Increasing the Selectivity of Optical Anion Sensors with Cationic Extracting Agents

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Anions play an important role in our life, from storing our genetic code on the polyanion DNA, to being the active ingredient in agricultural fertilizers and other industrial processes. Consequently, chemists have been designing systems that can sense anionic species through a variety of methods, such as unimolecular chromophores or sensor arrays. Nonetheless, most existing sensing approaches still have some drawbacks, particularly related to obtaining adequate selectivity and achieving sensing of anions in aqueous environments. In this manuscript, we report a liquid-liquid extraction (LLE)-based sensing approach that allows the conversion of non-selective optical anion sensors that only work in organic media, into selective sensing systems that allow detection of anions in water. We tested this approach on deprotonation-based anion sensors (alizarin, naphthol AS, 4-nitrophenol, BI-Lawsone, and chromophore 1) and hydrogen bonding-based anion sensors (1,2-diaminoanthraquinone and 4-nitro-1,2-phenylenediamine). In general, the deprotonation-based sensors could be converted from a non-selective sensor for basic anions (NCO^-, $H_2PO_4^-$, AcO^- and F^-) to a selective sensing system for NCO^- with the aid of carefully chosen tetraalkylammonium salts as extracting agents. On the other hand, the hydrogen-bonding based sensors could be converted to a selective sensing system for the hydrophobic anion ClO_4^- using similar tetraalkylammonium salts.

Keywords: Anion sensing, colorimetric detection, liquid-liquid extraction, sensing selectivity

Introduction

An estimated two thirds of all enzymes have anionic substrates or require anionic co-factors,[1] and some major environmental pollutants are also anionic species (e.g. arsenate,[2] nitrate,[3] fluoride,[4] perfluorooctanoic acid,[5] perchlorate[6]). It therefore comes as no surprise that supramolecular chemists have been trying to develop sensors that can monitor the presence and quantity of a variety of anionic species during the last 50 years.[7-13] Optical sensors, where binding of the analyte leads to a color change or a change in fluorescence, require no equipment or only cheap routine spectrometers and are therefore one of the most preferred type of sensor.[14] A good sensor should be selective (detect only a particular analyte), potent (detect the analyte in a wide range of concentrations), work in suitable media (usually water), and be inexpensive. Supramolecular chemists have tried to achieve these characteristics through chemical modifications of the sensor, or by using a combination of sensors and statistical analysis (multi-sensor arrays, artificial tongues).[15, 16] However, the first method can be very time-consuming with little chance of success, while the latter falls short when large amounts of competing background analytes are present.[15] Our group has therefore been trying to develop new approaches that can accelerate the discovery of new sensors with high selectivity towards a particular anion.

We have previously reported on a method to turn a non-selective, water-soluble anion sensor (lucigenin) into a selective sensor for iodide using our TLF approach (transporter-liposome-fluorophore).[17] In the TLF approach, a non-selective anion sensor is encapsulated into liposomes to protect it from possible interfering anions, and a synthetic anion transporter is added to the system. When an analyte is added, the TLF system will only give an optical response when the analyte is both transported into the liposomes *and* induces a change in fluorescence in the encapsulated fluorophore. This is essentially a type of orthogonal sensing, where the combination of a non-selective fluorophore and a transporter with orthogonal selectivity leads to a system with high selectivity. However, the TLF approach is only suitable for water-soluble fluorophores, and most reported optical anion sensors function in organic solvents. In this manuscript, we therefore wanted to develop a new approach where liquid-liquid extraction (LLE) is used instead of transmembrane transport to increase the selectivity of optical anion sensors.

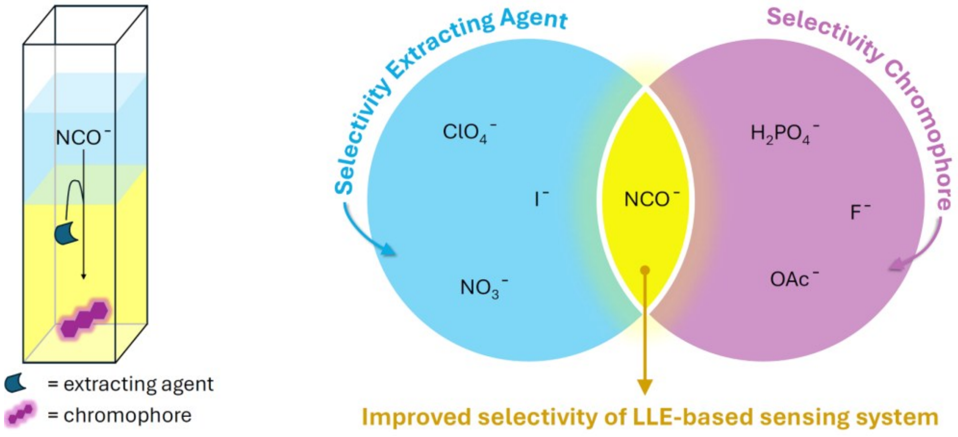


Figure 1. Schematic of the LLE-based sensing approach to increase anion selectivity. In this case, a non-selective chromophore that can detect basic anions (NCO^-, $H_2PO_4^-$, AcO^- and F^-) is combined with a non-selective extracting agent with preference for more hydrophobic anions (NO_3^-, I^-, ClO_4^- and NCO^-). The combined system becomes selective for detecting NCO^- in water, because this anion can be extracted *and* induces an optical change. Other interfering anions might be extracted but do not induce an optical change, or are not extracted at all.

For this LLE-based sensing approach, a fluorophore or chromophore is dissolved into an organic solvent, along with an extracting agent or ionophore that can transfer the desired anion into the organic layer upon the addition of an aqueous sample (Figure 1). This is again a type of orthogonal sensing, using the combination of a partially selective chromophore or fluorophore with an orthogonally selective extracting agent. In this case, only those anions that are both extracted into the organic layer *and* create an optical response in the fluorophore/chromophore are sensed by the LLE-based sensing system (Figure 1). Because neither the fluorophore/chromophore nor the extracting agent need to be highly selective, the development of selective sensors can be accelerated through the facile screening of various combinations of simple, unselective fluorophores/chromophores and extracting agents. Furthermore, this LLE-based sensing approach has the advantage that it provides a solution for both the selectivity *and* solubility problem of optical anion sensors.

