

Counterion Free Colorimetric Metal Cation Sensor Array

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Colorimetric chemosensors have been studied intensively for various ion species due to their simple detection through color changes.^{1,2} Most conventional chemosensor development relied on rational design and the synthesis of a target ion recognition moiety.^{3,4} A recent alternative approach is configuring a dye array consisting of several nonspecific sensors to attain suitable responses and analyze their pattern as a fingerprint. Metal cations were the most extensively studied targets in this field. For example, an eight fluorescent probe array for the discrimination of five metal cations was developed by Wolfbeis and co-workers in 2003.⁵ More recently, our group reported a 47 dye array system (New York Tongue-1 NYT1), and we successfully distinguished 43 metal cations in the aqueous phase.⁶ In solution, ions always exist in positive and negative ion pairs. It is always possible that each probe has cross reactivity to both ions, especially in aqueous solution; H^+ (H_3O^+) and OH^- will be other competing ion species whose concentration depends on the pK_a of the salt. In our previous cation discrimination, we used nitrate as the common counter anion for all cations due to its low response to dye array and high water solubility. With the successful cation study with NYT-1, we began to explore the anion effect in the same system hoping to achieve a dual ion sensing system.

NYT-1 is a colorimetric dye array system consisting of 47 off-the-shelf dyes⁶ (see the Supporting Information). Each dye's change in absorbance was collected using a 384 microtiter plate format, and we calculated the fold change in absorbance by attaining data at the λ_{max} wavelength. Fold change data were further analyzed by several data mining techniques. Before screening the anions with the NYT-1 system, we first checked the cations, which have the nitrate anion as their counter anion, to determine which cation has the minimum interference with the NYT-1 system. While tetrabutylammonium (TBA) and sodium seemed to show minimal response to NYT-1, we chose sodium salts to observe the anion effect, due to their water solubility. Finally, we selected 15 sodium anion salts that were fully soluble in water (1 mM final concentration) and collected the fold change data as described in the Supporting Information-(NaSCN, NaClO₄, NaHSO₄, NaOCN, NaI, NaF, NaBr, NaClO₃, Na₃citrate, NaH₂PO₄, NaOAc, Na₂HPO₄, Na₂SO₄, NaCl, and NaN₃). To visualize high dimensional data qualitatively, we used principal component analysis (PCA),^{7,8}

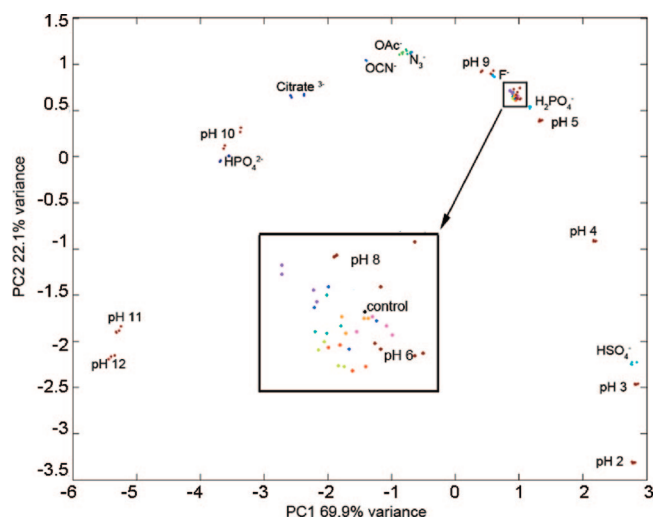


Figure 1. Principal component analysis of 15 anions: PCA results with 15 sodium anions at 1 mM (four repeated experiments). The first principal component has 69.9% variance, and the second principal component has 22.1% variance. The first two components cover an overall 92.0% variance. Anion labels in the magnification box: (purple) SO_4^{2-} , (red) SCN^- , (green) ClO_4^- , (blue) ClO_3^- , (pink) Cl^- , (orange) Br^- , (blue-green) I^- .

and the results were plotted with pH profiles in Figure 1. Principal component analysis is one of the popular data mining methods for reducing the original data dimensions. Utilizing PCA methods, the response pattern of 43 probes could be validated easily in a 2 or 3 dimensional graph.

