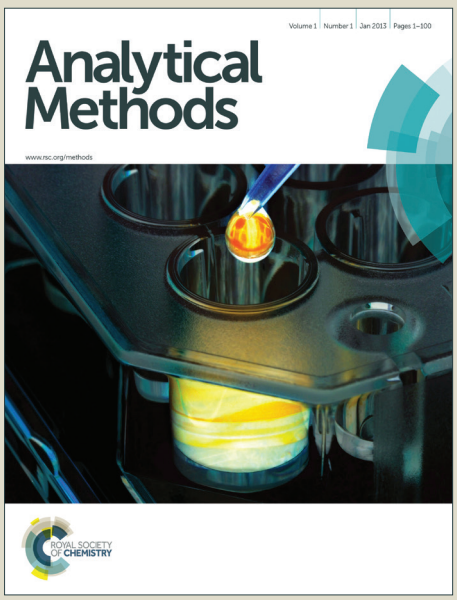
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods

RSCPublishing

COMMUNICATION

New Optical Boron Detection Method

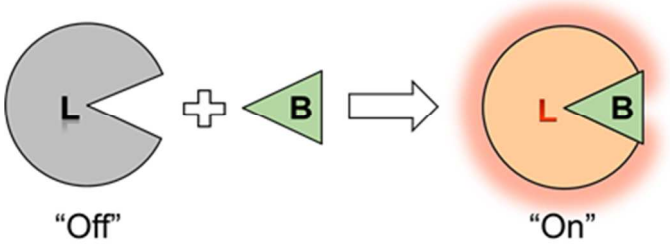
Cite this: DOI: 10.1039/x0xx00000x

Sérgio P. C. Alves,[a] Carlos Baleizão[a]* and José Paulo S. Farinha[a]*

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/



Analytical Methods

RSCPublishing

COMMUNICATION

New Optical Boron Detection Method

Cite this: DOI: 10.1039/x0xx00000x

Sérgio P. C. Alves,[a] Carlos Baleizão[a]* and José Paulo S. Farinha[a]*

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

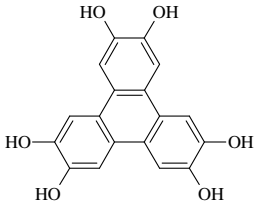
www.rsc.org/

The detection of boron, either as boric acid or phenylboronic acids, in natural or residual waters, still faces a number of challenges. Here, we propose the use of 2,3,6,7,10,11-hexahydroxytriphenylene as an optical sensor for boron, using spectrofluorimetry or UV-visible spectrophotometry detection. We evaluate this sensor for the quantification of both boric acid and phenylboronic acid in aqueous solution. The limit of detection of the sensor using spectrofluorimetry is 10 ppb of boron for boric acid and 6 ppb of boron for phenylboronic acid, with the interference of metal cations efficiently eliminated by using a chelating agent such as EDTA. The proposed method involves a very simple experimental procedure and is easily amenable for use in field work.

Boron is beneficial to human health and agriculture, but only in trace amounts.[1-5] In drinking water, boron is usually present in concentrations below 0.5 mg/L (0.5 ppm), with the World Health Organization recommending boron concentrations in water for human consumption below 2.4 ppm [3,4] and the European Union legislation establishing a maximum permissible value of 1.0 ppm of boron.[6] On crops, the tolerated boron level in irrigation water depends on the species, with adverse effects being observed even below 0.5 ppm of boron for some crops such as lemon.[5] Boron compounds are used in many industrial applications, including the fabrication of soaps and detergents, glass and ceramics, pesticides, fertilizers, semiconductors, flame retardants, high duress compounds, and pharmaceutical drugs.[2-4] High boron contents in water might result from residual waters discharge but also of leaching from rocks and soils containing borates and borosilicates.[2-4]

The most sensitive methods for the analysis of boron content in water are Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), with a detection limit of 6 to 10 ppb, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), with a detection limit of 0.15 ppb.[3,4,7] However, these methods have several disadvantages, such as high cost, non-portability, and the need of complex equipment. Alternatively, spectrophotometric and spectrofluorimetric detection using chemical sensors offer a simple and relatively low cost alternative, with equipment available for portable use. Generally, spectrofluorimetry methods allow greater sensitivity than methods based on spectrophotometric detection. However, relatively few examples of fluorescent boron sensors have been described.[8,9] Azomethine-H [1,4,10-15] and other molecules with similar structure [16-19] are the most common optical boron sensors, with reported detection limits around 10 ppb of boron.[4] However, these methods are not simple to use, involving the collapse of two chemical precursors (which have to be mixed or produced by hydrolysis of Azomethine-H). [13] Other detection methods have been described, although used in much lower scale.[1,7,8,10,12,15,20-29]

