"TOWARD DEVELOPMENT OF A FIBER OPTIC SPECTROELECTROCHEMICAL SENSOR FOR IN SITU METAL ION DETERMINATIONS"

Honors Project

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Abstract

This paper describes in detail the development, design, and performance of a fluorescence fiber optic spectroelectrochemical sensor for in situ determination of metal ions. The sensor design employs a bifurcated optical fiber bundle coupled to an electrochemical flow cell containing a large surface area reticulated vitreous carbon working electrode, and permits fluorescence detection of metal ions following their preconcentration by electrodeposition and subsequent anodic stripping and complexation with an appropriate ligand. Analytical figures of merit for fluorescence detection of aqueous copper (II) as the calcein complex are reported, and potential applications to process control and on-site environmental applications are presented.

Introduction

Fiber optic sensors continue to experience increased application in a variety of *in-situ* measurement scenarios, for example, on-site environmental analyses.[1] Various transduction schemes are typically employed in order to enhance the selectivity of the analysis, the most common approaches involving chemically specific reagents or biological recognition elements immobilized at the sensor's fiber terminus.[2] A promising but less frequently employed transduction scheme is to fabricate an electrochemical cell at the optical fiber tip, yielding a fiber optic spectroelectrochemical (FOSEC) sensor. This thesis presents preliminary results of work in the authors' laboratory that establishes calibration data for measuring fluorescence by using a spectrophotometer configured for use with a bifurcated bundle fiber optic probe, as well as data directed toward the development of a FOSEC sensor suitable for on-site environmental measurements.

Experimental

Reagents

All reagents were used as received from Fisher Scientific. Fluorescein, $C_{20}H_{12}O_5$ in 0.05M disodium hydrogen phosphate buffer made using 13.412 g of Na_2HPO_4 per liter of deionized, degassed water. Tris (2, 2'-bipyridyl) dichlororuthenium (II) hexahydrate, $C_{30}H_{24}Cl_2N_6Ru*6H_2O$; Calcein, $C_{30}H_{26}N_2O_{13}$; Copper (II) Sulfate, CuO_4S; and Potassium Chloride, KCl, were also used.

Apparatus

Many configurations of a Sciencetech Inc. spectrophotometer were used. For traditional calibration measurements, a Model 20-180 xenon lamp was used as the light source. Other measurements used an Ocean Optics tungsten-halogen lamp (HL-200-HP) as an excitation source. The xenon lamp source provided light which was focused by the Focusing Lens Unit, Model C7, then excited in a Model 9030 monochromater at an excitation wavelength specified by the user from a remote station. The tungsten halogen lamp bypassed the excitation monochromater and focusing lens unit, and used a linear variable filter (LVF-HL). The light excites fluorescing particles in either a cuvette chamber as shown in Figure 1, or the light travels through a bifurcated bundle fiber optic cable in which the light excites fluorescing particles in a solution suspended on an inverted column (apparatus shown in Figure 2) or a solution that contains the resting column (apparatus shown in Figure 4). The RBU220-1-SSS bifurcated bundle fiber optic cable assembly is functional at the range of 190-1100 nM. It has a 2.2 mm diameter and SMA905 connectors on both ends. A 2SU290-1-S/0.5 cable was used to connect the RBU220-1-SSS to the excitation monochromater. The input of the bifurcated bundle is

attached to the excitation monochromater using the 2SU290-1-S/0.5. The probe of the bifurcated bundle is attached to the columnator assembly. The LC-4N and LC-4U are columnator assemblies with the lens plano side out, and the lenses are recessed a few mm. The LC-4N measures 380-2200 nm (Vis-NIR) and the LC-4U measures 170-2400 nm (UV-Vis). The LC-4N-A and LC-4U-A measures the same as the corresponding columnator assemblies above, but the lens is not recessed at all. The output of the bifurcated bundle is attached to the emission monochromater. An alternate means of fluorescence measurements with the bifurcated bundle fiber optic cable was with a flow cell. An illustration of this data collection is shown in Figure 3. Solution was pumped into the flow cell for fluorescence measurements by either a programmable syringe pump (BS-8000, Braintree Scientific), or a Fisher Model 13-876-2 peristaltic pump. The light emitted by the fluorescing particles is collected by the bifurcated bundle probe and directed to the emission monochromater. In the case of the cuvette assembly, the light is reflected into the emission monochromater at an emission wavelength also set at the remote station. As the filtered light is received, the light is cast onto the photomultiplier tube (PMT), model PMH-02. The higher the number of fluorescing particles that are cast upon the tube, the greater the signal that is produced by the PMT. By using the gain and high voltage options of the PMT, the signal can be optimized for viewing at the remote station. A BAS CV-50 W Voltammetric Analyzer was used to provide stripping potentials for the spectroelectrochemical determination of copper.

