

Visualizing Redox Chemistry: Probing Environmental Oxidation–Reduction Reactions with Indicator Dyes

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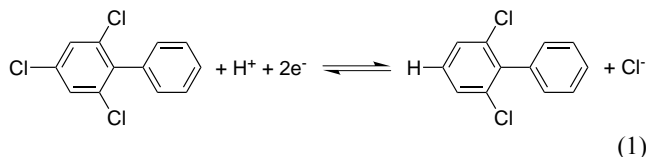
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Abstract: One of the important questions in the chemistry of pollutant degradation is the identity and distribution of chemical agents that are responsible for reduction reactions in the environment. Reduction occurs primarily in water-saturated environments, such as sediments, soils, and sludges. Redox indicators can be used (i) as chemical probes to obtain fundamental insights into biogeochemical processes and (ii) as the basis for demonstrations suitable for teaching aspects of environmental chemistry. This paper explores the latter with examples that involve a variety of indicators (indigo sulfonates, resazurin, etc.), environmental media (anaerobic sediments and granular iron metal), and physico-chemical processes (oxidation–reduction, adsorption, and diffusion). The results show that reduction by either media (sediments or iron metal) is primarily, although not always entirely, a surface reaction. This situation results in indicator behavior that is interesting and challenging for students with a wide range of backgrounds.

Background

Oxidation–reduction reactions are among the most important chemical processes in aquatic environments [1]. In natural anaerobic systems, reducing conditions are strongly linked to sediment microbial activity, and the combination of these factors controls the fate of many contaminants. In engineered systems, oxidizing or reducing conditions are sometimes created to achieve remediation of contaminated materials. Nevertheless, despite the importance of redox reactions in contaminant fate, this class of transformations has only recently begun to receive the recognition that has long been afforded other degradation pathways such as hydrolysis, photolysis, and biodegradation [2].

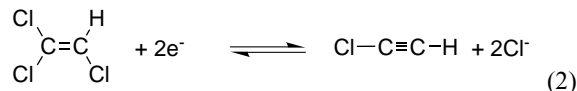


The importance of reduction reactions involving contaminants in natural aquatic environments is illustrated in Figure 1A for polychlorinated biphenyls (PCBs). Microbially mediated reductive dechlorination of PCBs can result in the natural attenuation of these contaminants in the sediments of ponds, rivers, and harbors [3]. A half reaction for

hydrogenolysis of 2,4,6-trichlorobiphenyl, one of 209 possible PCB congeners, as shown in eq 1.

Dechlorination generally results in less toxic products, and there are few alternative detoxification pathways available for PCBs under reducing conditions. As a result, sustaining and encouraging this degradation process is central to plans for restoring PCB contaminated sites such as the Hudson River [4] and New Bedford Harbor [5].

An example of a remediation technology that relies on reduction to degrade chlorinated solvents and other groundwater contaminants is shown in Figure 1B. The treatment zone is a permeable reactive barrier (PRB) containing granular zero-valent iron metal [6]. Fe^0 reduces a variety of groundwater contaminants (e.g., trichloroethylene, chromate, and nitrate) resulting in degradation (or immobilization) of the contaminant and dissolution of the Fe^0 to Fe^{2+} (i.e., corrosion). Trichloroethylene, or TCE, is reduced by Fe^0 via two dechlorination reactions: hydrogenolysis (analogous to eq 1) and reductive β -elimination [7]. The β -elimination of TCE yields chloroacetylene



which is rapidly transformed to nontoxic products [8]. PRBs made with Fe^0 (sometimes called “iron walls”) have become popular for groundwater remediation because of growing demand for technologies that are passive and in situ (as opposed to those that require long-term maintenance and pumping to above-ground treatment facilities).

Despite these and other environmental applications of reducing systems, relatively little is known about the

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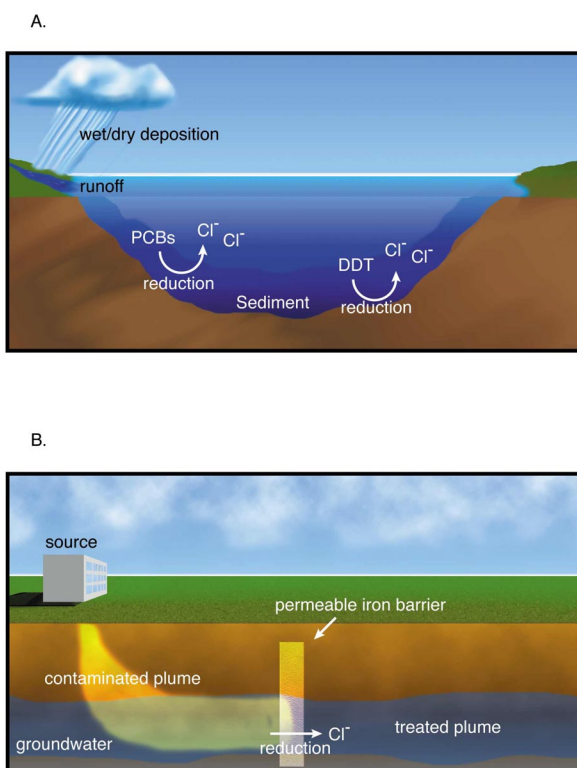


