MATERIALS AND STRATEGIES IN OPTICAL CHEMICAL SENSING

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A Dissertation

Submitted to the Graduate College of Bowling Green State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2008

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ABSTRACT

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The detection of chemical species that play an important role in biological systems, industrial processes or that are environmental pollutants demands the development of highly sensitive and selective chemical sensors capable of operating in various media, particularly in water. Reliable sensing in water is a difficult problem, thus analytical tests and sensor devices for detection in aqueous media remain rare. The present work describes the rational design and formulation of materials for the optical chemical sensing of anions and metal ions in aqueous solution. These materials consist of optical chemosensors embedded in hydrophilic poly(ether)urethanes, which mimic the synergy between proteins and cofactors in enzymes. The resulting materials are arrayed and used in tandem for the detection of single analyte and multianalyte samples, such as toothpaste, human blood serum and enhanced soft drinks. This work also aims to demonstrate that the discriminatory power of sensor arrays might be improved by the use of selective, yet cross-reactive sensing elements. The balance between the selectivity and the cross-reactivity allows for a significant reduction in the number of elements in the sensor array, thereby simplifying the pattern recognition protocols, training sets, and calibrations while maintaining the high overall reliability of the sensing process.
To my wife Lore
ACKNOWLEDGMENTS

There are many great people to whom I owe my gratitude and recognition. First, I would like to thank my family for all the support and for always believing in me. I have also been blessed with a lovely and caring wife Lorena Harris who has been my source of inspiration and support during the years of my Ph.D. adventure.

I would like to extend my heartfelt gratitude to my advisor Dr. Pavel Anzenbacher Jr. His encouragement and support helped me to grow as a scientist and as a person. Thanks to all my Committee members Dr. Phil Castellano, Dr. Deane Snavely, and Dr. John Laird for theirs continue help and advice and for helping me improving and sharpening my dissertation. Especial thanks to Kimberly Spallinger who’s great help, dedication and patient made the writing process a lot easier.

I would like to thank all my lab-mates, especially Karolina Jursikova, Dr. Grygori Zyryanov, Dr. Ryuhei Nishiyabu, Dr. Zhuo Wang, Dr. Dmitry Aldakov and Dr. Victor Montes, with whom I have collaborated in different projects over the past four years. Also, I am thankful to Cesar Perez, Selin (Dion) Ergun, Juan-li (Sensor) Liu, Sachin Vahile, Dr. Chris Gulgas, Dr. Marketa Schinkmanova and Dr. Shin-ya Takizawa. To all of you guys, it has been my pleasure working with you and learning from you. This journey has been so enjoyable thanks to the meaningful insights that very often you provided about science and life itself. I would like to thank the other members of the venezuelan crew Maria Luisa Muro and Leandro Estrada for their continuous support regardless the weather conditions.

Financial support from Mc Master Endowment, the Department of Chemistry, Graduate Student Senate, and Dr. Manuel Marquez from Philip Morris USA were greatly appreciated.
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CHAPTER I. INTRODUCTION.

Detecting the chemical species that surround us (neutral molecules, radicals, or ions) is of great importance due to the wide range of human activities in areas such as food manufacturing, health care, environmental studies, and the food supply chain all of which require detection. Sensing substances that are harmful to humans and animals, and toxic to the environment necessitates the development of reliable analytical tools for their identification and quantification.¹ Last but not least advancing fundamental knowledge regarding the chemical processes taking place in nature will allow us to understand and protect the environment.

Since the beginning of the chemical sciences, there have been several analytical tools and procedures designed for the identification and quantification of chemical species.² Over the last two decades, chemical sensors have become the tool of choice among analytical instruments when real-time monitoring of specific analytes in a sample is necessary.³ In contrast to sensing methods based on intrinsic analyte properties, such as GC-MS and IC-ICP, methods based on indirect sensing have expanded the range of analytes that could be determined. Chemical sensors can operate in the presence of the sample matrix, enabling the possibility of continuous monitoring.³

The term “chemical sensor” has been widely used to broadly describe various entities including molecular probes, sensing materials, sensing ensembles and devices.⁴ In this dissertation it is referred to a chemical sensor as a device that in the presence of a chemical entity of interest (analyte) is capable of changing a physical property generating an analytical useful signal.⁵ In order to avoid ambiguity we refer to a chemical sensor as a macroscopic device, which has a sensing layer (or sensing element) capable of transforming chemical information
into a form of energy to be converted to an output signal by a transducer. Czarnik has articulated a case for molecular devices (or “smart” molecules) capable of reversible recognition of analytes to be considered as *chemosensors*. It is important to point out that molecules that interact with analytes and yield a signal output but react irreversibly with the analyte are defined as *chemo-dosimeters* and are not to be mistaken as chemosensors.

Typically, chemosensors have two functional parts: a receptor and a transducer (or signal transduction unit) (Figure 1.1). The receptor is the part of the molecule capable of recognizing a specific kind of substrate or analyte. The receptor moiety can bind the analyte using different kinds of supramolecular interactions, such as Van der Waals forces, hydrogen bonding, and electrostatic. On the other hand, the transducer is the moiety capable of translating the presence of the analyte at the receptor into a form of energy and a useful analytical signal.

![Figure 1.1](image)

**Figure 1.1.** Schematic diagram of a chemosensor in a sensing process. The analyte is recognized by the receptor and the transducer regenerates a change in a physical property used as a signal output.

There are several signal (energy) transduction mechanisms that have been utilized in chemical sensing schemes. For instance, changes in the optical, electrical, mechanical and thermal properties of the sensing layers have been widely used to generate an output signal. In this regard, since the focus of this work is on chemosensors, I am going to focus on the forms of
energy that are typically relevant for chemosensors as in the case of radiant (UV, Visible and Infrared) and electrical energies. For example, chemosensors based on radiant energy can utilize the transduction of intensity, wavelength, time domain, polarization, and phase detection, while chemosensors based on electrical output can utilize among others the transduction of potentiometric, conductometric and amperometric signals.

The main focus of this study is on the utilization of optical (colorimetric and fluorometric) chemosensors incorporated in polymeric sensing membranes and their use as analytical tools. At the same time, these membranes have been integrated in multisensor-array platforms in order to generate useful information for the identification and potentially also the quantification of different kinds of analytes. Several optical chemosensors operating with different signal transduction mechanism have been reported and widely reviewed during the last years. This introduction will briefly review some examples from the literature on the detection of ions and neutral species, such as nitroaromates, i.e. explosives.

Our interest in metal ion detection was inspired by the heightened concern for human health and the environmental pollution that has stimulated active research on the potential impact of heavy metals and their toxic effects. Despite several years of research on the topic, analytical methods capable of accurate determination of metal ions are still widely sought mainly due to the fact that reliable sensing in competitive media (e.g. electrolytes, buffers) remains a major challenge. This is even more true in the case of anionic analytes because compared to isoelectric cations, anions often display tautomerism, adopt a wide range of geometries, and possess low surface-charge density. These features make the binding and sensing of anions less effective. Anions are a particularly interesting target for sensing because of their ubiquity in biological systems (DNA, ATP, and ADP to name a few) and their importance as components in
food, agricultural fertilizers, and industrial raw materials. This raises a number of safety concerns and necessitates the development of highly sensitive and reliable sensors for anions. Unfortunately, inexpensive materials capable of working with anions, administered in the form of purely aqueous solutions, are still rare, and this has become a major point of attention in the Anzenbacher research laboratory.

1.2 Optical Chemosensors

Optical chemosensors are molecules capable of displaying an observable optical event upon interaction with an analyte. During the past 20 years, optical chemosensors have been widely developed. According to Wolfbeis, and from the point of view of the instrumentation, this could be mainly due to several factors including (a) the availability of new light sources, including light-emitting diodes and laser diodes and (b) the availability of ultrasensitive light detectors as charge coupled devices CCDs, avalanche photodiodes and Peltier cooled photomultiplier tubes, to name few. From the molecular point of view, as articulated by Czarnik, scientists whose research agendas were focused on synthesizing and studying chemosensors (mostly organic chemists) became aware of the needs of analytical chemists and biologists. Supramolecular chemists took up the challenge and realized that they had the tools given by the multidisciplinary nature of supramolecular chemistry to study molecular recognition and analytical chemistry techniques to develop suitable chemosensors.

As mentioned earlier, optical chemosensors can operate by generating changes in the color of the absorption and/or photoluminescence. The characteristics associated with photoluminescence could be intensity, color or lifetime of the emission, among others. Colorimetric detection presents the advantage of simple instrumentation or even naked eye detection as in the case of pH indicator strips. On the other hand, fluorometric detection usually
requires more complex instrumentation due to the fact that excitation and emission are both required. Still, fluorometric schemes in optical sensing have been sought due to their sensitivity even when a colored background and signal-to-noise ratio are a problem. Colorimetric chemosensors usually require changes in their electronic structure to generate useful changes in the absorbance, while fluorometric chemosensor are more sensitive to slight changes in the molecular geometry and electronic structure of their ground and excited states to generate useful signaling.

Optical chemosensors design requires three main components to be considered. The first component is the analyte receptor affinity that is usually controlled by the supramolecular interactions between analytes and chemosensors, which can be evaluated by measuring the association constant \( K_a \), and corresponding enthalpic and entropic components.\textsuperscript{12} As previously mentioned, reversibility is the \textit{conditio sine qua non} requirement for a molecular probe to be considered a chemosensor. Thus, the affinity needs to be in such a range to allow for reversible binding. As a general rule, an association constant should be approximately the inverse of the centered median of the concentration dynamic-range of interest for a target analyte. Secondly and equally important, the signal transduction moiety, which is typically a chromophore or luminophore, should be designed to undergo an optical change when the analyte is bound by the receptor. Usually, the excitation and emission wavelengths are selected to avoid interference from the media. Finally, selectivity is a major component in all chemosensors regardless of the signal transduction scheme and it might be imposed by the receptor chemistry; it may also be affected by the signaling unit. To illustrate this point, consider a hypothetical colorimetric chemosensor A that displays a high affinity (and a association constant) towards analyte B over analyte C but yields a change in color that is virtually the same for both analytes B and C. When
B and C are present in the same sample at similar concentrations the signal displayed by the sensor will mainly originate from the interaction of A and B because B is selectively bound compared to C, thus giving to rise to a selective A-B association. Alternatively, the process may be driven by the signal transduction, for example when the colorimetric chemosensor A displays association constants similar between B and C, but just the complex A•B displays a change in color (Figure 1.2). The above are just academic examples representing the two extreme cases. Realistic experimental situations usually involve a number of less well-defined cases. In general, the combination of signaling and receptor selectivity is usually determined after the chemosensors have been prepared rather than designed. The disadvantage of chemosensors with selectivity driven by the signaling is that at high concentrations of competing interferences in the sample render the dynamic range and the detection limit compromised, particularly when the target analyte is at low concentration (compared to the interference).

**Figure 1.2.** A: Selectivity driven by the receptor. B: Selectivity driven by the signaling.

Also important is the fact that some chemosensor designs are based upon other interactions with analytes, such as collisional quenching. For instance, among the first chemosensors to find a major applications in functional sensing systems were colorimetric and fluorometric pH indicators covalently attached to a cellulose matrix. pH indicators are mostly
based on a proton transfer reaction, which is a very quick reversible reaction that in this case allows for continuous monitoring of pH levels. Also in the mid 1980’s some ruthenium complexes and metalloporphyrins were found to work as viable sensors for dioxygen determination based on the quenching of the luminescence. In the case of dioxygen chemosensors bearing transition metal complexes, the signal transduction is based on collisional quenching due to the alignment of the triplet energy manifold of the chemosensor and the dioxygen molecule.

Another signal transduction mechanism frequently utilized considered in the design of colorimetric and fluorometric chemosensor are ground and excited state proton transfers, and intermolecular and intramolecular charge transfers. Proton transfer is commonly used in pH indicators and, as explained above, it consists of protonation or deprotonation reactions that affect the electronic structure and distribution in the chromophore by extending the degree of conjugation or by inducing an internal charge transfer that changes the intrinsic dipole moment of the dye. This triggers a change in the absorption spectra, and potentially also in the emission spectra in the case of excited state proton transfer or where the chemosensor has intrinsic fluorescent properties as in Figure 1.3, top. Other sensing schemes are based on the disruption of excited state proton transfer, which in most cases results in a blue shift of the emission spectra (Figure 1.3, bottom).
Intramolecular or internal charge transfer (ICT) occurs in chemosensors bearing chromo-fluorophore containing electron-donating (EDG) and electron-withdrawing groups (EWG). Upon light excitation the chromophore undergoes a charge transfer from the donor to the acceptor group. The presence of an analyte, capable of enhancing or disrupting the electron distribution in the chromo-fluorophore, promotes a change in the absorption and emission properties of the chemosensor. ICT has been widely used in chemosensors for ion detection, mainly because it is easier to induce ICT when the analyte bears a net charge. Also, polarity probes have been designed based on ICT. Figure 1.4 show examples of ICT based chemosensors for calcium and fluoride ions. The complete understanding of the ICT phenomena still remains a challenge since it is not always clear how the perturbation caused by analytes can change the spectroscopic properties of the chemosensors and also if the electron-donating and withdrawing capabilities of the substituent groups will have the same electronic properties in the excited state.
Figure 1.4. Examples of ICT based chemosensors. Top: Crown-containing chemosensors in which the bound cation interacts with the donor group (Adapted from ref. 18). Bottom: Anion-hydrogen bonding results in partial ICT from electron-rich pyrrole to electron-poor tetracyanoethylene (Adapted from ref. 19).

Chemosensors based on intermolecular charge-transfer are less common. Mostly, these are based on a photoinduced charge transfer from (or to) the chemosensor to (or from) the analyte. This implies that the selectivity comes from a combination of supramolecular interactions and electronic coupling between the donor and acceptor. Figure 1.5 shows an example of an electron poor chemosensor that is decorated with binding motives for aromatic electron-rich diols.20

Figure 1.5. Example of CT based chemosensors. The addition of resorcinol to the chemosensor prompts the formation of CT colored complexes (Adapted from ref. 20).
Other types of signal transduction mechanisms, such as photoinduced electron transfer (PET) and resonance energy transfer (RET), are mostly relevant for luminescence chemosensors. Nevertheless, in colorimetric sensing schemes trivial energy transfer and inner-filter effect have successfully been implemented and they are closely related with energy transfer transduction mechanisms. Resonance energy transfer requires chemosensors functionalized with two chromo-fluorophores, a donor, and an acceptor. The theory behind resonance energy transfer is well understood and the details go beyond the scope of this introduction; however, it is important to stress that the efficiency of RET is proportional to the spectral overlap between the emission of the donor and the absorption of the acceptor and the distance between donor and acceptor. Thus, signal transduction mechanisms based on RET efficiency usually probe the conformational changes in the chemosensor in the presence of the analyte. Figure 1.6 shows one of the few examples in the literature of chemosensors for small analytes based on RET efficiency. The lack of examples in the literature is due to the difficulty to design RET based chemosensors that will display a large conformational change and low background emission. A more typical strategy is to use RET to induce ratiometric behavior in chemosensors and also to enhance the properties of the chemosensor, such as increasing quantum yield and dynamic range.

**Figure 1.6.** Example of RET based chemosensors. A flexible polyether based chemosensor bends in the presence of Lead ions. The complex yields the donor and acceptor pair closer enhancing the efficiency of the RET process (Adapted from ref. 22).
Due to its versatile and easy implementation, PET has been probably the most extensively utilized signaling mechanism in the design of chemosensors.\textsuperscript{16} Moreover, PET based chemosensors are among the most implemented in real-world applications.\textsuperscript{25} The PET process takes place between a donor and an acceptor of an electron. The relative energy manifolds of the frontier orbitals are responsible for the direction of the electron transfer. In other words, the thermodynamics of the process is determined by the energy levels (redox properties) of the orbitals involved in the electron transfer. There have been several types of PET based chemosensor designs reported in the literature.\textsuperscript{16} The most common design includes an electron donor moiety (e.g. nitrogen lone pairs from amine derivatives receptors) with a HOMO\textsubscript{D} energy level above the HOMO\textsubscript{A} energy level of an acceptor (e.g. a fluorophore). When the fluorophore is excited an electron is promoted from the MO\textsubscript{A} to a higher-level unoccupied MO\textsubscript{A} leaving a vacancy (unpaired electron) in the HOMO\textsubscript{A} orbital. When the fluorophore is the excited state an electron transfer is induced from HOMO\textsubscript{D} to the HOMO\textsubscript{A}. As a result the fluorophore acceptor emission is quenched. After the PET process occurs, a back electron transfer takes place and reestablishes the original ground state molecules (Figure 1.7, left). In the presence of the analyte the PET process is disrupted and the excited-state of the fluorophore is able to relax emitting light. This turn-on behavior was observed in Czarnik’s early designs and can generate up to a 500-fold amplification factor after recognition of the analyte (Figure 1.7, right).\textsuperscript{26}
Figure 1.7. Example of an “off-on” PET based chemosensor for Zn$^{2+}$ ions. Left: Frontier orbital scheme for a PET process quenching the excited state of the anthracene. Right: In the free / resting state the electron pairs on the amines partially quench the anthracene emission by PET. In the presence of Zn$^{2+}$ ions the PET process is disrupted yielding a 500-fold change in the fluorescence intensity (Adapted from ref. 26).

In addition, a PET based chemosensor could be obtained by targeting analytes capable of accepting (or donating) electrons. For instance, this is the case of electron poor analytes such as nitroaromatic compounds that have a very low LUMO manifold that decreases in energy with the addition of more nitro groups to the benzene ring. Swager and co-workers made use of this feature in nitroaromates to develop chemosensors based on amplified quenching.$^{27,28}$ These sensors are based in the inter- and intra- molecular delocalization and hopping of the excited state (exciton) in the solid state of the material. In the presence of the quencher the exciton is deactivated by PET (Figure 1.8).

Figure 1.8. Example of chemosensors based on amplified quenching. Left: Pentiptycene based polymer by Swager. Right: Based molecule self-assemble to yield nano-sized fibers. Both materials utilized quenching amplified due to exciton delocalization (Adapted from ref. 27a and 27c).
In summary, some of the most commonly used signal transduction mechanisms utilized in the design of optical chemosensors were presented to emphasize the importance of signal transduction in molecular sensing. There are other important signaling strategies, such as displacement assays,\textsuperscript{9,11,29} that have not been discussed here as they go beyond the scope of this dissertation.

1.3 Optical Sensor Arrays

All the previous examples describe chemosensor designs that display certain affinity and selectivity towards the analyte of interest. Regarding the molecular design of chemosensors, nature has taught us valuable lessons in molecular recognition. Unfortunately, attempts to emulate the affinity and selectivity of antibodies and enzymes have been difficult to achieve. Nevertheless, during the last 25 years, significant advances have been made in the design and synthesis of selective molecular receptors for small molecule targets and metal ions using the complementarities between the receptor and analyte (substrate), which is known as the classical Emil Fisher lock-and-key model. However, the difficulties and limitations presented by the rational design and complex syntheses, which have been only partly addressed by implementing combinatorial approaches to receptor syntheses,\textsuperscript{30} limit the practical utility of the lock-and-key approach. In terms of analyte recognition, the focus lies in the supramolecular properties of the analyte and the rational design of complementary receptors that are going to be part of the chemosensors. Sensor arrays utilizing cross-reactive sensor elements have been developed. Such devices do not rely on selective chemosensors, but on analyte-triggered perturbations in the chemosensor properties arising from a large number of less specific chemosensors (Figure 1.9). Cross-reactive sensor arrays display a wide range of nonspecific interactions resulting in the
formation of a pattern specific for a given analyte. For the purpose of analyte identification these patterns could be compared to the patterns stored in the device’s memory.

Figure 1.9. Schematic representation of host and guest interaction paradigms. (Left) Emil Fischer lock and key paradigm, which consists of the design of complementary analyte and receptor. (Right) Array of cross-reactive “differential” sensors. One analyte can interact with different sensors in the array and a response pattern could be generated (Adapted from ref. 30).

Perhaps the most important feature of array-based pattern-recognition sensors is that they are amenable to identification and quantification of multi-component analytes. The inspiration for this work is Nature’s use of “differential” receptors in the mammalian tongue and nose for the senses of taste and smell (Figure 1.10). For instance, sensor arrays can be understood as artificial tongues capable of developing response patterns to be analyzed by a computer.

The implementation of sensor arrays can improve the accuracy of the chemical sensor setup. Even the utilization of multiple redundant sensing elements can reduce the measurement of the standard deviation by a factor of $1/\sqrt{n}$, with $n$ being the number of replicates of the sensing element. However, the advantage of using sensor array setups is better explored when cross-reactive sensing elements are used, as implemented in artificial electronic noses (for vapor analysis) and tongues (for solution-phase analysis). Sensor arrays composed of cross-reactive sensing elements have the advantage of an expanded space response. The downside is that when the degree of cross-reactivity of the sensing elements is very high, the differences in response
pattern generated by the array might not be sufficient to discriminate among similar analytes. A routine way to circumvent the lack of discriminatory power is to add more sensing elements to the array. This is the usual case of electronic noses based on piezoelectric sensors, formed by coating quartz crystals with polymers. This kind of sensor usually contains up to hundreds of sensing elements in order to generate enough discriminatory data.

Figure 1.10. Top: Mammalian taste scheme. First, tongue fungiform papilla acts as individual sensor element. Second, the tongue acts as a multi-element array of sensors. Third, the central nervous system picks up the signal from all of the sensors and translates it into a flavor. Bottom: Artificial analog of mammalian taste. First, each sensor well is used as an individual sensor “taste bud”. Second, sensor chip supports array of sensor elements. Third, after digitalization the computer compares the patterns generated by an analyte and then with response patterns generated by the prescreened analytes.

Among the first examples of optical cross-reactive sensors arrays are the systems for vapor sensing introduced by Walt and Suslick in the 1990s. Later development of optical sensor arrays also included solution phase analytes such as metal ions, organic anions, beverages, proteins, nucleic acids, and biological fluids. The sensing platforms used often include microtiter plate-based assays, or the micro-bead based sensor elements similar to the Austin taste chip (developed by McDevit and Anslyn), among others.
1.4 Selective vs. Differential Sensor Arrays, Is There a Middle Ground?

Even in sensor arrays containing differential chemosensors, the sensing elements display a certain amount of selectivity towards different analytes, whether this partial selectivity is due to receptor-analyte binding or other interactions mechanisms affecting the signal transduction. As previously stated, the potential problem with highly cross-reactive sensing elements is that the information content that each individual sensor generates is in most cases very low and only the simultaneous analysis of a number of these sensor elements generates a differential response pattern. This increases the multiplicity of measurements and the amount of data, and as a result the complexity of the sensing process results in an accurate readout.

In order to illustrate the issues associated with selectivity and cross-reactivity in array responses, Figure 1.11 shows a hypothetical response space generated by sensor array of two sensors S1 and S2. First, in the case that S1 and S2 are 100% selective towards one analyte each, space resolution is achieved, but the sensor array can respond only to these two of the analytes. In the second case, S1 and S2 are 100% cross-reactive sensors. Here, several analytes can be detected, but the space resolution is compromised to a point where any one of the two sensors provides the same information as the two sensors, and the lack of resolution precludes the generation of useful information. The third case is a more realistic scenario and is probably the typical case in most cross-reactive sensor arrays. The cross-reactivity of sensors S1 and S2 leads to an expansion of the space response and an improved resolution might be achieved, and also additional analytes could fit in the space response. Finally, the fourth case presents the middle ground between the first and the third cases. When S1 and S2 are known to be cross-reactive in their response towards a certain groups of analytes biasing the selectivity of S1 and S2 to key
components in the group will expand the response-space. As a consequence the resolution of
the sensor array could potentially be increased.

The selectivity in the sensing elements could potentially be the key component to
increase the information density generated by the sensor array. Hence, the discriminatory power
might be improved by the use of selective, yet cross-reactive chemosensors. The increase in the
discriminatory capability of the sensor elements should allow for a significant reduction of the
number of array elements, while maintaining the reliability of the sensing process. Perhaps,
because such a potential reduction of sensor elements can be intuitively derived, there is no
explicit report of this approach in the literature, but it has become one of the cornerstones in the
design of our high-resolution minimal-size (number of elements) sensor arrays.

![Figure 1.11](image)

**Figure 1.11.** Schematic representation of the response-space of two sensors S1 and S2. (A) S1
and S2 are 100% selective. (B) S1 and S2 are 100% cross-reactive to a group of analytes. (C) S1
and S2 are cross-reactive, but have certain selectivity towards some analytes. (D) S1 and S2 are
cross-reactive and have enhanced selectivity towards key analytes, hence expanding the potential
response-space.
1.5 Support Materials for Optical Chemical Sensing

Support materials play an important role in the design and ultimately in the performance of a number of optical sensing systems. Several types of solid materials have been commonly used as part of chemical sensors, such as polymers, sol-gels, hydrogels, silica supports, glass, cellulose materials, composite materials and molecular imprints. Early applications include pH indicator dyes immobilized in cellulose matrices. The nature of the support matrix is usually sought to leverage certain characteristics of the analyte and the environment where the sensing process takes place. For instance, hydrophobic polymer matrices have been used in the detection of oxygen and CO$_2$, while the hydrophilic materials are often used for pH determination and ion detection schemes.

Generally speaking, optical probes and chemosensors can be immobilized into matrices in two ways. The first method is physical immobilization that consists of simply embedding or dissolving the probe in the host matrix. There are several examples of these materials in the literature where probes had been embedded in cellulose, silicone rubber, plasticized poly(vinyl chloride), supramolecular hydrogels, absorbed on silica, and last but not least polyurethanes. The second way to immobilize probes or chemosensors in a matrix is by chemically attaching the probes to the supporting matrices mentioned above. Covalent (or chemical) immobilization presents the advantage of preventing the leaching of the probes out of the sensing membrane, but it imposes additional synthetic steps required for their preparation.

The chemical nature of the host matrix can play a major role in the observed selectivity and response of the sensing materials. It has been shown in the literature that supramolecular hydrogels can act in synergy with chemosensors to improve the selectivity of the sensing process. On the other hand, in the case of ion selective optodes the polymeric matrix usually
acts as a medium where the recognition process takes place and the matrix does not effect the selectivity of the optode, which is driven solely by the ionosphere. The usual requirement for such inert matrices is that they are soft enough; for the diffusion processes to take place in an acceptable time window (seconds). In this regard, polyurethanes often display better mechanical properties than poly(vinyl chloride) matrices, even though the formulation of the ion selective optodes in both cases also includes plasticizers.45

Molecularly imprinted polymers (MIPs) represent the other end of the spectrum of matrices, where the polymeric matrix usually drives all the selectivity and response. The optical probes embedded in MIPs serve only as signaling units. The major setback for the implementation of MIPs in chemical sensors is that the recognition process is in most cases slow and irreversible.50

In most instances, the successful sensing process depends on the performance of both the chemosensor and the polymeric matrix. Our approach, in general, uses the synergy between these two components and seeks the development of materials consisting of very simple two-component bulk-optodes or sensing layers. Consequently, we utilize the propensity of highly hydrophilic polyurethanes to draw in water with ions.48,49,51,52 The affinity of hydrophilic polyurethanes to water results in a more or less homogeneous distribution of the water in the polymer. The newly established hydration equilibria between the water of the aqueous analyte and the polymer results in partial stripping of the solvating water molecules from the ionic analytes, thus rendering the ions available for an enthalpy-driven recognition process by the chemosensors. Finally, polyurethanes also lend mechanical support to the chemosensor molecules. This allows for the array of several sensors to be arranged on a solid platform for simultaneous analyses.
1.6 Goals of the Dissertation and Chapter Summaries.

The present studies aim to demonstrate that the discriminatory power of sensor arrays might be improved by the use of selective, yet cross-reactive sensing elements. This tuning between selectivity vs. cross-reactivity can be carried out by careful rational design of the sensing elements composed by a polymeric matrix and optical chemosensors that utilizes different types of signal transduction mechanisms. The balance between the selectivity and the cross-reactivity allows for a significant reduction in the number of elements in the sensor array thereby simplifying the pattern recognition protocols, training sets, and calibrations while maintaining the high overall reliability of the sensing process.

- Chemical sensing experiments require the development of a reliable analytical platform and detection schemes that allow consistent and reproducible quantification of the optical changes. Chapter II therefore presents the different tools developed and the statistical multivariate analysis techniques used for the data evaluation and comparison of the response patterns generated by the sensor arrays.

- Chapter III introduces the materials that compose the sensing elements (chemosensors and polymeric matrix) and describes their roles in the sensing process. In addition, Chapter III also provides the rationale and some theoretical aspects behind the utilization of (ICT) chromophores in the design anion chemosensors. Finally, the role of the polymeric matrix in the selectivity of the sensing process is also evaluated and discussed.

- Chapter IV and V describe a colorimetric and fluorometric sensor array used for detection and classification of aqueous anions, respectively. In Chapter IV a clear connection between the supramolecular behavior of the chemosensors forming the sensor array and the final
response of sensor array is established, while Chapter V explores the utility of combining two optical outputs to increase the discriminatory power in sensor arrays.

• Chapters VI and VII present fluorometric sensor arrays for metal ion detection. The sensor array shown in Chapter VI is based on only one type of coordination chemistry, while the selectivity of the chemosensor is driven by the signal transduction mechanism. Chapter VII presents a sensor array, where the sensor elements combine different types of coordination chemistries as well as different types of signal transduction mechanism to enable an accurate quantitative analysis of metal ions.

• Chapter VIII then describes how the processing of the polymeric matrix affects the response of small molecule-based fluorescent sensor for vapor detection of nitroaromatic explosives such as TNT.

• Finally, Chapter IX shows how the reliability of the sensing process can be improved when an optical and electrochemical signal are combined in the same chemosensor molecule.

1.7 References.

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CHAPTER II. METHODOLOGY: DETECTION SCHEME AND PATTERN RECOGNITION TECHNIQUES

2.1 Introduction

For optical sensor arrays, it is desirable to record the response from all sensing elements on a given platform simultaneously.\(^1\) While spectroscopic techniques are highly advantageous due to the high density of information that can be harvested from them, they also require time for the measurement of each sensing element (spot) and also complex equipment (scanning setups) to carry out the detection. In this regard, imaging techniques have the advantage of being capable of detecting several features in a given field of view. The fast development of sensitive CCD based cameras has promoted the utilization of imaging techniques for quantitation of fluorescence intensity. For both the spectral and imaging method, multiple responses produced by sensor arrays require tools from multivariate analysis for the interpretation and comparison of the patterns obtained from the data. In this chapter, the instrumentation and methodology utilized for the detection and evaluation of the response data taken from our sensor arrays is introduced.

2.2 Detection Schemes

In this section, two protocols were developed for the colorimetric and fluorometric reading of the sensor arrays. As mentioned earlier, these protocols are based on imaging techniques to facilitate the simultaneous quantification of the different sensing elements in the array. The platform used for the sensing experiments are in house-fabricated microtiter plates using microscope slides that are ultrasonically drilled to create the sample wells (Figures 2.1). Figure 2.1 (right) shows the ultrasonically drilled wells that are typically with radius of \(~ 500 \mu m\) and \(~ 255 \mu m\) in depth.
2.2.1 Colorimetric Protocol

For the detection of changes in the colorimetric output of the sensor arrays, a flatbed scanner is used. Suslick and coworkers first introduced the utilization of a flatbed scanner as an analytical tool for the detection of color intensities in sensor arrays. The general idea is that once a reliable assay is developed, the sensor array could be deployed and read on any PC. In this context, other groups have recently taken advantage of widely available tools to carry out ubiquitous chemical analysis. The utilization of different “consumer available” technologies, such as cellphone cameras by Whitesides at Harvard and DVD readers by Potyrailo at General Electric, were implemented for the optical detection of changes in sensing elements.