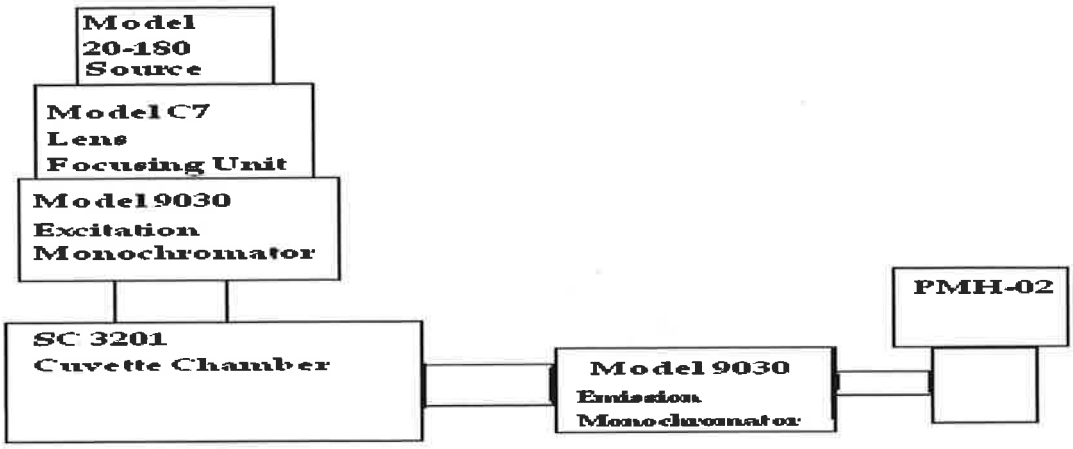


Figure 1. Layout of spectrophotometer with cuvette assembly.

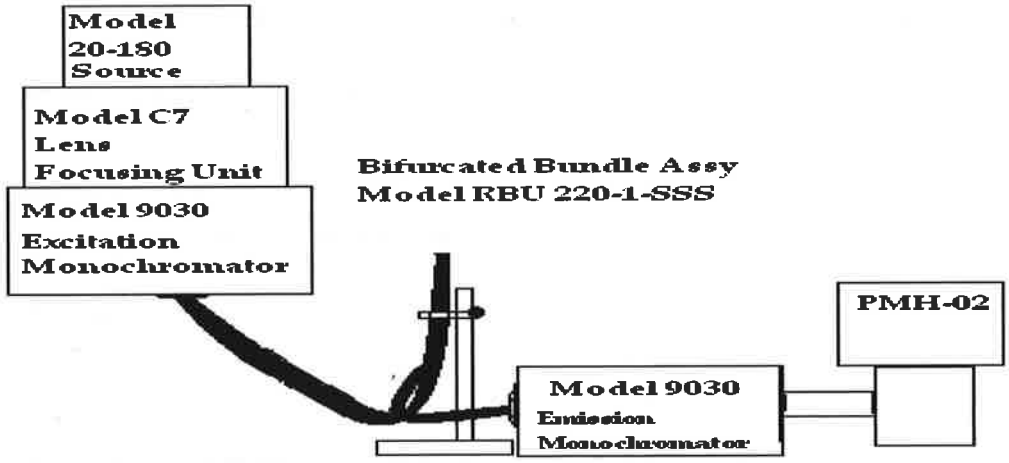


Figure 2. Layout of spectrophotometer with bifurcated bundle assembly.

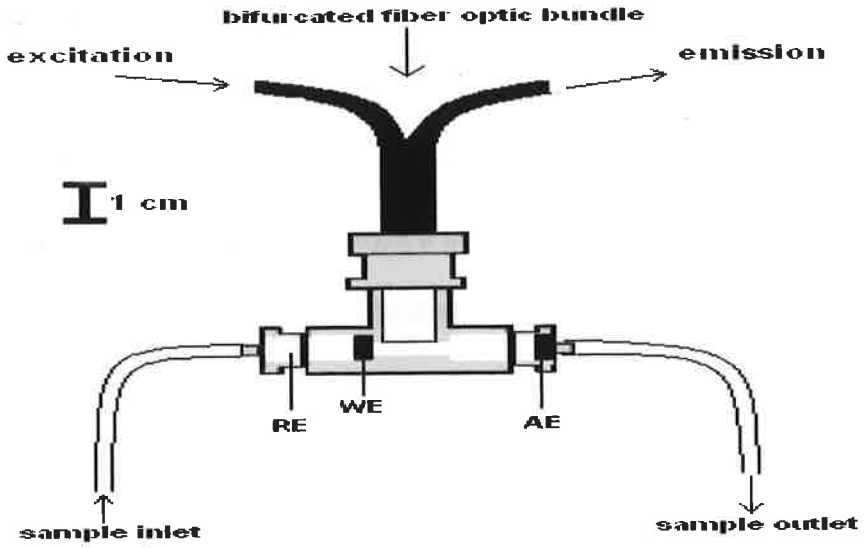


Figure 3. Layout of bifurcated bundle assembly (top) embedded in flow cell with electrodes for preconcentration measurements.