Figure 1. Schematics showing two types of environmental systems where reduction reactions are important determinants of contaminant fate. (A) Lake sediments and other saturated soils, which become anaerobic and reducing due to microbial metabolism and lack of oxygen recharge from the atmosphere. (B) Permeable reactive barriers containing zero-valent iron, in which contaminant reduction is driven by corrosion of iron as the contaminants flow through the treatment zone.

mechanisms of contaminant reduction or even the specific reducing agents responsible for these reactions. The case represented by eq 2 is different because the reductant, Fe⁰, is provided by design. Under these circumstances, the dominant reduction half reaction is certain to be $\text{Fe}^0 = \text{Fe}^{2+} + 2\text{e}^-$, and this information makes it easy to write a balanced, overall redox reaction for reduction of TCE by an iron wall. The situation in anaerobic sediments, however, is quite different because the reducing agents that are directly responsible for reactions such as eq 1 are not known. They could be Fe(II), Mn(II), S(II), any of a wide variety of complex materials that lack well-defined chemical structures, like “natural organic matter,” or transient species not yet identified. To develop a deeper understanding of the chemistry of reducing environments, recent studies have focused on what can be learned from model substrates that exhibit distinctive patterns of reactivity [e.g., 9–12]. Probe compounds, used in this way, have the potential to yield fundamental information about the chemistry of complex environments.

When redox dyes are used as the probe compounds, the probe response is indicated by changes in color that are visual and sometimes quite dramatic. These color changes can be quantified using common methods such as spectrophotometry, and the resulting data can be used to characterize both thermodynamic and kinetic properties of environmental systems [13]. Thus, the use of indicator dyes to probe the

redox properties of environmental systems provides an educational opportunity that is accessible using simple procedures but leads to challenging exercises in analysis and interpretation. A wide variety of educational experiments using redox indicators have been described previously [e.g., 14–18], but none have emphasized environmental applications. This paper provides a guide to the use of redox indicators to study environmental materials, with emphasis on how these experiments can be used to demonstrate fundamental concepts for chemical education.

Materials and Experimental Methods

Reagents. Commercially available indicator dyes are often of low purity (70 to 90%), but they are almost always used as received. The dyes used in this study were indigo carmine (5,5'-indigodisulfonic acid, disodium salt, 94%, Aldrich); 5,5',7-indigo trisulfonic acid, tripotassium salt (75%, Sigma); 5,5',7,7'-tetrasulfonic acid, tetrapotassium salt (TCI); methylene blue (87%, Aldrich); 2,6-dichloroindophenol, sodium salt hydrate (Aldrich); methyl viologen dichloride (Aldrich, 98%) resorufin, sodium salt (95%, Aldrich); resazurin (96%, Aldrich); and phenosafranine (80%, Aldrich). Selected properties of these indicators are summarized in Table 1. In all cases, mM stock solutions were prepared in deionized water (18 M Ω -cm NANOpure). Up to 5% (v/v) HPLC-grade methanol was added to stock solutions of some dyes to enhance solubility.

Various reducing materials with environmental relevance can be used for the experiments described below, including iron, aluminum, tin, and zinc metal; stream and pond sediments; and even sewage sludge (although the latter is not recommended unless appropriate safety precautions are taken). The iron metal used in this study (Fluka electrolytic iron, Catalog #44905) was selected to give manageable reduction rates in the experimental system described below. Prior to use, the Fe⁰ grains can be hand-sieved to constrain grain size (which alleviates spectroscopic interferences created by very fine grain particles). The surface area of our 18–20 mesh sieved iron sample, without acid treatment, was determined to be 0.2 m²/g by gas adsorption using BET analysis [19]. Acid washing of the iron is not necessary, but was performed because it results in faster and more reproducible kinetics of dye reduction. In most cases, this was done by immersing the iron in 1 M HCl for 10 minutes, followed by copious washing with deoxygenated water. Rapid drying to avoid reoxidation can be achieved by rinsing with methanol during vacuum filtration. The cleaned iron was weighed dry for use in experiments with the indicators.

The sediment samples used for this study were collected from the sediment/water interface of both freshwater and estuarine sources. Canning jars proved to be convenient for minimizing exposure to air after the sample had been removed from the site. Once in the laboratory, sediments generally were sieved (1 mm, 16 mesh) to remove irregular objects (vegetation, rocks, and insects), and sandy sediments were decanted to decrease the amount of coarse-grain material that could not be suspended by mixing with a magnetic stir bar. (The fraction removed in this way is mostly quartz sand, which exhibits little reactivity relative to other mineral and organic fractions). Aliquots of the sediment slurry were transferred from the stirred jar directly to reaction vials using a graduated pipette with the tip cut off to allow the sediment to pass without plugging. Reproducible sediment-to-water ratios were obtained most easily using an automatic pipettor (Pipet-Aid). Between sampling and use in the laboratory, all sediments were stored at 4 °C.

