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"Naked-Eye" Screening of Metal-Based Chemosensors for Biologically Important Anions

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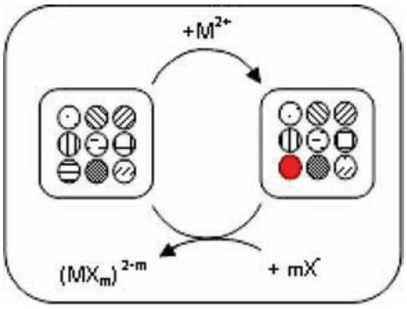
A two-step "naked-eye" screening of selective metal-based chemosensors for biologically important anions such as cyanide, phosphate, and oxalate from commercially available metal indicators and metal ions has been developed. Applications in food safety control have been demonstrated.

The development of selective colorimetric chemosensors for anions is traditionally based on a rational design and involves most often multistep chemical synthesis and the subsequent evaluation of an individual compound.[1] This time-consuming process can be accelerated by screening a library of potential candidates instead of a single compound.[2] Examples following this approach are still rare. They include the hydrogen-bond-assisted detection of inorganic anions with commercially available chromophores[3] and the detection of adenosyltriphosphate (ATP) with a guanidinium-modified hexasubstituted benzene scaffold.[4] Alternatively, nonselective interactions between an analyte and various differential receptors may lead to a characteristic diagnostic pattern that is useful for the differentiation of structurally related analytes as well as the analysis of complex mixtures.[5−7]

Pattern-based recognition using metal-containing indicator displacement assays and metal-based dynamic combinatorial libraries have been applied for the identification of carboxylic acids, amino acids, and dipeptides.[6,8,9] In contrast, a "naked-eye" screening of *selective* colorimetric anion chemosensors from mixtures of commercially available indicators and metal ions has not been reported so far.

Herein, we describe such a two-step experiment for the identification of novel metal-based chemosensors for biologically important anions. In this approach (Scheme 1), metal indicators were mixed at different pH values with metal ions having a high affinity toward the desired analyte. Metal-indicator complexes with a color distinct from the corresponding metal-free indicator were selected afterward. In the second round, these metal-indicator complexes were screened against different anions including the desired analyte. Metal-indicator complexes showing a color change due to an anion-induced decomplexation to the metal-free indicator were selected for further sensitivity as well as selectivity studies. First of all, we decided to test this approach for the selection of chemosensors of cyanide.[11−18] One of the most popular strategies for the optical detection of cyanide is based on the decomplexation of copper(II) complexes[12,19,20] including the

Scheme 1. Schematic Overview of the Optical Screening Procedure of Chemosensors from Mixtures of Commercially Available Indicators (left) and Metal Ions (M^{2+})[a]



[a] The first step includes the selection of metal-indicator complexes (red dot) with a color distinct from the corresponding metal-free indicator. Metal-indicator complexes (red dot) showing a color change due to back-formation to the metal-free indicator are selected afterward during an anion (X^-)-induced decomplexation reaction.

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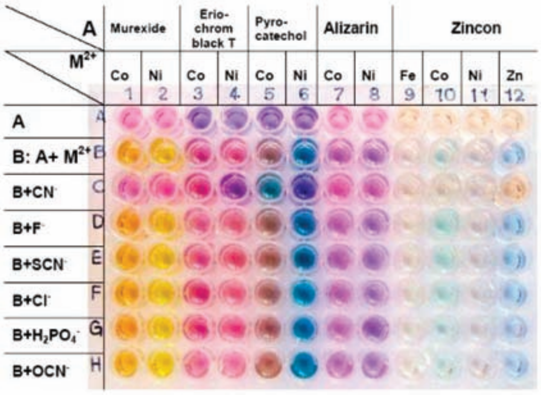

