C in a period of 4 h was observed. In the same period the signal decreased by 50%. This effect could be considerably reduced by water cooling of the flow cell compartment.

Tb–acac. With solutions of $1 \cdot 10^{-4}$ *M* Tb(III) and $3 \cdot 10^{-4}$ *M* acac, no solubility problems were observed, even in 100% water. Maximum luminescence intensity

TABLE I

SPECTROSCOPIC CHARACTERISTICS OF THE LANTHANIDE COMPLEXES Eu–ttac AND Tb–acac

Parameter	*Ion*	
	Eu(III)	*Tb(III)*
Ligand	ttac	acac
Maximum excitation wavelength (nm)	360	300
Emitting level of the ion	$^5D_0$	$^5D_4$
Energy of the emitting level[12] (cm^{-1})	17 200	20 500
Maximum emission wavelength (nm)	614	545
Typical luminescence lifetime of the complex in water–ethanol (50:50, v/v) (ms)	0.14	0.45

was observed at pH 7.0. As for Eu–ttac, at higher pH hydrolysis of Tb(III) will take place and at lower pH protonation of the β-diketonate will diminish the complexation with Tb(III).

The temperature dependence of the luminescence of Tb–acac was less dramatic than that for Eu–ttac and a stable signal could be maintained easily by using the water cooling of the flow cell compartment. Considering these results it seems that, for application in an HPLC system, Tb–acac has some advantages over Eu–ttac.

Dynamic quenching

The results of quenching experiments are the most important in drawing conclusions about the applicability of dynamic quenching of lanthanide luminescence as a detection method. Dynamic quenching of luminescence is described by the Stern–Volmer equation:

$$I_0/I = 1 + k_q\tau_0[Q] \quad (1)$$

In the absence of a quencher the luminescence intensity is I_0; in the presence of a dynamic quencher, the luminiscence intensity is decreased to I; k_q is the quenching constant (l mol^{-1}s^{-1}), τ_0 is the luminescence lifetime (s) in the absence of a quencher and [Q] is the concentration of the quencher (M).

A high value of the product $k_q\tau_0$ indicates that a low limit of detection for the quencher Q is possible. When the product has a value of $1.0 \cdot 10^5$ l mol^{-1}, a concentration of $1.0 \cdot 10^{-7}$ M induces a signal decrease of 1.0%. Higher values of $k_q\tau_0$ result in lower detection limits. Quenching of lanthanide luminescence by various compounds has been studied before[8,14–19]; most experiments showed that k_q is often low. We have studied the dynamic quenching of the luminescence of the Eu(III) and Tb(III) complexes by anions. From the results given in Table II, it is clear that only NO_2^-, CrO_4^{2-}, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ show dynamic quenching. It appears that some anions, when present at high concentrations, cause a decrease in signal in

TABLE II

LUMINESCENCE QUENCHING OF LANTHANIDE COMPLEXES: $k_q\tau_0$ VALUES FOR DIFFERENT ANIONS

Anion	$k_q\tau_0$ (l mol^{-1})	
	Eu–ttac[a]	*Tb–acac*[b]
NO_2^-	$1.4 \cdot 10^3$	$9.9 \cdot 10^4$
CrO_4^{2-}	$1.7 \cdot 10^3$[c]	$7.4 \cdot 10^5$
$Fe(CN)_6^{3-}$	$4.4 \cdot 10^3$	$1.3 \cdot 10^6$
$Fe(CN)_6^{4-}$	–[d]	$9.0 \cdot 10^5$
PO_4^{3-}, CO_3^{2-}, SO_4^{2-}, F^-	Signal decrease probably caused by ligand exchange	
NO_3^-, Cl^-, CN^-, SO_3^{2-}, $S_2O_3^{2-}$	No signal decrease observed[e]	

[a] $1 \cdot 10^{-4}$ M Eu(III) and $3 \cdot 10^{-4}$ M ttac in 5 mM aqueous Tris buffer (pH 7.0)–ethanol (50:50, v/v).
[b] $1 \cdot 10^{-4}$ M Tb(III) and $1 \cdot 10^{-4}$ M acac in 5 mM aqueous Tris buffer (pH 7.0).
[c] $1 \cdot 10^{-5}$ M Eu(III) and $1 \cdot 10^{-5}$ M ttac in 5 mM aqueous Tris buffer (pH 7.0)–ethanol (50:50, v/v).
[d] No linear Stern–Volmer plot.
[e] Maximum concentration of anion tested, $1 \cdot 10^{-4}$ M.

batch which is probably caused by a ligand-exchange process. When the β-diketonate is replaced with a non-donating ligand, a decrease in the Eu(III) or Tb(III) luminescence is observed. This also occurs with phosphate ions; for this reason phosphate buffers cannot be used.