Indicator Reduction Kinetics with Method 1. Semiquantitative studies of indicator reduction kinetics were done with simple equipment and a minimum of supplies using an adaptation of the method described previously by Tratnyek and Wolfe [13]. The sediment slurry was transferred to 13 × 100-mm screw-cap culture

Table 1. Selected Redox Indicator Dyes and their Properties^a

Oxidized form	$\lambda_{\text{max}}^{\text{ox}}$ ^b	$\lambda_{\text{max}}^{\text{red}}$ ^b	ϵ_{ox} ^c	E_0' ^d	E_m^7 ^d	pK _a ^e
2,6-Dichloroindophenol	601 blue	256 leuco	17.4	0.668	+0.228	8.1 (o1), 9.4 (r1), 10.6 (r2)
Methylene blue	663 blue	253 leuco	76.7	0.532	+0.011	11.0 (o1), 4.52 (r1), 5.30 (r2)
Resazurin/resorufin	600 purple	569 pink	40.1	NA ^f	NA ^f	6.93 (o1)
Resorufin/dihydroresorufin	569 pink	293 leuco	8.25	0.380	-0.051	6.93 (o1), 9.26 (r1), 10.00 (r2)
Indigo disulfonate	610 blue	407 yellow	20.0	0.291	-0.125	12.3 (o1), 12.8 (o2), 7.4 (r1), 12.1 (r2)
Indigo trisulfonate	600 blue	421 yellow	14.6	0.332	-0.081	11.7 (o1), 12.8 (o2), 7.2 (r1), 12.1 (r2)
Indigo tetrasulfonate	589 blue	433 yellow	18.5	0.365	-0.046	11.9 (o1), 12.9 (o2), 7.0 (r1), 11.8 (r2)
Phenosafranine	516 red	249 leuco	32.0	0.280	-0.252	4.96 (r1), 5.78 (r2)
Methyl viologen (paraquat) ^g	256 leuco	603 violet	17.0	NA	-0.446	

^aIncludes dyes used in this study and a few others that have been used previously [13] to extend the range of redox potentials covered. ^bAbsorbance maxima and color of oxidized and reduced forms of each dye determined in deionized water (nm). ^cextinction coefficients for the oxidized dyes ($\text{mM}^{-1} \text{cm}^{-1}$) determined in deionized water at the $\lambda_{\text{max}}^{\text{ox}}$. ^dFormal potential at pH 0 (E_0') and at pH 7 (E_m^7) in V versus SHE from [20, 46]. ^epK_as for the reduced (r1, r2) and oxidized (o1, o2) forms of the dyes from [20, 46]. ^fNot available because the couple is not reversible, but binary experiments with dyes indicate resazurin is reduced at potentials below methylene blue and above resorufin and I4S. ^gUnlike the other dyes used in this study, methyl viologen is quite toxic, so it is not recommended for student use.

tubes (e.g., Pyrex #9826-13), which were chosen because they can be inserted directly into the sample chamber of a fixed-wavelength spectrophotometer (Spectronic 20, Bausch & Lomb). A volume of 8.5 mL of slurry nearly filled these tubes, thereby minimizing the potential for indicator reoxidation by O₂ from the headspace. To initiate a reaction, 75 μL of 1-mM indicator stock solution was added to each tube, and then the tubes were capped and thoroughly mixed by hand. For contact times of more than a few minutes, tubes were mixed continuously and gently by rotation (at 7.5 rpm) on a mechanical shaker.

In order to obtain quantitative concentration versus time data, it is desirable to have a way to periodically measure absorbance of the indicator without opening the culture tubes and risking exposure to atmospheric O₂. To do this, each tube was centrifuged to produce a clear supernatant, and absorbance of the supernatant was measured by inserting the whole tube directly into the spectrophotometer. Small amounts of sediment formed a small pellet at the bottom of the tube, which did not interfere with the light path of the spectrophotometer; however, with cohesive sediments, large amounts of sediment could be accommodated by centrifuging the pellet into the top of the tube. After the measurement, the pellet was resuspended by vortexing. This procedure complicates the definition of reaction time because the dye has little contact with the sediment while the sediment is compressed in a pellet. This effect can be corrected by counting only time when the sediment is suspended.

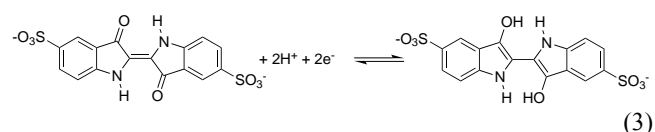
Indicator Reduction Kinetics with Method II. Quantitative determinations of indicator reduction kinetics were done with a new method that was developed to give more accurate results with a wider range of reducing sediments. In an anaerobic chamber, a wide-mouth pipette was used to dispense 10 mL of sediment slurry into large culture tubes. The reaction was initiated by adding 75 μL of the dye stock solution to a tube and mixing (briefly by hand). The mixture was immediately poured into the open end of a 10-mL Luer-Lok disposable syringe that contained a plug of glass wool and was fitted with a 0.45- μm syringe filter (25-mm Whatman Puradisc). This arrangement minimized plugging of the filter by the sediment. The syringe was used to force 5 mL of filtrate directly into a 10-mL screw-cap cuvette (10-mm-path-length optical glass) containing 75 μL of dilute 1% H₂O₂ (to quench dissolved reductants). The procedure was repeated for all of the desired contact times (measured from when the indicator and sediment were mixed together to when the filtrate was separated from the sediment into the cuvette). Finally, the sealed cuvettes were removed from the anaerobic chamber and the absorbance of the filtrates was measured from 340 to 700 nm with a

scanning spectrophotometer. Justification for the details of this method is provided in the discussion of Figure 7.

