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COMMUNICATION

A potential fluorescence detection approach to trace hexachlorobenzene *via* disaggregating with ethanol†

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A potential ultra-sensitive detection approach for hexachlorobenzene (HCB), based on the measurement of the intrinsic fluorescence of well-separated HCB molecules in ethanol, has been proposed. Owing to the strong intermolecular π–π stacking interaction of the planar aromatic rings, self-aggregated HCB shows almost no fluorescence. However, the intrinsic emission of HCB can readily be detected in ethanol due to the enhanced emission from the disaggregated HCB, which is related to the hydrogen bond formation between ethanol and HCB. By simply measuring the HCB intrinsic fluorescence, a HCB concentration a little bit higher than 10^{-14} M (~0.001 ppt) in ethanol can be detected; moreover, the fluorescence intensity of the HCB increases linearly with the HCB concentration ranging from 10^{-10} to 10^{-7} M. The approach might provide a simple, fast and efficient method for HCB quantification.

Introduction

Hexachlorobenzene (HCB), once used as a fungicide for crop seeds since 1945, has been found almost in all kinds of food. Its high toxicity and persistence have generated serious environmental and human health concerns.[1–3] The production of HCB was banned in the United States in 1966, and banned globally owing to the Stockholm Convention on persistent organic pollutants (POPs) in 2004. However, it still persists in the environment and can be found worldwide for its long life-time,[4] difficult degradation, and long-range transport. Thus the study on HCB's basic properties is important and exigent in both fundamental research and practical application, such as environmental protection.

So far, much effort has been directed towards HCB in environmental science and medical science.[5–9] However, little has been reported on the structural property of HCB.[10–12] HCB is a chlorocarbon with the molecular formula C_6Cl_6. The presence of a rigid planar aromatic π-electron system renders the molecule fluorescent (*i.e.*, HCB is intrinsically fluorescent),[5] and the presence of chlorine (Cl) enables intermolecular interaction with other functionalized monomers. Therefore, trace HCB might be detected by simply measuring the HCB fluorescence. Up to now, a variety of methods, such as quartz crystal microbalance,[5] dispersive liquid–liquid microextraction combined with gas chromatography-electron capture[6] and electrochemical DNA biosensor,[13] have been developed for the detection of HCB. However, these methods require sophisticated instruments, together with complicated and time-consuming sample preparation processes. Compared to the above-mentioned detection methods, the fluorescence analytical method is of high sensitivity, simplicity and convenience. However, as each HCB molecule has a planar aromatic ring structure, there exists strong π–π stacking interactions between the neighboring molecules, giving HCB molecules a strong tendency to self-aggregate into linear chain structures or to form hard agglomerations. The aggregation may quench the HCB intrinsic emission, so that the emission from HCB powders or aggregated HCB molecules can hardly be observed.

Therefore, to realize the fluorescence analysis of HCB, non-emission liquid media that can effectively disaggregate the aggregated HCB molecules must be found to release individual molecules. By measuring the intrinsic emission of the well-separated individual HCB molecules in the liquid medium of ethanol, HCB concentration can thus be quantified. The result showed that ethanol can indeed disaggregate the aggregated HCB molecules *via* hydrogen bonding interaction between the HCB molecules and ethanol, and further demonstrated that it is an effective sensing approach to HCB by simply measuring the HCB intrinsic fluorescence. Using this approach, a HCB detection limit near or a little bit higher than 10^{-14} M in ethanol has been achieved, demonstrating a new conceptual design route for the development of simple, low cost, and reliable trace detection approaches for some POPs with similar structure and properties.

Experimental

Materials

Ethanol (99.7%) and HCB (analytical grade) were purchased from Tianjin Guangfu Fine Chemical Research Institute, and n-hexane

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(99%) was purchased from Sigma-Aldrich. All the other chemicals were of purity above analytical grade, and were used as received without further purification.

Measurements

Fluorescence measurements were carried out on a FluoroMax-4 fluorescence spectrometer (Horiba Jobin Yvon Inc., France). 2 mL ethanol was added to a quartz cuvette with 10 mm path length followed by titration of 20 μL HCB solutions of different concentrations in ethanol, and the fluorescence spectra were recorded with the excitation of 360 nm at 25 °C. The excitation and emission slits were all set at 5 nm for all the fluorescent measurements.

