Selective sensing of competitive anions by non-selective hosts: the case of sulfate and phosphate in water

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Two sensing ensembles based on the common squaramide host **2** and the acid–base indicators Cresol Red (CR) and Bromocresol Green (BG) are described. Both couples were characterized in EtOH–H_2O mixtures by UV-Vis spectroscopy. Analysis of the binding curves and the corresponding Job plots indicate 1 : 1 binding between the indicators CR or BG and host **2**. The sensing solutions are responsive to sulfate and/or phosphate anions in water, changing the coloration of the solution. As in other indicator displacement assays (IDA), host **2** translates the chemical event into a colorimetric response that is analyzed by spectrophotometry. However, since other anions present in natural waters do not result in color changes, this method allows the quantification of sulfate and phosphate in water.

Introduction

The ubiquitous presence of anionic species in natural waters has triggered considerable research efforts into developing green analytical methodologies.[1] A sustainable assay system must avoid sample pretreatments such as preconcentration and/or separation and also the use of hazardous chemicals. Moreover, the possibility of obtaining numerical results in a short time with a minimum use of reagents is highly desirable for miniaturization.[2] As part of our ongoing effort to develop sensing assays for analytes of biomedical and environmental significance we are studying different hosts for the recognition of sulfate and phosphate in water. These two anions are present in many natural waters in appreciable concentrations and in excess, have deleterious effects on the environment. For example, a level higher than 0.1 mg L^{-1} of phosphate induces undesired algal growth in rivers[3] while sulfate is directly related to acid rain.[4] For these and other reasons, both species are subjected to restrictions[5] and simple assays for them would be of great interest for water quality assessment.

Of particular interest are the methods based on colorimetric detection of the analytes. Of these, the methods based on indicator displacement assays (IDA) are well established for the detection of inorganic as well as organic anions.[6] In colorimetric IDA experiments, a visual signal is detected when the target analyte displaces the indicator from an initial host-indicator couple. Ideally, a synthetic host with large affinities towards the analyte and the indicator is the key to the development of an efficient sensing device. In addition, the affinity of the host for the target anion must be higher than that for the indicator in order to induce appreciable spectroscopic changes. However, the realization of large K_as in aqueous solvents is an exceedingly ambitious goal. This is especially true for phosphate and sulfate, two kosmotropic[7] anionic species that show very high hydration energies[8] and, given their structural similarities, can easily compete for the same host.

Work in our laboratories has focused on a solution phase approach to the sensing of these two anions. Here, we report two sensing ensembles composed of a unique non-selective squaramide host in combination with two structurally related acid–base indicators, Cresol Red (pK_a = 8.25) and Bromocresol Green (pK_a = 4.66). We also demonstrate their use for measuring the individual concentrations of phosphate and sulfate in natural aqueous samples. The proposed method can be considered an example of differential sensing,[9] as it works with both IDA ensembles used in conjunction.

In this regard, the use of non-selective synthetic hosts is an apparent contradiction of the more conventional strategies based on selective recognition. However, it must be emphasized that for analytical applications selectivity must be claimed for the whole assay rather than for a particular host–guest complex.[10]

The squaramide host design is based on the robustness of the tetraalkylammonium group, a classic in anion recognition[11] and the hydrogen bond donor capability of the squaramide moiety.[12] By combining these two elements, the resulting host can bind oxoanions in aqueous solvents working under a principle of charge and hydrogen bond selection.[13]

Results and discussion

Squaramide **2** was synthesized according to Scheme 1 from diethyl squarate as described previously.[14] As expected, **2** is soluble in water and in alcohol–water mixtures (9 : 1 v/v). The linear Lambert–Beer plot at 290 nm indicates the absence of aggregation phenomena in the range 10^{-4}–10^{-6} M used in this study. Complex formation with Cresol Red (CR)[15] and Bromocresol Green (BG) was investigated by UV-visible