Figure 1. Representative examples of anion screening ([CHES] = 20 mM; pH 9.0 ± 0.1) with various combinations (B: A + M^{2+}) of metal indicators (A) and metal ions (M^{2+}) ([A] = 50 μM; [M^{2+}] = 50 μM; [anion] = 3 mM).[10]

demetalation of copper(II) zincon.[21] Unfortunately, Cu^{II} ions trigger the formation of highly toxic gaseous dicyan [$(CN)_2$],[22] which motivated us to search for alternative complexes with metal ions other than copper. We started from 12 commercially available metal indicators such as murexide, eriochrome black T, or pyrocatechol (50 μM) and added equimolar amounts of Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, or Zn^{2+} at pH 7 or pH 9 (Figure 1).[10] A listing of all combinations of metal indicators and metal ions used during the two rounds of this experiment is given in the Supporting Information. The color of 50 out of 120 combinations of metal indicators and metal ions differed unambiguously from the color of the corresponding metal-free indicator upon metal-ion coordination. This property was most pronounced for the yellow complex of murexide and Ni^{2+} (or Co^{2+}) in comparison with the pink color of the metal-free indicator (Figure 1; row 1 vs row 2). The anion screening of the 50 selected metal-indicator complexes (Figure 1; rows 3–8) was then performed at relatively high concentrations of CN^-, F^-, SCN^-, Cl^-, $H_2PO_4^-$, and OCN^- (3 mM). The latter five anions have been chosen to test the selectivity because they interfered with other chemosensors of cyanide.[17] After the addition of cyanide (3 mM) to the metal ion/indicator complexes (50 μM/50 μM), a change toward the characteristic color of the metal-free indicator was observed for 10 combinations within a few seconds at room temperature.

For instance, the yellow complex of murexide and Ni^{2+} (or Co^{2+}) converted to the pink color of metal-free murexide after treatment with cyanide (Figure 1; row 3 vs row 2).

Although all of the 10 selected metal complexes exclusively reacted with cyanide (3 mM) under the screening conditions, we decided to focus our attention on the murexide/Ni^{2+} (or Co^{2+}) and zincon/Zn^{2+} systems at pH 9 because of the most pronounced color changes at lower cyanide concentrations (Table 1). The most sensitive chemosensor was the nickel(2+) murexide complex with an optical detection limit (OD_{opt}) of as low as 40 μM cyanide and a limit of detection (LOD) below the maximum contaminant level of 0.2 mg/L (7.7 μM) of the U.S. Environmental Protecting Agency (Table 1).[23] The selectivity was tested in the presence of 13 different anions (F^-, Cl^-, Br^-, I^-, SCN^-, NO_3^-, CO_3^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, $H_2PO_4^-$, ClO_4^-, AcO^-, and OCN^-) at pH 9 in water ([CHES] = 20 mM). Only high concentrations of oxalate (0.5 mM) and HPO_4^{2-} (> 3 mM) induced the decomplexation of the murexide/Ni^{2+} complex (Table 1). However, the less sensitive zincon/Zn^{2+} complex was not affected by these anions. The absorption of murexide (H_5L) at λ_{max} = 522 nm indicated the existence of the purpureate anion (H_4L^-) at pH 9.[24] The stepwise addition of Ni^{II} (0–60 μM) to a solution of H_4L^- (62 μM, pH 9) shifted the absorption maximum to λ_{max} = 477 nm with an isosbestic point at 502 nm (Figure 2, right).

Table 1. Characteristic Data of the Selected Metal-Based Sensors

indicator/ metal ion[a]	OD_{opt}[b] [μM]	LOD[c] [μM]	color change	interf.$_{opt}$[d] [mM]
murexide/Ni^{2+}	40	6	yellow → pink	$C_2O_4^{2-}$/0.5 HPO_4^{2-}/3
murexide/Co^{2+}	60	14	yellow → pink	$C_2O_4^{2-}$/0.1 HPO_4^{2-}/1.5
zincon/Zn^{2+}	200	65	blue → slightly orange	

[a] [M^{2+}] = 20 μM, [murexide] = 36 μM, and [zincon] = 40 μM; pH 9.0 ± 0.1. [b] Optical detection limit of [CN^-]. [c] LOD of [CN^-]. [d] interf. = interferent.