While extraction mechanisms have been previously used in optical anion sensing, these systems invariably rely solely on the selectivity of either the extracting agent or the fluorophore/chromophore. For example, liquid-liquid extraction is sometimes used to allow sensing of anions in aqueous environments by fluorophores or chromophores that are not water soluble. In most cases, the extracting agent focuses on the counter cation (e.g. crown ether to extract Na^+ or K^+ ions) to generate a 'naked' anion.[18] This implies that the anion selectivity of the sensing system relies entirely on the selectivity of the fluorophore/chromophore, and not on the extracting agent. Alternatively, anion-selective optodes are sensing systems that involve the immobilization of a fluorophore/chromophore into a hydrophobic matrix (usually a polymer membrane or nanoparticle), combined with ionophores that can extract the ion from an aqueous solution into the organic polymer.[19-26] So far, there are few examples of optode-like systems that uses liquid-liquid extraction, and they have only been demonstrated for the detection of cations.[27, 28] For anion sensing, most optodes employ a pH-dependent dye as the fluorophore/chromophore, which relies on the co-extraction of H^+ along with the anion of interest (or sometimes a solvatochromic dye). To generate highly selective optodes it is therefore necessary to develop highly selective ionophores that can selectively extract one type of anion. In contrast, in the LLE-based sensing system discussed in this manuscript the overall selectivity depends on the partial orthogonal selectivity of both the extracting agent and fluorophore/chromophore. It is therefore more closely related to the three-component spectroelectrochemical sensors by Heineman and others,[29-36] which also use multiple selectivity filters. These sensors consist of an optically transparent electrode (OTE) coated with a polymer film, so that the device becomes selective for analytes that can partition into the polymer film, can undergo oxidizations or reduction at the correct potential, *and* have the correct optical properties in their reduced or oxidized form. Our LLE-based systems are simpler than these electrodes, can be made from materials and equipment that are present in every organic chemistry lab, and have the advantage that they can work for analytes that do not have reversible electrochemistry. We therefore envision that the development of highly selective optical anion sensors can be accelerated by our LLE-based approach.

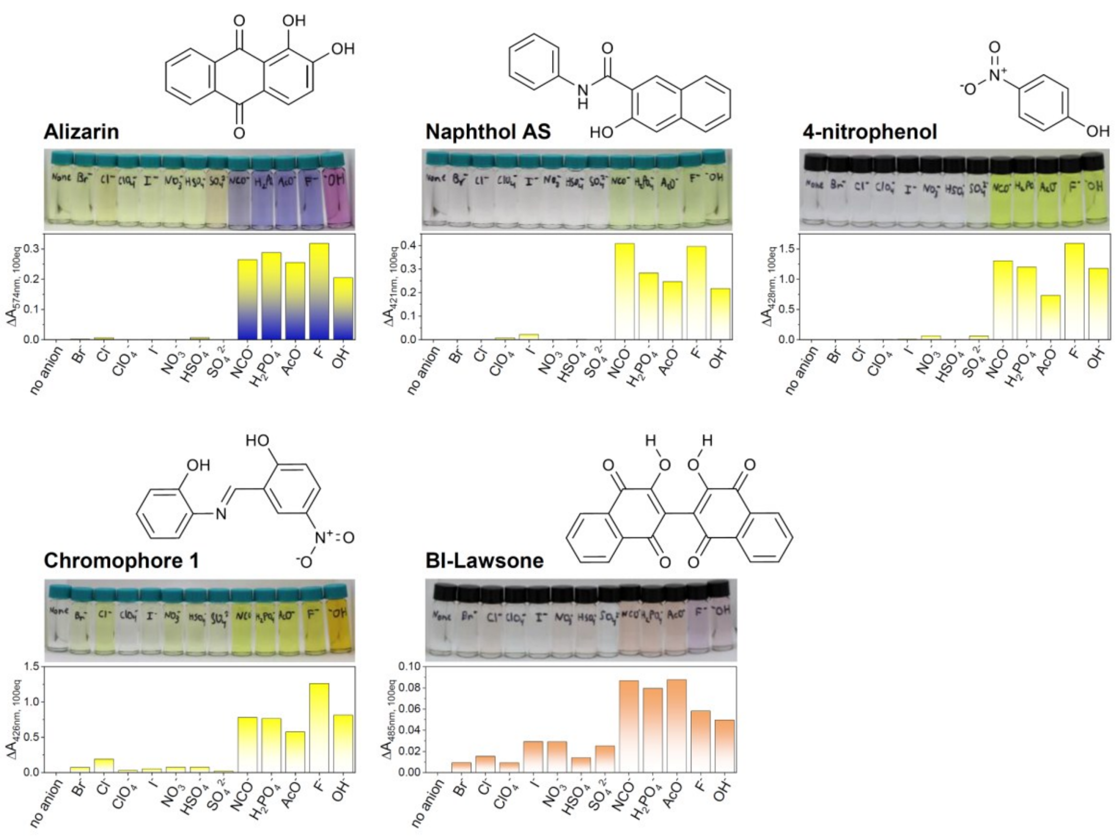
Results and Discussion

Choice of Chromophores

To provide proof-of-principle that liquid-liquid extraction can increase the selectivity of optical anion sensors, we needed compounds that have previously been shown to function as unselective anion sensors in solvents that are not miscible with water (e.g., dichloromethane). Furthermore, the aim is to show that selectivity can be achieved with very simple building blocks, and we were therefore interested in chemosensors that are commercially available or can be obtained in high-yielding 1-step syntheses. We took inspiration from a publication from Miyaji and Sessler that reported on off-the-shelf chromophores as anion sensors,[18] and picked those chromophores that functioned in dichloromethane: alizarin, naphthol AS, 4-nitrophenol, BI-Lawsone, 1,2-diaminoanthraquinone and 4-nitro-1,2-phenylenediamine (Figure 2). We also added a Schiff-base chromophore that had previously been shown to sense F^-, AcO^- and $H_2PO_4^-$ in organic solvents, and which can be prepared through a 1-step synthesis (chromophore 1, Figure 2).[37, 38]

