LUMINESCENT CHEMOSENSORS – ADVANCED TOOLS IN ANALYTICAL CHEMISTRY

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Abstract: Optical chemosensors represent smart analytical tools which become very popular in the last decades. They are advantageous due to the number of features such as non-invasiveness, absence of electromagnetic interferences, possibility of miniaturization, suitability for imaging etc. Optical chemosnesors are very versatile in their formats which include planar optodes, fiber-optic (micro)sensors, sensor paints, nanoparticles etc. This paper briefly reviews the main sensing principles and materials used in optical chemosensors and provides an overview of the novel luminescent chemosensors recently developed in the Institute of Analytical Chemistry and Food Chemistry in Graz University of Technology.

Luminiscenčni kemosenzorji – napredno orodje v analitični kemiji

Kjučne besede: luminiscentni kemosenzorji, napredno orodje, analitična kemija

Izvleček: Optični kemosenzorji predstavljajo pametno analitično orodje, ki je postalo priljubljeno v zadnjih desetletjih. Njihove prednosti so neinvazivnost, odpornost na elektromagnetne motnje, možnost miniaturizacije, ustreznost za slikanje itn. Optični kemosenzorji so zelo vsestranskih oblik kot so planarne optode, mikro senzorji na osnovi optičnih vlaken, senzorske barve, nanodelci.itn. V članku so na kratko opisani principi zaznavanja in uporabljeni materiali pri optičnih kemosenzorjih. Članek poda pregled novih luminiscentnih kemosenzorjev, ki so bili pred kratkim razviti na Inštitutu za analitično kemijo in prehransko kemijo na Tehnološki univerzi v Gradcu.

1 Introduction

Optical chemosensors became increasingly popular in the last decades /1-3/. They can be defined as devices capable of continuous monitoring of chemical or biological parameter by optically transforming the information into an analytically useful signal. Several features of optical chemosensors make them promising alternatives to other analytical techniques. Particularly, they are free of electromagnetic interferences, are non-invasive or minimally invasive, enable simultaneous measurements of several parameters, imaging over a surface or in volume, and, finally, they are very versatile and can be realized in a variety of formats. The most common sensor formats include planar foils, paints, fiber-optic sensors and microsensors and analytesensitive nano- and microparticles. The latter represent selfcontained analytical tools which can be used on their own (e.g. as an aqueous dispersion) or combined in a composite material, for example to enable multi-analyte sensing. Optical chemosensors rely on the use of indicators which respond to a particular analyte in altering their optical properties (absorption, emission intensity, decay time, anisotropy etc). The chemosensors based on luminescent indicators are particularly promising due their high sensitivity and versatility /1/. The requirements to the indicators include high selectivity to the analyte of interest, selectivity in the desired range, high luminescence brightness, preferably long Stokes' shifts and high photostability. However, the state-of-the-art indicators not always fulfill these requirements. Moreover, for many important applications indicators and sensors with tailor-made properties are required.

In this contribution we report the recent progress on optical chemosensors and novel sensing materials developed in our institute.

2 Results and discussion

2.1 Optical oxygen sensors

Oxygen belongs to one of the key analytes and its determination is of utmost importance in various fields of science and technology including biology, medicine, environmental monitoring, marine science etc., to mention only a few. Optical oxygen sensors rely on dynamic quenching of the luminescence of an indicator /4/. Upon the collision of an excited indicator molecule and molecular oxygen (in ground triplet state) the indicator is deactivated and the oxygen is promoted to the excited singlet state (Fig. 1). Since the singlet oxygen rapidly returns to the ground state, no consumption of the analyte occurs as e.g. in Clark electrode. In optical oxygen sensors the luminescence intensity and the decay time are directly related to oxygen concentration (Stern-Volmer equation).