In the Anzenbacher research laboratories, the images from the sensor array were recorded using a USB flatbed scanner (Canon, CanoScan LiDE 60) and the scanned images (24 bit, 8-bit per color) were acquired at 1200 dpi resolution. Particularly, the CanoScan LiDE 60 detects the colors by applying pulses of RGB colors and correlating position with the intensity of the light reflected from each pulse.
The image processing was carried out with NIH ImageJ software. The general approach for the analysis of the color intensity of the image consists of: (1) color (RGB) inversion, so the background has a zero-value, (2) RGB deconvolution, and (3) averaging of the gray value of the pixel for each sensing element (spot) in the array for each (RGB) component (Figure 2.2). In practice, there are several technical issues, which prevent a direct analysis of the images, such as the lack of uniformity in the size and distribution of the wells on the array. In order to facilitate and automate the image analysis, an ImageJ routine was written to overcome the problems of size distribution of the wells, uneven distances between the wells and differences in the original position of the array on the bed of the scanner. The actual code of the routine has been included in Appendix 2. The code first looks for features in the image that possess a certain threshold of color intensity. Then it assigns a label to the position of these features and creates a circular region of interest (ROI) that will create the selection. The average of the non-zero pixel intensity is measured in all three (RGB) color-components from the image and finally recorded in a table.

**Figure 2.2.** Image processing routine for colorimetric sensor arrays. (1) Color (RGB) inversion, so the background has a zero-value, (2) RGB deconvolution, and (3) averaging of the gray value of the pixel for each sensing element (spot) in the array for each (RGB) component.
2.3.2 Fluorometric Protocol

For the detection of the changes in the fluorescence intensity of the sensor arrays, a Kodak Image Station 440CF was used (Figure 3.2, left). The Image Station consists of an epifluorescence setup equipped with a UV broadband (300-400 nm, \( \lambda_{\text{max}} = 365 \) nm) lamp for excitation and a thermoelectrically cooled CCD camera. The resolution of the CCD sensor is 752 × 582 pixels and the image data is captured at 12-bit. The images of the sensor arrays were collected using a lens at 6× zoom that yields a field of view of 4.2 × 3.3 cm for a resolution of \(~57 \, \mu\text{m/pixel}\). In order to distinguish different color channels, a filter wheel is placed before the lens. The set of filters consists of 1) Blue: band-pass filter 380-500 nm \( \lambda_{\text{max}} = 435 \) nm, (2) Green: band-pass filter 480-600 nm \( \lambda_{\text{max}} = 525 \) nm, (3) Yellow: long pass filter >523 nm, (4) Red: long pass filter >580 nm and (5) Dark red: long pass filter >620nm. The utilization of different filters depends on the specific application, fluorescence emission color and the number of detection channels desired. The fluorescence of the molecules will typically present different features when excited with a broadband source and at a single wavelength. A way to emulate the excitation condition and the probable output signal is by measuring excitation-emission maps (Figure 2.3).

The analysis of the acquired images was carried out with the Kodak Molecular Imaging Software®. The automation made for the colorimetric images using ImageJ was not possible with the fluorescence setup since the 12-bit images cannot be exported at full dynamic range. The Kodak proprietary software one allows to manually generate ROIs grids and to center them on the fluorescent features in the image by a centering option that centers the ROI in the center of mass (of the pixel intensities) that usually yields very reproducible results.
Figure 2.3. Fluorometric detection. Left: Kodak Image Station 440CF and excitation-emission spectral maps are used to emulate the characteristic of the fluorescence features of a fluorophore using broad band excitation of the Image Station. Right: averaging (or integration) of the gray value of the pixel for each sensing element (spot) in the array for each component.

2.3 Data Matrix and Preprocessing of the Data

The recorded data are usually arranged in a response matrix containing a number of columns corresponding to the number of features detected and the number of rows corresponding to the number of recorded observation (Figure 2.4). An extra column with the classifier descriptor can be added, which will be used in supervised multivariate analyses.

Figure 2.4. Schematic representation of the data matrix.
The raw data in the response matrix can be preprocessed to obtain different types of information from the subsequent multivariate analysis or to simply clean up the data and achieve better resolution. The preprocessing of the data may have a significant impact on the final outcome of the multivariate analysis. Unfortunately, the power of the data preprocessing is usually underestimated due to the lack of a general rule or procedure to guide the preprocessing methods, which is the most appropriate for a given application. In most cases, the preprocessing algorithms can be systematically tested before the actual multivariate analysis method is applied to achieve the desired result.

Several methods of data preprocessing have been proposed in the literature\textsuperscript{5,6} including relative scaling, background subtraction, signal average, linearization, mean-center, autoscale and range scale. From all of these methods (and combinations) the ones that had yielded better results for a given set of data were applied to generate the response matrix. In the case of the colorimetric sensor array, the relative baseline subtraction was applied to the data set by subtracting the color intensities of the channels (RGB, see section 2.2.1) from the sensing elements ($C_0$) and after ($C$) the exposure to the analyte:

$$R_{cc} = C - C_0 \text{ with } cc \text{ color channel}$$

In the case of the fluorometric response, a combination of the relative baseline subtraction and relative scaling was used and it is called in this work relative intensity. The relative intensity consists of subtracting the fluorescence intensities of the color channels (up to four channels RGBY, see section 2.2.2) from the sensing elements before ($F_0$) and after ($F$) exposure to the analyte. The result is then divided by the intensity before the analyte exposure ($F_0$).
\[ R_{cc} = \frac{F - F_0}{F_0} = \frac{F}{F_0} - 1 \]

Also, in the case of fluorescence sensor arrays we have explored the utility of other preprocessing methods, such as range scaling, where after the background subtraction the response is compared with the dynamic range of the response for a given analyte in a given sensor.

\[ R_{cc} = \frac{F - F_0}{F_{\text{max}} - F_0} \]

Even though the range scaling has yielded interesting results in some applications, in the case of cross-reactive sensor arrays its implementation is rather inconvenient since the determination of \( F_{\text{max}} \) is relative to the dynamic range of each analyte, and is not possible in multianalyte environments. Nevertheless, in this chapter we have included, as an example, a dataset that has been preprocessed by both the relative intensity and range scaling to illustrate the impact the preprocessing methods on the final outcome of the multivariate analyses.

2.4 Pattern Recognition Protocols: Multivariate Analysis in Chemical Sensor Arrays

Pattern recognition techniques have been widely used for the interpretation multivariate data sets in chemistry.\(^5\) Notably, the intrinsic multivariate nature of the responses associated with sensor arrays requires the implementation of mathematical and statistical multivariate methods for the understanding and evaluation of the quality of the data.\(^6\) These methods are often based on the reinterpretation of the data into a lower dimensional space (dimensionality reduction) or on the comparison of the measurements of the vector responses direction and magnitude.\(^7\)
Response patterns can be analyzed by unsupervised and supervised multivariate analysis methods. In general, unsupervised methods are those where no additional information, e.g. the sample identity, is introduced in the data set. Unsupervised methods are often used to explore the actual clustering and dispersion of the data. There are several methods for unsupervised analysis of multivariate data sets; most of these are based on clustering analyses, e.g. as in hierarchical clustering analysis (HCA), and in statistical analyses, e.g. as in principal component analysis (PCA). On the other hand, supervised methods use the identity of the sample in the training data sets to generate models that can later be used for classification of unknowns, as it is in the case of discriminant analysis (DA). Several other multivariate analysis methods based on neural networks, data mining and machine learning algorithms have been also used in the evaluation of sensor arrays.

Here, the statistical multivariate methods specifically used in this dissertation will be qualitatively explained. In the case of principal component analysis we are going to pay more attention to the computational details since PCA is used for the optimization of the sensor array size (number of elements).

2.4.1 Principal Component Analysis (PCA)

Generally speaking, PCA is a statistical treatment that consists of the reinterpretation of a multidimensional data set in a dimensionally reduced space enclosing most significant characteristics of the data (the variance) originally contained in the data set. This is achieved by calculating orthogonal eigenvectors (principal components, PC) that lie in the direction of the maximum variance within that data set. The first PC contains the highest degree of variance, and
other PCs follow in the order of the decreasing variance. Thus, the PCA concentrates the
information in the dataset into a lower dimensional space.

The PCA typically departs from the covariance (or correlation) matrix $C$ of the original
preprocessed dataset (see above).

$$C = \begin{pmatrix}
    c_{1,1} & c_{1,2} & \cdots & c_{1,n} \\
    c_{2,1} & c_{2,2} & \cdots & c_{2,n} \\
    \vdots & \vdots & \ddots & \vdots \\
    c_{m,1} & c_{m,2} & \cdots & c_{m,n}
\end{pmatrix}$$

with $c_{m,n} = \frac{1}{n-1} \sum_{i=1}^{n} (x_{im} - \bar{x}_m)(x_{in} - \bar{x}_n)$

The basic premise is that the projection of the original $n$-dimensional data set into the
reduced space can be carried out by decomposing $C$ into a score matrix $S$ and a loading matrix $L$, so $C=S\cdot L$. Several methods could be used to decompose $C$. Among such methods, the singular
value decomposition (SVD) is the algorithm implemented by most commercial software. This is
because SVD has proven to be very robust in a number of applications.

When SVD is applied directly to the covariance matrix $C_{n,m}$ ($m$ trials, $n$ response
features), it results in a factorization of the form:

$$C = U \cdot \Sigma \cdot V^T$$ such that $CC^T = U$ and $C^T C = V$

Where $U_{m,n}$ and $V_{n,n}$ (score and loading matrices, respectively) are orthonormal and
formed by eigenvectors termed principal components $PC_i$ representing the projection of $C$ into the
new principal components eigenspace. The matrix $\Sigma$ (singular value matrix) is a diagonal
matrix containing singular values $\sigma_n$, which are associated with the root-square of the
eigenvalues \( \lambda_j = \lambda_n \) for each \( PC_i \). This implies that the minimum variance associated with the original variable is set to 1 and the summation of all \( j \) eigenvalues is equal to \( n \) the number of variables originally present in \( C \), \( \sum_{j=1}^{n} \lambda_j = n \). The variance contribution of each eigenvector \( PC_i^{\text{var}} \) is determined by the portion of the \( \lambda_i \) divided by the summation of all \( j \) eigenvalues. The first PC contains the highest degree of variance, and the other PCs follow in the order of decreasing variance.

\[
PC_i^{\text{var}} = \frac{\lambda_i}{\sum_{j=1}^{n} \lambda_j}
\]

The columns of the score matrix \( U_{m,n} \) can be used to project \( C \) into a lower dimensional space for exploration of patterns in the data (clustering). This representation is termed score plot and is usually associated with a certain amount of variance represented by the \( PC_i^{\text{var}} \) for each PC used in the plot.

The columns of the loading matrix \( V_{n,n} \) are composed of \( n \) vectors associated with each of the variables (or response features). When the correlation matrix is used instead of the covariance matrix, the loadings are nothing more than the correlation coefficients between the original variables and the newly derived principal components \( PC_n \). Moreover, the loading vectors are the projection of each feature \( n \) into the principal components eigenspace. Since these vectors are orthonormal, the relative contribution of the \( k \)-th variable on the principal component \( PC_n \) is taken as the square of the loadings (correlation coefficient, \( r_{PC_n,k} \)) between \( PC_n \) and the \( k \)-th variable (feature).
\[ k_{\text{Contribution} \, PC_n} = r_{PC,k}^2 \]

\[ \sum_{k=1}^{n} r_{PC,k}^2 = 1 \]

The PCA yields a lot of useful information when applied to the characterization of the response of sensor arrays. For instance, from the mathematical point of view, a successful PCA is one, in which the dimensionality reduction is maximized and several features are compressed down to two or three of the PCs. On the other hand, sensor arrays with a high discriminatory capacity will generate responses that will be so scattered in the \( n \)-dimensional space that the PCA fails to describe most of the variance in the first two PCs. In these situations, statistically relevant PCs could be utilized to explore the patterns in the data. Several methods have been proposed to determine the numbers of PCs statistically relevant for the description of a dataset.\textsuperscript{5-10} Here, we use Kaiser’s rules, which states that only the PCs with associated eigenvalues \( \lambda_i > 1 \) are relevant, since the minimum variance for a single variable was originally set to 1.

Thus, from a sensor array analysis point of view a successful PCA is a one, in which the score plot shows clear clustering of similar samples and the eigenanalysis shows the total variance (information), generated by the sensor array, widely dispersed over several PCs. Such results would attest to a high discriminatory power of the array. Unfortunately, having several statistically relevant PCs often implies that even a 3D representation might not be an accurate description of the sensor array response, and it would be quite difficult to correlate response features with distances in the 2D or 3D score plots. This is the downside of the PCA. Hence, it is very common to complement the PCA with another multivariate methods such as HCA.
As mentioned before, preprocessing the data might impact the final outcome of a PCA. In order to illustrate this point we have taken a set of data from one of our preview papers\textsuperscript{11} and applied a preprocessing routing(s) utilizing first the relative change in the fluorescence intensity and second the range scaling (see above). In the case of this dataset it is clear how the two preprocessing procedures yield very different score plots (Figure 2.5). For this data set range scaling shows better clustering of the data (figure 2.5, right). However, this is not necessarily always the case and judicious of preprocessing must be applied to obtain best clustering and resolution.

\[
\text{Relative intensity} = \frac{F}{F_0} - 1
\]

\[
\text{Range scaling} = \frac{F - F_0}{F_{\text{max}} - F_0}
\]

**Figure 2.5.** Impact of the preprocessing of a dataset on the PCA. The data is composed of the responses generated by the sensor array described in chapter VII to the presence of 9 different cations (250 μM) at pH 5. Left: relative change in the fluorescence intensity preprocessing. Right: range scaling preprocessing.

### 2.4.2 Hierarchical Clustering Analysis (HCA)

The HCA is an unsupervised method of multivariate analysis, which seeks classification of the observation by measuring the interpoint distances between all samples in the \(n\)-dimensional space resulting from \(n\)-numbers of studied features. The observations are aggregated (clustered) stepwise following the similarities (or distances) in their features.
There are several ways to calculate the distance and most of them are included in commercial version of statistical software. In our work using HCA, we have used the Euclidean distance \((d_{mn})\) between the sample \(m\) and \(n\) (or the square version), as follows:

\[
d_{mn}^2 = \sum_{k=1}^{K} (x_{mk} - x_{nk})^2 \quad \text{with} \ k \ \text{features}
\]

Also, there are several methods available to define the linkage between the clusters.\(^5\) We used Ward’s (minimum variance) method,\(^12\) which takes into consideration the minimum amount of the variance between the samples and analytes to define a cluster. For Ward’s method, first each sample is considered its own cluster, therefore, the variance is null. Then, each following step considers a pair of objects that can be fused while keeping the amount of variance as small as possible. This process repeats until a remaining supercluster is formed. The distance \((D_{MN})\) between two clusters \((M\) and \(N\)) is defined by

\[
D_{MN} = \frac{\| \bar{x}_M - \bar{x}_N \|^2}{\frac{1}{n_M} + \frac{1}{n_N}} \quad \text{where} \ n \ \text{is the number of members in a cluster}
\]

The clusters are then combined in such a way that the minimal increment in the within-group-distance is achieved by making the distance \((D_{ji})\) between a new cluster \(j\) and the observation \(i\), such that the distance from \(j\) and \(i\) to the clusters \(M\) and \(N\) results:

\[
D_{ji} = \frac{n_M + n_i}{n + n_i} D_{Mi} + \frac{n_N + n_i}{n + n_i} D_{Ni} - \frac{n_i}{n + n_i} D_{MN}
\]
Ward's method usually tends to generate well-structured dendrograms. The downside is that it also tends to join different clusters with a small number of observations, and it is strongly biased toward producing clusters with roughly the same number of observations, which is not a problem in most of our applications since we constantly accumulate the same number of observation per analyte.

In the context of chemical sensor arrays, the HCA has the ability to utilize the whole dimensionality to represent the patterns. This allows for the study of the responses including the influence of the errors intrinsic to the assay. However, in the case when the dataset is somehow noisy and does not present a clear structure, the HCA often produces a poor clustering of similar observations. On the other hand, the HCA produces dendrograms displaying in a mono-dimensional fashion the quantitative differences (or similarities) between the individual observations. This is valuable information because it makes for a straightforward correlation of chemical features with responses in the $n$-dimensional space.

As in the case of the PCA, the preprocessing of the data is likely to impact the final outcome of the HCA. Here, using the same dataset and preprocessing techniques, as in the previous example for the PCA, show how the two preprocessing procedures yield dendrograms with different structures (Figure 2.6). Similarly to the PCA, this dataset shows better clustering of the data when the range scaling is applied (figure 2.6, right).
Relative intensity \(= \frac{F}{F_0} - 1 \)

Range scaling \(= \frac{F - F_0}{F_{\text{max}} - F_0} \)

Figure 2.6. Impact of the preprocessing of a dataset on the HCA (displaying square Euclidean distance with Ward linkage). The data is composed of the responses generated by the sensor array described in chapter VII to the presence of 9 different cations (250 μM) at pH 5. Left: relative change in the fluorescence intensity preprocessing. Right: range scaling preprocessing.

2.4.3 Linear Discriminant Analysis (LDA)

LDA is a classical statistical approach for supervised dimensionality reduction. LDA is also a special case of discriminant analyses where the discriminant rule is based on linear combinations of features (e.g., sensors responses) that best separate two or more analytes. Using the defined group classes, LDA aims to maximize the ratio of the between-the-class distance to the within-the-class distance, thus maximizing the class discrimination. As in PCA, the linear combinations of the features are found by solving an eigenvalue problem.\(^5,7\) As described by Otto,\(^5\) the weights of the linear discriminant functions are determined from the eigenvector of the matrix \(D\).

\[ D = G^{-1}Hw = \lambda w \text{ where } \lambda \text{ is the eigenvalue} \]
The matrix $G$ is found from the covariance matrix $C$ of the different groups $g$

following:

$$G = (n - g)C = (n - g)\frac{1}{n - g} \sum_{j=1}^{g} (n_j - 1)C_j$$

and $$C_j = \frac{1}{n_j - 1} \sum_{i \in g_j}(x_i - \bar{x}_j)(x_i - \bar{x}_j)$$

for $n$ equal to the total number of observations, $n_j$ equal to number of observation in the group $j$ and $l$ is one observation of the $j$th group $g_j$.

The matrix $H$ explains the distribution of the group means $g_j$ over the total average $\bar{x}$,

$$H = \sum_{j=1}^{g} n_j(\bar{x}_j - \bar{x})(\bar{x}_j - \bar{x})^T$$

$$\bar{x} = \frac{\sum_{j=1}^{n} n_j \bar{x}_j}{n}$$

Solving the eigenvalue problem $D = G^{-1}Hw = \lambda w$ will derive a list of eigenvalues ($\lambda$) and eigenvectors ($w$). The eigenvector $w_1$ associated with the greatest eigenvalue $\lambda_1$ provides the first
discriminant function $s_1$:

$$s_1 = w_{11}x_1 + w_{12}x_2 + \ldots + w_{1p}x_p$$

Utilizing the rest of the x-data the following eigenvalue $\lambda_2$ is calculated along with
eigenvector $w_2$, to obtain the second discriminant function, $s_2$:

$$s_2 = w_{21}x_1 + w_{22}x_2 + \ldots + w_{2p}x_p$$

The calculation of the discriminant functions continues until all are determined. In order
to classify any observation, the vector response (in case of sensor arrays) is evaluated in the
discriminant functions in order to transform the vector of the raw data in the coordinates within the discriminant space. The observation is then assigned to the group to which it has the minimal Euclidean distance:

$$\min_j \| w^T (x_u - \bar{x}_j) \| \text{ with } j = 1, 2, \ldots, g$$

The results of the classification could be represented in a confusion matrix, which is a matrix that contains the numbers of correctly classified objects in each class on the main diagonal and the misclassified objects in the off-diagonal. The confusion matrix often overestimates the accuracy of the classification due to the bias imposed when a sample classification is attempted while using a training-set that contains the same observation. The cross-validation (leave-one-out) routine is used to test the predictability of the sensor array by leaving one observation out of the set at the time, and it uses the rest of the data as a training set to generate the linear discriminant function, which is then used to place the excluded observation (data point) within the correct cluster. This is performed for each observation, and the overall ability to classify the observations describes the quality and predictability of the array. As in the case of the confusion matrix, the cross-validated results can be represented in a jackknifed matrix.

The implementation of LDA is conveniently used for determination of classes by using the LD discriminant function, and it also provides a graphical output by plotting discriminant scores ($w_i$) against the canonical roots (or factors). These plots provide a graphical representation of how LDA is clustering similar patterns, and it attests to the degree of discrimination of the data, i.e., how good the resolution of the array is for a given group of samples.

As in the case of the two previous analyses the preprocessing of the data likely to have an impact on the final outcome of an LDA. Once more, the same dataset and preprocessing
techniques were used to generate two discriminant score plots from the same original response dataset as in the previous examples for PCA and HCA. These showed how the two preprocessing data procedures yield score plots with different clustering (Figure 2.7). Generally speaking, the LDA shows better clustering of the data when compared to the PCA. Interestingly, in the LDA this dataset shows better clustering of the data when the relative intensity is applied (Figure 2.7, left).

Relative intensity $= \frac{F}{F_0} - 1$

Range scaling $= \frac{F - F_0}{F_{\text{max}} - F_0}$

![Figure 2.7](image)

**Figure 2.7.** Impact of the preprocessing of a dataset on the LDA. The data is composed of the responses generated by the sensor array described in chapter VII to the presence of 9 different cations (250 μM) at pH 5. Left: relative change in the fluorescence intensity preprocessing. Right: range scaling preprocessing.

2.5 Optimization of the Number of Elements in Sensor Arrays

An important problem during the design of the sensor array is the question of how many sensor elements are required to achieve a certain level of discrimination. Unfortunately, without testing a training set it is hard to predict sensor array behavior. A typical approach is to overestimate the number of sensor elements by including more sensors than is necessarily needed (entire combinatorial libraries) and pre-screen the responses for the best set of sensors. On the other hand, in Chapter IV it is demonstrated that the observations made in a solution can
be extrapolated to the behavior of chemosensors in poly(ether)urethane optodes. This allows for the design of arrays biased towards certain analytes, while making it possible to circumvent the tedious pre-screening steps. Depending on the discriminatory power of the individual sensing elements in the array, it is possible to design sensor arrays with a reduced number of sensors still capable of identifying and quantifying certain group of analytes with high accuracy. An analytical procedure to determine the number of sensor necessary to “resolve” a given training set is highly desirable since it could result in less complex and custom platforms for a number of applications.

For any sensor array comprising more sensing elements than required for the accurate classification of a given group of analytes, the mathematical problem could be reduced to reveal which sensors have correlated responses. Thus, in the case of two sensors showing similar behavior in the $n$-dimensional space the contribution to the dispersion of the data of both sensors, will be virtually the same. We could think about the extreme case where the same sensor is used twice in the same array. In theory, the variables of both sensors should be highly correlated thus no additional information is gained, although the standard deviation of the measurement can be reduced by a factor of $1/\sqrt{2}$ (See chapter I, section 1.3). For instance, one approach could be to search for commonalities in the variables utilizing the HCA. Unfortunately, the HCA just yields similarities (or differences) between variables, and our sensing elements have more than one (response channel) variable. Hence, just studying the similarities of each individual variable makes it difficult to decide, which sensor to remove from the array. This is because the other channels (variables) associated with the same sensor, could still display unique features. Finally, while the HCA could be used to “clean” the data by eliminating the variables displaying
contribution of low significance, it is not convenient for the systematical elimination of sensors from the arrays.

Since the response of our sensor elements are \( c \)-dimensional, considering \( c \) as the number of (color) channels used for the detection, we need a tool that interprets the vectorial response in the \( c \)-dimensional (sensor space) and correlates it with the \( n \)-dimensional space response generated by the entire array. As mentioned before, the PCA can describe the variance (information) of a data set in a reduced space generated by the principal components. The contribution of each variable to each principal component is given by the projection of the original variable to the principal component. Hence, the contribution of a sensor \( S \) to a principal component \( p \) is the summation of the square of the loadings \( r_{iPC_p}^2 \) of the variables belonging to a channel \( c \) as shown in Figure 2.8.

![Figure 2.8](image)

**Figure 2.8.** Schematic representation of two not-normalized principal components (↑) showing the projections (---) of the centered RGB responses (↑↑↑) of a sensor \( S \) to the principal components.

As it has been reported in the case of monodimensional sensors,\(^{16}\) the idea is to select the sensors with the maximum contribution to each of the PCs of statistical significance. Thus, after
the PCA is applied to the original dataset, a sensor array with \( m \)-elements will be reduced to a maximum number of sensors equal to the number PCs with eigenvalues greater than one, according to Kaiser’s rule. After this, a second PCA is carried out on the reduced version on the dataset to study the amount of information lost in the previous reduction step. At this time, it is possible to employ another multivariate technique that can quantify predictability, such as LDA. If the predictability is still acceptable the contribution of the sensor can be estimated and another reduction step can be performed. The process can be repeated until a compromised threshold in the predictability (or clustering in the score plot) is reached (Figure 2.9).

**Figure 2.9.** Flowchart of the procedure for the sensor array size optimization. In this case the algorithm will yield a sensor array with accuracy greater than 95%, but the accuracy threshold can be adjusted to a desired level.

Finally, another method based on an iterative algorithm has also been proposed in the literature.\(^{17}\) While iterative methods are convenient since they study all possible combinations of sensor in a sensor array, it could tedious depending on the number of sensor elements and is not very systematic. Furthermore, the additional information gained through the systematic reduction
process, such as what sensors are shaping the space response, is not obtained as a direct consequence of the analysis.

2.6 Summary

The detection schemes for colorimetric and fluorometric detection have been presented. The details of each analysis, such as the specific number of detection channels are given in each chapter. Also, three different multivariate analysis methods were introduced as they have been implemented in the following chapters. It should be noted that other more advanced algorithms, such as artificial neural networks and support vector machines may and have been used previously in sensor array schemes. Fortunately the analytical challenges presented until now, have been successfully solved by the implementation of simpler tools, such as PCA, LDA and HCA. However, the analyses in multianalyte environments might require the utilization of more advanced multivariate analysis tools.

2.7 References


(8) The correlation matrix is used when the variables are heterogeneous or in indifferent scales. PCA starting from the covariance or correlation matrix having homogenous variables usually yield similar results.

(9) Non linear partial square decomposition.


(11) The origin of the data set is not important for the discussion in this chapter. The data is composed of the responses generated by the sensor array in chapter VII in presence of 9 different cations at 250 μM at pH 5.

(13) A dendrogram is a mono-dimensional representation that correlates the distances in the $n$-dimensional space between a series of observations (see refs. 5).


CHAPTER III. RATIONAL DESIGN OF MATERIALS FOR OPTICAL CHEMICAL SENSING

3.1 Introduction

The rational design of sensing materials relates to the understanding of the correlation between expected function and material composition. In order to establish this correlation, it is necessary to have a good knowledge of the function and mechanism operating in the individual parts of the sensing material. Even though the complex nature of the sensing processes makes the rational approach to the design of sensing materials often cumbersome it is still appealing since it may avoid synthesis and testing of several \(10^9\) possible candidates that could be generated in a typical combinatorial setup. In this chapter we introduce the materials that compose our sensing elements, to study their function and their expected role in the sensing process. Even though the knowledge gained through rational design is always limited, we will demonstrate in this chapter that the rational approach could still pave the road for the development of new and efficient sensing materials and deliver new chemical sensing paradigms suitable for practical application.

The design of our sensing materials is inspired by nature’s ability to use the synergy between protein and cofactors in the recognition process taking place in some enzymes. Thus far, the present materials consist of simple two-component bulk optodes or sensing layers. The first and key components of the sensing materials are the optical chemosensors playing the role of the co-factor. The second component is the polymeric matrix playing the role of the protein. Chemosensors are designed to display a change in their optical properties upon recognition of the analyte; in our research we use chemosensors utilizing various kinds of optical signal transduction modes. This chapter is going to focus largely on the internal charge transfer (ICT)
as signal transduction mechanism for colorimetric anion sensing. Other kinds of signal transduction mechanisms have also been explored, and they are described in other chapters, these include chelation-enhanced fluorescence (CHEF) (chapter V), resonance energy transfer (FRET) (chapter VI and VII) and photo-induced electron transfer (PET) (chapter VIII).

While the role of the chemosensor moiety is to generate signal output, the primary role of the second component, the polymeric matrix, is to lend mechanical support to the chemosensor and also provide compatibility between the water-insoluble chemosensor and the aqueous analyte. Our polymeric matrices consist of poly(ether)urethane (PEU) copolymers. The main characteristic of these polymers is that they possess hydrophilic segments that depending on their formulation can up-take water through a swelling process. In this chapter it is demonstrated how the formulation of the polymer can impact the sensitivity and selectivity of the sensing process and how this can be used to generate discriminatory information in sensor arrays.

3.2 Theoretical Aspects of Colorimetric Anion Sensor bearing Push-Pull Chromophores

In general, colorimetric sensors comprise a chromophore capable of undergoing intensive color change upon small perturbation of the electronic density induced by the analyte-chemosensor interaction. From the signaling point-of-view, it is important that the chromophore appended to the receptor presents a strong absorption ($\varepsilon \sim 10^4$). It is also important that the dipole moment (and electron density distribution) of the chromophore changes during the recognition binding event to the point that new and intensive electronic absorptions will appear in the visible (preferably) region of the absorption spectrum. A way to insure that the perturbation of the electronic density in the receptor moiety will affect the chromophore is to establish direct
electronic communication between the receptor and the chromophore, preferably via a π-conjugated bridge.

This idea of having electronic communication facilitated through conjugated systems has been widely explored in the past, in a number of fluorometric sensors and switches, and solvatochromic dyes. In most cases, an electron donor group (EDG) is attached to a conjugated backbone to provide direct electronic communication with an electron-withdrawing group (EWG). For instance, the \( \text{N,N-dimethyl-para-}((\text{para-nitrophenyl})\text{diazenyl})\text{benzamine (PNABA)} \) is a typical example of a solvatochromic dye comprising a pull-push chromophore (Figure 3.1). PNABA presents a positive solvatochromism (bathochromic shift) in the absorption spectrum of about ~60 nm upon transition from nonpolar (e.g. heptane) to polar (e.g. dimethyl sulfoxide) solvents.

![Figure 3.1](image)

**Figure 3.1.** \( \text{N,N-dimethyl-para-}((\text{para-nitrophenyl})\text{diazenyl})\text{benzamine (PNABA)} \) is a typical example of a solvatochromic dye with a pull-push chromophore. PNABA presents a positive solvatochromism (bathochromic shift) in the absorption spectrum when going from nonpolar to polar solvents probably due the stabilization of the first excited state in polar solution.

This solvatochromic effect in dyes with a pull-push chromophore shows that polar solvents stabilize better the first excited state relative to the ground state causing a bathochromic (red) shift. In this regard, the first excited state refers to the so-call Franck-Condon excited state, given by the molecular geometry with the same solvation pattern present in the ground state.
From studies of solvatochromic dyes in the literature, it is straightforward to draw a parallel: if dipole-dipole interactions (dye-solvent) can produce such a dramatic change in the absorption spectrum of the dye, the interaction of anions with a chemosensor bearing this kind of push-pull chromophore is likely to induce a colorimetric response. However, in order to make a feasible colorimetric chemosensor for anions a receptor should have an electronic structure compatible with the push-pull paradigm. In essence, the binding of the anion may result in a redistribution of the electronic density in the chromophore, when the electron density of the anion “pushes” the electron density of the receptor. For this reason, the chemosensor receptor should be electron rich in nature and it should have an EDG character (Figure 3.2). In this context, receptors based on pyrrole, as chelating agents, are ideal since pyrroles are electron-rich and prone to the push-effect. Following this design, in order to establish an efficient partial ICT the presence of an electron-rich anion (bound to an electron-rich aromatic receptor) needs an electron-poor moiety (EWG) attached to the receptor that then acts as an electron density acceptor. Figure 3.2 shows two examples of an effective push-pull chromophore (center) and one example of an ineffective one (right).

