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# Complex Samples Cyanide Detection with Immobilized Corrinoids

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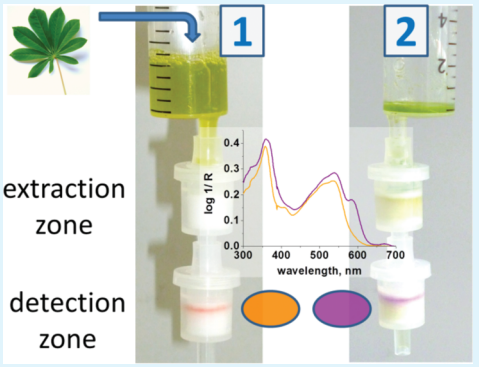
Supporting Information

**ABSTRACT:** Colorimetric solid phase with spatially separated extraction and detection zones as a rapid, effective and economic method for the optical detection of cyanide in complex samples is described. The system is seven times more sensitive for the optical detection of cyanide than the same class of chemical sensors used under homogeneous conditions. The application of the method in the detection of (i) endogenous cyanide in colored plant samples and of (ii) hydrogen cyanide in tobacco smoke is shown. The optical detection of multiple anions within a single sample has been demonstrated in principle for the detection of both $CN^-$ and $SCN^-$. Immobilized aquacyano-corrinoids and immobilized vitamin B12 are applied as chemical sensors, and cyanide is qualitatively identified by the violet color ($\lambda_{max}$ = 583 nm) of the corresponding dicyano-complex. Quantitative determinations with diffuse reflectance spectroscopy (DRUV−vis) are possible in the linear range up to 0.2 mg/L with a LOD of 1 $\mu$g/L. Alkyl-modified silica particles are employed for immobilization of the indicator on the surface of the solid phase (detection zone), and for removal of colored hydrophobic interferents (extraction zone).




**KEYWORDS:** *cyanide, optical detection, simultaneous sensing, immobilization, complex samples, solid phase*

## INTRODUCTION

Cyanide is toxic to mammals because it inactivates biologically important transition metal complexes. In particular, coordination of cyanide to the Fe(III) center of cytochrome C oxidase inhibits cellular respiration and leads to cellular hypoxia and acidosis.[1] Typical signs of acute cyanide intoxication include headache, vertigo, convulsion, and may result in coma or death.[2] Nevertheless, cyanide is produced in bulk quantities for industrially important applications[3] and is also frequently found in nature.[4] The enzymatic release of endogenous cyanide from cyanogenic glycosides is considered to be a natural self-defense mechanism of more than 2000 plants against animal predation.[5] Cassava (*Manihot esculenta* Crantz), a staple food for hundreds of million people in Africa and South America, represents the most important example for cyanide containing foods.[4,6] Cyanide uptake from insufficiently processed cassava products causes major health problems such as the paralytic disease *Konzo*.[7,8] The most frequent exposure to HCN in industrialized countries is probably the unintentional intake by cigarette smokers that leads to increased cyanide concentrations in blood.[9−11] One of the most important pathways for cyanide detoxification is the mitochondrial conversion of cyanide with thiosulfate to thiocyanate catalyzed by the enzyme rhodanase.[2,12] Therefore, urinary thiocyanate concentrations can be used as an indirect measure for cyanide uptake.[13,14]

The development of chemical sensors for the optical detection of cyanide is a current, active research area.[11,15−23] A few immobilized chemosensors have already been successfully applied for the detection of cyanide in water.[24−26] Corrin-based chemical sensors combine high sensitivity, selectivity, and fast binding kinetics,[15] and have been applied for the detection of cyanide in colorless plant samples,[19] industrial wastewater,[27] and blood.[28,29]

Herein, we report the sensitive optical detection of cyanide with immobilized corrinoids. We have successfully applied colorimetric solid phase[30−36] with spatially separated extraction and detection zones for the optical detection of cyanide and hydrogen cyanide in complex, colored samples without time-consuming sample preparation. Simultaneous optical detection of cyanide and thiocyanate is also possible following this approach.