Here we discuss the use of a new optical sensor for boron, 2,3,6,7,10,11-hexahydroxytriphenylene (**L**, Scheme 1), which features a sensitivity of the order of the best boron sensors, even at very low sensor concentration (1 μM), using a very simple measurement procedure, readily adaptable for field use. The sensor molecule **L** is readily available, having been used as a precursor in the synthesis of discotic compounds for liquid crystal mixtures [30] and in supramolecular structures and covalent organic frameworks.[31-34]



Scheme 1 Structure of 2,3,6,7,10,11-hexahydroxytriphenylene (**L**).

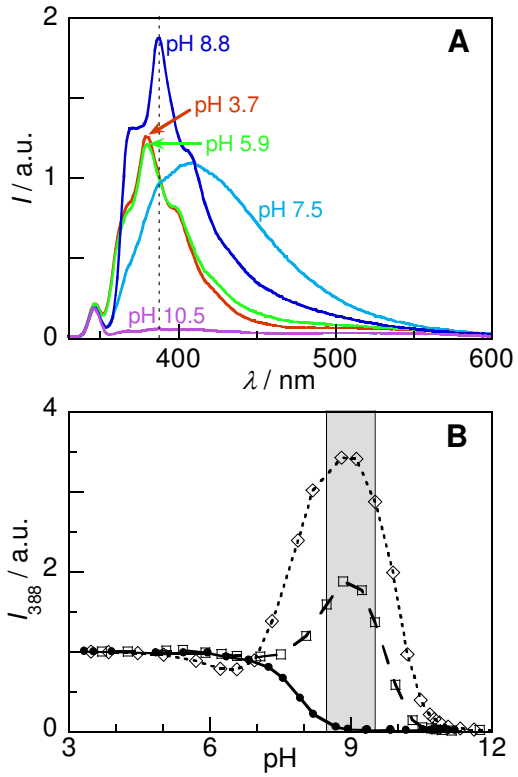


Fig. 1 (A) Fluorescence emission spectra of **L** (1 μM) in the presence of boric acid (1 mM, 11 ppm of boron) at different pH values (the *thin dotted line* at $\lambda = 388$ nm indicates the wavelength used in the titration curves); (B) Fluorescence emission titration curves of **L** (1 μM) in the absence of boron (–●–); in the presence of boric acid (1 mM, --□--); and in the presence of phenylboronic acid (1 mM, ··◇··), with the recommended analysis pH interval marked in *gray*. The spectra were recorded at $\lambda_{exc} = 310$ nm in NH_3 buffer (20 mM) in the presence of EDTA (10 mM), T ≈ 23 °C.

The absorption and emission spectra of **L** depend both on pH and on the amount of boron in solution. In fact, **L** can coordinate boron, in the form of boric acid or phenylboronic acids, with the resulting complexes featuring absorption and emission spectra different from free **L**. The change in absorption and emission spectra can be used for the quantification of boron at a fixed pH where coordination with boron occurs. To avoid interference of other metal ions we use EDTA, which chelates cations without interfering with boron (which is in neutral or anionic form in water).

The fluorescence spectra of **L** (1 μM) in aqueous solution (buffered with 20 mM ammonia and with 10 mM EDTA) at several pH values (Fig. 1A and Fig. S1, ESI) show that, while the fluorescence emission of free **L** is quenched at basic pH, a significant enhancement is observed for these conditions in the presence of both boric acid and phenylboronic acid. The effect is very clear in the fluorescence titration curves (pH 3 to 12) of **L** (1 μM) in the absence of boron and in the presence of boric acid or phenylboronic acid (Fig. 1B), showing that at basic pH, the presence of boric acid or phenylboronic acid causes a significant increase in fluorescence intensity at pH ≈ 8 – 10 (grey area in Fig. 1B). The pH interval 8.5 – 9.5 provides optimum conditions to use **L** as an off-on boron optical sensor: in the absence of boron the fluorescence of free **L** is almost quenched, while the addition of boron results in a strong increase in fluorescence intensity. The UV-visible absorption spectra of **L** in the absence of boron and in the presence of boric acid or phenylboronic acid (Fig. S2, ESI) also shows intensity changes (with pH and with the concentration of boric acid and phenylboronic acid) that can be used to quantify the amount of boron. We believe the interaction between **L** and boron happens in this pH range because while the medium is basic enough to partially deprotonate **L**, it is still sufficiently acidic to have boron in the non-hydroxylated form ($pK_a = 9.15$ for boric acid [4] and 8.8 for phenylboronic acid [35]).