Oxygen indicators are mostly represented by metal complexes which possess relatively long-lived luminescence (µs-ms). These mainly include ruthenium(II)-tris-polypyridyls and platinum(II) and palladium(II) porphyrins, but some



Fig. 1: Oxygen sensing scheme with an iridium(III) coumarin complex Ir(C_s)₂(acac) as an indicator

complexes of osmium(II), rhenium(I) or copper(I) were also reported. Unfortunately, the state-of-the-art complexes do not fully fulfill the requirements of luminescence brightness (defined as a product of molar absorption coefficient and luminescence quantum yield), photostability or spectral compatibility to the light sources (e.g. LEDs or laser diodes). Therefore, several novel indicators and oxygen sensing materials were developed in our laboratory to overcome these drawbacks.

2.1.1 Ultrabright oxygen optodes

These are based on the cyclometallated iridium(III) complexes with coumarin ligands (Fig. 1). Compared to conventional cyclometallated complexes the new indicators are distinguished by very high molar absorption coefficients in the blue part of the spectrum ($\epsilon \sim 90,000 \text{ M}^{-1}\text{ cm}^{-1}$ at 450-480 nm) and also possess high luminescence quantum yields approaching 60% /5/. This results in very high luminescence brightness which exceeds the brightness of the conventional indicators (ruthenium(II) polypyridyls and platinum(II) porphyrins) by ~10-fold (Table 1). Therefore, preparation of very thin fast responding optodes (response times < 1 s) became possible. The decay time of the complexes is ~ 10µs so that when embedded in polystyrene and similar materials the optodes show optimal sensitivity to oxygen at physiologically relevant concentrations.

Table 1: Photophysical properties of the iridium coumarin complex and two most common oxygen indicators

Indicator	λ_{abs} ,	ε,	QY	BS
	nm	$M^{-1}cm^{-1}$		
$Ir(C_s)_2(acac)$	472	92800	0.54	50000
Ru-dpp	463	28600	0.37	10600
PtTFPP	504	23200	0.2	4600

2.1.2 NIR-emitting oxygen indicators

The red-light excitable near-infrared emitting indicators and sensors are generally attractive for the following reasons: (i) they are less prone to interferences e.g. from backgroung fluorescence and light scattering; (ii) they can be compatible with cheap red-laser diodes and 632.8 nm line of the He-Ne laser (confocal imaging applications); (iii) they can be used for subcutaneous determination of oxygen - "smart tattoos" /6/. Since the optical glucose biosensors rely on oxygen transducers the NIR indicators can be used to subcutaneously monitor the glucose levels in blood (artificial pancreas) which may provide a convenient solution to diabetic patients. The state-of-the art NIR indicators included metal complexes of benzo- and naphoporphyrins /7-9/, Fig. 2. We developed platinum(II) and palladium(II) complexes with fluorinated benzoporphyrins which have improved photophysical properties (higher emission guantum yields and better photostability) /10,11/. Notably, these indicators can be excited both in the red (600-630 nm) and in the blue part of the spectrum (430-460 nm). The long Stokes' shift helps to eliminate the autofluorescence almost completely. Thus, measurements even in photosynthetically active biological media (which show intense chlorophyll fluorescence) become possible which makes the new materials especially attractive for a number of applications.

Additionally, molecular hybrids of benzoporphyrins and phthalocyanines were developed /12/. The novel azotetrabenzoporphyrin complexes occupy intermediate position between the two classes and combine the advantages of both which include high luminescence brightness, good solubility and extreme photostability. Particularly, photostability was found to increase by ~ 10 fold compared to the respective benzoporphyrins. Additionally, the platinum(II) and palladium(II) complexes possess bathochromically shifted absorption in the red part of the spectrum and become fully compatible with the red laser diodes (635 and 645 nm, respectively).

A palette of oxygen indicators with tailor-made spectral properties was also developed /13/. These represent the hybrids between benzo- and naphthoporphyrins. Both the absorption and the emission maxima are systematically tuned and shift bathochromically ~ 20 nm per each naphtho-group introduced in the porphyrin macrocycle. Thus, the excitation of the sensing materials can be performed at a preferred wavelength varying from 600 to 700 nm. The new indicators enable multiplexing – i.e. they can be separately excited to obtain several independent informations (e.g. oxygen and glucose concentration). Additionally, the benzo-naphthoporphyrin hybrids possess tunable decay times (which become shorter with naphthosubstitution) which makes possible to adjust the sensitivity to oxygen.

