Optical Sensors for Blood Analytes

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Introduction

Carrier-based ion-selective electrode (ISE) sensors are widely used for the selective detection of ionic species in a range of applications, including diagnostic medicine, environmental monitoring and process control. These sensors usually consist of a highly viscous ion-sensitive liquid membrane (such as PVC) that separates an aqueous sample containing the primary ion from an aqueous-based internal electrolyte. The membrane contains an ionophore that acts as a selective ion transporter or carrier and a lipophilic salt that acts as a charge exchanger. Changes in activity (concentration) of the primary ion in the sample solution result in, ideally, Nernstian changes in the potential at the surface of the membrane. Advantages of ISE’s over other sensors include high selectivity which is imparted by the ionophore and the other membrane components and, since the response to the primary ion is a change in surface potential, very rapid response. However, fabrication of ISE’s is problematic due to the need for compatibility between ionophore, membrane and the other components and in the final application, e.g., diagnostic instruments for blood analysis, ISE’s require continual calibration and utilize expensive electronics.

There is now a concerted industry effort to develop instrumentation, particularly for clinical applications, that is based on bulk optical sensors or bulk optodes. In these systems, an ionophore and a chromophore are deposited in a bulk polymer membrane and the interaction between these two components provides an optical signal upon complexation. Sensing relies on diffusion of the ion through the bulk polymer to the ionophore followed by chromophore response to complexation. While bulk optodes suffer from slower response times due to diffusion, their use can decrease both the chemical complexity of the sensor and the electronic complexity of the final sensing instrument. Also, unlike ISE’s, bulk optodes are not limited to a single physical configuration or shape, but rather can be tailored for specific applications or instrument designs.

Arguably, the most important component of either ISE’s or bulk optodes is the ionophore or ion complexation site, since it is this structure that to a large degree imparts the sensitivity and selectivity to the eventual sensor. In bulk optode sensors an additional component is required (the chromophore) to provide the optical signal for ion complexation. For the detection of cationic analytes, a wide variety of neutral or charged ionophores coupled with charged or neutral chromophores have been investigated. The operation of these systems depends on maintaining bulk neutrality or balance of charge in the bulk and this is usually achieved by the migration of protons into and out of the membrane in response to the movement of the primary ion. It is this protonation/deprotonation equilibrium that also often provides the optical response for ion complexation. For example, extraction of a cation from solution into the ionophoric site in the membrane will result in deprotonation of a chromophore in the membrane and in the process change its absorption, fluorescence or some other optical characteristic. One drawback of such a mechanism, however, is that sensors fabricated from such systems are obviously sensitive to pH in addition to the primary ion and measurements must be adjusted for changes in pH from sample to sample. Another approach is the incorporation

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From the Executive Director

D. C. Neckers, Executive Director, Center for Photochemical Sciences, Bowling Green State University

Mike Rodgers and I have had a number of conversations since September 11 about the vulnerability of countries and societies that are dependent on energy. The United States is exceptionally vulnerable because we rely on automobiles, which run on fuels derived from processed petroleum. America is not the only nation in this position. European countries, to a greater or lesser degree, also count on oil obtained mostly from the Middle East.

The automobile is not the only consumer of energies derived from fossil fuels. In the US many power generator plants consume large quantities of both oil and coal. I saw an estimate recently suggesting that 10% of the US power consumption is spent on energy for lighting. This sounds ridiculously high at first glance, but drive through any American industrial park near any community of any size and see how many buildings are fully lighted—offices included—in the middle of the night. We waste a lot of energy in America. We are not alone. The lights don’t often go out in Red Square either.

Americans would feel a lot safer at night if the United States were energy independent. Our job as scientists is to convince our political leadership that we need to achieve this energy independence. It will, however, take a major national commitment and more importantly, the necessary resources to accomplish energy independence in a decade or less. We could realize this, I’m sure, if our leadership makes it possible. After all, switching from incandescent lighting to LEDs and OLEDs would cut the America’s collective lighting bill by about 90% according to some estimates I’ve read.

Our Bowling Green conversations coincidentally coincided with an opinion piece written recently for The Blade, Toledo, Ohio, by Al Compaan. Professor Compaan is a member of the Advisory Board of the Center for Photochemical Sciences and leads a thin film photovoltaics effort in the Physics Department at our neighbors to the north, the University of Toledo. Compaan is closely connected to the former Solar Cells, Inc., now Northstar Solar, a company founded by long-term advisory board member and benefactor Harold McMaster. Compaan’s article is reprinted here in entirety and adds some interesting insights to the energy issues.

I’m interested in hearing from our readers—what do you think? How long would it take the US to become an energy independent society? What observations do you have about this that we can share with our political leaders? Where can the most savings be affected the most quickly? Let me know and I’ll publish your ideas in a future Spectrum.
It’s Time to Reassess Long-term Use of Energy

Alvin D. Compaan, University of Toledo
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9-11.

We’ve responded to the immediate shock. We have given blood, donated money, offered words of comfort, prayed for the loved ones of those killed and injured. The bombs are falling in retribution.

But what should be our longer-term response? How do we get at the roots of terrorism and guarantee our security? Many foreign policy issues appropriately are being re-examined, including economic and military aid. However, I would like to discuss here an internal, national response that can allow us to seize the ethical high ground, to be proactive rather than reactive, and to identify actions that can facilitate our own personal and national healing.

One of our greatest opportunities for effecting positive, long-term change is to re-evaluate our use of energy. Energy for transportation, energy for electricity generation, energy for manufacturing, energy for heating and lighting our living and working spaces.

Energy use is not the whole story, of course, but there is at least an indirect connection to terrorism. It is no accident that many terrorist groups are rooted in the Middle East, which just happens to be the source of much of the petroleum driving our economy.

Our insatiable thirst for foreign oil costs $200,000 per minute! Two and a half minutes of this addiction could have financed the estimated $500,000 it cost the terrorists to launch the Sept. 11 attacks. The longer we continue this transfer of wealth to the oligarchies, monarchies, and dictatorships of many of the oil-producing countries, the more intractable terrorism becomes. We are a society profligate in our use of energy.