Experiments & Results

Redox Indicators. A large number of redox indicators have been developed for various applications [20], but few of them are suitable for use with environmental materials due mainly to sorption and interfering background color. The color changes shown in Figures 2 and 3 illustrate some of the various effects that can be obtained when redox indicators are exposed to materials of environmental interest. Further interpretation of these effects is provided in the discussion that follows.

Indigo and its mono-, di-, tri-, and tetra-sulfonates, eq 3, constitute a homologous series of indicators, all of which provide strong contrast between oxidized (blue) and reduced (yellow) forms. Indigo is the original dye used to make blue denim, and indigo 5,5'-disulfonate (I2S) is the indicator commonly known as indigo carmine.



The sulfonated forms of indigo have high water solubility and low tendency to adsorb to solids, so color changes in the solution phase give a visible indication of redox conditions even in heterogeneous systems [13]. We prefer indigo 5,5',7,7'-tetrasulfonate (I4S) because it has the least tendency to sorb to sediments, but I3S and I2S generally give satisfactory results.

Figure 2 illustrates the behavior of I4S in the presence of a reducing sediment. All tubes were prepared identically by mixing and then centrifuging briefly to form a sharp sediment-water interface. However, tubes A through F were prepared at time intervals such that A represents the shortest contact time (< 1 day) and F represents the longest contact time (ca. 1 week). The effect of this procedure is to simulate the gradual progression of a front of reduced dye (yellow) up the tube starting from the sediment water interface. Reoxidation was

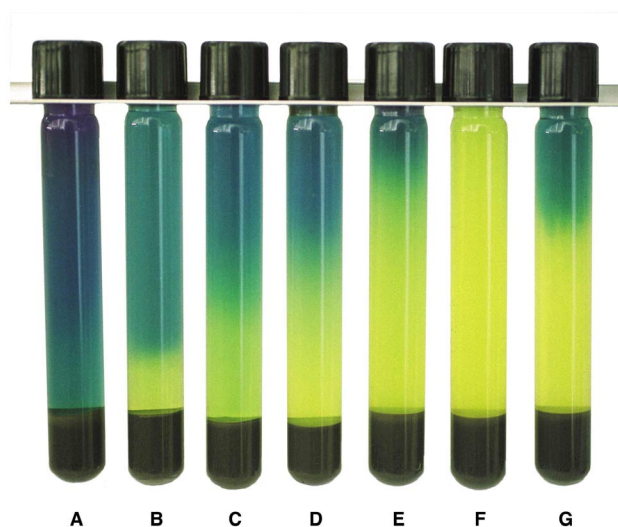


Figure 2. Reduction of indigo tetrasulfonate (I4S) by an anaerobic sediment. The oxidized form of I4S is blue; the reduced form is yellow. Tubes A through F show progression of the reduced front up from the sediment–water interface with increasing contact time. The indicators and solids were mixed briefly, allowed to settle, and then not disturbed until the photograph was taken. Tube G was fully reduced, but the I4S began to reoxidize from above after the cap was loosened.

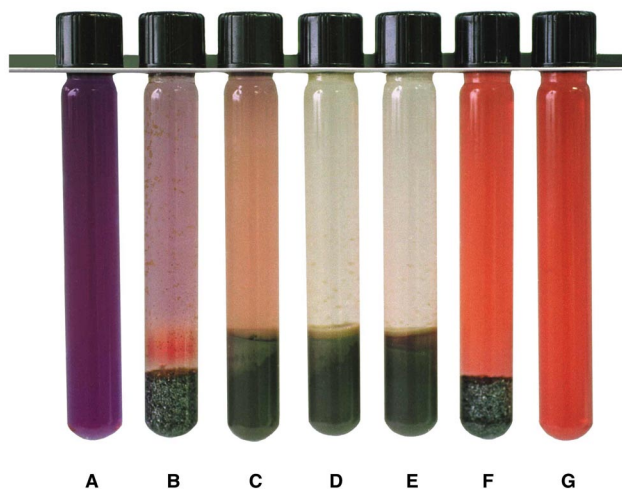
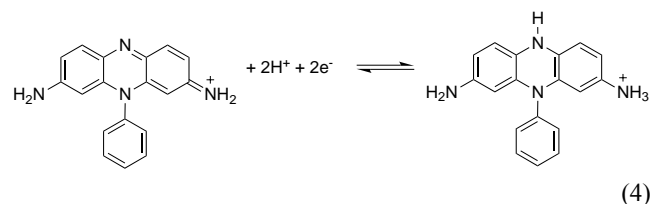


Figure 3. Color changes in two indicators (resazurin in tubes A through C and phenosafranin in tubes E through G) in the presence of two reductants (iron metal in tubes B and F and anaerobic sediment in tubes C and E). Controls are A (resazurin only), D (sediment only), and G (phenosafranin only). The indicators and solids were mixed briefly, allowed to settle, and then not disturbed for several days until the photograph was taken.

initiated by loosening the cap of tube G, thereby illustrating the reversibility of the color change corresponding to eq 3.

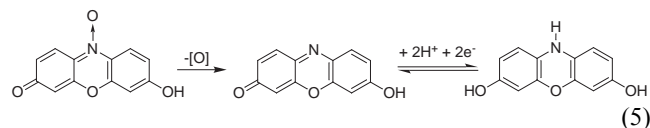
Phenosafranin, by contrast, is a red dye that bears a positive charge, as does its colorless reduced form, eq 4. The redox couple is reversible below pH 3 and above pH 7.5, but between pH 3 and 7.5 the indicator undergoes an irreversible, secondary reaction that leads to drift in the measured reduction potential of the couple [20].