Procedure

A) Calibration with Cuvette Chamber

Each calibration experiment with fluorescein in the cuvette chamber began with the preparation of new fluorescein standards in varying concentrations. Proceeding from the preparation of standards, the spectrophotometer and the remote station was powered up for usage. Concentrations of fluorescein ranged from 20.00 to 100.00 ppb. The highest concentrated fluorescein standard (100 ppb) was placed in a cuvutte and then placed in the sample chamber. The cuvette chamber was closed before proceeding with the optimization of signal using the PMH-02 photomultiplier tube (PMT). At this time, the PMT was adjusted for an optimal signal (5.0 volts/ 50% signal was considered optimal). This was done by adjusting the PMT gain to X1000 and the high voltage setting was set to 775. The emission monochromater was set to 520 nM, and the excitation monochromater was set to 492 nM.[3] Signals were not allowed to approach 10.0V, and

high voltages of no more than 1000 V were used. The Time Constant was set to 1000 msec, and the remote station was set to scan a range of 400- 700 nm in the "Wavelength Mode" of the spectrophotometer. After each scan, the emission monochromater was set to 200 nm, making the incandescent light a non-factor to the PMT. Peak heights were recorded by measuring the peak with the cursor from the remote station computer and subtracting an average of the baselines from 450 nm and 650 nm.

Tris (2, 2'-bipyridyl) dichlororuthenium (II) hexahydrate was scanned at a range of 400 to 850 nm, with λ_{ex} set to 467 nm, and the λ_{em} set to 604.5 nm.[4] Concentrations of Tris (2, 2'-bipyridyl) dichlororuthenium (II) hexahydrate ranged from 2.96 to 14.0 ppb, and the 14.0 ppb standard was inserted into the cuvette chamber. The PMT was adjusted to attain a signal of only 15% (1.47 volts). To attain this signal, the high voltage was set to 944 volts with the gain set to X1000.

Calcein was scanned using similar excitation and emission wavelengths as fluorescein measurements. λ_{ex} was set to 492 nm, and the λ_{em} was set to 517.65 nm. Standards from 100 to 500 ppb were used, and the PMT was adjusted to a 50% signal (5.01 volts) with the 500 ppb standards in the cuvette chamber. To attain this signal, the gain was set again to X1000 and the high voltage was tuned to 775 volts. Dwell time in this case was set to 330 msec.

At this time, the 500 ppb (8.03 X 10^-7 M) calcein standard was used as a platform to add copper. λ_{ex} was set to 492 nm, and the λ_{em} was set to 515.60 nm. Five standards were made, with varying mole ratios of calcein and copper. The standards ranged from only 8.03 X 10^-7 M calcein, 1 mole of copper to 5 moles of calcein, 2:5, 3:5, 4:5, and a 1:1 mole ratio. The 8.03 X 10^-7 M calcein standard was placed in the cuvette chamber,

and the signal was adjusted for a 50% signal (5.06 volts). The gain was once again set to X1000, and the high voltage was set to 795 volts. Dwell time remained at 330 msec.

B) Calibration Using the Bifurcated Bundle Fiber Optic Cable Assembly

In these experiments, a bifurcated bundle was used in the place of the cuvette chamber. Calibration was done with the bifurcated bundle using a series of columnator assemblies in two positions. Measuring the fluorescence of any of the three reagents used was done with the bifurcated bundle in either of two ways. The first position of the bifurcated bundle is shown in figure 2 as the probe of the bundle is inverted. A cuvette assembly was fabricated for this position by attaching a bushing over the top of the columnator assembly and holding it in place with parafilm. The second position used was achieved by dipping the columnator assembly in solution as illustrated in Figure 4. Measuring fluorescence with the bifurcated bundle was very similar in procedure to measurements made with the cuvette assembly, with only a few exceptions. Precautions were made to ensure that stray light did not enter the columnator assembly. The scan range could not include the 490-500 nm range due to large self-absorbance peaks that would occur, which was very dangerous to the PMT. Most scans were performed beginning at 500 nm to avoid this problem. When using columnator assemblies LC-4N and LC-4U, a small piece of polyethylene (usually a small piece of Siran Wrap) was attached to the end of the columnator assembly by using an O ring. Polyethylene was not needed when using the LC-4N-A and LC-4U-A. With each calibration run, the bifurcated bundle was adjusted to optimize the highest signal possible by the same manner in which cuvette chamber calibrations were done.

Calibration data was obtained for calcein with the fiber optic probe in the inverted position with use of the LC-4N-A columnator assembly. The excitation wavelength was set to 469nM, and the emission wavelength used was 521.45 nM. A signal of only 7% (0.70 volts) was obtained for a 500 ppb standard (range used was same for calcein in the cuvette assembly). This signal was achieved by tuning the PMT to a gain of X1000 and a high voltage of 998 volts. The dwell time was set to 330 msec.

Calibration was also obtained for calcein with the fiber optic probe dipped in solution, as shown in Figure 4. The range of standards used for this experiment was 0.5 ppm to 20 ppm. The 20 ppm standard was placed in a beaker and the excitation wavelength was set to 492 nM, with the emission wavelength set at 517.75 nM. Dwell time remained to be 330 msec, and the gain remained set to X1000. The high voltage was set to 971 volts, producing a 50% signal (5.02 volts).