To determine the inherent anion selectivity of the chromophores in organic solvents, we performed a series of UV-Vis titrations with a variety of anions as their tetrabutylammonium (TBA) salt. In general, a new red-shifted peak appeared upon interaction with anions. The increase in absorbance of this new peak in the presence of 100 equivalents of anion is given in the bar graphs of Figure 2, alongside photographs of the resulting color change (the full titration results can be found in the Supplementary Information). For all chromophores, the largest effect is seen for the most basic anions: NCO^-, $H_2PO_4^-$, AcO^- and F^- (as well as OH^-) (see Table 1 for the pK_a values of the anions).[39, 40] For the remainder of this manuscript, we focused our experiments on these four anions, with the addition of ClO_4^- as a control anion that does not induce a color change. Some of the chromophores, mainly 1,2-DAAQ and 4-NPA, also show a small color change with less basic anions such as Cl^- and Br^- (Figure 2). This is probably due to different mechanisms of sensing. The color shift seen for the phenolic compounds alizarin, naphthol AS, 4-nitrophenol, chromophore 1 and BI-Lawsone, is most likely due to the deprotonation of the OH functionality by the basic anions. This was confirmed by comparing the anion titrations with titrations performed with OH^- (as the TBA^+ salt) as a strong base. For these five chromophores, the new red-shifted peak that appears upon the addition of certain anions corresponds to the peak of the single deprotonated species. In the case of alizarin, chromophore 1 and BI-Lawsone two

Deprotonation-based anion sensors



Hydrogen bonding-based anion sensors

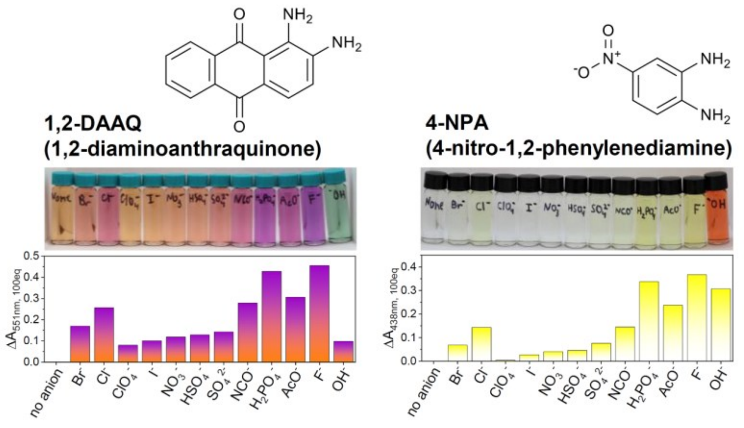


Figure 2. Structures of chromophores that function as non-selective anion sensors in dichloromethane *via* a deprotonation mechanism (top) or *via* hydrogen bonding to the anion (bottom). The photographs and bar charts show the change in color/absorbance induced by the addition of 100 equivalents of TBA salt in a dichloromethane solution of the chromophore. In each case, from left to right: no anion, Br^{-}, Cl^{-}, ClO_4^{-}, I^{-}, NO_3^{-}, HSO_4^{-}, SO_4^{2-}, NCO^{-}, $H_2PO_4^{-}$, AcO^{-}, F^{-} and OH^{-}.

deprotonations are possible, but this was not observed for any of the anions except OH^{-} (and sometimes F^{-}). Smaller shifts due to hydrogen bonding were sometimes seen for other anions as well, but these shifts were not responsible for the observed color change of these 5 anion sensors. In contrast, the amine-based sensors 1,2-DAAQ and 4-NPA did not show a red-shifted peak corresponding to deprotonation, indicating that the observed color changes are due to hydrogen bonding with the anions. As a result, these two chromophores show optical changes with a larger variety of anions than the deprotonation-based anion sensors (Figure 2). As a proof-of-principle, we aimed to show that our LLE-based sensing approach is compatible with any type of anion sensor and we therefore kept all 7 chromophores for further studies.

Choice of Extracting Agent

The chromophores selected allow naked-eye sensing of a variety of anions in dichloromethane. However, to be practically useful it is important that these optical sensors are selective and can sense anions in aqueous environments. To achieve this, we envision LLE-based systems where the chromophores in dichloromethane can sense anions in water with the help of an extracting agent. By carefully optimizing the extracting agent, it should be possible to also fine-tune the selectivity of the sensor. There are many extracting agents that could achieve this role, but for this proof-of-principle study we wanted to keep them as simple as possible. We therefore opted for various alkyl ammonium salts as the extracting agents, due to their low cost and easy availability. For these extracting agents we expect a simple anion exchange mechanism between the anion in the aqueous layer and the anion of the alkylammonium salt, and we do not expect selectivity beyond the lipophilicity of the anions (Hoffmeister bias). There are four anions that cause the most pronounced optical change in the chosen chromophores: NCO^-, $H_2PO_4^-$, AcO^- and F^- (see Figure 2). In theory, it should be possible to narrow the selectivity to the anion that is the easiest to extract, i.e. the most 'hydrophobic' anion. The hydrophobicity of an anion can be quantified by the Gibbs free energy of hydration (ΔG_{hyd}),[41] whereby more negative values indicate that the anion is more hydrophilic (less hydrophobic). Table 2 shows the ΔG_{hyd} of the anions tested in the UV-Vis titrations. Based on these numbers, NCO^- and AcO^- are more hydrophobic (and should therefore be easier to extract into an organic solvents) than $H_2PO_4^-$ and F^-. To increase the anion selectivity of our sensors, we thus need to find an extracting agent that can efficiently extract NCO^- (and AcO^-), without extracting $H_2PO_4^-$ and F^-. There are different ways in which the extraction efficiency of alkyl ammonium salts can be fine-tuned in order to find the extracting agent / chromophore combination that gives selective sensing in an LLE-based sensing system: (1) changing the anion of the alkyl ammonium salt, (2) changing the lipophilicity of the alkyl ammonium cation, or (3) changing the amount of alkyl ammonium extracting agent. All three of these potential optimization routes will be explored in the sections below.

Table 1. List of ΔG_{hyd} and pK_a values for the various anions studied in this manuscript.

Anion	ΔG_{hyd} at 25 °C (kJ/mol)[a]	pK_a[b]
ClO_4^-	-229	-2.4 to -3.1
I^-	-280	-9
NO_3^-	-286	-1.27
Br^-	-318	-8
Cl^-	-344	-6.1
NCO^-	-372	3.46
AcO^-	-374	4.76

F^-	-469	3.18
$H_2PO_4^-$	-473	2.15 (p$K_{a,1}$)
SO_4^{2-}	-975	-3 (p$K_{a,1}$), 1.96 (p$K_{a,2}$)

[a] values taken from reference 41. [b] pK_a values for the conjugate acid of each anion taken from references 39 and 40.

Increasing the selectivity of anion sensors by optimizing the counteranion of alkyl ammonium salts.