Because most minerals have a negative surface charge under environmental conditions, both forms of phenosafranin tend to adsorb to sediments by cation exchange. This makes loss of color from the solution phase difficult to interpret because it may be due to reduction or to sorption (or both). The challenge is illustrated by the tubes shown in the right half of Figure 3. In tube E, phenosafranin and sediment were mixed and allowed to settle, resulting in immediate decolorization of the water column. Tube F was mixed with iron metal, but this tube shows no sign of decolorization (c.f. the control, tube G). The combination of these results suggests that decolorization of phenosafranin in the presence of sediment is mostly due to adsorption, not reduction.

Adsorbed dyes may still be redox active, however, and the expected color change might be observed if enough indicator is provided to produce visible staining of the particles. The effect of sorption on the lability of real environmental contaminants to transformation reactions is an important practical issue that has received only a modest amount of study [21, 22]. Although adsorption is a complication when it occurs on sediments, the process can be turned to advantage when binding can be used to immobilize the indicator onto a physical probe, such as a fiber optic sensor [23].

Resazurin reduces in two steps, irreversibly to resorufin and then reversibly to dihydroresorufin, providing color changes from purple to pink to colorless, eq. 5. Reduction of resazurin (disappearance of purple) has been used to characterize bacterial contamination in milk [24], quality of semen [25], and to quantify microbial activity in sediments [26]. The reoxidation of dihydroresorufin (appearance of pink) is widely used in anaerobic microbiology to indicate contamination with oxygen [27]. In addition, dealkylation of resorufin ethers is used as an assay for enzyme activity [28, 29]



These uncharged molecules are moderately hydrophobic and therefore sorb to sediments by hydrophobic partitioning to organic matter. Changes from strongly colored to weakly or uncolored (leuco) forms can provide ambiguous results in heterogeneous media because loss of color may also be due to adsorption. Figure 3 illustrates the interpretation of color changes involving resazurin in the presence of reducing sediment and granular iron metal. In both cases, resazurin (purple) was reduced to resorufin (pink) before the particles settled (c.f., tubes B and C with the control, tube A). The intermediate color in the upper part of tube B probably reflects partial reduction due to more rapid settling and/or lower reactive surface area of the iron metal. The red band at the bottom of tube B was observed on several occasions and may be due to partially overlapping gradients of pH and redox potential

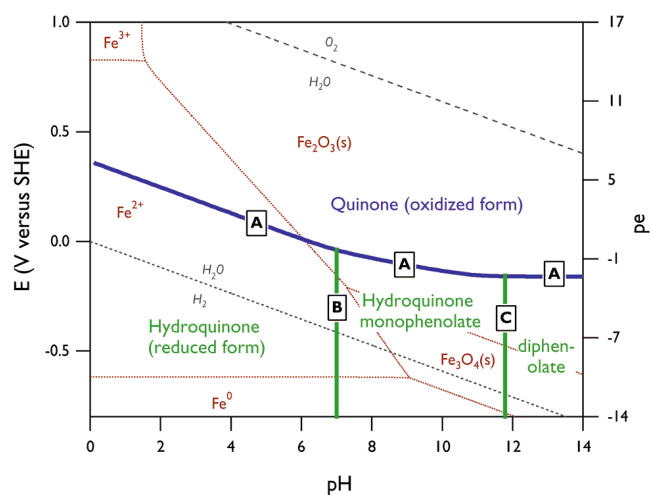


Figure 4. Stability (or Pourbaix) diagram showing equilibria involving water, iron, and indigo tetrasulfonate at conditions of natural waters (total $[\text{Fe}] = 10^{-6}$ M, 25°C). Hematite ($\alpha\text{-Fe}_2\text{O}_3$) and magnetite (Fe_3O_4) are assumed to be the controlling solid phase for iron speciation, but amorphous $\text{Fe}(\text{OH})_3$ would give qualitatively similar implications with respect to reaction with the indicators. Data were taken from [31, 32] for the inorganic species, and [20] for the indicators.

(recall that the corrosion of Fe^0 favors increased pH [30]). Clearly, even simple, qualitative experiments such as those illustrated by Figures 2 and 3, can pose challenging and edifying problems of interpretation for students.

Thermodynamics of Reduction. The speciation of most redox indicators is complicated by the presence of ionogenic functional groups in the oxidized and/or reduced forms. For example, the reduced form of I4S has two hydroxyl groups that are subject to deprotonation, resulting in three reduced species: the hydroquinone, the monophenoxide, and the diphenoxide. The relative stabilities of these species are represented as a function of potential (E) and pH in Figure 4.

