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Discrimination of Organic Acids Using a Three Molecule Array Based upon Cruciform Fluorophores

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Supporting Information

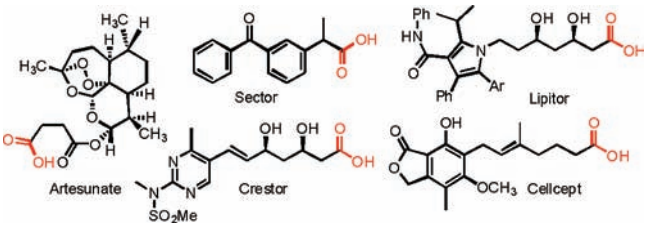
ABSTRACT: A small array was obtained from three reactive cruciform fluorophores in six different solvents. The array discerned 10 different aromatic carboxylic acids by protonation-induced fluorescence shifts, which were recorded by digital photography. This simple array can discern acids that have closely spaced pK_a values.

Tuning and enhancing the discriminatory and sensory responses of fluorophores or chromophores toward classes of compounds has emerged as an important topic at the interface of analytical, biological, organic, materials, and supramolecular chemistry.[1] The application of array-based sensing concepts by Suslick,[2] Lavigne,[3] and Anslyn[4] has provided material systems that recognize complex mixtures of similar compounds with surprising selectivity. Attention-grabbing examples include the identification of different whiskey brands[5] and selections of coffee grounds (Starbucks vs Folgers)[6] by small arrays of substrate-immobilized chromophores. The concept is known as chemical tongue or chemical nose and also works in discriminating among different amines.[7] Are there generalized conditions where chemical tongues are particularly successful? Chemical tongues work best when reporting the compositional deviation of an analyte, identifying (undesired) changes in a regulated/defined environment. For this reason, even complex mixtures (e.g., alcoholic spirits, coffee grounds, beers, etc.) can be "fingerprinted" and compared with each other by a specific set of sensory compounds or arrays. Chemical tongues are therefore particularly good for sophisticated quality control.

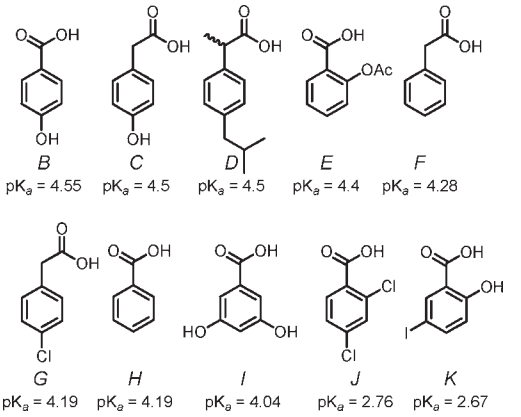
The detection of counterfeit drugs is critically important, as one wants to avoid ingestion of poisonous substances or the use of (for example) malaria medications that contain wrong or adulterated ingredients or have just expired.[8] A significant number of prescription drugs display either free amines (often in their ammonium form) or free carboxylic acids (occasionally in their salt form). Examples of the latter class are Artesunate, Lipitor, Crestor, Cellcept, and Sector (Scheme 1) as well as aspirin (*E*) and ibuprofen (*D*) (Scheme 2). A simple fluorescence-based test that can discern organic acids might therefore be of great interest, as it would have the potential to perform quality control of drug samples of questionable origin (e.g., Internet pharmacies, gray market, etc.). Such a tool would also be useful for public health applications.

Carboxylic acids are normally identified by spectroscopic methods after derivatization and subsequent chromatography,[9] but for volatile carboxylic acids, carbon black−poly(ethylenimine) composites have been used with some success.[10] In this communication, we describe the identification of 10 structurally related acids *B*−*K* derived from benzoic and phenylacetic acids (Scheme 2) using the small array of reactive cruciform (XF) fluorophores **XF1**−**XF3** (Scheme 3) in six different solvents.

Scheme 1. Some Drugs Displaying Carboxylate Groups



Scheme 2. Investigated Acids



In a first experiment, we investigated the interaction of phenylacetic acid (*C*) with **XF1**[11] to obtain the concentration-dependent response of the XF to the acid.[12] At concentrations above 500 mg of *C* in a 16 mL vial (31 g L^{-1}), the fluorescence started to shift from green to blue as a result of protonation of the aniline nitrogens (Figure 1). This experiment demonstrated that weak acids do elicit a response under these conditions. To evaluate whether different weak acids are differentially recognized, we exposed acids *B*−*K* (Scheme 2) to a small XF array containing 18 members (three XFs in each of six solvents) at an

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Scheme 3. Reactive Cruciform (XF) Fluorophores

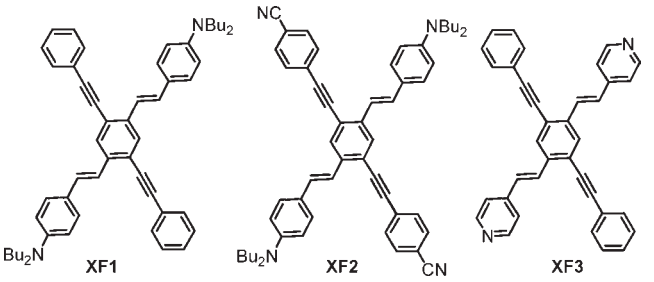

Figure 1. Photograph of solutions containing **XF1** and increasing concentrations of phenylacetic acid. Photographs were taken under a hand-held blacklight at a wavelength of 366 nm.