For our initial extraction studies, we chose tetrabutylammonium (TBA^+) salts as the extracting agents. They are commonly used as extracting agents or phase-transfer catalysts,[42-45] and many different types of TBA^+ salts are commercially available. We decided to use 10 equivalents of these extracting agents compared to chromophore, because UV-Vis titrations had shown that 10 equivalents is sufficient to induce a color change in most of the chromophores (see ‘choice of chromophores’). The extraction-based sensing experiments were thus performed by mixing 2 mL of a dichloromethane solution (containing the chromophore and 10 equivalents extracting agent) with 1 mL of an aqueous solution containing the sodium salts of the anions that commonly induce a color change (i.e., NaF, NaNCO, NaOAc, and NaH_2PO_4). An aqueous solution of $NaClO_4$ was also tested as a control experiment with an anion that does not induce a color change. Our initial hypothesis was that if ΔG_{hyd} of the counteranion of the extracting agent is similar or more negative than ΔG_{hyd} of the anionic analyte in the aqueous layer, an anion exchange would occur, and the analyte would be extracted from the aqueous layer to the organic layer.

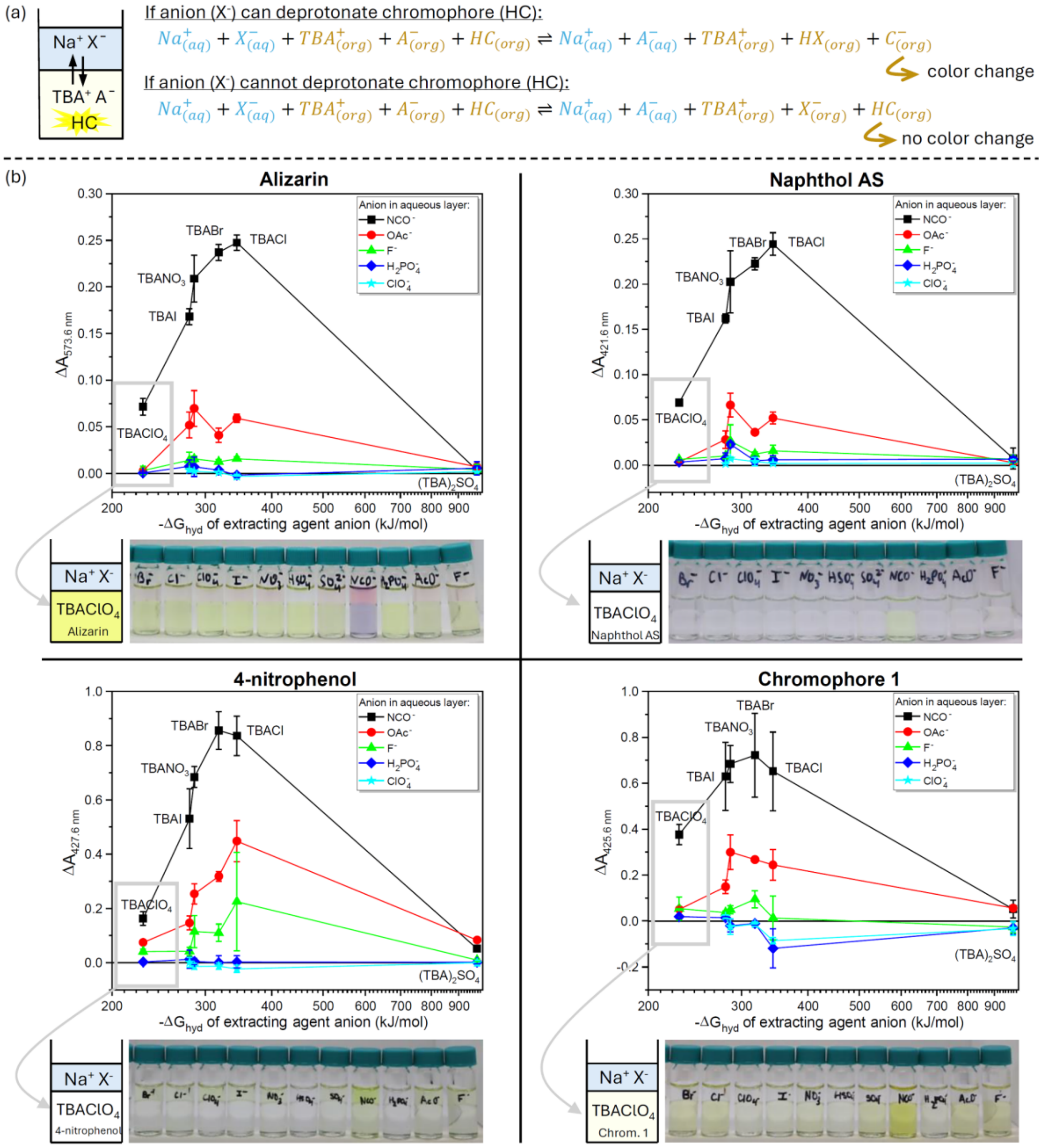


Figure 3. Selective sensing of aqueous NCO^- by deprotonation-based anion sensors and TBA^+ extracting agents. (a) Mechanism of LLE-based sensing systems using deprotonation-based anion sensors. The aqueous layer originally contains a sodium salt (Na^+X^-), and the organic layer contains an extracting agent (TBA^+A^-) and a deprotonatable chromophore (HC). Sensing depends on anion exchange between X^- and A^- and whether X^- can deprotonate the chromophore, as given by the equations. The position of the equilibrium is expected to depend on the difference in ΔG_{hyd} between X^- and A^-. (b) Results of the extraction experiments for alizarin, naphthol AS, 4-nitrophenol and chromophore 1. The graphs show the change in absorbance upon extracting an aqueous layer containing 10 mM sodium salt (NaOCN, NaOAc, NaF,

NaH_2PO_4 or $NaClO_4$) with a dichloromethane solution containing the chromophore (50 μM alizarin, 100 μM naphthol AS, 50 μM 4-nitrophenol, or 50 μM chromophore 1) and 10 equivalents TBA^+ salt. The x-axis is the $-\Delta G_{hyd}$ of the counteranion of the TBA^+ salt. The photographs show the result of the most selective LLE-based system for each [45]chromophore, consisting of a dichloromethane solution of chromophore and 10 equivalents $TBAClO_4$. For the photographs, extractions were performed with aqueous solutions containing 100 mM of the following Na^+ salts (from left to right): Br^-, Cl^-, ClO_4^-, I^-, NO_3^-, HSO_4^-, SO_4^{2-}, NCO^-, $H_2PO_4^-$, AcO^-, and F^-.

Deprotonation-based Sensors.

