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A chromoreactand for the selective detection of HSO_3^- based on the reversible bisulfite addition reaction in polymer membranes

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A new chromoreactand for the reversible optical detection of bisulfite anion is presented with a sensitivity in the range from 0.1 to 10 mM while no absorbance changes are observed for anions such as sulfate, chloride, phosphate or hydroxide.

The detection of sulfur dioxide and of bisulfite still is of considerable interest due to the use of bisulfite to stabilize wine during/after fermentation and due to a significant amount of sulfur dioxide produced and released in industrial processes. Optical sensors for sulfur dioxide or bisulfite mostly operate on the protonation of pH indicator dyes dissolved in thin polymer layers. Thus, a set of sensors was presented where lipophilic ion pairs of triphenylmethane dyes were dissolved together with tetraoctylammonium hydroxide in silicone. Sulfur dioxide interacted with the quaternary ammonium ion by forming the bisulfite ion pair while the residual H^+ formed in the layer caused protonation of the indicator dye. Usually fast and reversible colour changes from blue to yellow were observed but a severe limitation for the use of quaternary ammonium hydroxide in sensors is its limited operational and shelf life.[1]

Other optode membranes were constructed by entrapping within a plasticised poly(vinyl chloride) membrane a hydrogen sulfite selective carrier and a pH indicator dye. Due to selective transport of hydrogen sulfite into the membrane by the ligands, protons were co-extracted into the polymeric membrane phase leading to a change in the ratio of the protonated and deprotonated form of the pH indicator, and consequently, a change in the absorbance signal. However, the pH indicator dyes (and consequently the sensor layers) exhibited strong cross-sensitivity to acids and bases.[2]

Quenching of Rhodamine B isothiocyanate or of a covalent conjugate of the platinum(II) complex of coproporphyrin-I and bovine serum albumin by sulfur dioxide have also been reported but quenching processes can be unspecific because decreases in luminescence intensity are also caused by oxygen, heavy metal ions, halogenides, nitroaromatic compounds or humidity.[3] The enzyme sulfite oxidase was used for the optical determination of sulfite. The oxidation process was monitored *via* an oxygen sensor based on fluorescence quenching, or in a coupled enzymatic reaction *via* the consumption of NADH added to the system.[4]

In an approach comparable to enzymatic systems, we have described reversible chemical reactions of specific indicator dyes (chromoreactands) as the basis for the detection of electrically neutral analytes such as alcohols and amines and of fluororeactands for the detection of aldehydes.[5] In this work, we present the first use of a chromoreactand for the detection of ionic species, *i.e.* hydrogen sulfite, and the selectivity, sensitivity and response in plasticised polymer layers is discussed.

Formylazo dyes comparable to 4-*N*,*N*-dioctylamino-4′-formyl-2’-nitroazobenzene (**CR-514**) have initially been of interest as educts for the preparation of dyes in non-linear optics. They have been reacted first with malonodinitrile and subsequently with hydrogen cyanide to form the more long-wavelength absorbing dicyanovinyl and tricyanovinyl dyes.[6] However, the chemically reactive formyl group makes **CR-514**† an attractive candidate as a chromoreactand because

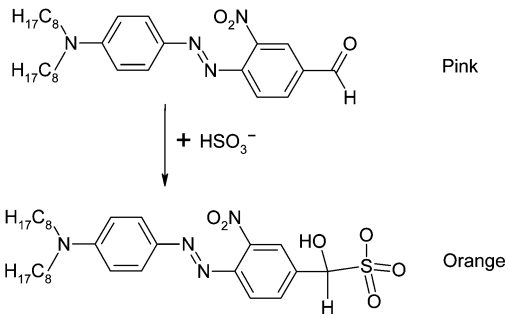

Fig. 1 Chemical structure of chromoreactand **CR-514** for sensing bisulfite, and corresponding bisulfite adduct.

