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Colorimetric sensor arrays for molecular recognition

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Abstract—The development of colorimetric sensor arrays for the detection of volatile organic compounds is reported. Using an array of chemo-responsive dyes, enormous discriminatory power is created in a simple device that can imaged easily with an ordinary flat-bed scanner. High sensitivities (ppb) have been demonstrated for the detection of biologically important analytes, including amines, carboxylic acids, and thiols. By the proper choice of dyes and substrate, the array can be made essentially non-responsive to changes in humidity.

1. Introduction

The generalized detection of volatile organic compounds (VOCs) is often referred to as electronic nose technology, and has generally been based on sensors that detect adsorption into a set of polymers or on electrochemical oxidations at a set of heated metal oxides.[1,2] In spite of some successes with such systems, the discrimination of compounds at low concentrations or within a given chemical class and the absence of interference from changes in humidity remain challenging goals. We have previously reported the colorimetric array detection of a wide range of odorants using metalloporphyrins.[3–6] The importance of including Lewis acids in such an array is emphasized by the recent indications that the mammalian olfactory receptors are in fact metalloproteins.[7] Here we review extensions of that concept to the development of an expanded colorimetric array detector.

2. The colorimetric sensor array

Fundamentally, molecular recognition involves the interactions between molecules: i.e. bond formation, acid–base interactions, hydrogen-bonding, dipolar and multipolar interactions, π–π molecular complexation, and last and least, van der Waals interaction and physical adsorption. Nearly all prior sensor technology relies essentially exclusively on van der Waals and physical adsorption, the weakest and least selective of forces between molecules. We believe this to be a fundamental flaw in the development of sensors with both high sensitivity and high selectivity. In many ways, our colorimetric sensor array revisits the earlier, pre-electronic era of analytical chemistry,[8] with the addition of modern digital imaging.

The design of the colorimetric sensor array is based on two fundamental requirements: (1) the chemo-responsive dye must contain a center to interact strongly with analytes, and (2) this interaction center must be strongly coupled to an intense chromophore. The first requirement implies that the interaction must not be simple physical adsorption, but rather must involve other, stronger chemical interactions. Chemoresponsive dyes are those dyes that change color, in either reflected or absorbed light, upon changes in their chemical environment. The consequent dye classes from these requirements are (1) Lewis acid/base dyes (i.e. metal ion containing dyes), (2) Brønsted acidic or basic dyes (i.e. pH indicators), and (3) dyes with large permanent dipoles (i.e. zwitterionic solvatochromic dyes) (Fig. 2). In addition, we have incorporated our own bis-pocketed metalloporphyrins[9] to provide an aspect of shape-selective sensing to the array.

Disposable colorimetric arrays of chemoresponsive dyes have been created by printing the dyes on various inert solid supports, e.g. reverse phase silica gel plates, acid-free paper, or porous membranes of various polymers (e.g. nylon, PVDF). The specific array used in this work (Fig. 1) was spotted on C2 reverse phase silica gel plates using 0.1 μL microcapillary pipettes. Arrays are commercially available from ChemSensing, Inc., Northbrook, Illinois (www.chemsensing.com), part number CSI.042 (Fig. 2).

For recognition of analytes with Lewis acid/base

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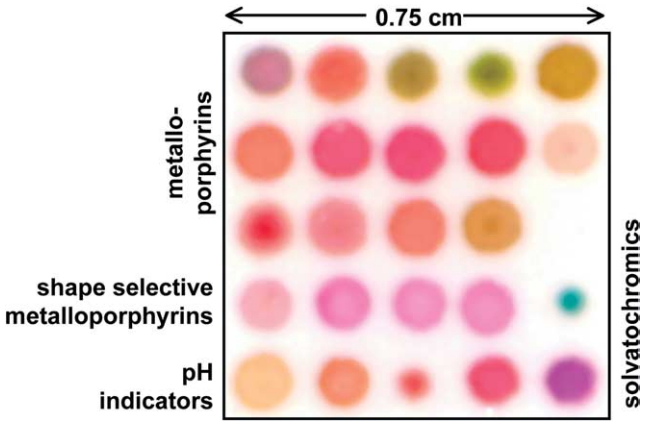

Figure 1. A colorimetric sensor array.