And because we waste so much, we are constantly seeking more oil - from the Alaska National Wildlife Refuge, from our Gulf Coast, from Saudi Arabia, Kuwait, even Papua New Guinea.

But petroleum supplies are finite; ANWR only buys a few more months. Petroleum imports account for most of our balance of payments deficits. Many argue that, with no viable challenge to our military strength, the main reason for keeping a large military is to be able to guarantee access to international oil supplies.

We must renew our commitment to sustainable energy practices that once, briefly, became a major concern of the U.S. After the dual oil price shocks of 1973 and 1978, we and the other industrialized nations became much more efficient in the use of energy.

Homes were reinsulated; corporate average fuel efficiency (CAFE) standards led to doubling of auto fuel efficiency to about 25 mpg; refrigerators today are bigger but more than twice as efficient as they were in the 1960s. However, as gas prices came down, we lost our focus.

The average fuel economy of today’s cars and light trucks, vans, and SUVs is the lowest in 20 years! Not because Detroit (and Toledo) built less efficient engines and transmissions, but because we individually chose to buy fewer sedans and roadsters, and ever-larger vans and SUVs.

We can immediately reverse the slide in CAFE by buying the more fuel-efficient vehicles already on the market. For the longer term we must challenge our governmental representatives to set high standards for the automakers to build lighter, still more fuel-efficient autos. The technology exists for safer and more efficient cars whose fuel savings will largely offset the extra purchase price. The technology exists to raise CAFE standards, not the measly 1 mpg of currently proposed legislation but to 40 mpg by 2010 and to 55 mpg a few years later. These goals need not cause disruptions if reasonable lead time is provided, but we must act now. It might require an increase in gas taxes to provide incentives, but I think we are all prepared to adjust our lives a bit in honor of those who lost theirs on Sept. 11.

Major long-term progress will require exploiting the exciting new technologies, such as hybrids and fuel cell/electrics, that will not only yield high efficiency but allow operation totally free from fossil fuels.

Renewable sources of electricity such as hydro, wind, geothermal, solar, which are available in abundance, can be used to generate the needed hydrogen from water. When the hydrogen is used in fuel cells or turbines or piston
engines the product is clean H2O and the recycling is complete. Radical? Not at all. Already wind generated and hydro-generated electricity is cheaper than coal-generated and much cheaper than electricity from fuel oil and natural gas.

Solar electric power (photovoltaic) costs are headed down but already provide power cheaper than many peak load generators do. And when the costs of distribution are included, rooftop PV looks even better!

History suggests that the move to a sustainable economy free from heavy dependence on carbon-based fuels may actually be inevitable. Energy production trends since the 1800s show that from primary dependence on wood, the industrialized countries moved primarily to coal by 1900, to oil by 1950.

This 50-year cycle of transitions is now due for another renewal. From another perspective, the carbon fraction of our energy sources from 1850 onward shows a steady decline through the present as the hydrogen content increases.

A renewables-dominated source for hydrogen-powered electricity can bring the carbon intensity almost to zero. For such stewardship of natural resources there is an unexpected bonus that is particularly meaningful in the post WTC era.

Wind and solar are universal resources distributed around the globe without regard to political and ethnic considerations. Renewables are great equalizers among nations.

Furthermore, with electricity production distributed across neighborhood rooftops and modest-sized generating fields, the need is decreased for ugly and vulnerable high voltage transmission lines, the tempting targets presented by giant, thousand megawatt nuclear and coal generating stations, and massive, polluting oil refineries.

Other bonuses accrue. Distributed generation can lead to more reliable and “cleaner” electricity free of voltage glitches that can damage sensitive microelectronics.

Best of all, less coal and gasoline combustion will reduce not only carbon dioxide but also sulfur dioxide, acid rain, particulates, and radioactive emissions.

Interestingly, the Toledo-Detroit region already hosts several businesses and universities that are leaders in the development of such alternative power sources for transportation and electricity generation.

Now is the time for us to step up to the challenge. As consumers, we have the power to start the changes immediately; we need not wait for new government standards or mandates; these can come later.

By taking such individual, active responses we can honor the memory of 9-11.

We have the means as a nation to set a compelling example for the world to emulate and in the process help provide long-term security for ourselves.

But do we have the will?
of chromophore and ionophore into one molecule, a chromoionophore. While charge balance and the migration of protons into and out of the membrane are still of importance in bulk optodes that incorporate such molecules, the chromoionophore itself can be designed to be immune or less sensitive to protonation making its optical response dependent directly on binding of the primary ion. Charge balance in such cases is maintained primarily by extraction of both the primary cation and a counter anion.

Over the past several years, there has been explosive growth in the development of chromoionophores based on a variety of optical effects. A comprehensive review of such systems has been published by de Silva et al.\(^2\) The preponderance of recent work has centered on the incorporation of an ion-selective binding site (ionophore) into a chromophore such that the electrostatic effect of ion complexation affects fluorescence emission. Thus, much effort has been spent on the covalent linking of ion-specific ligands with electron transfer-sensitive chromophores, and in particular, systems have been chosen that deliver an “off-on” switching effect. This mechanism depends on the disruption of an internal electron transfer quenching process in which the singlet excited state of the chromophore is quenched by electron transfer from an electron donating group in the chromoionophore. In the absence of complexed cation, the molecule emits little or no fluorescence, the amount depending on the relative magnitudes of the rate constants for fluorescence and electron transfer. Upon complexation, the electrostatic field of the cation makes electron transfer to the chromophore less efficient and leads to enhanced fluorescence emission, in effect turning the electron transfer off and turning the fluorescence on.

Over the past 18 months we have become involved in the development of optical sensors for the detection of a variety of cationic species in whole blood for diagnostic purposes. Specifically we have been interested in sensors for Na\(^+\), K\(^+\) and NH\(_4^+\), the latter being a marker for urea and various metabolites. Our research in this area spans a range of activities including the design of ionophore/chromophore systems and theoretical studies of ion docking energies, the synthesis and testing of optical response in solution, and the fabrication and testing of optodes. Below we outline some of the criteria that we have considered in the fabrication and testing of these sensors and discuss the results for two specific sensors.