2.1.3 Trace oxygen sensors

Sensing of low oxygen concentrations is also of much interest in many areas of science and technology. For example, continuous monitoring of dissolved oxygen (DO) is essential in breweries since high oxygen concentrations negatively influence the taste of beer (typical concentrations of DO in beer are in the range of 10-50 ppb). In water boilers the concentration of DO is kept at ~ 7 ppb to prevent corrosion. Monitoring of ultra-low concentrations of DO is of



Fig. 2: Chemical structures of the state-of-the-art and novel NIR emitting oxygen indicators

particular interest in aquatic biology and oceanography. For example, some marine ecosystems such as oxygen minimum zones and other oxygen-deficient environments contain less than 70 ppb of DO and sometimes as low as 0.06 ppb. Notably, conventional luminescent oxygen sensors do not provide such high sensitivities and are more suitable for monitoring of oxygen at physiologically relevant conditions. The sensors for trace oxygen determination usually rely on highly gas permeable polymers but often suffer from solubility (such as e.g. in case of Teflon AF) and stability (e.g. in case of poly(1-trimethylsilyl-1-propine) issues. Therefore, development of new materials for trace oxygen sensors is still of high importance.

We present a new approach based on covalent coupling of the highly photostable commercially available oxygen indicators to micrometer-sized silica-gel beads /14/. The palladium(II) and platinum(II) complexes with *meso*-pentafluorophenyl porphyrin can be easily covalently immobilized onto amino-modified silica-gel particles. These particles are dispersed in silicone. Notably, covalent coupling prevents aggregation and migration of the dye into silicone and also enables high dye loading. As demonstrated, the novel materials are suitable for monitoring of oxygen traces in ppb range and below.

2.2 Optical temperature probes

It is well known that the performance of all optical chemosensors (e.g. for oxygen, carbon dioxide, ammonia, pH) is compromised by temperature. In fact, temperature has pronounced effect on the diffusion of species inside the polymeric matrix, on their solubility, equilibrium constants etc. Moreover, the luminescence properties of the indicators are also affected by temperature. Thermal quenching (decrease of the luminescence quantum yields and the decay times) can be often very pronounced, e.g. in case of ruthenium(II) polypyridyl complexes. Fig. 3 provides an example of temperature effects in case of an oxygen sensor (extreme case).



Fig. 3: Oxygen calibration plots for a ruthenium(II) complex Ru-dpp embedded in polystyrene.

Evidently, reliable measurements are only possible if temperature is kept constant or is known. This lead to development of so called "dual sensors" which are capable of simultaneous monitoring of temperature and a chemical parameter /15,16/. The probes employed in such composite materials respond to temperature in altering their luminescent properties (intensity and/or decay time) and therefore are advantageous to other methods of temperature measurements. For example, simultaneous imaging of both parameters on surfaces becomes possible. These luminescent temperature probes are mostly represented by ruthenium(II) polypyridyl complexes and europium(III)tris-b-diketonates. Thermographic inorganic phosphors are

also used but they commonly respond at rather high temperatures. The sensitivity of these phosphors at ambient temperatures is rather low (~0.2% decay time change per K). Often the brightness of such phosphors is not sufficient for practical applications. We realized a new thermographic phosphor, chromium(III)-doped yttrium aluminium borate (YAB) which overcomes these drawbacks /17/, Fig. 4. The phosphor is excitable in the blue and in the red parts of the spectrum and emits in the NIR. The luminescence decay time decreases with increasing temperature and the sensitivity approaches 1%/K. Moreover, the luminescence intensity remains virtually constant in this temperature range so that the S/N ratio is not influenced. Compared to the luminescent probes based on metal complexes, Cr(III)-doped YAB also benefits from high chemical and photochemical stability. The phosphor can be easily obtained in large quantities via solution combustion technique.



