Chemsensing: A Colorimetric Array Detector

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Abstract
The development of an expanded colorimetric sensor array for the detection of volatile organic compounds is reported. Extremely high sensitivity (ppb) is demonstrated for the detection of biologically important analytes, including amines, carboxylic acids, thiols. This level of sensitivity is often better than human olfactory thresholds. In addition, highly selective discrimination of pure analytes and of mixtures has been demonstrated, even without the use of detailed chemometric analysis. Differentiation is shown for very closely related and even isomeric amines.

Keywords
array detection, sensors, colorimetric sensors, VOC, volatile organic compounds, porphyrins, metalloporphyrins, artificial olfaction, electronic nose.

INTRODUCTION
Array based vapor sensing has emerged as a powerful approach toward the detection of chemically diverse analytes. Electronic nose technology for the generalized detection of volatile organic compounds (VOC) has generally employed arrays of sensors based either on adsorption into a set of polymers or on oxidations at a set of heated metal oxides [1,2]. While such systems generally allow for distinction between analytes of different chemical functionality, the discrimination of compounds within a given chemical class remains a challenging goal. In addition, prior technology has generally proved to be relatively insensitive, especially to biogenically important analytes such as amines and thiols, and is usually extremely susceptible to interference from changes in relative humidity of analyte samples.

We have developed a unique chemical detection technology in which colorimetric changes in an array of dyes constitute a signal much like that generated by the mammalian olfaction system; each dye is a cross-responsive sensor. This electronic nose technology has been dubbed chemsensing™ [3-6]. This technology uses a disposable two-dimensional array of chemoresponsive dyes as the primary sensor elements, making it particularly suitable for detecting many of the most odiferous compounds.

Striking visual identification of a wide range of VOCs are easily made at part per billion (ppb) levels, for example to hydrogen sulfide, methylsulfide, formic acid, acetic acid, ammonia, and hexylamine (i.e., sensitivities comparable to or better than GC-FID or GC-MS detection).

THE COLORIMETRIC SENSOR ARRAY
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, i.e., bond formation, acid-base interactions, or strong dipoles. 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) Bronsted acidic or basic dyes (i.e., pH indicators), and (3) dyes with large permanent dipoles (i.e., zwitterionic solvatochromic dyes).

A colorimetric array of chemoresponsive dyes have been created by printing the dyes an inert solid support, e.g., reverse phase silica gel plates, acid-free paper, or porous membranes of various polymers (e.g., nylon, PVDF), as shown in Figure 1.

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Lewis acid/base dyes contain a Lewis acidic or basic center and change color in response to changes in the Lewis acidity or basicity of their environment. Lewis acid/base dyes include porphyrins and their metal complexes.

Metalloporphyrins are a natural choice 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 metallated tetraphenylporphyrins (TPP) was used to provide differentiation based on metal-selective coordination. Zinc-substituted, bis-pocketed porphyrins, based on ortho-substitution of the tetraphenylporphyrin core, were used to differentiate analytes based on size and shape. These compounds include a family of silyl ether porphyrins with large, medium, and small pockets on both faces of the porphyrin. These have been previously shown to exhibit extremely selective binding of organic ligands [5].

Common pH indicator dyes change color in response to changes in the proton (Brønsted) acidity or basicity of their environment. Sovtochromic 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 solvatochromic dyes are Reichard's Dye and Nile Red.

**DISCRIMINATION OF VOCs**

In order to demonstrate the ability of the sensor array to discriminate among different volatile organics, a series of 32 different volatiles representing the common organic functionalities: amines, arenes, alcohols, aldehydes, carboxylic acids, esters, halocarbons, ketones, phosphines, sulfides, and thiols. As shown in Figures 2 and 3, excellent discrimination among these analytes is observed. Even more impressively, hierarchical cluster analysis shows that 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.

