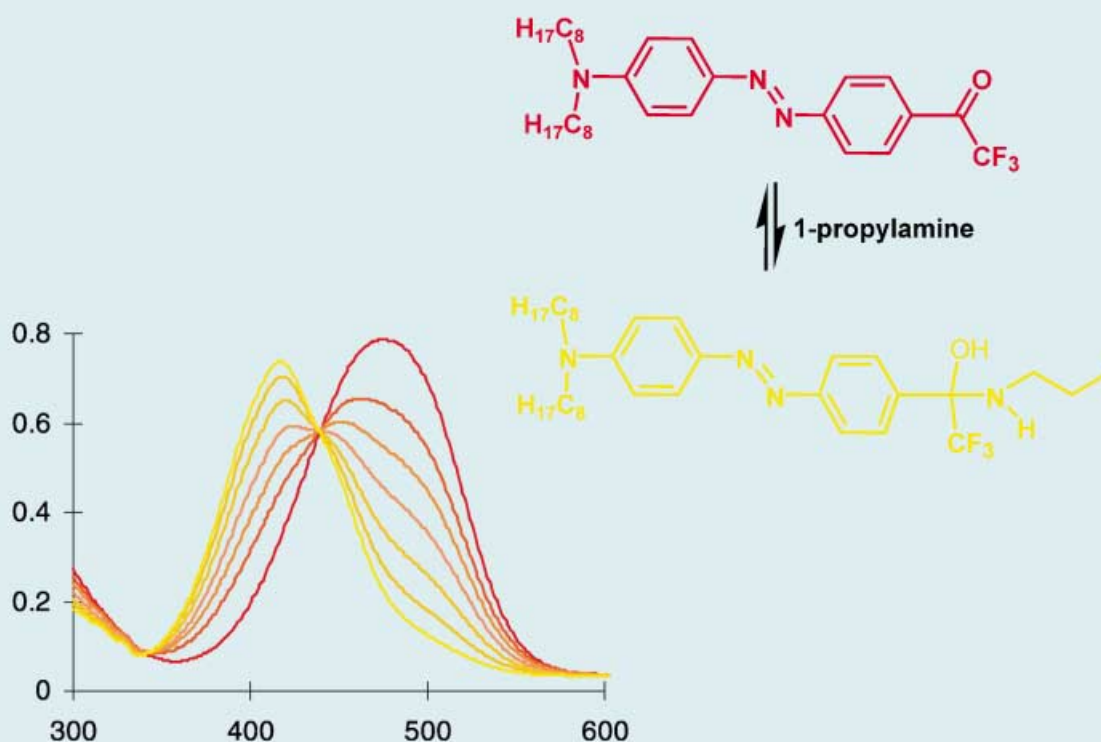


Synthetic Receptors for the Recognition and Detection of Neutral Analytes by Reversible Chemical Reactions



Reactands for neutral analytes
(analyte recognition by formation of a covalent bond)



Chromo- and Fluororeactands: Indicators for Detection of Neutral Analytes by Using Reversible Covalent-Bond Chemistry

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Abstract: Chromo- and fluororeactands are indicator dyes that allow the optical detection of electrically neutral analytes. Unlike complexing agents such as calixarenes, cyclodextrines or cyclophanes, reactands form a reversible covalent bond with the analyte molecule. This chemical reaction causes strong changes in absorbance or fluorescence. In this article reactands for analytes such as amines, alcohols, aldehydes, saccharides, carbon dioxide and sulfur dioxide are presented. Methods to enhance the sensitivity of the reactands as well as the operational and shelf lives of the corresponding optical sensors are discussed.

Keywords: dyes/pigments • fluorescence • reactand • sensors

Detection of neutral analytes

The detection of electrically neutral analytes through chemical and biochemical sensors is still a demanding task, since there are few ligands and indicator molecules that can recognise neutral molecules.^[1,2] A possible approach to continuous monitoring of neutral analytes such as alcohols, amines, thiols, aldehydes and the corresponding biomolecules with such functional groups is to use enzymatic conversion of analyte molecules and amperometric, potentiometric or optical detection. A limitation of enzymatic sensors is that their operational and shelf lives can be short and their performance is largely dependent on temperature, pH and inhibitors. Therefore, neutral analytes have been detected by ligands such as crown ethers, cyclodextrines or calixarenes.^[3,4] The recognition is based on hydrogen bonding, van der Waals interactions or hydrophobic interactions within ligand cavities, while the transduction of the recognition is then based on

mass changes, changes in conductivity or, for optical sensors, the analyte induced motion of fluorescent reporter molecules in/out of the cavity of the ligand.^[5,6] While there is good sensitivity for various analytes such as alcohols, terpenes and steroidal compounds the practical use of these ligands is somewhat limited by the reporter molecules, which usually have excitation wavelengths in the UV spectral range.

