

Potential analytical applications of differential fluorescence quenching: pyrene monomer and excimer emissions as sensors for electron deficient molecules

Kathy-Sarah Focsaneanu and J. C. Scaiano*

Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, ON, K1N 6N5, Canada. E-mail: tito@photo.chem.uottawa.ca

Received 14th April 2005, Accepted 18th July 2005

First published as an Advance Article on the web 18th August 2005

Quenching of the monomer and excimer emissions from pyrene allows excellent discrimination in the detection of electron-deficient molecules; this characteristic could be used to detect explosives and for rapid screening of complex samples suspected of containing explosives. The method responds to electron deficient molecules other than nitro compounds (e.g., 1,4-dicyanobenzene), giving some false positives; in this case, however, the method is unlikely to lead to false negatives, a desirable characteristic in security-related applications.

Introduction

Pyrene fluorescence quenching has been used as an effective analytical tool. Some recent applications in analytical chemistry include: detecting NO in NO-releasing oxygen-sensing polymer films,¹ probing the heterogeneous microenvironments of the C₁₈ stationary phase in capillary electrochromatography,² measuring intracellular molecular oxygen concentrations,³ and explosives detection,⁴ an application closely related to the present report. The majority of these applications involve very dilute concentrations of pyrene or pyrene-based derivatives and thus do not take advantage of excimer formation which occurs in moderately concentrated conditions (e.g. ≥ 0.001 M in solution). While the monomer–excimer equilibrium has been thoroughly investigated,^{5–8} studies on the effect of certain quenchers on the ratio of the two species remains limited. We report here on the potential application of differential excimer and monomer quenching as a method for detecting electron deficient substrates. The work is at the ‘proof-of-concept’ stage and early studies look very promising.

The fluorescence spectra of moderately concentrated solutions of pyrene show two distinct emission bands. The fine structured band below 400 nm corresponds to emission from the singlet excited state monomer, ¹M*. The broad band centered around 470 nm is emission due to the formation of ‘excited dimers’, or excimers. The excimer ¹E* is a relatively stable complex between one excited molecule of pyrene and another in its ground state,⁹ with a binding energy of 38–42 kJ mol⁻¹ in non-viscous solvents.^{10–12}

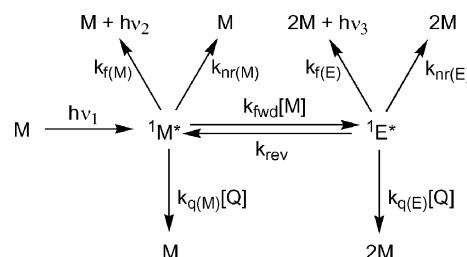
A variety of molecules are able to quench fluorescence emission from pyrene *via* charge-transfer or electron-transfer mechanisms.¹³ In the case of electron-deficient nitromethane, the mechanism of fluorescence quenching was rigorously explored *via* computational studies by McGuffin and coworkers.¹⁴ They concluded that the most likely mechanism is the formation of an excited state ion pair following electron transfer from pyrene to nitromethane. Nitromethane is also known to selectively quench alternant polycyclic aromatic hydrocarbons (by acting as the electron acceptor) whereas alkyl amines act as electron donors and quench non-alternant PAHs.^{15–18}

In this work we employ steady state and time-resolved emission techniques to examine pyrene fluorescence quenching as a way to discriminate between electron-donor and electron-acceptor quenchers. A series of compounds were used as quenchers, ranging from electron-rich to electron-poor molecules. We propose that the discrimination of these

quenchers between monomer and excimer quenching can find analytical applications in the area of explosive detection.

Results and discussion

The mechanism for the quenching of fluorescence from the monomer and excimer species of pyrene is shown in Scheme 1 (adapted from the literature).¹⁹



Scheme 1 Fluorescence quenching of pyrene. M = monomer form, E = excimer, k_f = fluorescence rate constant, k_q = bimolecular quenching rate constant, k_{nr} = sum of rate constants for non-radiative processes.