This hypothesis seemed to be valid for most of the deprotonation-based sensors, as shown in Figure 3. Figure 3a shows the equilibria present in the system, and Figure 3b shows the increase in absorbance of the new red-shifted peak that occurs upon deprotonation of the chromophore (y-axis). A value above zero indicates that extraction occurred and led to a color change. The results are plotted against the $-\Delta G_{hyd}$ of the counteranion of the extracting agent (x-axis), in order to determine the best extracting agent. As expected, the extracting agent with the most hydrophobic counteranion ($TBAClO_4$) often resulted in the least efficient extraction (a hydrophobic anion would prefer to remain in the hydrophobic organic layer, instead of exchanging into the aqueous layer). In fact, this extracting agent was only able to extract NCO^- and can therefore be used to develop selective sensing systems for this anion in aqueous solutions. This can be best seen from the photographs in Figure 3b, which show the result of mixing an aqueous solution containing different sodium salts with an organic solution containing a chromophore and 10 equivalents $TBAClO_4$. Only aqueous solutions of NaOCN are able to induce a color change under these conditions. The best results are seen for alizarin and naphthol AS, while 4-nitrophenol and chromophore 1 still had some minor color changes induced by other anions.

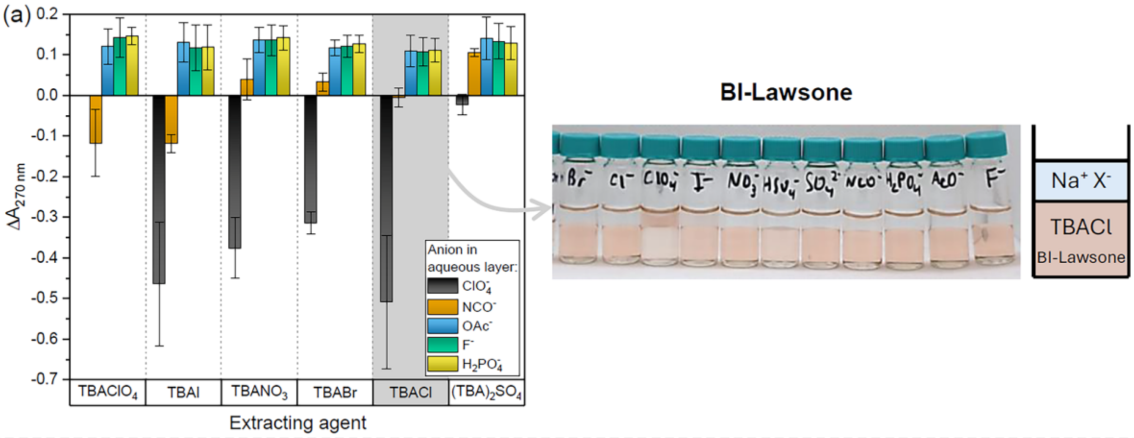
Figure 3b also reveals that as the counteranion of the TBA^+ extracting agent becomes more hydrophilic (larger $-\Delta G_{hyd}$), extraction becomes more efficient. However, the increased extraction efficiency also leads to a loss of selectivity and for most extracting agents color changes were observed with NCO^-, AcO^- and sometimes F^- (extraction of $H_2PO_4^-$ was never observed). The highest extraction efficiency (and lowest selectivity) was generally observed for TBABr and TBACl as the extracting agent. Interestingly, the most hydrophilic extracting agent (TBA_2SO_4) was not able to facilitate any extraction or color change. We assume that this extracting agent is so hydrophilic that both TBA^+ and SO_4^{2-} migrate to the aqueous layer, without any extraction of the original anion in the aqueous layer. This is corroborated by the fact that TBA_2SO_4 is highly hygroscopic and is only available commercially as an aqueous solution. In summary, most of the deprotonation-based sensors could be converted from a non-selective sensor for basic anions (NCO^-, $H_2PO_4^-$, AcO^- and F^-) to a selective sensor for NCO^- with the help of $TBAClO_4$ as extracting agent. It is important to note that when the aqueous layer contained ClO_4^-, a color change was never observed. ClO_4^-, which is a very hydrophobic anion (see Table 1), is most likely extracted into the organic layer but it is not capable of inducing a color change in the chromophores. This underlines the central concept of LLE-based sensing, where only those anions that are both extracted *and* cause a color change in the chromophores, are detected by the system.

BI-Lawsone.

Out of all the deprotonation-based sensors, BI-Lawsone was the only one that did not follow the general trend mentioned above. Instead, it was observed that the aqueous layer often would turn pink (the color of deprotonated BI-Lawsone), indicating that BI-

Lawsone is extracted into the aqueous layer rather than the analyte being extracted into the organic layer. In some cases, this migration of BI-Lawsone into the aqueous layer was so efficient that the organic layer became colorless and the UV-Vis spectrum of the organic layer showed no signal (see Supplementary Information). Because this did not happen for all extracting agents, we wondered whether we could use this loss of BI-Lawsone to achieve selective anion sensing. We therefore plotted the change in absorbance at 270 nm upon mixing BI-Lawsone solutions with various aqueous solutions, against the hydrophobicity of the extracting agent in the organic layer (Figure 4a). A negative value indicates that BI-Lawsone migrated to the aqueous layer. When the aqueous layer contained the basic anions that normally induce a color change (NCO^-, $H_2PO_4^-$, AcO^- and F^-), no migration of BI-Lawsone occurred. However, when the aqueous layer contained $NaClO_4$, efficient migration of BI-Lawsone to the aqueous layer was observed (Figure 4a). When TBACl was used as the extracting agent in the organic layer, selective sensing of ClO_4^- could be achieved (see Figure 4a). This is somewhat reminiscent of optodes that use sovatochromic dyes for sensing,[46-50] although in this case it is not pure solvatochromic sensing but involves protonation and deprotonation of BI-Lawsone.