**Figure 3.2.** The color transitions responsible for signaling are a result of an anion-induced intramolecular charge transfer (ICT) in the push-pull chromophore.
In recent years, our group has successfully prepared and studied anion colorimetric chemosensors based on pyrrolic receptors, such as dipyrrrolylquinoxaline (DPQ) or octamethylcalix[4]pyrrole (OMCP) bearing chromophores with an electron withdrawing moiety. Even though the operating principle in such anion chemosensors with *push-pull* chromophores is to some extent clear and intuitively understood, we wanted to shed some light on the actual electronic effects involved in the sensing process and quantify the extent of it by making use of quantum chemistry calculations. Specifically, density functional theory (DFT) has proven to be a very accurate tool for the calculation of anion-receptor interactions. In order to quantify and visualize the impact of the anion coordination to a chemosensor, the total electronic density can be calculated and mapped using the electrostatic potential (ESP). In this way, the electron distribution can be estimated and the electronic effect of the anion coordination determined. For our calculations we had utilized two OMCP derivatives 3.1 and 3.2 that resemble chromophores shown in figures 3.2.

![Figure 3.3](image)

Figure 3.3 shows the total electron-density (B3LYP | 6-31+G*) calculated for geometry optimized models of 3.1 and 3.2 in the *off* and *on* (complexed to chloride) states. The ESP surface shows that the *push-pull* effect is taking place by redistributing the electron-density in the chromophore. First, a qualitative inspection of the ESP surface of 3.1•Cl complex shows that the pyrrole moiety, which is a part of the extended chromophore, presents a less negative (lighter
Figure 3.3. Total electronic density (B3LYP | 6-31+G*) mapped with the ESP for the chemosensor in the 1,3-alternate conformation and bound to a chloride anion in the cone conformation. The color scale is relative and the charge increases from red to blue.
red) relative potential when compared to the non-substituted pyrroles. This attests to the electron withdrawing effect of the nitro group as an EWG in the chromophore.

On the other hand, the ESP surface of 3.2 in the off state shows that most of the charge is accumulated over the azo \((N=N)\) moiety. After chloride complexation, the chromophore in 3.2 is not capable of redistributing the electron-density. Furthermore, when the ESP is compared between the four pyrroles in the OMCP receptor of 3.2, the distribution of the charge between the pyrroles in the receptor moiety are virtually the same due to the presence of a EDG in the chromophore. Because the charge is not redistributed within the chromophore, the changes in the dipole moment of the chemosensor are smaller resulting in a only a very small change in the color output. The smaller magnitude of the response had been previously demonstrated in our labs,\(^5\) but there was not theoretical support for the experimental observations.

Originally,\(^5\) it was thought that the red shift observed in the absorption spectra in the azo-OMCP family of compounds was a direct consequence of the increment of the HOMO manifold mainly localized predominantly at the pyrrole moieties. At the same time, the LUMO manifold less affected since it is localized at the chromophore with a \(\pi^*\) character. In the case of 3.1 (Figure 3.4),\(^5\) the experimental data reveals that the change in color varies slightly with the kind of anion. The Uv-vis spectra also show that the anion-induced changes affects the complete shape of the spectra and it presents a small red shift (~30nm) of the absorption maxima.
This modest change in color does not seem to correlate with a simple decrease of the HOMO-LUMO gap since such a change in the electron-density would be expected to produce a deeper color change. An evaluation of the ground state calculations reveals that after the anion complexation both HOMO and LUMO increase their relative energy (Figure 3.5). Thus, the original idea about just the HOMO level being mostly responsible for the observed effects seems to be an oversimplified picture of the real origin of the color response.

Figure 3.5. B3LYP | 6-31+G* (PCM:DMSO) energy levels in hartrees (a.u.) calculated the LUMO and HOMO to HOMO-4 before and after complexation of chloride ion.
Interestingly, the estimated HOMO-LUMO gap from the ground state calculation, for chemosensor 3.1 (Figure 3.5) shows there is an actual decrease in the magnitude (~1 eV) of the HOMO-LUMO gap upon the anion complexation. Even though extrapolations of the energy levels of molecular orbitals in the excited state are not entirely accurate, a difference of 1 eV would translate into circa 250 nm shift, a magnitude not observed in the experiment (Figure 3.4).

Consequently, we decided to further explore the partial ICT mechanism by performing time dependent DFT (TD-DFT) on chemosensor 3.1. TD-DFT calculations expand the basic ideas of ground-state DFT to systems in a time-varying field. TD-DFT is one of the most widely used methods for the theoretical characterization of excited states. TD-DFT allows for the calculation of the vertical absorption energy ($E_{va}$), also known as Frank-Condon energy, and the changes (evolution) of the dipole moment of a molecule during the absorption of a photon (transition dipole moment). The square of the transition dipole moment determines the strength of the transition and it is usually represented as the oscillator strength. In other words, the oscillator strength ($f$) is directly proportional to molar absorptivity of a given molecule.

The theoretical vertical absorption energy (e.g. excitation energy) ($E_{va}$) and the oscillator strength ($f$) for the chemosensor 3.1 were calculated and plotted against the experimental data (Figure 3.6). As it can be seen in the figure 3.6 (left), TD-DFT is capable of accurately estimating the absorption maximum at 440 nm, as well as the shoulder feature around 375 nm.
A closer look at the oscillator strength shows that the first four excited states are actually weakly allowed transitions. Only the generation of S₅ is strongly allowed, thus confirming that the HOMO and LUMO manifolds are not the main contributors to the actual absorption spectrum.

TD-DFT was also applied to the complex 3.1•Cl. Unfortunately, the calculation was not able to reproduce the experimental absorption spectrum as it was in the case of 3.1 alone. This might be due to the fact that in solution the complex is actually a dynamic equilibrium between on and off states with multiple conformations coexisting at the same time. Such conditions are very hard to reproduce in a theoretical calculation, which explains the lower accuracy of such calculations. Nevertheless, the TD-DFT results for the complex clearly show the predicted bathochromic shift in the absorption spectrum in respect to the chemosensor 3.1 alone (Table 3.1).
Table 3.1 supports to some extent the estimation made from the ground state calculation regarding the decreasing the HOMO-LUMO gap after chloride complexation. The exact net change in the generation of $S_1$ after the chloride complexation is 0.43 eV (~150 nm), a magnitude not observed in the experiment. This can be explained by the HOMO to LUMO transitions being only weakly allowed in both (on and off) states. Together with these results, TD-DFT actually predicts a red shift of about ~ 0.1 eV (20 nm) in the absorption maximum ($S_0 \rightarrow S_5$), after chloride complexation. Also, TD-DFT provides a relative composition of the ground state molecular orbitals generating a specific excited state $S_n$. Figure 3.7 shows that after the complexation of the anion there are a multiple changes in the electronic distribution and the geometry of the MOs. These redistributions allow for diverse (even small) changes in the
absorption spectra that can be translated into qualitative information given the fact that these changes depend on the nature of the anion.

**Figure 3.7.** Graphical representation of the LUMO and HOMO to HOMO-4 of 3.1 and 3.1•Cl\(^{-}\). The green (\(f \sim 0.5\)) and blue (\(f \sim 0.1\)) highlights indicate the orbitals with allowed optical transitions to LUMO. Top: Chemosensor 3.1. Bottom: Complex 3.1•Cl\(^{-}\). The most allowed transition is due to an excitation of an electron from HOMO-3 \(\rightarrow\) LUMO (\(\pi \rightarrow \pi^*\) character) to generate S\(_5\).

Here, it has been shown that the anion-induced response in chemosensors comprising *push-pull* chromophores is more complex and rich than originally thought; they can generate more information than simply a spectral red shift. Furthermore, the nature of the interacting anion will have a strong influence in the chemosensor response, which depends on the anion electronegativity and structure as it influences the molecular dipole moment.

### 3.3 Bio-Inspired Sensor Material Design

As mentioned before, the design of the studied materials was inspired by Nature. The primary objective was to create a solid-state system capable of sensing ions in water utilizing
chemosensors that are insoluble in water. The sensing process should be carried out in a matrix that will also provide mechanical support to the sensing layer while accommodating the chemosensor in the inner lattice being able to absorb water thus helping the ion transport to the chemosensor embedded in the polymer matrix.

Over the past twenty years, ion-selective bulk optodes have utilized hydrophobic polymeric matrices, such as poly(vinyl chloride) PVC, operating typically under the ion exchange mechanism. These materials are designed to enable indirect sensing of ions by monitoring of smaller counter ions that can be easily co-extracted to the sensing membrane. For instance, Figure 3.8 shows a scheme representing an ion selective bulk-optode for anion detection; the high affinity of the neutral receptor to the anionic analyte drives the anion extraction into the sensing membrane, along with a proton that will trigger an optical signal in the pH sensitive dye, which is embedded in the optode.

![Figure 3.8](image)

Figure 3.8. Schematic view of the operating principle of a bulk optode membrane for anion detection containing a neutral receptor, neutral H⁺-selective chromoionophore and anion exchange salt.

The most efficient formulations of these materials usually provide high selectivity, but this advantage is compromised by a very complicated formulation (receptor, indicator, ion exchanger, plasticizer and polymer) and another technical drawbacks, such as indicator leaching.
out of the membrane, swelling of the polymer, which makes it difficult to establish accurate mathematical models to describe the optode behavior.

For our design, we planned to simplify the composition of the sensor material and take advantage of the synergy between a polymeric matrix and the chemosensor to improve the recognition process. This is particularly important in the anion detection since most of our chemosensors are based on hydrogen bonding to the anion. In a highly competitive media, such as water, chemosensors based on hydrogen bonding have to compete for the anion with highly negative solvation energies. Thus, an ideal polymeric matrix should: be able to accommodate the chemosensor and interact with water to help in the recognition process. The latter also implies that the polymer should not interact strongly, neither with the sensor nor the analyte, which render the anion-sensor association less effective. Nevertheless, we show that to a certain extent, these interactions could also be desirable and help in the selectivity recognition process.

The kind of synergy effect has been observed in nature, for instance, in enzymes where the proteins accommodate the cofactors in the active site. Specifically, the X-ray crystal structure of the porphobilinogen deaminase shows a dipyrromethane cofactor bound to the active site. The cofactor is primarily bound through the electrostatic interaction of the dypyrromethane carboxylate pendants (in the β-position of the pyrrole) and three different arginine residues in the protein. More importantly, the cofactor is engaged in a fourth interaction through a delicate hydrogen bond between both pyrrole N-Hs and the carboxylate moiety of the aspartate (Asp 84) of the active center (Figure 3.9). Particularly the last interaction should be sensitive to the presence of water molecules. However, the hydrophilic residues in the proximity of the active center can interact with water molecules and strip most of the solvent water from the active site, thus allowing such a weak interaction to occur. It is important to add that the replacement of the
aspartate-84 by a glutamate results in 99% loss of the enzyme activity. This attests to the importance of the interaction observed, in a natural system, between an anionic residue and pyrrolic-NHs. This interaction results highly effective even in an otherwise competitive (water solvation) environment.  

To draw an analogy between Nature (bio-mimetic approach) and the sensor materials, the polymer matrix could be seen as the protein capable of interacting with water and striping the solvate from the active site where the recognition process takes place, while the chemosensor acts as a cofactor and interacts with the substrate (anion).

**Figure 3.9.** X-ray structure of the enzyme porphobilinogen deaminase (cartoon representation) with the dipyrrromethane co-factor bound to a carboxylate from the Asp 84 (space filling representation) residue through a pyrrolic NH hydrogen bond. The red spheres are oxygens from water molecules. Left: Complete view. Right: detail of the active site.

As proposed above, the polymer matrix will be used to interact with water. Certain polymers have the ability to absorb water in equilibrium, as is the case of hydrogels and hydrophilic polymers. The main difference between these two kinds of materials is that the first are usually categorized as supersorbents and are capable of uptaking up to 5000 × of their weight in water by incorporating the water molecules as part of polymer network. Instead, hydrophilic
polymers tend to uptake lower amounts of water (up to 200% of their weight) by a swelling process that, in most of the cases, is related to regular hydrogen bonding interaction without compromising the polymer network. Moreover, both materials are insoluble in water and depending on their particular compositions, they could be soluble in most common organic solvents. The driving force for the water uptake by both the hydrogels and hydrophilic polymers is a prominent negative enthalpy of hydration with a small negative entropic contribution.\textsuperscript{15,16}

It has been reported that supramolecular hydrogels can uptake ions with water.\textsuperscript{17} Unfortunately, in the case of anion detection, the work by our group had shown that OMCP based chemosensors do not operate very well in hydrogels (polyacrylamide),\textsuperscript{18} probably because the anions remain solvated in micro domains in the polymer (gel) network. On the other hand, hydrophilic poly(ether)urethanes (PEUs, Tecoflex\textsuperscript{®}) have been previously employed in ion-selective bulk optodes (Figure 3.10).\textsuperscript{19} PEUs were first suggested as plausible materials for ion-selective electrodes and optodes because of their adhesion properties to glasses and their usually low transition glass temperature ($T_g \sim -60\,^\circ C$), which allows circumventing the use of plasticizers to achieve desired ion mobility in the membrane. For example, Tecoflex\textsuperscript{®} is in nature hydrophilic, it is only capable of 0.5 to 1% of water uptake, since swelling is not a desirable property for membranes in ion-selective electrodes and optodes.

![Figure 3.10](image_url)

**Figure 3.10.** General structure of Tecoflex\textsuperscript{®} a poly(ether)urethane co-polymer based on the copolymerization of diisocyanate aliphatic residue (hydrogenated methyl diphenyl diisocyanate (HMDI)) polybutylene glycol (PBG) and 1,4 butanediol chain extender.
In the bio-inspired sensor design, we were seeking materials with the right balance between hydrophilicity and hydrophobicity. Fortunately, hydrophilic polyurethanes (PEU) have the advantage that can be tailored to uptake certain amount of water in equilibrium by changing the ether segments based on polybutylene oxide (PBO) to a more hydrophilic polyether, such as polyethylene oxide (PEO), which by itself is soluble in water. The resulting PEU uptakes water proportionally to the percentage of the PEO comonomer in the polymer. Moreover, structure-function correlation in PEUs (containing PEO) has been well established and there are commercially available formulations that combine desirable water uptake with mechanical properties of the material such as durometry, transition glass temperature, tensile strength, elongation, elastic recovery, etc.

Lubrizol manufactures a line of hydrophilic thermo-plastic polyurethanes based on the chemistry of the PEO to tune the overall hydrophilicity of the Tecophilic® polymer (Figure 3.11). Unfortunately, the exact composition of commercial polymers remains a trade secret.

![General structure of Tecophilic®](image)

**Figure 3.11.** General structure of Tecophilic® a poly(ether)urethane co-polymer based on the copolymerization of diisocyanate aliphatic residue (hydrogenated methyl diphenyl diisocyanate (HMDI)) polybutylene glycol (PBG), polyethylene ether glycol (PEG) and 1,4 butanediol chain extender.

Seeking a better understanding of the chemistry of the Tecophilic® PEUs, spectral characterization of the series (SP-60D) was carried out by using $^1$H NMR. Figure 3.12 shows the $^1$H NMR of SP-60D-5 polymer, which is capable of circa 5% water uptake. From the NMR
spectra of this family of polymers, three groups of signals corresponding to the PEO soft
group (singlet, 3.65ppm, blue), PBO soft segments (multiplet, 3.42ppm, green) and PU-BO
hard segments (broadband, 4.82ppm, yellow) can be distinguished.

Figure 3.12. \(^1\)H NMR of Tecophilic® SP-60D-5 showing clearly resolved signals that can be
used for the quantification of the relative proportions of the co-polymer residues.

Within the series SP-60D the molecular weight is ~ 100 kDa with a polydispersity of 5
kDa.\(^{23,24}\) Also, from the NMR analysis (Figure 3.13), it is clear that the urethane content (hard
segments) of the polymer remains almost constant across the series. On the other hand, the ratio
between the soft segments (PEO:PBO) controls the water uptake. There appears to be a linear
correlation between the water uptake and the PEO:PBO ratio (Figure 3.13, right). This
correlation helps to achieve a right balance between hydrophilicity and hydrophobicity when
looking for the maximum synergy between the chemosensors and the matrix.
Figure 3.13. Left: $^1$H NMR of Tecophilic® SP-60D series. Right: the correlation of the PEO:PBO ratio with the water uptake for each polymer.

The sensor material consists of two components (chemosensor and polymer) fabricated by solution casting a THF solution of the chemosensors with a hydrophilic PEU. The proposed sensing mechanism is shown in scheme in Figure 3.14. During the water uptake the hydrophilic segments of the polymer matrix partially remove the hydrate water from the anion, leaving the analyte (anion or cation) with a reduced solvent sphere. The lower number of solvate molecules enables more effective recognition of the analyte by the receptor in a same way the protein does in enzymes. Finally, after the recognition event takes place the remaining water in the film is removed drying to fully develop the response and to make it analytically reproducible.
Figure 3.14. A general scheme of the proposed sensing mechanism. After the sensing layer is in contact with the aqueous sample (a) anions and cations are co-extracted during the water uptake process (polymer swelling). The PEU partially removes the hydrate from the analyte. The PEU-water interaction is the driving force that provides transport and ion partial dehydration. (b) The “semi-naked” ion can be recognized by the chemosensor. (c) The remaining water is removed by evaporation to reach analytical reproducibility.

The downside of a sensing layer operating under the above mechanism is that response relies on a non-equilibrium phenomenon that makes the elaboration of a mathematical model to describe the sensor behavior difficult. Another consequence is that under this sensing scheme does not allow for continuous analysis. The upside is that the amount of chemosensor and polymer needed in the films is very small (~ 50 ng of chemosensor /sensing element), which makes the development of inexpensive disposable assays possible.
3.4 Role of the Polymer Matrix in the Sensing Process

As stated before, the main role of the polymer matrix is to lend mechanical support to the chemosensor, while stripping the hydrate from the analyte. Beyond this, the nature of the polymeric matrix can also be utilized to induce additional selectivity features. This is shown on the example of PEU where the distribution of hydrophilic and hydrophobic sites can be engineered on demand. Furthermore, in the solid-state the hard segments (urethane and/or urea) of the PEUs self-assemble via inter- and intra-molecular hydrogen bonds producing a nanoscopic phase separation in the polymer network (Figure 3.15).

![Image of nanophase structure](https://example.com/figure3.15.png)

**Figure 3.15.** Highly schematic (2D) representation of the nanophase structure.

It is conceivable that the chemosensors have a different affinity expressed as a partition coefficient for the different nano-phases in the polymer network. Moreover, a similar partitioning could happen with the distribution and availability of the analyte. This could also mean that the sensing process could be dependent on the PEU chemical composition.

In order to investigate the impact of the polymeric matrix composition on the sensing process, we have used an OMCP derivative bearing a *push-pull* fluorophores, chemosensor 3.3 capable of detecting anions while displaying turn-on behavior. The sensing experiments were
carried out utilizing five different Tecophilic® PEUs matrices (SP-60D-5, SP-60D-10, SP-60D-16, SP-60D-20 and SP-93A-100) and six anions (acetate, benzoate, chloride, phosphate, fluoride and pyrophosphate as tetrabutylammonium) (Figure 3.16).

Figure 3.16. Left: $^1$H NMR for the five different Tecophilic® used in the sensing experiments. Right: Relative response of the sensing elements prepared using different polymeric matrices and chemosensor 3.3 to the presence of 200 nL of tetrabutylammonium salts (500 μM) of different anions in water at pH 7.

The response was recorded following the relative changes in the fluorescence (in the yellow channel) of the sensor films in the presence of aqueous solutions of anions. Figure 3.16 shows how different polymeric matrices impact the response produced by the embedded chemosensor. Interestingly, the response seems to increase with the water uptake until the uptake is 20% (w/w). When the polymer water uptake increases to 100% (w/w) the sensor response falls dramatically. As mentioned before, this is probably because the anion might
remain solvated in the hydrophilic domains in the highly hydrated polymer network, as in the case of polyacrylamide hydrogels.

In terms of selectivity, the response profiles show that the same chemosensor embedded in different matrices displays a different response fingerprint. In order to analyze if the patterns shaped by the polymeric matrices have enough discriminatory information for the classification of the six anions, a principal component analysis (PCA) was carried out for the responses consisting of 5 features (5 Tecophilic® grades). The PCA score plot showed a poor clustering of the data but still a high degree of dispersion, as seen from the amount of variance described by the principal components (Figure 3.17). Thus, changes in the polymeric matrix generate dispersion of the data, but there is not enough significant information to differentiate among the anions.

![Figure 3.17](image)

**Figure 3.17.** Left: 5-dimensional patterns (3.3 embedded in 5 different PEU matrices) generated by the 5-sensors array upon addition of 200 nL of tetrabutylammonium salts (500 µM) of different anions at pH 7. Right: PCA score plot of the first two PCs describing circa 86% of the total variance for all seven anion samples (4 trials each, 500 µM, 200 nL, pH 7).

Given the fact that PEUs copolymer can undergo nano-phase separation, we decided that to study also the effect of blending the two components (chemosensor and PEU) of the sensor
film at several different ratios. Different chemosensor:polymer ratios could result in a different distribution (partition) of the chemosensor in the polymer network and yield a differential response depending on the degree of dissimilarity in the interactions are between polymer, chemosensor, analyte, and solvate in the nano-phases in the polymer network.

In our experiment we prepared an array consisting of 16 different blends of chemosensor 3.3 and PEU Tecophilc® SP-60D-20. As expected, subtle interactions involved in the polymer-sensor-analyte relationship yielded a differential response of the sensor array. The corresponding PCA score plot showed an improved clustering of the data, but the discriminatory power is reduced and resembles those typical in electronic noses where most of the variance is displayed in just one principal component (Figure 3.18).

![Figure 3.18](image)

**Figure 3.18.** Left: Highly schematic representation of the possible interaction between the chemosensors and the polymer. The sensor array was prepared using 16 different blends of 3.3 and SP-60D-20. Right: PCA score plot for the responses of the 16-sensors array to the presence of all seven anion samples (4 trials each, 500 μM, 200 nL, pH 7). The score plot is describing circa 97% of the total variance in the first two principal components.

Even though the last two experiments show a poor discrimination among the set of anions, they confirm that the chemical nature of the polymer might influence the sensing process and contribute to the total information generated by the sensor by adding another layer of
information. It is worth noting that the last two sensor arrays utilized only one chemosensor (3.3) and that the polymer matrix somehow adds to the fine tuning of the sensing process by adding its own contribution to the selectivity of the sensor material.

We carried out our last experiment combining the two sets of data (5 PEUs × 16 different ratio blends) to generate an 80-sensors array, still using just one chemosensor. After combining the discriminatory features generated by the polymeric matrix, the PCA score plot shows clear clustering of the data and high discriminatory capacity (Figure 3.19). This experiment corroborates the hypothesis that the polymeric matrix plays a more important active role than to simply lending a mechanical support and shows that these intrinsic polymer properties could be used in sensor arrays protocols to generate information to discriminate among different samples when different chemosensors are not available.

**Figure 3.19.** Left: PCAs for the first two sensor arrays experiments (See Figure 3.17 and 3.18). Right: PCA score plot for the combined responses of the 80-sensors array (5 PEUs × 16 different ratio blends) to the presence of all seven anion samples (4 trials each, 500 μM, 200 nL, pH 7). The score plot is describing circa 97% of the total variance in the first two principal components.
3.5 Conclusion

In summary, first it was demonstrated that DFT and TD-DFT protocols could be used to gain a better understanding of the electronic processes involved in the sensing process in push-pull chromophores. Moreover, the addition of quantum chemistry calculations to the “rational design” toolbox opens the door for future implementation of theoretical calculations for the prediction of chemosensor response without having to synthesize it. Since molecular dynamics protocols will have to be coupled with DFT in order to obtain a better interpretation of the complex equilibrium when multiple conformations are present in the system, the computational cost of such a calculation is currently a limitation and makes this kind of calculation unrealistic even for state-of-the-art supercomputers.

Secondly, sensor materials were obtained through rational design by mimicking the protein-co-factor synergy in enzymes. The final sensing materials consist of only two components (chemosensor and hydrophilic polymer matrix) bulk optodes, a simplified version of traditional ion-selective bulk optodes operating with a coextraction mechanism. Also, it was shown that the role of the polymer in the sensing process extends beyond simply lending mechanical support and that the synergy between chemosensors and polymeric matrices can be utilized to extract additional information useful for the discrimination of potential analytes.

3.6 Experimental Section

3.6.1 Computational Details: All calculations were performed with the Gaussian 03 suite of programs, revision D.02. For geometrical optimizations the first guests were fully optimized by PM3 semi-empirical Hamiltonian. Next, the models were re-optimized at B3LYP | 6-31G* level of theory in vacuum. Finally, single point calculations were carried out on the optimized
geometries at the B3LYP | 6-31+G* level of theory. TDDFT calculation were performed as implemented in the Gaussian 03 code utilizing two different hybrid functionals B3LYP and PBE0 for comparative purposes. Even though it has been reported that the PBE0 functional yields more accurate excitation energies, in the case of chemosensor 3.1, B3LYP gave a deviation of 0.1 eV with the experimental absorption maxima, while PBE0 reported a 0.80 eV deviation. For the complex chemosensor 3.1•Cl and 3.2•Cl, tetramethylammonium was added to the molecular model to balance the charges and resemblance of solid-state (x-ray) binding motifs, while for solution calculations the complexes were treated as anions (i.e. without considering the counter ion). All single point calculations were carried out using polarizable continuum model (PCM) with DMSO as solvent as implemented in Gaussian 03 code. The molecular orbitals and total density cubes were generated in Gaussian 03 and visualized with GaussView 3.09.  

3.6.2 Chemicals and Solutions: Commercially available solvents and reagents were used as received from chemical suppliers. Tetrahydrofuran was distilled from a K-Na alloy under argon. 1H spectra were recorded using a 300 MHz Bruker instrument.

3.6.3 Preparation of Sub-microliter Sensor Arrays for Anion Sensing: The sensor materials were prepared by incorporating chemosensors 3.3 into poly(ether)urethane matrices, which were prepared by casting 200 nL of solutions containing the sensor (approx. 0.08% sensor in poly(ether)urethane, w/w) in a THF solution of different grades of Tecophilic™ (SP-60D-5, SP-60D-10, SP-60D-16, SP-60D-20 and SP-93A-100) (4 % w/w) onto a multi-well (sub-microliter) plate. For a typical assay, the solutions of the analytes were added (200 nL, 500 μM) as aqueous solutions of their tetrabutylammonium salts at pH 7.
3.6.4 Fluorescence Sensor Array Image Acquisition and Data Processing: Images from the sensor arrays were recorded using a Kodak Image Station 440CF. The scanned images (12 bit) are acquired with a resolution of 433x 441 pixels per inch and with grey levels over 1000 (12 second exposures). The sensor arrays are excited with a broadband UV lamp (300-400 nm, \( \lambda_{\text{max}} = 365 \) nm) and the emission detected through a yellow: long pass filter 523 nm. After acquiring the images, the integrated (non zero) grey pixel \( (n) \) value is calculated for each well of each channel. Images of the sensor chip were recorded before \( (b) \) and after \( (a) \) the addition of an analyte. The final responses \( (R) \) were evaluated as indicated in equation 1.

\[
R = \sum_{n} \frac{a_n}{b_n} - 1 \quad (1)
\]

3.7 References


(12) The oscillator strength is a dimensionless quantity that results from the transition dipole moment expression when divided by the charge.


(20) Unpublished results in polyacrylamide gel matrices carried out at Anzenbacher Research Group by Dr. Ryuhei Nishiyabu.


(24) The SP-60D-XX are PEU resins for solution processing with 60D shore hardness uptaking XX % (w/w) of water.

(25) Personal communication from Dr. Anthony Walters R & D director at Lubrizol.

(26) Molecular weight estimate by GPC against polystyrene standard.

(27) This is a slightly modify version of the original scheme. The diagram was originally developed by Dr. Robert Lomax and it is reproduced here with the author’s permission.

(28) The sensing properties of chemosensor 5.3 are not included here for the sake of clarity. These details are given later on chapter V.

(29) The 16 blends were prepared for all five PEUs, but in this example we refer just to the array containing the SP-60D-20 as it yielded the best results.


CHAPTER IV. A COLORIMETRIC SENSOR ARRAY FOR ANION DETECTION.

4.1 Introduction

As it has been proposed in Chapter I, the discriminatory power of sensor arrays might be improved by the use of selective, yet cross-reactive chemical sensors allowing for significant reduction of the number of array elements, while maintaining the reliability of the sensing process. In this chapter, we demonstrate the preparation of a sensor array for small inorganic anions that operate in water and in a multi-anion environment. For our proof-of-concept experiments we have used sensor elements that display certain selectivity for particular analytes, for example fluoride and pyrophosphate, while keeping a significant cross-reactive response for other anions, such as acetate, benzoate, bromide, chloride, nitrate, hydrogen phosphate, hydrogen sulfate and hydrogen sulfide. The practical utility of such a sensor array is demonstrated in the analysis of complex samples such as toothpaste, where, the sensor array utilizes the fluoride content as the main differentiating feature while the rest of the cross-reactive anions in the analyte are used for fine differentiation among toothpaste brands. We also confirm the utility of supramolecular observations of affinity and selectivity in the anion-sensor recognition process for the design of a high-resolution sensor array.¹

4.2 Selection of Chemosensors

The respective affinity constants of chemosensors 4.1-4.8 for different anions (in DMSO and MeCN solutions) recorded as part of previous studies\(^2\text{-}^5\) are listed in Table 4.1. In the following paragraphs, we show that the binding behavior observed in organic solvents is also observed in arrays where the chemosensors are embedded in polyurethane matrices and the anions are administered as strictly aqueous solutions. Thus, the classical methods of molecular recognition may be conveniently used to match the sensor-analyte pairs for array applications.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>F(^-)</th>
<th>Cl(^-)</th>
<th>AcO(^-)</th>
<th>H(_2)PO(_4)</th>
<th>HP(_2)O(_7)(^{3-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>7 240</td>
<td>&lt; 50</td>
<td>16 600</td>
<td>430</td>
<td>5 650</td>
</tr>
<tr>
<td>4.2</td>
<td>&gt; 10(^6)</td>
<td>652</td>
<td>125 000</td>
<td>8 050</td>
<td>274 000</td>
</tr>
<tr>
<td>4.3</td>
<td>&gt; 10(^6)</td>
<td>2 840</td>
<td>&gt; 10(^6)</td>
<td>160 000</td>
<td>512 000</td>
</tr>
<tr>
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<td>22 100</td>
<td>5 560</td>
<td>48 200</td>
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<tr>
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<td>8 540</td>
<td>3 330</td>
<td>92 200</td>
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<tr>
<td>4.6</td>
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<td>1 900</td>
<td>99 900</td>
<td>7 270</td>
<td>ND(^c)</td>
</tr>
<tr>
<td>4.7</td>
<td>&gt; 10(^6)</td>
<td>574</td>
<td>15 700</td>
<td>4 560</td>
<td>64 600(^c)</td>
</tr>
<tr>
<td>4.8(^b)</td>
<td>482</td>
<td>&lt; 50</td>
<td>1 200</td>
<td>&lt; 100</td>
<td>316 000</td>
</tr>
</tbody>
</table>

\(^a\)All errors are < 15 %. \(^b\)Affinity constants were obtained from Uv-vis titration experiments carried out in MeCN. \(^c\)The binding isotherm showed biphasic behavior indicating multiple equilibria.

**Table 4.1.** Affinity constants\(^a\) for compounds 4.1-4.8 (M\(^{-1}\)) calculated for tetra-\(n\)-butylammonium salts of anions in DMSO (0.5% of water) at 22 °C.

The relative affinity for chemosensor 4.1 is: acetate>fluoride>pyrophosphate. Chemosensors 4.2-4.7 show an affinity order: fluoride>pyrophosphate~acetate, and low affinity for phosphate, bromide, sulfate, and nitrate. In order to increase the selectivity in read-out information, we also included chemosensor 4.8, which shows almost equal affinity for fluoride.
and pyrophosphate, and which does not bind any other anion strongly. One can predict that the 8-sensor array composed of chemosensors 4.1-4.8 will have affinity bias for fluoride > pyrophosphate > acetate. Still, the chemosensors show certain degree of cross-reactivity to other anions.