**Sensor Design Considerations and Results**

The design of bulk optodes involves several important considerations, including the sensitivity/selectivity of the ionophoric site; the fluorophore and its photophysics and its sensitivity to internal electron transfer; the tether or linker between ionophore and chromophore in relation to both the synthetic feasibility of the target molecules and the efficiency of electron transfer; and the choice of bulk membrane material and its compatibility with the chromoionophore.

**Ionophores**

The literature is profuse with examples of both natural and synthetic ionophores\(^1\) that provide a wide range of desirable characteristics for optode fabrication. In our studies, the important characteristics include: high selectivity of the complexation site for the ion of interest; ease of synthetic coupling of the ionophore and chromophore; and the incorporation of an electron donor site into the molecule that can be oxidized by the excited chromophore.

Ionophore selectivity can be discussed in terms of the thermodynamic stability of the metal ion–ionophore complex, where more stable complexes are indicative of more selective interactions.\(^3\) Thermodynamic stability in these systems is a complex combination of enthalpic and entropic parameters that can be understood in part, by considering steric size-fit parameters and pre-organization of the ionophore. Table 1 gives a selection of thermodynamic values for alkali metal ion binding to a series of crown ethers as reported by Bradshaw and co-workers.\(^3\) In each case it is clear that complexation is an enthalpically favorable process and that there may be a modest correlation between the enthalpy of complexation and the size match between the ion and ionophore. Thus when the size matches closely, the metal ion is centered within the cavity and experiences the most symmetrical electrostatic interactions. A smaller ion will experience a proportionally smaller electrostatic interaction and thus will not be as tightly bound. Conversely an ion that is larger cannot fit within the pocket symmetrically and thus will not benefit from all of the electrostatic stabilizing interactions available from the ionophore.
Table 1. Thermodynamic values for alkali metal ion binding to a series of crown ethers as reported by Bradshaw and co-workers.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Cavity (pm)</th>
<th>Metal ion</th>
<th>Cation radius (pm)</th>
<th>-ΔG (kJ/mol)</th>
<th>-ΔH (kJ/mol)</th>
<th>TAS (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-crown-5</td>
<td>86-92</td>
<td>Na</td>
<td>95</td>
<td>19.7</td>
<td>20.9</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K</td>
<td>133</td>
<td>21.3</td>
<td>32.2</td>
<td>-10.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cs</td>
<td>169</td>
<td>12.6</td>
<td>49.0</td>
<td>-36.4</td>
</tr>
<tr>
<td>18-crown-6</td>
<td>134-143</td>
<td>Na</td>
<td>95</td>
<td>25.1</td>
<td>31.4</td>
<td>-6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K</td>
<td>133</td>
<td>34.7</td>
<td>56.1</td>
<td>-21.3</td>
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<tr>
<td></td>
<td></td>
<td>Rb</td>
<td>148</td>
<td>30.6</td>
<td>50.7</td>
<td>-20.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cs</td>
<td>169</td>
<td>27.2</td>
<td>47.3</td>
<td>-20.1</td>
</tr>
<tr>
<td>21-crown-7</td>
<td>~170</td>
<td>Na</td>
<td>95</td>
<td>10.0</td>
<td>43.5</td>
<td>-33.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K</td>
<td>133</td>
<td>24.3</td>
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<td>Rb</td>
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<td></td>
<td></td>
<td>Cs</td>
<td>169</td>
<td>28.5</td>
<td>46.9</td>
<td>-18.4</td>
</tr>
</tbody>
</table>

It is also clear from the data in Table 1 that the overall thermodynamic stability of the complexes, as indicated by the free energy, is not solely dependent on enthalpic factors. Rather, in each case there are significant, and likely, multiple entropic costs of complexation that contribute to decreased stability. For the ion alone, there are both favorable and unfavorable entropy changes that occur. Upon binding the ion undergoes an entropically-favorable loss of its solvation shell(s) but at the same time loses overall entropy, including a significant amount of translational entropy upon docking with the ionophore. Complexation also involves a decrease in entropy of the ionophore since it loses conformational freedom in the bound state. This overall entropic cost can be minimized however, and the resulting complex can be made more stable if the complexed and uncomplexed ionophore conformations are similar, i.e. if the ionophore conformation is pre-organized. A prime example of this effect is the natural antibiotic valinomycin in which six amide linkages force the depsipeptide structure into a pre-organized conformation through intramolecular hydrogen bonding. This leaves the six carboxyl carbonyls free to electrostatically complex K⁺ in an octahedral arrangement. (Figure 1)

From these considerations, it seems desirable for our work that suitable ionophores for Na⁺ and K⁺ should possess a pre-organized conformation and a suitably-sized pocket for the ion of interest. An additional factor is that the ionophore must have an available attachment site for the chromophore and also incorporate an electron donor site. Also, it should be sufficiently hydrophobic/lipophilic so as to not be extracted from the sensor during testing. (See Optode Fabrication on page 9.)

A general class of compounds that possesses such attributes are container compounds such as calixarenes and calixarene-crown ethers. There are numerous reviews on these compounds and as a result, their structure and some of their complexation behavior are fairly well understood. These calixarene molecules possess a pre-organized structure because the phenyl groups form a semi-rigid cone shape. In addition, various substituents may be placed along the lower rim to enhance complexation and thus increase selectivity. In comparison to monensin esters, which are traditional ISE Na⁺ ionophores (log $K_{Na,K}^{pot}$ -0.7), and ETH 2120 (log $K_{Na,K}^{pot}$ -1.5), some
very simple calix[4]arenes such as p-octylcalix[4]arene tetramethylester and p-tert-butylcalix[4]arene tetraethyl ester are able to attain higher selectivities, \( \log K_{\text{pot}} \) for \( \text{Na}^+ \), -2.5 and -3.1, respectively. Moreover, many unsubstituted calixarenes are commercially available allowing for simplified synthesis and providing convenient attachment points for chromophores.