Calculations using ChemAxon's online Chemicalize software suggest that BI-Lawsone has pK_a values of 4.46 and 6.63, with a charge of -1.8 at pH 7. These values are much lower than for the other deprotonation-based sensors and explain the anomalous behavior of BI-Lawsone (see Supplementary Information). When an organic solution of BI-Lawsone is mixed with an aqueous layer, the low pK_a values of this chromophore indicate that it will be deprotonated regardless of the anion in the aqueous layer. As a result, BI-Lawsone will be anionic and ion exchange can happen between the anion originally in the aqueous layer and the anionic BI-Lawsone. Interestingly, this exchange only happens in the case of the most hydrophobic anions in the aqueous layer, such as ClO_4^-, NO_3^- and I^-. This suggests that anionic BI-Lawsone is more hydrophilic than these anions and will migrate to the aqueous layer, yet is more hydrophobic than anions with a more negative ΔG_{hyd}, such as NCO^-, $H_2PO_4^-$, AcO^- and F^-. However, this view is a little bit too simplistic, since there are actually 3 anionic species that need to equilibrate between the aqueous and organic layer: the anionic species originally in the aqueous layer (the analyte), the counteranion of the TBA^+ extracting agent, and anionic BI-Lawsone (see Figure 4b). The complex interplay between the partitioning of all 3 anionic species will determine if BI-Lawsone will migrate to the aqueous layer, and this explains why selective sensing of ClO_4^- is only observed when TBACl is present in the organic layer.



(b)

$$Na^+_{(aq)} + X^-_{(aq)} + H_2O_{(aq)} + TBA^+_{(org)} + A^-_{(org)} + HC_{(org)} \rightleftharpoons$$

$$Na^+_{(aq)} + C^-_{(aq/org)} + H_3O^+_{(aq)} + TBA^+_{(org)} + X^-_{(org/aq)} + A^-_{(org/aq)}$$

color change in aqueous layer

Figure 4. Selective sensing of aqueous ClO_4^- by BI-Lawsone and TBA^+ extracting agents in dichloromethane. (a) The bar graph shows the change in absorbance upon extracting an aqueous layer containing 10 mM sodium salt (NaOCN, NaOAc, NaF, NaH_2PO_4 or $NaClO_4$) with a dichloromethane solution containing 20 µM BI-Lawsone and 10 equivalents TBA^+ salt. The photograph corresponds to the best LLE-based

system with TBACl as the extracting agent. (b) Putative equilibrium for the LLE-based sensing system with BI-Lawsone. The aqueous layer originally contains a sodium salt ($Na^+X^−$), and the organic layer contains an extracting agent ($TBA^+A^−$) and BI-Lawsone (HC). Upon mixing the layers, BI-Lawsone will be deprotonated by water and the resulting anion ($C^−$), as well as $X^−$ and $A^−$, will equilibrate between the organic and aqueous layers.

Hydrogen Bonding-based Sensors.

The hydrogen bonding-based anion sensors 1,2-DAAQ and 4-NPA also did not follow the same trend as the deprotonation-based sensors. When the same conditions were used as for the deprotonation-based sensors (10 equivalents of different TBA^+ salts), no color change was ever observed. However, this is not surprising because 10 equivalents of TBA^+ salt only produced a color change in the case of $F^−$ (see 'UV-Vis titrations in 'choice of chromophore'), and $F^−$ is difficult to extract (see $F^−$ extraction for the deprotonation based sensors – Figure 3). In order to use LLE-based sensing for hydrogen bonding chromophores, the extraction efficiency needs to be increased. To achieve this, we used more lipophilic extracting agents (tetrahexylammonium (THA^+) salts) at higher concentrations (100 equivalents compared to the chromophore). We also increased the concentration of the analyte in the aqueous layer from 10 mM to 100 mM. However, these new conditions posed a new challenge. Many of the THA^+ extracting agents already induced a color change themselves at 100 equivalents. Consequently, this color could either be lost if the anion extracted from the aqueous layer does not induce a color change, or could increase if the anion extracted from the aqueous layer induces a stronger color change (Figure 5a). These changes can be hard to observe with the naked eye, but can be easily detected with a spectrophotometer. Figure 5b shows the change in absorbance upon mixing the organic solutions of 1,2-DAAQ or 4-NPA containing

THA^+ extracting agents with various aqueous solutions. A positive value means an anion was extracted from the aqueous layer and induced a more pronounced color change than the original THA^+ extracting agent, whereas a negative value indicates that an anion was extracted from the aqueous layer that induces a less pronounced color change than the original THA^+ extracting agent. Values close to zero indicate that no extraction occurred. Figure 5b indicates that the hydrogen-bonding based anion sensors can be converted to a selective sensor for ClO_4^- using the LLE-based sensing system. ClO_4^- is the only anion that produces a strong negative change in absorbance (especially in the case of $THAHSO_4$ as the extracting agent), while the other anions either give no change or a positive change in absorbance. The reason that ClO_4^- is the anion that can be selectively sensed is due to its high hydrophobicity (making it easy to extract), combined with its low basicity and low propensity to form hydrogen bonds[51] (resulting in a low propensity to cause optical changes in anion sensors). These results indicate that it is possible to change both deprotonation-based and hydrogen bonding-based anion sensors from non-selective anion sensors to selective anion sensors by carefully optimizing the conditions of LLE-based sensing systems.