As mentioned before in Chapter III, the change in color of all chemosensors upon complexation is a result of partial intramolecular charge transfer (ICT) from the anion to the sensor. However, the actual color change and dynamic range of the response differ among individual chemosensors, thus contributing to the cross-reactivity of the sensor array. The nature of the response does not necessarily correlate directly with the binding affinity of the chemosensor, but with the efficiency (or degree) of electronic perturbation in the chemosensor’s chromophore when the anion is bound to the chemosensor.

4.3 Anion Sensing in Water

The polymeric sensor elements for the array were fabricated by blending the chemosensors 4.1-4.8 with a hydrophilic poly(ether)urethane (PEU, Tecophilic® SP-60D-20) in THF and solution-casting 400 nL of the chemosensor-PEU solution into a microwell array. The optimum composition of the materials were optimized through a combinatorial screening of several different PEU at different ratios chemosensors:PEU matrix. The role of the hydrophilic poly(ether)urethane is to lend mechanical support to the chemosensor molecules and to draw the bulk aqueous analyte into the sensor material and partially strip the hydrate off the anion, thus rendering the anion available for the recognition process by the receptor moieties (see Chapter III).
The qualitative response of the sensor array was tested using aqueous solutions of ten anions: acetate, benzoate, bromide, chloride, fluoride, nitrate, dihydrogen phosphate, hydrogen pyrophosphate, hydrogen sulfate, and hydrogen sulfide (Figure 4.1), (5 mM in water, 200 nL, 10 trials each; nitrate and hydrogen sulfate concentration 20 mM). The changes in color, which are also observable by the naked eye, were recorded using a USB 24-bit RGB scanner. The image was deconvoluted into RGB channels, and the grey pixel values in each color channel were averaged and subtracted from the image taken before exposure (blank) (See chapter II for details). Figure 4.1 shows color changes in the wells as well as the respective changes in the RGB values of chemosensors 4.1-4.8 upon addition of tetra-butylammonium acetate. The patterns (or response vectors) generated by each analyte consist of 24 dimensions given by 3 RGB channels × 8 sensor elements. Figure 4.1 (right panel) also shows the patterns generated from raw data in the green channel, which illustrates what is clear from naked eye observation: the 8-sensor array is generating unique patterns for each anion. A concentration of 5 mM was selected because it results in saturation of most sensors in the array by all anions. Naked eye inspection confirmed that the wells showing the deepest changes in color were those of fluoride, pyrophosphate, and acetate. Further evaluation was performed by methods of a multivariate analysis.8,9
Figure 4.1. Left: Typical 8-sensor array responses (raw data) to aqueous anions solutions (200 nL, 5 mM; NO$_3^-$ and HSO$_4^-$ concentration was 20 mM). Center: The net response profile of polymer sensors 4.1-4.8 to the addition of aqueous acetate (200 nL), appendix 3 lists response profiles (response vectors) for all analytes. Right: Patterns generated by the sensor array in the green channel in the presence of the same anion solutions as in the left panel.

Concentration dependant studies were also performed. While the majority of anions provided a response discernible by visual inspection at 100 μM concentrations and higher (Figure 4.2), the scanned images deconvoluted into the respective red-green-blue channels allowed for plotting response isotherms from 0.2 to 360 ppm anions (10 μM to 20 mM) (Figure 4.2). Figure 4.2, right panel, shows a quantitative representation of changes of the gray pixel value in the green channel of sensor elements doped with 4.6 and 4.7 at benzoate and chloride concentrations ranging from 10 μM to 20 mM, respectively. More examples are listed in Appendix 3.
Figure 4.2. Left: Response of the 8-sensor array to different benzoate (above) and chloride (below) concentrations. Right: Changes of the gray pixel value (green channel) of chemosensor 4.2 upon increasing benzoate (above) and chloride (below) concentrations.

The dynamic range and magnitude of response of the individual polymer sensors resembles the trend of the affinity of the chemosensors to anionic substrates in organic solvents shown in Table 4.1. Therefore, from a practical standpoint of view this means that chemosensors showing higher binding constants could address better analytes where the target anion concentration is low. This demonstrates why it is important to correlate the supramolecular behavior of the sensor elements, even though it was studied in organic solutions, to the array response where chemosensors are embedded in a polymer matrix. Figure 4.3 shows the response of two polymer sensors doped with 4.2 and 4.5, to acetate in different concentration ranges.
Here, polymer sensor doped with 4.2 reaches saturation at an acetate concentration below 10 ppm while polymer sensor doped with 4.5 reaches saturation at a significantly higher concentration, i.e. 300 ppm (> 10 mM). Of course, this may not be such a surprise considering that chemosensor 4.2 shows a binding constant for acetate of ca 100,000 M\(^{-1}\), while chemosensor 4.5 displays a modest binding constant of less than 10,000 M\(^{-1}\) in DMSO.

**Figure 4.3.** Changes in the gray pixel value (green channel) of polymer sensors 4.2 and 4.5 upon increasing acetate concentrations.

### 4.4 Statistical Analysis of Multivariate Response Patterns

The 8-member sensor array produces an output in the form of a multidimensional response vector (24-dimensional=3 RGB channels \(\times\) 8 sensor elements), *vide supra*. Hence, multivariate statistical evaluation of the array responses to aqueous solutions of anions was further explored using principal component analysis (PCA) and hierarchical clustering analysis (HCA). Here, the PCA of the dataset (10 trials for each anion) obtained from the 8-sensors array requires 9 dimensions (PCs) out of 23 to describe 95% of the discriminatory range (39% of all PCs). This attests to an exceptionally high degree of dispersion of the data generated by this sensor array consisting of just eight sensor elements. By the same token, this level of
discrimination contrasts with those reported for most electronic tongues, which have typically 95% of the discriminatory range in the first two PCs.\textsuperscript{10}

Furthermore, the PCA score plot (Figure 4.4, center) shows clear clustering of the data when only the first three PCs (representing 78.4% of variance) were plotted. More importantly, the high level of dispersion of the data shown by the PCA can be attributed to the selectivity of the chemosensors and a strong supramolecular interaction between the sensors and certain analytes ($F^-$, $\text{HP}_2\text{O}_7^{3-}$, $\text{AcO}^-$ and $\text{HS}^-$). At the same time the chemosensors also display a good degree of cross-reactivity as infer from the observable colorimetric response even to the anions that show lower affinity for the sensors. Generally speaking, it is this combination of a strong selective feature and high cross-reactivity of the sensor elements, which allows for better separation (resolution) of the clusters (analytes) in the PCA score plot. The latter finally translates in a sensor array with higher performance.

In addition, HCA was used to further explore the group relationship among the response patterns generated by different anions. In contrast to PCA, HCA considers the complete dimensionality of the data and provides a mono-dimensional graphic output in the form of a dendrogram (Figure 4.4, right). At 90% of similarity, HCA shows ten clusters, each comprising 10 samples. Interestingly, the HCA dendrogram shows clustering at approximately 1000 distance units (d.u.) for 6 anions: benzoate, chloride, phosphate, bromide, sulfate, and nitrate. This group of 6 anions are related by their similar relative affinity to calix[4]pyrrole derivatives 4.2-4.7 in organic solutions (Table 4.1). Moreover, both acetate and pyrophosphate that show strong affinity toward the chemosensors in solution are also separated by a very short distance (approximately 500 d.u.), which reflects similarities in their interaction with the sensors.
Finally, both PCA and HCA suggest that the array of chemosensors 4.1-4.8 embedded in Tecophilic® SP-60D-20 show a selectivity bias (in the sensing of anions in water solution) similar to that imposed by the supramolecular properties observed in organic solution for the chemosensors 4.1-4.8. Therefore, the solution-based observations performed by NMR, UV-Vis, and fluorescence spectroscopy can aid in constructing arrays comprising sensors with predictable analytical behavior. Particularly, the connection between supramolecular properties (e.g. changes in the conformation, binding stoichiometry, functional groups participation, and binding mode) on the atomic level observed by NMR and the discriminatory capability of the sensor array is important and cannot be easily established in arrays employing non-selective indicators.10,11,12

Two more aspects are worth noting: the sensing process is fully reversible and the array is reusable.

4.5 Toothpaste Brand Identification Based on Anion Content.

To demonstrate the utility of substrate-selective sensors for anions in an array aimed at complex analytes and provide a real-life example, we performed identification on toothpaste samples using their anion content for the analysis. Toothpastes are very complex analytes
composed of numerous known and unknown ingredients. A typical toothpaste comprises abrasives (e.g. hydrated silica, calcium phosphate), fluoride sources (sodium fluoride, stannous fluoride, or sodium fluorophosphate), polymers to thicken the paste and retain moisture (humectants), (carboxymethyl cellulose, polyethylene glycols), foaming agents (e.g. sodium dodecyl sulfate, sodium sarcosinate), tetrasodium pyrophosphate to remove Ca$^{2+}$ and Mg$^{2+}$ from saliva to prevent tartar calcification, whiteners (titanium dioxide), peroxide whiteners (sodium carbonate peroxide 2Na$_2$CO$_3$•3H$_2$O$_2$), sweeteners (sodium saccharin, xylitol), antibacterials (triclosan, zinc chloride), fragrances (e.g. peppermint, spearmint oils), sealants - sensitivity reducers (potassium nitrate, strontium chloride), preservatives (sodium benzoate, methyl paraben), buffering agents (sodium hydroxide), remineralization agents (calcium glycerophosphate, calcium ascorbate), etc. The approximate percentages of common ingredients are: water and humectants 75%, abrasives 20%, foaming and flavoring agents 2%, and buffering agents 2%, while fluoride is usually present in less than 0.25%. The fluoride content may differ significantly. Specialty toothpastes such as Fluoridex Daily Defense® contain 1.1% NaF (5,000 ppm). This paragraph illustrates that a toothpaste is a complex analyte comprising fluoride, pyrophosphate, and large concentrations of other anions. Array elements operating in such an environment should show partial selectivity toward fluoride and pyrophosphate as well as cross-reactivity to distinguish among the brands based on other anionic components.

For our demonstration, we chose three toothpaste brands: Aquafresh®, Colgate®, and Crest®, as well as Fluoridex® Daily Defense and sodium fluoride for comparison. PCA and HCA were carried out for the dataset generated by toothpastes with our 8-sensor array (Figure 4.5). The PCA score plot and HCA dendrogram for the 50 trials show clear clustering. Aquafresh, Colgate, and Crest appear to be more similar, and in the HCA they appear as one cluster at
approx. 700 d.u., while Fluoridex forms a cluster with NaF at approx. 1000 d.u. Interestingly, the HCA dendrogram shows Colgate moderately dissimilar to Aquafresh and Crest within the same cluster. This could be attributed to the fact that Colgate is the only toothpaste in this study that lists pyrophosphate as inactive ingredient (appendix 3 lists all ingredients for the toothpastes used). This supports our hypothesis that using sensor elements of known selectivity for the key analytes may allow for reducing the size of arrays while allowing for successful analysis of complex analytes.

Figure 4.5. Left: PCA score plot of the first two PCs for 50 trials of 4 toothpaste brands and NaF (10 each) and the trial clustering. The percentages on each axis account for the variance intrinsic to the axis. Right: HCA dendrogram with Ward linkage showing Euclidean distance between the trials.

To further support the hypothesis that fluoride is a major differentiation contributor to which the sensors react, we carried out control experiments by adding NaF to the less fluoridated toothpastes (Aquafresh, Colgate, Crest) and applied PCA to the dataset obtained using toothpastes and toothpastes+NaF samples. The addition of NaF (to the level of 5000 ppm) resulted in a clear outcome on the PCA score plot (Figure 4.6): The NaF addition renders the three toothpastes more similar to Fluoridex (5000 ppm NaF). And, as the NaF addition renders the toothpastes more similar, the resulting clusters appear much closer to each other, although the array can still separate them.
Figure 4.6. Left: PCA score plot for the 4 toothpaste brands and the results of the NaF-addition experiments. Right: Detail of the addition experiments showing that the addition of fluoride to the toothpastes results in increased similarity in response as shown by the clustering. Addition of Na\(^+\)-binding cryptand to Fluoridex generating more of naked fluoride resulted in a response shift to the top of the NaF addition cluster (gray arrow).

The second control experiment further illustrates the role of fluoride in an array response. Since NaF is not highly dissociated, we added a small amount of [2.2.1]-cryptand to bind some sodium cations to generate more of the ‘naked’ fluoride anion.\(^{15}\) The cryptand addition resulted in the shift of the Fluoridex cluster from the bottom of the group of clusters corresponding to the additive experiments (NaF+Aquafresh, NaF+Colgate, NaF+Crest) to its top (Figure 4.6, detail), confirming, once again, the major role of fluoride in the array response.\(^{16}\) Altogether, the control experiments confirm that fluoride is a major factor the 8-sensor array reacts to, with the other anions further differentiating among the samples.

4.6 General Performance of the Sensor Array in the Discrimination of 20 Different Samples.

In order to evaluate the overall discriminatory performance of the sensor array, we included all 190 samples corresponding to 19 analytes (anions, toothpastes, and toothpastes +NaF), as well as ten samples with cyanide\(^{17}\) as an analyte, were included in the same dataset and the PCA was performed. This PCA requires 10 PCs out of 24 to describe 95% of the variance (42% of the fraction of all PCs), which indicates that in the case when the number of
analytes (and samples) is increased to 200, our 8-sensor array still shows the same high discriminatory capability. According to Suslick,\textsuperscript{18} a “practical” discriminatory limit (the possible number of different patterns that the sensor array can probably resolve) of our 8-sensor array can be estimated by considering that the changes of the grey pixel values for all sensors (RGB channels) in our dataset average approx. ~30 units (of grey scale) and assuming that changes in at least 5 channels are needed to obtain a discriminatory pattern. The PCA shows that 10 PCs can describe 95% of the discriminatory data. We estimate that our sensor array should be capable of discriminating between \((30/5)^{10} = 6^{10}\) patterns. The latter approximation does not account for the dispersion of “all” of the data. PCA is prompted to “clean” the data from the errors intrinsic to the methodology, such as unevenness in the membrane casting, random errors in the analyte application on the array, border effects, etc. As a result, the “practical” discriminatory limit would tend to overestimate the real discriminatory capability of the sensor array.

Finally, for visualization of the clustering of the data, the HCA with Ward linkages was generated (Figure 4.7). The HCA showed clear clustering with 100% of correct classification for all analytes. Accordingly, HCA confirmed the highly discriminatory power display by the 8-sensors array and also observed by PCA.
Figure 4.7. HCA dendrogram with Ward linkage showing Euclidean distance between the trials, 200 samples (11 anions, 4 toothpaste brands and control experiments, 10 trials each).
4.7 Sensor Array Size Optimization

Considering that the “practical” discriminatory limit of the 8-sensors array estimates that the sensor array could differentiate among approximately ~60 millions patterns, it could be inferred that smaller versions of the 8-member sensor array should also be able to discriminate among the same group of 20 analytes. Therefore, in order to obtain the minimal size sensor array still capable of full resolution of the 20 analytes, we have utilized the method proposed in chapter II to optimize the number of elements of the sensor array.

Table 4.2 shows that the sensor array can be reduced to 5 sensors elements, while displaying a HCA classification accuracy of 95%. Interestingly, the size reduction methodology first eliminates the less cross-reactive (or more selective sensors) 4.1 and 4.8. Polymer sensor doped with 4.8 contains chemosensor 4.8 a DPQ derivative that displays high selectivity towards fluoride and acetate, but very low cross-reactivity toward other anions. This behavior does not come as a surprise given the fact the original rationale for the inclusion of sensor 4.8 in the array was to be able to asses the possible quantification of fluoride. In addition, polymer sensor is doped with 4.1 a N-confused OMCP derivative, which NC-OMCP displays selectivity similar to that of the regular OMCP, but a closer look at the association constants reveals the dynamic ranges of these interactions are rather modest. The latter could explain why most of the discriminatory information is generated by the regular OMCPs, which are selective and yet they display a high degree of cross-reactivity.

In addition to the HCA analysis, LDA was carried out for the data sets generated for all version of the array with all 20 analytes (Table 4.2). Since LDA is a supervised method it tends to give better results when dealing with data where group classes are known. In the final analysis, it is clear that even for a sensor array containing just 2 sensors (4.2 and 4.4) LDA with
The cross-validation routine can predict the analyte class with 94% accuracy. To the best of our knowledge, the sensor array containing just two sensing elements might be the only sensor array reported for anionic analytes that are capable of discriminating as many analytes as ten times the number of sensing elements.

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<td>0.0731</td>
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Table 4.2. Classification accuracy for the reduced versions of the original 8-member sensor array.
4.8 Conclusion

In summary, a supramolecular 8-member sensor array was prepared by embedding simple colorimetric sensors in polyurethane hydrogel. The studies were performed in organic solvents aimed at establishing the sensor-anion affinity and selectivity that allow estimating the response of the array to aqueous anionic analytes. The clear connection between supramolecular observations in non-aqueous solutions and analysis of aqueous anionic samples in solid state sensor arrays paves the road for using the numbers of the previously prepared sensors known from the literature, which, until now, could not be used due to low compatibility with aqueous environments. Furthermore, this could be an important tool to consider in the rational design of minimal-size high-resolution arrays because it allows for designing arrays with predictable analytical behavior. The general utility of these principles was demonstrated on a simple 8-sensor array that was shown to differentiate between 10 inorganic anions and to analyze toothpaste brands. Reduced versions of the original 8-member sensor array containing 6, 5, 4, 3 and 2 sensors displayed a classification accuracy over 94%, determined by LDA with cross-validation routing.

4.9 Experimental Section

4.9.1 Combinatorial Optimization of the Sensing Materials: In order to optimize the sensor materials, three different Tecophilic® (SP-60D-10, SP-60D-16 and SP-60D-20) materials were screened, while varying the polymer and chemosensor concentration. The responses of the arrays were evaluated by quantification of the none zero pixel in order to determine which material shows better responses (deepest change in color) in presence of chloride and acetate TBA salts at 5 mM concentration. Figure 4.9 shows an example of the combinatorial essay.
Figure 4.8. Example of a combinatorial essay showing three different materials. The polymer sensors were cast from solutions containing different concentration of Tecophilic® resin and fixed concentration of chemosensors (500 μM). The array in a dashed rectangle was the final candidate for this work.

4.9.2 Preparation of Sub-microliter Sensor Arrays for Anion Sensing: The sensor materials were prepared by incorporating chemosensors 4.1-4.8 into polyurethane matrices, which were prepared by casting solutions containing the sensors (approx. 0.08% sensor in the matrix, w/w after solvent evaporation) in a Tecophilic® SP-60D-20 THF solution (5 % w/w) onto a multi-well 10x8 (sub-microliter) size plate (Figure 4.9).

Figure 4.9. Multi-well 10x8 (sub-microliter) size plate containing ten times the sensor array.
4.9.3 Anion Sensing in Water Procedure: For a typical assay the anions were added (200 nL) as aqueous solutions of their TBA (tetrabutylammonium) salts. For the toothpaste analysis, 350 mg of toothpaste were added to 1 mL of water (nanopure). The sample was sonicated for 1h and centrifuged to remove the unsoluble solids. Finally, the supernatant was filtered and added (200 nL) into the assay. For the control experiments the NaF biased samples were prepared as follows: 2 mg of sodium fluoride were added to 1 mL of the supernatant of the toothpaste solution of Aquafresh®, Colgate® and Crest®. In the case of Fluoridex®, 20 mg of Kryptofix[221]® were added. After the addition of the analyte the multi-well plate was developed in a conventional oven (40 °C) for 50 sec, or 30 sec in a microwave oven. Even though the sensor array is reversible, a fresh array was used for every measurement.

4.9.4 Image Acquisition and Analysis: The images from the sensor array were recorded using an USB flatbed scanner (Canon, CanoScan LiDE 60). The scanned images (24 bit, 8-bit per color) were acquired at 1200 dpi resolution. The processing of the images consists of: (1) RGB inversion (so the background has a zero-value) (2) RGB deconvolution, and (3) averaging of the gray value of the pixels for each well in the array for each (RGB) component (Figure 4.10). Both images, before and after addition of the analyte, were recorded and their arithmetical difference were taken as the final response.
Figure 4.10. Image processing routine. The data in this example is taken from the response of the array to the tetrabutylammonium acetate anion (200 nL, 5 mM).

4.10 References


(6) The concentration of 20 mM was used for nitrate and hydrogen sulfate as these anions gave somewhat ambiguous responses at 5 mM.

(7) The gray pixel value is a numerical value of the grey shade that for a 8-bit pixel depth detector ranges between 0-255.


(15) Steed, J. W.; Atwood, J. L. In *Supramolecular chemistry*; Wiley: Chichester, New York.

(16) HCA dendrogram yields similar results; for more detail refer to appendix 3.

(17) Cyanide samples were not included before because the response of the 8-sensor array is not completely reversible for the whole set of sensors in presence of this anion.


CHAPTER V. A FLUORIMETRIC SENSOR ARRAYS FOR ANION DETECTION.

5.1 Introduction

As part of an ongoing project related to anion detection, we decided to further explore the utility of our sensing materials (see Chapter III) by incorporating fluorescent chemosensors into the poly(ether)urethane (PEU) matrices. We reasoned that we could use some of the benefits of a fluorescence output (see Chapter I) in order to tackle some challenging problems, such as the discrimination of structurally similar anions, e.g. carboxylate derivatives and also the detection of anions in highly competitive media, such as blood serum. Toward this end, we have carried out two preliminary studies intended as proof-of-concept.

First, it is demonstrated that chemosensors displaying both colorimetric and fluorometric response output can harness more discriminatory information. For this experiment, carboxylate non-steroidal anti-inflammatory drugs (NSAIDs) were targeted as analytes. Here, chemosensors displaying dual-signal outputs are combined in an array, in a hope that enough discriminatory data is generated to distinguish between five carboxylate drugs; diclofenac, ibuprofen, naproxen, ritalinate and salicylate.

Secondly, it is shown that chemosensors displaying anion-induced chelation-enhanced fluorescence (CHEF) and high affinity for phosphate related anions in an organic solution, could work in competitive media including biological fluids when embedded in a poly(ether)urethane matrix. Here, six tripodal chemosensors, displaying high biding affinities for hydrogen phosphate ($\log K_a \sim 7$) and fluorescence amplification of up to 200 ($I/I_0$), are utilized in an array in order to recognize and discriminate between four biological important phosphates (Pi, PPi, AMP and ATP) in human blood serum.
5.2 Detection of Carboxylate Drugs in Water

The discrimination of structurally similar anions is a challenging problem. This is particularly true in the case of carboxylate anions since most known synthetic receptors have been designed for recognition of the anionic moiety and give little consideration to other structural features.\textsuperscript{4,5} In the case of most OMCP based receptors, the selectivity towards different carboxylate anions is mostly driven by the electron density and basicity of the anionic moiety.\textsuperscript{4,6} That is the case of acetate and benzoate anions to which OMCP presents up to three times higher binding affinity constant for acetate ($K_a=668$ M$^{-1}$), a more basic anion ($pK_a$ 4.76), than for benzoate ($K_a=196$ M$^{-1}$ and $pK_a$ 4.21) in an organic solution (DMSO).

We were encouraged by the fact that even structurally similar carboxylate anions could have a very different electron density and basicity, especially in the case of the NSAIDs that we targeted as analytes (Figure 5.1). The first attempt to discriminate among carboxylate drugs was made using the OMCP-based colorimetric sensor array presented in chapter IV. To our surprise the sensor array consisting of 8 colorimetric sensors was not capable of discriminating among three NSAIDs sodium salts of ibuprofen, naproxen and salicylic acid (Figure 5.1, right).

The failure of the colorimetric sensor array to generate discriminatory data could be related with the fact that even though the basicity of the anion can affect at some extent the biding affinity, the discriminatory data comes from the signal transduction event (change in color) rather than the supramolecular recognition, and the affinity vs. basicity cannot always be directly correlated (see ref. 7). Even in the case that binding affinities vary among different analytes, the changes in color induced by the different carboxylate drugs applied to the sensor array may not result in a difference strong enough to generate discriminatory patterns.
Figure 5.1. Left: PCA score plot of the first three principal components of statistical significance for 30 samples (1mM, 200 nL, pH 7 HEPES 5mM of 3 carboxylate drugs, 10 trials each) produce by the 8-members colorimetric sensor array presented in chapter IV. The percentages on the axes account for the amount of variance to each PC axis for a total of 58.7% of variance. Right: Molecular structure of NSAIDs bearing carboxylate moeities.

One way to circumvent the problem of the lack of discriminatory information could be to add more sensors to the array. Another more desirable way is to be able to harvest more information from the same pool of sensors. For instance, a second optical signal, such as the luminescence response, could be recorded to complement the colorimetric data, thus expanding the dataset. Unfortunately, not all the sensors in our original 8-member sensor array were fluorescent. Thus, a new sensor array consisting of only OMCPs derivatives was assembled using sensors 5.6 and 5.7 from the previous colorimetric array complemented another five fluorescent chemosensors 5.3-5.7 that were synthesized in our laboratories.$^{2,9}$
The present chemosensors 5.1-5.7 use a common receptor OMCP, substituted with an extended conjugated fluorophore. Chemosensors 5.1-5.7 display a weak fluorescence in organic solution (Φ₅.₁ = 2.30 %, Φ₅.₂ = 0.29 %, Φ₅.₃ = 0.20 %, Φ₅.₄ = 0.15 %, Φ₅.₅ = 1.44 %, Φ₅.₆ = 3.34 % and Φ₅.₇ = 0.25 %). In the presence of anions, chemosensors 5.1-5.7 display changes in their fluorescence spectra. The nature of these changes varies with the chemosensor and the anion. For instance, chemosensors 5.1, 5.2, 5.5 and 5.6 display fluorescence quenching in presence of anions in DCM, while sensors 5.3, 5.4 and 5.7 display mostly a turn-on and ratiometric response depending on the anion (Figure 5.2).¹⁰

![Figure 5.2](image)

**Figure 5.2.** Top: UV-vis (left) and fluorescence (λₑₓₓ=395nm) titration spectra for chemosensors 5.3 in presence of increasing amount of TBA Acetate (0-5 eq) Bottom: Fluorescence excitation-emission map for 5.3, 5.3+AcO⁻, μM) with M²⁺ (5 eq) in dry DCM.

The above considerations suggest that chemosensors 5.1-5.7 can yield various changes and perturbations in the luminescence signal output. Here, it will be demonstrated that
Harnessing information from both the colorimetric and the fluorometric optical outputs can improve the discriminatory power of sensor arrays.

For the reassessment of carboxylic drug analysis, we tried the array consisting in chemosensors 5.1-5.7 embedded in a hydrophilic PEU matrix. This time also diclofenac sodium (NSAIDs) and sodium ritalinate (a metabolite of the attention deficit disorder drug Ritalin) were added to the pool of analytes consisting of the other three NSAIDs (ibuprofen, naproxen and salicylic acid). Figure 5.3 shows typical responses of the sensor array after the addition of water solutions containing the drugs (1mM, 200 nL, pH 7 HEPES 5mM).

![Figure 5.3](image)  
**Figure 5.3.** Typical array responses (raw data) to ten aqueous drug solutions (1mM, 200 nL, pH 7 HEPES 5mM). Left: Colorimetric response Right: Fluorimetric response.

From the inspection of the changes in color and fluorescence in the array, it is clear, that even by naked-eye observation, each drug is generating a distinctive optical response pattern. Interestingly, sensors 5.2 and 5.6 seem to display a change in the color of the emission (Figure 5.3), even tough they exhibit a fluorescence quenching behavior in presence of anions in organic solutions. The reason for this pseudo-ratiometric behavior in chemosensors 5.2 and 5.6 could be due to the high concentration at which the chemosensors are in the PEU matrix (approximately 10mM after solvent evaporation). At this concentration, there is a good chance for FRET to
occur between the off state (donor) and on state (acceptor) of the chemosensor. Also, trivial energy transfer or inner-filter effect could have a part in this particular response behavior. However, the origin of this intriguing optical response is currently under investigation in our laboratory.

The multidimensional response patterns obtained from 5.1-5.7 array consist of the colorimetric output, which provides 21 dimensions (7 sensors x 3 RGB color channels), and the fluorometric output provides 28 (7 sensors x 4 RGBY color channels). Hence, multivariate statistical evaluation of the array responses to aqueous solutions of carboxylate drug anions was further explored using PCA and HCA. Figure 5.4 shows the PCA score plot using the first three PCs (66.4% of variance). The PCA score-plot shows clear clustering of the data with less than 70% of variance. Other PCs of statistical relevance exceeding 20% (of the total variance) were left out of the plot and could be used for further discrimination if necessary. Since the variance described by the PCA score plot is relatively low (See chapter II), we also performed HCA to have further insight in the performance of the array. The HCA shows 100% accurate classification for all 40 samples.
To further support our hypothesis, a PC and HC analyses of the colorimetric and fluorimetric responses were evaluated separately. Figure 5.5 shows that the colorimetric output does not provide enough discriminatory data and both PCA and HCA show poor clustering. On the other hand, the PCA and HCA of the fluorimetric output shows good clustering and resolution. Nevertheless, it is the combination of both output signals that provides full-resolution of the drugs tested (Figure 5.4). The resolution improvement is not very evident because the list of analytes presented here is limited to 5 drugs. However, the increment of the information density, harvested from the dual-read out of the sensor arrays, may become beneficial when a greater number of analytes are tested or when quantitative analysis is the final goal.
In summary, all of the above attests for the improvement in the discriminatory capacity by harvesting more information from chemosensors without increasing the number of sensing elements in the array platform.

5.3 Detection of Phosphate Related Anions in Human Blood Serum

The detection of anions in biological milieu is a challenging problem and depends a great deal on the anion(s) targeted. For instance, detection of anions in blood serum is particularly difficult to accomplish due to high concentration of several anions (carbonates 27 meq/L, chloride,
phosphates 2 meq/L and carboxylates 6 meq/L, among others) already present in the media. Also, there are proteins, such as albumin, that when complexed with Ca\(^{2+}\) could compete for the anions.\(^{11}\) Thus, in order to assess anion detection in a highly competitive media, chemosensors should display a high binding affinity, sensitivity, and selectivity. Here, sensors based on anion-induced CHEF (\textit{turn-on}) are convenient as they have the potential to display high sensitivity and selectivity.\(^{12,13}\)

We have targeted phosphate anions due to their central role in biochemistry as they take part in many metabolic processes.\(^{14}\) Also, as mentioned in the introduction, recent studies have correlated high phosphate levels in blood serum to cardiovascular disease and acute renal failure,\(^{15}\) which reveals a need for the development of diagnostic tools for certain diseases.\(^{11}\) Despite the need, few fluorescent \textit{turn-on} phosphate sensors exist that function in water and in electrolyte solutions.\(^{16,17}\)

In order to attempt phosphate detection in blood serum, we reasoned that highly selective CHEF chemosensors embedded in PEU matrices could provide the sensitivity and selectivity needed for this task. Toward this end, we designed chemosensors 5.8-5.13 based on tripodal receptors, which can form arrays of up to six hydrogen bonds that are known to have high association constants for oxoanions.\(^{18,19}\)

As demonstrated by Zyryanov \textit{et al.},\(^{3}\) chemosensors 5.8-5.13 display the fluorescence amplification upon anion binding. The latter suggests that the anion binding to the thiourea-
moieties results in restriction of their conformational freedom, thus improving electronic coupling between the chromophore and hydrogen donors while limiting rotational and vibrational modes. The anion induced rotational restriction results in an up to a 200-fold increase in fluorescence intensity for phosphate, pyrophosphate, and acetate (Figure 5.6).

**Figure 5.6.** Left: Response of sensor molecules 5.11 and 5.13 to anions in solution (DMSO-H₂O 5%). Right: Fluorescence amplification of 5.11 in the presence of H₂PO₄⁻.