There have been several attempts to use chemical reactions between dyes and analyte molecules for analyte recognition (Figure 1). Changes in optical properties due to a change in the electron delocalisation within the dye molecule or due to photoinduced electron transfer were evaluated for the detection of saccharides by James et al., for ethanol by Simon et al. and for aldehydes by Narayanaswamy et al.^[7,8,9] Motivated by this research, reversible chemical reactions as a means to detect electrically neutral analytes have been investigated in more detail and new functional materials with improved performance have been developed. The results of this investigation, that is, adjustment of sensitivity and selectivity to practical requirements, spectral compatibility to small and cheap light sources and detectors, and enhanced shelf life and operational lifetime, are presented in this paper. Furthermore, related research of others and possible new materials and applications are discussed.

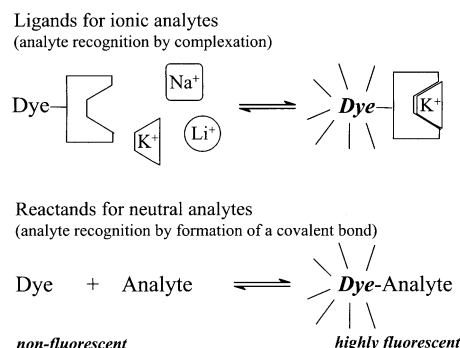


Figure 1. Principle of analyte recognition of ionic and neutral analytes using chromogenic and fluorogenic ligands and reactands. Apart from an increase in fluorescence upon interaction with the analyte, changes in absorbance can be observed as well.

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Detection of Neutral Analytes by Chromo- and Fluororeactands

Trifluoroacetyl reactands: The chromoreactand 4-*N,N*-di-octylamino-4'-trifluoroacetylazobenzene (**ETH^T 4001**) was the first dye in a series of trifluoroacetyl reactands that was synthesised and investigated for its chemical reaction with neutral analytes (Table 1).^[10,11] The chromoreactand is capa-

ble of performing a reversible chemical reaction with nucleophilic species, such as aliphatic and aromatic amines, alcohols and even with water (Table 2). The trifluoroacetyl group reacts with amines to give hemiaminals, with alcohols to give hemiacetals and with humidity to give diols. The trifluoroacetyl group is a strong electron acceptor (comparable in strength to a nitro group), while the *N,N*-dioctylamino group is a strong electron donor. The resulting electron de-

Table 1. Chemical structures of reactands and corresponding analyte functions, and absorbance maxima in different polymers and solvents before and after chemical reaction of the reactands with the respective analyte function.

Analyte function	Chromogenic or fluorogenic reactand	Solvent ^[a]	λ_{max} , [nm] before/after reaction	
-OH (humidity, primary, secondary, tertiary alcohols, phenols)		ETH 6022 ^[8]	PVC/DOS	decrease in absorbance at 305
-NH ₂ (primary, secondary and tertiary amines, aniline)		ETH ^T 4004 ^[10]	PVC/DOS	453 → 373
-SH		ETH ^T 4001 ^[10]	PVC/DOS	497 → 424
		CR-546 ^[18]	PVC/NPOE	556 → 482
		CR-573 ^[31]	PVC/NPOE	577 → 546
-NH ₂ (primary and secondary amines), OH		CR-593 ^[18]	PVC/NPOE	642 → 468
-NH-NH ₂ (hydrazines)		[20]	Sol-gel (TEOS)	increase in absorbance at 460
HSO ₃ ⁻ , SO ₂ , CN ⁻ , HCN		CR-514 ^[19]	PVC/NPOE	524 → 488
		CR-590 ^[18]	PVC/NPOE	582 → 524
cis-diols (glucose, fructose, mannose)		[24]	methanol/water (1:2, w/w)	564 → 509
-CHO (formaldehyde, acetaldehyde, acrolein, etc.), CO ₂		J-57 ^[26]	PVC/TOP	increase in fluorescence at 534, 576 and 613
		CR-418 ^[28]	PVC/NPOE	438 → 449
		pararosanine ^[9]	XAD-resin	increase in absorbance at 560
CO ₂		[29]	DMSO	increase in fluorescence at 380

[a] PVC: poly(vinyl chloride); DOS: bis(2-ethylhexyl)sebacate; NPOE: 2-nitrophenyloctyl ether; TOP: tris(2-ethylhexyl)phosphate; DMSO: dimethyl sulfoxide; TEOS: tetraethoxysilane.

