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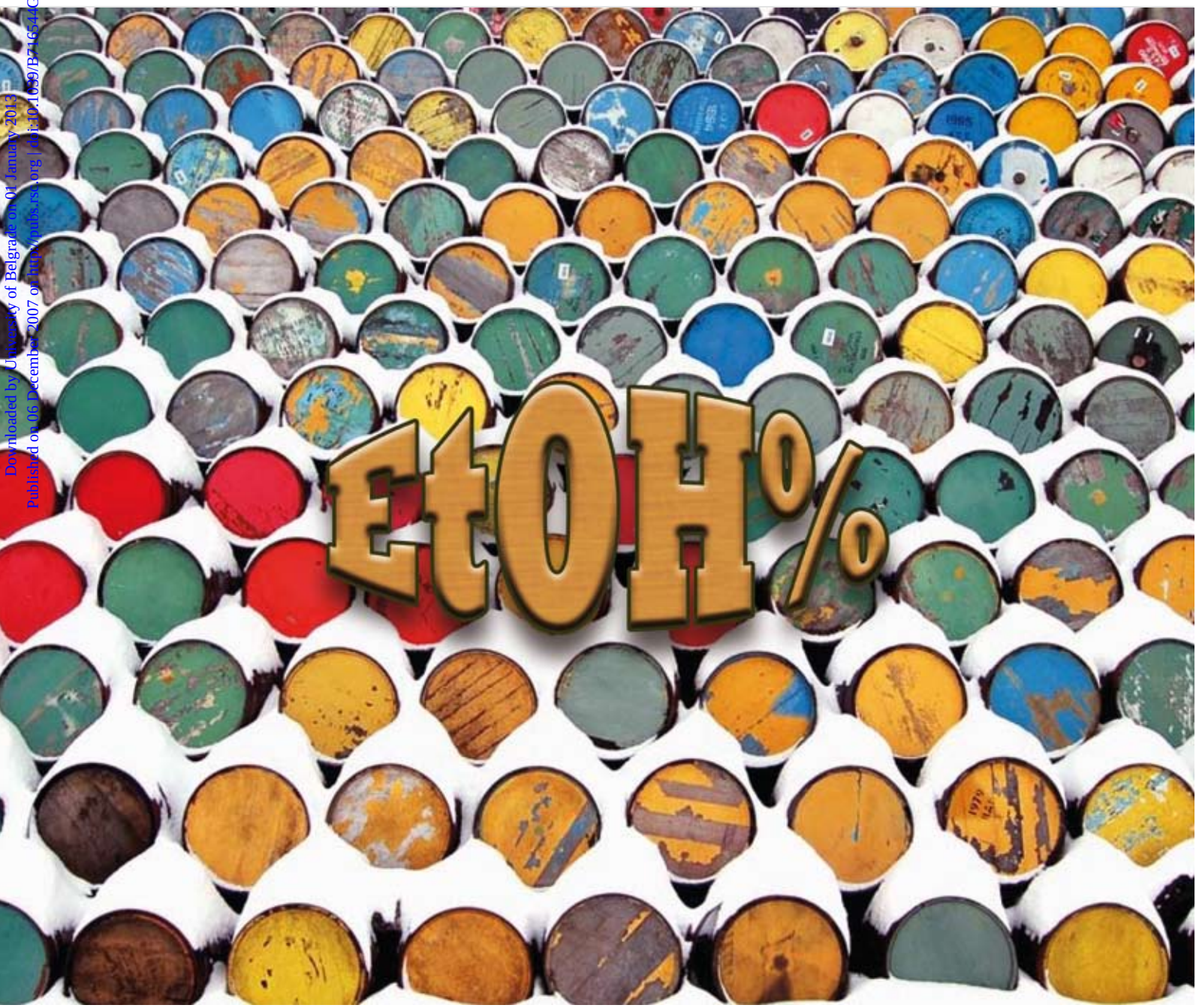

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LETTER
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A copper(II)-based multiphasic fluorous colorimetric ethanol assay