As stated by Zyryanov *et al.*,³ the anion binding affinities of 5.8-5.13 follow the general order: H₂P⁻ > HPP³⁻ > AcO⁻ >> Cl⁻ > Br⁻, which also corresponds with the relative degree of fluorescence signal amplification (I/I₀). The Anion affinities described by association constants (M⁻¹) range between 3.8-5.0×10⁶ for fluoride (F⁻), 10-100 for chloride (Cl⁻), 1.0-5.0×10⁶ for acetate (AcO⁻), 2.3-5×10⁶ for dihydrogen-phosphate (H₂P⁻), and 1.0-5.0×10⁶ for hydrogen pyrophosphate (HP₂O₇³⁻, HPP³⁻) determined in DMSO.¹⁸

From the binding studies in DMSO, we realized that due to the high anion affinity of the chemosensors 5.8-5.13 there was a possibility for detecting biological phosphates. Consequently, sensor arrays utilizing chemosensors 5.8-5.13 embedded in hydrophilic poly(ether)urethane Tecophilic® SP-60D-20 were prepared. A first set of experiments was carried out in aqueous solutions. Figure 5.6 shows how chemosensors 5.8-5.13 embedded in films reacted to the
presence of eight different anions by a dramatic increase in fluorescence intensity, a degree of which is specific to a particular analyte (Figure 5.7, left). The multidimensional response pattern (24-dimensional, 6 sensors × 4 RGBY channels) of the sensor array in the presence of the eight anions was statistically explored using principal component analysis (PCA) (Figure 5.7, right).

**Figure 5.7.** Left: Changes in the fluorescence intensity of the sensor-polyurethane films upon addition of aqueous anions (200 nL, 1 mM, except ATP and AMP, which were 5 mM). Right: PCA score plot of the first three principal components for 32 observations (8 anions, 4 trials each) showing clear clustering of the trials. The percentages on the axes indicate for the amount of variance to each PC axis for a total of 85.4% of variance.

The blood serum with its protein and electrolyte content behaves as a unique buffer, containing also phosphates and various carboxylates, and is therefore likely to give a unique response in the 5.8-5.13 array. In the next array (Figure 5.8, left), we applied water (control), serum, and serum samples with added anions (phosphate, pyrophosphate, AMP, or ATP). As expected, due to its intrinsic anion content, the serum itself turned on fluorescence of the array creating a fluorescence response, further modulated by the added anions. The PCA score plot (Figure 5.8, right) shows that 5.8-5.13 films allow discriminating between phosphate, pyrophosphate, AMP, and ATP. Interestingly, in the human serum analysis the nucleotide
(adenine) in AMP and ATP plays an important role in generating a unique response for these anions compared to inorganic phosphates.

Figure 5.8. Left: Qualitative changes in fluorescence of the sensor-polyurethane films after addition of human blood serum and serum with added anions (to increase the concentration to 5 mM). Right: Principal component analysis of the array response shows the quantified changes induced by the anions added to the blood serum, and compares the effect of anion to the pure serum and water.

In summary, it is clear that the benefits given by the utilization of CHEF chemosensors, embedded in hydrophilic PEU, allow for effective sensing of anions, including biologically important phosphates in a very competitive media such as human blood serum.

5.4 Conclusion

Preliminary experiments suggest that chemosensors embedded in polyurethane films may be used for the fabrication of optical sensor arrays that could allow the analysis of carboxylate drugs in water and phosphate related anions even in complex biological milieu such as blood serum.

Two proof-of-concept experiments were presented to demonstrate that the utilization of a fluorescent optical output could be beneficial when more information is needed to improve discriminatory capacity of sensor arrays and/or when more sensitivity is desirable.
Finally, the successful detection of various anions using fluorescence output suggests potential for the development of analytical platforms useful in real-world applications. However, further studies should comprise calibration schemes to carry out quantification of the analytes and the determination of the suitability of the assays when challenged with slightly different samples (e.g. blood serum from different individuals). Also, combinatorial assessment of sensor materials could yield sensor arrays with enhanced performance (e.g. sensitivity). This could be very important in the possible determination of pharmaceuticals in potable water since in most cases they are at trace levels (20-40 nM).

5.5 Experimental Section

5.5.1 Preparation of Sub-microliter Sensor Arrays for Carboxylate Drug Sensing: The sensor materials were prepared by incorporating chemosensors 5.1-5.7 into polyurethane matrices, which were prepared by casting solutions containing the sensors (approx. 0.08% sensor in poly(ether)urethane, w/w) in a Tecophilic® SP-60D-16 THF solution (5 % w/w) onto a multi-well 10x8 (sub-microliter) size plate.

5.5.2 Preparation of Sub-microliter Sensor Arrays for phosphate detection in blood serum: The sensor materials were prepared by incorporating chemosensors 5.8-5.13 into polyurethane matrices, which were prepared by casting solutions containing the sensors (approx. 0.08% sensor in poly(ether)urethane, w/w) in a Tecophilic® SP-60D-20 THF solution (5 % w/w) onto a multi-well 10×8 (sub-microliter) size plate.

5.5.3 Preparation of Carboxylate Drug Salts Samples: Carboxylate sodium salts were prepared by titration of the correspondent acids with sodium hydroxy in MeOH. The solvent was
evaporated to dryness and the salts recrystallized in EtOH. The salts were dissolved in nanopure water for analysis.

5.5.4 Sensing of Phosphate anions in Human Blood Serum: For a typical assay the solutions of the analytes were added (400 nL) as aqueous or serum solutions of their TBA (tetrabutyl-ammonium) salts for fluoride, chloride, acetate, benzoate, phosphate and pyrophosphate. AMP and ATP solutions were prepared from their sodium and disodium salts (from Aldrich), respectively. Charcoal stripped (pooled) human serum was acquired from Equitech-Bio, Inc. Lot #: SHS33-197. Certificate of analysis shows: calcium 3.73 mg/dL, phosphorus 1.4 mg/dL, glucose 10 mg/dL, bun 2 mg/dL, creatinine ~0.0 mg/dL, cholesterol 6 mg/dL, triglyceride 5 mg/dL, T. protein 6.2 g/dL, albumin 4.3 g/dL, sodium 126 mmol/L, potassium 3.8 mmol/L, chloride 105 mEq/L, uric acid 0.0 mg/dL and pH 7.28, bacteria less than 10 cfu/mL, no preservative and no anticoagulant. The sample was free of HIV and Hepatitis viruses.

5.5.5 Colorimetric Sensor Array Image Acquisition and Data Processing: The images from the sensor array were recorded using an USB flatbed scanner (Canon, CanoScan LiDE 60). The scanned images (24 bit, 8-bit per color) were acquired at 1200 dpi resolution. The processing of the images consists of: (1) RGB inversion so the background has a zero-value, (2) RGB deconvolution, and (3) averaging of the gray value of the pixel for each spot in the array for each (RGB) component. Both images, before and after addition of the analyte, were recorded and their arithmetical difference taken as the final response.

5.5.6 Fluorescence Sensor Array Image Acquisition and Data Processing: Images from the sensor arrays were recorded using a Kodak Image Station 440CF. The scanned images (12 bit) are acquired with a resolution of 433×441 pixels per inch and with grey levels over 1000 (12
second exposures). The sensor arrays are excited with a broadband UV lamp (300-400 nm, λmax=365 nm) and up to three channels were used for emission detection: (1) Blue: band-pass filter 380-500 nm λmax=435 nm, (2) Green: band pass filter 480-600 nm λmax=525 nm, (3) Yellow: long pass filter 523 nm. In order to generate a false color representation, images obtained using blue, green and red (long pass 580 nm) filters were merged in equal proportion using NIH ImageJ software. The RGB triplet is assigned to correspond with the color of the filter used. After acquiring the images, the integrated (non zero) grey pixel (n) value is calculated for each well of each channel. Images of the sensor chip were recorded before (b) and after (a) the addition of an analyte and their final responses (R) were evaluated as indicated in equation 1.

\[ R = \sum_n \frac{a_n}{b_n} - 1 \]  

5.5.6 PCA Analysis for Colorimetric|Fluorimetric Carboxylate Drugs essays: Since the colorimetric and fluorometric response have a different magnitude and range of values, PCA was carried out from the correlation matrix after autoscaling (See chapter II).

5.6 References

(1) The detection of pharmaceuticals in water has become a major concern, particularly since levels of several kinds of drugs have been detected in potable water supplies (a) Halford, B. *Chem. Eng. News* 2008, 86, 16.

(b) Ruhoy, I. S.; Daughton, C. G. *Science of the Total Environment* 2007, 388, 137.

(2) Anzenbacher, P., Jr.; Palacios, M. A.; Nishiyabu, R.; *paper in preparation*.


(5) There have been several studies showing that changes in the receptors can tune binding motifs to be more structure-dependant. For an example of OMCP derivatives see ref. 6.


(7) Even tough in most cases it is clear that higher association constant translate in stronger response this is not always the case. For examples see: Beer, P. D.; Gale, P. A.; Chen, G. Z., J. Chem. Soc., Dalton Trans. 1999, 1897.


(8) Sensors 5.1 and 5.2 are sensors 4.6 and 4.7 respectively in chapter IV.

(9) The synthesis of 5.3-5.7 were carried out by Dr. Ryuhei Nishiyabu at Anzenbacher Research Group laboratories.

(10) Fluorescence titration and excitation and emission mpas were carried out by Dr. Zhuo Wang at Anzenbacher Research Groups laboratories. See appendix 4 for examples of fluorescence titrations.


(18) The synthesis and anion binding studies of chemosensors **5.8-5.13** were carried out by Dr. Grigory V. Zyryanov at Anzenbacher Research Group laboratories.


CHAPTER VI. A FLUOROMETRIC SENSOR ARRAY FOR METAL ION DETECTION BASED ON 8-HYDROXYQUINOLINE COORDINATION CHEMISTRY.

6.1 Introduction

As has been articulated in the introductory Chapters I and III, the signal transduction could possibly be the key element for a balanced interplay between selectivity and cross-reactivity in sensor arrays. Here, the chemical sensing selectivity is not driven by the recognition chemistry but rather by the nature of the optical response. To test if the latter hypothesis is general, we decided to extend the focus of our study to other kinds of chemical species to include metal ions, since the same concept has been previously demonstrated with anionic analytes in Chapters IV and V. In this study we have made an array of optical sensors that bear the same 8-hydroxyquinoline (8-HQ) receptor moiety and various conjugated fluorophores to yield different sets of responses in the presence of various metal ions.

In the resting (off) state 8-HQ derivatives are partially quenched due to a proton transfer from the hydroxy group of the 8-HQ* to the solvent. This mechanism provides an efficient deactivation pathway for the excited state yielding a very weak fluorescence. After cation complexation, the formation of a new metalloquinolate fluorophores results in a change in the fluorescence. The nature of the final response is highly dependant on the cation. The combination of fluorescence enhancement, quenching and energy transfer results in a fingerprint-like pattern of responses for each sensor-cation complex. Here are presented arrays comprising various numbers of sensor elements for the qualitative identification of Ca²⁺, Mg²⁺, Cd²⁺, Hg²⁺, Co²⁺, Zn²⁺, Cu²⁺, Ni²⁺, Al³⁺, and Ga³⁺. The utility of these arrays were further explored in the identification of enhanced soft drinks based on their cationic content.¹⁻²
6.2 Selection of Chemosensors

The chemosensor utilized in the present study, design and synthesized by Dr. Montes and Dr. Anzenbacher, bear the same receptor, 8-HQ, substituted in the 5-position with a conjugated fluorophore such as pyrene or a fluorene moiety that emits blue fluorescence. 8-HQ does not have discernable emission above 300 nm. The idea is to tether a direct electronic communication between the receptor and the chromophore, preferably via a conjugated bridge.

In general, the corresponding quinolinolate anion displays luminescence modulated by the metal ion. Hence, upon metal ion coordination by 8-HQ, a second chromophore, metalloquinolinolate is formed. From the sensor array perspective, 8-HQ has a high potential for use in the design of fluorescence-based sensor arrays as it shows a turn-on signal and is highly cross-reactive, i.e. binds a number of metals while emitting light of a slightly different luminescence quantum yield and wavelength for each metal. The relative contribution of the two chromophores (e.g. pyrene and metalloquinolinolate, Figure 6.1) to the fluorescent output depends on the nature of the metal ion including its electropositivity, spin-orbit coupling, and the excitation wavelength. Metal electropositivity defines whether the quinolinolate emission will be more blue-emitting as it is in the case of Mg$^{2+}$, green as in the case of Al$^{3+}$, or rather yellow-emitting as in the case of Zn$^{2+}$. Spin-orbit coupling then defines whether the metalloquinolinolate complex will display fluorescence (e.g. Mg$^{2+}$, Ca$^{2+}$, Al$^{3+}$, Ga$^{3+}$) or rather red-shifted phosphorescence (Ir$^{3+}$, Pt$^{2+}$). However, not all phosphorescence quantum yields are high enough for the phosphorescence to be observed. Metals such as Hg$^{2+}$ or Ni$^{2+}$ usually quench the sensor luminescence, albeit with different efficiency.
Upon the cation coordination by the 8-hydroxyquinoline the metalloquinolinolate complex displays a change in fluorescence. The balance between the original fluorescence of the conjugated chromophore (A) and the newly established metalloquinolinolate complexes (B-E) provide for a unique ratiometric response.

Finally, the excitation wavelength determines, which part of the sensor is preferentially excited and, in the absence of energy transfer, emits light. A higher extinction coefficient ($\varepsilon$) of the organic fluorophore in the UV-region (250-370 nm) results in prevalent blue emission from the aromatic part while the excitation of the metalloquinolinolate $\pi-\pi^*$ transitions in the near-UV and Vis-region (350-410 nm) results in a turquoise-green emission of the quinolinolate complex. Since both the receptor and aromatic fluorophore are partly conjugated, excited state mixing and the corresponding emission may also be observed. One can also use a broad-band excitation source such as a UV-lamp or multiple LEDs to excite both types of absorption in the sensors.

From the above considerations, one can easily glean that the sensors utilizing an 8-HQ receptor with an attached fluorescent moiety can yield multiple changes and perturbations in the luminescence signal output. We show how the discriminatory power of the array utilizing the above fluorescent sensors could be increased by taking advantage of the cross-reactive, yet selective nature of the signaling.
Because metalloquinolinolates are weakly emitting ($\Phi<0.15$),\textsuperscript{6,8} it is desirable to attach a conjugated chromophore to enhance their luminescence output. Sensors S2-S6 with an extended conjugated chromophore show only weak fluorescence in solution ($\Phi_{S4} \sim 0.02$, $\Phi_{S5} \sim 0.05$) as the 8-HQ moieties exert some degree of intra-molecular quenching to pyrene (S2) and the fluorene bridges in S4-S6. However, with an extended fluorene, this quenching is not complete (Figures 6.2 and 6.3).

**Figure 6.2.** Upon the AlCl$_3$ cation coordination by 8-hydroxyquinoline, the Al$^{3+}$-quinolinolate complex of S2 (0.5 $\mu$M) displays a change in fluorescence. The balance between the original fluorescence of the conjugated chromophore and the newly established metalloquinolinolate complexes provides for a unique ratiometric response.

8-HQ is known to form luminescent chelates with a number of metal ions, including Cd$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Al$^{3+}$, Ga$^{3+}$, In$^{3+}$, Sn$^{4+}$, Ti$^{4+}$.\textsuperscript{9} In order to prove our signal transduction concept, we selected 10 metal ions known to form luminescent complexes, such as Ca$^{2+}$, Cd$^{2+}$, Mg$^{2+}$, Al$^{3+}$, and Ga$^{3+}$, and, to a lesser extent also Zn$^{2+}$, and also metal ions such as Hg$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, and Co$^{2+}$, that are known to quench the emission of the quinolinolate anion. The main question to be answered in this study was how many sensors were needed to achieve discrimination among the 10 cations of the above group. The success criterion is the 100% classification of the trials by the array.
Figure 6.3. Structures of sensor S1 (8-hydroxyquinoline, 8-HQ), and 8-HQ-based sensors S2-S5. R = rac-2-ethylhexyl, R’ = n-hexyl. The extended conjugated chromophore is shown in blue color.

6.3 Cross-reactive, yet Selective Response of S2-S6 in Organic Solution.

The cross-reactive, yet selective signal output was confirmed in organic solution prior to array fabrication. The method of excitation-emission maps was used to compare the emission from the sensors and the corresponding complexes. Figure 6.4 shows the excitation-emission maps of the changing ratios of the blue (turquoise) component of the extended chromophore, and the green (yellow) component of the metalloquinolinolate. Figure 6.4 illustrates how this ratiometric output, together with metal-specific attenuation or growth in the luminescence intensity, results in a high information density output signal that can be harnessed to provide the desired discriminatory power of such sensors.
Figure 6.4. Fluorescence excitation-emission map for S2, S2+Al³⁺, S2+Cd²⁺, S2+Cu²⁺, S2+Mg²⁺ and S2+Zn²⁺ (S2 (0.5 uM) with M²⁺ (50 equiv.)) in dry THF. The range of excitation wavelength is from 300 nm to 415 nm in 5 nm steps, and the range of emission wavelength is from 420 nm to 700 nm in 1 nm steps.

6.4 Metal Ion Sensing in Water

The solid-state array was fabricated using sensors S1-S6 dispersed in a hydrophilic poly(ether)urethane Tecophilic SP-93A-100 carrier (0.07 % S2-S6 in poly(ether)urethane, w/w). The purpose of the hydrophilic polyurethane is to draw in water together with the metal ions, while aiding in the formation of the metalloquinolinolate complexes, and to overcome the incompatibility in solubility of the lipophilic sensors and hydrophilic cations. The luminescence from the array was recorded upon exposure to ten metal cations: Ca²⁺, Mg²⁺, Cd²⁺, Hg²⁺, Co³⁺.
Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Al$^{3+}$, and Ga$^{3+}$, as their chloride salts in water (1 mM in water, 200 nL, 7 trials each). We tested pH=5, 6, and 7 of the analyte solution and decided to work with pH=5, as at pH=6 and 7 some metal ions started to precipitate as hydroxides. Figure 6.5 shows responses of S1-S6 in blue, green, and yellow channels to the cation solutions, and the corresponding changes in a yellow channel at pH=5 as an example of the raw data.

**Figure 6.5. Left:** Fluorescence responses of the S1-S6 sensor array to the presence of different cations (1 mM in water, 200 nL, pH 7). False color representation generated by superimposing of the equally weighed images corresponding to RGB channels. **Right:** Response patterns generated by the sensor array (just the green channel) by 10 different metal cations. The black tops in the graph indicate negative responses.

As predicted, each of the metal cations induced a different pattern of luminescence changes in the individual sensors of the array, thus creating a multidimensional response pattern (Figure 6.6, left). As an example, Figure 6.6 also shows the response pattern generated by the sensor array in the presence of Zn$^{2+}$. Additionally, appendix 5 shows the response patterns generated by all of the metal cations in all of the sensors. Inspection of the patterns reveals that S2 is the most sensitive sensor for Zn$^{2+}$ and a similar trend repeats for most of the cations. The right panel in Figure 6.6 shows a quantitative representation of changes of the relative intensity in the yellow channel of S4 at zinc concentrations ranging from 5 μM to 5 mM. The inset also
shows a biphasic behavior in the response probably due to the multiple stoichiometries given by the ditopic nature of the sensor \textbf{S4}, which has two 8-HQ sensors (two 8-HQ moieties are attached to the chromophore).

\textbf{Figure 6.6. Left:} BGY pattern (18-dimensional; 6 sensors \times 3 channels) generated by the 6-sensor array upon addition of ZnCl$_2$ (1 mM, 200 nL pH 5). \textbf{Right:} Changes of the relative intensity of \textbf{S4} (yellow channel) with increasing Zn$^{2+}$ concentrations. Inset: Detail of a low concentration (0-500 μM) region.

6.5 Statistical Analysis of Multivariate Response Patterns

The multidimensional response pattern (18-dimensional, 6 sensors \times 3 BGY channels) of the sensor array in the presence of ten cations (Ca$^{2+}$, Mg$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Co$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Al$^{3+}$, and Ga$^{3+}$) was statistically explored using the principal component analysis (PCA) and linear discriminant analysis (LDA). First, PCA was used to explore the quality of the data. The fact that the PCA correctly recognizes all of the data points that belong to one analyte (for all analytes tested) would attest to the high discriminatory ability of the array. Generally, the higher the number of PCs required to describe a certain level of discrimination, the better the sensor array discriminates between similar analytes.$^{12}$ Here, the PCA of the dataset (7 trials for each cation) obtained from the 6-sensor array requires 5 dimensions (PCs) out of 17 to describe 95% of the
discriminatory range (~30% of all PCs). This level of discrimination is in contrast to those reported for most electronic tongues, which have typically 95% of discrimination in the first two PCs.\textsuperscript{13}

In addition, each pattern generated by the 6-sensor array was reduced to a single score and plotted in the new space (PC space) generated using the PCs. The PCA score plot (shown in Figure 6.7) utilizes the first two PCs representing 80\% of variance, and it already shows clear clustering of the data. Furthermore, the high level of dispersion of the data shown by the PCA can be attributed to the high cross-reactivity given by the 8-hydroxyquinoline receptor and the specificity due to the unique photo-physical properties generated by the sensor-metal ion interaction. As we have articulated before, it is the combination of high variability in photonic output and high cross-reactivity in the metal binding by 8-HQ that generates a large difference in the sensor array output and allows for better separation (resolution) of the clusters in the PCA score plot.\textsuperscript{14}

Figure 6.7. Left: PCA score plot of the first two PCs describing ca. 80\% of the total variance. PCA score plot shows clustering for all 11 samples (7 trials each, 1 mM of their chloride salts, 200 nL, pH 5). Right: PCA score plot including the third PC describing ca. 8 \%. The percentage on each axis accounts for the variance intrinsic to the axis.
In order to test the predictability of the array $S_1$-$S_6$ LDA, coupled with a leave-one-out cross validation routine, was carried out using the same dataset. Based on the very successful PCA, we were not surprised to learn that the LDA yielded 100% correct classification for all 77 samples.

6.6 Sensor Array Size Optimization

Part of the motivation of this work was to use the molecular design to generate a significant amount of information with a minimal set of sensor elements in the array, an effort that could provide simple yet effective analytical devices in the near future. Hence, we attempted to select a subset of sensors that span the 18-dimensional ($6 \times 3$ channels) space generated by all sensors ($S_1$-$S_6$) while keeping discriminatory capacity. We have utilized the method proposed in chapter II to optimize the number of elements of the sensor array.

Figure 6.8 illustrates the size optimization of the $S_1$-$S_6$ sensor array (Array A). The PCA score plot still shows clustering with no evident overlap between the samples (Figure 6.8C). It is also important that this PCA obtained from the 2-sensor array (Array C: $S_2$ and $S_4$) requires 2 dimensions (PCs) out of 5 to describe 94% of the discriminatory range (~40% of all PCs), demonstrating that the reduction of the number of the sensor elements in the array has not significantly affected the discriminatory performance of the array for this dataset containing 11 analytes (10 cations and 1 water pH=5). Although the decrease in the magnitude of the eigenvalues (PCs) reveals that the response space generated by the array shrinks while reducing the number of sensing elements as it could have been expected. The decrease in the size of the response space could be counter productive, e.g. if the reduced version of the array is utilized for quantitative analysis, where more information is needed to avoid overlaps between similar analytes. At this point, it is important to stress that the array optimization procedure is unique to
each dataset, and any extrapolation to another dataset (group of analytes) is inappropriate, in statistical terms.

**Figure 6.8.** Schematic representation of the rational process for reduction of the number of sensor elements in an array. From top to bottom: (A) PCA for the complete set of sensors (S1-S6) shows that the main contributors for the dispersion are S4, S2, and S5 on the PCs with statistical significance. (B) Sensors S1, S3, and S5 were excluded from the data set and analyzed again with PCA. PCA shows that the main contributors were S2 and S4. (C) S3 was excluded from the data and PCA was carried out using the remaining data set. Qualitative inspection of the PCA score plot for the final set of two sensors (S2 and S4) shows clustering of the data without any evident overlap between different samples. Cross-validated LDA shows 100% accurate classification for all three arrays.

In addition, LDA using a cross-validation routine was also performed on both reduced arrays (S2, S4, S5 and S2, S4) showing 100% correct classification in all 77 cases. It is quite remarkable that just two sensors are capable of differentiating between eleven analytes.
6.7 Discriminatory Capacity of a Single Chemosensor S4

From Figure 6.8 it can be seen that S4 is the main contributor to the analyte discrimination in all three arrays. We decided to further explore the source of the “information” provided by S4. As mentioned before, each sensor contributes to the array with three color-emission-channels (BGY). Clustering in the three dimensions (BGY) can be explored without reduction of the dimensionality by PCA, i.e. we can just plot the raw data. Figure X.9 shows the sensor vector-response to different metal ions (left) and a scatter-plot (right) of the two dimensional raw data corresponding to the relative intensity in the S4-blue and S4-yellow emission channels.

From the scatter-plot (Figure 6.9, right) it is clear that just two channels have enough information to differentiate between all 11 analytes. It is important to realize that each of the four quadrants in the scatter plot corresponds to the nature of the signaling in S4. For example, the first and third quadrants show analytes that produced enhancement and quenching of the emission in both channels, respectively. Meanwhile, the second and fourth quadrants show the analytes displaying a ratiometric signaling.

Figure 6.9. Left: BGY Response profiles of S4 for all metal ions. Right: S4 Raw-data scatter-plot of blue and yellow channels shows clusters of 11 analytes (corresponding to 77 trials, 1 mM of their chloride salts, 200 nL, pH 5).
6.8 Enhanced Waters Brand Identification Based on Cation Content.

To illustrate the utility of the above arrays in potential practical application, identification of “enhanced water” drinks based on their Ca\(^{2+}\), Mg\(^{2+}\), and Zn\(^{2+}\) cation content was explored. The electrolyte and metal ion enhanced waters-beverages used in this test are complex analytes, comprising typically electrolytes (Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\)), flavoring agents, vitamins, artificial sweeteners, additives such as caffeine, and, last but not least, also Zn\(^{2+}\) ions. The list of ingredients for each beverage tested is included in the appendix 5, including pH values for all beverages.\(^{15}\) The fingerprint patterns were generated from the enhanced water samples without any pretreatment, utilizing chiefly the Ca\(^{2+}\), Mg\(^{2+}\), and Zn\(^{2+}\) cation content. Enhanced waters used were: VitaminWater Multi-v (Glaceau Co.), Antioxidant Water Strawberry Acai (Snapple Beverage Corp.), Propel Lemon (Gatorade Co.), Propel Calcium Mixed Berry (Gatorade Co.), Powerade Option Black Cherry (Coca-Cola, Co.), Owater Lemon & Lime (Obeverages, Co.), and flavored water Dasani Lemon (Coca-Cola Co.).\(^{16}\)

From Figure 6.10 it is clear that the S1-S6 array sorts the waters based on their cation content. The corresponding PCA score plot shows clear separation of all clusters. Thus, electrolyte waters that do not contain significant amounts of Ca\(^{2+}\) and are free of Mg\(^{2+}\) and Zn\(^{2+}\) (Dasani Lemon, Powerade Option and Propel Lemon) appear close to the nanopure water. The Ca\(^{2+}\) enhanced waters (Propel Calcium and Owater) appear together in the left upper corner, while the Ca\(^{2+}\), Mg\(^{2+}\), and Zn\(^{2+}\) supplemented waters (Vitaminwater and Antioxidant Water Snapple) appear together in the lower center of the PCA score plot. This pattern is highly consistent with the metal cation content and with the bias of the array sensors elements.
Figure 6.10. **Left:** Samples of enhanced water may be divided into groups by their metal ion content into three groups: A) electrolyte waters free of Mg$^{2+}$, Zn$^{2+}$ and with no or very low concentration of Ca$^{2+}$; B) Ca$^{2+}$-enriched electrolyte waters free of Mg$^{2+}$, Zn$^{2+}$; C) electrolyte waters enriched with Ca$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$ (nanopure water is included as a control). **Right:** PCA score plot of the first two PCs of the S1-S6 array describing ca. 90% of the total variance. PCA score plot shows clustering for all 8 samples (7 trials each), 200 nL of the sample were applied directly from the bottle to each element of the sensor array.

Likewise, the array optimization procedure was performed for the enhanced water samples dataset. PCA confirmed that sensors S2, S3, and S5 provide the highest contributions of statistical significance. Also, the S2, S3, S5 cross-validated LDA shows 100% accurate classification for all the trials. It is also noteworthy that the S2, S4, S5 array was also successful, and also showed 100% accurate classification (Figure 11).

Finally, following the statistical contributions in the PCA, we reduced the array into a two-member S3 and S5 array, and performed the PCA and LDA evaluations. Both PCAs corresponding to the S2, S3, S5 and S3, S5 arrays are shown in Figure 6.10. One can see that both analyses show clear clustering, although the discriminative power of the minimized arrays slightly decreases. This is in part due to the multi-analyte nature of the enhanced waters as well as the fact that the sensors can respond to only few ions in these complex samples (Ca$^{2+}$, Mg$^{2+}$,
and Zn\textsuperscript{2+}), which limits the principal component space utilized by the analysis. Nevertheless, the S\textsubscript{3}, S\textsubscript{5} cross-validated LDA shows 100% correct classification for all the trials.

**Figure 6.11.** Analyses of the enhanced water samples using arrays optimized with a reduced number of sensor elements. **Left:** PCA score plot for the S\textsubscript{2}, S\textsubscript{3}, S\textsubscript{5} array, describing ca. 87 \% of variance, showing all 56 trials corresponding to the 8 samples. **Right:** PCA for the S\textsubscript{3}, S\textsubscript{5}, array, describing ca. 95 \% of variance, showing all 56 trials corresponding to the 8 samples. The cross-validated LDA shows 100\% correct classification for all trials.

**6.9 Conclusion**

We have demonstrated that the rational design of optical signal transduction in simple luminescent sensors results in a dramatic enhancement of the analytical utility of such sensors and corresponding arrays. This approach utilizes one common receptor, 8-hydroxyquinoline, attached to conjugated fluorophores in such a way that the whole sensor is highly susceptible to changes in fluorescence based on the nature of the bound metal. The resulting sensors are highly cross-reactive and provide an information-rich fluorescence output in three (BGY) emission channels and may be used in both qualitative and quantitative analyses of metal ions. Pattern recognition methods (PCA and LDA) were used to evaluate the analytical utility of the described sensors in arrays. The discriminatory capacity of the arrays was tested on a set of 11 analytes, 10 of which were metal ions. The sensors that contribute most to the analyte discrimination were
identified and used to construct yet a smaller and smaller array. A two-member array was found to identify the 11 analytes with 100% accuracy. Finally, the best two of the sensors were tested alone, and both were found to be able to discriminate among the samples with 99% and 96% accuracy, respectively. To the best of our knowledge, this is the first time ever reported that one optical sensor element is capable of discriminating among 10 metal ions.

The discriminatory capacity of the described sensors and arrays was also tested on identification of complex analytes such as enhanced water samples comprising various compositions comprising electrolytes, Ca$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$, in various levels and proportions. Once again, the present sensors and arrays are capable of discriminating among these complex analytes that were used without any pre-treatment. It is noteworthy that the number of sensor elements in the arrays may be reduced using the same method described for metal ion solutions. These results strongly suggest that the sensor selection method is sufficiently general and may be used to generate minimal arrays for various analytes including complex multi-analytes such as beverages.

6.10 Experimental Section

6.10.1 Chemicals and Solutions: Commercially available solvents and reagents were used as received from chemical suppliers. Tetrahydrofuran was distilled from a K-Na alloy under argon. Sensors S2-S6 were originally synthesized by Dr. Victor Montes following the procedures described previously.$^2$ S1 is commercially available from Aldrich Chemicals.

6.10.2 Fluorescence Excitation-Emission Maps: These studies were originally carried out by Dr. Zhuo Wang and are included here in order to ease the understanding of the signal-transduction modes. Fluorescence excitation-emission maps were recorded by a single photon-counting
spectrofluorometer from Edinburgh Analytical Instruments (FL/FS 920). The range of excitation wavelength is from 230 nm to 415 nm in 5 nm steps, and the range of emission wavelength is from 420 nm to 700 nm in 1 nm steps. A 395 nm emission filter was used.