Table 2. Chemical reactions encountered with different types of functional reactand groups (R=chromophore or fluorophore, R¹, R²=H, alkyl, aryl).

Chemically reactive group	Analyte	Product	Reaction
			hemiaminal formation
			hemiacetal or diol formation
			I-amino-2,2-dicyanovinyl formation
			bisulfite addition reaction
			cyclic boronate ester formation
			hemiaminal formation
			carbamic acid formation

localisation along the azobenzene chromophore results in an absorbance that maximises at around 500 nm. The conversion of the trifluoroacetyl group into a hemiaminal, hemiacetal or diol causes a significant decrease in acceptor strength and, consequently, the product of the chemical reaction absorbs at around 420 nm.

By simply dissolving the reactand in thin layers of plasticised PVC, optical sensor layers are obtained. Figure 2 shows the colour change of such a sensor layer upon exposure to aqueous 1-butylamine, and the analyte dependent colour change from red to yellow. This colour change is fully reversible after exchange of aqueous 1-butylamine with

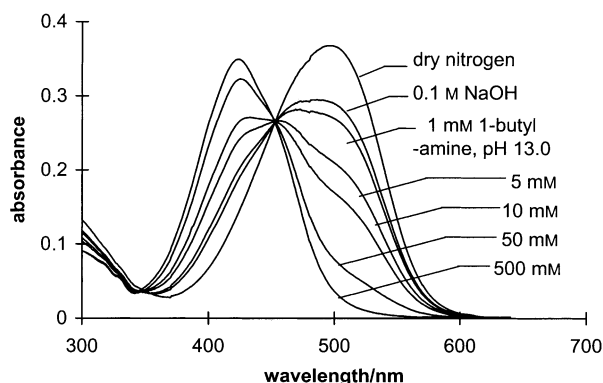


Figure 2. Absorbance spectra of a sensor layer composed of **ETH^T 4001**, PVC and bis(2-ethylhexyl)sebacate in contact with dry nitrogen and different concentrations of aqueous 1-butylamine at pH 13.0. When changing from nitrogen to 0.1 M sodium hydroxide solution, the diol is formed, whereas, when changing from plain buffer to aqueous 1-butylamine, the hemiaminal is formed. Both types of reactions are fully reversible.

plain buffer. The conversion of the trifluoroacetyl form of the reactand is also visible in the infrared spectral range in that the carbonyl vibration at around 1700 cm⁻¹ completely disappears upon interaction with amines and fully recovers after exposure to plain buffer. This confirms that the sensor membrane responds by means of a chemical reaction.^[12] The response time of the sensor layers is in the range of 5–15 min; this shows that reversible chemical reactions (and consequently the formation and breaking of covalent bonds) can proceed quite fast at room temperature.

The selectivity in the recognition process of reactand-based polymer sensor layers using chemical reactions is different to the selectivity encountered with complexing agents. Whereas Coulomb, van der Waals and hydrophobic interactions are re-

sponsible for the selective recognition of the analyte by a ligand or ionophore, reactands provide selectivity through their different chemical reactivity (nucleophilicity, electrophilicity) towards interfering species. Generally, the response of sensor layers based on reactands to analyte molecules is governed by two parameters, namely 1) different lipophilicity of the analyte and 2) the different nucleophilicity or electrophilicity of the analyte. More lipophilic analytes are more efficiently extracted from the aqueous phase into the organic membrane phase. This extraction process is described by the *n*-octanol–water partition coefficient.^[13] The different nucleophilicity/electrophilicity of analytes additionally contributes to the selectivity pattern. The selectivity of a sensor layer composed of 1% of **ETH^T 4001**, 33% of PVC and 66% of DOS for primary, secondary and tertiary amines is shown in Table 3. The overall equilibrium constants *K*^{opt} and the log*K*_{OW} values indicate that the sensor exhibits highest sensitivity for lipophilic primary amines. Although the lipophilicity of secondary and tertiary amines lies within the same range, the response is significantly lower. This difference is attributed to the fact that secondary and tertiary amines are sterically hindered in approaching the trifluoroacetyl group by their bulky alkyl groups. A similar discrimination is observed for primary amines with bulky substituents, such as *tert*-butylamine or 2-propylamine. The above sensor layer also responds to alcohols when a basic catalyst is added to the membrane composition,^[11] and to humidity when using highly hydrophilic polymer matrices.^[14] Nevertheless, the sensitivity toward alcohols and humidity is rather small because of their lower nucleophilicity compared to amines.