In order to further establish the extraordinary degree of selectivity that these colorimetric arrays possess, responses at a range of vapor concentrations were obtained at a set of very closely related analytes, specifically 12 amines: butylamine, hexylamine, octylamine, pyridine, cyclohexylamine, pyrrolidine (c-N(CH2)5), piperidine (c-N(CH2)6), homopiperidine (c-N(CH2)7), dipropylamine, triethylamine, diisopropylamine, and diethylamine [7]. The analytes were chosen to provide comparisons between linear, branched, and cyclic amines, as well as to compare compounds within each of these subdivisions. The ability to discriminate these compounds based on such subtle distinctions in electronics and molecular structure constitutes a stringent test of the colorimetric array performance.

![Figure 2. Colorimetric array responses for 18 common volatile organic compounds at full vapor pressure at 300K, shown in grayscale (differences are more obvious in color).](image-url)
Figure 3. Dendrogram of the colorimetric array responses to 32 common organic compounds at full vapor pressure at 300K. XLStat analysis package, v. 5.1, unweighted pair-group average agglomerative hierarchical clustering analysis.

Color difference fingerprints for equilibrium responses to saturated amine vapors are shown in Figure 4 for a 24 dye array [8]. As expected, these difference maps are more similar than those for differing chemical functionality (i.e., Figure 2). While the overall family resemblance among all the amines is clear, nevertheless, distinctions can readily be seen.

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 RGB color space, a hierarchical cluster analysis (HCA) was performed; the resulting dendrogram for the responses to saturated analyte vapors is shown in Figure 5. Remarkably, the clusters formed are in keeping with the qualitative structural and electronic properties of the amines: the branched amines produce one cluster, as do the cyclic compounds piperidine, homopiperidine, and cyclohexylamine. Pyrrolidine, the smallest cyclic compound tested, clustered loosely with the linear amines, which were tightly associated. Pyridine, the lone aromatic amine of this set (which also has the lowest basicity), was independent of any of the other clusters. In contrast to prior electronic nose technologies, the colorimetric array is able to easily resolve very similar compounds and even structural isomers. For example, the C₆ amines (n-hexylamine, homopiperidine, dipropylamine, diisopropylamine, and triethylamine) all give clearly distinct color patterns.

As may be seen in Figures 4 and 5, the closest analyte pairs were, not surprisingly, butylamine vs. hexylamine and di-
ethylamine vs. dipropylamine. Even in these cases, however, the array responses are differentiable, even without sophisticated chemometric analysis, as shown by a qualitative comparison of the difference maps.

Furthermore, the quantitative color differences are completely distinct. For example, if we define a goodness of fit parameter (i.e., 1 – variance) from the Euclidean distance between vectors representing these analytes normalized by the length of the vectors, we have 0.990 for butylamine vs. hexylamine and 0.989 for diethylamine vs. dipropylamine, whereas multiple analyses of the same analyte produce a goodness of fit above 0.995. For specific color value comparisons, the differences are again statistically significant; e.g., between the former analyte pair, there are six RGB values that differ by more than 3σ (the largest by 6σ, where σ is the mean standard deviation for the RGB values of the difference maps of replicate exposures: 2.6 color units out of 256.), and for the latter pair, eleven RGB values differ by more than 3σ (the largest by 5σ).

![Dendrogram representation of a hierarchical cluster analysis. Data for branched (light gray), cyclic (medium gray), and linear (black) amines are shown. HCA analysis was done using Ward’s method with squared Euclidean distance with MVSP 3.1 software (Kovach Computing Services).](image)

Our technology takes advantage of the large color changes induced in chemically responsive dyes to create an easy colorimetric technique that minimizes the need for extensive signal transduction hardware. We are able to obtain unique color change signatures for analytes for both qualitative recognition and quantitative analysis by simply taking the difference before and after exposure of scanned images of the array.