Samples of **L** (1 μM) in aqueous solution (with 20 mM ammonia and 10 mM EDTA) at pH = 9.1 and T ≈ 23 °C show increasing fluorescence intensity with the concentration of both boric acid (Fig. 2) and phenylboronic acid (Fig. S3, ESI). The limits of detection, defined as the lowest analyte concentration that can be reliably detected, were calculated as the concentration for which the difference between the spectra of **L** in the presence and absence of boron equals three times the peak-to-peak noise amplitude.[36] For the experimental conditions used here, the limits of detection are 10 ppb of boron for boric acid, and 6 ppb of boron for phenylboronic acid.

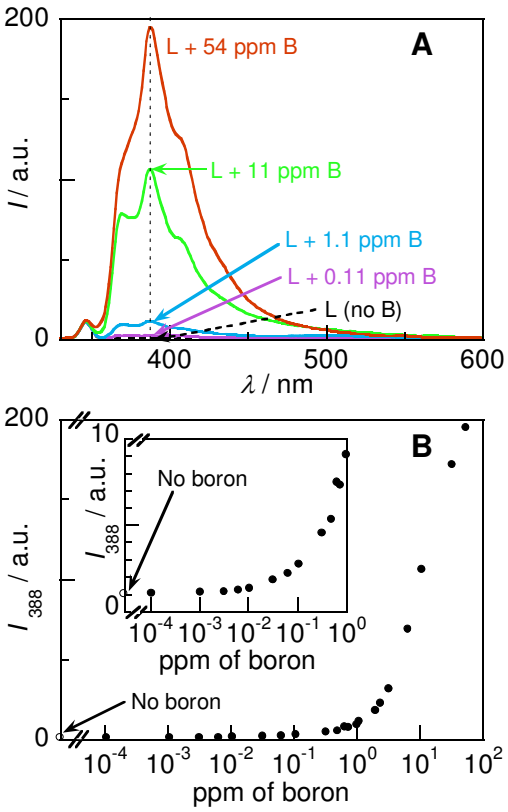


Fig. 2. (A) Fluorescence emission spectra (the *thin dotted line* at $\lambda = 388$ nm indicates the wavelength used in the titration curves) and (B) the corresponding fluorescence emission titration curves of **L** (1 μM) as a function of the concentration of boric acid (from 0 to 54 ppm of boron). The spectra were recorded at $\lambda_{exc} = 310$ nm and 23 °C, in NH_3 buffer (20 mM, pH = 9.1), in the presence of EDTA (10 mM). Coordination of **L** with boron at basic pH leads to an increase in emission intensity.

For UV-vis absorption detection (Figures S4 and S5, ESI), the spectral variations due to the concentration of boric acid and phenylboronic acid at pH ≈ 9 are less pronounced than for fluorimetric detection, and the limits of detection are 5 ppm of boron for boric acid and 0.3 ppm of boron for phenylboronic acid.

Although the selectivity of **L** towards boron in the presence of other metal ions is low (the interference of calcium is shown in Fig. 3), this can be easily circumvented by using an anionic chelating agent. The chelating agent should not affect the sensor response, and should not absorb or emit light in the wavelengths used to measure the sensor response. EDTA meets these requirements, since it complexes metal cations very efficiently, but does not affect boron in the forms of boric or phenylboronic acids since they have neutral or negative charge in water (a mixture of these forms is found in water at the working pH range of the sensor since their pK_a is close to 9).

The selectivity of **L** towards boric acid in the presence of EDTA was tested by measuring the fluorescence spectra of **L** in the presence of competing metal ions for several boron concentrations (Fig. 4): at the calculated limit of detection of the method (10 ppb of boron); at the maximum permissible limits in drinking water (1.0 ppm of boron in EU [6] and 2.4 ppm of boron according to WHO recommendations [3,4]); and at the FAO limits [5] for "sensitive" crops (0.5 – 1.0 ppm of boron) and "moderately sensitive" crops (1.0 – 2.0 ppm of boron).