Table 3. Apparent equilibrium constants K^{opt} of the sensor layer based on **ETH¹ 4001**/PVC/DOS for amines and alcohols calculated according to reference [10], and $\log K_{\text{OW}}$ values of the corresponding analytes.^[13]

Analyte	K^{opt} [M^{-1}]	$\log K_{\text{OW}}$
ammonia	0.35	[a]
methylamine	2	-0.57
ethylamine	4.9	-0.13
diethylamine	1.7	0.58
triethylamine	6	1.45
1-propylamine	20	0.48
2-propylamine	1.2	0.26
1-butylamine	70	0.86
<i>tert</i> -butylamine	1.2	0.40
1-hexylamine	600	2.06
pyridine	1.5	0.65
aniline	2	0.90
ethanol	0.11	-0.30
1-propanol	0.35	0.25
amphetamine	70	1.76
methamphetamine	90	2.07

[a] Data not available.

Tricyanovinyl reactands: Azobenzene dyes with tricyanovinyl groups as acceptors and dialkylamino groups as donors are among the longest-wavelength-absorbing monoazo dyes.^[15] The tricyanovinyl group exhibits a Hammett substituent constant σ_p of 0.98 which is significantly higher than that of the nitro group (σ_p of 0.78) or of the trifluoroacetyl group (σ_p of 0.80).^[16] The absorbance maximum of 4-*N,N*-diocetylamino-4'-tricyanovinylazobenzene (**CR-593**) in PVC plasticised by 2-nitrophenyloctyl ether is located at 642 nm. Interaction with aqueous 1-propylamine causes the long-wavelength absorbance to decrease until the tricyanovinyl group is completely converted into a 1-propylamino-2,2-dicyanovinyl group with an absorbance maximum at around 470 nm.^[17] Since hydrogen cyanide is released into the aqueous solution during the chemical reaction, the colour change is irreversible. Again the chemical reaction in the layer can also be evaluated by means of IR spectroscopy, because the interaction of primary amines with **CR-593** causes the formation of a broad amino valence vibration at 3334 cm^{-1} with a shoulder at 3270 cm^{-1} . Simultaneously, the nitrile valence vibrations of **CR-593** at 2231 cm^{-1} with a shoulder at 2223 cm^{-1} are shifted to a single band at 2211 cm^{-1} . The selectivity of the sensor layers towards amines is comparable to trifluoroacetyl reactands, because the chemical reactivity of the tricyanovinyl moiety and the nucleophilicity of the analyte is similar.^[18] Although the sensor layers are not applicable for a continuous monitoring of amines, they may be applied as optical test strips indicating, for example, food quality (volatile amines are released during degradation of meat and fish).

Aldehyde reactands: Aldehydes are known to interact with hydrogen sulfite by forming a bisulfite adduct. This reaction may be employed for the development of optical sensors if the aldehyde derivative exhibits absorbance in the visible spectral range (Figure 3). The chromoreactand 4-*N,N*-diocetylamino-4'-formyl-2'-nitroazobenzene (**CR-514**) dissolved in plasticised PVC shows a colour change from pink to orange (corresponding to a change in absorbance maxi-

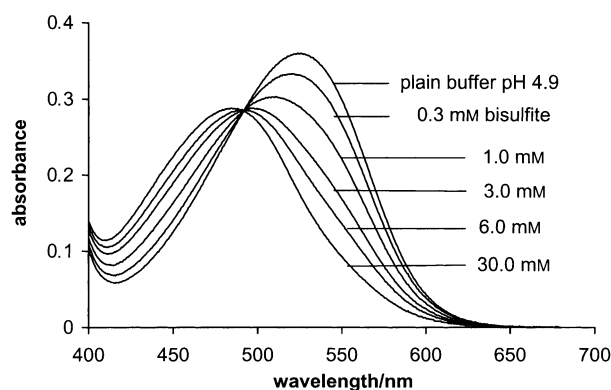


Figure 3. Absorbance spectra of **CR-514** embedded within a plasticised polymer layer in contact with plain citrate buffer and buffered solutions of bisulfite, all at pH 4.9. A similar optical response is observed for aqueous sulfur dioxide.

mum from 524 to 488 nm) when exposed to aqueous bisulfite solution.^[19] Similar to the reaction of trifluoroacetyl reactands, the carbonyl vibration vanishes almost completely at 1699 cm^{-1} and is recovered again upon exposure to plain buffer, clearly indicating the reversibility of the chemical interaction. The response time for both forward and reverse response is in the range of 10–20 min.