6.10.3 Preparation of Sub-microliter Sensor Arrays for Cation Sensing: The sensor materials were prepared by incorporating sensors S1-S6 into polyurethane matrices, which were prepared by casting 200 nL of solutions containing the sensor (approx. 0.08% sensor in polyurethane, w/w) in a THF solution of Tecophilic™ SP-93A-100 (4 % w/w) onto a multi-well (sub-microliter) plate as reported before.

For a typical assay, the solutions of the analytes were added (200 nL, 1mM) as aqueous solutions of their chloride salts. Dr. Zhuo Wang carried out a pH response screening to find the optimal condition for the analysis. Figure 6.12 shows pH screening for S2. It was determined that pH 5 was optimal for the analysis of all nine cations.

![Figure 6.12. Patterns generated by S2 in the presence of several metal cations at different pHs.](image-url)
6.10.4 Fluorescence Sensor Array Image Acquisition and Data Processing: Images from the sensor arrays were recorded using a Kodak Image Station 440CF. The scanned images (12 bit) are acquired with a resolution of 433×441 pixels per inch and with grey levels over 1000 (12 second exposures). The sensor arrays are excited with a broadband UV lamp (300-400 nm, \( \lambda_{\text{max}} = 365 \) nm) and up to three channels were used for emission detection: (1) Blue: band-pass filter 380-500 nm \( \lambda_{\text{max}} = 435 \) nm, (2) Green: band pass filter 480-600 nm \( \lambda_{\text{max}} = 525 \) nm, (3) Yellow: long pass filter 523 nm. In order to generate a false color representation, images obtained using blue, green and red (long pass 580 nm) filters were merged in equal proportion using NIH ImageJ software.\(^{17}\) The RGB triplet is assigned to correspond with the color of the filter used. After acquiring the images, the integrated (non zero) grey pixel \( n \) value is calculated for each well of each channel. Images of the sensor chip were recorded before \((b)\) and after \((a)\) the addition of an analyte. The final responses \((R)\) were evaluated as indicated in equation 1.

\[
R = \sum_n \frac{a_n}{b_n} - 1 \quad (1)
\]

**Figure 6.13.** Excitation-Emission map for the S2•Al\(^{3+}\) complex illustrating how the emission of the complex is recorded in the BGY channels upon excitation with a broadband near-UV source.
6.11 References


(3) Chemosensors S2-S6 were synthesis by Dr. Victor Montes at Anzenbacher Research Group laboratories. Detailed procedures of the synthesis are reported in reference (2).


(7) Figure courtesy of Dr. Pavel Anzenbacher Jr.


(10) Solution studies were carried-out at Anzenbacher Research Group by Dr. Zhuo Wang and are included here in order to ease the understanding of the signal-transduction modes.
CHAPTER VII. A FLUOROMETRIC SENSOR ARRAY FOR METAL ION DETECTION BASED ON MULTIPLE COORDINATION FEATURES.

7.1 Introduction

In the previous chapter a cross-reactive sensor array for metal ions utilizing chemosensor based on 8-hydroxyquinoline coordination chemistry was described. Such an array based on fluoro-ionophores bearing just one type of receptor depends mostly on their signal transduction mechanisms to generate discriminatory data. In this chapter a cross-reactive sensor array based on chemosensors bearing different kinds of coordination chemistries and different signaling schemes is presented. This approach provides the information needed for accurate detection of metal ions in a wide range of concentrations and identification of mineral and purified waters brands by their cation content.

7.2 Selection of Chemosensors

The criteria for the selection of chemosensors (fluoro-ionophores) were guided by the following requirements: The coordination chemistry of the chemosensor should allow for significant cross-reactivity (i.e. selective chemosensors were not particularly sought). The chemosensors should have strong absorption in the near UV (300-400 nm), for which LED light sources are widely available, as well as reasonably strong emission in the visible region. Also, the chemosensors should be commercially available or easy to synthesize. In general, it is desirable to generate a maximum discriminatory power with a minimal set of sensors. Guided by the above criteria, we have selected a set of 9 chemosensors (7.1-7.9) (Figure 7.1) to generate the array capable of analyte differentiation based on the metal-ion content. Chemosensors 7.1-7.9 comprise different kinds of receptors as well as different kinds of signal transduction schemes. Therefore, it is the
combination of different coordination chemistries and signal transduction schemes, such as fluorescent enhancement or quenching and ratiometric response, that generates enough discriminatory data for accurate classification of possible analytes, while keeping the number of sensing elements as low as possible. Here, the 7.1 and 7.2 were chosen based on the results obtained in a previous chapter (see chapter VI).1,3 Sensors 7.3 and 7.4 have been reported in the literature and their properties have been studied.4,5 Sensors 7.6, 7.7, and 7.9 have for the first time been synthesized for the purpose of this study; however, the coordination chemistry of similar compounds has been previously characterized in literature.6 The coordination chemistry of 7.6 is similar to that of 7.3, although the signal transduction in 7.6 and 7.7 is based on fluorescence quenching rather than its enhancement, while 7.9 displays a fluorescence turn-on behavior. Finally, 7.5 (Lumogallion) and 7.8 (Calcein Blue) are commercially available and their sensing properties have been characterized.7 Furthermore, incorporating chemosensor with different optical transduction schemes (turn-on, turn-off, and ratiometric) might increase the response space. Furthermore, if a metal ion can induce different kind of responses (turn-on, turn-off, and ratiometric) it might create an avenue for a potential increment of the variance within the \( n \)-dimensional vector response (generated by the array) for that given metal ion. In such a case, more information could be generated and the discriminatory power would be increased.
Figure 7.1. Structure of chemosensors (7.1-7.9) used in the array.

7.3 Configuration of the Array and Optical Sensor Membranes.

The solid-state array was fabricated as indicated in chapter VI using sensors 7.1-7.9 dispersed in a hydrophilic poly(ether)urethane carrier (~0.07 % chemosensor, in Tecophilic SP-93A-100, w/w) to yield a simple two-component optode.

The luminescence output from the array was recorded using four detection channels corresponding to the blue, green, yellow, and red region of the visible spectrum. Upon addition of the aqueous cation solutions to the wells, the emission of the array was re-recorded. The metal ions tested were Al³⁺, Cd²⁺, Ca²⁺, Co²⁺, Cu²⁺, Mg²⁺, Hg²⁺, Ni²⁺, Zn²⁺, and Ga³⁺ in the form of
their chloride salts in water (1 mM in water, 200 nL, pH 7) (Figure 7.2). Figure 7.2 shows a false color representation of the changes in the sensor array induced by the metal ions. A quick inspection reveals distinctive response patterns generated for each of the cations tested. Above all, Zn$^{2+}$ is an ion that presents the most distinct response pattern due to, among others, the high selectivity of 7.9 for this ion.

![Figure 7.2](image)

**Figure 7.2.** Fluorescence responses of the 7.1-7.9 sensor array to the presence of different metal ions (1 mM in water, 200 nL, pH 7). False color representation generated by superimposing of the equally weighed images corresponding to RGB channels.

In order to quantify the changes in luminescence, the non-zero pixels were integrated for each well. As predicted, each of the metal cations induced analyte-specific changes in luminescence in the individual chemosensors of the array, thus creating a multidimensional response pattern. The multidimensional response patterns (36-dimensional, 9 sensors × 4 BGYR channels) of the sensor array in the presence of ten cations (Ca$^{2+}$, Mg$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Co$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Al$^{3+}$, and Ga$^{3+}$) were further studied using LDA.
7.4 Effect of pH on the Sensor Array Response.

In the situation where the sensors comprise potentially pH sensitive chemical moieties, it is necessary to study the possible effect of water (blank) solutions at different pH on the overall response of the array. Therefore, we first studied the changes in the array fluorescence at a range of pH from 1 to 14, and applied LDA to the data generated as a response of the array to varying pH (Figure 7.3). From the canonical score plot, one can see that the responses could be divided into four clusters. The first cluster includes pH 1-4, a second cluster that includes the pH range 5-7, and the third cluster corresponding to pH 8-13. The fourth cluster then corresponds to pH of 14. The third cluster appears to cover a larger area of the canonical space, presumably due to the varying deprotonation processes taking place in different chemosensors in the pH range between 8 and 13.

![Figure 7.3](image.png)

Figure 7.3. LDA canonicals score plot for the response of the array to blanks at different pH levels.

Because of the potential applications, we decided to work in the pH region between 5 and 7, which is more appealing for potential biological and environmental applications. In this pH range the response of the array does not show significant differences in response, presumably
due to the cation coordination chemistry of the receptors within this range. Also, at low pH (pH < 4), protonation of the coordination sites of the receptors can affect the photophysical and coordination chemistry properties of the chemosensors. On the other hand, at high pH (pH > 8), the receptor deprotonation might affect the chemosensor properties, and the solubility of the metal salts/hydroxides decreases. Hence, we decided to evaluate the array performance in cation classification within the pH “comfort zone” at three different pH levels: 5, 6, and 7 where all metal salts are soluble at concentration 2 mM. The cations affected by pH are Al$^{3+}$ or Cu$^{2+}$, albeit only at high concentrations. Due to high sensor-cation affinities, sensors 7.1-7.9 show saturation at 1mM (see appendix 6). Thus, the lower solubility of metal salts at pH 7 does not appreciably affect the analyses. For each pH, a data set corresponding to 10 cations (8 trials) was generated. LDA was first applied separately to each dataset (Figure 7.4). Figure 7.4 shows LDA canonical score plots for the first 3 factors (10 different metal ions) at three different pH conditions. Three factors were necessary to describe at least 85% of the total information (variance) contained in the dataset. Here, the cross-validation routine shows 100% accuracy for the classification of all cations at all three pH levels. Even though the pH does not seem to have a significant effect on the predictability of the overall array behavior, the individual cations show a major difference between the pH levels.
Figure 7.4. LDA canonical score plots describe the response of the 7.1-7.9 array to 10 cations (1 mM) at three different pHs (5, 6, and 7). The first 3 factors were used in order to describe at least the 85 % of the total variance. Data sets (10 cations + blank, 8 trials) recorded at different pH levels were evaluated. For each pH, the cross-validation routine shows 100 % correct classification.
In order to determine whether the data obtained using ten cations at three different pHs could be used for their determination at a particular pH or for pH independent cation detection within the range of pH (5-7), a LDA was carried out including all 240 trials. First, LDA was used to investigate the data describing the array response to cations at a particular pH as a grouping variable to study the ability of the array to distinguish the 30 classes (10 metal ions × 3 pHs). LDA cross-validated (leave-one-out) classification shows 99% accuracy for all ten cations at pHs 5, 6, and 7. This result is remarkable given the fact that some cations, such as Cd$^{2+}$, present a very similar response profile at different pHs (Figure 7.5, left). Secondly, LDA was used to investigate the data set, in which the cation class is used as a grouping variable (Figure 7.5, right) to test if regardless of the pH (5, 6 or 7) the LDA can accurately identify the metal ion. Here, the LDA shows 97% accurate classification, thus showing that the accurate pH independent classification of the cation can be predicted even though there are cations such as Al$^{3+}$ and Zn$^{2+}$ that yield very different response patterns at each pH.

**Figure 7.5.** LDA canonical score plots for the response of the 7.1-7.9 array to 10 cations (1mM) at three different different pH level (5, 6, and 7). Left: LDA was performed using “M$^{n+}$ at pH X” as a grouping variable. Right: LDA performed using just the cation class as a grouping variable.
7.5 Array Performance at pH = 7.

The limit of a detection concept in arrays can be translated into the limit of discrimination, i.e. the concentration at which the sensor array is capable of discriminating between different analytes. Therefore, we studied the array performance at various concentration ranges. For this reason, a data set comprising 10 cations at 8 different concentrations (5 µM - 5000µM, 8 trials each) at pH 7 was generated. The LDA was applied to all 640 trials using cation concentration or cation class as a grouping variable (Table 7.1). First, the cation class is used as a grouping variable to test whether regardless of the concentration it is possible to qualitatively identify the cations. As seen in Table 7.1, a LDA cross-validation routine shows overall 96% of correct classification. The accuracy of the classification differs for each cation.

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<th>Correct Proportion for Prediction of Metal Ion Concentration</th>
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**Table 7.1.** LDA cross-validated (leave-one-out) classification accuracy for 10 different metal ions at different dynamic ranges of concentration (upper limit is 5 mM, in all cases).

For example, 88% classification accuracy was observed for Cu^{2+} and 100% for Zn^{2+}, respectively. This behavior correlates with dynamic ranges of the different sensing elements for different cations. For example, in the case of Cu^{2+} the overall dynamic range of the array lies between 20 µM and 500 µM, while for Zn^{2+} the overall dynamic range is approximately 5 µM –
5000 μM. Thus, it was necessary to evaluate the dependence of the discriminatory capacity of the array at different dynamic ranges (Table 1. \( \geq 5 \mu M: 5 - 5000 \mu M; \geq 10 \mu M: 10 - 5000 \mu M; \geq 50 \mu M: 50 - 5000 \mu M; \) and \( \geq 100 \mu M: 100 - 5000 \mu M \)). The classification accuracy data corresponding to these four dynamic ranges are listed in Table 7.1. The discriminatory capacity for cation identification exceeds 95% overall accuracy at ca. 5 μM and 99% overall accuracy at the concentration \( \geq 50 \mu M \).

On the other hand, when the cation concentration is used as a grouping variable instead of the cation class, the analysis will allow for determining the array’s capability to discriminate between 80 groups of analytes (10 cations \( \times \) 8 concentrations) and perform a quantitative analysis.

Even though LDA is not a regression technique, it can be used to plot the concentration response dependence (trajectory) in the LDA canonical space (Figure 7.6). Figure 7.6 shows trajectories composed of the average scores for each concentration of a given cation. From the discriminant score plots it is clear that at lower concentration (close to the origin of the plots) it is harder to discriminate between different cations in different concentrations. In the case of quantitative determination of cations the limit of discrimination depends even more on the overall dynamic range of the sensor array. This is because not only the general trends in the response pattern (within a class of cation) are important but also mostly the magnitude (of the change in the response) in these trends affects the assignment/interpretation of the concentration by the array. This implies that all of the patterns will tend to be more similar at a low concentration where the sensors are close to their LODs.
As in the case of qualitative determination of cations, we applied LDA to the array response at different concentrations and calculated the percentages for the correct classification of cation concentration (Table 7.1). LDA using a cross-validation routine shows 85% classification accuracy at a threshold of 5 μM. Here, the misclassified data correspond to the 15%, corresponding to 97 trials out of 648 trials. Interestingly, just one trial was misclassified as another cation (Cu$^{2+}$ 5 μM misclassified as Al$^{3+}$ 5 μM); the remaining 96 trials were classified as a correct cation, but at incorrect concentration. For example, a Ni$^{2+}$ 5 μM trial was misclassified as Ni$^{2+}$ 10 μM, Al$^{3+}$ 1000 μM was misclassified as Al$^{3+}$ 5000 μM, etc. Also, 38 misclassifications are evenly distributed between Cu$^{2+}$ and Hg$^{2+}$ ions. This is because the sensors 7.1-7.9 show a similarly limited dynamic range between 20 μM and 500 μM for these two cations. Thus, 13 out of 22 misclassifications for Cu$^{2+}$ are between 5 μM and 10 μM, and 9 out of 21 misclassifications for Hg$^{2+}$ appear in the same concentration range.
Figure 7.6. LDA canonicals score plots describing the response of the 7.1-7.9 array to 10 different metal ions at a concentration range between 5 and 5000 μM. Concentration trajectories are derived from/calculated as the average of the scores for each concentration of a given cation.

At the concentration range of 10 - 5000 μM, where the cation concentrations are out of the LOD zone for most of the sensor elements, the accuracy of the concentration prediction increases to 91%. The same trend is observed with the concentration increased to 50 and 100 μM corresponding to the overall classification accuracy of 93 and 95 %, respectively. In contrast, the correct quantitative determination of Ni²⁺ and Cu²⁺ seemed to decrease at the concentration range of 50 - 5000 μM and higher, presumably due to the fact that most sensors display saturation around 1 mM for these two cations. However, the 7.1-7.9 array still shows a limit of
discrimination of 10 μM for quantitative determination of the cation concentration with more than 90% of classification accuracy, while for qualitative determination the limit of discrimination is 5 μM.

Within the training sets defined by a cation type, concentration, and pH, the array performance is very good (in most cases >95% correct classification) considering that it consists of only 9 sensor elements. This is due to the known and predictable coordination chemistry of the receptors and the luminescence signaling displayed by these sensors, which is different from previous contributions. The chemosensors used in this work are cross-reactive but maintain certain predictable selectivity in binding, while the fluorescence signaling, which is individual for each probe and cation, seems to add yet another layer of information utilized in the analysis. Taken in concert, the intrinsic binding profiles and fluorescence signaling features generate enough discriminatory data to distinguish among 10 cations in a wide range of concentrations. This supports the hypothesis that cross-reactive, yet selective sensing elements might help increasing the response space and as a consequence the discriminatory power of sensor arrays (see chapters IV-VI). While these results appear to be promising, it should be noted that the performance and practicability of the array are limited to the generated training sets. Nonetheless, we believe that this kind of sensor arrays could, perhaps, be utilized in multi-ion detection schemes, particularly if aided by advanced classification algorithms, such as support vector machines or artificial neural networks.

7.6 Mineral Water Brand Identification Based on Metal Ion Content.

Encouraged by the results above, we decided to explore the utility of the 7.1-7.9 sensor array by exploring a potential application: Identification of mineral and purified (Aquafina) waters based on their cation content (mostly Ca\(^{2+}\), Mg\(^{2+}\)). For mineral water analysis the
responses for nine commercial brands along with two controls/blanks (nanopure water and tap water) were collected. Figure 7.7 lists the calcium and magnesium ion contents for all of the mineral water brands. The pH levels of all these brands are in the range of 5-7 where the sensor array presents a rather flat response. From the list (Figure 7.7) it is clear that all 8 brands contain different kinds and concentrations of cations and also in different proportions. Furthermore, from the range of cation concentrations and their kinds, it could be expected that our sensor array could generate a fingerprint-like response pattern for each brand of water based on their cation content. Cross-validation routine shows 100% correct classification for all 88 trials (Figure 7.7, right). Interestingly, Aquafina® brand, due to its low electrolyte content, presents a very weak response, owing to the fact that it is closest to the nanopure water by cation content; Aquafina® is actually not commercialized as a “mineral water,” but as “pure water.”

As a control experiment, we tried to determine the consistency in the cationic fingerprint of the mineral water Evian®. For this brand, three different bottles from different lots were selected randomly and tested using the 7.1-7.9 sensor array. The responses were recorded and evaluated in the discriminant function generated from the analysis of the mineral waters. LDA classified the three Evian bottles as Evian (100% correct classification).
### Figure 7.7. Left: Metal ion content for different brands of mineral and purified water samples. Right: LDA canonicals score plots corresponding to the response of the 7.1-7.9 array to 9 different water brands. The data set contains 9 brands and 2 blanks, 8 trials each. LDA shows 100% correct classification for all water brands.

#### 7.7 Conclusion

A sensor array containing nine selective, yet cross-reactive, sensing elements was developed. The interplay between the selectivity and cross-reactivity given by a different kind of cation-receptor coordination chemistry, along with different signaling schemes, was successfully exploited/utilized to provide highly cross-reactive and provide an information-rich fluorescence output in four (BGYR) emission channels. This easy-to-observe luminescence output may be used for both qualitative and quantitative analyses of metal ions. A pattern recognition method (LDA) was used to evaluate the analytical utility of the described sensors in the array. The discriminatory capacity of the array was tested using a set of 10 metal ions at different ranges of pH and at different concentrations. Qualitative identification of cations can be determined with over 96% of accuracy in a concentration range covering 3 orders of a magnitude (5 – 5000 μM). Quantitative analysis can be achieved with over 90% accuracy in the concentration range between 10 and 5000 μM.
The discriminatory capacity of the described sensors and arrays was also tested in the identification of 9 different mineral water brands utilizing their various electrolyte compositions and their Ca\(^{2+}\), Mg\(^{2+}\) and Zn\(^{2+}\) levels. The present sensor array is capable of discriminating among these complex analytes that were used without any pre-treatment (directly from the bottle). Preliminary results suggest that similar arrays could be used in testing the consistency of the purification and/or manufacturing process of purified and mineral waters.

7.8 References


(2) The experimental data presented in this chapter has been recorded by Dr. Zhuo Wang in Anzenbacher Research Group Labs. All the sensing data is on the appendix 6.

(3) Sensors 7.1 and 7.2 are sensors S2 and S4 respectively in chapter VI.


(9) The electrolyte contents of the water samples are listed in the appendix 6.

(10) Evian® was selected for the ease to find different production lots.
CHAPTER VIII. SMALL MOLECULE-BASED FLUORESCENT SENSORS FOR VAPOR DETECTION OF NITROAROMATIC EXPLOSIVES.

8.1 Introduction

Several materials have been used for the detection of nitroaromatics, most of them fluorescent polymers that are capable of fluorescence quenching in the presence of electron-poor nitroaromatic derivatives. In most cases, the signal transduction mechanism consists of a photoinduced electron transfer (PET) from the SOMO-2 (the LUMO in the resting state) of the chemosensor-excited state to the LUMO of the nitroaromates (See Figure 8.1).¹

![Figure 8.1](image)

**Figure 8.1.** Schematic representation of a PET process, where the excited-state of the chemosensor (CS) is the electron donor and the nitroaromates (NA) is the electron acceptor.

Examples of materials capable of detecting explosives are iptycene-based polyphenyleneethynlenes,²ᵃ poly-acetylenes,²ᵇ polysiloles,²ᶜ polymetaloles,²ᵈ and silica-supported metalloporphyrins.²ᶜ Swager and coworkers have used pentiptycene phenylene-ethynylene polymers in the detection of TNT vapors at ppb levels.²ᵃ,³ In these previous studies, it was proposed that the origin of the chemosensor high performance was mainly due to (a) an electronic effect (amplified quenching),⁴ (b) an electrostatic interaction between the electron-rich polymer and electron-poor nitroaromates and last but not least, (c) a structural effect (porosity of
the material and the thickness of the film). We wanted to explore the possibility of using our chemosensor-polymer system (Chapter III) to detect nitro-aromatic vapors at ppb levels. We reasoned that given the intrinsic breathability of poly(ether)urethanes, the strength of the interaction of nitroaromatic compounds with the polymeric matrix (physisorption) should suffice for the PET process to take place between the chemosensors (embedded in the polymeric matrix) and nitroaromates even in the case when (not amplified) small fluorescent chemosensors are used as probes.

In order to explore the utility of the chemosensor-polyurethane system in the detection of explosive vapors in air, we use non-polymeric pentiptycene-derive chemosensors embedded in poly(ether)urethane matrices as bulk optodes. In our proof-of-principle experiment, we first utilized film casted membranes to yield the sensing layer. Finally, we demonstrate that the magnitude of the response and the kinetics of the sensing process can be enhanced by changing the material processing. For instance, we use electro-spinning to generate nanofiber mats (of the chemosensor-polyurethane materials) as a sensing layer in order to increase the surface to volume ratio and the contact area between the analyte and the chemosensor. Therefore, it is feasible to investigate the possibility of generating an efficient sensing platform without the need for redesign and synthesize new chemosensors.

8.2 Selection of Chemosensors

The present chemosensors 8.1 and 8.2 have been synthesized by Dr. Zyryanov for the detection of nitroaromates and they are simple small pentiptycene derivatives functionalized with electron-rich thiophene moieties. In 8.1 and 8.2 the signaling chromophore is located close to the binding cavity while having a sufficiently high LUMO level to prompt an exergonic PET.

On
the other hand, it is important not to exceed the energy of the LUMO manifold to avoid falling into the Marcus inverted region that might decrease the rate of the PET.⁹

For our design we considered that one of the ways to manipulate the LUMO energy level could be by increasing the HOMO-LUMO gap and this can be achieved by limiting the degree of conjugation in the sensor. With this in mind, fluorogenic residues were attached directly to the 1,4-positions of the pentiptycene central benzene ring. Since a complete planar conformation is restrained by unfavorable steric interactions between the iptycene bridge hydrogens and the beta-hydrogen of the thiophenes, the conformation results in a perpendicular arrangement of the central benzene ring and the thiophenes in the 1- and 4- positions in the benzene ring (Figure 8.2). Consequently, this effect results in a greater HOMO-LUMO gap in 8.1 and 8.2.

**Figure 8.2.** Left: X-ray crystal structure of 8.2 (Hs not shown for clarity). Right: Electron density of 8.2 mapped with the electrostatic potential calculated at the B3LYP/6-31G** level of theory from the X-ray crystal structure geometry.
The photophysical properties of 8.1 and 8.2 were investigated by UV-Vis and fluorescence spectroscopy by Dr. Zyryanov. The quantum yields of 8.1 and 8.2 are relatively modest, $\Phi_{8.1} \sim 0.16$ and $\Phi_{8.2} \sim 0.17$. This is maybe due to the poor conjugation of the system and the presence of sulfur atoms that promote intersystem crossing to the non-radiative triplet states.

When titrated with nitroaromatic compounds, chemosensors 8.1 and 8.2 showed dramatic fluorescence quenching. The Stern-Volmer constants for 8.1 and 8.2 are summarized in Table 8.1. Interestingly, the TNT-quenching displayed by 8.1 and 8.2 suggests that the detection effectiveness of these small-molecule chemosensors is comparable to the iptycene-based polyphenyleneethynylenes amplifying polymers ($K_{SV}=1,170 \text{ M}^{-1}$) or polasilole sensors ($K_{SV}=4,340 \text{ M}^{-1}$) in solution. Even though the latter results are encouraging, they have to be seen in light of the fact that in the case of the iptycene-based polyphenyleneethynylenes it has been previously articulated that the amplifying mechanism operates efficiently only in the solid state.\textsuperscript{10} Furthermore, this finding seems to suggest that the amplifying effect is due to the transfer (hoping) of the exciton between the polymer chains rather than within the chain. This explains why the amplification mechanism does not operate in solutions.\textsuperscript{11,12}

<table>
<thead>
<tr>
<th>Nitroaromate</th>
<th>8.1</th>
<th>8.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhNO$_2$</td>
<td>2 160</td>
<td>91</td>
</tr>
<tr>
<td>2,4-DNT</td>
<td>4 500</td>
<td>1 290</td>
</tr>
<tr>
<td>TNT</td>
<td>3 330</td>
<td>1 100</td>
</tr>
<tr>
<td>DMDNB</td>
<td>81</td>
<td>18</td>
</tr>
</tbody>
</table>

\textbf{Table 8.1}. Values of the apparent Stern-Volmer constants ($K_{SV}$). $K_{SV}$ were recorded in solution (5.0 $\mu$M in CH$_2$Cl$_2$); Fit errors were <10%. The samples were excited in their absorption maxima.\textsuperscript{13}
8.3 Nitroaromates Sensing with Film Casted Optodes

The polymeric films were first fabricated by blending chemosensors 8.1 and 8.2 with a hydrophilic poly(ether)urethane (PEU, Tecoflex® 80A) in THF and solution-casting 200 nL of the chemosensor-PEU solution into a microwell array. The arrays were exposed to equilibrated vapors of 2,4-DNT (180 ppb) and TNT (7.70 ppb) and imaged before and after exposure. Figure 8.3 shows the response after 30 min exposure to nitroaromates vapors. The emission of both 8.1 and 8.2 appeared to be quenched (>80%) while the red emission from tetraphenylporphyrin (HTPP) that was used as internal standard was barely affected. Also, blowing air (2 min) over the sample can regenerate the original state of the array. Finally, the non-zero pixels can be integrated from the image in real time to yield data, which may be directly used for quantitative evaluation.

Figure 8.3. Top: CCD images of sensor chips using 8.1 and 8.2 embedded in polyurethane illuminated by black light. Bottom: Quasi-3D interpretation of the data using a x-y-fluorescence intensity matrix.

We wanted to study the impact of the polymeric matrix in the sensing process. Toward this end we carried out a second set of experiment utilizing polymeric films containing the
chemosensors **8.1** and **8.2** with a mixture 1:4 of hydrophilic poly(ether)urethane and high molecular weight polystyrene PS. PS polymers are known for having a high transition glass temperature and very poor breathability. The experiment was designed to explore if the composite could tune the degree of fluorescence quenching of the chemosensors. The new arrays were exposed to equilibrated vapors of 2,4-DNT and TNT and imaged before and after exposure. Figure 8.4 shows the response after 30 min exposure to nitroaromate vapors. The emission of both **8.1** and **8.2** appeared to be quenched to a lesser degree compared to the first experiment. Moreover, the recovery time of the array is about 5 times longer. The latter demonstrates that the sensing capability of the chemosensor is limited by diffusion of the nitroaromates into the bulk of the material. Moreover, once the analyte is in the matrix, the limited diffusion of the nitroaromates in the bulk materials slows down the desorption of the analyte from the film.

**Figure 8.4.** Top: CCD images of sensor chips using **8.1** and **8.2** embedded in 20% polystyrene in polyurethane illuminated by black light. Bottom: Quasi-3D interpretation of the data using a x-y-fluorescence intensity matrix.
8.4 Nitroaromate Sensing with Non-Woven Electrospun Mats

As seen in the previous section, the efficient detection of nitroaromates depends a great deal on the vapor exchange between the air and the bulk optode (sensor film). Thus, there is a clear dependence on the film thickness; thicker films will require more time and analyte in order to show a response. In this regard, our solution film casting process usually yields films that are \( \sim 10 \, \mu\text{M} \) thick, approximately 4,000 times thicker than the films used by Swager and coworkers.\(^3\) Unfortunately, due to the modest quantum yields of the chemosensors 8.1 and 8.2, it is rather difficult to obtain reliable results from thinner films.

Encouraged by the findings described above, we decided to explore another kind of material processing to generate the sensing membrane. The idea was to create nanostructured materials that would yield sensing membranes with a high surface to volume ratio. Towards this end, an electrospinning process was used to fabricate polymer nanofiber mats.\(^{15}\) Electrospinning of a Tecoflex® solution can produce fibers with 200-400 nm in diameter. The fibers resulting from the electrospinning process were characterized by SEM (Figure 8.5, left).\(^{16}\)

The assay consisting of electrospun fiber resembles that of Figures 8.2 and 8.3. Nanofibers containing chemosensor 8.2 were collected along with nanofibers containing HTPP as an internal standard. The dual-component nanofiber mats were exposed to equilibrated vapors of TNT. The fluorescence of the fibers was imaged using a fluorescence microscope (Figure 8.5, center).
As seen in Figure 8.4, either spectroscopic or imaging techniques could be used for quantification of the fibers quenching. However, we were eager to show that imaging techniques could be successfully used for this purpose. The response was followed by taking color (RGB) images of the nanofiber mat. The observable quenching (20%) of X.1 (blue nanofibers) in presence of TNT vapors (7.7 ppb) took place within 1 minute, while the HTPP (red fibers) were not significantly quenched (Figure 8.5).

The deconvolution of the RGB channels into separate red and blue channels allows for integration of non-black (i.e. non-zero) pixels (Figure 8.6). The ratio of blue (5)-to-red (HTPP) pixel counts serves as an output value. Here, the blue to red pixel ratios were as follows: at 0 min ~ 2.1835, at 1 min ~ 1.8024, at 3 min ~ 0.7866, and at 5 min ~ 0.1108. These measurements allow for the construction of a quenching isotherm for the sensing process (Figure 8.6, right). The blue to red ratios do not change significantly between 10 randomly selected sites in the mat. Finally, the response time of the membrane produced by electrospinning is up to ~6 times faster compared to the one produced by the film casting.
Figure 8.6. Left: Images of the nanofiber mat in presence of TNT (7.7 ppb). The deconvolution of RGB images to obtain the pixel ratios to construct the quenching isotherm (right).

8.5 Conclusion

In summary, we showed the utility of non-polymeric pentiptycene-derived chemosensors embedded in poly(ether)urethane matrices as bulk optodes and nanofiber mats for the detection of explosive vapors in air. Furthermore, it was shown that the relative degree and timeline of the sensor response might be tuned by using various polymer matrices and the manner these matrices are processed, without the need for redesigning receptors and synthesizing new sensor moieties. The use of polymer nanofiber mats seems to be suitable for production of such sensor assays.