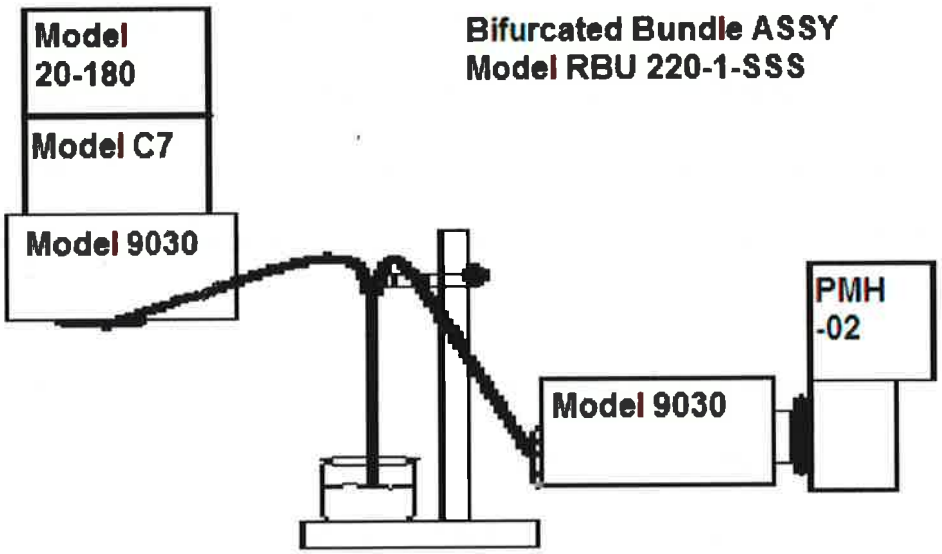


Figure 4. Layout of spectrophotometer with bifurcated bundle assembly with columnator assembly in solution.

C) Calibration Using the Bifurcated Bundle Fiber Optic Cable Assembly Inverted in Flow Cell

Solution was placed in a beaker and a variable speed pump was attached to the flow cell. The pump cell pumped solution from the beaker (which was usually placed on a stir plate to allow for spikes of standard) into the flow cell and then back into the beaker. Before beginning a scan, the beaker was filled with DDH_2O, and the flow cell was adjusted to ensure that no air bubbles existed.

For calcein calibration, a stir bar was placed in a 250mL beaker on a stir plate. After flushing the flow cell with DDH_2O, 100.00 mL of 0.05 M $Na_2HPO4*7H_2O$ in 0.1 M KCl was placed in the beaker. The pump was set to medium speed (~1 mL/min), and the flow cell was adjusted to ensure that no air bubbles were present. The spectrophotometer was changed from wavelength scan to time scan, and the time frame was set to 800 seconds. With the gain set to X1000, the high voltage was tuned to 950 volts. The excitation wavelength was set to 492 nM, and the emission wavelength was set to 517.75 nM. After beginning the time scan, a 1.00 mL spike of 0.01 M calcein was added at 100 seconds, and every 100 seconds after. This resulted in concentrations from 1.028 μM to 6.791 μM.

With the highest concentrated solution of calcein mentioned previously in the beaker (6.791 M), copper was added sequentially in the same manner to produce the copper/calcein complex calibration. Every 100 seconds (starting at 100 seconds), 0.52 mL of 0.2 mM copper (II) sulfate was added. This resulted in copper concentrations from 0.924μM to 6.327 μM. Wavelengths of importance remained the same from the above calcein calibration, because the experiments were performed back to back.

D) Copper Concentration Determination By Use of Fiber Optic Spectroelectrochemical (FOSEC) Sensor

In this phase of experimental work, the light source was changed from the xenon lamp to the tungsten-halogen lamp. Also, the excitation monochromator and lens focusing unit were bypassed and a linear variable filter was used. The filter was tuned manually to ~ 492 nM, and the emission wavelength was set to 517.75 nM. This change in source allowed a gain of X100 to be used, and again a high voltage of 950 volts was used. 1 μM copper (II) sulfate was pumped through the flow cell (after the cell had been flushed with DDH2O and the cell was relieved of air bubbles) via a programmable syringe pump. The pump was set to 1.000 mL/min. A voltammetric analyzer was used to apply a potential of -500 mV to the working electrode of the flow cell for 180 seconds while the copper solution was being pumped through the cell. After 180 seconds, the syringe pump was stopped and the copper solution was replaced with 5μM calcein. The calcein was injected manually (~10 mL) to flush out the copper, and fresh calcein was placed on the syringe pump, and the pump was set to 1.000 mL/min. With calcein in the flow cell, the potential on the working electrode was set to 200 mV for 60 seconds.

E) Depth Study

A study was done with the bifurcated bundle assembly to determine if the depth of solution in contact with the columnator assembly affected the level of signal that is a function of fluorescing particles. The columnator assembly LC-4U-A was used for this study with a solution of 20 ppm calcein. This experiment was done in two different configurations of the bifurcated bundle: with the fiber optic probe in the inverted position (see Figure 2), and with the probe dipped in solution (see Figure 4). With the probe

dipped in solution, a 25ml graduated cylinder was used to house the solution. The probe was started out flush with the bottom of the graduated cylinder, and the signal was recorded. This process was repeated as the probe was moved up in recorded distances from the bottom of the graduated cylinder. With the probe inverted, measured amounts (0.04 ml) of calcein were added to the bushing parafilmed to the top of the columnator assembly. The distance of the solution from the tip of the columnator assembly was calculated using the radius of the bushing (depth= volume/π*radius^2).