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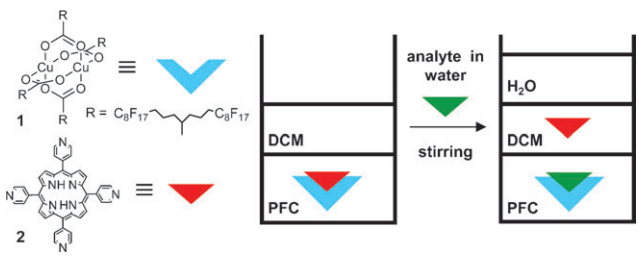
A copper(II)-based multiphasic colorimetric assay exhibiting selectivity for ethanol over water and linear aliphatic alcohols has been developed and applied in the titration of aqueous, organic and gasoline–ethanol blends (bioethanol).

Transition metal based indicator-displacement assays (IDAs),[1] in particular those involving copper(II) complexes, have received considerable interest in recent years affording practical sensing/titration methodologies for a range of anionic analytes such as carbonate,[2] pyrophosphate,[3] phosphate,[4] histidine,[5] or citrate.[6] The discrimination between small peptides using dynamic combinatorial libraries of Cu(II)/Ni(II)–dye complexes,[7] and the colorimetric enantiodiscrimination of α-amino acids using chiral copper(II)–dye complexes represent other major achievements in this area.[8] However, competition from water binding to the receptor can render such methods incompatible with an aqueous environment. Recently, an IDA for nitric oxide has been reported in which application of a polymeric membrane impermeable to water,[9] or encapsulation of the sensor into a polymer film,[10] eliminated water competition for the receptor.

We previously reported an alternative strategy in which the sensing ensemble was compartmentalized into a perfluorocarbon (PFC).[11,12] We employed the highly fluorophilic complex **1** as a receptor (Scheme 1). Extensive EPR studies confirmed that **1** belonged to the well-known family of the dimeric copper(II)–carboxylate complexes.[13] These complexes are known to retain their dimeric structure upon coordination of monodentate ligands such as pyridine,[14,15] THF,[15] CH_3CN,[15,16] acetone,[16] EtOH,[16] or water,[17] to the available apical coordination sites. The principle of the multiphasic IDA employing **1** as the receptor, and the 5,10,15,20-tetrapyridyl porphyrin **2** (TPyP) as the indicator, is presented in Scheme 1. Binding of the pyridyl groups of **2** to the accessible coordination sites of **1** led to complete extraction of **2** from an organic phase into a fluorous phase. In the presence of an excess of competing ligand, such as coordinating solvents (THF, EtOH, MeOH, CH_3CN, *etc.*), the TPyP was released into the dichloromethane (DCM) phase.[11] The detection/titration was carried out by measuring either the appearance of strong visible light absorption or the fluorescence of the released TPyP into the DCM phase.[11] It is noteworthy that the TPyP is released in a separated phase which is, in principle, free of any other absorbing/emitting species. An attractive feature of the PFC/DCM compartmentalization is that alteration of the optical properties of the released indicator is not required. This is necessary when carrying out IDAs in a one-phase system. We now report that such a multiphasic IDA can be used as a practical assay for EtOH in aqueous and organic solutions.

The general procedure employed for the EtOH assays is outlined herein. A batch solution of the PFC phase (typically 15 mL) containing the sensing ensemble was prepared by stirring a biphasic system consisting of a perfluorodecalin (PFD) solution of **1** (15 mL, 0.6 mM) with a DCM solution of **2** (45 mL, 0.01 mM). After 30 min of vigorous stirring almost complete extraction of the porphyrin (~99%) into the PFD phase was achieved, resulting in a fluorous phase containing **1** and **2** at 0.6 and 0.03 mM, respectively. After standing for 1 h the two phases were separated and aliquots of 1 mL of the fluorous phase were subsequently used for the assays described below.

