ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 115 (1998) 157–161

Journal of Photochemistry and Photobiology A:Chemistry

A fluorescent sensor for Cu^{2+} at the sub-ppm level

Kerry A. Mitchell [a], Robert G. Brown [a,*], Dongwu Yuan [b], Shao-Chieh Chang [b], Ronald E. Utecht [b], David E. Lewis [c,1]

[a] *Chemistry Department, University of Central Lancashire, Preston, Lancs PR1 2HE, UK*
[b] *Department of Chemistry and Biochemistry, South Dakota State University, Brookings, SD 57007-0896, USA*
[c] *Department of Chemistry, University of Wisconsin-Eau Claire, Eau Claire, WI 54702, USA*

Received 10 November 1997; accepted 24 November 1997

Abstract

Two 4-(aminoalkyl)aminonaphthalimide compounds which exhibit fluorescence enhancement and associated spectral changes in the presence of Cu^{2+}, Mn^{2+} and Ni^{2+} are reported. The compounds are believed to undergo intramolecular quenching by the distal nitrogen on the 4-substituent. When metal ions are present, this quenching mechanism is partially or wholly removed due to complexation of the metal ions with the 4-substituent. The two compounds are particularly sensitive to Cu^{2+} such that concentrations in the 0–1 ppm range are sufficient to effect complete restoration of the naphthalimide fluorescence.

Keywords: Fluorescent sensor; Cu^{2+}; Metal ions

1. Introduction

Fluorescent materials are now used for a wide variety of sensing applications [1] and there is considerable current interest in the development of sensors for metal ions. In developing such systems, one seeks both specificity and sensitivity toward the ion of interest. In addition, compounds which exhibit an increase in fluorescence intensity (chelation enhanced fluorescence, CHEF) in the presence of the ion and/or a spectral change (so that a ratiometric sensor not requiring frequent calibration becomes possible) are attractive.

Many of the fluorescent ion sensors reported to date are based on a design which uses a crown ether as the ionophore, attached to a fluorescent species whose properties change when a metal ion is captured by the crown ether. An example of this type of sensor is Coumarin 153 linked to monoaza-15-crown-5 [2]. Unfortunately, these systems do not tend to be very selective. An alternative strategy, developed by Czarnik [1], involves a fluorescent molecule chemically attached to a quencher (usually an amine), e.g., 9,10-bis-(TMEDA)anthracene. In this example, the quencher reduces the fluorescence intensity and lifetime of the anthracene moiety until such time as it becomes protonated or acts as a ligand to a metal ion when the normal emission properties of the anthracene are restored. These systems can display all of the required characteristics with the exception of a spectral shift so calibration will be required.

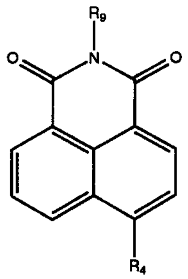
This sensing strategy has been further exploited by the work of Bissell et al. [3] who have synthesised a number of novel fluorescent probes and sensors involving photo-induced electron transfer (PET) as their mode of action. Included among the molecules they have studied are a number of naphthalimides. Ghosh et al. [4] have recently reported an elegant trianthrylcryptand which displays excellent fluorescence enhancement in the presence of various metal ions which is quite sensitive toward Zn^{2+} and appears to be quite selective but which is not capable of use in ratiometric mode. There are also many reports of systems which are both specific and sensitive to particular metal ions (e.g., Fabbrizzi and Poggi [5] and Fabbrizzi et al. [6] for Cu^{2+}) but where the sensing action involves fluorescence quenching. We feel that although such systems can inform sensor design, the ubiquitous nature of fluorescence quenching reduces their practical utility.

---

[*] Corresponding author. E-mail: r.g.brown@uclan.ac.uk

[1] Email: lewisd@uwec.edu

1010-6030/98/$19.00 © 1998 Elsevier Science S.A. All rights reserved.
PII S1010-6030(97)00335-3



1a: R_4= $NH(CH_2)_2NH_2$; R_9= C_6H_{13}
1b: R_4= $NH(CH_2)_3N(CH_3)_2$; R_9= C_4H_9
1c: R_4= $NH(CH_2CH_2O)_2CH_2CH_2NH_2$; R_9= C_6H_{13}

Very recently, Ramachandram and Samanta [7] have reported CHEF from some 4-aminophthalimides. Enhancement factors of up to 55 for sub-mM amounts of metal ions were achieved for various transition metals. Here, we report similar observations for two related molecules–4-(aminoalkyl)aminonaphthalimides (**1a,b**) which display similar properties, especially for Cu^{2+} as the analyte, and which offer exciting development possibilities.