Fig. 4: Calibration plot for the new Cr(III)-doped YAB temperature phosphor. The inserts show the photographic images of the two phosphor forms.

Recently we demonstrated the application of the novel phosphor for temperature compensation of the pH chemosensors /18/. The composite material relied on the fluorescent red light-excitable pH indicator dissolved in a polyurethane hydrogel where the phosphor particles were also dispersed. The phosphor not only served as a temperature probe but also as a reference for the pH indicator with help of Dual Lifetime Referencing technique.

2.3 Luminescent nanochemosensors for various analytes

Nanosensors belong to smart analytical tools which became popular in the last decade /19,20/. They are rather small ($\Phi < 1 \mu m$ and more often <100 nm) and therefore respond to the analytes of interest virtually in real time. Many of them can be used for intracellular monitoring of important parameters. The nanosensors are simply dispersed in the analyzed media in which they behave similarly to dissolved indicators with notable exception that the indicator inside is protected from undesired interferences (e.g. from other analytes) and is much less prone to nonspecific binding. Many typical nanosensors are designed by immobilizing an indicator into polymeric nanoparticles, inorganic solgel particles or hybrid Ormosil materials. Notably, the indicator behaves similarly to the balk optodes since the material also serves a solvent and a support for the indicator, and as a permeation-selective membrane. Many other types of nanosensors exist (based on quantum dots, metal particles etc. /20/) but they are more or less limited to specific analytes.

Recently we developed a versatile material for designing optical nanosensors /21/. A block polymer of styrene and vinylpyrrolidone is used as a matrix. The polymer is commercially available in form of the nanobeads ($\Phi \sim 220$ nm) which in aqueous dispersion possess core-shell structure, Fig. 5.





As we have demonstrated this determines the unique stability of the particles which do not aggregate even at very high concentrations in presence of inorganic salts and other substances, and can be freeze-dried and redispersed afterwards. The lipophilic indicators are easily immobilized by swelling (the beads are swollen in the mixture of an organic solvent and water, an indicator is added and the organic solvent is slowly removed under reduced pressure) into the core or into the shell of the nanobeads. Particularly, immobilization into the core is performed from tetrahydrofurane:water mixtures, and the shell of the beads can be stained from ethanol:water. A palette of oxygen nanosensors was prepared by staining into the core, which possessed desirable spectral properties and sensitivity /22/. Temperature probes were also manufacturing by immobilizing a europium(III) complex into the core /21/. On the other hand, the nanosensors for pH and ions were prepared by immobilizing lipophilic indicators into the shell of the beads /21/. Particularly, the nanosesnors covering the dynamic ranges of interest (pH 5-9) were prepared and are likely to be suitable for applications in biotechnology, biology, medicine and marine science /23/.

On the basis of the oxygen nanosensors we designed the first water dispersible and washable Pressure Sensitive Paint (PSP) /24/. The state-of-the-art PSPs are widely used in aeronautics, aircraft and automobile industries for determining pressure distribution on the models. A relatively new technique relies on use of oxygen sensors to measure total pressure via oxygen partial pressure. In this case the whole model is usually imaged with a CCD camera. Unfortunately the materials used require expensive and environmentally harmful solvents for polymers and indicators. Our novel material represents an aqueous dispersion of dye-doped poly(styrene-block-vinylpyrrolidone) beads which is sprayable on various surfaces including e.g. aluminium. Notably, upon drying the dense mechanically stable layer is formed and enables imaging of oxygen partial pressure and temperature (in case of the temperature probes dispersed along with the oxygen-sensitive nanospheres). The layer can be removed simply by washing with water.

We also developed a simple method for preparation of optical nanosensors via precipitation /25/. Briefly, a polymer and an indicator are dissolved in organic solvent (miscible with water) and water is added to the solution. The size of the particles is determined by the nature of the polymer but can be also tuned by the concentration of the components, the solvents used, speed of water addition etc. This highly versatile technique works with many indicators and polymers. For example, nanosensors for oxygen, pH, temperature were manufactured from neutral, cationic and anionic polymers. The positively charged RI-100 beads, particularly, are rather small ($\Phi \sim 40$ nm) and are found to penetrate through the cell membranes to enable intracellular monitoring of various parameters.

