Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

Rapid Visual Detection of Cyanide in Blood

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Contents

Material and Methods

General information.

Potassium cyanide, dicyano-cobyrinic acid heptamethylester (**1-CN**), hydroxocobalamin, potassium hydroxide, Ches, antifoam A concentrate, sodium citrate, and phosphoric acid were obtained from Fluka (Buchs, CH). Aquacyano-cobyrinic acid heptamethylester **1** was synthesized as pairs of diastereomers from their corresponding dicyano-form through non-selective displacement of either the ("upper") β- or the ("lower") α-cyanide as described elsewhere.[1, 2] KCN stock solutions (10^{-3} M) were prepared freshly before use. The desired pH values of the stock solutions of the buffer Ches (1M; pH 9.6) was adjusted by the addition of a solution of 2 N NaOH. All measurements with blood were performed at a final buffer concentration of 0.5 M. The pH values of solutions were measured with a Metrohm 827 pH lab.

Chromabond *C18ec* polypropylene columns (100 mg) and a Chromabond adapter PP were obtained from Machery-Nagel AG Schweiz. Syringes (V = 10 ml) were connected via the adapter to the columns.

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

The blood of hog was received freshly from the slaughterhouse of the Veterinary Clinic of the University of Zürich. To avoid blood coagulation, it was immediately diluted (V= 9:1) with a solution of Na-citrat (3.8% in water). The blood was stored at 5°C and was used maximal 3 days upon slaughter.

Potentiometric measurements were performed with a crystal membrane electrode 6.0502.130 (CN-sensitive electrode) from Metrohm/Schweiz, using an Ag/AgCl reference electrode 6.0726.100 and a Methrom pH 713 Meter (in mV).

CSPE-method.[3-5]

General.

Methanol (0.5 ml) and water (10 ml) were pressed with the help of a syringe through the columns in order to condition the *C18ec silica* material before further use.

Blood cyanide detection

1. Preparation of the blood sample. Blood (0.5 ml) was spiked with cyanide ($[CN^-]$ = 0- 100 µM). Fifteen minutes later, the blood was adjusted to pH 9.6 with Ches buffer (0.47 ml, 1M) and **1** (30 µl; 1.4 mM) was added.

2. Adsorption of the chemosensor.

After one minute, the sample was pressed through the *C18ec* column (1 ml). The column was subsequently washed with water (3 ml) to remove adhering blood from the C18ec material. Thereby a red to violet coloured ring of **1-CN** was visible on the top of the C18ec material (Figure 1, right). The colour depends on the concentration of cyanide (Figure 3 inset).

3. Analysis.

Method I. The top layer of the *silica C18ec* material (~ 2 mm) containing immobilised **1-CN** was used for spectroscopic measurements.

Method II. Immobilised **1-CN** was eluted with MeOH (400 µl) from the C18ec column. The volume was adjusted to 500 µL, transferred to a quartz cuvette and analysed with UV-vis spectroscopy.

Blood cyanide detection in the presence of hydroxocobalamin

Blood (0.5 ml) was spiked with cyanide ($[CN^-]$ = 40 µM) and after 5 minutes with hydoxocobalamin (20 µl; 1mM). After another 5 minutes, the blood sample was adjusted to pH 9.6 with Ches buffer (0.45 ml, 1M) and **1** (30 µl; 1.4 mM) was added. The analysis was performed as described above. The characteristic maxima of **1-CN** at 583 nm did not overlap with that of hydroxocobalamin (λ_{max} = 536 nm) and vitamin B12 (λ_{max} = 550 nm).

Cyanide detection in water

Control experiments in water (0.38 % vol. of sodium citrate) instead of blood were performed as described for the detection of cyanide in blood.

Spectroscopic measurements.

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.

A handheld spectrophotometer CM-2900d from Minolta was used to measure the differences of colour in the CIELAB-system with Specular Component Excluded (SCE).[6] All values are averaged from at least 8 measurements. Cyanide detection was performed with three different blood samples.

