Supplementary Information:

An array sensor consisting of a single indicator with multiple concentrations and its application in ions discrimination†

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1. Experimental

Deionized water was used throughout the whole work. The indicators and metal salts (NiCl$_2$, CoCl$_2$, CuCl$_2$, ZnCl$_2$, PbCl$_2$, CdCl$_2$, and HgCl$_2$) used were analytical-reagent grade, obtained from Alfa Aesar Co., and used without further purification unless otherwise specified. All anion salts (NaF, NaCl, NaBr, NaI, Na$_2$S, Na$_3$PO$_4$, Na$_2$CrO$_4$, and Na$_2$C$_2$O$_4$) were purchased from Shanghai Jingchun Reagent Co., P. R. China, and were metals basis (99.99%). UV-Vis spectra were obtained from TU-1901 UV/Vis spectrometer (Beijing Perkinje General Instrument Co., Ltd, P. R. China).

All stock solutions of anions were prepared using deionized water and were adjusted to pH 6.5 by the addition of NaOH or HCl utilizing a Titrator T50 (Mettler Toledo Co.) and all the solutions were used immediately after preparation. The heavy-metal ions stock solutions were prepared using acetic acid buffer (0.01 M, pH=5.6).

The sensor array composition is listed separately as below.

<table>
<thead>
<tr>
<th>Sensor array for SO$_4^{2-}$ and PO$_4^{3-}$ sensing</th>
<th>Sensor array for eight anions sensing</th>
<th>Sensor array for seven heavy-metal ions sensing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicator #</td>
<td>1-(2-pyridylazo)-2-naphthol (μM)</td>
<td>Pb$^{2+}$ (mM)</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>0.35</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>0.40</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
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</tr>
<tr>
<td>11</td>
<td>35</td>
<td>0.55</td>
</tr>
<tr>
<td>12</td>
<td>35</td>
<td>0.60</td>
</tr>
</tbody>
</table>

198 μL aqueous solutions (for anion sensing: ethanol/water = 50%, v/v, pH 6.5; for heavy-metal ions sensing: ethanol/acetic acid buffer (0.01 M) = 50%, v/v, pH 5.6) of 12 indicators were loaded in a 6×2 polystyrene well plate (from 96-well plate). Each well was an individual cylinder with 10 mm tall (the inner diameter = 6 mm; the out diameter = 8 mm). The wells were then sealed immediately by plastic film to prevent the solvent evaporation. The “before” image was acquired. The Epson V200 flatbed scanner with a built-in transparency unit was utilized to collect the full digital images of the array from the bottom of the 6×2 well plate. The transparency scan unit was used (usually for negative scan) to avoid the light reflection of solutions. Uniform images of solutions were obtained.

2 μL individual corresponding anions or heavy-metal ions stock solutions were separately added to the corresponding 6×2 array to reach the desired concentrations. After three minutes mixing, the “after” image was acquired.

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Digitization of the color differences was performed using Adobe Photoshop software package. Difference maps were obtained by taking the difference of average red, green, and blue (RGB) values from the center (φ= 4 mm, 80% of the total size) of each individual well from the “before” and “after” images. The chemometric analysis was carried out on the color difference vectors using the Multi-Variate Statistical Package; in all cases, minimum variance (i.e., “Ward’s Method”) was used for hierarchical clustering analysis (HCA).

2. Schemes

Scheme S1. The deduction of the initial concentration of indicator A.

\[
A + S \xrightleftharpoons{\ rate k} B
\]

let 0 and 1 denote different chemical species (analytes) with different equilibrium coefficients; let x and y denote the same indicator at different concentrations; let \( k \) denote the equilibrium coefficient when the indicator reacts with an analyte; let \([A]\) denote the initial concentration of the indicator \( A \); let \([B]\) denote the concentration of product produced by the reaction of the indicator and the analyte; let \([S]\) denote the initial concentration of the analyte;

For the same indicator at different concentrations \( A_x \) and \( A_y \)

\[
k = \frac{[B]}{([A] - [B])([S] - [B])}
\]

Thus, the concentration of indicator \( A \) could be deduced as follow:

\[
[A] = \frac{[B]}{k([S] - [B])} + [B]
\]

If the final color of analyte 0 and 1 are same after the reaction with \( A_x \), that is, \([B]_{0x} = [B]_{1x} = [B]_x\), then,

\[
[A]_x = \frac{[B]_{0x}}{k_0([S]_0 - [B]_{0x})} + [B]_{0x} = \frac{[B]_{1x}}{k_1([S]_1 - [B]_{1x})} + [B]_{1x}
\]