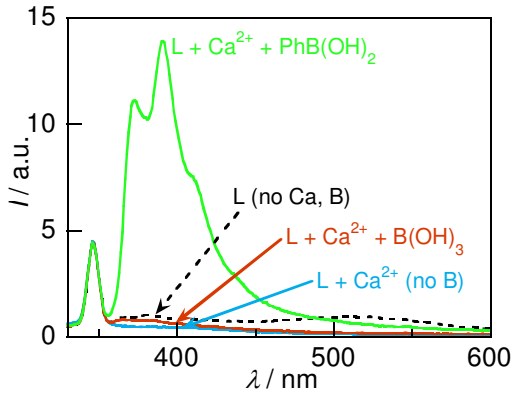


Fig. 3. Fluorescence emission spectra of **L** in the absence of both Ca^{2+} ions and boron (*black dotted line*), **L** in the presence of Ca^{2+} ions with no boron (L+Ca^{2+}(no B), *cyan*), and **L** in the presence of Ca^{2+} ions and either boric acid (L+Ca^{2+}+$B(OH)_3$, *red*) or phenylboronic acid (L+Ca^{2+}+$PhB(OH)_2$, *green*). The spectra were recorded at λ_{exc} = 310 nm, with pH = 8.9, T ≈ 23 ºC, 1 μM of **L**, 1 mM of Ca^{2+}, and 1 mM of either $B(OH)_3$ or $PhB(OH)_2$. The remaining fluorescence emission of free **L** at this pH (*black dotted line*) is further quenched in the presence of Ca^{2+} (L+Ca^{2+}(no B), *cyan*), as well as in the presence of both Ca^{2+} and $B(OH)_3$ (L+Ca^{2+}+$B(OH)_3$, *red*). Some emission is still observed in the presence of both $PhB(OH)_2$ and Ca^{2+} (L+Ca^{2+}+$PhB(OH)_2$, *green*), although this is lower than the observed emission in the absence of Ca^{2+} (Fig. S3, ESI, *green curve* (1 mM = 11 ppm B)). The fluorescence intensity (at 388 nm) of **L** in the presence of Ca^{2+} and phenylboronic acid is about 200 times that of free **L** when EDTA is used, but only about 14 times that of free **L** if EDTA is not used.

We observed fluorescence intensities very similar to those previously obtained in the absence of interfering ions, proving that EDTA effectively eliminates the interference of metal cations without affecting the performance of the sensor.