F) Signal Intensity/Peak Wavelength as a Function of Dwell Time

The dwell time is set at the remote station to control the time interval that the monochromator remains at a fixed wavelength. If a large time constant is used (for our purposes, a large time constant of 1000 msec was usually used), peaks may appear dampened with a short dwell time. This is due to the detector signal having inadequate time to rise before the monochromater has moved to the next wavelength. A study was done to see the effects of the dwell time on the signal intensity as the bifurcated bundle's probe reflected light off a palladium mirror (bifurcated bundle used in place of the cuvette chamber with a LC-4N columnator assembly). The λ_{ex} was set to 498 nm, and the λ_{em} was set to the same wavelength so that a reflection signal was established. A 50% signal was established with a selected dwell time 110 msec set at the remote station (the λ_{em} adjusted to 499.30 nm to achieve an optimal signal), and the PMT was set to a gain of X100 with a high voltage of 789 V. A scan was performed over the range of 475-525 nm. Due to the number of scans that were to be performed, a multiscan option was selected at the remote station to record 14 scans on one file. The peak height was

recorded and graphed as a function of time. The same experiment was used to determine the effect of dwell time on the wavelength of the peak produced by the reflected light.

Results & Discussion

A) Calibration with Cuvette Chamber

Calibration data was achieved from four different systems (fluorescein, ruthenium, calcein, and the calcein/copper complex) using the cuvette chamber in the spectrophotometer system. All peaks were measured with respect to the baseline. The fluorescein solution was used first and was found to produce very linear results. Figure 5 displays the data recorded, and the sensitivity was found to be 50.8 mV/ppb. The R^2 value for this data plot was 0.9997. Calibration data was slightly less accurate using Tris (2, 2'-bipyridyl) dichlororuthenium (II) hexahydrate as the fluorescing standard. Figure 6 shows the data recorded for ruthenium with an R^2 value of 0.9976, and with a sensitivity of 73.7 mV/ppb. Calcein produced even less linear data than Tris (2, 2'-bipyridyl) dichlororuthenium (II) hexahydrate. With similar settings to the fluorescein complex, Figure 7 is graph of data produced by calcein. The R^2 value was recorded to be 0.9887, and the sensitivity was found to be 6.17 V/μM. When complexed with copper, the R^2 value dropped to 0.935 and the sensitivity was found to be -4.63 V/μM of copper. This data was recorded in Figure 8.

B) Calibration Data with Bifurcated Bundle

Measurements with the bifurcated bundle had the most success in the inverted position (refer to Figure 2). Linearity was lost with the fiber optic probe when compared with the cuvette assembly. Fluorescence was measured for calcein with the fiber optic probe in two different conformations: with the probe inverted or dipped in solution. Figure 9 is a

calibration curve for calcein with the fiber optic probe in the inverted position. With a R^2 value of 0.9936 and a sensitivity of 451.8mV/μM, it can be said that the fiber optic probe inverted is more linear but less sensitive than the cuvette chamber. With the probe dipped in solution, the results were very poor. This can be viewed in Figure 10.

C) Calibration Using the Bifurcated Bundle Fiber Optic Cable Assembly Inverted in Flow Cell

Signal transients and calibration curves can be viewed for calcein and calcein/copper complexes in Figures 11 and 12. The sensitivity was calculated to be 291 mV/μM for calcein determination, and likewise, the sensitivity for copper was shown to be 412 mV/μM. The success that we found in these experiments led to the development of a FOSEC sensor, and those results are shown below.

D) Copper Concentration Determination By Use of Fiber Optic Spectroelectrochemical (FOSEC) Sensor

Preliminary results for the spectroelectrochemical determination of copper are shown in Figure 13. Duplicate signal measurements following preconcentration and stripping of copper Figure 13. Based on these measurements, a sensitivity of 2500 mV/μM and a detection limit of 10 nM may be estimated. When compared to the sensitivity with other methods used, sensitivity with the FOSEC sensor provided an increase five-fold. The detection limit provided an estimated five-fold improvement from detection limits published by similar methods.[5]

E) Depth Study

In the instance of the inverted columnator assembly, the depth of solution could be increased in much smaller increments than in the case of the columnator assembly dipped

in solution (mm vs. cm). Therefore, the inverted columnator showed a steady rise in signal as the depth was increased. The dipped columnator assembly showed very small deviation from the signal established at initial depth. This is most likely due to the fact that the depth of solution was already providing maximum signal. The inverted columnator assembly showed signs of the signal leveling off near the initial depth of the dipped columnator. Results can be viewed in Figure 14. The steady rise of signal leveled off at ~ 4.25 cm, which is assumed to be the point of maximum signal.