In alcohols at room temperature, the rate constant for excimer dissociation, k_{rev} , has values around 10^6 s⁻¹.^{8,12} Since the monomer and excimer singlet excited state lifetimes are ~ 100 ns and ~ 50 ns, respectively, under our conditions (*vide infra*), we expect excimer dissociation (k_{rev}) to be unimportant. A concentration of pyrene of 3 mM was used throughout, since comparable emission intensities for monomer and excimer (that this concentration achieves) ensure the best sensitivity in dual quenching experiments; further, under these conditions the criterion of negligible excimer dissociation is readily met.

The electron-rich compounds (*i.e.* tribenzylamine and *p*-dimethoxybenzene) displayed no significant quenching of pyrene, even at high quencher concentrations ([Q]), due to their small values of k_q . Anilines (e.g. DMA shown in Fig. 1, top),

$$\frac{\Phi_f^0}{\Phi_f} \approx \frac{F_0}{F} = 1 + k_q \tau_f^0 [Q] = 1 + K[Q] \quad (1)$$

displayed a moderate degree of quenching of both emissions, illustrated as Stern–Volmer plots in the inserts in Fig. 1. This dynamic quenching is described by the Stern–Volmer expression, eqn (1),²⁰ where Φ refers to quantum yields and F to emission intensities in arbitrary units.

DMA and other amines showed equivalent monomer and excimer quenching. There are in fact two situations in which

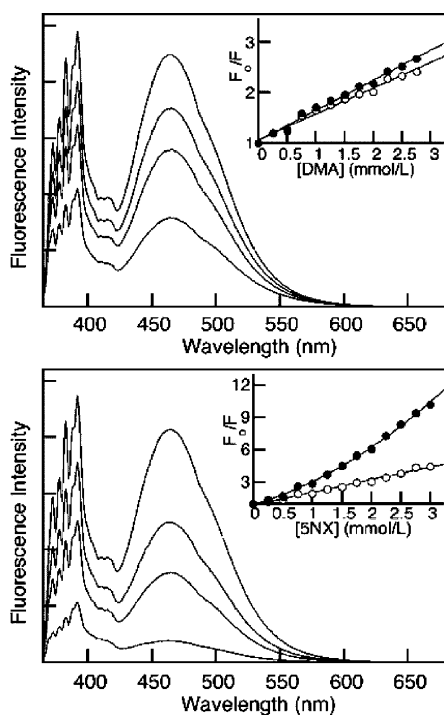


Fig. 1 Steady-state fluorescence quenching of pyrene (3 mM in 99% ethanol, $\lambda_{ex} = 355$ nm). Top: Q = *N,N*-dimethylaniline (DMA); Bottom: Q = 5-nitro-*m*-xylene. Insets: Stern–Volmer plots taken at 392 nm to represent the monomer form (○) and 464 nm to represent the excimer form (●); bandpath ~ 3 nm. Some spectra omitted for clarity. Note the differences in the F_0/F scale in the inserts.

the slopes for monomer and excimer quenching are expected to be the same: either (a) the monomer–excimer equilibrium is maintained at all times, or (b) the monomer form is being quenched exclusively, and the observed excimer quenching merely reflects the reduced monomer population. Since we have already discarded the presence of an equilibrium, the latter situation must apply for systems where both (monomer and excimer) quenching plots have the same slope. Thus, identical Stern–Volmer plots (*i.e.*, K_M and K_E) are expected, as shown in the inset graph. This behavior reflects that excimers are “stabilized” against electron-transfer quenching.¹⁹ Selective monomer quenching by aromatic amines has also been observed by Ottolenghi and coworkers, who measured the monomer-to-excimer fluorescence ratio of pyrene-labeled lipid probes to examine lipid miscibility in bilayers.²¹

Interestingly, the electron-deficient compounds (5-nitro-*m*-xylene shown) had monomer fluorescence quenching which followed the Stern–Volmer relationship. However, when plotted, the excimer form showed a positive deviation from the expression. In all cases, the excimer fluorescence, while attenuated, shows no significant spectral changes.

This deviation indicates a combination of two-state quenching resulting from a reduced $^1M^*$ population, and dynamic fluorescence quenching of the excimer itself. In other words, these quenchers are strong enough to deactivate the excimer complex. Those quenchers which displayed the strongest deviation were nitroaromatics, with bimolecular quenching rate constants approaching diffusion control (*vide infra*).