UV-vis spectra were measured at T = 21 ±1°C with a Cary 50 spectrometer using quartz cells with a path length of 1 cm. Compound **1-CN** was eluted from the C18ec material with methanol. The concentration of **1-CN** was determined with a calibration curve obtained from different concentrations of **1-CN** (10- 100 µM) in methanol. Concentrations of cyanide were determined from a calibration curve obtained from the titration of **1** (30 µl; 1.4 mM) with different concentrations of cyanide (5 to 25 µl, 10^{-3} M).

Quantification/ Kubelka Munk/ 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 (ε) and scattering coefficient (s) for a given wavelength[4]:

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

$$F(R) = \varepsilon \cdot C / s.$$

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.

Cyanide detection with potentiometric measurements using microdestillation

The microdiffusion cascade consisted of 4 flasks (10 ml), closed with septa and connected to each other with syringes and PVC-tubes. The first flask was filled with water. The second one contained blood (1.0 ml) spiked with cyanide ($[CN^-]$ = 0- 50 µM), ascorbic acid (1 ml; 60 mM)[7] , water (850 µl), 3 drops of antifoam A concentrate and was subsequently adjusted to pH 3.3 with phosphoric acid (10%; 150 µl). The third and fourth flask contained KOH (0.1 M; 3 ml). The second flask was heated to 80°C for 40 min, while a stream of N_2 (3 bubbles per second) was passed through the solutions. Afterward, an aliquot of flasks three and four (300 µl) was diluted with aqueous KOH (0.1 M, 9.7 ml) to a final volume of 10 ml. The amount of cyanide was determined with a CN-sensitive electrode and the help of a calibration curve.

The calibration curve was obtained by adding various concentrations of cyanide (10- 100 µl, 10^{-4} M) to an aqueous KOH solution (0.1 M; V_{final} = 10 ml). The corresponding voltage was measured with a CN-sensitive electrode.

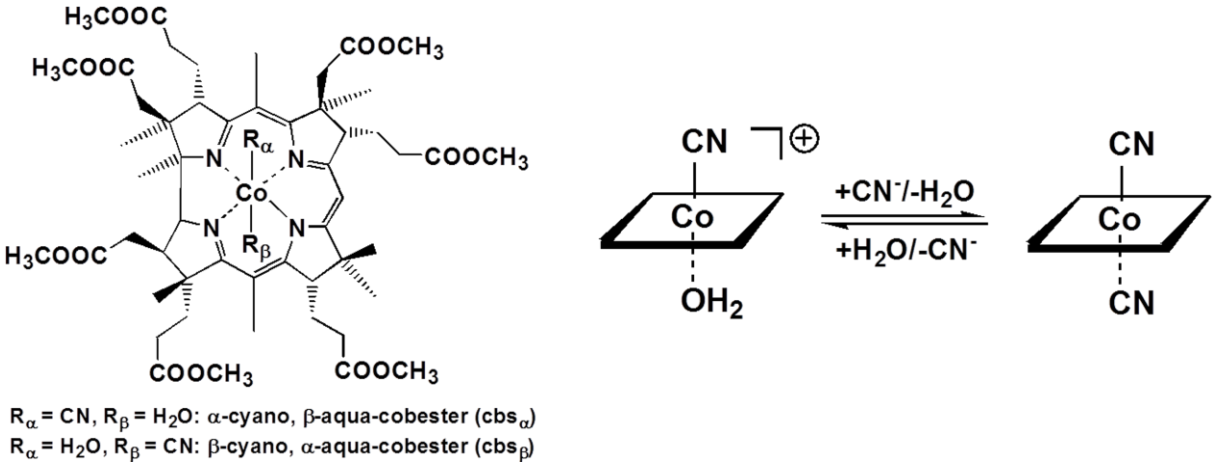

Figure S1. Structural formula of **1** (*left*) and its reaction with cyanide to **1-CN** (*right*; the reaction is only shown for one diastereomer).