F) Signal Intensity/Peak Wavelength as a Function of Dwell Time

In this experiment, dwell time had a significant effect on the signal intensity. There was no point, as the dwell time increased, that signal intensity appeared to level off. The rise in signal intensity as a result of dwell time provided a very linear relationship as evidenced by the graph in Figure 15. Figure 16 shows that no such relationship existed between signal wavelength and the dwell time. The signal wavelength remained virtually unchanged as the dwell time was increased.

Conclusion

Work in this area is continuing, with present efforts aimed at exploring several signal transient anomalies (a major signal deviation is found at the time the syringe pump is turned on after calcein injection) believed to be related to poor flow dynamics within the sensor body. Flow cells with better flow dimensions will be constructed in the future. The effects of electrodeposition time, flow rate, and electrode positioning will be explored as this method continues to be optimized. The effect of interferants will also be looked at.

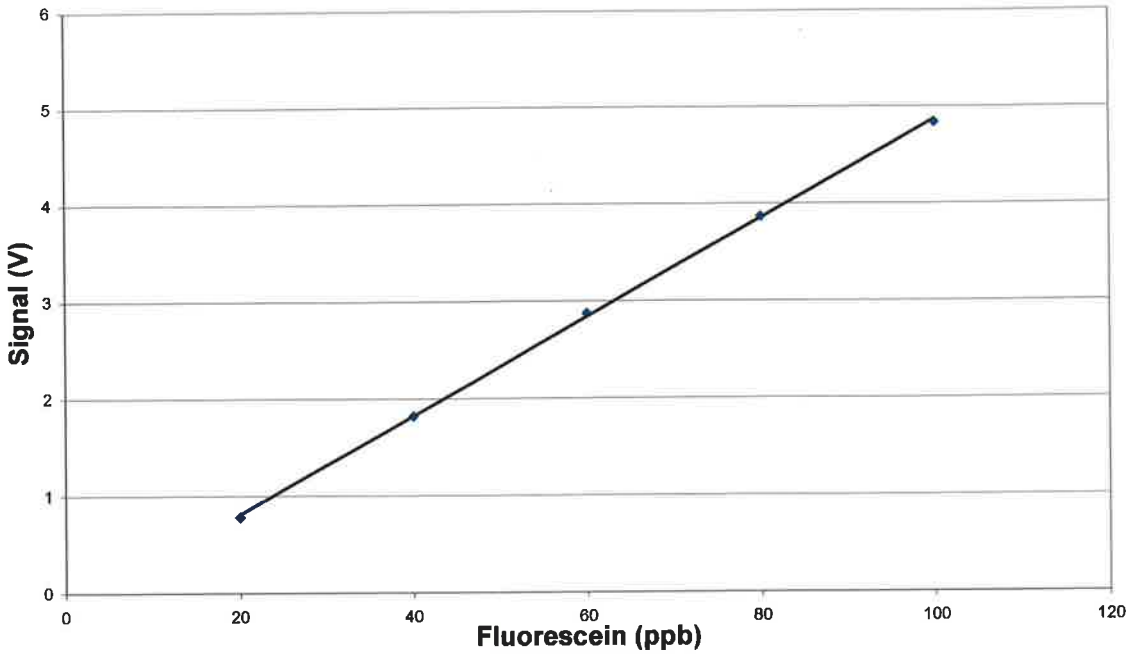


Figure 5. Calibration curve for Fluorescein using cuvette chamber.

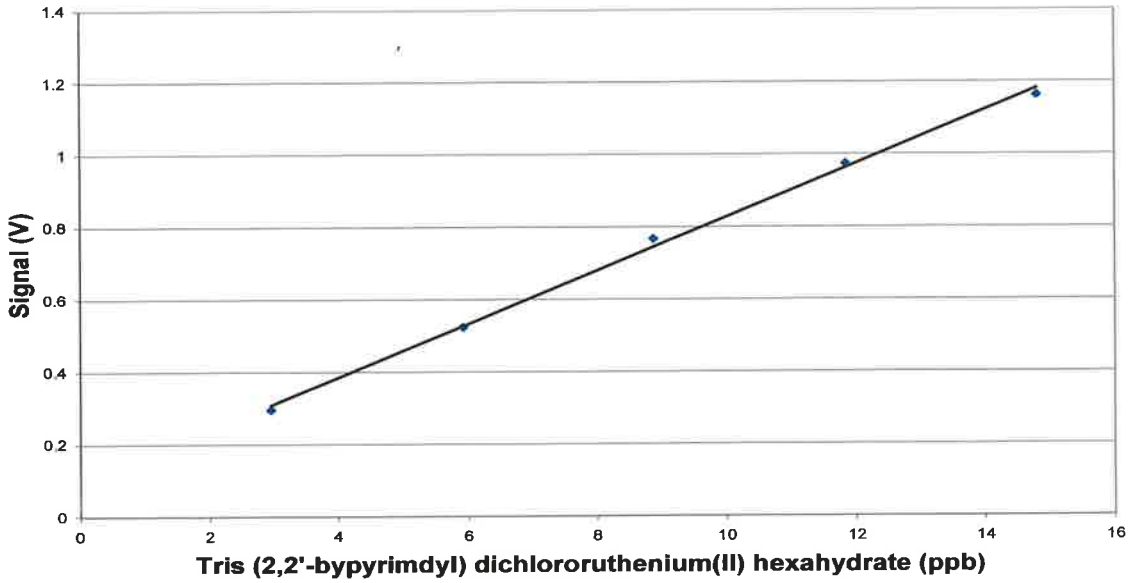


Figure 6. Calibration curve for Tris (2, 2'-bipyridyl) dichlororuthenium (II) hexahydrate using cuvette chamber.