The concepts outlined above add a new dimension to the application of pyrene fluorescence quenching to analyze electron-deficient molecules, particularly nitroaromatics. By recording the *ratio* of monomer-to-excimer emission intensities, it is possible to have a detection method that is only sensitive to electron-deficient molecules, suggesting possible methodologies for explosives detection.

Simultaneous monomer and excimer quenching allows the generation of calibration curves by plotting the ratio of the

Table 1 Slope values from the plots of F_M/F_E for various quenchers^a

Substrate	Slope/ M^{-1}
Nitrobenzene, NB	618
<i>p</i> -Dinitrobenzene, pDNB	595
5-Nitro- <i>m</i> -xylene, 5NX	588
<i>m</i> -Dinitrobenzene, mDNB	572
Nitromethane, NM	191
Nitroethane, NE	170
<i>N,N</i> -Dimethylaniline, DMA	11.1
Triethylamine, TOA	6.0
<i>N,N</i> -Dimethyl-2,6-diisopropylaniline, DDA	−5.3

^a Typical errors are <5%.

fluorescence intensities of the two forms (F_M/F_E) as a function of quencher concentration. As a result, quantification of unknown samples will be possible. Table 1 includes the values of the slopes of the calibration curves constructed for the various quenchers. From this, three clear groupings can be discerned. Aromatic amines do not differentiate between monomer and excimer and hence their graphs are nearly horizontal. The nitroaliphatics show some discrimination and thus their slopes are larger (around 150 to 200 M^{-1}). Again, the nitroaromatics display the greatest degree of quenching, leading to slopes in the 550 to $-625 M^{-1}$ range; therefore, assuming a value of $590 \pm 40 M^{-1}$ will allow the estimation of an approximate concentration, even for molecules where a sample of the authentic material is not available. Representative example curves of these three groups, DMA, NE, and 5NX, respectively, are shown in Fig. 2.

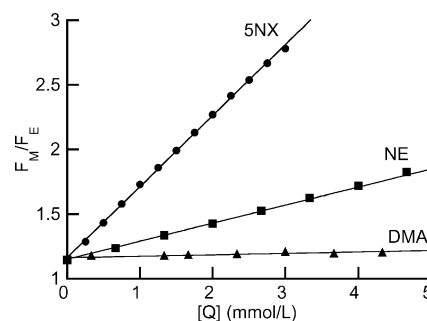


Fig. 2 Left: representative calibration curves constructed by taking the ratio of fluorescence intensities of 3 mM pyrene (in ethanol) at 392 nm and 464 nm (F_M/F_E) as quencher is added: (●) Q = 5-nitro-*m*-xylene; (■) Q = nitroethane; (▲) Q = *N,N*-dimethylaniline. Right: F_M/F_E “ruler” indicating the values of the slopes of the calibration curves for each of the quenchers used in this study.

In order to establish mechanistic details, our results include time-resolved studies of monomer and excimer quenching. However, we wish to note that these measurements would not be required for analytical applications, where only steady state fluorescence at two separate wavelengths would be required.

Time-resolved studies show that quenching is a dynamic process, and that at the concentrations employed pyrene–quencher preassociation is not important. Monomer fluorescence (monitored at 392 nm) can be fitted with a monoexponential decay, confirming that dissociation from the excimer is negligible. Fig. 3 (top) shows the quenching plot for mDNB, where the observed rate constant (τ^{-1}) is plotted against the quencher concentration. The bottom panel shows Stern–Volmer plots based on emission intensity (F_0/F) and on lifetimes (τ_0/τ). The excellent agreement observed is normally accepted as a good indicator that only dynamic quenching is involved.¹³ Similar results were obtained with other quenchers. The time-resolved studies such as that of Fig. 3 lead, from the slope, to the rate constants for excited state quenching, k_q , these values are reported in Table 2. Not

Table 2 Quenching rate constants for pyrene monomer fluorescence in ethanol at room temperature^a

