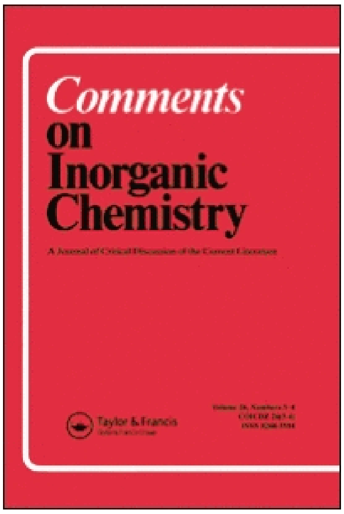

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Chemical Signal Enhancement by Chemical Amplification

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A catalytic cycle can be viewed as an amplifier for a signal represented by introduction of its catalyst to a system. Examples of catalytic signal amplification taken from the photographic literature are presented. Issues in the design of catalysts and system stability will be discussed and some ideas for modifying a photographic system for non-imaging applications are presented.

Keywords: catalysis, sensors, amplification, cobalt chemistry, imaging

INTRODUCTION

The detection of chemicals in the environment is a topic of current interest. There is a need for systems that can detect the presence of very low levels of ambient toxins, both natural (e.g., aflatoxin, bacterial toxins) and man-made (e.g., solvents, PCBs, industrial process wastes), as well as more specific applications such as the detection of unexploded land mines[1] or of arsenic in drinking water.[2] While there are well-established instrumental methods for detection of extremely small amounts of these materials, they depend on the availability of expensive, sophisticated laboratory instrumentation, and the costs associated with sample collection, transport, handling, and the actual analyses are substantial. The principal current need is for analytical methods that lend themselves to on-site analyses with minimal costs for instrumentation, analyst training, and the analyses themselves.

Color-forming chemical reactions have been used as analytical tools for about as long as chemistry has existed. Such reactions can be the basis for simple and robust test systems such as test strips (e.g., pH paper) and portable test kits (e.g., soil analysis kits). These test systems generally meet the

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requirements of low cost and simplicity, but are by nature relatively insensitive and of limited precision.

The sensitivity of a colorimetric test strip or kit based on the stoichiometric generation of a colored molecule (dye) for each molecule of analyte reacted is fundamentally limited by the extinction coefficient of the dye chromophore produced. Common organic chromophores have extinctions on the order of 10^4–10^5 $\mathrm{mol}^{-1}\mathrm{cm}^{-1}$, while inorganic chromophores are often much lower.

An estimate of the maximal sensitivity test strip, using a 1:1 stoichiometric reaction to produce a color change, can be arrived at as follows: The human eye can, under good illumination conditions, distinguish a 0.1 increase in dye density over a nominally white starting color, although there is some dependence on the color being perceived; the eye is less sensitive to intensity changes in yellow than in darker colors. For a blue or red color, this 0.1 increase in optical density represents a shift from white to a light but distinct pastel shade. Typical test strip coating thicknesses range from around 5–150 µm, somewhat more for indicators imbibed into paper or nonwovens, but optical effects are harder to analyze in the latter type of system than in a separately coated layer. For a clear film coating on an impervious plastic base, a 100 µm thickness would be on the high side, being more expensive to coat and harder to control, but not implausibly so.

Application of Beer's Law to the above parameters, 0.1 OD (optical density) change, 100 µm coating thickness, and 10^5 extinction coefficient, gives a dye concentration of 1×10^{-4} m in the reacted test layer. For an analyte of molecular weight 100 that reacts completely with the indicating chemistry to produce the chromophore, this color change, read in transmission, indicates the presence of 1×10^{-7} g analyte per cm^2 of test strip. This result assumes that the coating is pure indicator chemical, an unlikely scenario, but one that sets a limit on maximum test strip sensitivity. In general, sensing chemistry needs to be diluted in a film-forming binder, both to produce a physically robust coating and if it is a dip test, to prevent loss of indicating chemistry into the solvent in which the analyte is dissolved.