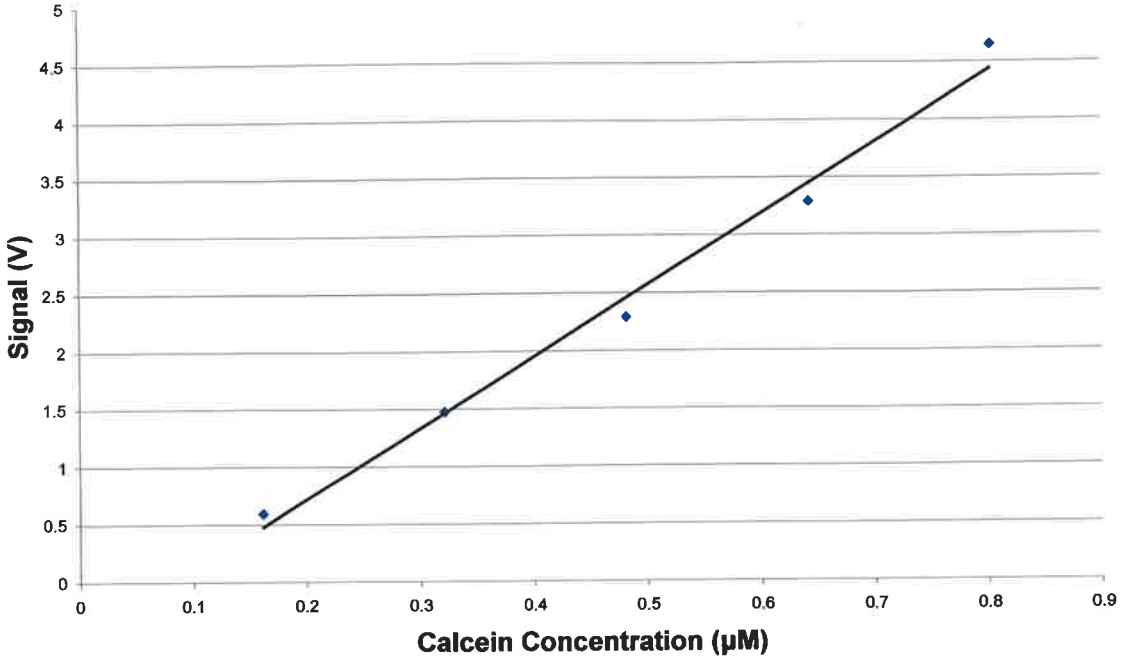


Figure 7. Calibration data for Calcein using cuvette chamber.

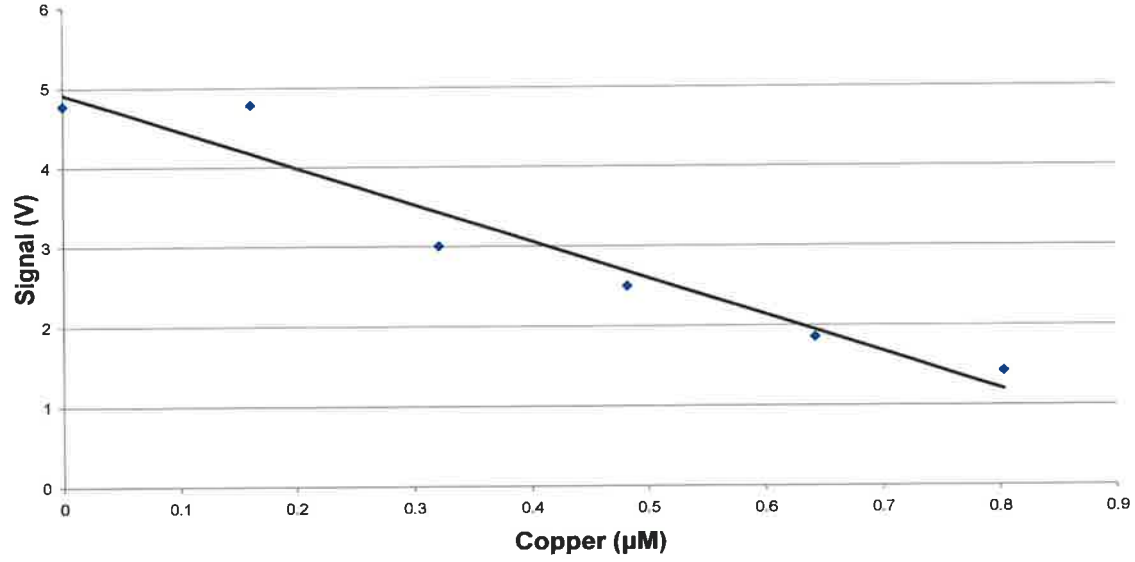


Figure 8. Calibration curve for Copper/Calcein complex using cuvette chamber.