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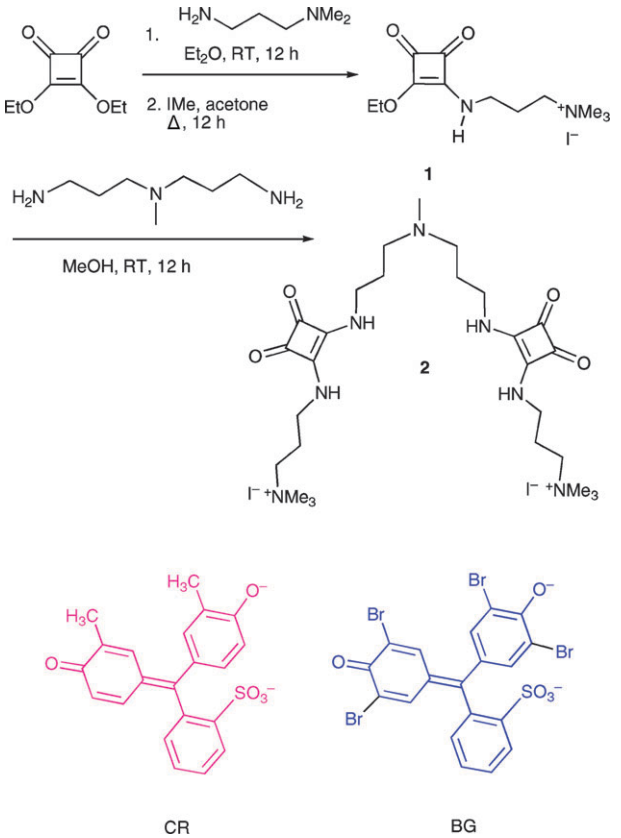

Scheme 1 Synthesis of flexible squaramide host **2** and structures of the two indicators involved in this work, Cresol Red (CR) and Bromocresol Green (BG) in their dibasic forms.

spectroscopy. Since host **2** is positively charged and CR and BG display a phenolic and a sulfonic acid groups, it is reasonable to expect the strongest binding to occur in the pH range where both groups exist as dianions, that is, when the acidity of the solutions are above their respective pK_as.

Characterization of IDA ensembles

Interaction of **2** with CR and BG was followed by UV-Vis spectroscopy. Initially, the apparent pH of the starting solutions was kept low enough to ensure the occurrence of the monobasic (yellow) form of the indicator, $[IH^-] > 10\,[I^{2-}]$.

Upon addition of **2** to the pH 8.9 (TRIS, 10 mM) buffer solution containing CR (3.9×10^{-5} M) in 96% EtOH–H_2O (9 : 1 v/v) the intensity of the absorption band at around 430 nm gradually decreased and that at 580 nm increased. These bands were ascribed to the monoanionic and dianionic states of CR, Fig. 1(a).[16]

A similar trend is observed upon the addition of **2** to a pH 4.5 (AcOH, 10^{-4} M) solution containing BG (4.0×10^{-5} M). The band at 428 nm decreased and that around 615 increased, Fig. 1(b). Remarkably, in both cases complex formation is accompanied by a hypochromic effect and a slight bathochromic shift relative to the indicator alone.[17] Since the apparent pH remains essentially constant during the whole titrations, the variation of intensity of these two bands are assignable to the formation of a complex between each indicator and host **2**. Thus, it is reasonable to propose a model where addition of **2**

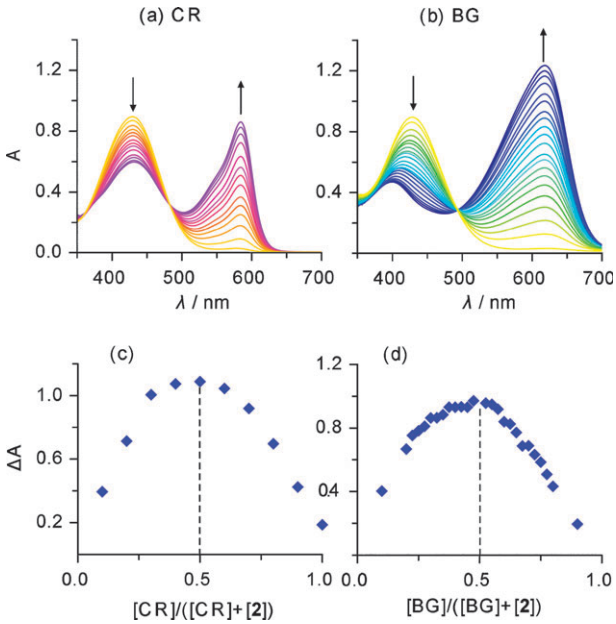

Fig. 1 (a) and (b) UV/Vis spectra of additions of 0.2–10 equivalents of **2** to CR (a) or BG (b). (c) and (d) Job plots obtained at [**2**] + [I] = 2.0×10^{-4} M, constant by registering the absorption changes observed at 580 nm for CR (c) and at 618 nm for BG (d).

displaces the acid–base equilibrium of CR or BG towards the basic side by preferential complexation with the dianionic form, Fig. 2.