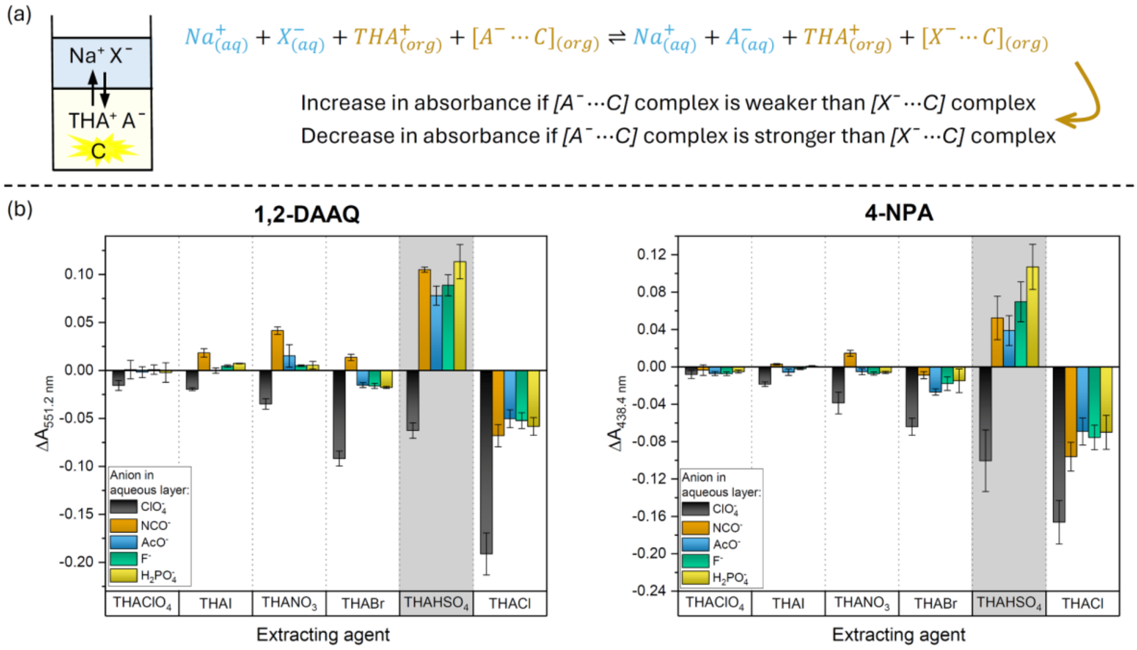


Figure 5. Selective sensing of aqueous ClO_4^- by hydrogen bonding-based sensors and THA^+ extracting agents in dichloromethane. (a) Putative equilibria present. The aqueous layer originally contains a sodium salt (Na^+X^-), and the organic layer contains an extracting agent (THA^+A^-) and a hydrogen-bonding based chromophore (C), which will be complexed with the anion of the extracting agent. Upon mixing the layers, anion exchange can occur between X^- and A^-, and the chromophore will be complexed to the new anion. The position of the equilibrium is expected to depend on the difference in ΔG_{hyd} between X^- and A^-, and the difference in association constant between the hydrogen bond complexes with the chromophore. (b) The bar graphs show the change in absorbance upon extracting an aqueous layer containing 100 mM sodium salt (NaOCN, NaOAc, NaF, NaH_2PO_4 or $NaClO_4$) with a dichloromethane solution containing 50 µM 1,2-DAAQ or 4-NPA and 100 equivalents THA^+ salt.

Increasing the selectivity of anion sensors by optimizing the lipophilicity of the alkylammonium cation.

The equilibrium of the extraction process, and thus the sensing selectivity, could also be altered by changing the cation of the extracting agent. To illustrate this, we used alizarin as an example because it gives a yellow-to-purple color change that is easily detectable by the naked eye and because it was one of the best-behaved chromophores in the counteranion study discussed above (Figure 3). As extracting agent, we chose alkylammonium bromide salts of different chain length, because TBA bromide salts consistently gave one of the highest extraction efficiencies in the counteranion study (Figure 3). By altering the alkyl chain length, it is possible to affect the lipophilicity of the cationic center, which should affect extraction efficiency. To help interpretation, we calculated the log*P* values of the cationic center using ChemDraw 19.1.0.8 (Clog*P*). Figure 6a shows the change in absorbance upon mixing organic solutions containing alizarin and various alkylammonium bromide salts with aqueous solutions containing $NaClO_4$, NaF, NaOCN, NaOAc or NaH_2PO_4. Extraction efficiency increases as the lipophilicity of the cation increases (due to longer chain lengths), which leads to an increase in absorbance. However, this increase is noticeable for all anions and the highly lipophilic extracting agents, such as tetraoctylammonium bromide (TOABr), show a color change with NCO^-, AcO^-, F^- and even $H_2PO_4^-$. Figure 6a also clearly shows that the easiest anion to extract is NCO^-, followed by $AcO^- > F^- > H_2PO_4^-$, which agrees with the ΔG_{hyd} of these anions (Table 1). As a result, by lowering the lipophilicity of the cationic center, alizarin can be converted to a selective sensor for NCO^- when tetrapropylammonium bromide (TPrABr) is used as extracting agent (Figure 6). Note again that ClO_4^- never causes a change in absorbance, even though it is a highly lipophilic anion and therefore easier to extract than NCO^-. This is because ClO_4^- cannot deprotonate alizarin and the LLE-based sensing system will only detect anions that are both extracted and cause an optical change.