Each line in Figure 4 represents a transition from predominance of one species to predominance of another. For example, the oxidized (blue) form of I4S is stable above line A, and the various reduced (yellow) forms are stable below line A. The lines dividing stability fields are drawn from the Nernst and Henderson-Hasselbach equations. For example, line A derives from the Nernst equation for the indigo dyes, eq 6 [20],

$$E = E'_0 + \frac{RT}{2F} \ln \frac{C_0}{C_r} + \frac{RT}{2F} \ln \left([\text{H}^+]^2 + K_{r1}[\text{H}^+] + K_{r1}K_{r2} \right) \quad (6)$$

using the formal potential (E'_0) and dissociation constants of the reduced form (K_{r1} , K_{r2}) of I4S (Table 1), and assuming that the total concentration of all oxidized forms (C_0) equals the total concentration of all reduced forms (C_r). Line B corresponds to the first $\text{p}K_a$ for reduced I4S ($\text{p}K_{r1}$) and is derived from the Henderson-Hasselbach equation using K_{r1} for

$$\text{pH} = \text{p}K_{r1} + \log \frac{C_b}{C_a} \quad (7)$$

I4S from Table 1 and assuming equal concentrations of the protonated (C_a) and deprotonated (C_b) forms. Line C reflects the second dissociation of the reduced form of I4S and is drawn from eq 7 using the corresponding value of $\text{p}K_{r2}$ (Table 1).

Superimposed on the stability fields for I4S, we have drawn the stability limits for various forms of iron using data from standard aquatic chemistry texts [e.g., 1, 31]. Comparison of the two reveals that the iron couple has a lower formal potential than the I4S couple at pH values greater than 6.1 (where line A crosses the line dividing Fe^{2+} from Fe_2O_3). Therefore, reduction of I4S by $\text{Fe}(\text{II})$ is thermodynamically favorable in this region, and it may be contributing to the color change observed in the presence of anaerobic sediment (recall Figure 2). The stability region for iron metal lies below -0.61 V (assuming the total $[\text{Fe}] = 10^{-6}$ M) at all pHs, so Fe^0 is a possible reductant of I4S under all experimental conditions. The resazurin/resorufin couple could not be added to Figure 4 because the half reaction is not reversible and a standard potential is not available; however, the rapid reduction of resazurin by anaerobic sediments and iron metal suggests that the half reaction has a potential that is higher than that of I4S. In fact, we found that resazurin is reduced by the reduced form of I4S but not the reduced form of methylene blue indicating that the reduction potential for resazurin falls between that of I4S and methylene blue.

Diagrams of E versus pH, such as Figure 4, are used quite commonly to summarize the thermodynamic properties of complex chemical systems [32–34]; however, they can contribute to misconceptions, particularly with respect to interpretation of the boundaries between stability fields. Sharp lines suggest sharp boundaries to many students, but these lines should be interpreted as continuous transitions from predominance of one species to predominance of another. This subtlety can be visualized with redox indicators such as I4S because the transition from the oxidized (blue) to the reduced (yellow) forms results in a transition zone that is green (this can be seen in Figure 2). Of course, the green transition zone also reflects a degree of disequilibrium due to kinetic controls on reactions in heterogeneous systems.

Kinetics of Reduction. The kinetics of reduction vary widely with the indicator, reducing medium, and other experimental variables. In fact, qualitative (visual) characterizations of the rate of indicator color change can be sufficient to provide students with evidence for the effects of reactant concentrations, mixing, etc. The importance of mixing is very apparent just by comparison of the color in sealed tubes set up with the same indicator and Fe^0 but subject to various methods of shaking. More subtle manifestations of mixing effects can be seen in the zones of color in Figure 2 and Figure 3. For example, progression of the color transition up tubes A through F in Figure 2 could be due to diffusion of reduced I4S or some soluble reductant released from the sediment. The red band in tube B of Figure 3 is an even more challenging manifestation of diffusion effects on redox chemistry.

Diffusion-controlled effects such as these take days to develop, but they can be destroyed in minutes by moving the tubes from a laboratory bench to a sunny window. The resulting convection currents due to solar heating are particularly dramatic with the color change afforded by the indigo sulfonates.

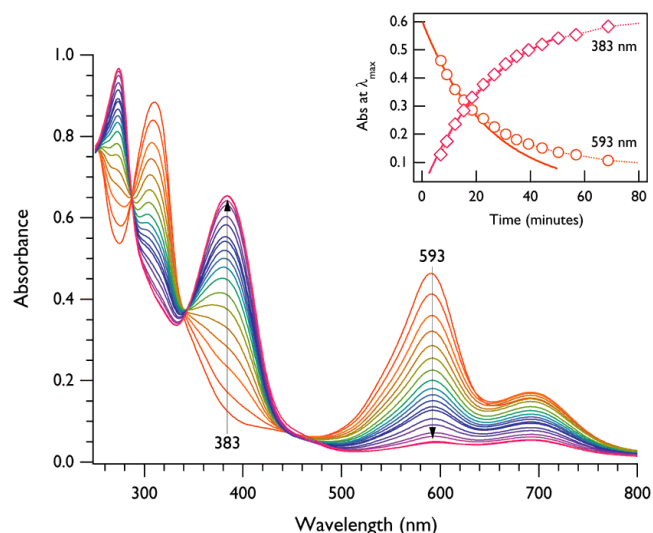


Figure 5. Kinetics of indigo tetrasulfonate reduction by Fluka Fe^0 (143 g/L) in a sealed, quartz cuvette with hand mixing between scans. Inset shows time courses for absorbance at the λ_{max} for I4S in its oxidized (593 nm) and reduced (383 nm) forms. The exact λ_{max} for each form is slightly affected by the presence of Fe^{2+} from corrosion of Fe^0 , and interactions between the dye and Fe^{2+} are also responsible for the peak at 700 nm. Fitting initial time course data to a first-order kinetic model (eqs 8 and 9) gives the smooth curves shown in the inset and rate constants given in the text.