If the amount of analyte just calculated was contained in 1 ml of fluid, and 100% of the analyte was adsorbed into a 1 cm^2 test strip, this test strip would have a sensitivity limit of around 100 parts per billion (ppb). Although a reaction that converted one molecule of analyte into two molecules of dye would have double the sensitivity, virtually all the other assumptions made in this calculation result in overestimating the sensitivity of the test strip. For example, analyte is partitioned between the sample and the test strip, and most test strips do not have a substantially higher affinity for analyte than does the matrix the analyte is dissolved in. This leads to an approximately linear dependence of color signal on analyte concentration (rather than on total amount of analyte present) over a wide range of analyte concentrations. In the case above, the test strip might adsorb only one-tenth to one one-hundredth of the analyte in the 1 ml sample, leading to at least a 1–2 order of magnitude loss in sensitivity.

This apparent sensitivity level does not allow for sampling losses that are inherent in analyses such as volatile organics in soil by headspace analysis of solvent extracts. Adventitious analyte adsorption onto test strip components other than the active sensor component (e.g., colorimetric reagent) also produces a reduction in the calculated sensitivity by reducing the analyte available for reaction. The net result of these impacts on potential test strip sensitivity is that although there are a few commercially available test strips that can be read in the 20–50 ppb range (with very limited precision), most test strips have a lower readability limit of 0.5 ppm or higher.

The standard approach to attaining maximal analyte sensitivity is to take advantage of instrumental methods that involve photon or ion counting combined with electronic amplification. Inherent in this approach is the use of an electronic instrument. While great strides have been made in producing smaller, more portable and rugged analytical instruments, the costs of their purchase, maintenance, and user training remain fairly high, and ultimate sensitivities still require large, very expensive laboratory instrumentation. The continued use of current test strips, in spite of their sensitivity and accuracy limitations, is one indication among others that there is a market need for simple, low-unit-cost chemical sensors that would combine the convenience of test strips with sensitivity approaching that of instrumental methods.

A purely chemical approach to increasing the sensitivity of colorimetric chemistry is chemical amplification. Perhaps the broadest definition of chemical amplification is any chemical mechanism by which the presence of a single molecule is translated into the presence of multiple detectable molecules. By this definition, a chemical reaction that uses one molecule of analyte to produce two chromophores would be a chemically amplified system. Much higher sensitivity can be obtained if one molecule of analyte generates many molecules of a dye, for example, a mechanism in which analyte or its reaction product serves as a catalyst for a color-forming reaction. This could produce an orders-of-magnitude increase in sensitivity.

CHEMICAL AMPLIFICATION

There are several examples of chemical amplification mechanisms in the literature. While some of these have been applied to chemical sensing, for example enzymatic assays based on inhibition of catalysis, perhaps the most dramatic examples of chemical amplification involve the sensing of photons rather than chemical analytes. Modern photographic film imaging is an example of a detection system that undergoes an approximately 10^6-fold chemical amplification of an initial photon-induced chemical event.

"Imaging" in its broadest sense is the generation of detectable molecules in response to some event. If the event is a photon at a point in a plane and the response is the generation of dye at a corresponding point in the image plane having visual absorption spectra appropriately related to the wavelength of the photon event, we have a color photograph.

Viewed as a photon detection system and considering the extinction coefficients of available dyes, the sensitivity of color photography is limited by the number of dye molecules produced per photon. If only one dye molecule were produced per photon, color photography would require several minutes of exposure to bright sunlight or UV arc lamps to get a decent image, but silver chloride and bromide can be reduced autocatalytically, enabling dye/photon ratios of 10^6 and exposure times often well under 0.01 seconds.