Most of the data from the four repeated experimental trials were closely clustered and overlapped with each other, demonstrating a low error range and high reproducibility. Most halide anions (Cl^- , Br^- , and I^-) except for the fluoride ion (F^-) showed a minimal response to the NYT-1 array, and their spots were located close to the control spot (no response), whereas other anions showed relatively high responses. Interestingly, most anions' spots were located at a similar region of the pH profile in the PCA plot. The HSO_4^- ($pK_a = 1.99$) spots were near to the acidic area in the PCA plots, and Br^- , Cl^- , SCN^- , SO_4^{2-} , I^- , ClO_4^- , and ClO_3^- were plotted close to pH 6 or the neutral region. Unlike other halide species (Br^- , Cl^- , and I^-), F^- ($pK_{aHF} = 3.15$) had a unique pattern close to pH 9.0. The OCN^- ($pK_{aHOCN} = 3.92$), OAc^- ($pK_{aHOAc} = 4.76$), and N_3^- ($pK_{aHN_3} = 4.72$) spots were closely plotted around pH 9–10, and the HPO_4^{2-} ($pK_a = 12.32$) spots were closely plotted in the basic region as well. From the correlation between the PCA patterns of the anions and the pH, we concluded that the anion effect in NYT-1 might be a reflection of their pK_a values.

The origin of the anion effect in NYT-1 pointed out an important issue for an anion sensing strategy using small molecules in aqueous solution. The color change of the colorimetric sensor was induced by an electronic environment change of the chromophore. The protonation or deprotonation of popular anion recognition functional groups (e.g., $-OH$, $-NH$) can interfere with the chromophore's electronic

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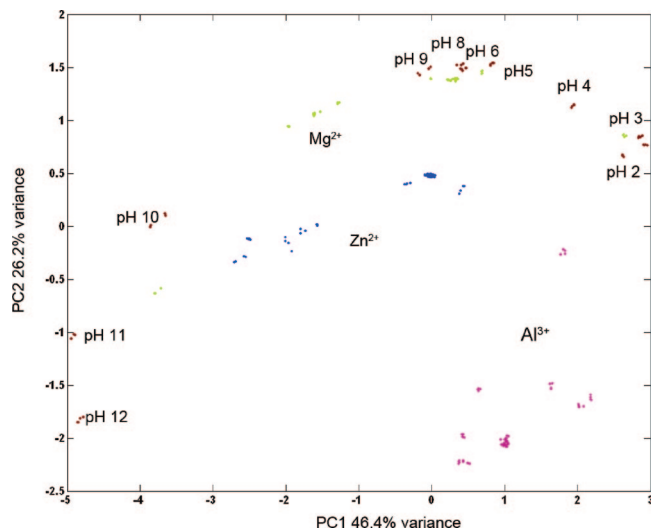


Figure 2. Principal component analysis result with the series of sodium anions and metal cation complex in nonbuffer condition. Green spots are the Mg^{2+} nitrate and 15 sodium anion mixture. Purple spots are the Al^{3+} nitrate and 15 sodium anion mixture. Bright-blue spots are the Zn^{2+} nitrate and 15 sodium anion mixture. Dark-brown spots are the pH profiles.

environment more dramatically than hydrogen bonding or a Lewis acid type electron donation. Since most experiments were performed in an organic solvent without any buffer conditions, direct anion sensing should address more elaborate validation. Presumably, the previous anion indicator's response pattern reflected more anion basicity (i.e., OH^-) rather than specific anion recognition in aqueous solution.^{9,10} In other words, the changes observed with anions in organic solvents are mostly due to acid–base reactions.

At this point, it was apparent that the NYT-1 system could not discriminate anions directly due to their pH response. To turn this negative observation into an advantage in the NYT-1, we decided to configure a universal cation sensing system, in which the anion effect was effectively suppressed. Because much of the anion effect was due to a pH response in the NYT-1 system, we expected that a unique cation profile could be extracted out through the use of a proper pH buffer condition. Due to the difficulties of collecting 15 anion salts of each metal ion, we used a mixture of metal nitrate and sodium anions. Sodium and nitrate salts were least responsive to NYT-1 in any combination with other counter ions. It is also believed that the mixture solution of metal nitrate and a series of sodium anions are close to a real world sample. We prepared a 1 mM final concentration of metal nitrates (Zn^{2+} , Mg^{2+} , and Al^{3+}) mixed with the same concentration of 15 sodium anions (SCN^- , ClO_4^- , HSO_4^- , OCN^- , I^- , F^- , Br^- , ClO_3^- , citrate, H_2PO_4^- , OAc^- , HPO_4^{2-} , SO_4^{2-} , Cl^- , and N_3^-).