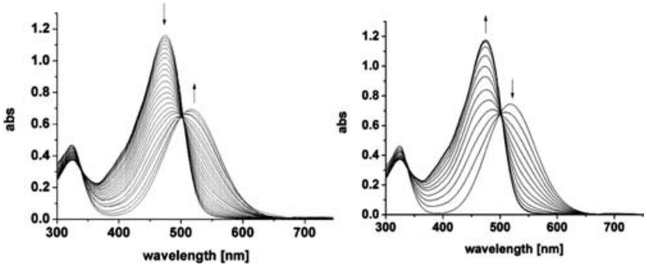

Figure 2. Left: UV–vis spectra of the titration of a mixture of murexide (62 μM) and Ni^{2+} (55 μM) with increasing amounts of CN^- (0–240 μM) in water ([CHES] = 20 mM; pH 9.0 ± 0.1; 21 °C). Right: UV–vis spectra of the titration of a solution of murexide (62 μM) with Ni^{2+} (0–60 μM) in water ([CHES] = 20 mM; pH 9.0 ± 0.1; 21 °C).

A metal-to-ligand ratio of 1:1 of the nickel(2+) purpureate complex was determined using the method of continuous variations, suggesting the formation of a $(Ni-H_4L)^+$ complex (Figure 3).[25,26] When cyanide (0–240 μM) was added to this metal complex, a reversible reaction to the violet metal-free purpureate anion (H_4L^-) took place (Figure 2, left). We assume that a ligand displacement reaction of $(Ni-H_4L)^+$ to a metal cyano species under liberation of the corresponding metal-free indicator is favored by the higher thermodynamic stability of the latter complex (Figure 3). For instance, the binding constant (log β) of $[Ni(CN)_4]^{2-}$ is around 30 and that of $(Ni-H_4L)^+$ only 6.34.[27,28] Cyanide is stored in the form of cyanogenic glycosides in more than 2000 plants like cassava, flax seeds, or bamboo.[29] Our group demonstrated recently

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Table 2. Characteristic Data of the Selected Metal-Based Sensors

indicator/metal ion[a]	analyte	OD_{opt}[b] [μM]	LOD[c] [μM]	color change	interf.$_{opt}$[d] [mM]
calcon/Ca^{2+}[e]	HPO_4^{2-}[f]	80	35	violet → blue	CO_3^{2-}/0.5 OCN^-/0.4
eriochrom black T/Mg^{2+}[g]	$C_2O_4^{2-}$	1000	95	violet → blue	HPO_4^{2-}/2

[a] [Mg^{2+}] = 20 μM, [Ca^{2+}] = 120 μM, [eriochrom black T] = 20 μM, and [calcon] = 40 μM. [b] Optical detection limit of [analyte]. [c] LOD of [analyte]. [d] interf. = interferent. [e] pH 12.3 ± 0.1 (4% MeOH). [f] A 1:1 mixture of HPO_4^{2-}/PO_4^{3-} is expected at pH 12.3 [pK_a(HPO_4^{2-}) = 12.3]. [g] pH 9.0 ± 0.1.