Classic silver halide–based photography achieves chemical amplification via the direct generation of a catalytic center, referred to as the "latent image." If a sufficient number of photons are absorbed by a silver halide crystal in a short enough time period, a cluster of silver atoms is formed while bromine atoms are released to the surrounding environment.[3] These clusters, which can ideally contain as few as three or four atoms,[4] can serve as catalytic sites for further chemical reduction of the rest of the crystal. This is done by treating the exposed film to a mild reducing agent, the "developer," that preferentially reduces those silver halide crystals containing silver atom clusters. The result is a black and white negative image, which can then be used to expose another sheet of silver halide crystals to produce a black and white positive. Alternatively, the reduced silver can be discarded, and the developer oxidation products turned into dyes to produce a color negative.[5] Figure 1 shows both imaging chemistries.

Because four photons can lead to the complete reduction of a micron-sized silver halide crystal, a tremendous chemical amplification factor is attained in

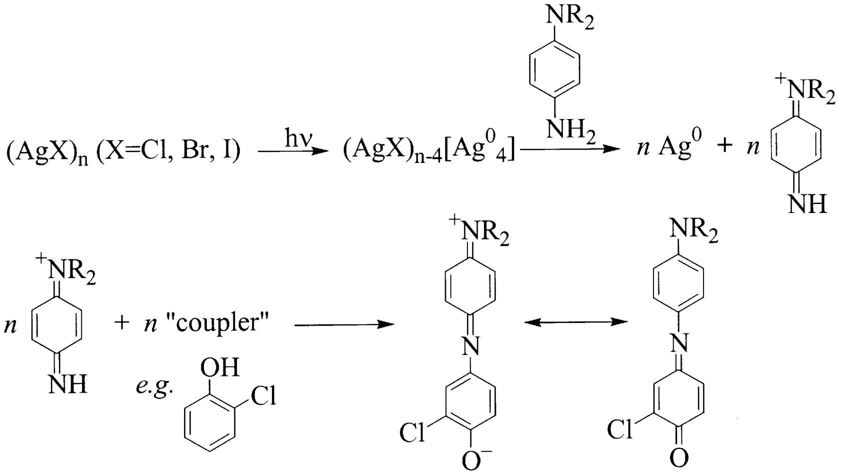


FIGURE 1 Photographic development mechanisms.

modern photographic films. Photographic chemists have been searching for other photon-amplifying chemistries for over a century. While none have come close to silver halide's amplification factor, there are a number of other examples of significant chemical amplification. Amplified photoresist photolithography is an important recent example.[6] In one form of amplified photoresist, a proton is generated by photodecomposition of an iodonium or sulfonium salt. The proton serves to catalyze the hydrolytic cleavage of a monomer unit side chain in a polymer. Loss of the side chain results in a change in solubility such that either the original polymer or the hydrolyzed polymer can be selectively removed by exposure to an appropriate solvent. Since the formation of one proton can result in the cleavage of many side chains and can thereby effect a change in solubility of several polymer molecules, there is a net amplification of the initial photoevent. Amplification factors of 10^3 have been reported.[7]

Related amplification chemistry has been used by Polaroid Corporation to produce a non-silver photographic image.[8] In this system, a proton photogenerated as above is used to catalyze the hydrolysis of an ester to produce more protons. The result is a pH change in the film that can be registered by specially designed indicator dyes. By use of multiple layers sensitive to different photon wavelengths, a full-color image can be produced. Maximum attained amplification has not been reported, but is presumably not high enough to use in camera film since the amplifying chemistry is dissolved in a polymer binder rather than being highly concentrated in a crystal.

A different approach to non-silver chemically amplified photon detection was taken by Eastman Kodak during the silver price crisis of the 1970s. Kodak produced a series of patents that rely on photoreduction of a cobalt(III) complex to produce cobalt(II) imagewise.[9] The Co(II) centers then react with a ligand present in the film to produce a Co(II) complex that can catalyze ligand exchange in nearby Co(III) complex molecules, as shown in Figure 2. With an appropriate ligand, the new Co(III) complex formed can be the image. For example, $Co(NH_3)_6^{3+}$ is a pale tan color, but the Co(III) complex of 2′-pyridylazo-2-naphthol (PAN) (1) is deep green-black, and the Co(II) PAN complex is a catalyst for the exchange of PAN for NH_3.[10] Alternatively, the image can be formed by reaction of the released NH_3 with pH indicator dyes or molecules that trap ammonia to produce color.[11]

Simple models suggest that amplification factors of 10^2–10^4 should be attainable using this chemistry.