Quantitative treatment of the reaction kinetics is possible by measuring absorbance as a function of time, either at one of the λ_{max} values given in Table 1 or by performing full scans of absorbance from ca. 300 to 800 nm. In Figure 5, we illustrate how the former method is related to the latter, using data for the reduction of I4S by Fe^0 . The inset shows the resulting disappearance plot for the blue color of the oxidized form of I4S ($\lambda_{\text{max}} = 593$ nm). The curve shows agreement between the data and a first-order kinetic model for decrease in absorbance, A ,

$$A_t = A_0 e^{-k_{\text{obs}} t} \quad (8)$$

and a disappearance rate constant (k_{obs}) equal to $0.041 \pm 0.002 \text{ min}^{-1}$ ($t_{1/2} = 17$ min). Essentially identical values were obtained by non-linear regression using eq 8 and by linear regression using ln-transformed absorbance data ($r^2 = 0.995$). The rate constant could also have been evaluated without recourse to regression [35].

Note that we have only fit data for the first 20 minutes ($n = 5$), because substantial tailing is apparent after one half-life. The major reason for this tailing is probably that the tube was mixed intermittently and only when it was not in the spectrophotometer. For advanced students, it may be informative to contrast the kinetics of indicator reduction in this system (where the reaction is far from equilibrium) to the tailing observed with acid–base indicators in buffered solutions (where reversibility of the reaction is important) [36]. The validity (both fundamental and pedagogical) of analogizing between redox and acid–base processes has been discussed in detail previously [37–39]. Another extension of the treatment given here would be to develop a kinetic model that includes terms for sorption, as well as reduction, of the dye.

Appearance of the yellow color associated with reduced I4S (at $\lambda_{\text{max}} = 383$ nm) is also shown in the inset to Figure 5. The initial data show good agreement to first-order appearance kinetics (eq 9)

$$A_t = A_0 (1 - e^{-k_{\text{obs}} t}) \quad (9)$$

using nonlinear regression to obtain k_{obs} equals $0.038 \pm 0.001 \text{ min}^{-1}$. The approximate agreement between the rate constants for appearance and disappearance of the two forms of I4S suggest that eq 3 accounts for most of the observed color change. Additional evidence that eq 3 alone is responsible for the color changes can be found in the consistent isosbestic points (where all spectra have the same absorptivity). Sharp isosbestic points are expected in simple systems where all absorbing components are linked by a single reaction [40]. To demonstrate material balance directly, absorbance data would have to be converted to molar concentrations using appropriate extinction coefficients or calibration curves.

Because values of k_{obs} can be determined quickly and routinely, teams of students can design their own experiments to compare the effects of various factors on k_{obs} . Some experimental variables that will influence k_{obs} include mixing rate and grain size of the solids in the medium. The net effect of pH may be challenging for students to interpret because pH can influence the indicator (e.g., most indicators have one or more pK_{a} s, which makes their color and absorbance spectra pH-dependent), the reductant (e.g., corrosion of Fe^0 is greatly accelerated as pH decreases), or the kinetics of reaction between the two. Figure 6 illustrates the effect of two other variables (degree of sulfonation and mass of reductant) on k_{obs} for reaction between derivatives of indigo and Fe^0 . For all three indicators, k_{obs} appears to vary linearly with the concentration of iron (mass of Fe^0 per volume of solution), as has been observed previously for the reduction of pollutants such as carbon tetrachloride and vinyl chloride [41, 42].

Regression on the three sets of data in Figure 6 gives estimates of the slope for each line, and these slopes represent mass-normalized rate constants, k_{m} , for each indicator. The results, summarized in Table 2, indicate that the order of reactivity with Fe^0 is I3S > I2S > I4S. The differences in reactivity are small, however, and this is consistent with previously reported results obtained with anaerobic sediment as the reducing medium [13]. Dividing each value of k_{m} by the specific surface area of the Fe^0 used in this study gives surface-area-normalized rate constants, k_{SA} . The resulting values (Table 2) are comparable to those for dehalogenation of many chlorinated hydrocarbons [c.f., 19, 43].

The intercepts in Figure 6 are not significantly different from zero (statistical data not shown), suggesting that there is no reduction of the indicators in the absence of Fe^0 . Corrosion of Fe^0 involves dissolution of Fe^{2+} , and this species could serve as a dissolved phase reductant of other solutes [41]; however, studies with immobilized substrates suggest that contact with the metal surface is the dominant reduction pathway under conditions that are environmentally relevant [44]. With anaerobic sediments, results similar to Figure 6 have been obtained by varying the ratio of solids to water and measuring k_{obs} for the reduction of various contaminants [21, 45]. The conclusion from such studies is that most contaminant reduction reactions in sediments are predominantly surface

Table 2. Rate Data for Reduction of Indigo Dyes by Zero-Valent Iron

Oxidized form	k_m^a	k_{SA}^b	r^2	n	E_m^7 (V)
Disulfonate	0.059 ± 0.007	0.29 ± 0.04	0.957	5	-0.125
Trisulfonate	0.074 ± 0.003	0.37 ± 0.01	0.996	5	-0.081
Tetrasulfonate	0.047 ± 0.011	0.23 ± 0.06	0.769	7	-0.046

^aIn $\text{hr}^{-1} \text{g}^{-1} \text{L}$. ^bIn $\text{hr}^{-1} \text{m}^{-2} \text{L}$ assuming specific surface area of the iron was $0.2 \text{ m}^2/\text{g}$.