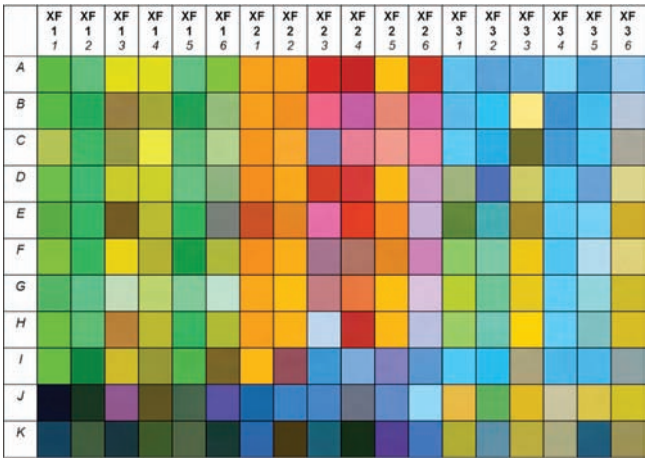

Figure 2. Photograph of an array formed by exposing **XF1**−**XF3** in six different solvents (1 = DCM, 2 = EtOAc, 3 = MeCN, 4 = DMF, 5 = iPrOH, 6 = MeOH) to 10 different carboxylic acids (*A* = reference; *B* = 4-hydroxybenzoic acid, pK_a = 4.55; *C* = 4-hydroxyphenylacetic acid, pK_a = 4.5; *D* = ibuprofen, pK_a = 4.5; *E* = aspirin, pK_a = 4.4; *F* = phenylacetic acid, pK_a = 4.28; *G* = 4-chlorophenylacetic acid, pK_a = 4.19; *H* = benzoic acid, pK_a = 4.19; *I* = 3,5-dihydroxybenzoic acid, pK_a = 4.04; *J* = 2,4-dichlorobenzoic acid, pK_a = 2.76; *K* = 5-iodosalicylic acid, pK_a = 2.67). Photographs were taken row by row under a blacklight at an excitation wavelength of 366 nm.

acid concentration of 31 g L^{-1} (Figure 2). The results were striking, as all of the carboxylic acids could be discerned even though the pK_a differences were small in some cases. Not unsurprisingly, the most acidic analytes [dichlorobenzoic acid (*J*) and iodosalicylic acid (*K*)] showed the largest changes in the response patterns. Extraction of the RGB values from a photograph of the array (Figure 2) using Colour Contrast Analyzer[13] was followed by statistical evaluation of the response patterns.

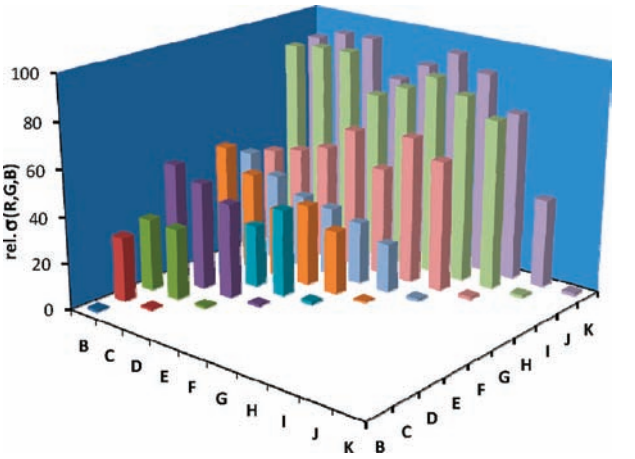

Figure 3. Differential autocorrelation plot of the fluorescence responses of the carboxylic acids *B*−*J* by the 18-membered XF + solvent array. The *z* axis represents the relative standard deviation (σ) of the R, G, and B values relative to carboxylic acid *B*. Only when identical acids were correlated (diagonal series) did the σ value vanish, and therefore, all of the acids were correctly identified.