2.4 Novel optical pH sensors

Most state-of-the-art optical pH sensors rely on use of fluorescent indicators such as fluoresceins, 8-hydroxypyrene-1,3,6-trisulfonate and naphthalimide derivatives. All these indicators have some disadvantages. Fluoresceins, particularly, are prone to self-quenching and possess low photostability. The brightness of the naphthalimides is not sufficient for many applications due to low molar absorption coefficients. Therefore, development of new materials with improved photophysical properties is still of much importance. Photoinduced electron transfer (PET) is widely used for designing pH and ion sensors (mainly on the basis of naphthalimides). We recently developed the new optical pH sensors based on perylene dyes and relying on PET. Perylenes are known as chemically and photochemically robust chromophores that possess high molar absorption coefficients and fluorescence quantum yields approaching 100%. The perylene structure can also be modified to enable dyes with different spectral properties. Moreover, NIR analogous (terrylenes) are available and, in principle, can be used to design pH sensors. We have shown that preparation of the pH sensors on the perylene basis is feasible /26/. The pKa values can be tuned over a broad range to enable different applications. This is achieved by varying the nature of amine substituent (primary, secondary or tertiary) responsible for PET. Covalent immobilization of the indicators also is possible and will be realized in future work.

2.5 Trace ammonia sensors

Ammonia is known to be very harmful for aquatic organisms even in very small quantities. Ammonia concentration is also an important parameter in biotechnology. Therefore, its precise monitoring is particularly important in many biological applications and in fish farming. Our group developed novel optical sensors for detection of trace ammonia. They rely on lipophilic xantene dyes (eosin and 2,7dichlorofluorescein) embedded into a gas permeable polymeric matrix /27,28/. The trace quantities in the ppb range can be reliably monitored. Referenced sensors based on nanoparticle materials also were prepared /29/.

2.6 Sensing materials with magnetic properties

Optical sensing materials with magnetic properties become increasingly popular in the last few years. Compared to the conventional materials they provide possibility of manipulation. For example, the sensors based on micro- or nanoparticles can be collected in the region of interest, manipulated there and easily removed from the analyzed media with magnetic forces. The signal intensity of the sensor spot formed from magnetic particles is comparable to conventional planar spots. We developed a variety of magnetic sensing materials ranging from nanoparticles $(\Phi \sim 100 \text{ nm})/30/$ to microparticles (2< Φ <100 μ m) /31/ and to magnetic macrospheres (Φ of few mm) /32/, Fig. 6. Basically, these materials bear similar sensing chemistry as the conventional ones but also incorporate magnetite to render them magnetic. The primary applications of magnetic nanoparticles are those in microscopy and microfluidics, however, photodynamic therapy, drug delivery etc. are also considered. The magnetic microparticles are collected much fasted and manipulated more easily due to their larger dimensions. Finally, magnetic macrospheres

(produced by coating of iron balls with sensing composition) are collected virtually instantaneous and can be easily moved in reactors /32/. They can also be used in 24channel plate readers to substitute the sensor spots. Special magnetic adaptors for collecting the magnetic sensors directly in front of an optical fiber also were developed /33/. So far, magnetic materials fro sensing pH and oxygen were realized but sensing of other analytes is certainly feasible.



Fig. 6: Photographic image of the optical magnetic spherical sensors

3 Conclusions

Novel luminescent indicators and optical sensing materials were developed which substantially improve the performance of optical chemosensors and open new frontiers for their application is science and technology. Particularly, ultrabright oxygen sensors, novel NIR oxygen and pH sensors, nanoparticle-based sensors, sensors with magnetic properties as well as optical temperature-sensitive materials were described. They are expected to find numerous applications in biology, biotechnology, marine science, medicine etc.

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