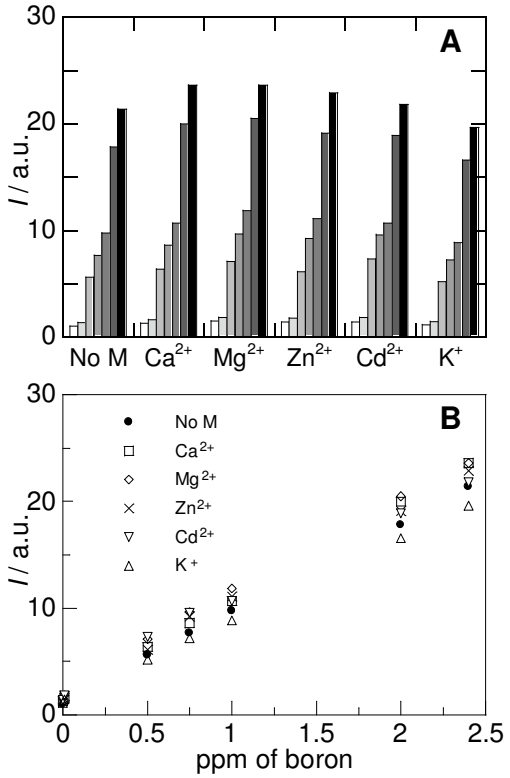


Fig. 4. (A) Fluorescence intensity at 388 nm (λ_{exc} = 310 nm) of **L** (1 μM) upon addition of $B(OH)_3$ in NH_3 buffer (20 mM, pH = 9) with EDTA (10 mM), T ≈ 23 ºC, in the absence of interfering metal ions (*no M*) and after addition of 1000 equiv. of various metal cations: Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, K^+. For each metal, left to right (*white* to *black*): no boron added; 10 ppb B; 0.5 ppm B; 0.75 ppm B; 1.0 ppm B; 2.0 ppm B; and 2.4 ppm B. (B) Fluorescence intensity at 388 nm in the absence of interfering metal ions (●) and after addition of 1000 equiv. of Ca^{2+} (□), Mg^{2+} (◇), Zn^{2+} (×), Cd^{2+} (▽), and K^+ (△). A linear increase of the fluorescence intensity with the concentration of $B(OH)_3$ is observed for all cases. The fluorescence intensity does not change significantly by addition of the metal cations in the presence of EDTA, with the small differences observed being due to changes in pH from the deprotonation of EDTA upon coordination of the metal cations.

The small fluctuations observed in the fluorescence intensities are due to minor differences in pH arising from the deprotonation of EDTA amine groups upon coordination of the metal cations (the solution becoming slightly more acidic in the cases of Ca^{2+}, Mg^{2+} and Zn^{2+}, which bind more strongly to EDTA). Similar results have been found for phenylboronic acid (Fig. S6, ESI).

We also addressed the possibility of determining boron in solutions containing both boric and phenylboronic acids. We first quantify the concentration of phenylboronic acid from its absorption spectra (ESI, Fig. S7). Afterwards, we prepare solutions with the concentration of phenylboronic acid determined previously and increasing concentrations of boric acid. This is then used as a calibration line from which the amount of boric acid can be determined (ESI, Figs. S8 and S9). It should be stressed that the endogenous form of boron is boric acid,[4] and therefore, it is unlikely that a mixture of boric and phenylboronic acid (or other boronic acids) occurs in nature.

Conclusions

Using 2,3,6,7,10,11-hexahydroxytriphenylene we are able to detect boric acid and phenylboronic acid in aqueous solution, based on its enhancement of fluorescence (or changes in the absorption spectrum) at basic pH (preferably at pH ≈ 8.5 – 9.5). The proposed method involves very simple experimental procedures and has limits of detection of the order of the best chemical sensors for boron (10 ppb of boron for boric acid and 6 ppb of boron for phenylboronic acid at pH ≈ 9.1, using spectrofluorimetric detection at 23 ºC) even at very low sensor concentration (1 μM). Using spectrophotometric detection, the limits of detection at pH ≈ 9.1, T ≈ 23 ºC are 5 ppm of boron for boric acid and 0.3 ppm of boron for phenylboronic acid. Although the sensor itself can interact with metal cations, if used in combination with a chelating agent such as EDTA, these interferences are efficiently eliminated. This new fluorescent boron sensor is part of an ongoing project to develop a smart platform for boron sensing and removal from natural and residual waters.

Acknowledgments

This work was partially supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) and POCI 2010 (FEDER) within project PTDC/CTM-NAN/115110/2009. S.A. acknowledges a postdoctoral grant from FCT, Portugal (SFRH/BPD/74654/2010).

Notes and references

[a] CQFM – Centro de Química-Física Molecular and IN – Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, University of Lisbon 1049-001 Lisboa, Portugal. E-mail: carlos.baleizao@tecnico.ulisboa, farinha@tecnico.ulisboa.pt.

† Electronic Supplementary Information (ESI) available: Details of experimental methods and supplementary data. See DOI: 10.1039/c000000x/