We first tested the selectivity of the assay for EtOH over water and other linear aliphatic alcohols. DCM solutions (3 mL) of the alcohols (0.164 M, 0.493 mmol) were added to the fluorous phases (1 mL) obtained as described above. In the case of water, 8.8 μL (0.493 mmol) of water was added to a biphasic DCM/PFD (3 mL : 1 mL) system. After 15 min of stirring at 1250 rpm followed by 1 h standing, the extent of TPyP release was determined by measuring the absorption of the DCM phase at 415 nm (Soret band, $\varepsilon = 25 \times 10^4$ M^{-1} cm^{-1}). The results obtained for assays carried out in triplicate are presented in the Fig. 1. To our surprise this IDA displayed a remarkable selectivity for EtOH. The response to water was very low (1–2% compared to that of EtOH). Even in the presence of a large excess of water (up to 3 mL added to the biphasic system) a maximum TPyP release of 2% was measured, showing that the PFC can efficiently isolate the receptor from the aqueous phase. Interestingly, the measured response for EtOH was *ca.* double that of MeOH, whilst a *ca.* 10-fold increase was observed over *n*-octanol. This selectivity between



Scheme 1 Principle of the compartmentalized fluorous IDA.

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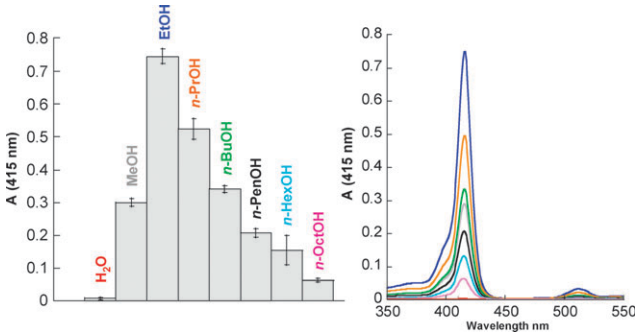

Fig. 1 Comparison of the TPyP release in the DCM phase for linear alcohols and water (experimental conditions given in the text). On the right is shown the absorption spectra of the released TPyP recorded in the DCM phase for the various alcohols.

alcohols might be explained by considering several parameters such as their partitioning in the DCM/PFC biphasic system and their coordinating properties (electronic properties and steric hindrance). We first considered the partition coefficients of the alcohols in the DCM/PFC biphasic system. Under the conditions of the selectivity tests, the residual concentrations of MeOH, EtOH, *n*-pentanol and *n*-octanol in perfluorodecalin were estimated at around 1.3, 2.2, 4.2 and 1.8 mM, respectively. Such values, measured by GC analysis using α,α,α-trifluorotoluene as internal standard (see Experimental), clearly showed that the observed selectivity cannot be attributed only to the partitioning of the alcohols. However, it could explain why EtOH gave a higher response than MeOH due to its significantly higher concentration in the PFC. EtOH (pK_a = 29.8 in DMSO) might also be a better ligand than MeOH (pK_a = 29.0) as reflected by its slightly higher basicity.

We suggest that steric hindrance might also play a role. Ohmura and co-workers recently reported the preparation of a metal–organic framework which could serve as a good structural model for our system. Starting from TPyP and an excess of $Cu_2(AcO)_4$, a bidimensional grid was obtained in which the porphyrins were metallated by copper(II) ions and surrounded by four $Cu_2(AcO)_4$ dimers.[14] As suggested by the space-filling model presented in Fig. 2, in the [TPyP:(**1**)$_4$] complex anticipated to form in the fluorous phase, the porphyrin might be completely shielded by the surrounding 32 perfluorinated chains ($(CH_2)_3C_8F_{17}$, green in Fig. 2).

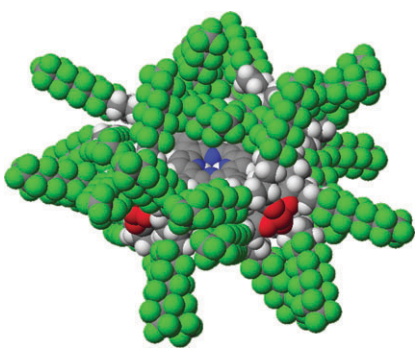

Fig. 2 Space-filling model (MM2, CaChe 3.1) of the [TPyP:(**1**)$_4$] complex.