Substrate	$k_q/M^{-1} s^{-1}$
1,4-Dicyanobenzene, DCB	9.7×10^9
5-Nitro- <i>m</i> -xylene, 5NX	8.0×10^9
Nitrobenzene, NB	7.7×10^9
<i>p</i> -Dinitrobenzene, pDMB	1.0×10^{10}
<i>m</i> -Dinitrobenzene, mDNB	9.9×10^9
Nitromethane, NM	3.6×10^9
<i>N,N</i> -Dimethylaniline, DMA	4.6×10^9

^a Typical errors are <5%.

was fixed to values calculated according to the linear fit shown in Fig. 3, top, to minimize error from the actual experimental data points. All three sets of values (k_1 expt'l, k_1 calc'd, and the resulting k_2 from eqn (2)) are shown in Table 3.

Since the integrals under the emission curves shown in Fig. 4 are proportional to the emission intensity under steady state conditions, a Stern–Volmer plot, analogous to Fig. 3 (bottom) can also be constructed for the excimer, as shown in Fig. 4 (bottom). The plot based on τ_0/τ , is as expected, since it only reflects excimer quenching. In contrast, the plot of I_0/I (based on the integrals under the curves) is expected to be a parabola,¹³ and is fitted as such in Fig. 4 (bottom). Only small curvature is introduced by the excimer term in this case.

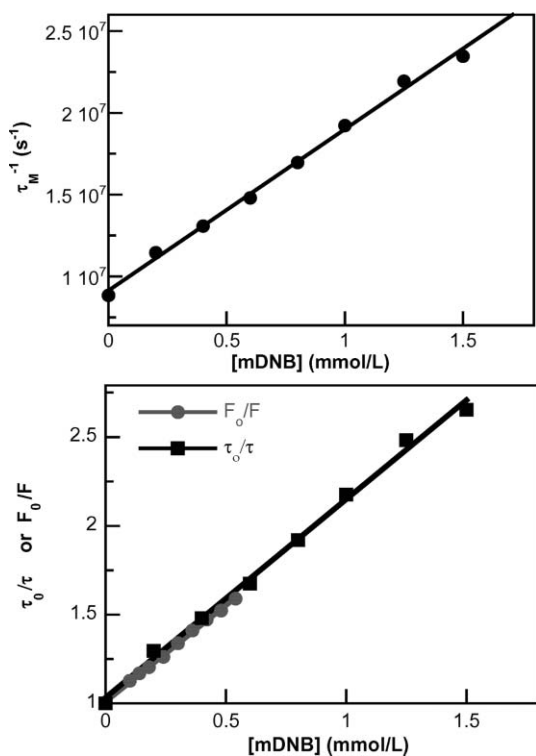


Fig. 3 Quenching of fluorescence at 392 nm from 3 mM pyrene in ethanol by mDNB, following excitation at 355 nm. Top: Quenching data from time-resolved studies; Bottom: Stern–Volmer plots based on steady state measurements (●) and time-resolved studies (■).

surprisingly, other electron deficient substrates (such as 1,4-dicyanobenzene) are also excellent quenchers.

$$I_t = A_0 \frac{k_1}{k_2 - k_1} (\exp(-k_1 t) - \exp(-k_2 t)) \quad (2)$$

Fig. 4 (top) shows the growth and decay of the excimer luminescence monitored at 464 nm. The data can be fitted with a series of two exponentials (eqn (2)) to yield k_1 (τ_M^{-1}) and k_2 (τ_E^{-1}). In order to improve the fitting, the growth component (k_1)