^{1}H nuclear magnetic resonance (NMR) spectra were acquired on a Bruker AVANCE 400 (400 MHz) Fourier transform NMR spectrometer with chemical shifts recorded in parts per million (ppm) relative to tetramethylsilane; coupling constants (J) are expressed in Hz. Peak multiplicities are recorded as s (singlet), d (doublet), t (triplet), m (multiplet), q (quartet), quint (quintet), and qd (quartet of doublets).

Results and discussion

Fluorescence measurements

The fluorescence spectra of HCB of different concentrations in ethanol solution were shown in Fig. 1, where the peak at 403 nm belongs to the Raman spectrum of the HCB/ethanol system,[14] and the peak at 430 nm belongs to the fluorescence emission spectrum of HCB. As shown in Fig. 1A, the emission intensity (at 430 nm) of HCB increases with the HCB concentration in ethanol, and a detection limit near or a little bit higher than 10^{-14} M (~0.001 ppt) was achieved, given that the criterion of 10% fluorescence enhancement is enough to define the sensitivity of the system. However, with the increase of HCB concentration, there appears a turning point at around 10^{-7} M HCB concentration (Fig. S1†). The fluorescence intensity decrease at higher HCB concentrations may be ascribed to the fluorescence quenching effect resulting from the aggregation of HCB molecules. In the aggregated HCB system the intermolecular energy transfer dominates, leading to non-radiative decay and thus the fluorescence quenching effect.[15]

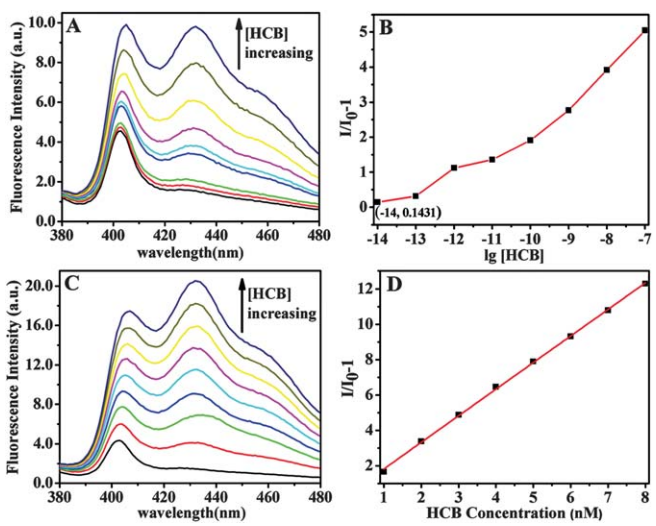


Fig. 1 (A) Fluorescence emission spectra of HCB in ethanol solution with HCB concentrations of 0, 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M (showing arrowhead), respectively. (B) Plot of relative fluorescence enhancement ($I/I_0 - 1$) *versus* lg[HCB]. (C) Fluorescence emission spectra of HCB in ethanol solution with HCB concentrations of 0 (the lowest one) and from 1 nM to 8 nM, respectively. (D) Approximate linear relationship between ($I/I_0 - 1$) and HCB concentration in the range of 1 nM to 8 nM (λ_{ex} = 360 nm, λ_{em} = 430 nm).

Fig. 1B is the plot of relative fluorescence enhancement ($I/I_0 - 1$) *versus* lg[HCB], where it can be seen that there exists a linear relationship between the fluorescence intensity enhancement and the HCB concentration ranging from 10^{-10} to 10^{-7} M. In order to quantify this relationship, we measured the fluorescence intensity of HCB in ethanol with the concentration from 1 nM to 8 nM (Fig. 1C and D). A linear relationship (R^2 = 0.99926) between fluorescence intensity I and HCB concentration c in the range of 1 nM to 8 nM is given by

$$I = I_0 \times (1.31676 + 1.50391c)$$

where, I_0 and I correspond to the fluorescence emission intensity in the absence and presence of a given HCB concentration in ethanol, respectively.