## EXPERIMENTAL SECTION

**Materials. General Information.** Potassium cyanide, dicyano-cobyrinic acid heptamethylester (**1-CN**), dicyano-cobinamide (**3-CN**), Cyano-cobalamin (vitamin B12) **4**, Ches and Hepes were obtained from Fluka (Buchs, CH). Aquacyano-cobyrinic acid heptamethylester **1**, aquacyano-cobyrinic acid **2**, and aquacyano-cobinamide **3** were synthesized as pairs of diastereomers from their corresponding dicyano-forms through nonselective displacement of either the ("upper") $\beta$- or the ("lower") $\alpha$-cyanide as described elsewhere.[19,37] KCN stock solutions ($1 \times 10^{-3}$ M) were prepared freshly before use. The desired pH values of the stock solutions of the buffers Ches (0.1 M; pH 9.5) and Hepes (0.1 M; pH 7.5) were adjusted by the addition of either a solution of 2 N NaOH or 1 N HCl. All measurements were performed at a final buffer concentration of 20 mM. The pH values of solutions were measured with a Metrohm 827 pH lab.

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Chromabond *C18ec* polypropylene columns (100 mg) and Chromafix *C18ec* cartridges (270 mg) were obtained from Macherey-Nagel AG Schweiz. In this paper we will use the terms *C18ec* columns for the former, *C18ec* cartridges for the latter, and *C18ec* material for both types, respectively.

Solvents of HPLC grade or of highest purity and doubly distilled water were used.

Cigarettes were purchased at local supermarkets in Zurich, Switzerland. Leaves from *Manihot esculenta* Crantz were a generous gift from the botanical garden of the University of Zurich.

**CSPE Method.** [30−33,38]

*General.* The colorimetric solid phase extraction (CSPE) device consisted of a 10 mL syringe that was either connected directly or via a Chromabond adapter PP (Macherey-Nagel) to *C18ec* columns or *C18ec* cartridges. The procedure is exemplified for indicator **1**.

1. *Conditioning.* The *C18ec* column was washed with methanol (0.5 mL) and water (10 mL).
2. *Adsorption of Indicator.* The indicator was adsorbed on the top of the *C18ec* material while an aqueous solution of **1** (0.5 mL; 40 $\mu$M) was passed through the filter material. The immobilized indicator $\mathbf{1}_{SP}$ (SP for "solid phase") was visible as a red colored ring (height ∼1 mm).
3. *Analysis.* A sample solution (usually 5 mL) was passed through the filter with a rate of approximately 2 mL/min. Detection with $\mathbf{1}_{SP}$ was indicated by a change of color.
4. *Regeneration.* $\mathbf{1}_{SP}$ was regenerated from $\mathbf{1}_{SP}$**-CN** while passing 1% of an aqueous acid (3 mL) and water (20 mL) through the filter.

**Spectroscopic Measurements.** UV−vis spectra were measured at $T = 21 \pm 1$ °C with a Cary 50 spectrometer using quartz cells with a path length of 1 cm. DRUV−vis spectra were recorded on a Perkin-Elmar Lambda 50 spectrometer equipped with an integrating sphere setup (diameter 110 mm) and pure $MgSO_4$ as reference material.

**Sample Preparation for Diffuse Reflectance (DRUV−Vis) Spectroscopy.** For DRUV−vis measurements of immobilized reagent and immobilized reagent-analyte complexes, the cartridges were opened under greatest care. The top layer of the *silica C18ec* column (∼ 1 mm) containing the immobilized reagent-analyte complex was removed from the cartridges or columns, dried (10 min at 45 °C and 30 min on air) and used for DRUV−vis measurements.

**Quantification/Kubelka−Mulk/Limit of Detection.** The percentage of reflectance (R) was measured with respect to silica *C18ec* as standard white. The Kubelka−Munk equation gives the relation between the percentage of reflectance ($R$) and the concentration of the analyte under assumption of a constant molar absorptivity ($\varepsilon$) and scattering coefficient ($s$) for a given wavelength.[30]

$$F(R) = (1 - R)^2/2$$

$$RF(R) = \varepsilon C/s$$

The limit of detection (LOD) was determined as the mean of the blank measures at 583 nm plus three times the standard deviation of the blank from at least 10 measurements.

**CIELab Measurements.** A hand-held spectrophotometer CM-2900d from Minolta was used to measure the differences of color in the CIELAB-system with Specular Component Excluded (SCE).[39] All values are averaged from at least 8 measurements. The differences in color $\Delta E$ have been estimated according to

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The major sphere of $\Delta E$ was used for the description of color differences.[39]

**Detection of Endogenous Cyanide in Colored Plant Material.** A cassava leaf extract was prepared by (i) grinding of about 2 g of the cassava leaf with mortar and pistil, (ii) dilution with water (5 mL; pH 9.5, [Ches] = 0.1 M) and (iii) centrifugation at 6000 U for 10 min. The green colored supernatant was used directly for CSPE.