According to this model, the formation of CR·**2** or BG·**2** depend on the apparent pK_a of the indicator (I), yet host **2** competes with H^+ for CR^{2-} or BG^{2-}. The titration data for the interaction of **2** with CR and BG were analyzed with regard to the three colored species, IH^- (CR or BG, monobasic), I^{2-} (CR or BG, dibasic forms) and the 1 : 1 complex (S·**2**). Adjusting the whole data to a simple 1 : 1 binding model with Specfit[18] gave a reasonable fitting and provided the association constants, $K_{11} = 9.3 \times 10^3$ M^{-1} and $K_{11} = 8.4 \times 10^4$ M^{-1} for CR and BG, respectively. It is known that in protic solvents the presence of complexes with stoichiometries greater than 1 : 1 is feasible.[19] Nevertheless, attempts to modify the proposed model by including species such AS $(IH{\cdot}\mathbf{2})^+$ or $(S{\cdot}\mathbf{2}_2)^{2+}$ did not produce any improvement. Moreover, although for host **2** a 1 : 1 stoichiometry is always observed with different guests, techniques and solvents,

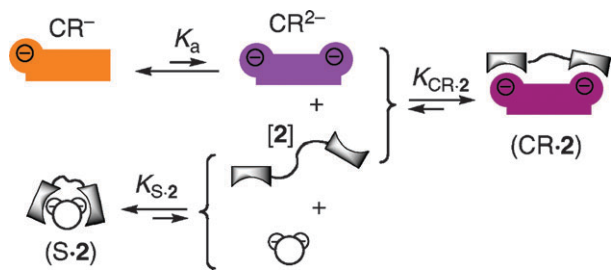

Fig. 2 Illustration of the overall equilibrium between CR and **2** including the three colored species mentioned in the text. The addition of strong competing guests SO_4^{2-} and HPO_4^{2-} (S in the figure) modulates the concentration of the sensing ensemble (CR·**2**).

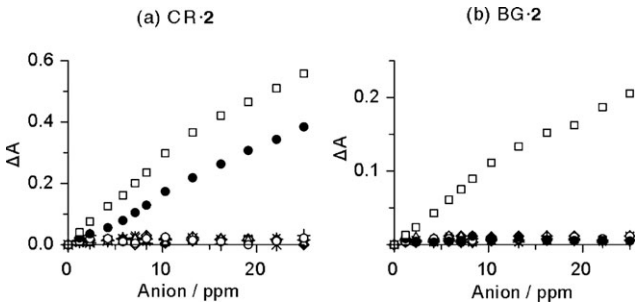

Fig. 3 Changes in the absorption of the two sensing ensembles: (a) at 580 nm for CR·**2** and (b) at 628 nm for BG·**2** upon incremental addition of 0–25 ppm of the following anions: SO_4^{2-} (□); $HPO_4^{2-}/H_2PO_4^-$ (●); CO_3^{2-}/HCO_3^- (○); NO_3^- (△); NO_2^- (×); F^- (+); Cl^- (◇); Br^- (*); I^- (◆).

we further confirmed the 1 : 1 stoichiometry by independent Job plot analysis, Fig. 1(c) and (d).

The interaction between host **2** and a sulfate anion is evident by ^1H NMR even in competitive solvents such as DMSO or MeOH. Thus, upon addition of tetramethylammonium sulfate, the resonances of the NH squaramido groups of **2** in DMSO-d_6 show a large downfield shift (>2 ppm) indicating the participation of hydrogen bonds. In MeOH-d_4, the resonances of the N–CH protons also display diagnostic CIS effects.[13] In EtOH–H_2O mixtures, changes were studied by UV-Vis. The addition of sulfate and/or phosphate to a sensing ensemble composed of CR (4.0×10^{-5} M) and **2** (2.0×10^{-4} M) produced the restoration of the typical yellowish color of the monoacid form of the indicator as it was released from the complex. The couple CR·**2** is selective for sulfate and phosphate (as HPO_4^{2-}) in the presence of other anions that are common in natural waters, Fig. 3(a). Relative to this, the couple BG·**2** is even more selective since the band at 618 nm only diminishes upon addition of sulfate, but not with phosphate. This effect is due to the lower pH of the solution. At the pH of the experiment, phosphate occurs as $H_2PO_4^-$ and does not compete with SO_4^{2-} for the same host, Fig. 3(b). However, the relative change in intensity of the absorption is smaller than that observed for the couple CR·**2**. This is in agreement with the higher association constant measured for **2** *vs.* BG which is roughly one order of magnitude higher than that to CR. In a extreme case, where $K_{I\cdot 2} \gg K_{S\cdot 2}$, the sensing ensemble would be insensitive to changes in the concentration of the analyte. In practical terms, it means that changes in concentration of sulfate will result in a smaller change of absorption intensity. Overall, the two sensing ensembles have an excellent colorimetric response and the selectivity between sulfate or phosphate with CR·**2**, or sulfate alone with BG·**2**, compared to other potentially competitive anions is better than 100 : 1, Fig. 3.