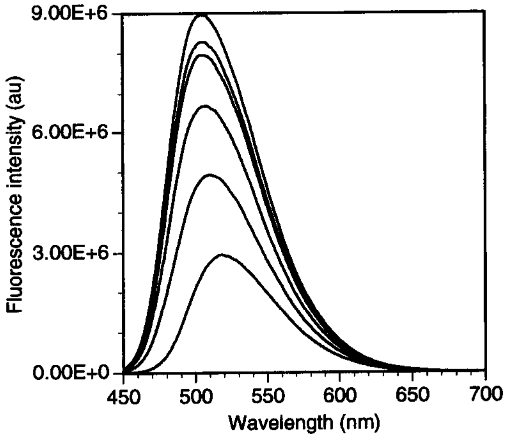


Fig. 1. Fluorescence emission spectra of **1b** in 2-propanol (10 μM) with added Cu^{2+} (as the nitrate). Cu^{2+} concentrations (with increasing emission intensity): 0, 0.3, 0.4, 0.5, 0.75, 1.0 ppm.

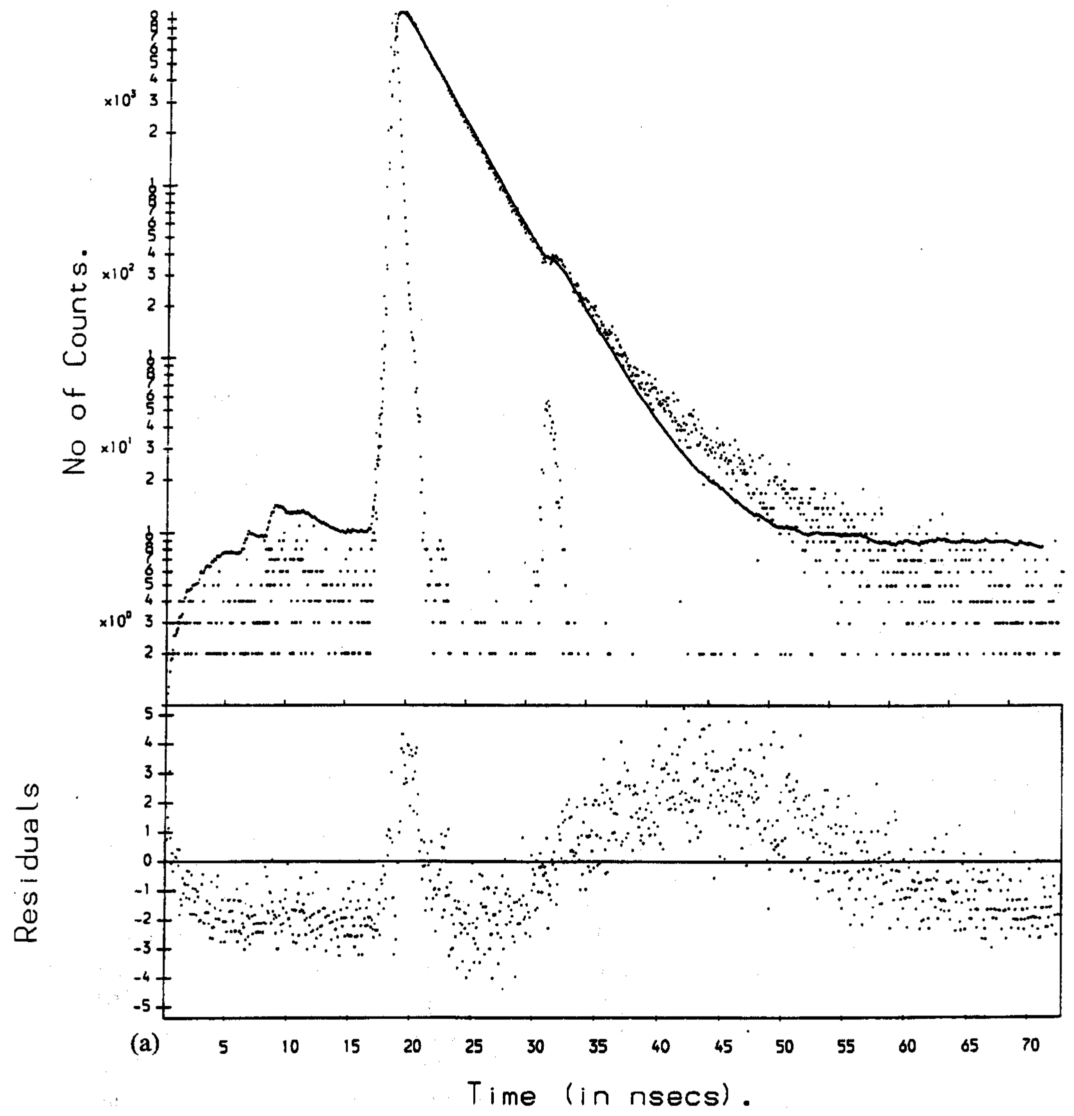


Fig. 2. *(continued)*

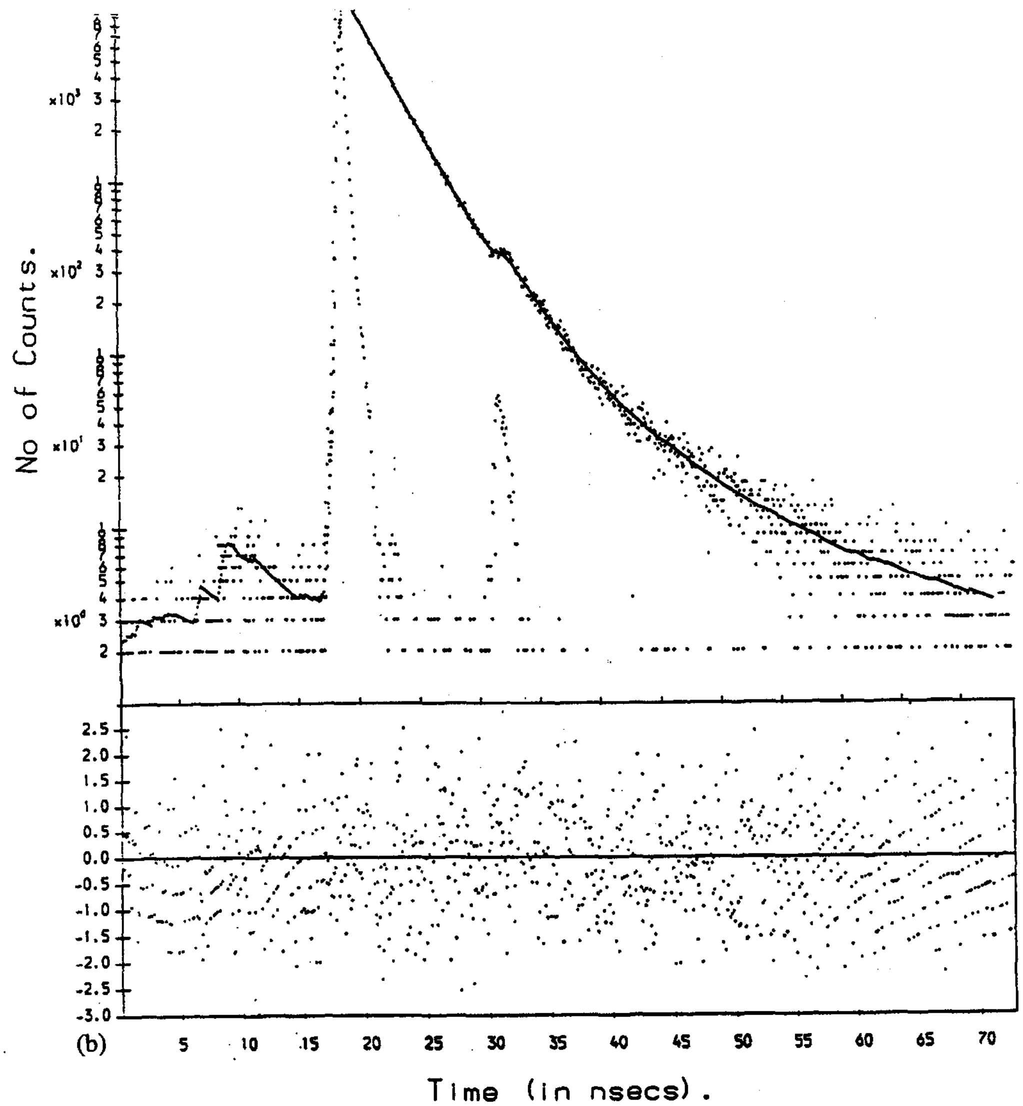


Fig. 2. Experimental and calculated fluorescence decay profiles for **1b** in 2-propanol (10 μM): (a) monoexponential fit, (b) biexponential fit. The instrument response function is also shown.

2. Experimental

Naphthalimides **1a–c** were prepared by standard methods [8–10] from 4-chloro- or 4-nitro-1,8-naphthalic anhydride by reaction with firstly hexylamine (**1a,c**) or butylamine (**1b**) and then ethylenediamine (**1a**), *N,N*-dimethylpropylenediamine (**1b**) or 1,8-diamino-3,6-dioxaoctane (**1c**). All three compounds were purified by recrystallisation and/or column chromatography and gave satisfactory NMR spectra and elemental analyses. All the metal salts used were Analar grade.

Absorption and fluorescence spectra were measured on a Hewlett-Packard 8451A diode array spectrometer and a SPEX Fluoromax spectrofluorimeter, respectively, in 2-propanol (Aldrich spectrophotometric garde, chosen as a solvent in which both the naphthalimides and metal salts have reasonable solubility and in which both fluorescence enhancement and spectral shifts are observed). Fluorescence decay profiles were measured by the time-correlated, single photon counting technique [11] using the Daresbury Laboratory Synchrotron Radiation Source for excitation. The experimental set-up has been described elsewhere [12]. The decay profiles were analyzed by computer convolution and the 'goodness of fit' evaluated on the basis of the χ^2 value and the distribution of residuals.

3. Results and discussion

The three naphthalimides presented here (**1a–c**) exhibit very similar absorption and emission properties to other related 4-aminonaphthalimides [8,13,14] in that they all have