CHEMICALLY AMPLIFIED CHEMICAL SENSING

All of these systems use a photon to produce a catalyst. The catalyst then proceeds to catalyze a chemical reaction involving materials present in

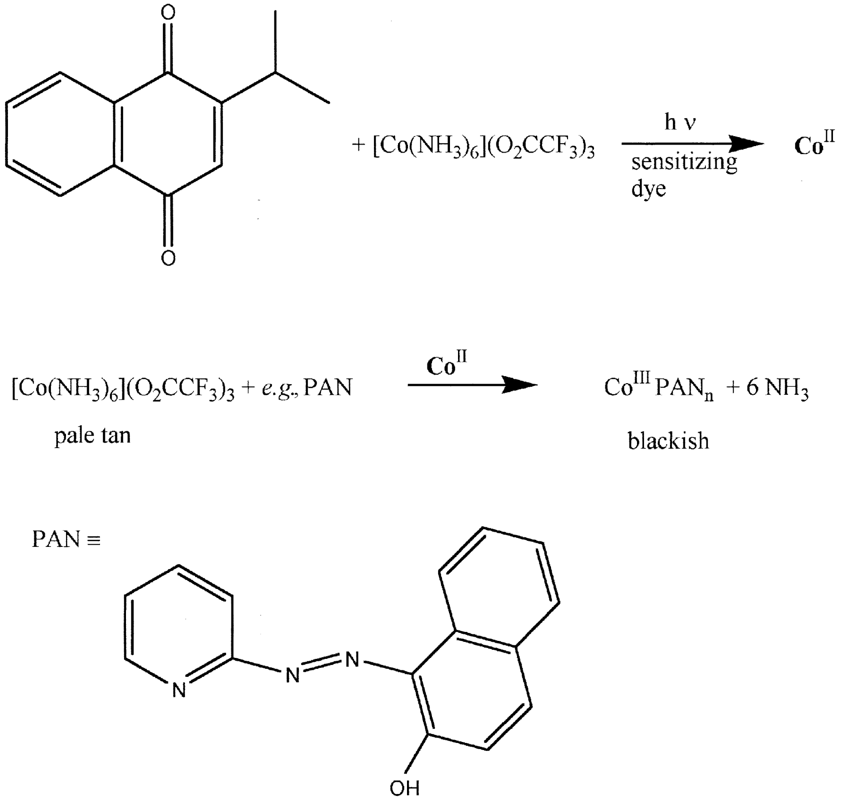


FIGURE 2 Kodak Co-based amplified imaging scheme.

proximity to the catalyst that ultimately results in the formation of an intensely colored molecule in numbers substantially greater than the number of photons absorbed in producing the catalyst. Another view of the Kodak system is that the Co(III) chemistry detects and amplifies the presence of a reducing agent. Therefore it can be used to amplify any system that forms either a strong enough reducing agent to reduce some Co(III) to Co(II) or a redox catalyst for Co(III) ligand exchange. In later work, Kodak workers have noted that this chemistry can be applied to the amplified detection of reducing agents generated in a chemical, non-imaging process, e.g., in chemical analyses using enzymatic redox chemistry.[12] A general detection scheme could be one in which exposure to an analyte triggers the formation or release of a reducing agent that then catalyzes the Co(III) ligand exchange reaction, as shown schematically in Figure 3.