Plate reader determinations

These results enable the quantitative analysis of sulfate and phosphate by comparison with calibration curves. In order to demonstrate the viability of the anions determination we implemented our IDA method on a UV-Vis microplate reader. Experiments were performed by adding a small volume of the water sample to be measured (20 μl) to 220 μl of a measuring solution containing a mixture of CR or BG (8.0×10^{-4} M) and **2** (2.0×10^{-4} M), see Experimental section. Seven calibration solutions of SO_4^{2-} (Na_2SO_4) and a blank solution were also incorporated in each plate.

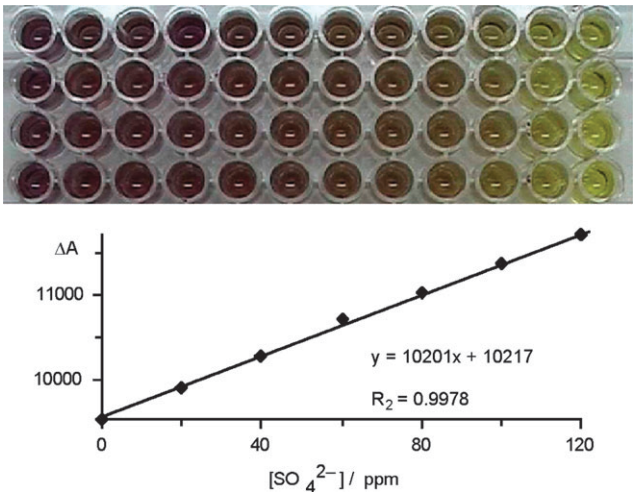

Fig. 4 Top: part of a microplate showing the gradation in coloration produced upon addition of sulfate or phosphate dianions in a sensing solution composed of CR·**2** Bottom: representative calibration curve obtained for SO_4^{2-} (CR·**2**) over the concentration range 0–120 ppm. Data points are the mean of four samples from one experiment. ΔA is the difference in absorbance, in relative counts, measured between blank and sample solutions.

The working calibration curves were linear (minimum correlation coefficients, 0.995) in the range of study 0–120 ppm and were verified on each plate. The limits of detection were 3 ppm for SO_4^{2-} and 5 ppm for HPO_4^{2-}. In a representative experiment, quadruplicate samples of water were added to 220 μl of the measuring solution. The only difference among the wells was the different composition of water added into the wells: natural water, solutions with known concentrations, and pure water as sample, calibrating solutions and blank, respectively.

Fig. 4 shows the gradation of color due to the increased concentration of sulfate in a microplate using the sensing system composed of the host **2** and the indicator CR. This method was applied to the direct determination of sulfate in a series of samples of potable water lacking phosphate. The results in Table 1 indicate that there is no significant difference between our IDA methods when compared with a standard determination by ionic chromatography (IC).

Table 1 Concentration of SO_4^{2-} in different samples of water[a]

	SO_4^{2-}/ppm		
Sample	CR·**2**	BG·**2**	IC
Water well no. 1	55.5	48.0	61.6
Water well no. 2	74.2	72.8	75.2
Water well no. 3	40.4	44.2	41.5
Spring water no. 1	13.8	11.1	11.5
Spring water no. 2	17.9	16.7	15.9
Certified water[b]	54.9	52.6	53.2[c]

[a] Estimated errors are approximately 10% at the 95% confidence limit. [b] Certified Reference Material LGC6020 Riverwater, River Thames. [c] Certified value.