When covering the sensor layer with a microporous PTFE layer permeable only to gaseous but not to ionic species, then the detection of sulfur dioxide in aqueous solution becomes feasible as well.^[18] Under the experimental conditions (sulfur dioxide is present in aqueous solution only under acidic conditions due to its $\text{p}K_a$ of 1.8) there is neither cross-sensitivity to amines (because they are protonated), nor to alcohols or acetic acid. Consequently, the sensor layers can be applied for the detection of sulfur dioxide in beverages. A similar response to bisulfite/sulfur dioxide is also observed for the chromoreactand 2-(4-*N,N*-diocetylaminophenylazo)-4-chloro-5-formylthiazol (**CR-590**), whose absorbance maximum is at longer wavelengths and is shifted from blue to pink upon interaction with sulfur dioxide (maxima at around 580 nm and 520 nm, respectively). Preliminary experiments have shown that there is also an interaction of aldehyde reactands with cyanide/hydrogen cyanide, but this interaction was found to be irreversible.^[18]

A sensor for the detection of hydrazine makes use of the reaction of 4-*N,N*-dimethylaminobenzaldehyde with hydrazine to form a coloured benzalazine, which has its absorbance maximum at around 460 nm in sol-gel glass.^[20] The aldehyde is either physically entrapped or covalently immobilised in the matrix. The forward response with aqueous hydrazine is in the range of 15–120 min and regeneration is necessary in order to recover initial absorbance values. This is achieved by heating the sensor to 50°C in 1 M nitric acid.

Boronic acid reactands: A wide range of boronic acid derivatives of chromophores and luminophores for the detection of diols has been described recently, mainly by Shinkai and James et al.^[21] Specifically, the interaction between boronic acids and saccharides (i.e., the formation of a cyclic boronate ester) has been used to create a photoinduced electron-

transfer (PET) sensory system. The interaction of the boronic acid (Lewis acid) and a neighbouring tertiary amine (Lewis base) is strengthened on saccharide binding. The strength of this boronic acid/tertiary amine interaction modulates the PET from the amino nitrogen to the fluorophore. Consequently, the indicator molecule shows increased fluorescence through suppression of the PET from nitrogen to the fluorophore on saccharide binding.^[22]

The selectivity of the indicator dyes for saccharides is governed by the structural rigidity of the vicinal diols and is highest for D-fructose, followed by D-galactose, D-glucose, D-mannose and ethylene glycol. However, when attaching two boronic acid moieties with correct spacing onto one fluorophore, then the pre-organised system exhibits enhanced selectivity for D-glucose over D-fructose. This is due to the fact that D-glucose forms 1:1 cyclic complexes with diboronic acids, while D-fructose tends to form 2:1 acyclic complexes.^[23]

James et al. have also described an azobenzene dye in which changes in absorbance are caused by the change in acidity of the boronic acid (and thus the aniline moiety of the azobenzene dye in close proximity) upon interaction with saccharides and the subsequent effect on the electron delocalisation within the azobenzene chromophore.^[24] The colour change with D-glucose in methanol/water (1:2) is from purple to red (corresponding to a shift in absorbance maximum from 564 nm to 509 nm). Although the dye has not been investigated in polymer layers, it is certainly an interesting candidate for glucose sensing. Optical sensor layers for saccharides based on boronic acid moieties have been prepared by Pringsheim et al., who used polyaniline boronic acid derivatives for sensing of fructose in aqueous solution.^[25] Colour changes are in the NIR spectral range with maximum signal changes between 650 nm and 750 nm. The layers are fully reversible although a slow response has been reported.

Amino reactands: Chromogenic and fluorogenic reactands with chemically reactive amino groups such as *N*-amino-*N'*-(1-hexylheptyl)perylene-3,4:9,10-tetracarboxylbisimide (**J-57**) can be used for the detection of aldehydes and ketones. The lone pair of the amino nitrogen quenches the fluorescence of the perylene dye, but when the amino group, upon reaction with aldehydes/ketones, is converted into a hemiaminal the quenching of fluorescence is reduced and an increase in fluorescence takes place.^[26] Consequently, the optical transduction of the recognition process is comparable to the detection of saccharides, because changes in photoinduced energy transfer are observed. The fluorescence of sensor layers based on **J-57** in plasticised PVC increases at the emission maxima of 534 nm, 576 nm, and 613 nm when exposed to aqueous aldehydes (Figure 4). A significant effect of sample pH on the response time of the sensor layers is observed, indicating acid catalysis for the chemical interaction of the reactand with aldehydes in the membrane. The response time in changing from plain buffer to 137.4 mM propionaldehyde and vice versa is in the range of 2–6 min at pH 2.0, 15–45 min at pH 4.0 and 50–180 min at pH 6.0. However, the magnitude of the signal changes is not affected by pH. The selectivity pattern is comparable to that already observed with sensors for amines and alcohols based on tri-