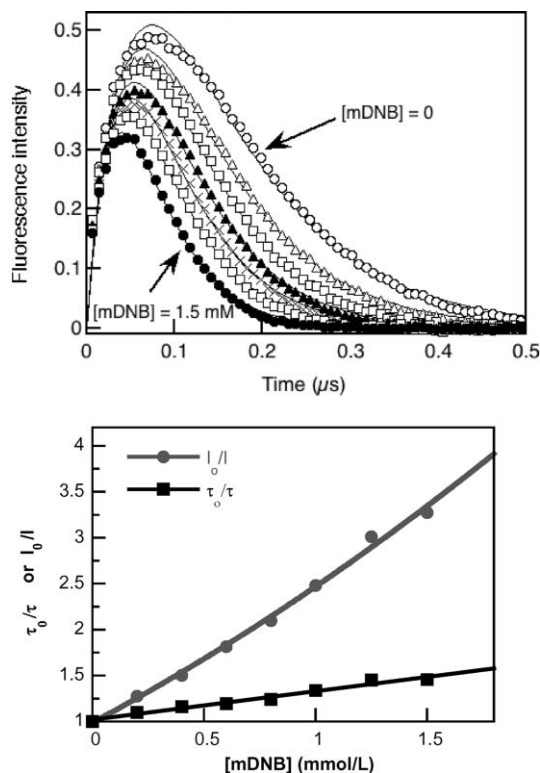


Fig. 4 Top: Time-resolved excimer fluorescence quenching of 3 mM pyrene in ethanol by mDNB, monitored at 464 nm following excitation at 355 nm. Bottom: Stern–Volmer plots based on steady state measurements (●) and time-resolved studies (■).

Potential analytical applications

The use of fluorescence spectroscopy to detect explosives is not new; an example is modification of environmental or forensic sample analysis: currently, samples are generally analyzed according to the EPA8330 protocol developed by the U.S. Environmental Protection Agency. In this method, samples are injected into a reverse-phase HPLC with UV-vis detection.²²

Table 3 Experimental and calculated rate constants for pyrene fluorescence quenching by mDNB in ethanol at room temperature

[mDNB]/mmol L ⁻¹	k_1 (expt'l) ^a , $\times 10^7 s^{-1}$	k_1 (calc'd) ^b , $\times 10^7 s^{-1}$	k_2 (expt'l) ^c , $\times 10^7 s^{-1}$
0.00	0.88	0.91	1.89
0.20	1.14	1.11	2.08
0.40	1.31	1.31	2.20
0.60	1.48	1.50	2.25
0.80	1.70	1.70	2.35
1.00	1.92	1.90	2.53
1.25	2.19	2.15	2.74

^a Data shown in Fig. 3, top. ^b Values calculated according to the linear fit shown in Fig. 3, top. ^c Values obtained by fitting Fig. 4, top with eqn (2) and k_1 (calc'd).

Similar methods employ capillary liquid chromatography to separate and identify sample mixtures.²³ Although sensitivity is high, it offers little selectivity towards explosives (electron-deficient compounds), especially when these compounds are part of complex mixtures. The addition of a fluorescence spectrophotometer as a detection system has previously been explored and has been proven to be an effective method: a fluorophore (pyrene) is introduced post-column *via* a mixing tee and the resulting fluorescence quenching by separated components is monitored,⁴ resulting in a significant increase of the signal-to-noise ratio. We propose that recording the monomer fluorescence quenching, as reported by Goodpaster and McGuffin,⁴ concurrent with acquisition of the ratio F_M/F_E will allow clear discrimination between general and specifically electron-deficient quenchers. Experimentally this would require the use of a multiwavelength fluorescence detector (such as a diode array), rather than a single wavelength detector.

Application of the ideas illustrated here to conventional LC analysis (*i.e.* F_M/F_E acquisition) should lead to chromatograms that highlight those peaks which are due to electron deficient molecules such as nitrated compounds, including explosives. While complete quantitation would be possible with authentic samples, approximate values ($\pm 15\%$) could be obtained by assuming a representative slope for the F_M/F_E plot. The fact that nitrated aromatics cluster with slopes around $590 \pm 40 \text{ M}^{-1}$ is partly due to the fact that monomer quenching approaches diffusion control for these samples. Finally, it should be noted that while this technique would require higher concentrations of fluorophore (to facilitate excimer formation), quenching does not require significantly larger concentrations of substrate. For typical molecular weights the detection limit should be below 10 ppm. The method may respond to electron deficient molecules other than explosives, thus being prone to some false positives; however, more importantly, the method is very unlikely to lead to false negatives, a desirable characteristic in security-related applications.

Conclusion

In conclusion, two-wavelength analysis of the quenching of pyrene fluorescence by a range of molecules shows that the monomer-to-excimer fluorescence ratio unequivocally senses electron deficient molecules and provides approximate concentration information even in the absence of authentic samples. We propose that this interesting characteristic could be employed in the detection of explosives.