Table 2 Comparison of results[a] obtained from IDA and IC assays[ab]

Nominal concentration[c]		IDA[d]		IC[d]	
SO_4^{2-}	HPO_4^{2-}	SO_4^{2-}	HPO_4^{2-}	SO_4^{2-}	HPO_4^{2-}
73.2	0	71.1	0.5	72.9	0
100	40	96.8	40.3	97.5	37.0
20	15	13.5	14.4	18.4	13.3
0	80	3.3	74.6	0	77.1

[a] ppm. [b] Estimated errors are approximately 10% at the 95% confidence limit. [c] Amount of each anion expected to be present in the sample. [d] Experimental concentration found by the indicated method.

The use of the two sensing ensembles working in parallel allows the quantification of sulfate and phosphate present simultaneously in water samples due to the linear response of both systems. The combined method requires three calibration curves, namely: phosphate with the couple CR·**2** and sulfate with CR·**2** as well as BG·**2**. First, the concentration of SO_4^{2-} is determined directly from BG·**2** as described. The resulting concentration is transformed in a virtual absorbance with CR·**2** that stands for the contribution of SO_4^{2} to the overall change of absorbance observed with CR·**2**. Finally, the contribution of HPO_4^{2-} is calculated from the difference:

$$\Delta A(HPO_4^{2-}) = \Delta A_{obs} - \Delta A_{calc}(SO_4^{2-})$$

Table 2 shows the concentrations obtained with four test samples of water containing both SO_4^{2-} and HPO_4^{2-}. The values obtained with the IDA combined method are in reasonable agreement with those obtained by ionic chromatography.

Conclusion

Colored complexes of the commercial indicators CR and BG with the receptor **2** are very sensitive to the addition of SO_4^{2-} and/or HPO_4^{2-} and have proved to be suitable IDA ensembles for anion sensing. Importantly, we implemented our IDA method on a microplate reader using visible spectroscopy. The new protocol is faster and environmentally safer than any classical method and compares well with ionic chromatography. These results open up new opportunities in the chromogenic sensing of highly hydrophilic anions by poorly selective synthetic receptors.

Experimental

UV-Vis titrations

All titrations were performed at room temperature in 96% EtOH–H_2O (9 : 1 v/v) on a Cary 300 UV-Vis instrument (Varian) using Teflon stopped quartz cells with pathlength of 1.0 cm. For CR, a cuvette with TRIS buffered solution (10^{-2} M, apparent pH 8.9) served as the blank. The second cuvette was filled with 2 ml of the buffered solvent mixture containing the indicator, CR (4.0×10^{-5} M). The titrant solution was prepared by dissolving host **2** (8.0×10^{-4} M) in 3 ml of the above solution thus, the concentration of the indicator remained constant during the titration. Portions of the titrant (10–300 μl) were added by syringe to the measuring cuvette at 4 min time intervals. After stirring, the UV/Vis spectrum was recorded in the range 350 to 700 nm. The data obtained by UV-Vis spectrophotometric titrations were analyzed by fitting the whole series of spectral data at 0.5 nm intervals by using the SPECFIT/32 program.[16] Additionally the spectra of CR containing only the monobasic or the dibasic forms were imported and fixed. An apparent acidity constant (K_a = 9.19) of CR, obtained by separate pH titrations in a similar solvent mixture, was also fixed. The fitting procedure using a three colored species model allowed globally optimized parameters to be obtained.

Titrations of BG *vs.* **2** were carried out in a similar fashion as for CR and the apparent pH of 4.5 was adjusted by adding AcOH (4.0×10^{-2} M). The apparent acidity constant for BG (K_a = 5.5) was also measured and fixed as above.

Microplate reader determinations

Quantitative determinations were performed using a transparent polystyrene UV 96 well plate with a clear flat bottom. Two measuring solutions were prepared, (A) containing CR (8.0×10^{-4} M), **2** (2.0×10^{-4} M), TRIS (1.0×10^{-2} M) in 96% EtOH–H_2O (9 : 1 v/v) and (B) BG (8.0×10^{-4} M), **2** (1.0×10^{-4} M), AcOH (4.0×10^{-2} M) in 96% EtOH–H_2O (9 : 1 v/v). In a typical experiment, 200 μl of the measuring solution A or B were introduced into the wells with a multichannel pipette; to these solutions, 20 μl of pure water (blank), calibrating solutions or water samples to be determined were added according to the measurement. The microplate was automatically shaken for 30 s before reading the absorbance on a Hidex Plate Chameleon 425–104 microplate reader equipped with 580 nm (CR) or 620 nm (BG) filters. The reading cycle was repeated at least five times.