Calixarene-based counterparts for \( \text{K}^+ \) are also well known. One of the most promising is the 1,3-alternate calix[4]arencrown-5. Although the molecule possesses the basic calix[4]arene structure, the addition of the crown-5 moiety creates a fixed pocket that is now too large to stabilize \( \text{Na}^+ \), thereby illustrating the importance of size-fit interactions. In comparison to the industry standard, valinomycin, an enhanced selectivity was found for this calixcrown, \( \log K_{\text{pot}} \) for \( \text{K}^+, \text{Na}^+ \) and \( \text{K}^+ \), -3.9 vs. -4.2, respectively. The calixcrown ethers also offer several other attractive characteristics. First, the synthetic flexibility offered by these structures allows the incorporation of a variety of chromophores. Also, electron donor groups can be incorporated into the crown structure in positions that are potentially in close proximity to both the ion docking site and the chromophore. For example the phenyl group in the crown structure of calix-benzocrown ethers is comparable to 1,2-dialkoxybenzenes in its electron donor behavior.8

Fluorophores

The choice of fluorophore depends first of all on the transduction mechanism that will be employed in the optode. We have chosen to utilize the “off-on” fluorescence emission mechanism (photoinduced electron transfer (PET) mechanism) of signal transduction.2 The most important considerations for this mechanism are the thermodynamics of the electron transfer process and its rate relative to other deactivation processes, particularly fluorescence. A thermodynamic prediction of the feasibility of electron transfer can be made by calculating the free energy of the process using the Rehm-Weller equation.

The “off-on” mechanism is illustrated by 9-anthryl-azacrown-5 in which 9-methyl anthracene is covalently-linked to the secondary amine of the azacrown moiety. The free energy change for electron transfer in this molecule is \( \Delta G_{\text{PET}} = -0.41 \text{ eV} \). Upon complexation of \( \text{K}^+ \) cations a 40 fold increase in the fluorescence quantum yield was observed. The anthryl chromophore also satisfies other requirements for a usable optode, namely a large extinction coefficient in a (possibly) convenient region of the spectrum \( (\varepsilon_{360} \sim 7000 \text{ M}^{-1} \text{cm}^{-1}) \) and a high fluorescence quantum yield, absent any electron transfer quenching \( (\phi_{\text{fl}} = .30) \). In addition, anthracene is available in a large number of substituted forms allowing the use of different synthetic linking strategies.

Based on these ionophore and chromophore considerations, we have assembled a prototype chromoionophore, \( I \), (see Scheme 1 for synthetic details) which consists of a 1,3-alternate calix[4]arene structure coupled with an azacrown ether and a methyl anthracene chromophore. Compound \( I \) was subjected to computational analysis with emphasis on determining the changes in charge density that occur as a result of complexation. Figure 2(a) and 2(b) shows the charge density in \( I \) before and after complexation. Clearly, these calculations indicate that there is a significant redistribution in charge density in the calix-azacrown binding pocket following ion binding. The results for \( I \) in solution have been very promising from both sensitivity and selectivity standpoints and fabrication of \( I \) into an optode has also yielded positive results. The preliminary solution phase results follow.
However, the utility of I in a practical sensor application may be limited because 350-400 nm light emitting diodes that would be required for photoexcitation of the anthryl group in the sensor are, at this point, still quite expensive. For this reason we have also investigated other possible chromophores. Rhodamines, for example, can be photoexcited at wavelengths greater than 540 nm where many inexpensive LED’s emit, they have high extinction coefficients ($> 1 \times 10^4$ M$^{-1}$ cm$^{-1}$) and reasonable quantum yields ($\Phi_{fl} \sim .60 - .90$). In addition, like anthracenes, rhodamines are available with a wide range of substituents making linking strategies more straightforward. For example, in synthesizing II, (see Scheme 2) we chose the mixed 5,6 isomer of amino rhodamine B because the free primary amine allows linking to the ionophore through an amide bond. In this case electron transfer quenching is also provided by the amide linkage created. This molecule nicely points out the trade-offs between excitation wavelength, chromophore choice, electron donor choice and synthetic ease. For example, while linking the chromophore and ionophore in II is synthetically simple, the conversion of amine to amide increases the oxidation potential. When combined with the lowered excited state energy of rhodamine (compared to anthracene), spontaneous electron transfer demands a significantly lower reduction potential for the chromophore. Fortunately, such is the case for rhodamine and the free energy for electron transfer is $\Delta G_{PET} = -0.128$. However, this is obviously not always the case. For example, whereas the azacrown/anthracene donor/acceptor pair provides a thermodynamically favorable driving force for electron transfer, the benzocrown/anthracene pair does not. For benzocrown systems, cyanoanthracene with its significantly less negative reduction potential compared to anthracene has been utilized. Results for II (shown on page 10) were quite encouraging in an optode configuration.
**Linkers**

The linkers we have used in the two systems we describe here were chosen primarily for the availability of synthetic precursors and ease of synthesis. Because these are prototypes, we have not paid particular attention to the linkers and their possible effects on the “off-on” mechanism. However, in future versions of these chromoionophores, in which we will be attempting to optimize their optical response, linker length and composition will be an important consideration. Some work has already been reported on the effect of linker length on the “off-on” fluorescence mechanism.

**Optode Fabrication**

The polymer membrane is also crucial to the functioning of the optode. In particular it is desirable to use a matrix that is sufficiently hydrophobic so as to insure the long-term mechanical stability of the membrane in aqueous environments. However, it must also be sufficiently hydrophilic to allow for water uptake and ion transport from the aqueous phase into the organic phase of the membrane. In addition, the membrane must be optically transparent at the excitation and emission wavelengths of the chromophore. Copolymers of acryl amides and alkyl acrylates have been applied in similar applications and in our preparation of an optode we have chosen a copolymer of 40% hydroxybutylacrylate and 60% isopropylacrylamide. The copolymer and II were solubilized and a small aliquot of the solution was deposited onto glass microscope slides. The slides were spun to produce sensors with membrane thickness of ca. 4 µm.

