Journal of Chromatography, 439 (1988) 165–170
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20232

Note

Dynamic quenching of europium(III) and terbium(III) luminescence: a potential detection method in ion chromatography

R. A. BAUMANN, D. A. KAMMINGA, H. DERLAGEN, C. GOOIJER*, N. H. VELTHORST and R. W. FREI

Department of General and Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)

Indirect detection methods based on UV absorption and fluorescence measurements are well known in single-column ion chromatography[1]. The detector responds to the chromophoric or fluorophoric eluent ions; a negative signal is observed, as the eluting analyte displaces an equal amount of eluent at the detector. As a consequence, low detection limits are attainable only if the analytical separation is performed at low eluent concentrations.

In our laboratory, we have developed an indirect detection method based on dynamic quenching of luminescence[2–5]. Here displacement effects and therefore eluent concentrations play no role. A decrease in signal is observed for analytes able to reduce the luminescence quantum yield of a luminescing compound present in the detector cell.

Previously we made use almost exclusively of the phosphorophore biacetyl, present as a solute in the eluent, and successful experiments have been reported[2–5]. Unfortunately, the need to remove oxygen completely from the whole high-performance liquid chromatographic (HPLC) system makes the experimental set-up more complicated than for HPLC with UV absorption or fluorescence detection. To reduce or even eliminate this problem we have followed two approaches. First we developed a detection technique based on an immobilized phosphorophore, covalently bound via an alkyl spacer on glass or silica gel beads[6]. As a second approach, reported here, we utilized rare earth metal ions as luminescent probes. Europium (III) and terbium (III) emit long-lived luminescence in fluid aqueous solutions that is hardly or not influenced by the presence of oxygen.

Europium and other lanthanide ions have been applied as luminescence probes in studies on the structures of biological macromolecules[7] and polymeric materials[8] and as NMR shift reagents[9]. Several non-radioisotopic immunoassays make use of Eu^{3+} chelates as luminescent markers[10,11]. Because of their long luminescence lifetimes (10^{-5}–10^{-3} s), the combination with time-resolved luminescence detection techniques results in very sensitive methods[12]. The use of Eu^{3+} and Tb^{3+} as detection luminophores in LC in a sensitized luminescence mode has been described by DiBella *et al.*[13]. The organic compounds are excited by UV radiation, energy transfer from the triplet state of the organic compound to an excited state of the lanthanide ion occurs and luminescence of the lanthanide is detected. Interesting results were ob-

0021-9673/88/$03.50

tained provided that careful deoxygenation was applied; obviously this is due to the influence of oxygen on the lifetime of the excited state of the donor. The role of oxygen was also considered by Sabbatini *et al.*[14], who studied the mechanism of dynamic luminescence quenching of the europium cryptate [Eu C 2.2.1]$^{3+}$ by $M(CN)_6^{4-}$ (M = Fe, Ru or Os).

In this work the potential of dynamically quenched lanthanide ion luminescence for detection in flow injection analysis (FIA) and HPLC was examined. Europium(III) was taken as a representative luminescence probe as this element is the most widely studied lanthanide. A screening test for a number of anions was performed and the influence of buffers and organic modifiers was investigated. It is shown that by replacing H_2O by ^2H_2O in the mobile phase, the sensitivity of the detection system is greatly improved. Because of the financial aspects, miniaturization of the HPLC system would make this replacement feasible. The possibility of utilizing time resolution was investigated for two different commercially available luminescence detectors. Finally, the applicability of the method is illustrated by means of chromatograms for nitrite.

EXPERIMENTAL

Apparatus

Batch experiments were performed on a Perkin-Elmer (Norwalk, CT, U.S.A.) Model MPF-44 A fluorescence spectrophotometer provided with two Hamamatsu R777-01-HA photomultiplier tubes, a DCSU-2 differential corrected spectra unit and an XBO 150-W xenon lamp (OSRAM, Munich, F.R.G.).

The HPLC system consisted of a Gilson 302 HPLC pump (Gilson, Villers le Bel, France) equipped with a Gilson 802c manometric module, a Rheodyne 7010 six-port injection valve, a stainless-steel column (20 cm × 2.1 mm I.D.) packed with 5-μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) and an LS 2 filter fluorimeter (Perkin-Elmer, Beaconsfield, U.K.) for the europium luminescence measurements or a 1046A fluorescence detector (Hewlett-Packard, Böblingen, F.R.G.) for the terbium luminescence measurements. In the latter experiments the luminescence signal obtained was inverted by the electronic inverter described by Donkerbroek *et al.*[15].