We previously proposed that such steric hindrance was responsible for the unusual solution behavior of the [TPyP:**1**] ensemble by preventing the copper(II) ions access to the macrocycle core.[11] Indeed, metallation of the TPyP macrocycle by Cu^{2+} ions was never observed during the extraction/release process (even in the presence of a large excess of **1**). Similarly, the access to the pyridine–copper(II) coordination bonds might be limited for the large aliphatic alcohols. Overall, EtOH might thus represent the best compromise in terms of partitioning, affinity for copper(II) and steric hindrance to account for the observed selectivity.

As a potential application of the fluorous IDA we first tested this assay for ethanol titration in commercial beverages. A calibration curve over the range 10–45% (v/v) was obtained by adding aliquots (70 μL) of aqueous ethanolic standard solutions to biphasic systems consisting of 3 mL of DCM and 1 mL of PFD containing **1** at 0.6 mM and **2** at 0.03 mM (prepared as described above). The resulting calibration curve (Fig. 3) was subsequently used for the quantification of EtOH in beverages such as vodka, gin, rum and whiskey with alcohol contents of 37.5, 37.5, 40 and 40%, respectively. The curved shape of the plot,[18] was most probably due to the fact that **1** was in excess over **2**. As we add EtOH it will first bind to free **1** before competing with **2**. In a typical experiment, an aliquot (70 μL) of the beverage (newly opened bottle) was added to the biphasic chemosensing ensemble which was then stirred at 1250 rpm for 15 min. After 1 h of decantation, the absorbance of the released porphyrin in the DCM phase was measured and the concentration of EtOH estimated graphically from the calibration curve. EtOH contents of 39.0, 37.5, 39.4, and 39.4% were obtained for vodka, gin, white rum and whiskey, respectively, in good agreement with the expected values.

We also tested the IDA for determination of the alcohol content of EtOH–gasoline blends. We hypothesized that *n*-octane could serve as a chemical model for gasoline and thus could be used to generate the calibration curve. The calibration curve depicted in Fig. 4 was obtained over the range 1–8% (v/v) by adding aliquots (500 μL) of the *n*-octane–ethanol standard solutions to the biphasic DCM/PFD (3 mL : 1 mL) system. Two solutions of unleaded gasoline containing 3 and 5% of EtOH (v/v) were prepared and submitted to the

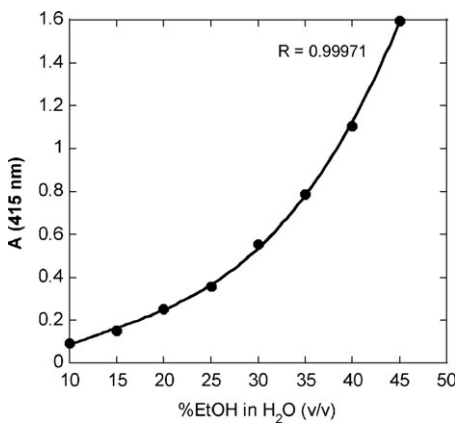

Fig. 3 Variation of the TPyP absorption intensity in the DCM phase as a function of EtOH concentration of the hydroalcoholic solutions added to the biphasic assay.

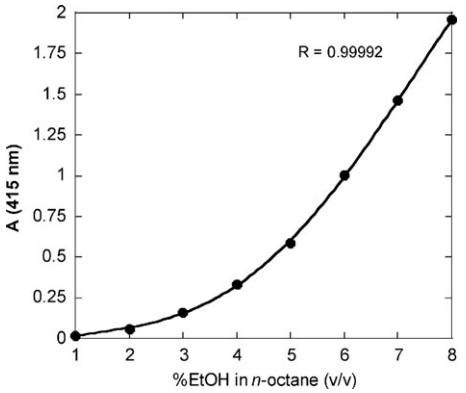

Fig. 4 Variation of the TPyP absorption intensity in the DCM phase as a function of EtOH concentration of the EtOH–*n*-octane solutions added to the biphasic system.

assay by using aliquots of 500 μL of these solutions. Values of 3.2 and 5.2% (average of two assays) were obtained from the calibration curve by the graphical method.