**Sensitivity and Selectivity Studies**

Figure 3 shows the fluorescence emission spectra for I in dichloromethane obtained at \( \lambda_{ex} = 355 \text{ nm} \) at room temperature in the absence and presence of added potassium acetate. This figure clearly shows the “off-on” switching behavior of this chromoionophore in solution. This chromoionophore is sensitive to protonation at the azacrown nitrogen atom and unless an organic base is added to prevent protonation the fluorescence quenching mechanism is disrupted leading to intense fluorescence emission in the absence of K⁺. It can be inferred from the inset in Figure 3 that in solution the chromoionophore and K⁺ form a 1:1 complex. This is indicated by the linear dependence of fluorescence intensity on K⁺ concentration up to a level that is roughly equivalent to the chromoionophore concentration. It should be noted that studies in solution are not performed at K⁺ concentrations in the normal physiological range because chromoionophore extinction coefficients prevent such large concentrations from being used (assuming 1:1 complexation). However, in bulk films that typically measure a few microns in thickness, absorption will not be a limiting factor for chromoionophore concentration, meaning that the chromoionophore can potentially provide sensitivity for ions in the physiological range.
Selectivity studies in solution are typically performed when other ions are expected to be present. The number of potentially competing ions is variable from application to application with environmental analysis presenting large numbers of potential competitors but relatively few found in whole blood analysis. Our selectivity studies involve measuring the relative response to a series of ions including, \( \text{Li}^+ \), \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Cs}^+ \), \( \text{Rb}^+ \) as well as divalent ions such as \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \). The inset in Figure 3 also shows the results of selectivity measurements for \( \text{I} \) with \( \text{Na}^+ \) and \( \text{K}^+ \). These ions are usually the focus of selectivity studies for blood measurement applications because they are found in relatively high concentrations and can often compete for binding due to their similar size.

The selectivity of a chromoionophore to the primary ion in the presence of an interfering ion can be calculated by measuring the fluorescence intensity versus ion concentration for each ion independently. The maximum fluorescence response to the interfering ion defines the minimum detection limit for the primary ion; i.e., an independent response to the primary ion is obtained only in the concentration range for the interfering ion where there is a flat fluorescence response. This approach is comparable to a fixed interference method (FIM)\(^{18}\) that is employed for ion selective electrodes where the selectivity ratio is determined by the ratio of the limit of detection (in the presence of a constant interfering ion concentration) to the concentration of the interfering ion. It is clear from the inset of Figure 3 that we observed no response from the addition of \( \text{Na}^+ \) and therefore we can only estimate a lower limit of the selectivity value. Based on this, we estimate that the selectivity ratio of \( \text{I} \) is at least \( 1 \times 10^3 \).

Figure 4 shows the fluorescence emission spectra obtained for \( \text{II} \) in optode format for various concentrations of \( \text{Na}^+ \). The fluorescence intensity was a linear (\( R^2 = 0.9996 \)) function of the sodium ion concentration (Inset, Figure 4). Figure 5 shows the dynamic response to sodium within the clinical range (0-0.2 M). The data indicate that response times of the optode are on the order of two minutes with a maximum 30% increase in the fluorescence intensity. In this solid optode format we have measured the \( \text{Na}^+ / \text{K}^+ \) selectivity (\( \log K_{\text{Na,K}}^{\text{opt,SSM}} \)), based on the recommended procedure of Bakker et al.\(^{18}\) Using this procedure, we obtain a selectivity value of \( \log K_{\text{Na,K}}^{\text{opt,SSM}} = -.6 \). We note that this value meets the required selectivity of a sodium sensor for whole blood analysis.\(^{19}\)
Conclusions

The encouraging preliminary results presented here for I and II point out the feasibility of taking a rational approach to the assembly of functioning optical sensors from their individual components. Through this approach, we have successfully produced a new optical potassium fluoroionophore with K$^+$/Na$^+$ selectivity of at least $1 \times 10^3$ and >8-fold response to K$^+$. We have also shown the development of a novel sodium optode which could be integrated into clinical diagnostics systems for the determination of Na$^+$ within the human physiological range.

References

12. For other examples of amide linkers, see Fluorescent Chemosensors of Ion and Molecule Recognition; Cazarnik, A. W., Ed.; ACS Symp. Ser. 538; American Chemical Society: Washington DC, 1993.

About the Authors

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Solution Chemistry and DNA Cleavage by Photogenerated Rh$_2$(O$_2$CCH$_3$)$_4^+$

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Introduction

Photoexcitation of bridged bimetallic d$^7$–d$^7$ complexes with visible light is known to result in the formation of reactive diradical species and of excited states capable of undergoing radical reactions. Prominent examples include Mn$_2$(CO)$_{10}$ and Re$_2$(CO)$_{10}$ (structures shown in Figure 1), which undergo metal-metal bond cleavage upon near-uv irradiation resulting in a metal-centered σ → σ* transition. A generalized MO diagram is shown in Figure 1 for d$^7$–d$^7$ bimetallic systems. Rh$_2$(CH$_3$CN)$_4^{4+}$ is a recent example of this type of photoreactivity, since irradiation with $\lambda_{irr}$ > 435 nm results in the initial formation of •Rh(CH$_3$CN)$_2^{2+}$ radicals, which were shown to recombine to regenerate the starting material following several steps.

In contrast, in bridged d$^7$–d$^7$ bimetallic complexes light excitation often results in the formation of excited states that possess significant radical character centered on the metals. Recent examples are Rh$_2$(dfpma)$_2$Cl$_4$ (dfpma = bis(difluorophosphino)methylamine) and Rh$_2$(MeN[P(OEtF$_3$)$_2$]$_2$Cl$_4$ (MeN[P(OEtF$_3$)$_2$]$_2$ = bis(bis(trifluoroethoxy)phosphine)methylamine), which are emissive as solids at room temperature with phosphorescence maxima at 821 nm ($\tau$ = 250 µs) and 819 nm ($\tau$ = 350 µs), respectively. These complexes were recently shown to photocatalytically produce H$_2$ from HX solutions.