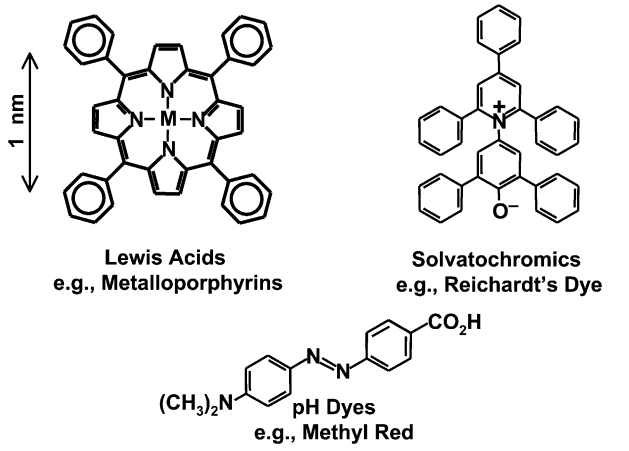

Figure 2. The components of colorimetric sensor arrays are inherently nanoscale.

capabilities, the use of porphyrins and their metal complexes are a natural choice. Metalloporphyrins are nearly ideal for the detection of metal-ligating vapors because of their open coordination sites for axial ligation, their large spectral shifts upon ligand binding, and their intense coloration. A series of metalated tetraphenylporphyrins (TPP) was used to provide differentiation based on metal-selective coordination.

Common pH indicator dyes change color in response to changes in the proton (Brønsted) acidity or basicity of their environment. Solvatochromic dyes change color in response to changes in the general polarity of their environment, primarily through strong dipole–dipole interactions. To some extent, all dyes inherently are solvatochromic, although some are much more responsive than others; among the most responsive are Reichardt's Dye and Nile Red.

In addition, our array incorporates a series of Zn-substituted, bis-pocketed porphyrins, based on *ortho*-substitution of the tetraphenylporphyrin core to differentiate analytes based on size and shape. These compounds include a family of silylether porphyrins with large, medium, and small pockets on both faces of the porphyrin (Figs. 3 and 4). These have been previously shown to exhibit extremely selective binding of organic ligands (Fig. 5).[9]

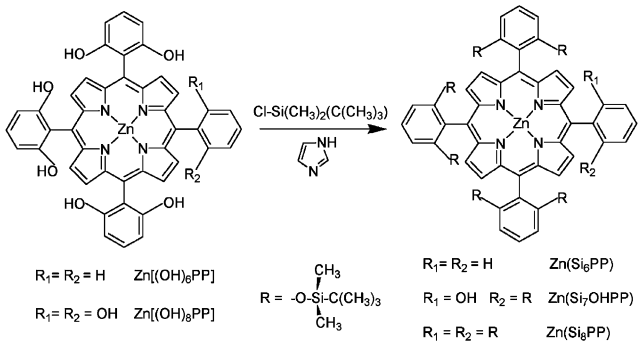

Figure 3. Silylether bis-pocket porphyrin synthesis.