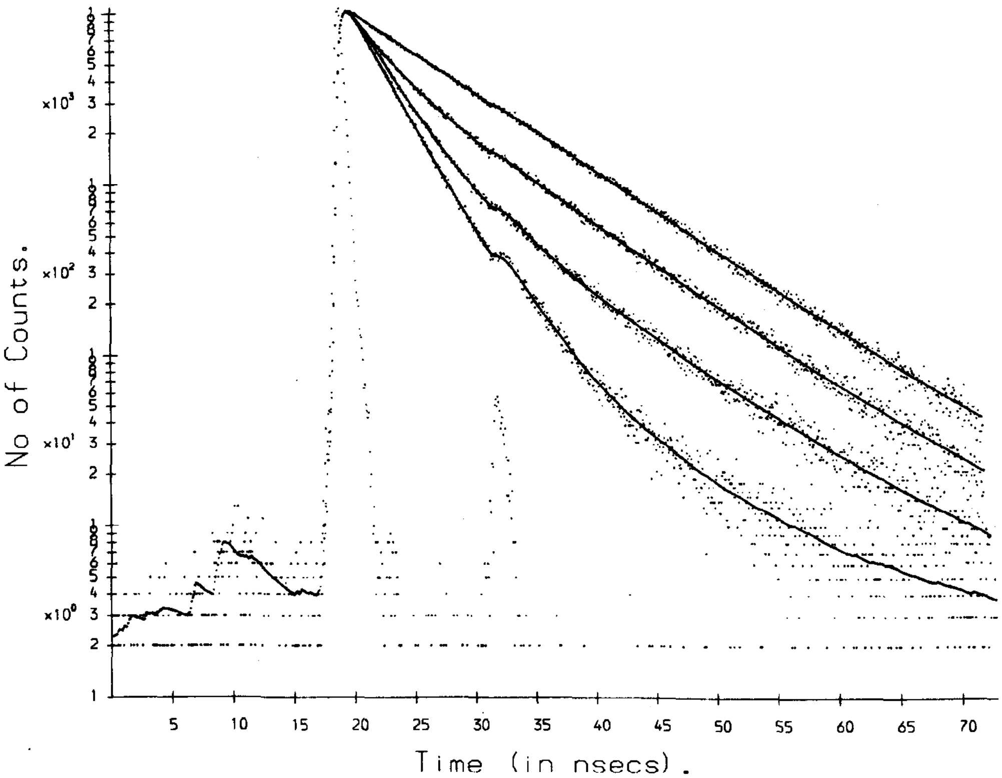


Fig. 3. Experimental and calculated fluorescence decay profiles for **1b** in 2-propanol (10 μM) with added Cu^{2+} (as the nitrate). Cu^{2+} concentrations (in order bottom to top): 0, 0.75, 1.0, 10.0 ppm. The instrument response function is also shown.

absorption maxima near 440 nm and maximum fluorescence emission intensity in the region of 520–530 nm; **1a** and **1c** exhibit a secondary emission maximum near 490 nm.

The addition of selected metal ions to solutions of **1a** and **1b**, however, causes both the absorption and fluorescence maxima to undergo a 10–30 nm hypsochromic shift. In **1a**, the intensity of the emission band at 490 nm increases to the point that this emission band becomes dominant. When the metal ion added is Cu^{2+}, only sub-ppm amounts are required to effect this enhancement in fluorescence intensity as Fig. 1 shows. Most other metal ions from the first transition series (such as Ni^{2+} and Mn^{2+}) also cause this increase in fluorescence but the concentrations required to achieve a similar effect to that observed with Cu^{2+} are much higher for certain of these metal ions (10–100 ppm for Ni^{2+} and Mn^{2+}). This behaviour appears to be restricted to metal ions that readily form ammine complexes: metal ions that do not (Zn^{2+}, Mg^{2+}, Al^{3+} and Ca^{2+}) do not lead to the observed changes in the fluorescence spectrum.

In contrast to this, the addition of Cu^{2+} ions to a solution of **1c** has no effect on the wavelength of either the absorption or the emission maximum, but it results instead in a *reduction* in the intensity of both emission bands without affecting the relative intensity of either. This behaviour is much more in accord with the behaviour expected when a paramagnetic metal ion becomes complexed to the fluorophore (it is axiomatic that paramagnetic species quench the fluorescence). It is also clear that when the 4-substituents used here are present on the imide bridge of a 4-amino-1,8-naphthalimide, the terminal amine of the side chain appears to be completely insulated from the naphthalimide [7] (presumably because the distal nitrogen is too spatially remote from the fluorophore to interact with it) and it is only when the 4-amino substituent is, for example, protonated that changes in absorption and fluorescence are observed [13].

Measurement of fluorescence decay profiles for **1a** and **1b** in the presence of varying amounts of Cu^{2+} provides further information to assist in determining the mechanism that leads to the enhancement of the fluorescence. In the case of **1a**, the fluorescence decay profiles on initial analysis appear to be uniformly single exponential with a lifetime of approximately 8.2 ns at low Cu^{2+} concentrations which increases to some 9.5 ns at concentrations of 1 ppm+. This increase in the lifetime is far smaller than that in the fluorescence intensity. These two observations appear mutually incompatible.

However, when the decay profiles for **1b** are analysed they are found to be best described by a sum of two exponentials (Fig. 2) with lifetimes of some 3.2 ns and 9.0–10.0 ns for Cu^{2+} concentrations in the 0–1 ppm range and a single exponential with a lifetime of 9.0–10.0 ns above that. The decays are dominated by the 3.2 ns component at low Cu^{2+} concentrations (e.g., Fig. 2) but the longer-lived component provides an increasingly greater contribution to the overall decay as the concentration of the metal ion increases (Fig. 3).

The same lifetime parameters are observed at all emission wavelengths although their relative proportions change. In the light of these observations, on re-analysis the decay profiles for **1a** are also found to be more adequately described by a biexponential model.