Sensing mechanism

The HCB molecule contains a rigid planar aromatic structure with a large conjugated π bond, which endows HCB with a high fluorescence quantum efficiency. So the HCB free monomer is strongly fluorescent. However, HCB is apt to aggregate due to its strong intermolecular π–π stacking interactions,[16] and shows a strong tendency to self-aggregate even in some aqueous solutions, which obviously quenches the HCB fluorescence emission.[17] It has been reported that the aggregation can be disaggregated effectively by the addition of ethanol,[17,18] which, as a strong hydrogen bond donor,[19] interacts with HCB *via* hydrogen bonds and effectively disrupts HCB aggregation, thus enhancing HCB fluorescence emission.

The formation of hydrogen bonds between HCB and ethanol can be characterized by ^{1}H NMR spectroscopy. The hydrogen bonding interaction causes a de-shielding effect on a proton and hence a down-field shift of the chemical shift. In the HCB/ethanol system, the Cl atom on the HCB molecule, as a hydrogen bond acceptor, forms a hydrogen bond with the hydroxyl group (–OH) of the ethanol molecule. This interaction leads to the proton chemical shift of the hydroxyl group (–OH) of ethanol to shift down-field, and therefore the strength of the hydrogen bond can be correlated to the chemical shifts of ^{1}H directly.[20,21] We acquired the ethanol proton spectra from pure ethanol, as shown in Fig. 2, where the proton chemical shift at 3.42 ppm corresponds to the ethanol hydroxyl proton. However, the ethanol hydroxyl proton chemical shift changes to 3.43 ppm in the presence of HCB, namely in the HCB/ethanol system. This down-field shift of the ethanol hydroxyl proton in the NMR spectra indicates the formation of a hydrogen bond between the HCB molecule and the ethanol molecule.

As the hydrogen bond interaction is temperature-dependent, temperature fluctuation may also affect the HCB fluorescence intensity. We studied the effect of temperature on the HCB fluorescence emission in ethanol at a given concentration of 10^{-14} M. As expected, the fluorescence enhancement of HCB increases with the

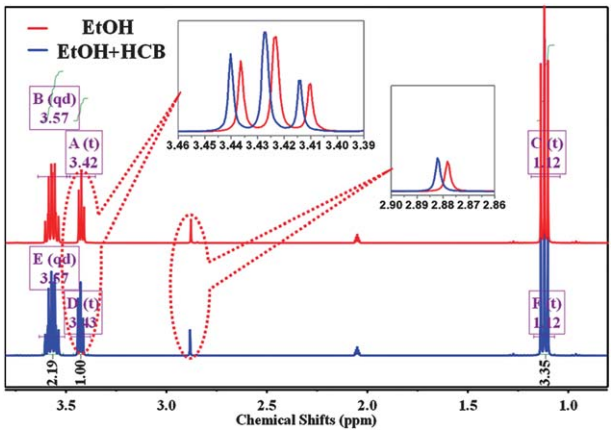


Fig. 2 ^{1}H NMR spectra of ethanol before and after adding HCB (^{1}H NMR, CD_3COCD_3, 400 MHz, 27 °C). Chemical shift changes are labeled by the red dotted line. A(t) and D(t) refer to hydroxyl group (–OH); B(qd) and E(qd) refer to methylene (–CH_2–); C(t) and F(t) refer to methyl (–CH_3); $\delta = 2.05$ ppm refers to the methyl group of CH_3COCH_3.

decrease of temperature (Table 1), suggesting that stronger hydrogen bonding between ethanol and HCB at lower temperature further weakens the intermolecular π–π stacking interaction between HCB molecules, thus leading to a higher HCB fluorescence quantum efficiency.

A down-field shift at 2.878 ppm (Fig. 2 insert) is also observed, which refers to the proton of residual H_2O,[22] indicating the formation of hydrogen bonds between HCB and H_2O. However, no fluorescence emission peak of HCB can be observed in water (Fig. S2†), which could be ascribed to the fact that the water molecule contains two O–H bonds and can form hydrogen bonds with two HCB molecules. In the water molecule, the two hydrogen atoms bound to one oxygen atom to form a 'V' shape rather than a straight line, so the formation of the HCB–H_2O hydrogen bond cannot effectively disrupt the π–π stacking of HCB.