A differential fluorescence correlation plot obtained by multivariate analysis of variance (MANOVA) can be used to identify unknowns by their fluorescence signatures (Figure 3). All 10 acids were reliably discerned using this autocorrelation tool. In the MANOVA approach, standard deviations (σ) of the individual RGB values relative to a particular compound are calculated. This approach reveals properties and structure relationships of the cruciform fluorophores **XF1**, **XF2**, and **XF3** with the solvents and analytes. There are correlations between the σ values of different XFs dissolved in the same solvent and the same XF dissolved in different solvents, but the σ values are characteristic, making possible an unambiguous assignment of the analytes through the use of an orthogonal fluorophoric sensor array. This allows the prediction of σ values for unknown analytes. We found that the σ values for **XF2** in EtOAc correlated well with the pK_a values of the analytes (relative to *B* with the highest pK_a) (Figure 4a). **XF3** gave a clustered response correlated to the acids' chemical structures (Figure 4b). All of the nonfunctionalized acids along with 4-chlorophenylacetic acid (*G*) were clustered in the region indicated by the green circle. Acids featuring free hydroxyl groups (red circle) were grouped together, while the two most acidic halogenated benzoic acids (*J* and *K*) showed the greatest σ values (blue circle). Here too, the presence of the hydroxyl group in *K* lowered the σ value relative to that of *J*. **XF3** apparently recognizes functional features, while **XF2** displays responses that strictly correlate with the pK_a values of the acids. Attempts to convert the spectroscopic (as opposed to the photographic) responses (for response spectra of **XF2** and **XF3** with *B*−*J*, see the Supporting Information) to RGB values and functionally map the information from the spectra onto those obtained from the photographs is currently not possible. There is no simple algorithm that converts fluorescence spectra directly into RGB values.

In an effort to achieve unambiguous detection of analytes with the smallest number of fluorophore−solvent combinations, we analyzed the obtained three-dimensional (3D) data sets to find the largest differences in the σ values. With only three different fluorophore−solvent combinations (Figure 5), the minimal set, the analytes *B* to *K* were identified.

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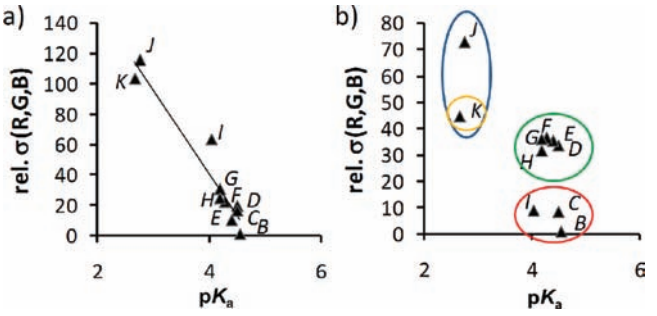

Figure 4. Correlation plots of pK_a values and the relative standard deviation σ of the RGB values. (a) **XF2** dissolved in EtOAc. (b) **XF3** dissolved in EtOAc. The red circle indicates acids with a hydroxyl group. The blue circle groups acids with a halogen substituent (except for *G*). The green circle groups acids without any functionality along with acid *G*. The yellow circle indicates an acid with both hydroxyl and halogen substituents.

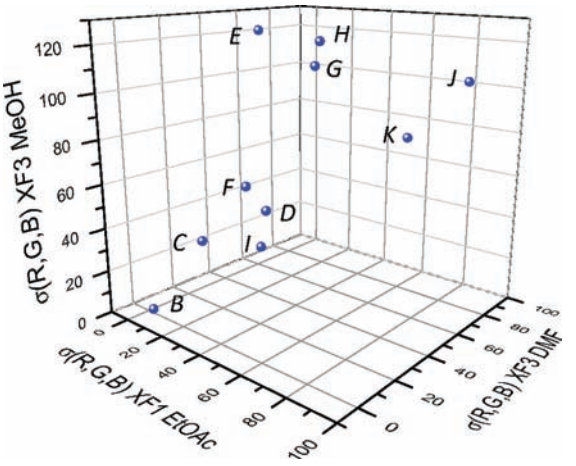

Figure 5. 3D plot for two cruciform fluorophores **XF1** and **XF3** in three solvents. The data points represent well-separated standard deviations of the individual analytes *B* to *K*.

In conclusion, a fluorophore array [three fluorophores (**XF1**−**XF3**) in each of six solvents] discerned 10 different carboxylic acids by differential fluorescence response using digital photography. Some of the acids had pK_a values that are close to each other, but this array was considerably more selective than a simple "pH paper". Its selectivity should be further tuned when other more basic or hydrogen-bonding XFs are included. The apparent disadvantage that one has to use high concentrations of carboxylic acids to elicit a response is not an issue. Drug formulations (i.e., tablets, gel caps, etc.), the final target, can be crushed and used in concentrations necessary to elicit a robust fluorescence response. One would then only have to compare the (photographically reproduced) profile of an original tablet with that of the sample at hand or use a statistical algorithm. This concept should be well-suited for identifying and discerning drug formulations that are acidic in nature.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details, photographs of all color responses, selected fluorescence spectra, statistical evaluation of the experiments, and complete ref 8c. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Colour Contrast Analyzer is a downloadable freeware program. See: http://www.visionaustralia.org.au/info.aspx?page=628 (accessed March 24, 2011).