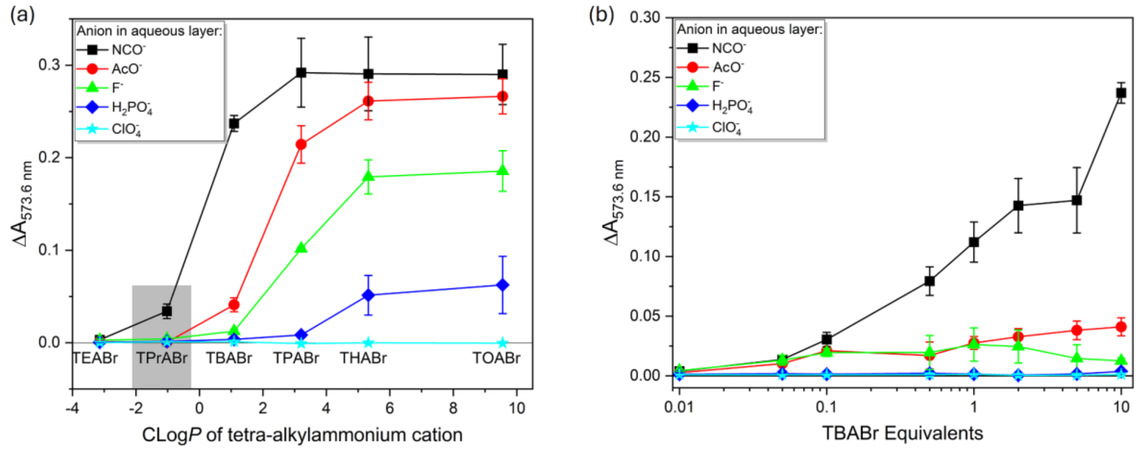


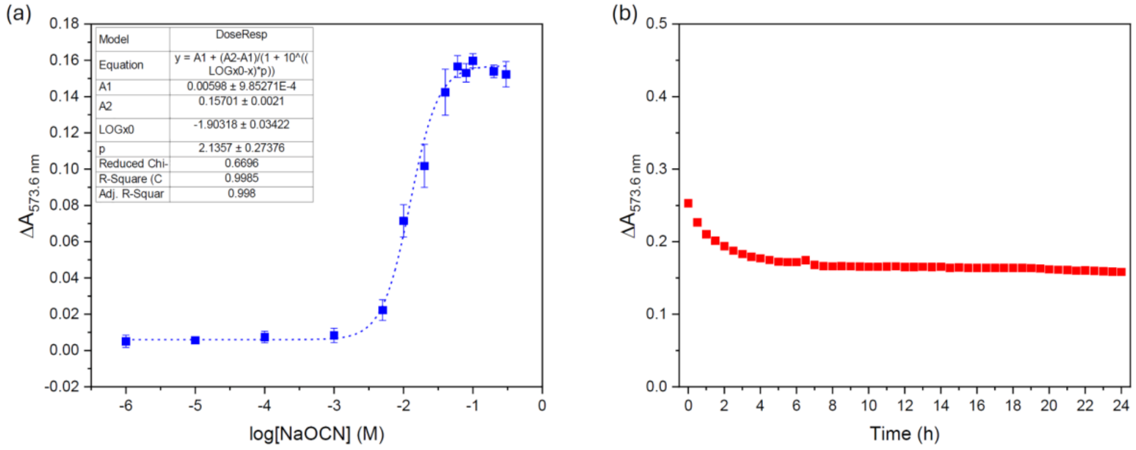
Figure 6. (a) Change in absorbance of alizarin (573.6 nm) upon mixing an aqueous layer containing 100 mM sodium salt (NaOCN, NaOAc, NaF, NaH_2PO_4 or $NaClO_4$) with a dichloromethane solution containing 50 µM alizarin and 100 equivalents tetraethylammonium bromide (TEABr), tetrapropylammonium bromide (TPrABr), tetrabutylammonium bromide (TBABr), tetrapentylammonium bromide (TPABr), tetrahexylammonium bromide (THABr), or tetraoctylammonium bromide (TOABr). The gray box highlights selective sensing of NCO^- using TPrABr as extracting agent. (b) Change in absorbance of alizarin (573.6 nm) upon mixing an aqueous layer containing 100 mM sodium salt (NaOCN, NaOAc, NaF, NaH_2PO_4 or $NaClO_4$) with a dichloromethane solution containing 50 µM alizarin and various equivalents of tetrabutylammonium bromide (TBABr).

Increasing the selectivity of anion sensors by optimizing the amount of extracting agent.

So far, we were able to show that non-selective anion sensors can be converted to selective sensors by optimizing the anion extraction efficiency through variations of the anionic or cationic center of the extracting agent. In theory, extraction efficiency should also be dependent on the amount of extracting agent. Once again, we used alizarin combined with TBABr as an example. Figure 6b shows the change in absorbance upon mixing organic solutions containing alizarin and different equivalents of TBABr with aqueous solutions containing $NaClO_4$, NaF, NaOCN, NaOAc or NaH_2PO_4. The change in absorbance decreases as the amount of TBABr is decreased. However, the magnitude of the decrease is not the same for all anions and as a result there is no single point where selective sensing of NCO^- can be achieved. Altering the anion or cation of the extracting agent is therefore a better way of optimizing the selectivity of anion sensors than altering the amount of extracting agent.

Dynamic Range and Response Time.

To establish the usefulness of the LLE-based sensing approach, we determined the dynamic range and response time. For the dynamic range, we used the system consisting of alizarin and 10 equivalents $TBAClO_4$ as extracting agent, because this system gave a pronounced and selective color change when used to extract a NaNCO solution (Figure 3). We measured the change in absorbance of the organic solution of alizarin and $TBAClO_4$ upon mixing with aqueous solutions of varying concentrations of NaNCO. The results are shown in Figure 7a. A sigmoidal relationship is observed between the change in absorbance and the logarithm of cyanate concentration, with the biggest change in absorbance observed in the range of 1 mM to 60 mM [NaOCN]. Such sigmoidal calibration curve is common for other extraction-based sensing systems such as optodes and ion selective electrodes, and does not hinder the usefulness of our LLE-based sensing approach.[52] However, optodes are often limited by slow response times, because there are multiple equilibria that need to be established. To investigate the response time and equilibration time of our LLE-based systems, we vortexed a biphasic mixture of an aqueous solution containing 10 mM NaOCN and a dichloromethane solution of alizarin and 10 equivalents TBABr, and then measured the change in absorbance of the dichloromethane layer over time (Figure 7b). Although this confirmed that it can take a few hours for these systems to equilibrate, the largest change in absorbance is obtained immediately after vortexing and phase-separating. Throughout this manuscript, we therefore measured the change in absorbance immediately after vortexing the aqueous and organic layers, because this is the most practical approach for sensing and because it gave repeatable results and satisfying calibration curves. We therefore do not think that the slow equilibration time is a limiting factor for LLE-based anion sensing systems.



Model	DoseResp
Equation	y = A1 + (A2-A1)/(1 + 10^((LOGx0-x)*p))
A1	0.00598 ± 9.85271E-4
A2	0.15701 ± 0.0021
LOGx0	-1.90318 ± 0.03422
p	2.1357 ± 0.27376
Reduced Chi-	0.6696
R-Square (C	0.9985
Adj. R-Squar	0.998

Figure 7. (a) Change in absorbance of alizarin (573.6 nm) upon mixing an aqueous layer containing various concentrations NaOCN (log[NaOCN]) with a dichloromethane solution containing 50 µM alizarin and 100 equivalents $TBAClO_4$. The data is fitted to a sigmoidal dose-response curve to obtain a calibration curve and estimate the dynamic range. (b) Change in absorbance of alizarin (573.6 nm) at different times after mixing an aqueous layer containing 10 mM NaOCN with a dichloromethane solution containing 50 µM alizarin and 100 equivalents TBABr.

Conclusions

In this work, we have shown how nonselective anion sensors can be made selective by the use of a liquid-liquid extraction system with orthogonally selective extracting agents. As proof-of-principle, we used a series of deprotonation-based and hydrogen-bonding-based anion sensors, combined with tetraalkylammonium salts as extracting agents. Most deprotonation-based sensors could be converted from a non-selective sensor for basic anions, to a selective sensing system that allows the detection of NCO^- in aqueous solutions. In this case, NCO^- is the only anion that is lipophilic enough to be extracted *and* basic enough to deprotonate the anion sensor in the organic layer. On the other hand, BI-Lawsone and the hydrogen-bonding-based anion sensors could be converted from non-selective anion sensors to selective sensing systems for the highly hydrophobic ClO_4^- anion in aqueous solutions. The simplicity of the LLE-based anion sensing approach presented in this manuscript is expected to encourage researchers working on optical anion sensors to try this approach to increase the selectivity of their systems.

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Disclosure Statement

No potential conflict of interest was reported by the author(s).

Data Availability Statement

Electronic supplementary information (ESI) available: materials and methods, full UV-Vis spectra of the UV-Vis titrations and all extraction-based experiments, additional photographs of the extraction experiments, calculated physicochemical properties of the chromophores, and determination of the stability of the chromophores in the presence of $TBAClO_4$.

Full datasets (.csv and .xls files and lab book entries) can be found here:

https://doi.org/10.25833/5pms-hn14

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