The fluorescence emission spectrum of HCB in 1-butanol was also observed, where there is also a fluorescence enhancement. However, compared to the fluorescence enhancement of HCB in ethanol, less enhancement in the level of intensity was shown (Fig. S3†), which should be ascribed to the fact that 1-butanol has a larger alkyl group than that of ethanol, so the shielding effect on a proton is larger than that of ethanol. Thus the hydrogen bonding interaction between HCB and 1-butanol is weaker than that with ethanol.

In order to validate the effect of ethanol on the HCB intermolecular π–π stacking interaction, we purposely added ethanol to HCB/n-hexane solution, as n-hexane is one of the commonly-used organic solutions without hydroxyl groups. By subtracting the contribution of the HCB dilution to the fluorescence, we calculated and recorded

Table 1 Relationship of detection sensitivity and temperature (with HCB concentration of 10^{-14} M in ethanol)

T/°C	Fluorescence enhancement (%)
~25	14.31
~20	27.01
~15	65.27
~10	76.43

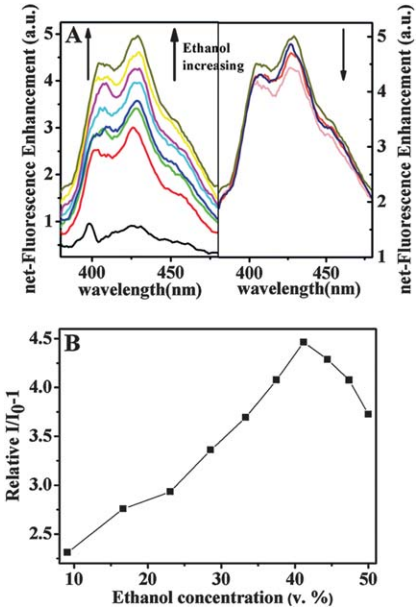
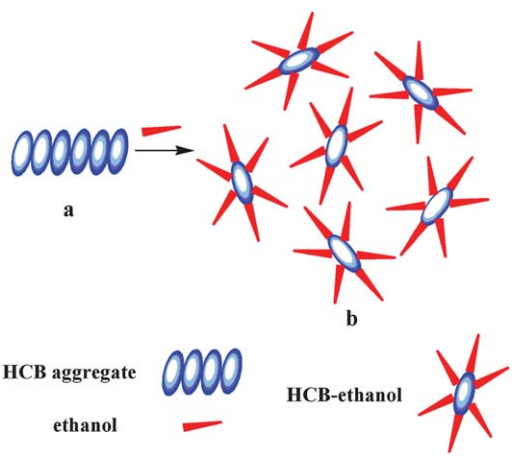


Fig. 3 (A) Net-fluorescence enhancement of HCB in n-hexane (~10^{-5} M) with the addition of ethanol; (B) relationship between the net-fluorescence enhancement of HCB at 430 nm and the volume percentage of ethanol added ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 430$ nm).

the net-fluorescence enhancement. The addition of ethanol to the HCB/n-hexane solution (with the HCB concentration of 10^{-5} M) results in the enhanced fluorescence of HCB, and the net-fluorescence enhancement increases with the ethanol concentration (Fig. 3A). When the ethanol volume concentration was increased to 40%, the relative net-fluorescence enhancement reached a plateau (at about 4.5), and further increase of the ethanol volume concentration causes little change in the relative net-fluorescence enhancement (Fig. 3B). These results clearly indicate that HCB aggregates are gradually converted into free HCB monomers with the increase of ethanol concentration as shown in Scheme 1, and almost all HCB aggregates were converted into free HCB monomers at 40% ethanol concentration. This may be related to the factor that aggregated HCB molecules are not fluorescent and the free HCB monomer is highly fluorescent.



Scheme 1 Strategy for the sensing mechanism of HCB in ethanol: (a) HCB only; (b) HCB/ethanol system.

In order to validate the high sensitivity of HCB to ethanol, the fluorescence quantum yield of HCB was calculated by a relative method where R6G was selected as the standard fluorophore. The quantum yield of HCB was estimated as 0.78 in ethanol (see details in the ESI, fluorescence quantum yield of HCB), which means that HCB exhibits high fluorescence quantum efficiency in ethanol. This could result from the reduction in non-radiative processes, which can be attributed to the well-separated HCB monomers.