aldehydes are known to interact with hydrogen sulfite by forming a bisulfite adduct (Fig. 1). This process is usually employed to purify aldehydes[7] but may also be used to analytically detect bisulfite, because the conversion of the aldehyde into the bisulfite adduct can bring about a change in the electron acceptor strength. Consequently, a colour change of the formyl dye upon interaction with bisulfite is to be expected. Indeed, when reacting a sensor layer **M1** (composed of the chromoreactand and dioctadecylmethylamine in PVC plasticised by 2-nitrophenyloctyl ether) first with plain buffer of pH 4.9 and then with buffer containing 30 mM bisulfite, a shift in absorbance maximum from 524 to 484 nm was observed (Fig. 2). The buffer pH of 4.9 was chosen in order to provide the analyte anion as the chemically reactive bisulfite and not as sulfite or sulfur dioxide. Membrane **M1** exhibited the highest sensitivity to bisulfite in the 0.1–10 mM range and the LOD was found to be 0.02 mM (Fig. 3). The overall equilibrium constant for the interaction of **M1** with bisulfite at pH 4.9 was calculated to be 580 M^{-1}. The forward response time, t_{95} (time needed for 95% of the total signal change to occur) was in the range of 10–15 min, whereas the time for the reverse response was slower and was in the range of 15–18 min (Fig. 4). Dioctadecylmethylamine was added to the membrane composition because, without the lipophilic amine, no signal changes upon exposure to bisulfite were observed. This is due to the fact that

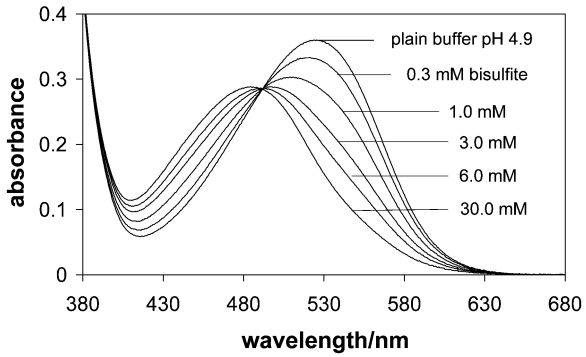

Fig. 2 Absorbance spectra of **CR-514** embedded within the polymer layer **M1** in contact with plain citrate buffer and buffered solutions of bisulfite, all at pH 4.9.

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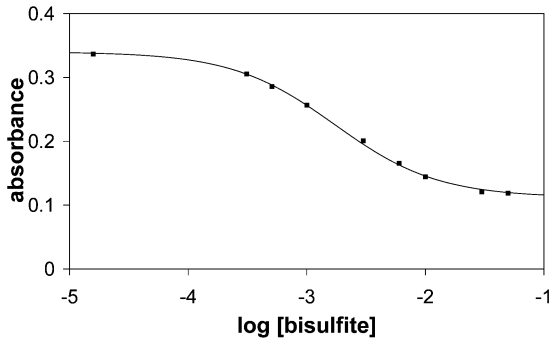

Fig. 3 Calibration plot of the sensor layer **M1** measured at 540 nm upon exposure to different concentrations of bisulfite, all at pH 4.9.

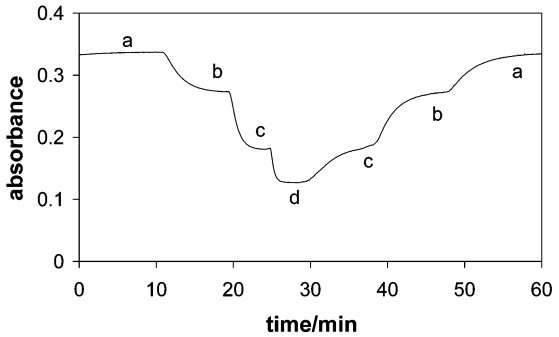

Fig. 4 Response and reversibility of the sensor layer **M1** measured at 540 nm upon exposure to: a, plain buffer; b, 1.0 mM bisulfite; c, 6.0 mM bisulfite; d, 30.0 mM bisulfite, all at pH 4.9.

co-extraction of the bisulfite anion with a proton into the layer is necessary in order to provide electroneutrality within the polymer layer, a mechanism frequently encountered for ion diffusion into plasticised polymers.[2] The layer **M1** exhibited high selectivity over other anions such as sulfate, phosphate or chloride in that only a signal change for bisulfite (due to the selective interaction) was observed and none at all for 50 mM concentrations of the other anions at pH 4.9. The layer did not show cross-sensitivity to 50 mM 1-propylamine and to ethanol in concentrations as high as 16 vol% at pH 4.9. The layer also did not change colour upon exposure to 0.1 M sodium hydroxide solution and only a small increase in absorbance of 4% at 524 nm upon exposure to 0.1 M hydrochloric acid was visible, which was fully reversed upon exposure to plain buffer. However, the layer was cross-sensitive to cyanide concentrations higher than 1 mM at pH 4.9, but such high amounts of hydrogen cyanide are not expected to be present in beverages.