As far as we know, efficient dynamic quenching of Tb(III) luminescence by chromate has not been reported before. The results in Table II indicate that quenching of Tb–acac luminescence by chromate seems very interesting.

Chromatographic experiments

Experimental set-up. Because of its relevance, we shall demonstrate the detection method for the selective determination of chromate in water samples in an HPLC system. We previously tested the chromatographic system with nitrite as a model ion. The experimental set-up for HPLC experiments is shown in Fig. 2. The Tb–acac solution is added post-column. In this way the chromatographic separation of the anions is not interfered with the presence of the Tb–acac complex.

Ion separations were carried out by ion-pair reversed-phase chromatography. Tetrabutylammonium bromide (TBABr) was used as the ion-pairing reagent. This reagent had no negative effect on the Tb–acac luminescence.

From the Stern–Volmer expression (eqn. 1), it is clear that not the signal decrease $(I_0 - I)$ but $(1/I_0 - 1/I)$ is proportional to [Q]. We used a signal converter which converts the intensity I into a signal $1/I$, so that the peak height is proportional to [Q]. A description of the signal converter has been given before[2].

Nitrite solutions. In order to test the experimental set-up, chromatographic experiments with nitrite as model compound were performed with an LC mobile phase consisting of aqueous $5.0 \cdot 10^{-3}$ *M* Tris buffer (pH 7.0)–methanol (90:10); for anion separation $5.0 \cdot 10^{-4}$ *M* TBABr was added. For the post-column solution, which had the same composition as the mobile phase, concentrations of $2.0 \cdot 10^{-4}$ *M* of Tb(III) and acac were used. Nitrite solutions were prepared in the eluent and injected (the injection volume was 100 μl). A chromatogram is shown in Fig. 3. The detection limit was $2.5 \cdot 10^{-7}$ *M* and the repeatability was 1.3% (at $5 \cdot 10^{-6}$ *M*, n = 6). Linearity was observed in the range $5 \cdot 10^{-7}$–$1 \cdot 10^{-4}$ *M*, with r = 0.9994 (n = 10).

The detection limit is five times higher than that reported by Baumann *et al.*[8] who used aqueous Tb(III) for luminescence. Although the excitation of Tb(III) has

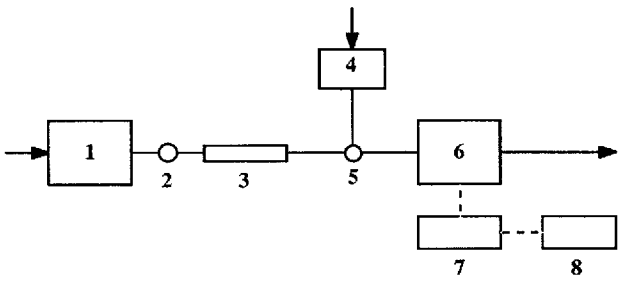

Fig. 2. Schematic diagram of the HPLC system. 1 = LC pump; 2 = injection valve; 3 = analytical column; 4 = post-column pump; 5 = mixing tee; 6 = detector; 7 = signal converter; 8 = recorder.

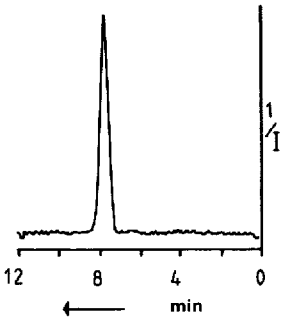

Fig. 3. Chromatogram of a solution of $5.0 \cdot 10^{-6}$ *M* nitrite in the eluent (injection volume 100 μl).

been improved, the efficiency of quenching by nitrite decreased after complexation of Tb(III).

Chromate solutions. For chromate experiments, acetonitrile was used as organic modifier in order to obtain reasonable retention times. The composition of the eluent was $5 \cdot 10^{-4}$ *M* TBABr in aqueous $5 \cdot 10^{-3}$ *M* Tris buffer (pH 7.0)–acetronitile (90:10). The post-column solution additionally contained $1 \cdot 10^{-4}$ *M* Tb(III) and $1 \cdot 10^{-4}$ *M* acac. Chromate solutions were prepared in the eluent and injected; the injection volume was 20 μl. From the chromatogram in Fig. 4, it is clear that slightly asymmetric peaks result. Tailing is also observed when direct UV absorption detection is applied and is probably caused by interaction of chromate with metal parts. The detection limit for 20-μl injections in this system was $1.1 \cdot 10^{-7}$ *M*. The calibration graph was linear from the detection limit up to $1.0 \cdot 10^{-5}$ *M* ($r = 0.9997$, $n = 7$) and the repeatability was 1% (at $3.0 \cdot 10^{-6}$ *M*, $n = 6$).