Conclusions

In summary, the fluorescence emission of HCB can be markedly enhanced *via* disaggregation induced by hydrogen bond formation with ethanol, making it suitable for HCB trace detection based on its intrinsic fluorescence. There exists a strong HCB aggregation due to π–π stacking, and ethanol can disrupt this π–π stacking *via* hydrogen bonding, thus ethanol can lead to the effective enhancement of HCB fluorescence. By measuring the HCB intrinsic fluorescence, 10^{-10} to 10^{-7} M HCB in ethanol can be quantified. Based on this phenomenon, an effective fluorescence sensing approach to HCB might be developed.

Acknowledgements

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Notes and references

1 J. L. Barbera, A. J. Sweetman, D. v. Wijk and K. C. Jones, *Sci. Total Environ.*, 2005, **349**, 1–44.
2 M. Suwalsky, C. Rodríguez, F. Villena, F. Aguilar and C. P. Sotomayor, *Pestic. Biochem. Physiol.*, 1999, **65**, 205–214.
3 X. Gao, W. Wang and X. Liu, *J. Hazard. Mater.*, 2009, **169**, 279–284.
4 N. R. d. Nascimento, S. M. C. Nicola, M. O. O. Rezende, T. A. Oliveira and G. Öberg, *Geoderma*, 2004, **121**, 221–232.
5 K. Das, J. Penelle and V. M. Rotello, *Langmuir*, 2003, **19**, 3921–3925.
6 R. R. Kozani, Y. Assadi, F. Shemirani, M.-R. M. Hosseini and M. R. Jamali, *Talanta*, 2007, **72**, 387–393.
7 W. W. Brubaker and J. a. R. A. Hites, *Environ. Sci. Technol.*, 1998, **32**, 766–769.
8 Y. Su, H. Hung, P. Blanchard, G. W. Patton, R. Kallenborn, A. Konoplev, P. Fellin, H. Li, C. Geen, G. Stern, B. Rosenberg and L. A. Barrie, *Environ. Sci. Technol.*, 2006, **40**, 6601–6607.
9 D. J. Cripps, H. A. Peters, A. Gocmen and I. Dogramici, *Br. J. Dermatol.*, 1984, **111**, 413–422.
10 M. A. El-Sayed, A. A. Gwaiz and C. T. Lin, *Chem. Phys. Lett.*, 1972, **16**, 281–286.
11 C. T. Lin and M. A. EL-Sayed, *Chem. Phys.*, 1974, **4**, 161–180.
12 G. A. George and G. C. Morris, *Mol. Cryst. Liq. Cryst.*, 1970, **10**, 187–217.
13 L. Wu, X. Lu, J. Jin, H. Zhang and J. Chen, *Biosens. Bioelectron.*, 2011, **26**, 4040–4045.
14 E. L. Inman and J. a. J. D. Winefordner, *Anal. Chim. Acta*, 1982, **138**, 245–252.
15 S. Fery-Forgues, J.-P. Fayet and A. Lopez, *J. Photochem. Photobiol., A*, 1993, **70**, 229–243.
16 W. Wang, J. J. Han, L.-Q. Wang, L.-S. Li, W. J. Shaw and A. D. Q. Li, *Nano Lett.*, 2003, **3**, 455–458.
17 B. Wang, F. Wang, H. Jiao, X. Yang and C. Yu, *Analyst*, 2010, **135**, 1986–1991.
18 B. Wang and C. Yu, *Angew. Chem., Int. Ed.*, 2010, **49**, 1485–1488.
19 A. Rachkov, S. McNiven, A. El'skaya, K. Yano and I. Karube, *Anal. Chim. Acta*, 2000, **405**, 23–29.
20 H.-J. Schneider, F. Hacket and V. Rüdiger, *Chem. Rev.*, 1998, **98**, 1755–1785.
21 R. V. Pinjari, K. A. Joshi and S. P. Gejji, *J. Phys. Chem. A*, 2007, **111**, 13583–13589.
22 H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512–7515.