1 L. Zaijun, C. Zhengwei and T. Jian, *Food Chem*., 2006, **94**, 310.
2 A. T. Batayneh, *Int. J. Environ. Sci. Technol*. 2012, **9**, 153.
3 World Health Organization, *Guidelines for drinking-water quality*, fourth ed., World Health Organization, 2011.
4 World Health Organization, *Boron in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality*, World Health Organization, 2009.
5 R. S. Ayers and D. W. Westcot, *Water quality for agriculture. FAO Irrigation and Drainage Paper 29 Rev. 1*; Food and Agriculture Organization of the United Nations, Rome, Italy, 1985.
6 Council Directive 98/83/EC of 3 November 1998, *Official Journal of the European Communities*, p. 330/32, 5/12/98.
7 R. N. Sah and P. H. Brown, *Plant Soil*, 1997, **193**, 15.
8 A. M. G. Campaña, F. A. Barrero and M. R. Ceba, *Analyst*, 1992, **117**, 1189.
9 C. E. White, A. Weissler and D. Busker, *Anal. Chem*., 1947, **19**, 802.
10 Z. Marczenko and M. Balcerzak, *Separation, preconcentration, and spectrophotometry in inorganic analysis*, Elsevier Science Ltd, Amsterdam, The Netherlands, 2000.
11 R. Capelle, *Anal. Chim. Acta*, 1961, **24**, 555.
12 F. J. Lopez, E. Giménez and F. Hernández, *Fresenius Journal of Analytical Chemistry*, 1993, **346**, 984.
13 D. L. Harp, *Anal. Chim. Acta*, 1997, **346**, 373.
14 E. Merdivan, Y. Benibol and S. Seyhan, *Spectrochim. Acta Part A*, 2009, **71**, 2045.
15 T. Takahashi, S. Yawata and H. Hoshino, *Anal. Bioanal. Chem*., 2008, **391**, 1101.
16 A. A. Zhirkov, O. V. Razvazhnaya, T. A. Kazakova, D. B. Petrenko, M. A. Proskurnin, Y. M. Dedkov and B. K. Zuev, *J. Anal. Chem*., 2011, **66**, 1180.
17 X. Deng, C. Fan, F. Li, Z. Liu and Z Luo (China Inst. Atomic Energy), CN101713740, May 26 2010.
18 K. Hiroshi, F. Shinji and N. Emiko (Chugoku Electric Power Co Inc., Chuden Kankyo Technos Co Ltd.), JP2005291971, October 10 2005.
19 Y. Kazuhisa, T. Ko, I. Kei, and, H. Takashi (Takaoka Kasei Kogyo Kk, Kyushu Univ), JP2011163888, August 25 2011.
20 S. Thangavel, S. M. Dhavile, K. Dash and S. C. Chaurasia, *Anal. Chim. Acta*, 2004, **502**, 265–270.
21 G. L. Shuhua, G. Zhengrang, C. Yun, Z. Jing, W. Haimin and L. Wuyi (Bai Li), CN1766561, CN100487426, CN1766561 (A), March 05 2006.
22 J. Lapid, S. Farhi and Y. Koresh, *Anal. Lett.*, 1976, **9**, 355.
23 D. F. Kuemmel, M. G. Mellon and *Anal. Chem*., 1957, **29**, 378.
24 J. Iwata, K. Watanabe, M. Itagaki and Bunseki Kagaku 2006, **55**, 473.
25 S. Sakata, J. Iwata, I. Shitanda, M. Itagaki and K. Watanabe, *Tetsu To Hagane*, 2011, **97**, 65.
26 L. Ma, Z. Zhang and Q Li, *Spectrochim. Acta Part A*, 2011, **79**, 599.
27 G. Springsteen, C. E. Ballard, S. Gao, W. Wang and B. Wang, *Bioorg. Chem.*, 2001, **29**, 259.
28 S. Arimori, C. J. Ward and T. D. James, *Chem. Commun.*, 2001, 2018.
29 D.-H. Lee, S.-C. Han, T.-H. Kim and J.-I. Yun, *Anal. Chem*., 2011, **83**, 9456.
30 J. W. Goodby, M. Hird, D. R. Beattie, P. Hindmarsh, G. W. Gray, D. G. Mcdonnell, J. C. Jones and T. J. Phillips (Secretary Of State For Defence Brit., Defence Research Agency, United Kingdom), EP0702668, WO9429263, US5750050, JPH08511512, GB2294046, DE69320655, March 27 1996.
31 M. Dogru and T. Bein, *Nat. Nanotechnol*., 2011, **6**, 333.
32 H. M. El-Kaderi, J. R. Hunt, J. L. Mendoza-Cortés, A. P. Côté, R. E. Taylor, M. O'keeffe and O. M. Yaghi, *Science*, 2007, **316**, 268.
33 F. Thébault, L. Öhrström and M. Haukka, *Acta Crystallogr., Sect. C*, 2011, **67** (4), o143.
34 M. C. T. Fyfe, J. N. Lowe, J. F. Stoddart and D. Williams, *Org. Lett*., 2000, **2**, 1221.
35 J. Yan, G. Springsteen, S. Deeter and B. Wang, *Tetrahedron,* 2004, **60**, 11205.
36 ACS (American Chemical Society), Committee on Environmental Improvement and Subcommittee on Environmental Analytical Chemistry, *Anal. Chem*., 1980, **52**, 2242.