Experimental

Reagents

Pyrene (99%) was obtained from Aldrich (Oakville, Ontario, Canada) and recrystallized in methanol before use. Nitromethane (NM), nitroethane (NE), nitrobenzene (NB), *p*-dicyanobenzene (DCB), 5-nitro-*m*-xylene (5NX), trioctylamine (TOA), tribenzylamine (TBA), *N,N*-dimethylaniline (DMA), and *p*-dimethoxybenzene (DMB) were all obtained from Aldrich and used as received. *N,N*-dimethyl-2,6-diisopropylaniline (DDA) from Carbolabs, Inc. (Bethany, Conn., USA), *m*-dinitrobenzene (mDNB) from Avocado Research Chemicals (Heysham, UK), and *p*-dinitrobenzene (pDNB) from Eastman Organic Chemicals (Rochester, NY, USA) were also used as received.

Instruments

Steady-state fluorescence spectroscopy was carried out using a Photon Technology International version 1.2X luminescence spectrometer.

Time resolved fluorescence was recorded using the third harmonic of a Surelite Nd:YAG laser generating pulses at

355 nm of 6 ns duration and $\sim 15 \text{ mJ}$ energy. The signals from the monochromator/photomultiplier system were initially captured by a Tektronix 2440 digitizer and transferred to a Power Macintosh computer that controlled the experiment with a software developed in the LabVIEW environment from National Instruments.²⁴ A total of four shots were averaged when obtaining the decay traces. For fluorescence lifetimes above 50 ns, typical errors are $< 3\%$ for the main emission component, while shorter lifetimes can have as much as 10% error.

Sample preparation

Fluorescence of nitrogen-saturated solutions of 3 mM pyrene in 99% ethanol or spectroscopic-grade acetonitrile were measured in fused silica cuvettes from Luzchem Research with a 10 mm or 7 mm optical path, for steady-state or time-resolved measurements, respectively. Deaerating the samples is important, since oxygen is a good excited state quencher; oxygen concentrations above 1% of saturation (5% relative to air) can interfere with the quenching plots. Quenchers were added either as neat liquids or as solutions in ethanol or acetonitrile in 5 to 10 μL increments to pyrene samples using a 10 μL Hamilton syringe.

Acknowledgements

The Natural Sciences and Engineering Research Council of Canada (NSERC) has generously supported this research through a Canada Graduate Scholarship (KSF) and a discovery grant (JCS). The authors would like to thank a particularly helpful reviewer.