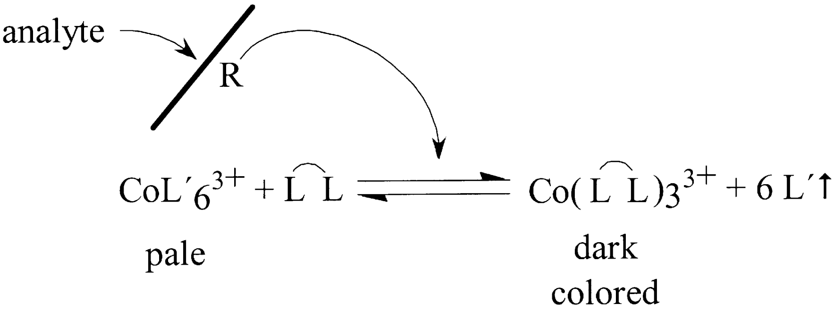


FIGURE 3 Schematic representation of Co amplification applied to chemical detection.

Our laboratory has been interested in developing simple chemical sensors with high sensitivity, and thus we were interested in pursuing and understanding the Kodak cobalt amplification system.

CHEMICAL AMPLIFICATION USING COBALT CHEMISTRY

Figure 4 shows the visible spectra of (a) the PAN ligand, (b) the reaction product of PAN with the N-cyclohexylsalicylaldimine complex 2 (Figure 5), and (c) the result of reacting (b) with excess PAN and $Co(NH_3)_6^{3+}$.[13]

Spectrum (c) is what produces the final color of the PAN ligand system, which changes from the yellow-orange of PAN itself to a dark greenish black. The limited spectral overlap between PAN and its Co(III) complex allows for the study of the stoichiometry and kinetics of these reactions.

We have examined whether the choice of Co(II) source has any effect on the rate of the ligand exchange reaction at Co(III). As shown in Table 1, there is only a slight rate difference between Co(II) complexed to three very different ligands. This is most likely a result of ligand-dependent small differences in the rate of PAN exchange at Co(II).

The spectroscopic data is consistent with the Co(II)-PAN complex being the active catalyst regardless of which of the three starting Co(II) complexes is used. Qualitatively, the bis(N-phenylsalicylaldimine) complex also seems very similar to its cyclohexyl analogue although we have not performed precise kinetic studies on it yet. These data are consistent with a mechanism in which the displacement of the starting ligand by PAN or its analogues is very rapid compared to the rate of catalysis.

An interesting observation not noted in the Kodak patents is that excess PAN inhibits the rate of formation of the PAN-Co(III) complex, as shown in Table 2.

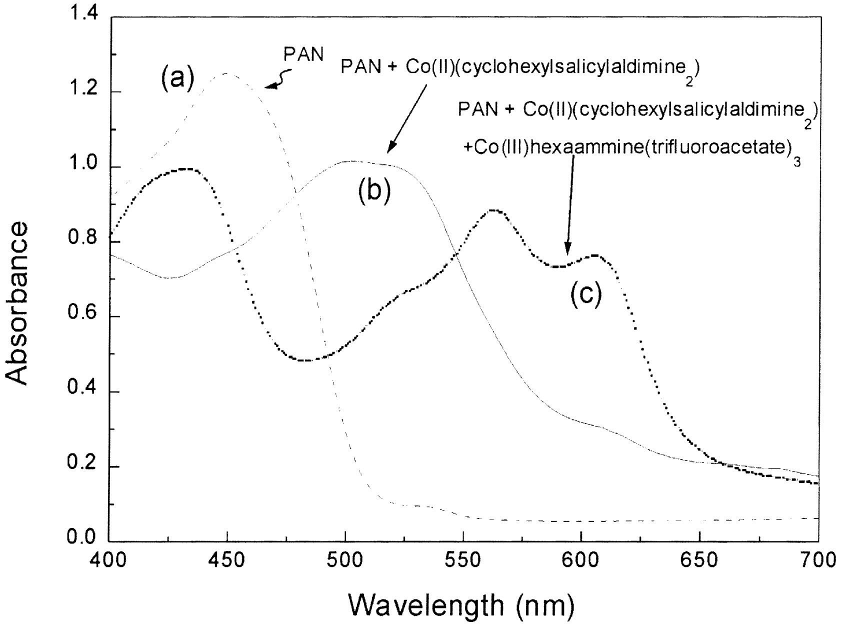


FIGURE 4 Visible spectra of starting materials for Co-based amplification.