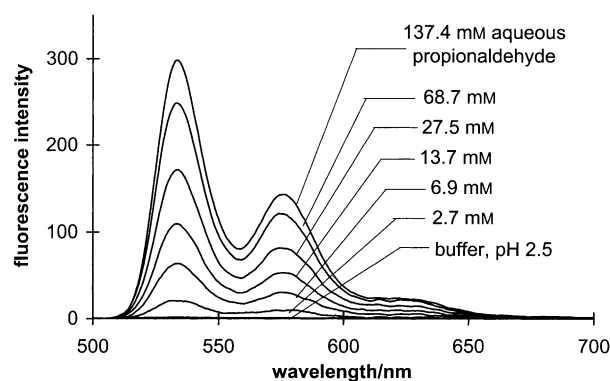


Figure 4. Fluorescence emission spectra of a sensor layer composed of plasticised PVC and *N*-amino-*N'*-(1-hexylheptyl)perylene-3,4:9,10-tetracarboxylbisimide in contact with plain buffer and buffered solutions of propionaldehyde, all at pH 2.5. The excitation wavelength was set to 485 nm.

fluoroacetyl dyes. Again a dependence of the response on the lipophilicity of the respective aldehyde is found in that highest sensitivity is observed for butyraldehyde, followed by propionaldehyde, acetaldehyde, glutaraldehyde, formaldehyde and glyoxal. The sensitivity to ketones is negligible, because they are less electrophilic and have sterically demanding structures. A similar selectivity pattern was also observed by Yang et al. who used 3,3',5,5'-tetramethyl-*N*-(9-anthrylmethyl)benzidine in plasticised PVC as the reactand for aqueous aldehydes.^[27]

Narayanaswamy et al. have obtained an optical colour sensor for formaldehyde by immobilizing pararosanine hydrochloride on cellulose phosphate.^[9] The reflectance changes of the resulting resin particles towards aqueous formaldehyde were largest at around 560 nm with forward response times in the range of 6–15 min, but with no reversibility of the reaction. The particles were responsive only to formaldehyde and unsaturated aldehydes, such as crotonaldehyde and acrolein, while acetaldehyde and butyraldehyde did not produce any signal changes.

The chromoreactand 1-amino-4-(4-decylphenylazo)naphthalene (**CR-418**) exhibits a comparable selectivity pattern to pararosanine in that it also shows higher sensitivity to formaldehyde than to acetaldehyde or propionaldehyde.^[28] The sensor membrane based on **CR-418** responds to aqueous formaldehyde in the 1–100 mM range with a limit of detection of 0.2 mM. Unfortunately, the response time of this sensor layer, even if acid-catalysed, is much slower than in the case of the perylene dye and is in the range of 15–20 min for the forward reaction, but 120–150 min for reverse response at pH 3.0. Furthermore, colour changes are comparably weak relative to the other reactands with shifts in maxima from 438 nm to 449 nm in plasticised PVC. The response to aqueous formaldehyde is due to the formation of a hemiaminal, as indicated by the IR spectral changes, because the primary amino group of the reactand (3480 cm^{-1} , 3378 cm^{-1} , and 3248 cm^{-1}) is converted into a secondary amino group (3410 cm^{-1}) and the vibration at 1630 cm^{-1} disappears.

Rudkevich et al. have presented an interesting approach to the detection and sensing of carbon dioxide based on the

reversible reaction of the amino group of 1-methylaminopyrene or 1-aminomethylnaphthalene with carbon dioxide to form carbamic acid in polar aprotic organic solvents.^[29] As a consequence of the chemical reaction a strong fluorescence enhancement at 336 nm for the naphthalene derivative and at 380 nm for the pyrene derivative is observed. This is due to the fact that PET from the lone pair of the nitrogen atom is smaller after the formation of carbamic acid. The authors have covalently linked the fluorophores to different polymer surfaces in order to allow continuous monitoring of carbon dioxide. This approach is a new step toward stable sensors for carbon dioxide that are less prone to drift than optical sensors based on pH indicator dyes in combination with chemically instable quaternary ammonium hydroxides.^[30]

Improving sensitivity and spectral properties of reactands:

The synthesis of azo and stilbene dyes is usually very convenient and straightforward, because the dyes are synthesised from highly functional reactants to give multifunctional products. In the case of azobenzene dyes, anilines are easily synthesised to exhibit chemically reactive functions and different acceptor or donor moieties, while the coupling components exhibit functional groups for dissolving reactands in various solvents, or allow covalent coupling.^[12]