With a 6x6 array, as currently finalized, we represent each analyte as a 108-dimensional vector (36 red, green, and blue values) each of which can take on one of 256 possible values (for inexpensive 8 bit digital scanners and cameras). The theoretical limit of discrimination, then, would be the number of possible patterns, i.e., (256)^108. 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 4 is needed in the R, G, or B value (we can actually easily discriminate with changes of 2). From principal component analysis, not all of the 108 dimensions are equally important. In fact, roughly 95% of all information needed to discriminate among the 32 test analytes of Figure 2 is contained in ~15 specific dimensions (i.e., linear combinations of the 108 different R, G, and B values). These 15 dimensions are the most important eigenvectors out of a total of 31 needed to describe the 32 analytes precisely. This implies a ‘practical’ limit of discrimination that is still immensely large: more than (40/4)^15 = 10^15 distinct patterns should be recognizable in a simple 6x6 array.

**IMPERVIOUS TO HUMIDITY**

One of the most serious weaknesses in current electronic nose technology is sensitivity to changes in humidity. 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 6, the dyes are essentially unresponsive to water vapor. The water-vapor insensitivity of our technology gives it a substantial advantage over polymer-based electronic noses, which are very susceptible to changes in humidity.

![Image showing the colorimetric array's insensitivity to changes in relative humidity.](image)
SENSITIVITY AND LIMITS OF DETECTION

Chemsensing 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 chemoselective sensor arrays is that they are able to provide unique patterns for the identification of odors even at extremely low vapor concentrations. Chemsensing relies on strong interactions between analyte and sensor dyes.

Metal-ligand (i.e., metal-analyte) bonds range in their bond enthalpies from ~40 to ~200 kJ/mol. In non-coordinating solvents (e.g., alkanes), equilibrium binding constants are often ~10^9 M^-1. For pyridines, the vapor pressure is 0.02 atm at room temperature, so we have a Raoult’s constant of ~2 x 10^-3 atm M^-1. For a binding constant of ~10^6 M^-1, this is equivalent to ~2 ppb vapor. In contrast, the enthalpy of physical adsorption (e.g., into polymers) is only ~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 x 10^-5 as large as that for ligation to metal ions. Therefore, ligation is intrinsically ~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 Figures 7 and 8, this expectation of improved sensitivities is realized experimentally. High sensitivities are particularly difficult to achieve with permanent gases and low molecular weight (i.e., low boiling point) compounds. The chemical potential or fugacity of such compounds is low relative to less volatile analytes, making low ppm sensitivities highly problematic. Even GC-MS achieves typically only ppm to 100 ppb sensitivities for such compounds in the absence of pre-concentration. Figure 7 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, which also are important volatiles produced by bacterial growth. Figure 8 shows multiple examples of such sensitivities, many of which are comparable or exceed human olfactory thresholds [9]. One should note that these are not even our limits of recognition, much less limits of detection, but rather indicate the lower limits readily available by serial dilution of permeation tube sources. We extrapolate that our true limits of detection for many thiols, amines, and carboxylic acids will actually prove to be well into the parts per trillion region.

REAL WORLD APPLICATIONS

The absence of interference from humidity combined with extremely high sensitivities to biologically important analytes makes real world applications entirely feasible for our chemsensing technology. Current development of a portable, battery powered imaging unit is progressing rapidly.

For example, classification of different coffees is easily done, as shown in Figure 9. Figure 10 shows the excellent reproducibility of the patterns seen for multiple runs of ground coffee. Amusingly, one can even distinguish the differences in aroma of individual whole coffee beans, simply by placing a single bean in a closed volume with the array and imaging with a flat bed scanner.

Given the high sensitivity of these colorimetric arrays to biogenic analytes, it should be no surprise that even body malodor is easily monitored by chemsensing. As shown in Figure 11, the growth of skin bacteria after showering was monitoring by the simple expedient of placing an array under the arm for exposure and then rapidly imaging it on a

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**Figure 7.** Colorimetric array sensitivity to low molecular weight analytes, shown in grayscale. Limits of recognition are well below 1 ppm.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2S</td>
<td>1 ppm</td>
<td>Human detection limit</td>
</tr>
<tr>
<td>NH3</td>
<td>1 ppm</td>
<td></td>
</tr>
<tr>
<td>HCO2H</td>
<td>0.7 ppm</td>
<td></td>
</tr>
<tr>
<td>CH3CO2H</td>
<td>0.7 ppm</td>
<td></td>
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</tbody>
</table>