Excess ligand is not generally expected to inhibit catalysis that is redox-based, since ligand dissociation is not usually a requirement in redox catalysis. A possible explanation is that a complex of higher ligand:metal stoichiometry is formed that is less catalytically active. We have so far been unable to find evidence for such a complex. Job's plots, shown in Figure 7, of Co(II) versus the ligands shown in Figure 6, PNS (PAN-sulfonic acid, 3) and PAR (2-pyridylazoresorcinol, 4), indicate primarily formation of 1:2 complexes for both. We have observed that the PAR does not display this inhibitory behavior.

Interestingly, the end state of the Co(II) catalyzed $Co(NH_3)_6^{3+}$ /PAR reaction is a Co(II)-PAR complex, rather than a Co(III) one. This implies that the Co(III)-PAR complex is a much stronger oxidizing agent than is the Co(III)-PAN complex. Preliminary cyclic voltammetric data at 1 mm Pt electrodes shows that the redox behavior of these systems is complex. None of the species present in the (complex 2)|$Co(NH_3)_6^{3+}$ |(PAN or PAR) system is part of a reversible couple in acetone under these conditions. We are continuing to examine the electrochemistry of this system.

Another interesting observation about this chemistry is that the rate of reaction is solvent dependent. In fact, there is no catalysis of Co(III)-PAR

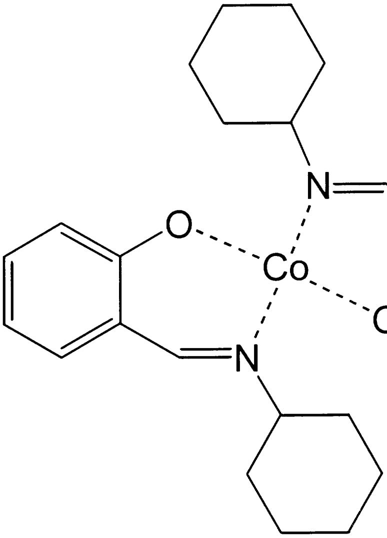


FIGURE 5 Structure of Compound 2.

formation from PAR and $Co(NH_3)_6^{3+}$ in water, and reaction is faster in acetone than in acetonitrile.

MAKING AN AMPLIFIED CHEMICAL SENSOR

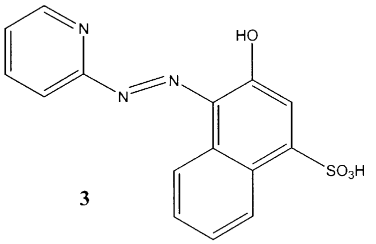
For the ultimate user, the simplest format that a chemical sensor can be presented in is that of a test strip. While simple test strips such as pH paper are made by simply imbibing a reagent solution into a paper or porous polymer strip and hence are limited in their precision and chemical complexity, a great deal of work has been done on applying the precision multi-layer coating technologies developed for the photographic film industry

TABLE 1 Reaction of 0.013 M Co(III) Hexaammine Trifluoracetate and PAN with Co(II) Catalyst in MeOH at Instrument Ambient Temperature

Co(II) catalyst	Rate constant ($s^{-1} \times 10^3$)
bis[4,4,4-trifluoro-1-(2-naphthyl)-1, 3-butanedionate]	2.76 ± .14
bis[N-cyclohexylsalicylaldimine]	1.09 ± .05
bis[acetylacetonate](2,2′-bypyridine)	2.73 ± .14

TABLE 2 Kinetics of formation of PAN-Co(III) complex

PAN	$k_1 s^{-1}$	$t_{1/2}$ s
0.0005	0.0081 ± 0.0004	82 ± 4
0.001	0.0072 ± 0.0004	96 ± 5
0.002	0.0053 ± 0.0003	133 ± 6.7
0.0036	0.0036 ± 0.0002	218 ± 11
0.01	0.00083 ± 0.00004	710 ± 36



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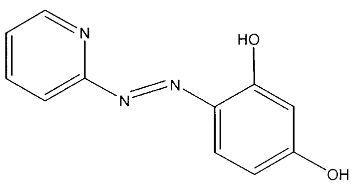

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FIGURE 6 Structures of Compounds 3 (PNS) and 4 (PAR).