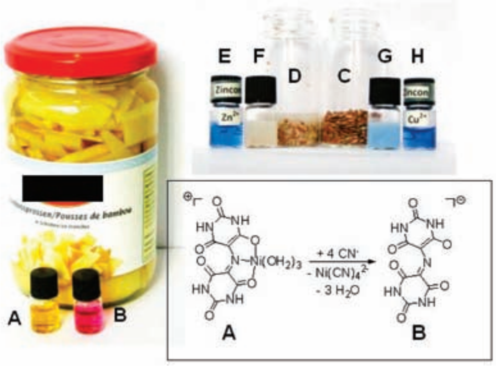

Figure 3. Color of murexide/Ni^{2+} (36 μM/20 μM) at pH 9.0 ± 0.1 ([CHES] = 20 mM) (A) as well as after treatment of A with 40 μL from the preserving solution of commercially available pickled bamboo shoots (B). Flax seed (C) and a crude aqueous suspension of flax seed mush (D). Color of zincon/Zn^{2+} (40 μM/20 μM; E) and zincon/Cu^{2+} (40 μM/20 μM; H) at pH 9.0 ([CHES] = 20 mM) as well as after the addition of an aliquot of D to either E or H (F and G). Bottom right: Proposed structures of the decomplexation of murexide/Ni^{2+} [A; $(Ni-H_4L)^+$] with endogenous cyanide to the purpureate anion (B; H_4L^-).

the optical detection of endogenous cyanide from cassava using a vitamin B12 derivative.[30] The three novel metal-based chemosensors obtained from the screening represent alternatives for applications in biological matrixes. We used the murexide/Ni^{2+} sensor (Figure 3A) successfully for the detection of endogenous cyanide in the preserving solution of commercially available pickled bamboo shoots, indicated by a color change from yellow (Figure 3A) to pink (Figure 3B). An average concentration as high as 55 mg/L of cyanide was detected in agreement with an independent quantification carried out with aquocyanocobyrinic acid from two different jars and four measurements.[30] The zincon/Zn^{2+} system (Figure 3E) was successfully applied for the detection of endogenous cyanide in the crude aqueous suspension of crushed flax seeds (Figure 3C−F; $\Delta\lambda_{max}$ = 133 nm, color change from blue to slightly orange; [CN^-] = 63 ± 5 mg/L) as well as on the surface of a freshly cut cassava slice.[25] Interestingly, the zincon/Cu^{2+} system[21] (Figure 3H) did not work under the reaction conditions (G vs H in Figure 3).[25]

In a second screening experiment, we decided to search for the chemosensors of other biologically important anions like phosphate and oxalate. Increased levels of the latter anion are linked to inborn metabolic diseases such us primary hyperoxalury or the intestinal disease Morbus Crohn and analyzed in clinical settings using enzymatic tests or ion chromatographic methods.

Figure 4. From left to right: calcon (40 μM), calcon/Ca^{2+} (40 μM/120 μM) without any additional anion as well as after the addition of either $H_2PO_4^-$, $C_2O_4^{2-}$, F^-, Cl^-, Br^-, I^-, SCN^-, NO_3^-, SO_4^{2-}, ClO_4^-, or AcO^- (2 mM) at pH 12.3 (water/4% MeOH).

We started from 36 combinations of metal indicators and metal ions (Ce^{4+}, Zr^{4+}, Mg^{2+}, and Ca^{2+}) possessing a high affinity toward these anions and performed a search according to the procedure proposed in Scheme 1. The screening was followed by thorough selectivity as well as sensitivity studies, as described in the Supporting Information and summarized in Table 2. This procedure led to identification of the complexes calcon/Ca^{2+} and eriochrom black T/Mg^{2+} as chemosensors for the optical detection of micromolar phosphate and millimolar oxalate, respectively (Figure 4).[25,31−33]

In summary, a "naked-eye" two-step screening procedure for the rapid selection of metal-based chemosensors for biologically important anions has been developed. Starting from mixtures of different metal ions and commercially available metal indicators, we successfully identified chemosensors for the selective detection of micromolar cyanide, micromolar phosphate, and millimolar oxalate. The detection of endogenous cyanide in common foodstuff has also been demonstrated. We anticipate that multistep optical screening procedures are not limited for the selection of metal-based chemosensors for anions but will also help to identify sensors for other important chemical and biological compounds in the near future.

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Supporting Information Available: Experimental data for experiments discussed but not illustrated in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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