**Figure 8.** Colorimetric sensitivity in ppb range to various volatiles produced by bacterial growth, shown in grayscale. Concentrations shown are above both limit of detection and limit of recognition.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formic acid</td>
<td>140 ppb (2800)*</td>
<td>Human detection limit</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>170 ppb (150)*</td>
<td></td>
</tr>
<tr>
<td>Iso-valeric acid</td>
<td>280 ppb</td>
<td></td>
</tr>
<tr>
<td>3-Me-2-hexenoic</td>
<td>12 ppb</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2S</td>
<td>14 ppb (18)*</td>
<td>Human detection limit</td>
</tr>
<tr>
<td>CH3SH</td>
<td>22 ppb (1)*</td>
<td></td>
</tr>
<tr>
<td>n-hexylamine</td>
<td>100 ppb</td>
<td></td>
</tr>
<tr>
<td>Cadaverine</td>
<td>1 ppm</td>
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</tbody>
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flat bad scanner. While there are clearly similarities in the patterns, it also appears that there are individual differences detectable as well, which is certainly in keeping with our everyday experience.

Figure 9. Comparison of various ground coffee samples from direct head gas monitoring. Shown in grayscale; differences in color patterns are more dramatic.

Figure 10. Ground vs. whole bean coffee, blue channel only shown in grayscale. Upper triplicate set: sample-to-sample reproducibility of ground coffee samples gives a variance of 0.5% (Euclidean distance between analyte vectors normalized by length of vector). Lower pair: bean to bean variation for whole bean coffee determined from head gas of single beans.

The monitoring of bacterial growth can also be accomplished under more controlled conditions. Diagnosis of infections still relies on cell culturing, a technology unchanged in more than a century. Cell cultures are relatively slow because they rely on visual observation of individual bacterial colonies; $\sim 10^6$ bacteria are required for visualization of any single colony. For fast growing bacteria, this means an incubation period of $\sim 24$ to 48 hours, but for other, slower growing organisms (e.g., tuberculosis), incubation of a week or more is required before cultures can be analyzed. It may be argued, however, that bacterial growth is better monitored by the VOCs produced by their metabolism because this is an inherently integral of growth. In addition, rapid evaluation of bacterial growth may permit rapid evaluation of antibiotic resistance, an increasingly problematic aspect of diagnosis and treatment.

As shown in Figure 12, the high sensitivities of our colorimetric arrays to biogenic analytes permits rapid evaluation of the growth of E. coli. This promising, preliminary result is under further study for quantitative analysis, discrimination among different bacteria, and the influence of growth media on volatile metabolites.

Figure 11. Monitoring the volatiles produced in body malodor versus time, shown in grayscale.

Figure 12. Monitoring the volatiles produced by bacterial growth versus time, shown in grayscale.

CONCLUSIONS

The development of an expanded colorimetric sensor array for the detection of volatile organic compounds has been achieved. Extremely high sensitivity (ppb) is demonstrated for the detection of biologically important analytes, including amines, carboxylic acids, thiols. In addition, highly selective discrimination of pure analytes and of mixtures has been demonstrated, even without the use of detailed chemometric analysis. Differentiation has been shown for very closely related and even isomeric amines. Relative humidity has essentially no effect on the response of the arrays, so real world applications become straightforward. For one example, discrimination of different brands of coffee and even measurement of differences in individual coffee beans has been achieved. As another example, the volatile metabolites of bacteria have been characterized by the colorimetric arrays both in well-defined cultures and in body malodor studies.
ACKNOWLEDGMENTS
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REFERENCES


[7] 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 data from Yaws, C. L.; Handbook of Vapor Pressure; Gulf Publishing: Houston, 1994. All liquid analytes were obtained from Aldrich or Fisher Scientific and used as received.

[8] Images were obtained at 1200 d.p.i. in RGB color mode using an ordinary flatbed scanner (EPSON Perfection 1200S). RGB images after and before exposure to analytes were subtracted using a 314 pixel average from the center of each dye spot.