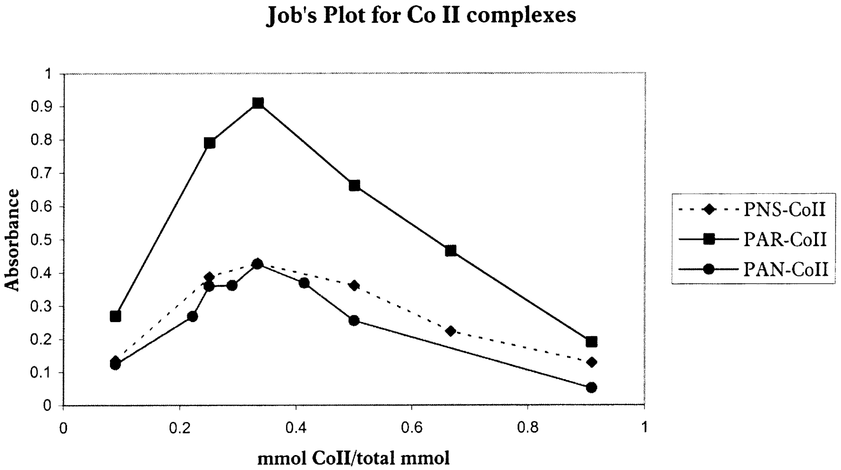

FIGURE 7 Stoichiometry of Co(II) complexes; 1 cm cell, 1.5×10^{-5}m Σ(ligand + Co).

to high accuracy and sensitivity test strips.[14] There are several issues that need to be considered in designing a multi-layer integral test strip using a chemical amplification system such as the Kodak Co/PAN system.

Issue number one is what chemistry would go into each layer. In the general scheme of Figure 3, it is clearly simplest to put the actual analyte detecting chemistry at the top of the layer stack. That way, application of a sample to the top of the device can release the signaling molecule such that it can only diffuse through the stack in the useful direction. The signaling molecule, be it catalyst or a catalyst precursor, must encounter both Co(III) and PAN. Therefore, either coating conditions must be found where Co(III) and PAN display long-term stability in the absence of catalyst, or various layer binders must be chosen so that not only can the catalyst diffuse to the layer where reaction will actually occur, but one of Co(III) or PAN must be able to find its way there too, either as a direct result of sample exposure or as a consequence of a secondary treatment such as heating or illumination.

A corollary to this issue is that each of the layer compositions must be produceable as a fluid that can be coated onto a moving web, preferably using standard wet-coating techniques and equipment. This implies that not only must a suitable polymer binder and solvent be found, but that coating additives like surfactants must be found that do not affect the reactive components. The reactive components must be soluble in or evenly dispersible in the coating fluid.

These layer compositions must be formulated such that each layer can be coated over the preceding one without mixing with it or having components diffusing between the layers. The polymer binders and coating additives are the reaction medium, and since we have shown that the Co/PAN system and its relatives are solvent sensitive, there are likely to be additional constraints on the selection of layer components. Shelf-life issues will also have a major impact on system design.

It is clear then that converting the Co(II/III) amplification system into a test-strip form chemical sensor is a nontrivial task, which is presumably why it has not yet been commercialized. Continuation of the studies described herein will give us the understanding necessary to achieve the stability, reproducibility, and sensitivity that will lead to commercial chemical sensors incorporating chemical amplification.

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