After complexation, the luminescence intensity of Tb(III) increased and quenching by chromate was still efficient enough to obtain interesting detection limits. Except for the tailing, the previously reported chromatographic problems with chromate[8] were not observed in this instance.

Chromate in surface and drinking water. Chromatographic experiments with surface and drinking water were performed with a newly packed column of the same

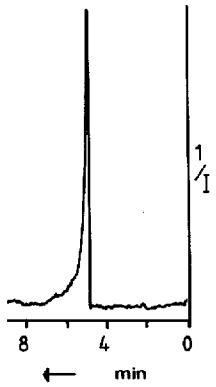

Fig. 4. Chromatogram of a solution of $2.5 \cdot 10^{-6}$ *M* chromate in the eluent (injection volume 20 μl).

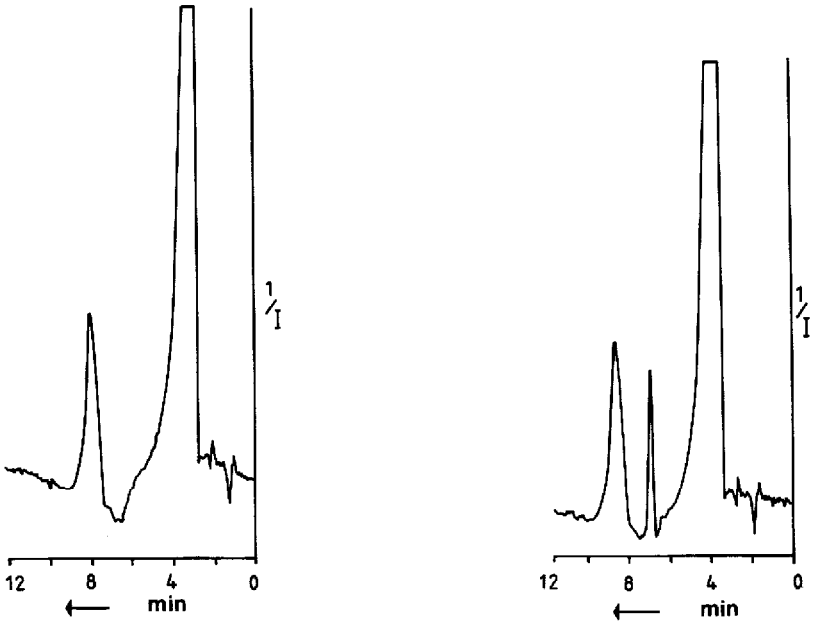

Fig. 5. Chromatogram of a blank surface water sample (left) and of a surface water sample spiked with $1.0 \cdot 10^{-6}$ *M* chromate (right) (injection volume 20 μl).

dimensions as used for the artificial sample (150 × 3.1 mm I.D.). However, the retention characteristics of this column were different from that used previously; when the mobile phase contained 12% of acetonitrile, the retention time of chromate was still longer than in the previous instance.

Tap water and surface water were spiked with chromate, the samples were filtered over a Millipore filter and the surface water samples were subjected to further clean-up by filtering over an octadecyl extraction column. In Fig. 5, chromatograms of a blank and a spiked sample of surface water are depicted. The two peaks in the blank arise from hydrogencarbonate (4 min), which is present in water at high concentrations, and sulphate (>8 min), which is present at a concentration of *ca.* $4 \cdot 10^{-4}$ *M*. These peaks are also observed when drinking water is injected. A decrease in intensity (owing to the converter, positive peaks are registered) is caused by ligand exchange; both carbonate and sulphate may form complexes with lanthanide(III) ions. In spite of the presence of these ions, chromate can be detected fairly well. The analytical data for chromate in surface and drinking water were similar to the results for the artificial solution; a limit of detection of $1.1 \cdot 10^{-7}$ *M* (13 ppb) was found, which is comparable to those with other detection techniques[5].

CONCLUSION

The results show the potential of dynamic lanthanide luminescence quenching for detection in ion chromatography. In particular, solutions of Tb–acac can be used as a post-column flow without compatibility problems. The intensely luminescent

Tb–acac complexes are simply obtained by mixing equimolar solutions of Tb(III) and acac. Oxygen removal is not necessary. The method has a high inherent selectivity as only a few inorganic anions induce efficient dynamic quenching, although some anions induce a decrease in the luminescence intensity of the complex by ligand exchange. Dynamic luminescence quenching is an appropriate detection method for chromate in water samples. The applicability of the method to the detection of other compounds, *e.g.*, hexacyanoferrate(II) and hexacyanoferrate(III) complexes, is still under study.

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