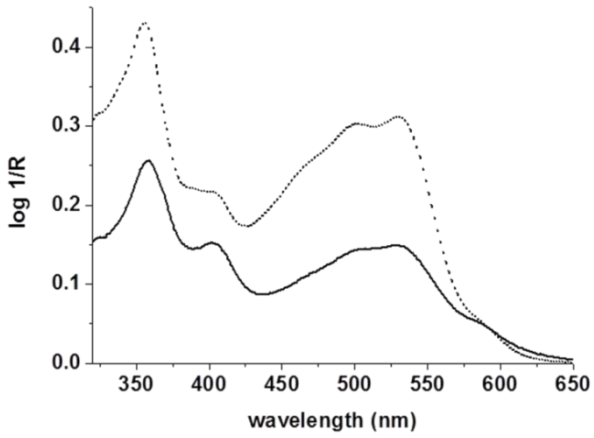

Figure S2. DRUV-vis spectra of **1** extracted on the C18*ec* material from either water (*dashed line*, [Ches] = 0.5 M, pH 9.6) or from blood (*solid line*, [Ches] = 0.5 M, pH 9.6).

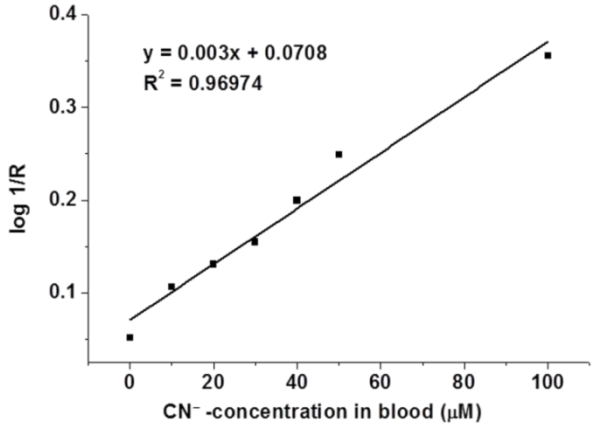

Figure S3. Correlation of the reflection maxima at 583 nm of immobilised **1-CN** versus different concentrations of cyanide in spiked blood.

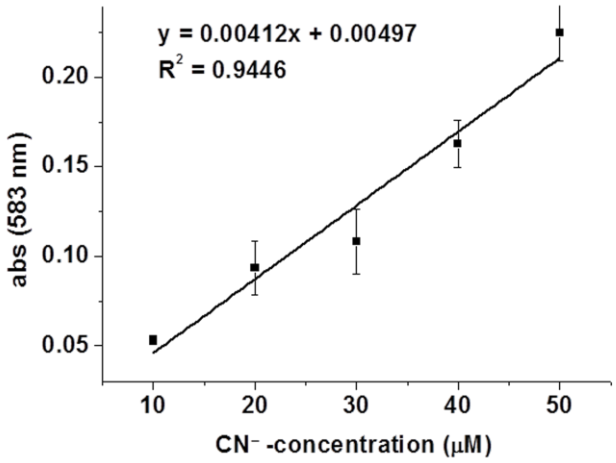

Figure S4. Correlation of the absorption maxima at 583 nm of **1-CN** versus different concentrations of cyanide in spiked blood. **1-CN** was eluted from the column prior to its detection as described in *method II*.

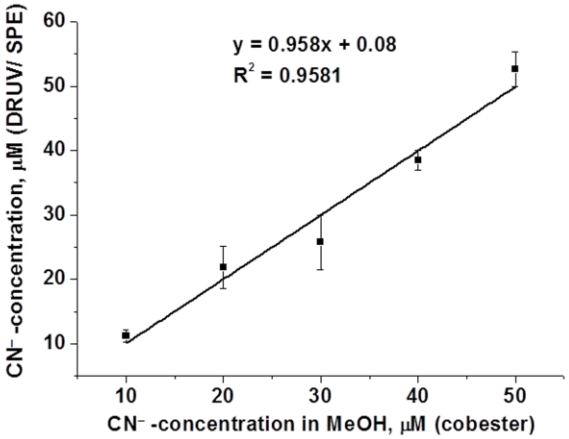

Figure S5. Results of the determination of different concentrations of cyanide with DRUV-Vis spectroscopy using the described SPE-method (Method I) versus absorbance measurements using UV-Vis spectroscopy (Method II).

References

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