8.6 Experimental Section

8.6.1 Nitroaromates sensing by X.1 and X.2 embedded in polymer matrices: Studies utilizing polyurethane matrices were performed using polymer films with incorporated sensors (8.1 and 8.2), which were prepared by casting THF solutions containing sensors 8.1 and 8.2 (0.4% w/w
sensor and 0.2% w/w for tetra-phenylporphirin HTPP films as internal standard) in Tecoflex®
80A and Tecoflex® 80A /Polystyrene 1:4 (3.5% w/w) onto multi-well chip.

The fluorescence quenching of the polymer films with nitroaromate was carried out by
placing the sensor chip in a glass chamber where the analyte vapors had been equilibrated. The
quenching of the fluorescence was estimated by comparison against the fluorescence of the
internal standard (HTPP). The analytical measurements of the optical density were carried out
using and CCD camera and ImageJ software.

8.6.2 Chemosensor 8.2 embedded in electrospun polyurethane matrices for TNT sensing:
Polymer solution (8%, 2:1 THF/EtOH w/w, Tecoflex® and 0.03% w/w, 8.2 or HTPP) was loaded
into a 1 mL disposable plastic syringe. A Hamilton® stainless steel needle with a 21 gauge was
used. The needle was connected to a high-voltage power supply (ES30P-5W, Gamma High
Voltage Research Inc.). The polymer-sensor solution was continuously dispensed using a syringe
pump (Genie Plus, Kent Scientific) at a rate of 3 μL/min. In a typical electrospinning
experiment, a voltage of 5 kV was applied on the needle. The distance between the needle tip
and the collector was 10 cm. The collector consists in two independent aluminum rods connected
to ground with a microscope slide (75 x 25 mm) between them. The typical collection time to
achieve the 3:1 optical composition was 2 min for the HTPP polymer solution and 5 min for the
8.2 polymer solution (Figure 8.6). The characterization of the fibers was carried out using an epi-
fluorescence optical microscope (DIALUX 20, Leitz/Leica) with CCD cooled color camera
(Penguin 150CL, Pixera), and scanning electron microscope SEM images were taken using a
scanning electron microscope (Inspect S, FEI Company, Portland, OR) operated at an
accelerating voltage of 10 kV. The samples for SEM were coated in a sputter-coater (E500,
Poloron) with Palladium.
In a typical sensing experiment, microscope slides containing electrospun fibers with
8.2 and fibers containing tetraphenylporphyrin as an internal standard were prepared for the TNT sensing. The slides were exposed to TNT vapors (7.7 ppb) and DNT vapors (180 ppb) at 23 °C. Micrographs were taken at different times during the experiment. For this we used an epi-fluorescence optical microscope and excitation at 365nm. In order to prevent false positives from photobleaching, every measurement was taken in a fresh spot in the nanofiber mat. Also, control experiments show that non-significant photobleaching takes place in the first 2 min of exposition to UV light. The RGB deconvolution and all the pixel-density calculation were carried out using ImageJ.

![Figure 8.7](image)

**Figure 8.7.** Different ratios of chemosensor to internal standard HTPP can be achieve by collecting the fibers for different period of time.

8.7 References


(4) See chapter I.


(7) Sensors X.1-X.2 and their photophysical characterization were carried out by Dr. Grigory V. Zyryanov at Anzenbacher Research Group laboratories.

(8) Other derivatives were also prepared (see ref. 5) although they have not been tested embedded in PEU films.


(13) Because the UV-Vis spectra of the 8.1 and 8.2 did not change in the presence of nitroaromates, the excitation could be performed at a wavelength where the 8.1 and 8.2 absorb light and the nitroaromates do not (absorb light).

(14) PS films were also studied, but they did not present a discernible response.


(17) Recently, the fast development of sensitive CCD based cameras has promote the utilization of imaging techniques for quantization of fluorescence intensity. Moreover, detection setups based on CCD color cameras have the advantage of being less complex, since they do not require filters for the simultaneous detection of the fluorescence in the three RGB channels. On the other hand, spectrofluoromeers require monochromators or filter sets for detection of different channels and they cannot be detected at the same time (see ref. 1).
CHAPTER IX. SIMPLE ELECTROOPTICAL CHEMOSENSORS FOR ANION DETECTION IN SOLUTION

9.1 Introduction

In order to upgrade the reliability of the sensing process, we decided to investigate the possibility of using two independent output signals (change in color and current/redox potential) by integrating a chromophore (light-absorption) and an electrochemically active unit that reacts to the presence of an anionic analyte.\(^1\) Although previously demonstrated, such materials are still rare.\(^2,3,4\)

Proof-of-principle experiments were conducted by observing the electrooptical response of chemosensors 9.1, 9.2 and 9.3 (Figure 9.1) to seven different anions in organic solution. Chemosensors 9.1, 9.2 and 9.3 comprise a chromophore capable of undergoing a dramatic change in color as well as changes in redox behavior in the quinone moiety. In order to generate strong colorimetric and electrochemical signals, the redox-active moiety is fused directly to the chromophore and to the pyrrolic receptors comprising hydrogen bond donors.

![Figure 9.1. Structures for chemosensors 9.1, 9.2 and 9.3.](image)
9.2 Synthesis of 9.1, 9.2 and 9.3

Chemosensors 9.1 and 9.2 were prepared (Scheme X.1) by condensation of dipyrroylethane-1,2-dione (a) with 1,2-diaminoantraquinone (b) and 2,3-diamino[1,4]naphthoquinone (e), respectively. We prepared chemosensor 9.3 containing sulfonamide moieties as described previously by Crabtree and Kavallieratos. All compounds were characterized by NMR and mass spectrometry (see Appendix 7).

Scheme 9.1. Synthesis of chemosensors 9.1, 9.2 and 9.3. Reaction conditions: (i) AcOH, 100 °C, 12 hr; (ii) TsCl/pyridine, RT, overnight; (iii) Potassium phthalimide, MeCN, reflux; (iv) N₂H₄•H₂O, 60 °C, 8 hr.

9.3 Colorimetric Response of 9.1, 9.2 and 9.3 to the Presence of Anions

Visual inspection of the solutions of chemosensors 9.1, 9.2, and 9.3 (25 μM in MeCN with 0.5% water) before and after the addition of anion salts (50 equiv.) showed a dramatic change in color in case of fluoride, cyanide, acetate, and pyrophosphate suggesting a strong binding, while the addition of dihydrogenphosphate, benzoate, or chloride did not result in appreciable change in color (Figure 9.2).
Figure 9.2. Left panel: solutions of chemosensors 9.1-9.3 (25 μM in MeCN) in the presence of anions (50 eq.). Central panel: examples of UV-vis-spectra of chemosensors 9.1-9.3 (9.1/F⁻, 9.2/AcO⁻, 9.3/HP₂O₇³⁻). Right panel: Affinity constants (Kₘ) for compounds 9.1, 9.2 and 9.3 (M⁻¹) and anionic substrates in MeCN (25 μM, 0.5% water at 22 °C).

We believe that the selectivity trend is due to a difference in sizes of binding sites (9.1, 9.2 vs. 9.3) as well as the direct result of the acidity of protons involved in hydrogen bonding the anions, as well as the basicity of the anions. In order to quantitatively determine the nature of the interaction of our chemosensors with anions, UV-vis titrations with different anions (F⁻, Cl⁻, HP₂O₇³⁻, H₂PO₄⁻, CN⁻, benzoate, and acetate) were carried out for chemosensors 9.1, 9.2 and 9.3 (Figure 9.2) in MeCN, both with and without tetrabutylammonium perchlorate (TBAClO₄) as the supporting electrolyte. The chemosensor–anion affinity was investigated by calculating the association constants (Figure 9.2). The association constants corroborate what was qualitatively observed during the visual inspection. Chemosensor 9.1 and 9.2 show similar behavior towards
similar anions due to the same nature of the receptor while chemosensor 9.3 shows remarkable preference towards small anions.

9.4 Electrochemical Response of 9.1, 9.2 and 9.3 to the Presence of Anions

The electrochemical responses of chemosensors 9.1, 9.2, and 9.3 were studied using cyclic voltammetry (Figure 9.3). In the case of chemosensors 9.1 and 9.2, the electrochemistry experiments revealed one electron quasi-reversible reduction at around -0.640 V. These reductions are associated with the injection of an electron into the LUMO of the anthraquinone moiety. Chemosensor 9.3 shows two independent reduction processes. The first one (-0.490 V) is a quasi-reversible process probably associated with the anthraquinone moiety, while the second one (-0.720 V) is non-reversible and could be associated with the sulfonamide moieties.

![Cyclic voltammograms of chemosensor 9.1, 9.2 and 9.3](image)

**Figure 9.3.** Cyclic voltammograms of chemosensor 9.1, 9.2 and 9.3. All experiments were carried out in distilled anhydrous and degassed MeCN at 50 μM concentration of the sensor and 0.100 M TBAClO₄ was present as the supporting electrolyte.

In order to obtain the second “validating” signal output to supplement the UV-Vis spectroscopy data, the anion binding by chemosensors 9.1, 9.2, and 9.3 was also investigated by cyclic (CV) and square-wave voltammetry (SWV) (Figure 9.4). Both methods confirm that the
anion binding is accompanied by anion-specific change in the redox potential as well as by
decrease in current, a behavior attributed to the formation of the chemosensor-anion complex
with a lower diffusion coefficient.\textsuperscript{7,8} Also, reduction waves of 9.1, 9.2, and 9.3 show cathodic
shifts due to the internal charge transfer caused by the complexation to the anion, which makes
the reduction of the anion-chemosensor complex more demanding in energy.\textsuperscript{9}

The data summarizing both colorimetric and electrochemical responses upon addition of
anions is shown in Table 9.1. Inspection of the data in Table 9.1 reveals that the SWV titrations
show measurable changes in the peak current and reduction potential even in the case of anions
that induce only a weak color change insufficient for reliable determination by absorption
spectroscopy (e.g. H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−}).

\textbf{Figure 9.4.} Example of changes in redox properties of 9.1 (50 μM) upon addition of TBA
H\textsubscript{2}PO\textsubscript{4} observed by SWV.
Table 9.1. Changes in color and current at saturation in chemosensors 9.1-9.3 in the presence of anions in MeCN (25 μM, 0.5% water, 22 °C). b ●●●● indicates strong color change; ○○○○ indicates no color change (determined as visual observation). c The data suggest passivation of the electrode. d The data was obtained from the CV titration. e Partial decomposition of acetate coincides with the reduction of chemosensor 9.3.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Color change</th>
<th>( \Delta I_p ) (μA)</th>
<th>( \Delta E_p ) (mV)</th>
<th>Color change</th>
<th>( \Delta I_p ) (μA)</th>
<th>( \Delta E_p ) (mV)</th>
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<tr>
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<tr>
<td>HP₂O₇⁻³</td>
<td>●●●●</td>
<td>3.7 84</td>
<td></td>
<td>●●●●</td>
<td>3.6 58</td>
<td></td>
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<tr>
<td>H₂PO₄⁻</td>
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<td></td>
<td>○○○</td>
<td>3.8 53</td>
<td></td>
<td>○○○</td>
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<td>0.6 &lt; 2</td>
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<td>AcO⁻</td>
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<td>5.6 34</td>
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<td>●●●</td>
<td>12.47</td>
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<td>●●●</td>
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The binding isotherms obtained from titration experiments carried out by UV-Vis spectroscopy (\( \Delta \lambda = f(\text{anion}) \)) and SWV (\( \Delta I = f(\text{anion}) \)) show remarkable similarity and also yielded similar affinity constants (within the 15% error margin). For example, the affinity constant for chemosensor 9.2 and fluoride anion by UV-vis and SWV was 28,300 and 33,400 M⁻¹, respectively (Figure 9.5).

Figure 9.5. Examples of binding isotherms for 9.2 titrated with TBAF obtained from UV-Vis and SWV measurements, both in MeCN/TBAClO₄.
While agreement over the course of the electrochemical and spectrophotometric titration experiments suggests that both types of changes originate from chemosensor-anion interaction, the data in Table 9.1 reveal that the magnitude of the association constant does not necessarily correspond to the actual magnitude of anion-induced changes in current and reduction potential. For example, the magnitude in changes of the redox properties in 9.1, 9.2, and 9.3 recorded for H$_2$PO$_4^-$ matches the magnitude of changes in electrical properties recorded for F$. However, the affinity constants calculated from absorption spectra for F$^-$ are higher than for H$_2$PO$_4^-$. The same behavior was also reported for pyrrole-ferrocene systems.\textsuperscript{8} The phenomenon that cause this disparity is still not entirely clear.\textsuperscript{10,11,12}

Because the anionic analytes are administered predominantly as aqueous solutions, we have investigated the sensing process with anions added as a solution in water (25 mM). In these experiments, the sensing process is rendered more complex as a result of multiple equilibria including hydration:

\begin{align*}
  \text{Sensor} &+ \text{H}_2\text{O} \rightleftharpoons \text{Sensor} \cdot \text{H}_2\text{O} & (1) \\
  \text{Sensor} &+ \text{Anion} \rightleftharpoons \text{Sensor} \cdot \text{Anion} & (2) \\
  \text{Sensor} \cdot \text{H}_2\text{O} &+ \text{Anion} \rightleftharpoons \text{Sensor} \cdot \text{H}_2\text{O} \cdot \text{Anion} & (3) \\
  \text{Sensor} &+ \text{Anion} \cdot \text{H}_2\text{O} \rightleftharpoons \text{Sensor} \cdot \text{Anion} \cdot \text{H}_2\text{O} & (4) \\
  \text{Sensor} \cdot \text{Anion} &+ \text{H}_2\text{O} \rightleftharpoons \text{Sensor} \cdot \text{Anion} \cdot \text{H}_2\text{O} & (5)
\end{align*}

Given the timescale of the voltammetric experiments and the fast and reversible nature of the association/hydration processes, the ternary species, composed of chemosensors, anions, and water (i.e. Equations 3, 4, and 5), would appear as one (Equations 3-5) (Figure 9.6). From the equilibria proposed for chemosensor 9.1, we should observe three different redox-active species in the voltammogram: a free chemosensor, a hydrated chemosensor (Equations 1), and a
hydrated anion-chemosensor complex (Equations 3-5). Furthermore, we assumed that in order for the hydration process to be energetically favorable, the hydrated chemosensor-anion complex (Equations 3-5) should show a lower reduction potential compared to the non-hydrated anion-chemosensor complex (Equations 2). Indeed, the SWV traces show three peaks as expected.

![Figure 9.6](image-url) Example of changes in redox properties of 9.1 (50 μM) upon addition of aqueous H₂PO₄⁻ observed by SWV.

9.5 Qualitative Insight in the Binding Mode and Signal-Transduction Process for 9.1 in the Presence of Anions

From the results described above and density functional theory (DFT) calculations (B3LYP/6-31G(d) level),¹³,¹⁴ we have inferred the qualitative insight into the binding mode and signal transduction process in chemosensor 9.1. In the resting state, the pyrrole moieties prefer a coplanar arrangement with the aromatic chromophore. The highest HOMO density is localized on the pyrroles, while the highest LUMO density is localized at the anthraquinone. The anion-induced red shift in the UV-Vis spectra suggests that the anion binding results in a conformational change affecting the HOMO level ($\Delta E_{\text{HOMO}} \sim 0.65 \text{ eV}$) and the partial negative
charge transfer from pyrrole to the quinoxaline-anthraquinone. This is confirmed by the electrochemical experiments, where smaller increments were observed in the LUMO energy corresponding to the cathodic-shift ($\Delta E_{\text{LUMO}} \approx 0.10$ eV) (Table 9.1). These observations were supported by DFT calculations for the 9.1•F•TBA$^+$ complex that show surprising agreement with the recorded data supporting our binding model.

Figure 9.7. Electronic picture of the sensing process. Left panel: Experimental estimation from the electrooptical response of chemosensor 9.1 of the HOMO and LUMO manifolds. Right panel: Density functional theory calculation of the proposed binding mode of chemosensor 9.1 to fluoride.

Even though our design of electroptical chemosensors has shown remarkable performance, a thorough thermodynamic analysis of the electrochemical sensing could show that more effective chemosensors are theoretically possible. For instance, chemosensors 9.1, 9.2 and 9.3 show a cathodic shift upon the addition of an anion to the chemosensor solution. This fact reveals that once the chemosensor is reduced, its radical anion should show a diminished association constant compared to its electroneutral form. This feature can be described
quantitatively because the electrochemical sensing process is a closed thermodynamic cycle in which the total change of free energy in the system is equated to zero. This allows us to describe the thermodynamic processes by the equations (Equations 6 and 7). Equation 8 shows that the ratio between the binding constant of the reduced form the chemosensor and the binding constant of the neutral chemosensor are exponentially related to the change (shift) in the redox potential.

\[
\sum \Delta G = \Delta G_s + \Delta G_{na} + \Delta G_c + \Delta G_{ wa} = 0
\]  

(6)

\[ nF (E - E_s^0) - RT \ln (K^{red}_a) + nF (E_c^0 - E) + RT (K_a) = 0 \]  

(7)

\[ nF (E_c^0 - E_s^0) = RT \ln \left( \frac{K^{red}_a}{K_a} \right) \]  

(8)

Equation 8 implies that future designs of chemosensors capable of undergoing an oxidation will result in a radical cation after an anodic shift in an enhanced response due to fact that the oxidized form of such a chemosensor will be binding anion more strongly than its neutral form. Recently Fabbrizzi and coworkers have reported a tripodal electrochemical sensor for chloride ion based on the redox properties of the Co$^{2+}$.$^{15}$ In their study it is clear that the sensing process can benefit from having a redox moiety present in the sensor undergoing an oxidation. Nevertheless, a new design should be performed very carefully because this expected enhancement in the binding properties could be due to electrostatic interactions, which could result in a less selective binding process.

9.6 Conclusion

In summary, our efforts have focused on two interesting problems in anion sensing. First, we have demonstrated the viability of easy-to-produce anion chemosensors that respond to the
presence of anions by both changes in color and redox properties. We demonstrated that these two methods (UV-Vis and SWV) may be used to cross-examine the anion sample while providing two independent output datasets used to distinguish between different analytes in the cases when either colorimetric or redox-based measurements alone may provide an ambiguous output signal. The preliminary experiments suggest that this approach may yield anion chemosensors potentially useful for sensing of anions in the presence of water and electrolytes. Finally, the combination of multiple signal outputs in just one molecule may result in a “lab on a molecule” chemosensor capable of generating discriminatory data for qualitative and quantitative identification of anions.

9.7 Experimental Section

9.7.1 General: \(^1\)H and \(^{13}\)C NMR spectra were recorded using a Varian Unity 400 (400 MHz) spectrometer. The chemical shifts (\(\delta\), ppm) are referenced to a solvent. EI-DIP mass spectra and were recorded using a Shimadzu QP5050A spectrometer. MALDI mass spectra were recorded using Brucker Daltonics Omniflex. Absorption spectra were recorded using a Hitachi U-3010. Electrochemical measurements were performed using a CH-Instruments-430 potentiostat interfaced with Pentium PC. A platinum wire was used as an auxiliary electrode and a Ag/Ag\(^+\) reference electrode was used for the measurements. A scan rate of 100 mV/s was typically employed. Under these experimental conditions, the ferrocene/ferrocenium couple was determined to be +0.121 V vs. Ag/Ag\(^+\) reference electrode.

9.7.2 Cyclic Voltammetry (CV) studies: All experiments were carried out in distilled anhydrous and degassed MeCN at 50 \(\mu\)M concentration of the chemosensor and 0.1 M TBAClO\(_4\) was present as the supporting electrolyte. All CVs for compounds 9.1, 9.2, and 9.3 did not show any changes in \(E_p\) or \(I_p\) upon changes in the scan rate over the range of 100-400 mV/s.
9.7.3 Square Wave Voltammetry (SWV) titration: All experiments were carried out in distilled anhydrous and degassed MeCN at 50 μM concentration of the chemosensor and 0.1 M TBAClO₄ was present as the supporting electrolyte. The typical setting for SWV experiments was as follows: freq. = 15 Hz, increment = 4 mV, amplitude = 25 mV. Hydrated tetrabutylammonium (TBA) salts of the anions were used in this study: fluoride (x6H₂O), cyanide (x6H₂O), chloride, phosphate (x2H₂O), and pyrophosphate (x2H₂O). The degree of hydration was estimated from elemental analyses.

9.7.4 Examples of square-wave-voltammetry titration data

Figure 9.8. Chemosensor 9.1 and dihydrogen phosphate. Changes in the square wave voltammogram of the chemosensor 9.1 (50 μM) upon the addition of phosphate (0-150 μM).

Figure 9.9. Chemosensor 9.1 and hydrogen pyrophosphate. Changes in the square wave voltammogram of the chemosensor 9.1 (50 μM) upon the addition of pyrophosphate (0-70 μM).
Figure 9.10. Chemosensor 9.1 and Acetate. Changes in the square wave voltammogram of the chemosensor 9.1 (50 μM) upon the addition of acetate (0-75 μM).

Figure 9.11. Chemosensor 9.1 and benzoate. Changes in the square wave voltammogram of the chemosensor 9.1 (50 μM) upon the addition of benzoate (0-100 μM).

9.7.5 Density Functional Theory (DFT) Calculations for Chemosensor 9.1: The Spartan 2004 was used for all the calculations presented in this chapter. The calculations were carried out at the B3LYP level of theory with 6-31G* as the basis set as it was reported in the literature for the same family of compounds. For geometrical optimizations the first guests were fully
optimized by PM3 semi-empirical Hamiltonian. Next, the models were re-optimized at 
B3LYP/6-31G* level of theory. For the complex chemosensor $9.1\cdot F$, tetra-butyl-ammonium was 
added to the model to balance the charges.

Chemosensor $9.1$: HOMO and LUMO for the optimized structure in gas-phase.

\[ E^{\text{HOMO}} = -5.51 \text{ eV} \]
\[ E^{\text{LUMO}} = -3.04 \text{ eV} \]
\[ E_{\text{Fermi}} = -3.71 \text{ eV} \text{ (Calc. from the $E_{\text{red}}$ in solution)} \]

\[ \Delta E^{\text{HOMO-LUMO}} = 2.47 \text{ eV} \approx 501 \text{ nm} \]

Complex Chemosensor $9.1\cdot F\cdot TBA$: HOMO and LUMO for the optimized structure gas-phase.
(The TBA$^+$ is not shown for clearness).

\[ E^{\text{HOMO}} = -4.76 \text{ eV} \]
\[ E^{\text{LUMO}} = -2.96 \text{ eV} \]
\[ E_{\text{Fermi}} = -3.48 \text{ eV} \text{ (Calc. from the $E_{\text{red}}$ in solution)} \]

\[ \Delta E^{\text{HOMO-LUMO}} = 1.80 \text{ eV} \approx 688 \text{ nm} \]
9.8 References


(6) Karolina Jursikova originally carried out the UV-vis titration experiments presented in this work.


(17) *Spartan’04* Wavefunction, Inc. Irvine, CA.

### APPENDIX 1. LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>ampere</td>
</tr>
<tr>
<td>AC</td>
<td>alternating current</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ANN</td>
<td>artificial neural networks</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BzO⁻</td>
<td>benzoate</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>C</td>
<td>concentration</td>
</tr>
<tr>
<td>CHEF</td>
<td>chelation-enhanced fluorescence</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>DFT</td>
<td>density functional theory</td>
</tr>
<tr>
<td>DIP</td>
<td>direct insertion probe</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPQ</td>
<td>Dipyrrrolilquinoxaline</td>
</tr>
<tr>
<td>E</td>
<td>energy</td>
</tr>
<tr>
<td>EDG</td>
<td>electron-donating group</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>Ep</td>
<td>electric potential</td>
</tr>
<tr>
<td>ESP</td>
<td>electrostatic potential</td>
</tr>
<tr>
<td>eq.</td>
<td>molar equivalent</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>eV</td>
<td>electron-volt</td>
</tr>
<tr>
<td>EWG</td>
<td>electron-withdrawing group</td>
</tr>
<tr>
<td>FRET</td>
<td>Foster resonant energy transfer</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GCMS</td>
<td>gas chromatography coupled with mass-spectrometry</td>
</tr>
<tr>
<td>HCA</td>
<td>hierarchical clustering analysis</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>HTPP</td>
<td>hydrogen tetraphenylporphyrin</td>
</tr>
<tr>
<td>I</td>
<td>electrical current</td>
</tr>
<tr>
<td>IC-ICP</td>
<td>ion chromatography coupled with inductively coupled plasma</td>
</tr>
<tr>
<td>ICT</td>
<td>internal charge transfer</td>
</tr>
<tr>
<td>IRF</td>
<td>instrument response function</td>
</tr>
</tbody>
</table>


$i$-PrOH  isopropanol

$K_a$  association constant

$\lambda$  wavelength

LDA  linear discriminant analysis

LoD  limit of detection

LUMO  lowest unoccupied molecular orbital

m  meter

M  molar (mol/lt)

MA  multivariate analysis

MALDI  matrix-assisted laser desorption

$\mu$A  microamper ($10^{-6}$ A)

$\mu$m  micron ($10^{-6}$ m)

$\mu$M  micromolar ($10^{-6}$ M)

MeCN  acetonitrile

MeOH  methanol

MHz  megahertz

MS  mass spectrometry
mV millivolt \((10^{-3} \text{ V})\)

nm nanometer

NMR nuclear magnetic resonance

ns nanosecond

NSAID Non-steroidal anti-inflammatory drug

ORTEP Oak Ridge thermal ellipsoid plot

OMCP meso-octamethylicalix[4]pyrrole

ps picosecond

PC principal component

PCA principal component analysis

PET photo-induced electron transfer

PEU polyetherurethane

Pi dihydrogen phosphate

\(pKa\) -log of the acidity constant

ppb parts per billion

PPI hydrogen pyrophosphate

ppm parts per million
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU</td>
<td>polyurethane</td>
</tr>
<tr>
<td>RGB</td>
<td>red, green and blue</td>
</tr>
<tr>
<td>RGBY</td>
<td>red, green, blue and yellow</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>SOMO</td>
<td>singly occupied molecular orbital</td>
</tr>
<tr>
<td>SWV</td>
<td>square wave voltametry</td>
</tr>
<tr>
<td>TBACl</td>
<td>tetrabutyl ammonium chloride</td>
</tr>
<tr>
<td>TBAClO₄</td>
<td>tetrabutyl ammonium perchlorate</td>
</tr>
<tr>
<td>TD</td>
<td>time dependent calculation</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>UV-vis</td>
<td>ultraviolet-visible</td>
</tr>
<tr>
<td>V</td>
<td>volts</td>
</tr>
</tbody>
</table>
APPENDIX 2. NIH IMAGEJ ROUTINE FOR THE READING OF IMAGES FROM
COLORIMETRIC SENSOR ARRAYS.

As presented the code is written for sensor arrays displaying 80 spots in 8 columns and
10 rows (or viceversa):

```java
name=getTitle();
n_points=80;
    posx = newArray(n_points);
    posy = newArray(n_points);
    wi = newArray(n_points);
    hi = newArray(n_points);
    rv = newArray(n_points);
    gv = newArray(n_points);
    bv = newArray(n_points);

run("Rotate 90 Degrees Right");
run("Arbitrarily...", "angle=2 interpolate");
selectWindow("A");
run("8-bit");
setAutoThreshold();
setThreshold(0, 205);
run("Threshold", "thresholded remaining black");
run("Set Measurements...", " bounding display redirect=None decimal=3");
run("Set Scale...", "distance=1 known=1 pixel=1 unit=");
run("Analyze Particles...", "size=100-Infinity circularity=0.40-1.00 show=Outlines display");
rename("Selected ROIs for_"+ name);
selectWindow("A");
close();

selectWindow(name);
setTool(1);
for (i=0;i<=n_points-1;i++){
    posx[i]=getResult("BX", i);
    posy[i]=getResult("BY", i);
    wi[i]=getResult("Width", i);
    hi[i]=getResult("Height", i);
}
run("Set Measurements...", " mean redirect=None decimal=3");
run("Invert");
run("RGB Split");
selectWindow(name+" (red)");
for (i=0;i<=n_points-1;i++){
    makeOval(posx[i], posy[i], wi[i], hi[i]);
    run("Measure");
    rv[i]=round(getResult("Mean", i));
}
selectWindow("Results");
close();
selectWindow(name+" (green)");
for (i=0;i<=n_points-1;i++){
```
makeOval(posx[i], posy[i], wi[i], hi[i]);
run("Measure");
  gv[i]=round(getResult("Mean", i));
}
selectWindow("Results");
run("Close");
selectWindow(name+" (blue)";

for (i=0; i<=n_points-1; i++){
  makeOval(posx[i], posy[i], wi[i], hi[i]);
  run("Measure");
  bv[i]=round(getResult("Mean", i));
}
selectWindow("Results");
run("Close");
close();
close();
close();

print("ROI", "red", "green", "blue");
for (j=0;j<=n_points-1;j++){
  print(j+1, rv[j], gv[j], bv[j]);
}
selectWindow("Log");
saveAs("Text", "/Users/manuel/Desktop/Colorimetric Chip Read/Results for "+name);
run("Close");
selectWindow("Selected ROIs for_"+ name);
saveAs("Jpeg", "/ Selected ROIs for "+name);
}
APPENDIX 3. EXAMPLES OF SENSOR ARRAY RESPONSE VECTORS FOR DIFFERENT ANALYTES AND DETAILED MULTIVARIATE ANALYSES FROM CHAPTER IV.

Anion Analysis: Changes in the RGB values upon addition of solutions of tetrabutyl ammonium salts of each anion (200 nL, 5mM). Each profile represents the average response over 10 observations.

Acetate

![Acetate graph]

Benzoate

![Benzoate graph]

Bromide

![Bromide graph]

Chloride

![Chloride graph]
The response of the sensor array might not be completely reversible for the whole set of sensors.

\[ \text{Hydrogen Sulphate} \quad \text{Hydrogen Sulfide} \]

\[ \text{Cyanide}^a \]

\(^a\)The response of the sensor array might not be completely reversible for the whole set of sensors.
**Toothpaste Analysis:**

List of ingredients contained by the toothpastes used in this study as it appears on the product boxes. Find in bold ingredients known to be detectable by our sensor array.

<table>
<thead>
<tr>
<th>Toothpaste</th>
<th>Active Ingredient</th>
<th>Inactive Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aquafresh®</strong></td>
<td>Sodium Monofluorophosphate (15% W/V of Fluoride Ion)</td>
<td>Calcium Carbonate; Calcium Carrageenan; Cellulose Gum; D&amp;C Red #30 Lake; D&amp;C Yellow #10 Lake; FD&amp;C Blue #1 Lake; FD&amp;C Green #3; Flavor; Glycerin; Hydrated Silica; Peg-8; Sodium Benzoate; Sodium Bicarbonate; Sodium Lauryl Sulfate; Sodium Saccharin; Sorbitol; Titanium Dioxide; Water.</td>
</tr>
<tr>
<td><strong>Cavity Protection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Colgate®</strong></td>
<td>Sodium Monofluorophosphate (15% W/V of Fluoride Ion)</td>
<td>Dicalcium phosphate dehydrate, water, glycerin, sodium lauryl sulfate, cellulose gum, flavor, tetradsodium pyrophosphate, sodium saccharin.</td>
</tr>
<tr>
<td><strong>Cavity Protection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Crest®</strong></td>
<td>Sodium Fluoride (15% W/V of Fluoride Ion)</td>
<td>Sorbitol, water, hydrated silica, sodium lauryl sulfate, trisodium phosphate, flavor, sodium phosphate, cellulose gum, carbomer 956, sodium saccharin, blue 1.</td>
</tr>
<tr>
<td><strong>Cavity Protection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fluoridex®</strong></td>
<td>1.1% Neutral Sodium Fluoride</td>
<td>Cellulose Gum, D&amp;C yellow No.10, FD&amp;C blue No. 1, Flavor (Mint, Thymol and Eucalyptus Oil,) Glycerin, Mica (and) Titanium Dioxide, Poloxamer 234, Potassium Nitrate, Silica, Sodium Lauryl Sulfate, Sodium Saccharin, Sorbitol, Water, Xylitol.</td>
</tr>
<tr>
<td><strong>DailyCare</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** All ingredients are listed as they appear on the product boxes.
Examples of Sensor Array Responses at Different Analyte Concentrations.