Chemicals

$EuCl_3 \cdot 6H_2O$ (99.9%) and $TbCl_3 \cdot 6H_2O$ (99.9%) were obtained from Aldrich (Milwaukee, WI, U.S.A.). ^2H_2O (99.8 atom-% ^2H) and ion-pair reagents were purchased from Janssen (Beerse, Belgium). Tris buffer (Trizmabase, analytical-reagent grade) was obtained from Sigma (St. Louis, MO, U.S.A.) and acetonitrile (HPLC grade) from Baker (Deventer, The Netherlands). All analytes and buffers were used as sodium salts (analytical-reagent grade). Deionized water was distilled twice before use.

RESULTS AND DISCUSSION

Batch experiments

The UV absorption maximum of $TbCl_3$ in water is at 225 nm, which is too low to perform batch experiments with the Perkin-Elmer MPF 44 A luminescence

spectrometer. For this reason we utilized Eu^{III} solutions (absorption maximum at 394 nm) for out batch screening experiments. We consider Eu^{III} to be a representative luminescent probe as the rare earth metal ions have similar chemistry.

When dynamic luminescence quenching occurs, the relationship between the original luminescence signal I_o, the resulting signal I and the quencher concentration [Q] is given by the Stern–Volmer equation:

$$I_o/I = 1 + k_q\tau^o\,[Q] \qquad (1)$$

where k_q is the bimolecular quenching constant (l mol^{-1} s^{-1}) and τ^o the lifetime (s) of the luminophore in the absence of the quencher. The sensitivity of a detection method based on dynamic luminescence quenching is proportional to $k_q\tau^o$. For a number of inorganic anions $k_q\tau^o$ values were determined in batch as described previously[16]. Table I gives values determined in water without applying deoxygenation procedures. The measured lifetime of the Eu^{3+} luminescence in the absence of quenching ions, denoted by τ^o, was 106 μs, which is in good agreement with previously reported values[17,18]. The results are promising especially for $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$. Anions that do not quench the luminescence of Eu^{3+} are F^-, Cl^-, Br^-, I^-, SCN^-, SO_4^{2-}, NO_3^- and S^{2-}, so the method has an inherent selectivity.

We found that quenched Eu^{III} luminescence detection is completely compatible with methanol and acetonitrile, solvents frequently used in FIA and HPLC. Quenching of Eu^{III} luminescence is particularly effective in ^2H_2O owing to the long emission lifetime in this medium (3.2 ms in ^2H_2O *vs.* 0.11 ms in H_2O)[18]. This can be attributed to the difference in O–H and O–D vibrational energies, as the main depopulation route of the excited ^5D levels is radiationless deactivation via these vibrations of the surrounding solvent molecules. As a consequence of this difference in lifetime, the product $k_q\tau^o$ in eqn. 1 and hence the sensitivity of detection ^2H_2O is 30 times higher than that in normal water. Preliminary flow injection experiments showed that the improvement in detection limit is even more favourable (*viz.*, a factor of about 100) because the detector can be operated at a lower amplification, thus producing a lower noise level.

Because Eu^{3+} forms complexes with a large number of compounds, one has to be careful with the choice of a buffer system. As a rule, when complexes of Eu^{3+}

TABLE I

$k_q\,\tau^o$ VALUES FOR DIFFERENT ANIONS MEASURED IN WATER

$[EuCl_3] = 2 \cdot 10^{-3}$ *M*; excitation wavelength, 394 nm; emission wavelength, 591 nm; spectral band passes, 10 nm.

Anion	$k_q\,\tau^o$ *(l mol^{-1})*
NO_2^-	$9.9 \cdot 10^3$
SO_3^{2-}	$1.0 \cdot 10^4$
$S_2O_3^{2-}$	$5.5 \cdot 10^2$
CrO_4^{2-}	$2.2 \cdot 10^4$
$PtCl_4^{2-}$	$4.8 \cdot 10^3$
$Fe(CN)_6^{3-}$	$4.0 \cdot 10^5$
$Fe(CN)_6^{4-}$	$5.5 \cdot 10^5$

with increasing stability are formed, the quenching for a given species decreases and even becomes zero eventually. Trizmabase was found to be the most suitable buffer system. As europium phosphate is insoluble in water, phosphate buffers cannot be used.