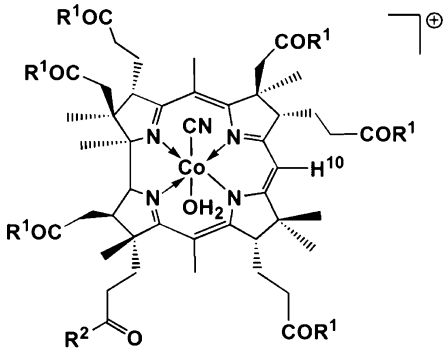
**Detection of HCN in Tobacco Smoke.** To detect hydrogen cyanide (HCN) in tobacco smoke, a cigarette was connected to two attached *C18ec* cartridges. The first cartridge was used to remove tar and other potential interferents from tobacco smoke, whereas the second column containing $\mathbf{1}_{SP}$ or $\mathbf{4}_{SP}$ was used for HCN detection.

A vacuum (500 mbar) was applied to pull the tobacco smoke through the cartridges. Afterward the top layer of cartridge 2 containing the immobilized reagent-analyte complex was carefully removed, dried (10 min at 45 °C and 30 min on air) and used for DRUV−vis measurements.

## RESULTS AND DISCUSSION

**Cyanide Detection with Immobilized Aquacyano-Corrinoids.** Immobilization of corrinoids on alkyl modified silica surfaces is caused by attractive hydrophobic interactions between the surface of the solid support and the chemical sensor. *Silica C18ec* showed the best adsorbing properties for corrinoids compared to other silica materials (*C4*, *C8*, and *C18*) and was therefore chosen as matrix material for all experiments.

**Scheme 1. Structural Formula of Corrinoids 1-3 (Only the $\beta$-Cyano, $\alpha$-Aqua Diastereomer Is Depicted)**



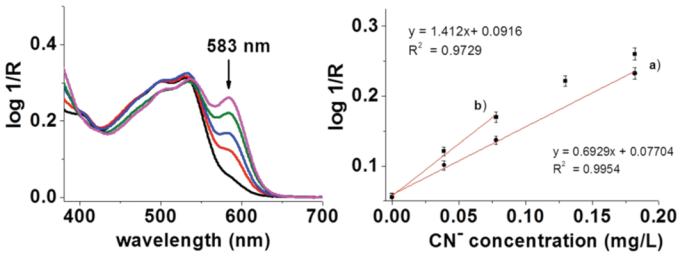

1: $R^1 = R^2 = OCH_3$
2: $R^1 = R^2 = OH$
3: $R^1 = NH_2$, $R^2 = NHCH_2CH(CH_3)OH$

The mode of cyanide detection with immobilized aquacyano-corrinoids (**1**- **3**) is outlined in eq 1.[18] Substitution of Co(III) coordinated water with cyanide leads to the corresponding dicyano complexes (**1-CN**-**3-CN**) and causes a color change of the chemical sensors from orange to violet with a characteristic maximum at ∼583 nm in the reflection spectrum.[19]

$$[H_2O - Co - CN]^+ + CN^- \rightleftharpoons [NC - Co - CN] + H_2O \quad (1)$$

Aquacyano-cobester (**1**) was immobilized on a white colored *C18ec* column as described in the Experimental Section. Indicator **1** was thereby adsorbed as an orange colored ring of $\mathbf{1}_{SP}$ on the top of the solid phase. When buffered aqueous solutions of cyanide (0.04−1.04 mg/L; pH 7.5 or 9.5) were passed through the $\mathbf{1}_{SP}$-column, the color of the indicator changed from orange to violet.

The violet colored complex at the top of the *C18ec* material was subsequently investigated with DRUV−vis spectroscopy. The reflection spectrum is identical to the UV−vis spectrum of **1-CN** under homogeneous conditions (Figure 1 left).[18] Calibration curves for the binding of cyanide to $\mathbf{1}_{SP}$ were generated using Kubelka−Munk transformations at 583 nm




| c ($CN^-$) mg/L | L* | a* | b* | ΔE |
|---|---|---|---|---|
| 0 | 69.9 | 32.4 | 8.1 | ref. |
| 0.04 | 53.3 | 34.1 | 3.5 | 17.3 ± 0.2 |
| 0.08 | 51.8 | 31.5 | 1.5 | 20.5 ± 0.2 |
| 0.13 | 45.5 | 32.0 | 7.3 | 28.9 ± 0.6 |
| 0.18 | 44.6 | 25.6 | 9.3 | 31.4 ± 1.3 |