Similarly, if the final color of analyte 0 and 1 are same after the reaction with \( A_y \), that is, \([B]_{0y} = [B]_{1y} = [B]_y\), then,

\[
[A]_y = \frac{[B]_{0y}}{k_0([S]_0 - [B]_{0y})} + [B]_{0y} = \frac{[B]_{1y}}{k_1([S]_1 - [B]_{1y})} + [B]_{1y}
\]
Scheme S2. The calculation for equilibrium constants.

\[ A + S \rightleftharpoons k B \]

Then the following equation is valid \(^{[1]}\),

\[
\frac{1}{\delta_0 - \delta} = \frac{1}{[A]_0 \Delta \varepsilon L} + \frac{1}{[A]_0 \Delta \varepsilon L k [S]_0}
\]

Where \( \delta_0 \) and \( \delta \) are the absorbance of \( A \) and mixed solution, respectively. \([A]_0 \) and \([S]_0 \) are initial concentration of \( A \) and \( S \). \( L \) is the path length. \( k \) is the equilibrium constant, which can be very approximately calculated from the slope of the straight line obtained by plotting \( \frac{1}{\delta_0 - \delta} \) vs \( \frac{1}{[S]_0} \).

For sulphate anions, \( k=995 \text{ M}^{-1} \). (\( \delta_0=0.4247 \))

For phosphate anions, \( k=76543 \text{ M}^{-1} \). (\( \delta_0=0.4247 \))

3. Figures and tables

**Fig. S1.** (a) Absorption spectra of 1-(2-pyridylazo)-2-naphthol (3.5×10⁻⁵ M) after 3 min upon the addition of Pb²⁺ at increasing concentrations in ethanol/acetic acid buffer solution (50% v/v, 0.01 M, pH 5.6); (b) Absorption spectra of 1-(2-pyridylazo)-2-naphthol (3.5×10⁻⁵ M) - Pb²⁺ (5×10⁻⁴ M) chelating compound after 3 min upon the addition of increasing concentrations of sulfate anion in ethanol/water solution (50% v/v, pH 6.5); (c) Absorption spectra of 1-(2-pyridylazo)-2-naphthol (3.5×10⁻⁵ M) - Pb²⁺ (5×10⁻⁴ M) chelating compound after 3 min upon the addition of increasing concentrations of phosphate anion in ethanol/water solution (50% v/v, pH 6.5).
**Fig. S2.** The final color of 1-(2-pyridylazo)-2-naphthol-Pb²⁺ displacement reaction versus response time for sulfate and phosphate at different concentrations in ethanol/water solution (50% v/v, pH 6.5). As shown, three minute is enough to get equilibrium for both sulfate and phosphate anions.

![Graph showing absorption over time for different anions](image)

**Fig. S3.** Images of 6x2 sensor array after 3 min upon the addition of SO₄²⁻ and PO₄³⁻ anions.
**Fig. S4.** The color changes (defined as $\sqrt{\Delta R^2 + \Delta G^2 + \Delta B^2}$) of each individual sensing unit for 1-(2-pyridylazo)-2-naphthol-Pb$^{2+}$ sensor array. All experiments were run in quintuplicate.

**Fig. S5.** Images and color difference maps of eight anions at their wastewater discharge standard concentrations after 2 min of exposure. For display purposes, the color ranges of these difference maps are expanded from 4 to 8 bits per color (RGB range of 4-19 expanded to 0-255).
**Fig. S6.** Images and color difference maps of seven heavy-metal ions at 50 μM after 2 min of exposure. For display purposes, the color ranges of these difference maps are expanded from 4 to 8 bits per color (RGB range of 4-19 expanded to 0-255).

**Fig. S7.** Hierarchical cluster analysis for seven heavy-metal ions at equal concentrations (50 μM) and a control. All experiments were run in quintuplicate; no confusions or errors in classification were observed in 40 trials.
Table S1. The Chinese wastewater discharge standard concentration for eight anions. [2]

<table>
<thead>
<tr>
<th>Anions</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻</td>
<td>$5.60 \times 10^{-4}$</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>$7.05 \times 10^{-3}$</td>
</tr>
<tr>
<td>Br⁻</td>
<td>$5.00 \times 10^{-5}$</td>
</tr>
<tr>
<td>I⁻</td>
<td>$5.00 \times 10^{-5}$</td>
</tr>
<tr>
<td>S²⁻</td>
<td>$3.00 \times 10^{-5}$</td>
</tr>
<tr>
<td>CrO₄²⁻</td>
<td>$2.88 \times 10^{-5}$</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>$1.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>C₂O₄²⁻</td>
<td>$5.00 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

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|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|