For comparative purposes, the concentration of SO_4^{2-} and HPO_4^{2-} was also measured by ionic exchange chromatography on an IonPac column for anion analysis (Dionex) at a constant flux of 1.01 mL min^{-1}.

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References

1 L. H. Keith, L. U. Gron and J. L. Young, *Chem. Rev.*, 2007, **107**, 2695.
2 K. H. Yea, S. Lee, J. B. Kyong, J. Choo, E. K. Lee, S. W. Joo and S. Lee, *Analyst*, 2005, **130**, 1009.
3 G. Hanrahan, M. Gledhill, P. J. Fletcher and P. J. Worsfold, *Anal. Chim. Acta*, 2001, **440**, 55.
4 D. W. Schindler, *Science*, 1988, **239**, 149.
5 See, EU Directives 96/61/EC and 98/83/EC.
6 (*a*) C. Schmuck and M. Schwegmann, *Org. Biomol. Chem.*, 2006, **4**, 836; (*b*) R. G. Hanshaw, E. J. O'Neil, M. Foley, R. T. Carpenter and B. D. Smith, *J. Mater. Chem.*, 2005, **15**, 2707; (*c*) Y. Kubo, T. Ishida, A. Kobayashi and T. D. James, *J. Mater. Chem.*, 2005, **15**, 2889; (*d*) R. Martínez-Mañez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419; (*e*) S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963.

7 Kosmotropic anions are those anions that contribute to the long-range structure of water, they are ''water structure makers'', see: D. J. Tobias and J. C. Hemminger, *Science*, 2008, **319**, 1197.

8 (*a*) J. W. Steed and J. L. Atwood, *Supramolecular Chemistry*, Wiley, Chichester, 2000; (*b*) A. Bianchi, K. Bowman-James and E. García-España, *Supramolecular Chemistry of Anions*, Wiley-VCH, Chichester, 1997.

9 S. L. Wiskur, P. N. Floriano, E. V. Anslyn and J. T. McDevitt, *Angew. Chem., Int. Ed.*, 2003, **42**, 2070.

10 (*a*) E. V. Anslyn, *J. Org. Chem.*, 2007, **72**, 687; (*b*) B. E. Collins, A. T. Wright and E. V. Anslyn, *Top. Curr. Chem.*, 2007, **277**, 181.

11 (*a*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486; (*b*) K. Bowman-James, *Acc. Chem. Res.*, 2005, **38**, 671; (*c*) T. Clifford, A. Danby, J. M. Llinares, S. Mason, N. W. Alcock, D. Powell, J. A. Aguilar, E. García-España and K. Bowman-James, *Inorg. Chem.*, 2001, **40**, 4710; (*d*) E. Garcia-España, P. Diaz, J. M. Llinares and A. Bianchi, *Coord. Chem. Rev.*, 2006, **250**, 2952; (*e*) F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609; (*f*) M. A. Hossain, S. O. Kang, D. Powell and K. Bowman-James, *Inorg. Chem.*, 2003, **42**, 1397.

12 (*a*) R. Prohens, G. Martorell, P. Ballester and A. Costa, *Chem. Commun.*, 2001, **16**, 1456; (*b*) R. Prohens, S. Tomas, J. Morey, P. M. Deya, P. Ballester and A. Costa, *Tetrahedron Lett.*, 1998, **39**, 1063.

13 C. Schmuck and V. Bickert, *J. Org. Chem.*, 2007, **72**, 6832.

14 M. N. Piña, C. Rotger, B. Soberats, P. Ballester, P. M. Deyà and A. Costa, *Chem. Commun.*, 2007, **9**, 963.

15 M. N. Piña, M. C. Rotger, A. Costa, P. Ballester and P. M. Deya, *Tetrahedron Lett.*, 2004, **45**, 3749.

16 D. Heger, J. Klánová and P. Klán, *J. Phys. Chem. B*, 2006, **110**, 1277.

17 Similar changes are described for the association of anionic dyes with ammonium salts, see: A. A. Ishchenko and S. A. Shapovalov, *J. Appl. Spectrosc.*, 2004, **71**, 605.

18 R. A. Binstead, B. Jung and A. D. Zuberbühler, *Specfit/32, © 2000*, Spectrum Software Associates, Marlborough, MA, USA, 2000.

19 M. Rekharsky, Y. Inoue, S. Tobey, A. Metzger and E. Anslyn, *J. Am. Chem. Soc.*, 2002, **124**, 14959.