**Figure 1.** Left: DRUV–vis spectra of $\mathbf{1}_{SP}$ (20 nmol) and $\mathbf{1}_{SP}$ (20 nmol) upon passing solutions (5 mL, [Ches] = 20 mM, pH 9.5) of $CN^-$ (0.04 mg/L, 0.08 mg/L, 0.13 mg/L and 0.18 mg/L) through the column. Middle: Calibration curves for the quantification of $CN^-$ at 583 nm and pH 7.5 (a) or pH 9.5 (b; only the linear range is fitted). Right: Corresponding CIELab values (pH 9.5): *L**, a*, *b**, and Δ*E*. Δ*L**, Δ*a**, Δ*b** = ±0.1.

(Figure 1 middle). Quantitative determination of cyanide is possible in the linear range up to 0.075 mg/L at pH 9.5 and 0.2 mg/L at pH 7.5 with a LOD of 1 μg/L.

Differences in color were analyzed with a hand-held spectrophotometer CM-2900d and were expressed as Δ*E* values[39] in the CIELab-system (Figure 1, Figure S1 in the Supporting Information).

The Δ*E* values observed for the reaction of $\mathbf{1}_{SP}$ with 0.04 mg/L of cyanide is 17.3 and 8.0 at pH 9.5 and 7.5, respectively (Figure 1 right, Figure S1 in the Supporting Information). These values are significantly above the minimum value of 2.0 that allows visual detection ($\Delta E_{vis}$).[40]

Concentrations as low as 0.04 mg/L of cyanide (*V* = 5 mL; 7.5 nmol; pH 7.5 or 9.5) have been identified with the naked-eye, a value below the guideline value of 0.05 mg/L in drinking water as suggested by the WHO (Table 1; entries 3, 4; Figures S1 and S2 in the Supporting Information).[2]

**Table 1. Comparison of Homogenous and Heterogenous Visual Detection of Cyanide**

| entry | chemosensor | pH | $c_{(CN)}$ [mg/L][f] | $n_{(CN)}$ [nmol] | RQ[g] |
|---|---|---|---|---|---|
| 1[a,b] | **1** | 7.5 | 0.3 | 10 | 0.25 |
| 2[b,c] | **1** | 9.5 | 0.3 | 10 | 0.25 |
| 3[d] | $\mathbf{1}_{SP}$ | 7.5 | 0.04 | 7.5 | 0.375 |
| 4[e] | $\mathbf{1}_{SP}$ | 9.5 | 0.04 | 7.5 | 0.375 |

[a][**1**] = 40 nmol, [Hepes] = 20 mM, V = 1 mL. [b]ref 18. [c][**1**] = 40 nmol; [Ches] = 20 mM, V = 1 mL. [d][$\mathbf{1}_{SP}$] = 20 nmol, [Hepes] = 20 mM, V = 5 mL. [e][$\mathbf{1}_{SP}$] = 20 nmol; [Ches] = 20 mM, V = 5 mL. [f]>10% over the minimum visual detectable concentration of cyanide [g]RQ: Ratio of quantities, RQ = $n_{[CN]}/n_{[CS]}$, CS: chemosensor. The semiqualitative results are average values of at least 8 measurements.

The immobilized system $\mathbf{1}_{SP}$ is seven times more sensitive for the optical detection of cyanide than **1** applied under homogeneous conditions.[18] This effect results from the extraction of cyanide from the five times larger sample volume as indicated by comparable ratio of quantities (RQ) of cyanide per quantity of chemical sensor (Table 1; entries 3 vs 1, 4 vs 2).

The selectivity of $\mathbf{1}_{SP}$ was tested with the following fourteen different anions $F^-$, $Cl^-$, $Br^-$, $I^-$, $NO_3^-$, $H_2PO_4^-$, $SO_4^{2-}$, $ClO_4^-$, $SCN^-$, $C_2O_4^{2-}$, $HCO_3^-$, $OAc^-$ (0.1 M; 5 mL; [Ches] = 20 mM, pH 7.5 and 9.5). Only $SCN^-$ (>0.6 mM; 35 mg/L), $OCN^-$ (>1 mM; 42 mg/L) and $I^-$ (>3 mM; 381 mg/L) interfered. The reflection spectra of $\mathbf{1}_{SP}$-**X** (X = $SCN^-$, $I^-$, $OCN^-$) are in good agreement with the absorption spectrum of **1**-**X** in organic solvents (see Table S1 in the Supporting Information).