In order to elucidate the mechanism responsible for the colour changes of the chromogenic aldehyde with bisulfite, a membrane layer with enhanced amount of **CR-514** in plasticised PVC (**M2**) was investigated *via* FT-IR spectroscopy. The layer was fixed in the photometer and measured before and after exposure to aqueous bisulfite solutions. The spectra showed that in the case of 30 mM buffered bisulfite, the carbonyl vibration vanished almost completely at 1699 cm^{-1} and was recovered again upon exposure to plain buffer, clearly indicating the reversibility of the chemical interaction.

In summary, chromoreactands are known to offer the possibility for detecting electrically neutral analytes with significant absorbance changes in the visible spectral range. However, the present paper shows that chromoreactands can also be used for the detection of ionic species such as bisulfite that performs a reversible chemical reaction with the formyl group of the indicator dye. The resulting sensor layers exhibit good operational and shelf life, especially if compared to enzymatic sensors. Although a chemical reaction is responsible for the selective recognition process, the response is relatively fast. These findings are generic in that other analytically relevant anions such as cyanide or nitrite may also be detected *via* chemical reactions in polymer layers and these are under current investigation in our laboratory.

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Notes and references

† 4-*N*,*N*-Dioctylamino-4′-formyl-2′-nitroazobenzene (**CR-514**) was obtained by diazo-coupling 4-formyl-2-nitroaniline[6] to *N*,*N*-dioctylaniline[5] in acetic acid as the solvent at 10 °C for 3 h. The resulting lipophilic formylazo dye **CR-514** was precipitated by adding aqueous sodium acetate, extracted into dichloromethane and dried over magnesium sulfate. The azo dye was then purified on silica gel using dichloromethane/hexane (4 : 1) as the eluent. ^{1}H NMR (250 MHz, $CDCl_3$) of **CR-514**, δ (ppm): 10.08 (s, 1H), 8.29 (s, 1H), 8.08 (d, 1H), 7.86 (m, 3H), 6.68 (d, 2H), 3.40 (t, 4H), 1.65 (m, 4H), 1.31 (m, 20H), 0.88 (t, 6H). Analysis: Calculated for $C_{29}H_{42}N_4O_3$ (494.68): C, 70.41; H, 8.56; N, 11.33. Found: C, 70.28; H, 8.44; N, 11.24%; mp 78–80 °C. λ_{max} (MeCN): 514 nm, ε_{514} = 39200 M^{-1} cm^{-1}.

Membrane **M1** was obtained by dissolving 1.0 mg of the chromoreactand **CR-514** together with 1.2 mg of dioctadecylmethylamine, 40 mg of the plasticizer 2-nitrophenyloctyl ether and 80 mg of PVC in 0.75 ml of tetrahydrofuran, and spin-coating 0.3 ml of the solution on a rotating glass plate (serving as a mechanical support for the sensor layer). The resulting sensor layer with a thickness of 3–5 μm was placed in ambient air for drying. Then, the sensor layer was fixed in a home-made flow-through cell.[5] The measurements were performed by placing the flow-cell in the spectrometer and pumping the sample solutions through the cell at a flow rate of 1.7 ml min^{-1} using a peristaltic pump. The absorbance spectra were recorded on a Lambda 16 UV-VIS spectrometer (Perkin Elmer) at 25 ± 2 °C.

M2 was prepared by dissolving 1.0 mg of **CR-514**, 1.2 mg of dioctadecylmethylamine, 16 mg of PVC and 32 mg of the plasticiser in 0.3 ml of tetrahydrofuran, and pipetting 0.05 ml on a glass disk to give a layer of 1 cm diameter. The layer was peeled off and fixed in the FT-IR photometer. Anion solutions were obtained by dissolving the appropriate amount of each analyte in 0.1 M citrate buffer adjusted to pH 4.9 by addition of sodium hydroxide solution. Due to the pH of 4.9, the investigated sodium sulfite was present in the chemically reactive bisulfite form. Bisulfite solutions were prepared freshly and used within 6 h.

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