As a control, the response pattern of metal cations (Zn^{2+} , Mg^{2+} , and Al^{3+}) with 15 anions in nonbuffer conditions was visualized with the pH curve in Figure 2. As expected, the patterns of metal cations (especially Zn^{2+} and Mg^{2+}) with different counter anions spread out along the pH profile curves. The cation spot location in the PCA plot is determined by a balance between the metal cation effect and the major anion effect which seems to stem from its pH.

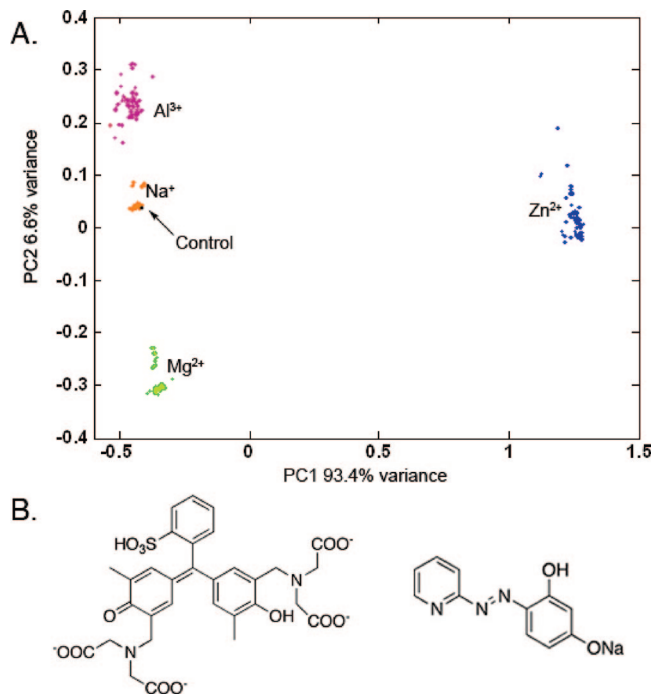


Figure 3. (A) Principal component analysis of universal metal cation discrimination. Four metals were distinguished independent of 15 counter anions in the phosphate buffer condition by 2 selected dyes. (B) Two selected probes. Xylenol Orange is a sodium salt, and 4-(2-Pyridylazo) resorcinol is a monosodium salt.

To determine the ideal condition, we tested several combinations of buffer composition and concentrations (carbonate, PBS, HEPES, and phosphate buffer). It was obvious that higher buffer capacity helped to suppress pH fluctuation, but many analytes had low solubility in high concentration buffer conditions. From extensive evaluation of buffer conditions in terms of salt solubility and anion effect suppression, we finally selected a phosphate buffer (80 mM, pH 7.4). The anion effects were successfully suppressed in the 80 mM phosphate buffer condition, and cation spots were clustered by only their cation identity regardless of the anions (see Figure S2 in the Supporting Information).

To determine the minimum number of required dyes for full discrimination, we applied linear discrimination analysis (LDA) to evaluate the data and select the minimum number of probes needed.¹¹ LDA was performed using a 47 probe set, and probe selection was performed using a variable selection algorithm using SYSTAT (version 11.0) to evaluate which probes are crucial for accurate discrimination. The selected dyes' distinguishing power was validated by a jack-knifed classification matrix, which is a cross-validation routine (see the Supporting Information). We finally selected two probes, Xylenol Orange and 4-(2-Pyridylazo) resorcinol, which are known metal indicators. Xylenol Orange changes its color from orange to red due to the response of metal cations at basic pH.^{12,13} 4-(2-Pyridylazo) resorcinol sodium salt is known to be selectively responsive to Zn^{2+} cations at a pH of 7.4.^{14,15} From the total 240 element data set (15 counter anions \times 4 experiments \times 4 metal cations), 100 % accurate discrimination was achieved by the jack-knifed classification matrix (see the Supporting Information). The two selected dyes were able to show clear discrimination between metal cations independent of the 15 counter anions

and have a similar discriminating power as the 47 dye probe set (Figure 3). The same procedure can be performed to prepare a sensor array for any kind of cation discrimination sensor array.

In summary, by a systematic study in NYT-1, we found that the anion effects are mainly due to their pH. This result cast a warning for careful validation for any other anion sensing system, especially for sensor arrays. By positively utilizing this observation, we effectively suppressed the anion effect using a proper pH buffer condition and devised a practical cation sensor system.

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Supporting Information Available. Detail of experimental procedure and data analysis results. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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