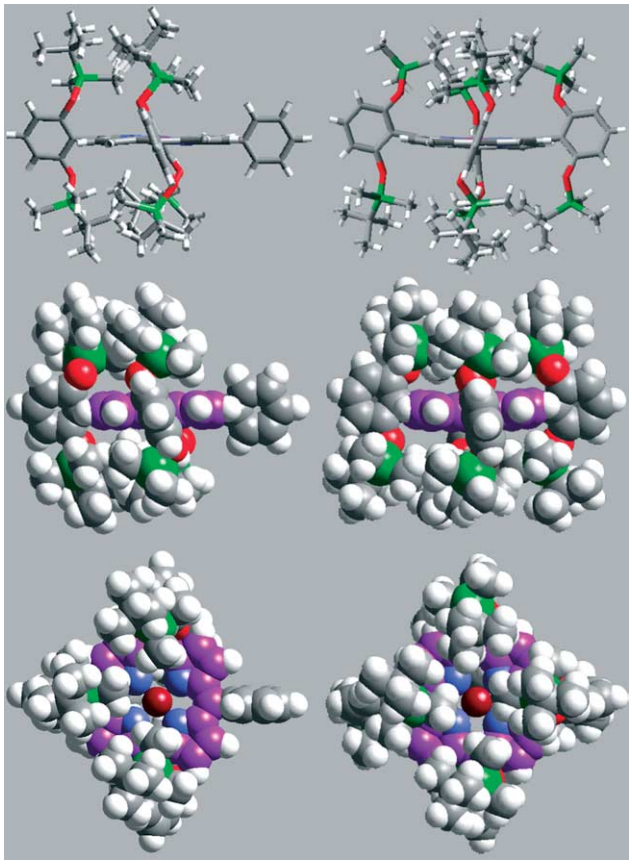

Figure 4. Molecular models of Zn(Si_6PP) (left column) and Zn(Si_8PP) (right column). The pairs of images from top to bottom are cylinder side-views, side-views, and top-views, respectively; space filling shown at 70% van der Waals radii; with the porphyrin carbon atoms shown in purple, oxygen atoms in red, silicon atoms in green, and Zn in dark red. The X-ray single crystal structure of Zn(Si_8PP) is shown;[9] for Zn(Si_6PP), an energy-minimized structure was obtained using Cerius 2 from MSI.

3. Discrimination of VOCs

In order to demonstrate the ability of the sensor array to discriminate among different volatile organics, the sensor array responses were determined for a series of different volatiles representing the common organic functionalities: amines, arenes, alcohols, aldehydes, carboxylic acids, esters, halocarbons, ketones, phosphines, sulfides, and thiols. These patterns are shown in Figure 6. Excellent discrimination among these analytes is observed even without any chemometric manipulations.

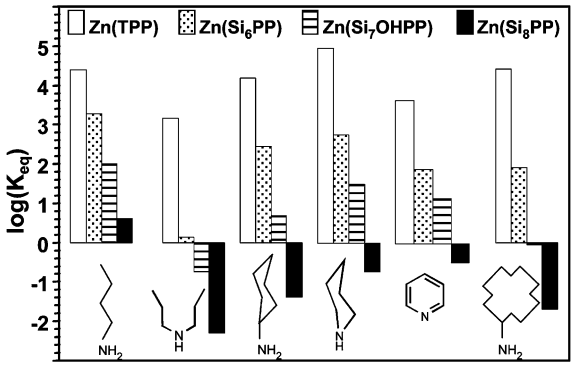

Figure 5. Ligand binding constants for siloxyl porphyrins compared to Zn(TPP) for a series of amines. The binding constants of silylether porphyrins are remarkably sensitive to the shape and size of the substrates relative to Zn(TPP) and can be controlled over a range of 10^1 to 10^7 relative to Zn(TPP). These selectivities originate from strong steric repulsions created by the *t*-butyldimethylsiloxyl substituents. The steric congestion caused by these bulky silylether groups is pronounced even for linear amines and small cyclic amines.

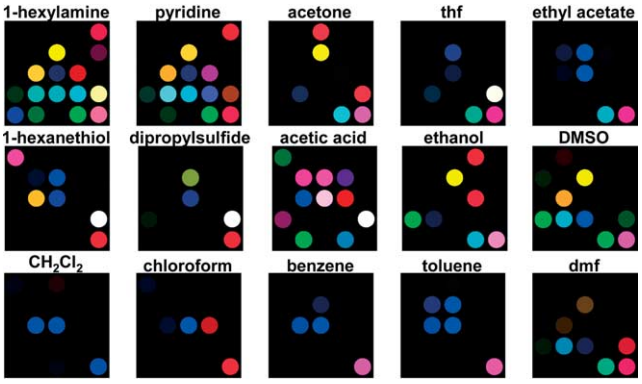

Figure 6. Colorimetric array responses for 18 common volatile organic compounds at saturation vapor pressure at 300 K.

An Epson Perfection 1250 flatbed scanner was used for scanning. The 'before' image was first acquired on the flatbed scanner; an array was then exposed to a flowing stream of N_2 containing the analyte of interest, and the array was then scanned again after equilibration. The center of each dye spot (~700 pixels, 15 pixel radius) was averaged and the value from the before image subtracted; this can be done with Photoshop™ or with a customized software package, ChemEye™ (ChemSensing, Inc.). The arrays are disposable and are not meant for re-use, especially after exposure to high analyte concentrations.

Gas streams containing the vapor of interest were generated by flowing nitrogen through the neat liquid analyte in a thermostated, glass-fritted bubbler. To vary analyte concentrations, serial dilution in nitrogen using digital mass-flow controllers was utilized. Vapor pressures were calculated using published data.[10] All liquid analytes were obtained from Aldrich or Fisher Scientific and used as received.