Our research focuses on the utilization of these radical excited states as possible reagents in photochemotherapy, with desired photoreactivity that parallels that observed for the related d$^8$–d$^8$ diplatinum(II) complex Pt$_2$(pop)$_4^{4-}$ (structure shown in Figure 1). Pt$_2$(pop)$_4^{4-}$ has been extensively investigated, and its lowest excited state with a $(d\sigma^*)^1(p\pi)^1$ electron configuration is reactive towards various substrates, including DNA. The long-lived triplet excited state of Pt$_2$(pop)$_4^{4-}$ is able to abstract hydrogen atoms from C-H, P-H, Si-H, and Sn-H bonds, where the axial Pt$_2$(pop)$_4^{4-}$H$_4$ intermediate was detected. The α-hydrogen atom abstraction by *Pt$_2$(pop)$_4^{4-}$ from isopropanol takes place with $k_q = 1.0 \times 10^9$ M$^{-1}$s$^{-1}$, which is the initial step in the catalytic conversion of isopropanol to acetone and H$_2$. Furthermore, hydrogen abstraction from the DNA backbone upon photoexcitation of Pt$_2$(pop)$_4^{4-}$ was reported, which results in DNA photocleavage. The reaction with calf-thymus DNA proceeds via hydrogen atom abstraction, with the formation of

Figure 1. (a) Structures of M$_2$(CO)$_8$ (M = Re, Mn), Pt$_2$(pop)$_4^{4-}$, and Rh$_2$(O$_2$CCH$_3$)$_4(L)_2$ complexes (L = H$_2$O, py, PPh$_3$) and (b) general MO diagram for $D_{4h}$ ML$_8$ d$^7$–d$^7$ bimetallic complexes.
Pt\(_2\)(pop)\(_4\)H\(^+\) and Pt\(_2\)(pop)\(_4\)H\(_2\)\(^+\), where the latter reacts with H\(_3\)O\(^+\) to form the observed diplatinum(III) Pt\(_2\)(pop)\(_4\)(H\(_2\)O)\(_2\)^{2-} complex and H\(_2\). The cleavage of duplex DNA by the triplet excited state of Pt\(_2\)(pop)\(_4\)^{2-} is not enhanced by piperidine treatment, consistent with hydrogen abstraction rather than with a mechanism involving guanine oxidation. However, the electrostatic repulsion between the complex with overall negative charge and the anionic DNA backbone results in a slow bimolecular rate of photocleavage (k ~ 10\(^{-5}\)-10\(^{-6}\) M\(^{-1}\) s\(^{-1}\)).

Our work in this area has focused on the excited state properties and photoreactivity of the d\(^7\)-d\(^7\) dirhodium(II) tetraacetates, Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(L)\(_2\) with various axial ligands, L (CH\(_3\)OH, H\(_2\)O, PPh\(_3\) = triphenylphosphine, py = pyridine, THF = tetrahydrofuran), as well as the exploration of the potential role of these molecules in photochemotherapy. The Rh\(_2\)(O\(_2\)CR)\(_4\)(L)\(_2\) complexes (structures shown in Figure 1) have long been known, and their structure, bonding, and reactivity have been extensively investigated. Dirhodium tetracarboxylates are known to catalyze many reactions including cyclopropanation, alkyne cyclopropenation, C-H insertion, and carbonoid initiated C-C bond formation. However, these are examples of ground state reactions that are not initiated by light. The Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(L)\(_2\) complexes were chosen for our initial studies because they are known to bind DNA and nucleic acids and are insensitive to water and O\(_2\). In addition, the related Rh\(_2\)(O\(_2\)CR)\(_4\)(N-N)\(_2\)\(^+\) (R = PhCH(OH), CH\(_3\)CH(OH); N-N = 1,10-phenanthroline, 2,2'-bipyridine) systems have recently been shown to act as antibacterial agents and to exhibit cytostatic activity against human oral carcinoma.

**Photophysical Properties**

Variation of the axial ligands, L, leads to a dramatic shift in the position of the lowest energy electronic transition of the Rh\(_2\)(O\(_2\)CR)\(_4\)(L)\(_2\) complexes, which has been previously assigned as RhRh \(\pi^*\) to RhRh \(\sigma^*\). The shift is explained by significant mixing between the axial Rh-L \(\pi\) interactions and the Rh-Rh \(\sigma\) and \(\sigma^*\) molecular orbitals (MOs), as well as mixing between the low-lying carboxylate \(\pi\) and \(\pi^*\) systems and the metal-centered MOs of the Rh\(_2\)(II,II) bimetallic core (see Figure 1b).

No emission was observed from methanol solutions of Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(L)\(_2\) (L = CH\(_3\)OH, PPh\(_3\), py) in the 500 nm to 1,100 nm spectral region at room temperature, and at 77 K in methanol or as a solid between 500 and 850 nm. To our knowledge, there have been no previous reports of emission from Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\).

Although the Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\) complexes do not emit light, upon excitation with a short laser pulse a transient absorption signal is observed on the microsecond timescale. Figure 2 presents the transient absorption spectrum of Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(PPh\(_3\)) in CH\(_3\)OH collected ~500 ns following the 532 nm (FWHM ~10 ns, 5 mJ/pulse) excitation laser pulse, similar to that obtained with 355 nm excitation. The return of the transient signal at ~760 nm to the baseline (\(\Delta OD = 0\)) could be fit to a monoexponential decay with lifetime of 5.0 \(\mu\)s. Similar spectral profiles with a peak at ~760 nm were collected for Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(CH\(_3\)OH) in CH\(_3\)OH (\(\tau = 4.4\) \(\mu\)s) and in CH\(_4\)Cl\(_2\) (\(\tau = 4.6\) \(\mu\)s). The transient absorption spectra of the Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(L)\(_2\) (L = THF, py) complexes in methanol exhibit similar spectral profiles to that shown in Figure 2 with monoexponential lifetimes of 3.5 \(\mu\)s and 4.8 \(\mu\)s, respectively, measured at 760 nm.