Color changes upon addition of different concentrations of solutions of tetrabutyl ammonium salts of anions (200 nL, 0.1 – 20 mM). The experiments were made by triplicate. Isotherms were plotted using the changes of the gray pixel value of a specific RGB channel for a given sensor in the array. Each point in the graph represents the average of three measurements.
<table>
<thead>
<tr>
<th>Acetate</th>
<th><strong>Sensor 6</strong> (green channel)</th>
<th><strong>Sensor 5</strong> (green channel)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Sensor 6 (green channel) graph" /></td>
<td><img src="image2.png" alt="Sensor 5 (green channel) graph" /></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Benzoate</th>
<th><strong>Sensor 6</strong> (red channel)</th>
<th><strong>Sensor 4</strong> (blue channel)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Sensor 6 (red channel) graph" /></td>
<td><img src="image4.png" alt="Sensor 4 (blue channel) graph" /></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chloride</th>
<th><strong>Sensor 6</strong> (red channel)</th>
<th><strong>Sensor 7</strong> (green channel)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Sensor 6 (red channel) graph" /></td>
<td><img src="image6.png" alt="Sensor 7 (green channel) graph" /></td>
<td></td>
</tr>
</tbody>
</table>
Fluoride Sensor 1 (red channel)\(^a\) | Sensor 8 (green channel)
---|---
| 1 2 3 4 5 6 7 8 Sensors | ![Graph showing sensor response to fluoride](image)
| ![Sensor 1 response](image) | ![Sensor 8 response](image)

\(^a\) Biphasic behavior probably due to the deprotonation of pyrrole.\(^b\)

Dihydrogen Phosphate Sensor 5 (green channel) | Sensor 6 (red channel)
---|---
| 1 2 3 4 5 6 7 8 Sensors | ![Graph showing sensor response to dihydrogen phosphate](image)
| ![Sensor 5 response](image) | ![Sensor 6 response](image)

Pyrophosphate Sensor 3 (red channel) | Sensor 7 (green channel)
---|---
| 1 2 3 4 5 6 7 8 Sensors | ![Graph showing sensor response to pyrophosphate](image)
| ![Sensor 3 response](image) | ![Sensor 7 response](image)
### Statistical Multivariate Analysis

#### Anion Analysis PCA:

Eigenanalysis of the Correlation Matrix

<table>
<thead>
<tr>
<th>Eigenvalue</th>
<th>8.9696</th>
<th>6.4992</th>
<th>3.3443</th>
<th>1.5657</th>
<th>0.7777</th>
<th>0.5847</th>
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Hydrogen Sulfide Sensor

Sensor 3 (red channel)

Sensor 8 (blue channel)
### Toothpaste Analysis PCA:

Eigenanalysis of the Correlation Matrix

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**Toothpaste Biased with NaF Analysis PCA:**

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Toothpaste Biased with NaF Analysis HCA:
Dendrogram showing Euclidean Distance with Ward Linkage
Anion and Toothpaste Combined Analysis (General Performance of the Array) PCA:
Eigenanalysis of the Correlation Matrix

| Eigenvalue | 7.212 | 5.453 | 3.395 | 2.436 | 1.487 | 1.302 | 0.614 | 0.478 |
| Proportion | 0.300 | 0.227 | 0.141 | 0.102 | 0.062 | 0.054 | 0.026 | 0.020 |
| Cumulative | 0.300 | 0.527 | 0.771 | 0.833 | 0.887 | 0.912 | 0.932 |

| Eigenvalue | 0.301 | 0.255 | 0.223 | 0.161 | 0.139 | 0.104 | 0.085 | 0.071 |
| Proportion | 0.013 | 0.011 | 0.009 | 0.007 | 0.006 | 0.004 | 0.004 | 0.003 |
| Cumulative | 0.945 | 0.955 | 0.965 | 0.971 | 0.977 | 0.982 | 0.985 | 0.988 |

| Eigenvalue | 0.067 | 0.050 | 0.042 | 0.039 | 0.027 | 0.026 | 0.023 | 0.013 |
| Proportion | 0.003 | 0.002 | 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 |
| Cumulative | 0.991 | 0.993 | 0.995 | 0.996 | 0.997 | 0.999 | 0.999 | 1.000 |

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APPENDIX 4. EXAMPLES OF UV-VIS AND FLUORESCENCE TITRATION OF COMPOUNDS FROM CHAPTER V.

Chemosensor 5.1 with TBA•Chloride in DCM

UV-Vis Titration

Fluorescence Titration

Isotherm
Chemosensor 5.1 with TBA•Acetate in DCM
UV-Vis Titration

Fluorescence Titration

Isotherm

Isotherm
Chemosensor 5.2 with TBA•Chloride in DCM

UV-Vis Titration

Isotherm

Fluorescence Titration

Isotherm
Chemosensor 5.2 with TBA•Acetate in DCM
UV-Vis Titration

Fluorescence Titration
Chemosensor 5.3 with TBA•Chloride in DCM

**UV-Vis Titration Isotherm**

Fluorescence Titration

**Isotherm**
Chemosensor 5.3 with TBA•Acetate in DCM

UV-Vis Titration

Isotherm

Fluorescence Titration

Isotherm
Chemosensor 5.4 with TBA•Chloride in DCM

UV-Vis Titration Isotherm

Fluorescence Titration Isotherm
Chemosensor 5.4 with TBA•Acetate in DCM
UV-Vis Titration Isotherm

Fluorescence Titration Isotherm
Chemosensor 5.5 with TBA•Chloride in DCM

UV-Vis Titration

Fluorescence Titration

Isotherm

Isotherm
Chemosensor 5.5 with TBA•Acetate in DCM

UV-Vis Titration Isotherm

Fluorescence Titration Isotherm
Chemosensor 5.6 with TBA•Chloride in DCM

UV-Vis Titration Isotherm

Fluorescence Titration Isotherm
Chemosensor 5.6 with TBA•Acetate in DCM

UV-Vis Titration

Isotherm

Fluorescence Titration
Chemosensor 5.7 with TBA•Chloride in DCM

**UV-Vis Titration**

Absorbance vs. Wavelength (nm)

**Fluorescence Titration**

Fluorescent Intensity vs. Wavelength (nm)

**Isotherm**

Absorbance vs. [Cl⁻] / M

Fluorescent Intensity vs. [Cl⁻] / M
Chemosensor 5.7 with TBA•Acetate in DCM

UV-Vis Titration

Fluorescence Titration

Isotherm
APPENDIX 5. EXAMPLES OF SENSOR ARRAY RESPONSE VECTORS FOR DIFFERENT ANALYTES AND DETAILED MULTIVARIATE ANALYSIS FROM CHAPTER VI.

*Cation Analysis:* Changes in the RGYB values upon addition of solutions of chloride salts of each anion (200 nL, 1mM). Each profile represents the average response over 7 observations.

**Aluminum**

**Calcium**

**Cadmium**

**Cobalt**
Examples of Sensor Array Responses at Different Analyte Concentrations.

Fluorescence changes upon addition of different concentrations of solutions of chloride salts of cations (200 nL, the concentrations are 0mM, 0.005mM, 0.01mM, 0.05mM, 0.1mM, 0.25mM, 0.5mM, 1mM and 5mM). Isotherms were plotted using the changes of the gray pixel value of a specific RGB channel for a given sensor in the array. Each point in the graph represents the average of eight measurements.

**Aluminum (S2)**

**Calcium (S2)**

**Cadmium (S2)**

**Cadmium (S4)**
Statistical Multivariate Analysis (PCA and LDA).

Analysis Sensor Array S1-S6:

PCA:

**PCA Score Plot**

Variables (and Sensors) Contributions in the PCs

<table>
<thead>
<tr>
<th>Sensor</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
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</thead>
<tbody>
<tr>
<td>S1</td>
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<td>S2</td>
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<td>S3</td>
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Eigenanalysis of the Correlation Matrix

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<th>Eigenvalue</th>
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<tr>
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LDA:

**LDA Factor Score Plot**

Jack-knifed Classification Matrix

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<thead>
<tr>
<th>Sensor</th>
<th>Al</th>
<th>Blan pH 5</th>
<th>Ca</th>
<th>Cd</th>
<th>Co</th>
<th>Cu</th>
<th>Ga</th>
<th>Hg</th>
<th>Mg</th>
<th>Ni</th>
<th>Zn</th>
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<tbody>
<tr>
<td>S1</td>
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<tr>
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<td>S3</td>
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<tr>
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Linear Discriminant Function for Groups

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<th>Cu</th>
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<th>Zn</th>
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Sensor Array S2, S4 and S5:

### PCA:

**PCA Score Plot**

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<th>0.116</th>
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<tr>
<td>Cumulative</td>
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**Eigenvalues of the Correlation Matrix**

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<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
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**Variables (and Sensors) Contributions in the PCs**

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<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
</tr>
</thead>
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### LDA:

**LDA Factor Score Plot**

**Jack-knifed Classification Matrix**

<table>
<thead>
<tr>
<th>Al</th>
<th>Blan</th>
<th>Ca</th>
<th>Cd</th>
<th>Co</th>
<th>Cu</th>
<th>Ga</th>
<th>Hg</th>
<th>Mg</th>
<th>Ni</th>
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| N = 77 | N Correct = 77 | Proportion Correct = 1.00 |

**Linear Discriminant Function for Groups**

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Sensor Array S2 and S4:

PCA:

Eigenanalysis of the Correlation Matrix

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LDA Factor Score Plot

Jack-knifed Classification Matrix

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Total N = 77
N correct = 77
Proportion Correct = 1.000

Linear Discriminant Function for Groups

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Sensor S2:

**PCA Score Plot**

**Eigenanalysis of the Correlation Matrix**

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**LDA Factor Score Plot**

**Jack-knifed Classification Matrix**

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| N correct | 7  | 7   | 7  | 7  | 7  | 7  | 5  | 7  | 5  | 5  |
| Proportion | 1  | 1    | 1  | 1  | 1  | 1  | 1  | 1  | 0.9| 1  | 0.7|

N = 77  N Correct = 74  Proportion Correct = 0.961

**Linear Discriminant Function for Groups**

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-962  0  -7.1  -115  -133  -188  -76  -14  -177  -145  -490
-757  -4.3  -78  -404  -494  -870  -279  -148  -504  -508  -406
175  6.1  96.8  167  119  80.3  -125  8.8  500  88.8  -499
884  -11  -3  133  -50  -6.5  304  61.1  -144  -21  1142
Sensor S4:

**PCA Score Plot**

Eigenanalysis of the Correlation Matrix

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Variables (and Sensors) Contributions in the PCs

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**LDA Factor Score Plot**

Jack-knifed Classification Matrix

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<th>Hg</th>
<th>Mg</th>
<th>Ni</th>
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<tr>
<td>AI</td>
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<td>0.0</td>
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<td>Blank pH 5</td>
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<td>Co</td>
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Total N: 77
N correct: 77
Proportion Correct: 0.987

Linear Discriminant Function for Groups

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<th>Co</th>
<th>Cu</th>
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<th>Zn</th>
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<td>-6.3</td>
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<td>0.16</td>
<td>0.23</td>
<td>0.78</td>
<td>0.53</td>
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N = 77  N Correct = 77  Proportion Correct = 0.987
Enhanced Waters Analysis.

In a typical assay, enhanced waters were applied (200 nL) directly without any treatment to the sensor chip.

Enhanced waters Ingredients:

<table>
<thead>
<tr>
<th>Name</th>
<th>Ingredients</th>
<th>Cation Reported Amounts</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propel Lemon Flavor</td>
<td>• Water&lt;br&gt;• Sugar&lt;br&gt;• Sucrose syrup&lt;br&gt;• Natural lemon flavor with other Natural flavors&lt;br&gt;• Citric acid&lt;br&gt;• Sodium citrate&lt;br&gt;• Potassium citrate&lt;br&gt;• Sucralose</td>
<td>• VitaminC (ascorbic acid)&lt;br&gt;• Vitamin E acetate&lt;br&gt;• Niacinamide (vitamin B3)&lt;br&gt;• Calcium disodium EDTA&lt;br&gt;• Calcium pantothenate (vitamin B5)&lt;br&gt;• Pyridoxine HCl (vitamin B6)&lt;br&gt;• Acesulfame potassium&lt;br&gt;• Vitamin B12.</td>
<td>3.5</td>
</tr>
<tr>
<td>Propel Calcium Mixed berry</td>
<td>• Water&lt;br&gt;• Sucrose syrup, Malic acid&lt;br&gt;• Calcium citrate&lt;br&gt;• Natural and artificial flavors&lt;br&gt;• Citric acid</td>
<td>• Calcium chloride&lt;br&gt;• Sucralose niacinamide (vitamin B3)&lt;br&gt;• Calcium disodium EDTA&lt;br&gt;• Calcium pantothenate (vitamin B5)&lt;br&gt;• Pyridoxine HCl (vitamin B6)&lt;br&gt;• Acesulfame potassium&lt;br&gt;• Vitamin B12</td>
<td>Calcium 400 mg / L&lt;br&gt;Magnesium 71 mg / L&lt;br&gt;Zinc 5 mg / L</td>
</tr>
<tr>
<td>Vitaminwater Multi-v</td>
<td>• Vapor distilled, deionized, and/or reverse osmosis water&lt;br&gt;• Crystalline fructose&lt;br&gt;• Cane sugar&lt;br&gt;• Citric acid&lt;br&gt;• Calcium lactate gluconate&lt;br&gt;• Natural flavor&lt;br&gt;• Ascorbic acid (vitamin C)&lt;br&gt;• Magnesium chloride&lt;br&gt;• Gum acacia&lt;br&gt;• Zinc picolinate</td>
<td>• Ester gum&lt;br&gt;• Vitamin E acetate&lt;br&gt;• Vitamin A palmitate&lt;br&gt;• Monopotassium phosphate&lt;br&gt;• Niacin (B3)&lt;br&gt;• Pantothenic acid (B5)&lt;br&gt;• Pyridoxine hydrochloride (B6)&lt;br&gt;• Cyanocobalamin (B12)&lt;br&gt;• Folic acid</td>
<td>3.2</td>
</tr>
<tr>
<td>PowerRade Option Black-Berry</td>
<td>• Water&lt;br&gt;• High fructose corn syrup&lt;br&gt;• Citric acid&lt;br&gt;• Natural flavors&lt;br&gt;• Salt&lt;br&gt;• Potassium citrate&lt;br&gt;• Sucralose</td>
<td>• Sodium citrate&lt;br&gt;• Potassium phosphate&lt;br&gt;• Acesulfame potassium&lt;br&gt;• Niacinamide (vitamin B3)&lt;br&gt;• Pyridoxine hydrochloride (vitamin B6)&lt;br&gt;• Cyanocobalamin (vitamin B12).</td>
<td>3.1</td>
</tr>
<tr>
<td>Antioxidant Water Snapple</td>
<td>• Purified water&lt;br&gt;• Sugar&lt;br&gt;• Citric acid&lt;br&gt;• Natural flavors&lt;br&gt;• Potassium citrate (electrolyte)&lt;br&gt;• Calcium lactate (electrolyte)&lt;br&gt;• Calcium gluconate (electrolyte)&lt;br&gt;• Magnesium lactate (electrolyte)&lt;br&gt;• Modified corn starch&lt;br&gt;• Ginseng extract&lt;br&gt;• Caffeine&lt;br&gt;• Guaran seed extract&lt;br&gt;• Vegetable juices (for color)&lt;br&gt;• Acacia gum</td>
<td>• Calcium disodium EDTA&lt;br&gt;• Ribose&lt;br&gt;• Niacinamide (vitamin B3)&lt;br&gt;• Vitamin E acetate&lt;br&gt;• Calcium pantothenate (vitamin B5)&lt;br&gt;• Zinc gluconate (electrolyte)&lt;br&gt;• Pyridoxine hydrochloride (vitamin B6)&lt;br&gt;• Manganese gluconate (electrolyte)&lt;br&gt;• EGCG (epigallocatechin gallate)&lt;br&gt;• Vitamin A palmitate&lt;br&gt;• Cyanocobalamin (vitamin B12).</td>
<td>Calcium 68 mg / L&lt;br&gt;Magnesium 28 mg / L&lt;br&gt;Zinc 1.1 mg / L</td>
</tr>
<tr>
<td>Owater Lemon-Lime</td>
<td>• Purified water&lt;br&gt;• Natural lemon &amp; lime flavors&lt;br&gt;• Electrolytes:&lt;br&gt;• Calcium chloride</td>
<td>• Magnesium chloride&lt;br&gt;• Potassium bicarbonate&lt;br&gt;• Potassium sorbate (preserves natural flavor).</td>
<td>4.2</td>
</tr>
<tr>
<td>Dasani Lemon Flavor</td>
<td>• Filtered water&lt;br&gt;• Citric acid&lt;br&gt;• Natural flavors&lt;br&gt;• Potassium sorbate&lt;br&gt;• Potassium benzoate (to protect taste)</td>
<td>• Sodium citrate&lt;br&gt;• Sucralose&lt;br&gt;• Magnesium sulfate&lt;br&gt;• Acesulfame potassium&lt;br&gt;• Potassium chloride&lt;br&gt;• Salt</td>
<td>3.2</td>
</tr>
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APPENDIX 6. EXPERIMENTAL SECTION AND EXAMPLES OF SENSOR ARRAY RESPONSE VECTORS FOR DIFFERENT ANALYTES AND DETAILED MULTIVARIATE ANALYSIS FROM CHAPTER VII.

Preparation of Sub-microliter Sensor Arrays for Cation Sensing.

The sensing experiments here presented were carried out by Dr. Wang in Anzenbacher Research Group’s labs. The sensor materials were prepared by incorporating sensors 7.1-7.9 into poly(ether)urethane matrices, which were prepared by casting solutions containing the sensors in a poly(ether)urethane (Tecophilic®, SP-93A-100, Thermedics division from Lubrizol, Cleaveland, OH) THF solution (4 % w/w) onto a multi-well 10x8 (sub-microliter) size plate made by ultrasonic drilling. The concentrations of sensors 7.1-7.9 in THF vary (7.1-1 mM, 7.2-1 mM, 7.3-1 mM, 7.4-1 mM, 7.5-0.5 mM, 7.6-0.1 mM, 7.7-1 mM, 7.8-0.5 mM, 7.9-1 mM). The chloride salts of the cations were administered as aqueous solutions. In order to evaluate responses of different cations at different pHs, the concentration of cation solution was 1 mM. pH was adjusted in solution by addition of NaOH (0.01 M) or HCl (0.01 M utilizing a Titrator T50 (Mettler Toledo Co.) with an accuracy of pH ±0.1 and the solutions were used immediately after preparation. Mineral water analysis was carried-out by applying 200 nL (directly from the bottle) to the array elements.

Fluorescence Sensor Array Image Acquisition and Data Processing.

Images from the sensor arrays are recorded using a Kodak Image Station 440CF. The scanned images (12 bit) are acquired with a resolution of 433 x 441 pixels per inch and with grey levels over 1000 (12 second exposures). The sensor arrays are excited with a broadband UV lamp (300-400 nm, λ_max=330 and 365 nm) and up to four channels are used for emission detection: (1) Blue:
band pass filter 380-500 nm $\lambda_{\text{max}}=435$ nm, (2) Green: band pass filter 480-600 nm $\lambda_{\text{max}}=525$ nm, (3) Yellow: long pass filter 523 nm, (4) Red: long pass filter 580 nm. In order to generate a false color representation, images obtained using blue, green and red filters were merged in equal proportion using NIH ImageJ software. The RGB triplet is assigned to correspond with the color of the filter used. After acquiring the images, the integrated (non zero) grey pixel ($n$) value is calculated for each well of each channel. Images of the sensor chip were recorded before ($b$) and after ($a$) the addition of an analyte. The final responses ($R$) were evaluated as follows.

$$R = \sum_{n} \frac{a_n}{b_n} - 1$$

---

*Cation analysis and quantitative analysis at pH 7:*

Changes in the RGYB values upon addition of solutions of chloride salts of each anion (200 nL, 1mM). Each profile represents the average response over 7 observations.
Aluminum

Cadmium
Calcium

Cobalt
Copper

Mg^2+ mM

Relative Intensity (a.u.)

Relative Peak value (Red Channel)

Magnesium

Mg^2+ mM

Relative Intensity (a.u.)

Relative Peak value (Green Channel)

Relative Peak value (Yellow Channel)
Mercury

![Graph for Mercury]

Nickel

![Graph for Nickel]
Zinc

Gallium
Cation analysis at pH 6:

Aluminum

Cadmium

Calcium

Cobalt

Copper

Magnesium
Cation analysis at pH 5:
Dynamic Ranges of cations with different sensors at pH 7:

<table>
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<tr>
<th>Cations</th>
<th>Dynamic Range</th>
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<td>Al$^{3+}$</td>
<td>S1: 10—1000 uM</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>S3: 15—5000 uM</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>S3: 200—5000 uM</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>S6: 35—1000 uM</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>S3: 200—500 uM</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>S1: 35—2000 uM</td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>S3: 50—2000 uM</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>S1: 30—2000 uM</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>S3: 20—5000 uM</td>
</tr>
<tr>
<td>Ga$^{3+}$</td>
<td>S1: 5—5000 uM</td>
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Mineral waters content as reported on a bottle:

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<td>-</td>
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<td>0944TG040382</td>
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<tr>
<td>Bonaqua</td>
<td>$\text{Na}^+$ 2.5 mg/L, $\text{Ca}^{2+}$ 62.1 mg/L, $\text{K}^+$ 0.6 mg/L, $\text{Mg}^{2+}$ 41 mg/L</td>
<td>6.1</td>
<td>12.10.2005 L12.04.05LN23:12</td>
</tr>
<tr>
<td>Gerolsteiner</td>
<td>$\text{Li}^+$ 0.13 mg/L, $\text{K}^+$ 10.8 mg/L, $\text{Mg}^{2+}$ 108 mg/L, $\text{Ba}^{2+}$ 0.014 mg/L, $\text{Sr}^{2+}$ 2.9 mg/L, $\text{Mn}^{2+}$ 0.39 mg/L</td>
<td>6.6</td>
<td>NOV 2005</td>
</tr>
<tr>
<td>Fiji</td>
<td>$\text{Ca}^{2+}$ 17 mg/L $\text{Mg}^{2+}$ 13 mg/L</td>
<td>7.5</td>
<td>01 08 07 19:00</td>
</tr>
<tr>
<td>Biovive</td>
<td>$\text{Na}^+$ 18.6 mg/L, $\text{Ca}^{2+}$ 42 mg/L, $\text{K}^+$ 2.4 mg/L, $\text{Mg}^{2+}$ 3.8 mg/L</td>
<td>7.5</td>
<td>28:05:2007 12:27</td>
</tr>
<tr>
<td>Magnesia</td>
<td>$\text{Na}^+$ 5.4 mg/L, $\text{Mg}^{2+}$ 234 mg/L, $\text{Ca}^{2+}$ 37.6 mg/L, $\text{Mn}^{2+}$ 0.2 mg/L</td>
<td>7.0</td>
<td>20.01.2004 16:14</td>
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<tr>
<td>Naleczowianka</td>
<td>$\text{Na}^+$ 12.7 mg/L, $\text{Ca}^{2+}$ 114.5 mg/L, $\text{Zn}^{2+}$ &lt; 0.03 mg/L, $\text{Pb}^{2+}$ &lt; 0.006 mg/L, $\text{Mn}^{2+}$ &lt; 0.05 mg/L</td>
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<td>Perrier</td>
<td>$\text{Na}^+$ 11.5 mg/L, $\text{Mg}^{2+}$ 7 mg/L</td>
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<tr>
<td>Evian</td>
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<td>7.4</td>
<td>10 08 07 19:29</td>
</tr>
<tr>
<td>Evian - A</td>
<td>-</td>
<td>7.3</td>
<td>09 11 07 19:51</td>
</tr>
<tr>
<td>Evian - B</td>
<td>-</td>
<td>7.3</td>
<td>01 26 08 16:55</td>
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<td>Evian - C</td>
<td>-</td>
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<td>09 29 07 17:23</td>
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* Information about mineral content was obtained from the manufacturers.
Mineral waters analysis:

In a typical assay, mineral waters and other waters were applied (200 nL) directly without any treatment to the sensor chip.

Aquafina

Bonaqua

Gerolsteiner

Fiji

Biovive

Magnesia
APPENDIX 7. PREPARATION AND CHARACTERIZATION AND EXAMPLES OF
UV-VIS TITRATIONS OF COMPOUNDS FROM CHAPTER IX

Synthesis of Compounds 9.1, 9.2, and 9.3:

The following compounds were prepared originally by Dr. Pavel Anzenbacher and synthesized later following the described procedure.

2,3-di(1H-pyrrol-2-yl)naphtho[2,3-f]quinoxaline-7,12-dione (9.1) was synthesized by condensation of 1,2-diaminoanthracene-9,10-dione (commercially available) and di-(1H-2-pyrrolyl)-ethanedione in acetic acid as described in the literature.\(^1\) M.p. >260 °C. \(^1\)H NMR (DMSO-\(d_6\), \(\delta\)): 6.20 (ddd, 1H, \(J=2.4, 3.7, 4.6\) Hz), 6.23 (ddd, 1H, \(J=2.6, 3.7, 4.9\) Hz), 6.52 (ddd, 1H, \(J=1.5, 2.3, 3.7\) Hz), 6.65 (ddd, 1H, \(J=1.5, 2.4, 3.7\) Hz), 7.11 (ddd, 1H, \(J=1.5, 2.4, 4.6\) Hz), 7.15 (ddd, 1H, \(J=1.5, 2.6, 4.1\)), 7.88-7.99 (m, 2H), 8.18-8.22 (m, 2H), 8.25 (d, 1H, \(J=8.7\)), 8.40 (d, 1H, \(J=8.7\)), 11.14 (brs, 1H), 11.86 (brs, 1H). \(^{13}\)C APT NMR (DMSO-\(d_6\), \(\delta\)): 109.44 CH, 109.44 CH, 113.07 CH, 114.02 CH, 123.07 CH, 123.75 CH, 125.40 CH, 126.17 CH, 126.47 CH, 128.05 C, 128.17 C, 128.57 C, 131.91 C, 133.44 CH, 133.71 C, 133.77 CH, 134.68 CH, 134.79 C, 136.73 C, 142.12 C, 144.84 C, 145.84 C, 182.48 C, 182.82 C. EI/MS (70 eV): 390 (100) [M\(^+\)].

2,3-di(1H-pyrrol-2-yl)benzo[g]quinoxaline-5,10-dione (9.2) was synthesized by condensation of 2,3-diaminonaphthalene-1,4-dione and di-(1H-2-pyrrolyl)-ethanedione in acetic acid as described in the literature.\(^1\) 2,3-diaminonaphthalene-1,4-dione was synthesized from 2,3-dichloronaphthalene-1,4-dione as described in the literature.\(^2\) M.p. >260 °C. \(^1\)H NMR (DMSO-\(d_6\), \(\delta\)): 6.20 (ddd, 2H, \(J=2.6, 3.8, 4.9\)), 6.60 (ddd, 2H, \(J=1.5, 2.4, 3.8\) Hz), 7.09 (ddd, 2H, \(J=1.5, 2.6, 4.3\) Hz), 7.49 (m, 2H, AA’XX’), 8.23 (m, 2H, AA’XX’), 11.66 (brs, 2H). \(^{13}\)C APT NMR
(DMSO-$d_6$, δ): 109.71 CH, 113.36 CH, 123.80 CH, 126.68 CH, 127.75 C, 133.15 C, 134.45 C, 140.39 C, 145.76 C, 180.87 C. EI/MS (70 eV): 340 (100) [M$^+$.]

1,2-bis(tosylamino)anthracene-9,10-dione Compound (9.3) was synthesized from 1,2-diaminoanthracene-9,10-dione (commercial available) and tosyl chloride in ethanol as described in the literature.$^3$ M.p. = 247 °C. $^1$H NMR (DMSO-$d_6$, δ): 2.47 (s, 6H), 6.88 (d, 1H, $J = 8.0$ Hz), 7.35 (d, 1H, $J = 8.0$ Hz), 7.50 (m, 4H), 7.72 (m, 4H), 7.86-7.96 (m, 2H), 8.15 (ddd, 1H, $J = 8.3, 1.7, 0.8$ Hz), 8.23 (ddd, 1H, $J = 7.3, 1.4, 0.8$ Hz). $^{13}$C APT NMR (CDCl$_3$, δ): 21.77 CH$_3$, 115.30 CH, 115.56 C, 125.70 C, 126.89 CH, 127.05 CH, 129.09 CH, 132.84 C, 133.49 CH, 134.28 CH, 134.63 C, 135.82 C, 138.18 CH, 145.89 C, 150.57 C, 182.97 C, 184.96 C. MALDI-TOF: 547 [M+1]$^+$.  

**Anion Binding Studies by UV-vis titrations**

The following titration procedures were originally carried out by Karolina Jursicova at Anzenbacher Research Group.

A typical UV-vis titration was carried out in dry acetonitrile without and in the presence of TBAClO$_4$ as a supporting electrolyte used for electrochemical studies. The concentration of the chemosensor was 25 mM and 50 mM, respectively. Hydrated tetrabutylammonium (TBA) salts of the anions were used in this study: fluoride (x6H$_2$O), cyanide (x6H$_2$O), chloride, phosphate (x2H$_2$O), and pyrophosphate (x2H$_2$O). The degree of hydration was estimated from elemental analyses. The supporting electrolyte was TBAClO$_4$.

Data fitting was performed using a quadratic equation for 1:1 binding model

$$
\Delta A = \frac{\Delta A_{\text{max}} KL}{1 + KL}
$$
L = \frac{K[Anion] - K[Sensor] - 1}{2K} + \frac{\sqrt{(K[Sensor] - K[Anion] + 1)^2 + 4K[Anion]}}{2K}

where \( L \) is a corrected anion concentration, \( A_{max} \) is the maximum absorbance change, \([Sensor]=\) [Chemosensor] and \([Anion]\) are respective concentrations, and \( K \) is a binding constant.

The fitting errors were <15%. The 1:1 stoichiometry for the binding events was confirmed by Job plots and selected examples are shown below.

**Examples of UV-vis titration data**

**Sensor 9.1 and Pyrophosphate**
Changes in absorption spectra of the sensor **9.1** (25 \( \mu \)M) upon the addition of pyrophosphate (0-200 \( \mu \)M).

**Sensor 9.1 and Acetate**
Changes in absorption spectra of the sensor **9.1** (25 \( \mu \)M) upon the addition of pyrophosphate (0-12 mM).

\( K_a = 316 \, 123 \pm 35 \, 761 \, M^{-1} \)

\( K_a = 1 \, 200 \pm 54 \, M^{-1} \)
**Sensor 9.2 and Acetate**
Changes in absorption spectra of the sensor 9.2 (25 μM) upon the addition of pyrophosphate (0-9 mM).

512 nm
\[ K_a = 5200 \pm 541 \text{ M}^{-1} \]

**Sensor 9.2 and Benzoate**
Changes in absorption spectra of the sensor 9.2 (25 μM) upon the addition of pyrophosphate (0-60 mM).

512 nm
\[ K_a = 400 \pm 28 \text{ M}^{-1} \]

**Sensor 9.3 and Cyanide**
Changes in absorption spectra of the sensor 9.3 (25 μM) upon the addition of cyanide (0-4.0 mM).

551 nm
\[ K_a = 11900 \pm 2500 \text{ M}^{-1} \]

**Sensor 9.3 and Fluoride**
Changes in absorption spectra of the sensor 9.3 (25 μM) upon the addition of cyanide (0-3.7 mM).

464 nm
\[ K_a = 3.4049 \times 10^{17} \pm 1.7837 \times 10^{16} \text{ M}^{-1} \]
References