FIA and HPLC experiments

The absorption band of Eu^{3+} in water at 394 nm is very weak, *i.e.*, it is only a few nanometers wide and the maximum absorptivity is only 3 l mol^{-1} cm^{-1}. Therefore, the luminescence signal of Eu^{3+} in normal water (in ^2H_2O the situation is much more favourable) is too noisy to achieve useful detection limits. The Tb^{3+} UV absorption band is much wider and higher ($\varepsilon \approx 350$ l mol^{-1} cm^{-1}), but its wavelength (225 nm) falls outside the radiation output range of various luminescence spectrometers (such as the Perkin-Elmer MPF 44 A and LS 2). Fortunately, the Hewlett-Packard 1046 A luminescence detector, equipped with a pulsed source and a gated photomultiplier like the Perkin-Elmer LS 2, proved to be applicable for excitation in the 225 nm region. For both the FIA and HPLC experiments a one-pump system with $5 \cdot 10^{-3}$ *M* $TbCl_3$ dissolved in the mobile phase, which consisted of $5 \cdot 10^{-3}$ *M* Tris buffer and, $5 \cdot 10^{-4}$ *M* tetrabutylammonium hydroxide (pH 6.5) in water, was used. The measured lifetime of Tb^{3+} luminescence in the mobile phase in the absence of quenchers, denoted by τ^o, was 261 μs.

Optimal results were obtained by choosing a detector delay time, t_d, of 30 μs and a gating time, t_g, of 2.0 ms. Screening experiments showed that Tb^{3+} luminescence could be quenched by NO_2^-, $S_2O_3^{2-}$, SCN^-, $PtCl_4^{2-}$, $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$ and CrO_4^{2-}; SO_3^{2-} precipitated in the mobile phase and could not be determined in this way.

The FIA experiments provided for nitrite a detection limit of $3 \cdot 10^{-8}$ *M* (at a signal-to-noise ratio of 3). The linearity of response was more than three orders of magnitude when the electronic signal inverter was used. Injection of $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$ or chromate solutions in this system resulted in broad, irreproducible peaks. Addition of 10% (v/v) acetonitrile to the mobile phase greatly improved the

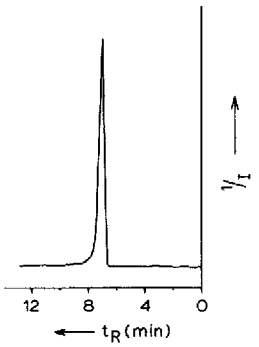

Fig. 1. Chromatogram of $5 \cdot 10^{-6}$ *M* nitrite standard solution. Column: LiChrosorb RP-18 (5 μm) (20 cm × 2.1 mm I.D.). Mobile phase: $5 \cdot 10^{-3}$ *M* $TbCl_3$; $5 \cdot 10^{-3}$ *M* Tris buffer; $5 \cdot 10^{-4}$ *M* tetrabutylammonium hydroxide in 100% water, pH 6.5. Injection volume, 8 μl; flow-rate, 300 μl min^{-1}. Detector settings: excitation wavelength, 225 nm; emission wavelength, 553 nm; t_d = 30 μs; t_g = 2.0 ms. The inverted luminescence signal is monitored.

peak shape but the FIA peaks where still too broad to permit quantitative calculations.

The influence of ^2H_2O on the Tb^{3+} luminescence was examined. Plug injections of Tb^{3+}–NO_2^- mixtures in H_2O and ^2H_2O gave calibration graphs with slopes of 0.06 and 0.25, respectively. This means that the use of ^2H_2O instead of H_2O gives a four-fold increase in sensitivity.

To show the potential of the Tb^{3+} system for quenched luminescence detection in HPLC, an ion-pairing reversed-phase system was used for the detection of nitrite. Fig. 1 shows a chromatogram for a $5 \cdot 10^{-6}$ *M* nitrite standard solution. The limit of detection is $5 \cdot 10^{-8}$ *M* (28 pg absolute), which indicates that this method is very sensitive. The repeatability of the method was 1.1% at the $5 \cdot 10^{-6}$ *M* level and 3.6% at the $5 \cdot 10^{-7}$ *M* level, both based on six injections.