**Energy and Electron Transfer**

Unequivocal evidence for the formation of an excited state of the Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(L)\(_2\) complexes upon excitation is imparted by the observed energy transfer from \(\ast\)Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(PPh\(_3\)) to the \(3\pi\pi^*\) of various organic acceptors. Upon excitation of \(\ast\)Rh\(_2\)(O\(_2\)CCH\(_3\))\(_4\)(PPh\(_3\)) with \(\lambda_{exc} = 532\) nm in the presence of tetracene (\(E_{\text{exc}}(\ast\pi\pi^*) = 1.24\) eV), the spectrum of the \(3\pi\pi^*\) excited state of tetracene is observed with its characteristic millisecond lifetime. Excitation of tetracene alone with 532 nm
does not result in the production of its triplet excited state, however, it can easily be generated with 355 nm excitation. Energy transfer was also observed from *Rh$_2$(O$_2$CCHO)$_4$(PPh$_3$)$_2$ to perylene ($E_{\text{thr}}$(ππ*) = 1.30 eV), but not to diphenylantracene ($E_{\text{thr}}$(ππ*) = 1.77 eV) or phenothiazine ($E_{\text{thr}}$(ππ*) = 2.62 eV).

Electron transfer to 4,4'-dimethyl viologen (MV$_2^+$; $E_{1/2}$(A$^{0/-}$) = -0.46 V vs NHE), takes place upon 532 nm excitation of Rh$_2$(O$_2$CCHO)$_4$(PPh$_3$)$_2$ in methanol with a quenching rate constant, $k_q$, of 8.0 x 10$^6$ M$^{-1}$s$^{-1}$, where the absorption peaks of MV$_2$$^{*+}$ at 395 nm and 605 nm are observed in transient spectrum. In addition, the broad spectral feature at ~540 nm can be assigned to the one electron oxidized complex, Rh$_2$(O$_2$CCHO)$_4$(PPh$_3$)$_2^{*+}$. Similarly, the oxidative quenching of the *Rh$_2$(O$_2$CCHO)$_4$(PPh$_3$)$_2$, transient by chloro-p-benzoquinone ($E_{1/2}$(A$^{0/-}$) = -0.10 vs NHE) in methanol proceeds with $k_q$ = 1.2 x 10$^{10}$ M$^{-1}$s$^{-1}$, and $k_q$ = 5.0 x 10$^7$ M$^{-1}$s$^{-1}$ was measured for Ag$^{+}$.

The energy and electron transfer experiments place the excited state energy of Rh$_2$(O$_2$CCHO)$_4$(PPh$_3$)$_2$ between 1.34 eV and 1.77 eV.

**Solution Photochemistry**

No overall photochemistry was observed for Rh$_2$(O$_2$CCHO)$_4$(PPh$_3$)$_2$ in methanol either in the presence or absence of electron acceptors. In contrast, when methanol solutions of Rh$_2$(O$_2$CCHO)$_4$(CH$_2$OH)$_2$ were utilized in the quenching experiments with the electron acceptors described above, irreversible photochemistry took place. No chemical reactions were observed under same conditions in the dark or upon irradiation of Rh$_2$(O$_2$CCHO)$_4$(CH$_2$OH)$_2$ in methanol in the absence of electron acceptors. This photochemical reaction in the presence of electron acceptors was also observed when ethanol (EtOH) and isopropanol (i-PrOH) were used as solvents.

It was recently shown by $^1$H NMR that photolysis ($\lambda_{\text{irr}}$ > 515 nm) of benzene-d$_8$ solutions of Rh$_2$(O$_2$CCHO)$_4$(CH$_2$OH)$_2$ in the presence of various electron acceptors and i-PrOH results in the photocatalytic production of acetone. Although the mechanism of this reaction is still under investigation, it likely proceeds via the photoinduced formation of the one electron oxidized Rh$_2$(O$_2$CCHO)$_4$$^{*+}$ complex. We prepared Rh$_2$(O$_2$CCHO)$_4$$^{*+}$ independently through bulk electrolysis, followed by isolation and purification. The $^1$H NMR spectra of a benzene-d$_8$ solution of Rh$_2$(O$_2$CCHO)$_4$$^{*+}$ in the presence of i-PrOH results in the quantitative formation of acetone relative to the starting Rh$_2$ complex in the dark after ~2 hours at room temperature.

**DNA Photocleavage**

In addition to the reactivity of Rh$_2$(O$_2$CCHO)$_4^+$ with alcohols in solution, the DNA cleavage by photogenerated Rh$_2$(O$_2$CCHO)$_4$(L)$_2$$^{*+}$ (L = CH$_2$OH, H$_2$O) was also observed. The imaged ethidium bromide-stained agarose gel in Figure 3 reveals that the excited state of Rh$_2$(O$_2$CCHO)$_4$(H$_2$O)$_2$ itself does not cleave DNA. Lane 1 (Figure 3) shows that 100 µM (bases) of pUC18 plasmid alone is found mostly as a supercoil (Form I) with a small amount of nicked, circular DNA (Form II). Relative to Lane 1, irradiation ($\lambda_{\text{irr}}$ ≥ 395 nm) of 100 µM plasmid alone (Lane 2) or in the presence of 40 µM Rh$_2$(O$_2$CCHO)$_4$(H$_2$O)$_2$ does not result in DNA cleavage. Excitation of Rh$_2$(O$_2$CCHO)$_4$(H$_2$O)$_2$ with visible light in the presence of electron acceptors, such as 3-cyano-1-methylpyridinium tetrafluoroborate (py$^+$) results in the formation of the one-electron oxidized complex, Rh$_2$(O$_2$CCHO)$_4$(H$_2$O)$_2^{*+}$. Lane 6 (Figure 3) shows the efficient cleavage of the plasmid by 40 µM Rh$_2$(O$_2$CCHO)$_4$(H$_2$O)$_2$ irradiated with $\lambda_{\text{irr}}$ ≥ 395 nm (10 min) in the presence of 2 mM py$^+$, evidenced by the disappearance of Form I and the formation of Form II DNA. This cleavage is not observed.
when the mixture is kept in the dark (Lane 5). Furthermore, irradiation of 100 µM plasmid with 2 mM pyO− alone (Lane 4) does not result in DNA cleavage.