Regeneration of immobilized $\mathbf{1}_{SP}$ from $\mathbf{1}_{SP}$-**CN** is achieved by washing the filter with 1% of diluted aqueous acids (3 mL) and subsequently with water (20 mL). The adsorption of the indicator on the surface of the alkyl modified silica particles is not affected by this procedure, and at least ten consecutive runs of cyanide detection and regeneration can be performed without loss of sensitivity.

**Practical Situations.** Applications of immobilized corrinoids were tested for optical detection of cyanide in proof-of-principle studies. We tested the identification of endogenous cyanide in raw extracts of green colored cassava leaves (Figure 2 left) and hydrogen cyanide in tobacco smoke, respectively.

When a freshly prepared green-colored sample of a cassava leaf was passed through the device with spatially separated extraction and detection zones as shown in Figure 2, the colored interferents remained adsorbed in the hydrophobic extraction zone of the *C18ec* material. Endogenous cyanide was

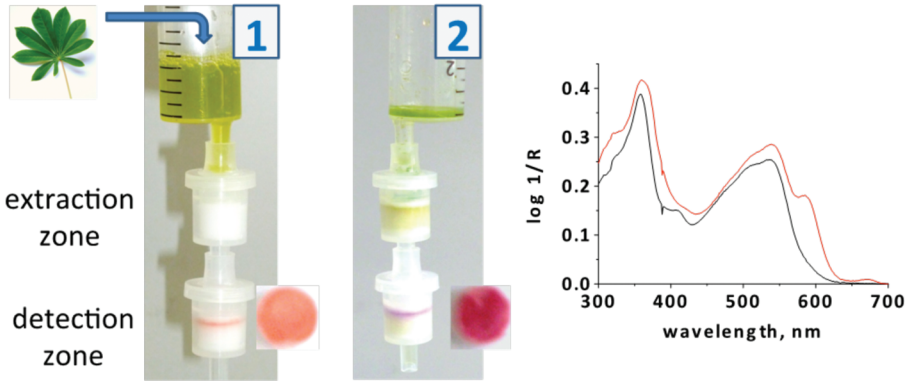



**Figure 2.** Left: Experimental setup for the detection of cyanide in a raw green-colored extract of a cassava leaf. Right: DRUV–vis spectra of $\mathbf{1}_{SP}$ before (black) and $\mathbf{1}_{SP}$-**CN** (red) after passing the crude solution through the device.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afterward indicated in the detection zone by a color change of $\mathbf{1}_{SP}$ from orange to violet (Figure 2).

The reflection spectrum of the violet colored product with a maximum at 583 nm is similar to the UV−vis spectrum of **1-CN** under homogeneous conditions (Figure 2).[18,19]

The application of colorimetric solid phase with spatially separated extraction and detection zones allows also the qualitative detection of gaseous HCN in tobacco smoke as described in the Experimental Section.

The formation of $\mathbf{3}_{SP}$**-CN** from $\mathbf{3}_{SP}$ and tobacco smoke was unambiguously shown with DRUV−vis-, ($\Delta\lambda_{max}$ = 578 nm; see Figure S3 in the Supporting Information) and $^1$H NMR spectroscopy after elution of the violet colored product from the cartridges. The signal for the proton H10 at 5.85 ppm is indicative for **3-CN** and is downfield shifted compared to those of the starting material ($\Delta\delta$ = 0.54 ppm, 0.62 ppm; Figure 3).

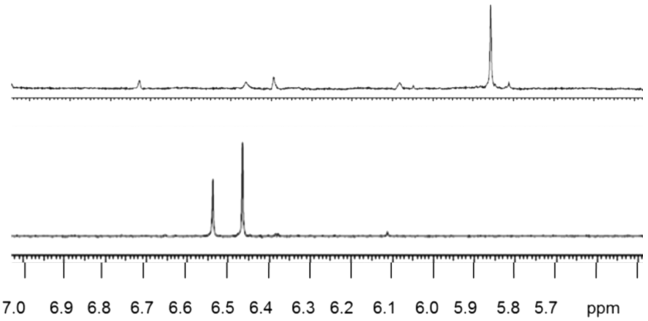


**Figure 3.** Left: $^1$H NMR signals of the H10 proton of **3** before (bottom) and of **3-CN** after reaction of **3** with tobacco smoke (top). (The existence of two signals for the H10 proton of **3** are due to the application of **3** as a diastereomeric mixture of the $\alpha$, $\beta$- aquacyano isomers).