Each analyte response is represented as the red, green, and blue values of each of the 24 dyes, i.e. a 72 dimensional vector. To examine the multivariate distances between the analyte responses in this 72-dim. RGB color space, a hierarchical cluster analysis (HCA) was performed; this analysis makes use of only the digital data representing the observed difference maps—no chemical information is included. The resulting dendrogram for the responses to saturated analyte vapors is shown in Figure 7. Remarkably, the clusters formed are in keeping with the qualitative structural and electronic properties of the VOCs. The familial similarities among compounds of the same functionality are exceptional: amines, alcohols, aldehydes, esters, etc. are all easily distinguished from each other. The only exception to this appears to be the pair of ketones, methyl ethyl ketone and 4-hexanone (methyl amyl ketone), which overlap into the ester and ether subgroups, respectively. This slight intergroup confusion may reflect the inclusion of too few ketones (i.e. two) to form a recognizable class out of sample of 32 VOCs.

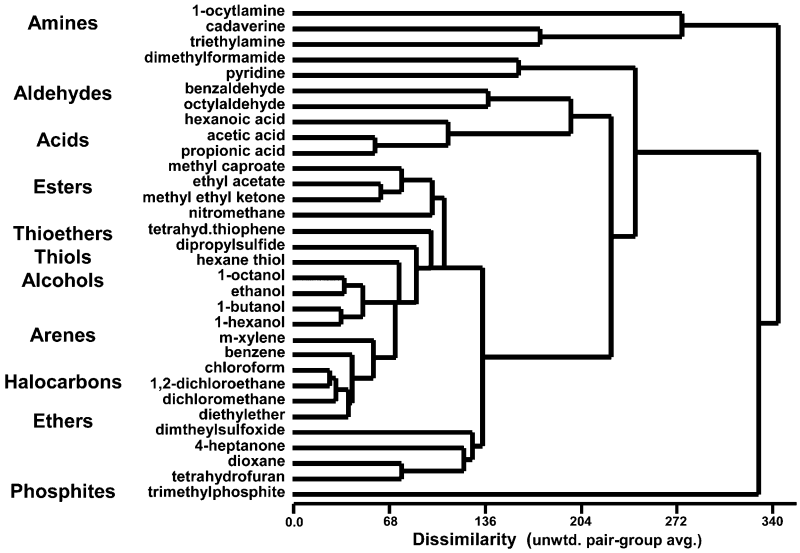

Figure 7. Dendrogram of the colorimetric array responses to 32 common organic compounds at full vapor pressure at 300 K. XLStat analysis package, v. 5.1, unweighted pair-group average agglomerative hierarchical cluster analysis.

A principal component analysis (PCA) of the full set of the VOC patterns reveals a surprisingly high degree of dispersion among the independent dimensions created by linear combinations of the RGB responses of the 24 dyes used in these arrays. The PCA shows that 95% of the discriminatory range requires 12 dimensions and 99% requires 20 dimensions. This extremely high dispersion reflects the very wide range of chemical-properties space being probed by our choice of chemoresponsive dyes. In comparison, most prior electronic nose technology is dominated by only a two or three independent dimensions (one of which, hydrophobicity, often accounts for 90% of the discrimination);[1,2] this is the inherent result of relying on van der Waals interactions for molecular recognition.

With a 24-dye array, each of the 72 dimensions (i.e. 24 RGB's) can take on one of 256 possible values (for inexpensive 8 bit scanners or digital cameras). The theoretical limit of discrimination, then, would be the number of possible patterns, i.e. $(256)^{72}$. Realistically, however, the RGB vector components do not range over the full 256 possible values; we do observe R, G, and B values vary over a range of 40. To discriminate patterns, let us assume a change of at least four is needed in the R, G, or B value (we can actually easily discriminate with changes of two). From the PCA, not all of the 75 dimensions are equally important. In fact, roughly 95% of all information is contained in $\sim$12 specific dimensions (i.e. linear combinations of the 72 different R, G, and B values). This implies a 'practical' limit of discrimination that is still immensely large: $(40/4)^{12}=10^{12}$ distinct patterns should be recognizable in a 24-dye colorimetric sensor array.

4. Interference from humidity

One of the most serious weaknesses in current electronic nose technology is sensitivity to changes in humidity. Water vapor ranges in the environment from $<$2000 to $>$20,000 ppm; if one is interested in few ppm concentrations of VOCs, even a very low level of interference from water is therefore intolerable. Because the colorimetric array has been selected from hydrophobic, water insoluble dyes, these arrays are essentially impervious to changes in relative humidity. As shown in Figure 8, the dyes are essentially unresponsive to water vapor. The water–vapor insensitivity of our technology gives it a substantial advantage.