When the trifluoroacetyl reactand **ETH^T 4001** was used for the detection of ethanol in beverages,^[11] the absorbance changes of the reactand at around 500 nm were compromised by the intrinsic absorbance of red wine. Only after decolourisation with active carbon was it possible to correctly quantify ethanol concentration. As a consequence, reactands with absorbance (or fluorescence) at longer wavelengths are required for real measurements. The possibility to synthesise bisazo as well as monoazo dyes has resulted in the preparation of the dye 4-[4-(4-trifluoroacetylphenylazo)-1-naphthylazo]-*N,N*-dioctylaniline (**CR-573**). This reactand is obtained by increasing the length of the chromophore by the insertion of a naphthylazo moiety and has its absorbance maximum at around 570 nm.^[31] More importantly, the signal changes with amines and alcohols are largest at around 630 nm. The dye is also more sensitive than the monoazo dye, because the trifluoroacetyl group is chemically more reactive. In the case of the bisazo dye the bisazonaphthalene moiety in the *para*-position to the trifluoroacetyl group is a stronger electron acceptor than the 4-*N,N*-dioctylaminophenylazo group of the monoazo dye. Consequently, the chemical reactivity of the trifluoroacetyl group is enhanced.

The chemical reactivity can further be improved by introducing a nitro group in *meta*-position to the trifluoroacetyl group of the monoazo dye.^[18] The resulting *N,N*-dioctylamino-4'-trifluoroacetyl-2'-nitroazobenzene (**CR-546**) exhibits a sensitivity that is 20-fold higher than that of the reactand without the nitro group (Table 1). Furthermore, the absorbance is shifted by around 40 nm to longer wavelengths, making the sensor membranes compatible with the green LED as the light source for a miniaturised sensor device. The colour change from purple to orange upon interaction with amines corresponds to a shift in maximum from 556 nm to 482 nm.

In a similar manner, the chromoreactand 4-*N,N*-dioctylamino-4'-formyl-2'-nitroazobenzene (**CR-514**) rather than 4-*N,N*-dioctylamino-4'-formylazobenzene is used for the detection of bisulfite/sulfur dioxide. Not only is the sensitivity of 4-*N,N*-dioctylamino-4'-formylazobenzene significantly smaller (more than tenfold), but also its absorbance maximum (around 470 nm in plasticised PVC compared to 524 nm of **CR-514**) is at significantly shorter wavelengths.^[18]

Improving operational and shelf lives of sensor materials: In order to provide sensors with a fast response (and analyte diffusion), mostly plasticised polymers are used as the polymer matrix for optical sensor layers. This practice is founded on the use of plasticised polymers in potentiometric sensors, where similar to optical sensors, ligands and ionophores are dissolved in polymer layers. However, electrode membranes are usually quite thick (up to mm) because the aqueous-organic interface and not the whole bulk of the layer is responsible for the potentiometric signal. Optode membranes have to be thin, because the whole layer reacts with the analyte, and, consequently, for reasons of fast response, optode layers often have a thickness of only 2–10 μm . As a result, leaching and evaporation of components (especially of plasticisers) is a severe problem for optical sensor layers.

One approach to more stable sensors is to use plasticiser-free polymers with low glass transition temperatures such as polysiloxanes, polyurethanes, poly(alkylmethacrylates), etc.^[32] However, the search for appropriate polymer materials is very much a case of trial and error, because it is not possible (unlike in the case of using plasticisers for PVC) to tailor the properties of plasticiser-free materials.^[33] Furthermore, the solubility of such polymers in organic solvents (in order to allow spin-, dip- or spray-coating) is often rather poor as well as is the solubility of ligands and dyes in these polymers.

An alternative is to develop materials in which all components (polymer, plasticiser and indicator dye) are covalently linked to each other. Such materials, namely copolymers made of acrylates and reactands, can be prepared by using reactands with a methacrylate group attached to the *N*-alkyl chain (e.g., **ETH^T 4012**, and **4014**^[34,35]) and co-polymerising them with methyl methacrylate and butylacrylate.^[36] The response behaviour of such membranes incorporating reactands linked to the polymer is comparable to the structurally related reactand dissolved in plasticised PVC. However, covalently immobilizing the reactands causes response times to be 2–5 times slower. This indicates that both chemical reactivity/mobility and diffusion are affected by covalently linking the reactand to a more rigid polymer backbone rather than dissolving it in a plasticised "solvent polymeric membrane". In addition, covalent immobilisation of the receptor to the polymer backbone may also affect the selectivity of the recognition process. Accordingly, James et al. observed a decrease in selectivity of a diboronic acid fluorophore immobilised on a poly(styrene-co-maleic acid) partial isopropyl ester for D-glucose over D-fructose.^[37] They assumed the close proximity of the receptor molecule to the polymer backbone to be responsible for the difference in se-

lectivity compared to the dye molecule dissolved in aqueous methanol.