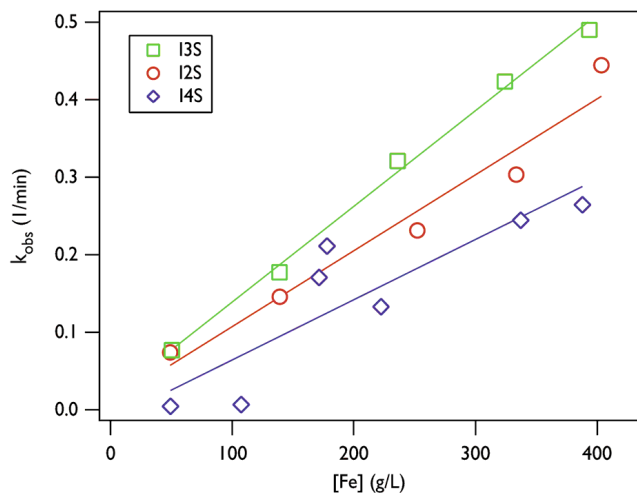


Figure 6. The effect of iron loading on the pseudo-first-order rate constants (k_{obs}) for reduction of indigo sulfonates. Each data point represents an independent experiment equivalent to the one shown in Figure 5. Linear regression on the k_{obs} versus $[\text{Fe}]$ data gives the lines shown and mass-normalized rate constants (k_m) given in Table 2.

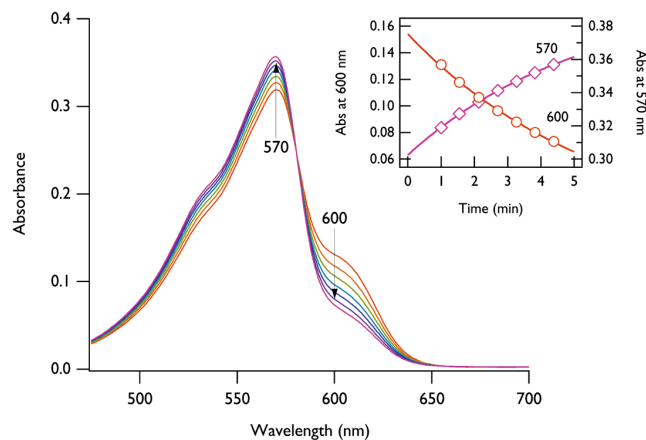


Figure 7 Kinetics of resazurin reduction by unfiltered pore water in a sealed, quartz cuvette with hand mixing between scans. Inset shows time courses for absorbance at the λ_{max} of resazurin (600 nm) and resorufin (570 nm). Fitting initial time-course data to the first-order kinetic model (eqs 8 and 9) gives $k_{\text{obs}} = 0.172 \pm 0.002 \text{ min}^{-1}$ for resazurin disappearance and $0.19 \pm 0.01 \text{ min}^{-1}$ for resorufin appearance.

reactions, and that there is little if any reaction occurring in the pore water [22]. Leading students to think about the chemistry of heterogeneous systems is one of the major benefits of the experiments described in this manuscript.

Some redox indicators appear to be more labile to reduction than most contaminants, however, so they might provide a more sensitive probe for dissolved-phase reducing species in

environmental systems. In fact, while testing the syringe filtration method (method II) on indicator/sediment combinations that gave complete reaction in as little as 1 min, we noted that the color of resazurin was not stable in $0.45\text{-}\mu\text{m}$ filtered pore water. The absorbance spectra in Figure 7 were taken on one of these samples, and they confirm that the observed color change was due to reduction of the indicator. Subsequent control experiments (not shown) proved that addition of H_2O_2 was effective at quenching the reduction of resazurin, and that H_2O_2 does not oxidize resorufin back to resazurin (recall from eq 5 that this reaction is generally not reversible). Therefore, we adopted a protocol that involved filtering roughly 5 mL of sediment pore water directly into a cuvette containing 75 μL of dilute H_2O_2 . The method proved to be robust as long as the amount of dilution was controlled by taking care to add consistent amounts of filtrate to the cuvette.

Conclusions

Anaerobic sediments and permeable reactive barriers of granular iron metal are two examples of environmental systems where the characteristic pathway for contaminant transformation is reduction. Redox indicator dyes can be used to probe the physico-chemical properties of these reducing systems in research on the environmental fate of contaminants, but also to form educational exercises that illustrate some of the many facets of environmental chemistry. Key concepts that can be highlighted with the methods described in this manuscript include (i) thermodynamic versus kinetic control on reactivity, (ii) mass transport versus chemical control on reaction kinetics, (iii) initial rates and pseudo-first-order kinetics, (iv) the relationship between chemical structure and reactivity, (v) reversibility, (vi) stability analysis with E and pH as master variables, (vii) adsorption versus reaction at surfaces, (viii) chemical versus biologically mediated reactions in natural systems, (ix) spatial and temporal variability in environmental samples, and (x) the “chemical” nature of both pristine and contaminated environments. The methods can be adapted to provide lessons at levels that range from high school to graduate school, as reflected in the diversity of people that have contributed to this work.

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