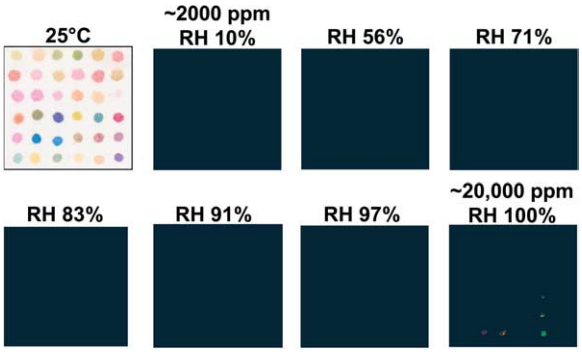

Figure 8. The colorimetric array is insensitive to changes in relative humidity. Similarly, changes in humidity do not significantly affect the color fingerprints of other analytes.

5. Sensitivity

Our colorimetric array sensor technology is based on strong and relatively specific interactions between the analytes and a chemoresponsive dye library. This is in marked contrast to prior electronic nose technology that relies on weak and extremely non-specific interactions (e.g. physical adsorption into polymers) between the analytes and the detectors. For example, the prior use of adsorption into polymer arrays (e.g. conductive polymer arrays, quartz microbalance or surface acoustic wave detectors coated with a variety of polymers, or polymers doped with single indicating fluorophore) depends upon weak matrix–analyte interactions to provide limited selectivity and relatively poor sensitivity. A major advantage of our sensor arrays is that they are able to provide unique patterns for the identification of odors even at extremely low vapor concentrations because we rely on strong interactions between analyte and sensor dyes for molecular recognition.

Metal–ligand (i.e. metal–analyte) bonds range in their bond enthalpies from $\sim$40 to $\sim$200 kJ/mol. In non-coordinating solvents (e.g. alkanes), equilibrium binding constants are often $>10^6\,M^{-1}$. For pyridine, the vapor pressure is 0.02 atm at room temperature, so we have a Raoult's constant of $\sim 2\times10^{-3}$ atm M^{-1}. For a binding constant of $\sim 10^6\,M^{-1}$, this is equivalent to $\sim$2 ppb vapor. In contrast, the enthalpy of physical adsorption (e.g. into polymers) is only $\sim$5 to 20 kJ/mol (i.e. roughly a tenth of a metal bond). Therefore, the equilibrium constant for adsorption will typically be only about 5×10^{-5} as large as that for ligation to metal ions. Therefore, ligation is intrinsically $\sim$20,000-fold more sensitive than adsorption into polymers. Differences in the sensitivity of detection techniques, of course, can either enhance or diminish this intrinsic advantage of ligation and other strong interactions over physical adsorption and other weak interactions (e.g. van der Waals).

As shown in Figure 9, this expectation of improved sensitivities is realized experimentally. Even GC-MS achieves typically only ppm to 100 ppb sensitivities for VOCs in the absence of pre-concentration. Figure 9 shows, however, that sub-ppm discrimination is not a problem with our colorimetric array detection for functionalized analytes such as thiols, amines and carboxylic acids. In fact, we can extend our sensitivities down to the low ppb regime for many such analytes. Extension of sensitivity by improved imaging technology (e.g. true 16-bit or 24-bit color resolution in place of our currently used 8-bit scanners) may improve our sensitivity by a thousand-fold.

It is worth noting that for these colorimetric arrays, every analyte at a different concentration is best thought of as a different analyte. For each analyte, a specific dye will begin to change color at some concentration and as the concentration of the analyte increases, the color change will saturate asymptotically; some dyes change at low concentrations of any given analyte but some only at very high concentrations. Quantitative analysis of single analytes