CONCLUSIONS

Eu^{3+} and Tb^{3+} can be used as luminophores for the quenched luminescence detection of a number of anions in FIA and HPLC. Deoxygenation of the mobile phase is not necessary, which greatly simplifies the practical application of the method. Methanol and acetonitrile can be employed as organic modifiers in the mobile phase without changing the sensitivity of the method drastically. The use of ^2H_2O instead of H_2O increases the sensitivity of detection owing to the long lifetime of the luminescence of the rare earth ions in this medium. Miniaturization of the HPLC system would permit the use of an HPLC solvent such as ^2H_2O. The choice of a buffer is critical; Tris buffer is the most suitable. Luminescence detectors with a pulsed source-gated photomultiplier system improve the sensitivity of detection.

The sensitivity of the Eu^{3+} detection system is low owing to the low absorptivity of Eu^{3+}. This problem might be solved by employing a Eu^{3+} complex, with indirect excitation via a ligand. The Tb^{3+} detection system, which has much better absorption characteristics than Eu^{3+}, has a high sensitivity.

Future work should cover the use of parallel systems, in which a column packed with solid $TbCl_3$ is placed parallel to the analytical column and the mobile phase is pumped through both columns simultaneously, to achieve the post-column addition of Tb^{3+} with only one pump operating. Further, the applicability of the method to the detection of a number of metal cyanides and the detection of cyanide itself via complexation with a suitable metal ion should be examined. Also, the use of ion exchangers or certain ligands for the immobilization of Tb^{3+} or Eu^{3+} in a solid-state detector cell seems interesting in both quenched and sensitized luminescence detection.

ACKNOWLEDGEMENT

We are indebted to Dr. K. P. Hupe of Hewlett-Packard (Waldbronn, F.R.G.) for the use of the HP 1046 A detector.

REFERENCES

1 J. S. Fritz, *Anal. Chem.*, 59 (1987) 335A.
2 C. Gooijer, A. C. Veltkamp, R. A. Baumann, N. H. Velthorst, R. W. Frei and W. J. F. van der Vijgh, *J. Chromatogr.*, 312 (1984) 337.

3 R. A. Baumann, N. H. Velthorst, R. W. Frei, I. Klein and W. J. F. van der Vijgh, *J. Pharm. Biom. Anal.*, 5 (1987) 165.
4 J. J. Donkerbroek, *Thesis*, Free University, Amsterdam, 1983.
5 R. A. Baumann, M. Scheurs, C. Gooijer, N. H. Velthorst and R. W. Frei, *Can. J. Chem.*, 65 (1987) 965.
6 R. A. Baumann, C. Gooijer, N. H. Velthorst, R. W. Frei, I. Aichinger and G. Gübitz, *Anal. Chem.*, in press.
7 J. M. Brewer, L. A. Carreira, R. W. Irwin and J. I. Elliott, *J. Inorg. Biochem.*, 14 (1981) 33.
8 Y. Okamoto, Y. Ueba, N. F. Dzanibekov and E. Banks, *Macromolecules*, 14 (1981) 17.
9 C. C. Bryden and C. N. Reilley, *Anal. Chem.*, 54 (1982) 610.
10 H. Déchaud, R. Bador, F. Clanstrat and C. Desuzinges, *Clin. Chem.*, 32 (1986) 1323.
11 R. Bador, H. Déchaud, F. Clanstrat and C. Desuzinges, *Clin. Chem.*, 33 (1987) 48.
12 E. Soini and H. Kojola, *Clin. Chem.*, 29 (1983) 65.
13 E. E. DiBella, J.B. Weissman, M. J. Joseph, J. R. Schultz and T. J. Wenzel, *J. Chromatogr.*, 328 (1985) 101.
14 N. Sabbatini, A. Bonazzi, M. Ciano and V. Balzani, *J. Am. Chem. Soc.*, 106 (1984) 4055.
15 J. J. Donkerbroek, A. C. Veltkamp, C. Gooijer, N. H. Velthorst and R. W. Frei, *Anal. Chem.*, 55 (1983) 1886.
16 J. J. Donkerbroek, C. Gooijer, N. H. Velthorst and R. W. Frei, *Anal. Chem.*, 54 (1982) 891.
17 C. C. Bryden and C. N. Reilley, *Anal. Chem.*, 54 (1982) 610.
18 N. Sabbatini, S. Dellonte, M. Ciano, A. Bonazzi and V. Balzani, *Chem. Phys. Lett.*, 107 (1984) 212.