However, the copolymer-based sensor layers exhibit several advantages over PVC-based membranes, namely: 1) all components are covalently linked to each other and thus no leaching is observed; 2) due to the covalent linkage, no crystallisation, migration or aggregation of the reactands is found; and 3) the copolymers do not require plasticisers, thus enhancing the operational and shelf lifetimes. The polarity of copolymer membranes (usually composed of alkyl acrylates) can easily be tailored by making use of 2-cyanoethyl, *N,N*-diethylaminoethyl or 2-alkoxyethyl derivatives of acrylates and methacrylates.

Implications for future research: Indicator dyes that perform reversible chemical reactions with analyte molecules are highly promising candidates for the preparation of molecularly imprinted polymers.^[38] First, the covalent pre-organisation between dye and analyte guarantees a large content of highly specific recognition sites in the imprinted polymer. Second, the chemical interaction between dye and analyte causes significant changes in absorbance or fluorescence. However, the chemical preparation of such reactands is challenging, because the reactands not only have to have absorbance preferably at wavelengths above 500–600 nm, but also have to yield strong signal changes with analyte molecules and must have functional groups for covalent attachment to the imprint polymer (Figure 5).

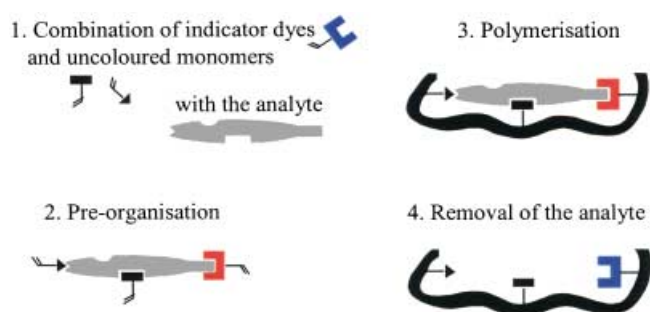


Figure 5. Preparation of molecularly imprinted polymers using reactand monomers for reversible chemical reactions and uncoloured functional monomers for additional noncovalent interactions.

Wang et al. have already shown the feasibility of combining reactands with imprinted polymers by preparing fructose-imprinted particles through polymerisation of an anthracene boronic acid derivative with 2-hydroxyethylmethacrylate and ethylene glycol dimethacrylate as co-monomers.^[39] The formation of a cyclic ester of the anthracene boronic acid with fructose causes significant increases in luminescence. Unfortunately, the absorbance of the anthracene dye is in the UV region; this makes the sensor incompatible with optical fibers and light-emitting diodes. Furthermore, the analyte-induced changes in fluorescence may be compromised by intrinsic sample absorbance and autoluminescence.

In a different approach, Zimmerman et al. have prepared imprinted polymer materials with flexible cavities by using cross-linkable dendrimers with chromogenic trifluoroacetylazobenzene moieties as reporters of the analyte recognition.^[40] They have prepared polymer materials with two recognition sites in one cavity in order to selectively detect diaminoalkanes and have observed significantly enhanced sensitivities compared to monoaminoalkanes.

Electronic noses and tongues frequently are based on commercially available polymers and their different physical response to the enrichment of analyte molecules (i.e., changes in temperature, mass, conductivity); these are detected by the respective physical microsensors (quartz crystal microbalances, thermopiles, interdigital electrodes). The signal evaluation is performed by means of chemometric methods, because the polymers show rather unselective response to different analyte molecules. Reactands may overcome this limitation and may be used for the development of more selective and sensitive calorimetric and capacitive microsensors, because chemical reactions can provide changes in reaction enthalpy as well as in dipole moment.^[41]

Chemically reactive functional groups may also be introduced into conjugated polymers. The resulting materials might be used for the investigation of intra and intermolecular energy transfer (due to a change in the electron acceptor strength of the functional groups via chemical reactions) as well as for the preparation of supramolecular conjugates with chemically complementary macromolecules.

Conclusion

Chromogenic and fluorogenic reactands have introduced reversible chemical reactions into analytical chemistry. However, these multifunctional materials may also find application for the development of advanced electronic noses, functional liquid crystals, conjugated polymers or nanomaterials.

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