Finally, we also tested this system as a potential visual colorimetric assay for EtOH detection in chlorinated solvents for which EtOH is commonly employed as a stabilizing agent.

For these experiments we employed the 5,10,15-tripyridyl-20-phenylporphyrin–Mn(III) metalloporphyrin **3** (Fig. 5) as the indicator, showing that metalloporphyrins can be very efficiently phase-switched with this system. To improve the color contrast, the concentration of **3** in the fluorous phase was increased to 0.09 mM whilst the concentration of **1** was kept at 0.6 mM. This was achieved by preparing the fluorous phase starting from a DCM phase with an initial porphyrin **3** concentration of 0.03 mM instead of 0.01 mM. The assays were carried out by adding aliquots of 250 μL of commercial chloroform stabilized either with amylene (0.02% v/v) or ethanol (0.6% v/v) in a vial containing 250 μL of the fluorous phase. The mixtures were hand-shaken for a few minutes giving, after decantation, the biphasic systems presented in the photograph in Fig. 5. As clearly seen, a strong response is obtained with the chloroform stabilized with ethanol (sample a), the metalloporphyrin being completely released in the chloroform (upper phase) whilst the dicopper complex **1** (blue color) is left in the fluorous phase. No response is observed for the chloroform stabilized with amylene (sample d). When diluting 10 times the commercial chloroform stabilized with EtOH a faint green color was observed in the upper phase, affording a visual detection limit at around 0.06% v/v.

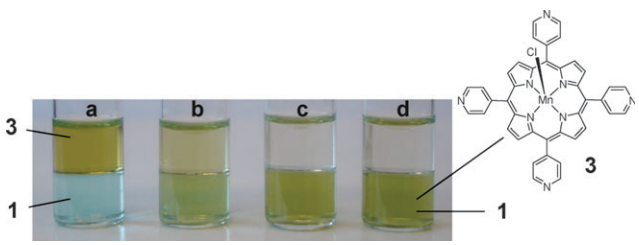

Fig. 5 Colorimetric response to commercial chloroform solutions containing EtOH at 0.6% v/v (a), 0.06% (b) and 0.006% (c), or amylene at 0.02% (d).

In summary, we have developed a multiphasic EtOH assay in which the receptor–indicator ensemble is compartmentalized into a perfluorocarbon. This assay was found to be effective and quite sensitive for titration of EtOH in organic solutions. Maybe more importantly, these results clearly demonstrate that the compartmentalization of a receptor into a fluorous phase could be considered as a valuable strategy to prevent adventitious water competition, a problem often encountered in supramolecular-based sensing/titration assays.

Experimental

The residual concentrations of the alcohols in the perfluorodecalin phase were determined by GC analysis using α,α,α-trifluorotoluene as an internal standard, a compound which is fully soluble in perfluorocarbons. The response factors between methanol, ethanol, *n*-pentanol, *n*-octanol and α,α,α-trifluorotoluene were first estimated using THF solutions of the alcohols and the standard. Response factor values of 0.16, 0.29, 0.61 and 1.02 were obtained for methanol, ethanol, *n*-pentanol and *n*-octanol, respectively. The residual concentrations of the alcohols in the fluorous phase were determined by stirring (1250 rpm, 1 h) a biphasic system of CH_2Cl_2 (2 mL) containing the alcohol (0.16 M) and perfluorodecalin (2 mL). After standing for 24 h, 500 μL of the fluorous phase was withdrawn, to which was added a controlled amount of α,α,α-trifluorotoluene for titration by GC.

Acknowledgements

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