**Individual Kit for the Visual Detection of Cyanide and Thiocyanate.** The selective visual detection of multiple analytes within one sample has not yet been shown. For example, cyanide and thiocyanate cannot be detected simultaneously with corrin-based chemical sensors.[17,18] We tested the simultaneous detection of both anions by the consecutive extraction into spatially separated detection zones, and thus applied vitamin B12 (**4**) for the detection as well as removal of cyanide prior to the detection of thiocyanate with indicator $\mathbf{1}_{SP}$. Vitamin B12 was chosen because detection of cyanide occurs without $SCN^-$ interference.[17,41]

The prototype of the device consists of three filter cartridges as shown in Figure 4. Cartridge 1 (cyanide detection zone) contains $\mathbf{4}_{SP}$ for the detection of cyanide. The same indicator was also immobilized on the top and bottom of cartridge 2 (cyanide extraction zone) for the complete removal of excess cyanide. Cartridge 3 (thiocyanate detection zone) contains immobilized indicator $\mathbf{1}_{SP}$ for the visual detection of $SCN^-$ in the absence of potentially interfering cyanide.

When a sample containing $\mu$M of cyanide and mM of $SCN^-$ ($V$ = 8 mL, pH 9.5) was passed through this device, both anions could be detected with the naked-eye (Figure 4). Cyanide and thiocyanate were indicated by a color change from red ($\mathbf{4}_{SP}$) to violet ($\mathbf{4}_{SP}$-CN) in cartridge 1 and from orange ($\mathbf{1}_{SP}$) to brown-violet ($\mathbf{1}_{SP}$**-SCN**) in cartridge 3 as indicated by accompanying DRUV−vis measurements. Of note, the sensitivity of sensor **4** for cyanide is not influenced by the presence of thiocyanate.[42]

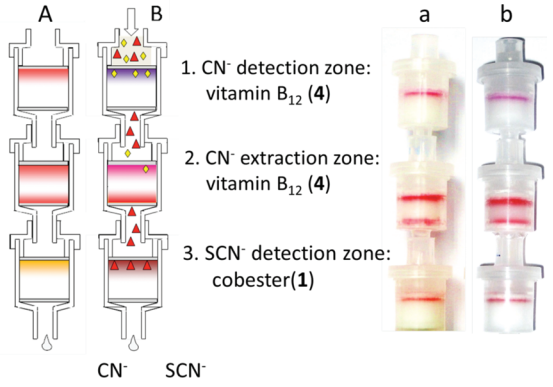


**Figure 4.** (A, B) Schematic representation and (a, b) pictures of the anion detection kit. Cartridge 1: cyanide detection zone: $\mathbf{4}_{SP}$ (50 nmol). Cartridge 2: cyanide extraction zone: $\mathbf{4}_{SP}$ (200 nmol (top)/50 nmol (bottom). Cartridge 3: thiocyanate detection zone: $\mathbf{1}_{SP}$ (50 nmol). (B) Application of a mixture of $CN^-$ and $SCN^-$. b: upon passing a mixture (8 mL, [Ches] = 20 mM, pH 9.5) of $CN^-$ (0.52 mg/L) and $SCN^-$ (0.13 g/L) through the cartridges shown in a.

## CONCLUSION

Immobilized aquacyano-corrinoids exhibit a 7-fold higher sensitivity for the optical detection of cyanide than their homogeneous counterparts. This behavior allows optical sensing below the guideline value for cyanide in drinking water of the World Health Organization (WHO; 0.05 mg/L). Quantitative determinations are possible in the linear range up to 0.2 mg/L with a LOD of 1 $\mu$g/L. Colorimetric solid phase with spatially separated extraction and detection zones has been applied for the straightforward optical detection of cyanide and hydrogen cyanide in complex samples such as colored plant material and tobacco smoke. Following the same strategy, the optical detection of multiple anions within a single sample has been demonstrated in a proof-of-concept study for the detection of both $\mu$M $CN^-$ and mM $SCN^-$.

The design of the device was shown to be modular and its application is not necessarily limited to the analyzed examples and/or corrin based chemical sensors.

Applications of such easy-to-use and low-cost devices for environmental, medical, and food safety control were proposed.

## ASSOCIATED CONTENT

### Supporting Information

Additional information, including pictures of chemosensors before and after cyanide exposure, CIELab values, DRUV−vis spectra of the chemosensors before and after exposure to tobacco smoke, comparison with solution studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(42) Higher concentrations of reagents and anions have been chosen to ensure a better visibility in Figure 4.