These data suggest that in naphthalimides **1a** and **1b** an interaction occurs between the terminal amine moiety of the (aminoalkyl) amino group at the 4-position and the naphthalimide ring (possibly involving PET [15]) which quenches the excited naphthalimide. This quenching interaction is adequately described by exciplex-type kinetics [16] which predict the observed biexponential decay profiles. Complexation of the distal amino nitrogen of this side chain with a metal ion in both the ground and excited states removes the possibility of this interaction and thus leads to an increase in both fluorescence intensity and lifetime. Ramachandram and Samanta [7] have recently reported similar observations for two derivatives of 4-aminophthalamide. We also note that the paramagnetism of the metal ion appears to have a minimal impact on the observed changes in the fluorescence spectrum: even Mn^{2+}, which has five unpaired electrons in the high spin state, still exhibits the same behaviour (it is interesting to note that as the number of unpaired electrons in the metal ion increases, the amount of the metal ion required to produce the same effect on the fluorescence spectrum increases).

The conclusion that the complexation of the distal nitrogen eliminates exciplex formation and internal quenching of the excited state of the naphthalimide is not novel but the observation that the quenching by the distal nitrogen is more effective than by a paramagnetic metal ion the same (or similar) distance from the chromophore is unprecedented. In addition, the low level of Cu^{2+} required to effect these fluorescence changes is exciting and offers the promise that we can design and synthesise other naphthalimides which will offer even lower detection levels for such metal ions.

Acknowledgements

We thank EPSRC, the University of Central Lancashire, the South Dakota Governor's Office of Economic Development CITE Program, and MicroBioMed for support.

References

[1] A.W. Czarnik (Ed.), Fluorescent sensors for ion and molecule recognition, ACS Symposium Series #538, American Chemical Society, WA, 1992.
[2] J. Bourson, M.-N. Borrel, B. Valeur, Anal. Chim. Acta 257 (1993) 189–193.
[3] R.A. Bissell, A.P. de Silva, H.Q.N. Gunaratne, P.M.L. Lynch, G.E.M. Maguire, K.R.A.S. Sandanayake, Chem. Soc. Rev. (1992) 187–195.
[4] P. Ghosh, P.K. Bharadwaj, S. Mandal, S. Ghosh, J. Am. Chem. Soc. 118 (1996) 1553–1554.
[5] L. Fabbrizzi, A. Poggi, Chem. Soc. Rev. (1995) 197–202.
[6] L. Fabbrizzi, M. Licchelli, P. Pallaicini, A. Perotti, A. Taglietti, D. Sacchi, Chem. Eur. J. 2 (1996) 75–82.
[7] B. Ramachandram, A. Samanta, Chem. Commun. (1997) 1037–1038.
[8] M.S. Alexiou, V. Tychopoulos, S. Ghorbanian, J.H.P. Tyman, R.G. Brown, P.I. Brittain, J. Chem. Soc., Perkin Trans. 2 (1990) 837–842.
[9] D.E. Lewis, R.E. Utecht, M.M. Judy, J.L. Matthews, US Patent No. 5,308,773, 1994.
[10] S.-C. Chang, B.J. Archer, R.E. Utecht, D.E. Lewis, M.M. Judy, J.L. Matthews, BioMed. Chem. Lett. 3 (1993) 555–556.
[11] D.V. O'Connor, D. Phillips, Time-Correlated, Single Photon Counting, Academic Press, London, 1984.
[12] R. Sparrow, R.G. Brown, E.H. Evans, D. Shaw, J. Chem. Soc., Faraday Trans. 2 82 (1986) 2249–2262.
[13] D. Yuan, R.G. Brown, J. Chem. Soc., J. Chem. Res. (S) (1994) 418–419.
[14] D. Yuan, R.G. Brown, J. Phys. Chem. A 101 (1997) 3461–3466.
[15] R.A. Bissell, A.P. de Silva, H.Q.N. Gunaratne, P.M.L. Lynch, G.E.M. Maguire, C.P. McCoy, K.R.A.S. Sandanayake, Topics in Current Chemistry, Springer-Verlag, Berlin, 1993, vol. 168, pp. 223–264.
[16] C. Lewis, W.R. Ware, Mol. Photochem. 5 (1973) 261–285.