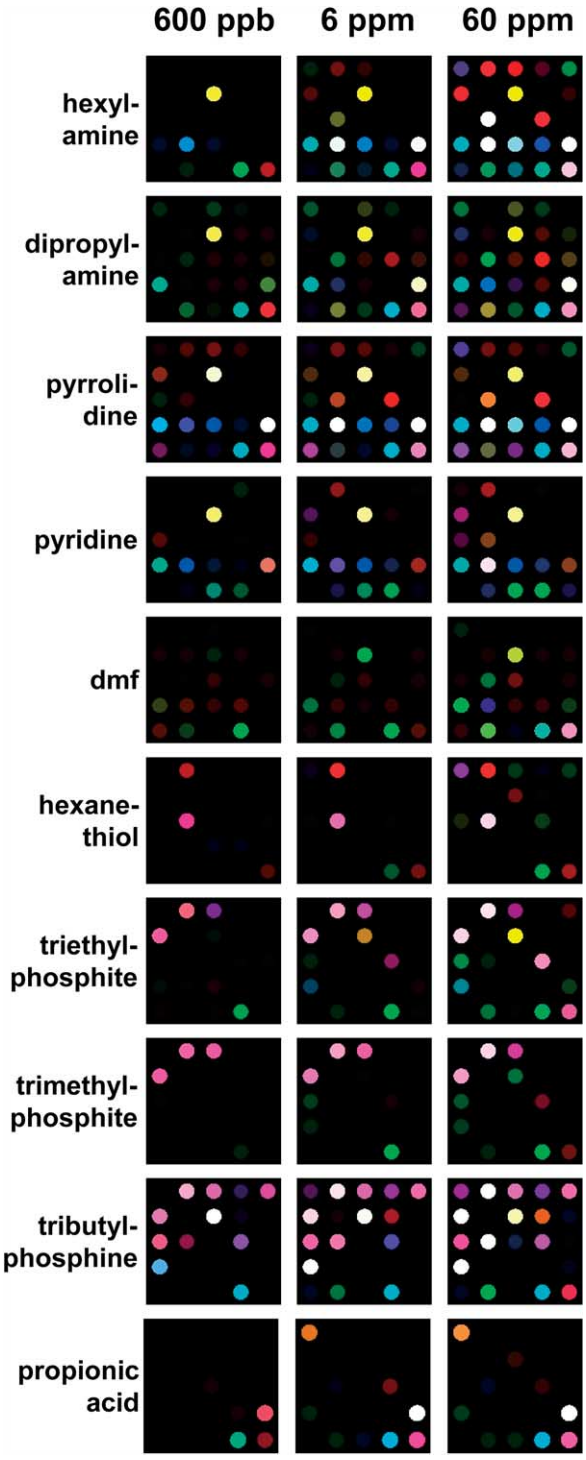

Figure 9. Colorimetric array sensitivity to low molecular weight analytes. Limits of recognition are well below 1 ppm.

(or of a single analyte changing in a constant background) can be done easily by comparison with a library of analyte patterns as a function of concentration.

6. Complex mixtures

Electronic nose techniques, in general, give a composite response to complex mixtures, as indeed does the mammalian olfactory system. Chemists very naturally tend to assume that for any complex mixture, the single analytical goal is a complete component-by-component analysis. But in fact, multiple analytic goals for complex mixtures are conceivable: comparison to a standard (e.g. quality control, counterfeit detection), identification of chemical class or family of an unknown VOC, correlation of odors to properties (e.g. 'good smelling' as determined by a human sensory evaluation panel), changes in concentrations of a few components against a constant background, and of course, a complete component by component (CBC) analysis. Separation techniques (e.g. GC, HPLC, CE, etc.) excel at CBC analyses. For many other applications, however, a composite response may not only be sufficient, but actually preferred. Too much data is often only slightly better than not enough.

For rapid determination of identity of a complex mixture, for example, we find that colorimetric array sensors can fill an important niche. As a closing example, consider the results of Figure 10 as an interesting comparison among complex odorants.

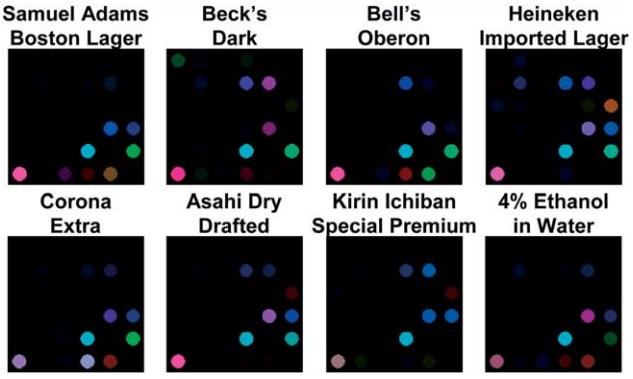

Figure 10. Colorimetric array analysis of a complex mixture: headspace analysis of various beers compared to 4% ethanol in water.

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