To ensure that the cationic electron acceptor was not taking part in the cleavage the anionic 1,8-antraquinone disulfonate (AQ−) was utilized, whose negative charge precludes its binding to the polyanionic double helix. Cleavage of 100 µM plasmid by photoproduced (λirr ≥ 455 nm, 15 min) Rh₂(O₂CCH₃)₃(PPh₃)₂⁺ (25 µM) in the presence of 20 mM AQ− is also observed. Irradiation of 20 mM AQ− under the same conditions with 100 µM plasmid (Lane 3) does not result in DNA cleavage. Similar DNA cleavage was observed when 200 µM Ag⁺ and 200 µM FeO⁺ were utilized as the electron acceptors.

The photoinduced DNA cleavage by Rh₂(O₂CCH₃)₃(H₂O)₂ in the presence of electron acceptors is more effective under N₂ atmosphere than in air, consistent with the quenching of the excited state of the rhodium complex by O₂ resulting in a decreased production of the Rh₂(O₂CCH₃)₃(H₂O)₂⁺ electron transfer product. In addition, the wavelength dependence shows that the cleavage remains efficient up through λirr ≥ 590 nm, and a significant amount of cleavage is also observed for λirr ≥ 610 nm. No photocleavage is evident at λirr ≥ 630 nm, consistent with the electronic absorption spectrum of the dirhodium complex.

No cleavage of 100 µM pUC18 plasmid was observed when 10 µM of Rh₂(O₂CCH₃)₃(L)₂ with py and PPh₃, axial ligands, L, were employed up to 30 min irradiation time (λirr > 395 nm) in the presence of 200 µM Ag⁺. These results are important because they point at the necessity of a labile axial ligand, such as H₂O, for the DNA cleavage to take place. The need for a labile axial ligand may indicate that the presence of an open coordination site is either required for the reactivity of the complex or for its binding to DNA. The radical Rh₂(O₂CCH₃)₃(H₂O)₂⁺ complex may be able to abstract a hydrogen atom from the DNA backbone, thus resulting in the observed cleavage. Alternatively, hydroxyl radicals formed by Rh₂(O₂CCH₃)₃(H₂O)⁺ may participate in the DNA cleavage. Guanine oxidation (E'O/2 = 1.14 V vs NHE) is slightly unfavorable, however, it cannot be ruled out as a means for initiation of the DNA cleavage at this time.

Conclusions

The Rh₂(O₂CCH₃)₃(L)₂ (L = CH₃OH, THF, PPh₃, py) complexes possess a long-lived non-emissive excited state with lifetimes that range from 3.5 to 5.0 µs. The transient absorption spectra indicate that excited state exhibits an optical transition at ~760 nm independent of axial ligand, and energy transfer from excited Rh₂(O₂CCH₃)₃(PPh₃)₂ to tetracene and perylene takes place upon excitation of the Rh₂ complex to form the π π* excited state of each acceptor. Electron transfer from *Rh₂(O₂CCH₃)₃(PPh₃)₂ to MV₂⁺ and Cl-BQ in methanol takes place with quenching rate constants of 8.0 × 10⁶ M⁻¹ s⁻¹ and 1.2 × 10⁷ M⁻¹ s⁻¹, respectively. The observations are consistent with the production of an excited state of Rh₂(O₂CCH₃)₃(PPh₃)₂ with energy, E'O/2 between 1.34 eV and 1.77 eV.

The excited state of Rh₂(O₂CCH₃)₃(L)₂ is not able to undergo hydrogen abstraction chemistry. It is likely that, unlike Pt₂(pop)L₂⁺, the excited state of the Rh₂(O₂CCH₃)₃ complexes is not diradical (or radical) in nature, possibly owing to the mixing of the acetate low-lying orbitals with metal-centered MO’s. However, the photoproduced one-electron oxidized complex, Rh₂(O₂CCH₃)₃⁺, is able to convert isopropanol to acetone and to efficiently cleave DNA, which can be generated with λirr ≤ 610 nm.

References

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Electronic Versus Paper Journals

D. C. Neckers, Executive Director, Center for Photochemical Sciences, Bowling Green State University

In the summer *Spectrum* I asked our readers what they thought about the future of printed journals and books in light of the electronic communication revolution. What triggered my question was the observation that my students were reading articles found on electronic journals before my hard copy of JACS or Macromolecules had crossed my desk.

This needs a bit of further explanation. Bowling Green’s library subscribes to OhioLINK, a consortium of the libraries of 78 Ohio colleges and universities and the State Library of Ohio. OhioLINK has an electronic journal center. Therefore, my students sitting in their laboratory offices, not in the science library, have direct access to almost all the journals of interest to us.

Approximately 40 readers responded to my questions. As you can imagine, individual opinions ran the gamut, and I suspect that age is a factor in the results. Following are summaries of the differing views:

• Both the electronic version and the hard copy are necessary for scientists.
• The electronic version will be mainly used for instantaneous access to current research and “News”.
• The electronic versions will provide easier archival literature searches.
• Electronic versions will save storage space in libraries, offices and laboratories.
• Archives will consist almost exclusively of the hard copies.
• The more generic “literature search” with which we are all familiar will never give way to searching and reading from the hard copy.
• Nothing will ever replace holding a printed journal in one’s hand.
• Electronic journals will never be a substitute for knowing exactly where a journal is located in an office.

I want to thank our readers for their input. It helped me personally gauge how I would deal with my ever increasing journal bill from the American Chemical Society and elsewhere. I hope it will help some of the younger among you think about how to position your own libraries now and for the future. My own decision was to continue to collect those hard copies, and to share them with my colleagues in order to assist them in their studies. Of course this begs the issue of what to do with what is now a rather imposing library when I retire, but I guess that decision will wait for a further day.

I write this on Christmas Eve 2001. As I do so, I listen, as I have for years, to A Festival of Nine Lessons and Carols broadcast live from Kings College Chapel, Cambridge. If ever there was a need for a Prince of Peace, by whatever name and in whatever religion, it is Christmas 2001. From each of us to all of you we wish you a very happy holiday season. May the year 2002 bring health, happiness and good science to us all.


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