References

- 1 M. H. Schoenfish, Z. Huiping, M. C. Frost and M. E. Meyerhoff, Nitric Oxide-Releasing Fluorescence-Based Oxygen Sensing Polymeric Films, *Anal. Chem.*, 2002, **74**, 5937–5941.
- 2 Y. He and L. Geng, *In Situ* Time-Resolved Fluorescence Spectroscopy in the Frequency Domain in Capillary Electrochromatography, *Anal. Chem.*, 2002, **74**, 1819–1823.
- 3 J. Ji, N. Rosenzweig, I. Jones and Z. Rosenzweig, Molecular Oxygen-Sensitive Fluorescent Lipobeads for Intracellular Oxygen Measurements in Murine Macrophages, *Anal. Chem.*, 2001, **73**, 3521–3527.
- 4 J. V. Goodpaster and V. L. McGuffin, Fluorescence Quenching as an Indirect Detection Method for Nitrated Explosives, *Anal. Chem.*, 2001, **73**, 2004–2011.
- 5 J. B. Birks, D. J. Dyson and I. H. Munro, 'Excimer' Fluorescence II. Lifetime Studies of Pyrene Solutions, *Proc. R. Soc. London, Ser. A*, 1963, **275**, 575–588.
- 6 J. B. Birks and H. G. Seifert, Double-Photon Excitation of Excimer Fluorescence of Pyrene Solutions, *Phys. Lett.*, 1965, **18**, 127–128.
- 7 J. B. Birks and A. J. H. Alwattar, Influence of Environment on the Radiative and Radiationless Transition Rates of the Pyrene Excimer, *Chem. Phys. Lett.*, 1971, **11**, 89–92.
- 8 J. B. Birks, *Photophysics of Aromatic Molecules*, Wiley-Interscience, New York, 1970.
- 9 A. Gilbert and J. Baggott, *Essentials of Molecular Photochemistry*; Blackwell, Oxford, 1991.
- 10 B. Stevens, Influence of Environment on the Radiative and Radiationless Transition Rates of the Pyrene Excimer, *Adv. Photochem.*, 1971, **8**, 161.
- 11 F. M. Winnik, Photophysics of Preassociated Pyrenes in Aqueous Polymer Solutions and in Other Organized Media, *Chem. Rev.*, 1993, **93**, 587–614.
- 12 J. Duhamel, M. A. Winnik, F. Baros, J. C. André and J. M. G. Martinho, Diffusion Effects on Pyrene Excimer Kinetics: Determination of the Excimer Formation Rate Coefficient Time Dependence, *J. Phys. Chem.*, 1992, **96**, 9805–9810.
- 13 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd edn, Kluwer Academic, New York, 1999.
- 14 J. V. Goodpaster, J. F. Harrison and V. L. McGuffin, *Ab Initio* Study of Selective Fluorescence Quenching of Polycyclic Aromatic Hydrocarbons, *J. Phys. Chem. A*, 2002, **106**, 10645–10654.
- 15 E. Sawicki, T. W. Stanley and W. C. Elbert, Quenchofluorometric Analysis for Fluoranthenic Hydrocarbons in the Presence of Other Types of Aromatic Hydrocarbon, *Talanta*, 1964, **11**, 1433–1441.

-
- 16 J. V. Goodpaster, S. B. Howerton and V. L. McGuffin, Forensic Analysis of Commercial Petroleum Products Using Selective Fluorescence Quenching, *J. Forensic Sci.*, 2001, **46**, 1358–1371.
 - 17 S. Pandey, K. A. Fletcher, J. R. Powell, M. E. R. McHale, A.-S. M. Kauppila, W. A. Acree, J. C. Fetzer, W. Dai and R. G. Harvey, Spectrochemical Investigations of Fluorescence Quenching Agents Part 5. Effect of Surfactants on the Ability of Nitromethane to Selectively Quench Fluorescence Emission of Alternants PAHs, *Spectrochim. Acta, Part A*, 1997, **53**, 165–172.
 - 18 S. B. Howerton, J. V. Goodpaster and V. L. McGuffin, Characterization of Polycyclic Aromatic Hydrocarbons in Environmental Samples by Selective Fluorescence Quenching, *Anal. Chim. Acta*, 2002, **459**, 61–73.
 - 19 R. A. Caldwell, D. Creed, D. C. DeMarco, L. A. Melton, H. Ohta and P. H. Wine, Charge-Transfer Quenching of Singlet Excited Complexes, *J. Am. Chem. Soc.*, 1980, **102**, 2369–2377.
 - 20 O. Stern and M. Volmer, Uber Die Abklingzeit Der Fluoreszenz, *Phys. Z.*, 1919, **20**, 183–188.
 - 21 Y. Barenholtz, T. Cohen, E. Haas and M. Ottolenghi, Lateral Organization of Pyrene-Labeled Lipids in Bilayers as Determined from the Deviation from Equilibrium between Pyrene Monomers and Excimers, *J. Biol. Chem.*, 1996, **271**, 3085–3090.
 - 22 C. A. Weisberg and M. L. Ellickson, Practical Modifications to US EPA Method 8330 for the Analysis of Explosives by High Performance Liquid Chromatography (Hplc), *Am. Lab.*, 1998, **30**, 32N–32V.
 - 23 J. V. Goodpaster and V. L. McGuffin, Separation of Nitramine and Nitroaromatic Explosives by Capillary Liquid Chromatography, *J. Liq. Chromatogr.*, 2001, **24**, 1965–1978.
 - 24 J. C. Scaiano, Solvent Effects in the Photochemistry of Xanthone, *J. Am. Chem. Soc.*, 1980, **102**, 7747–7753.