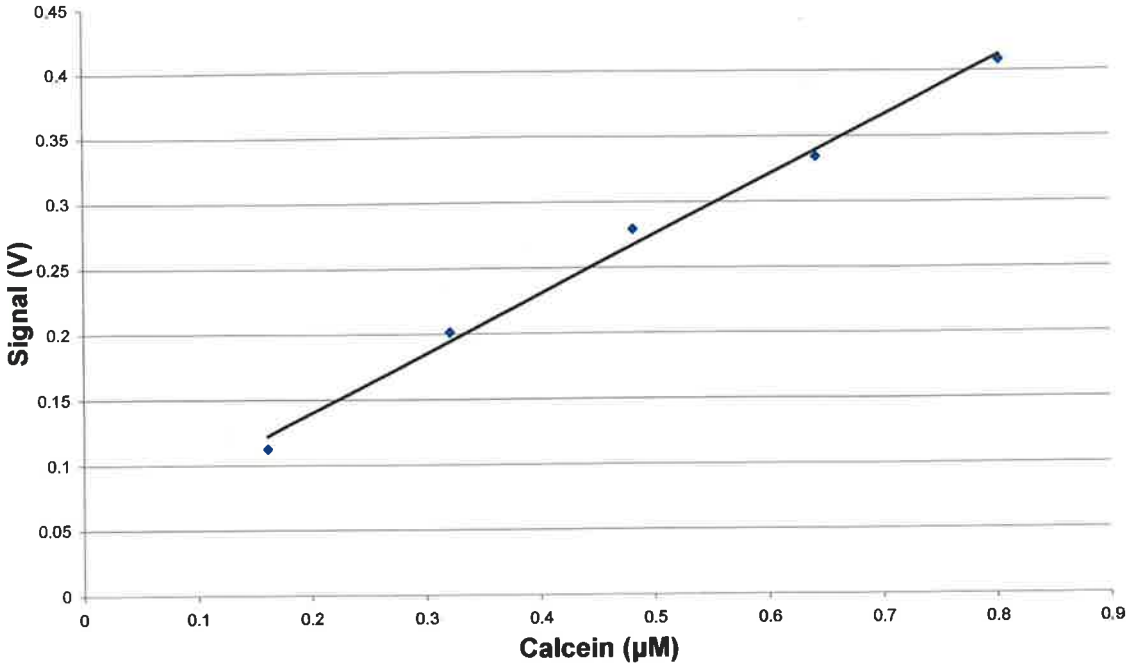


Figure 9. Calibration data for Calcein using bifurcated bundle in the inverted position.

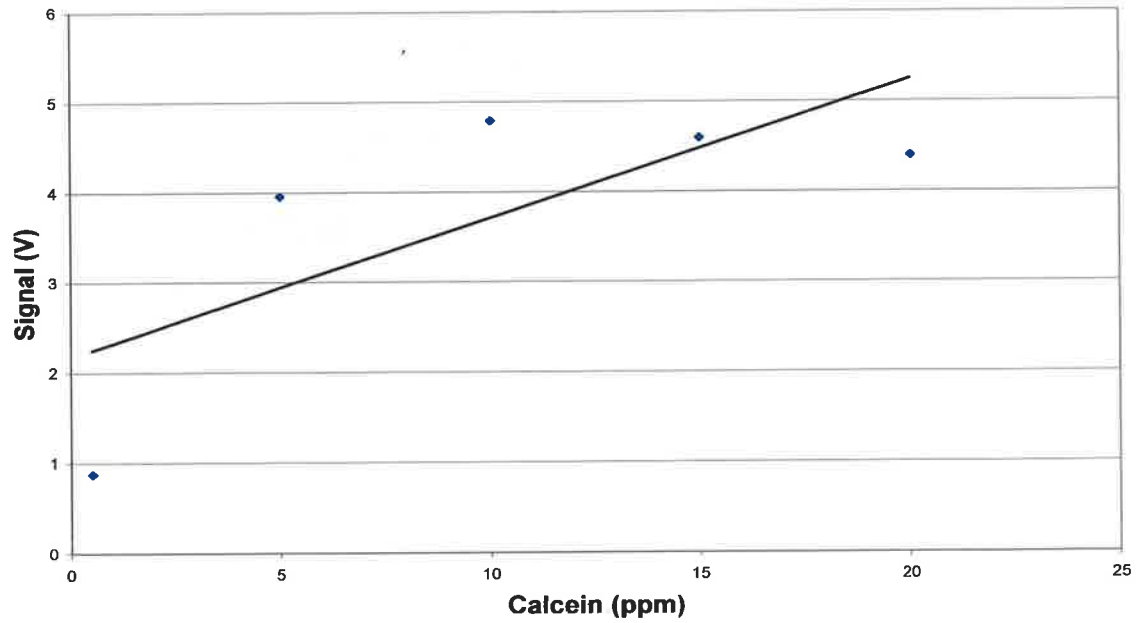


Figure 10. Calibration data for calcein using bifurcated bundle in the dipped position.

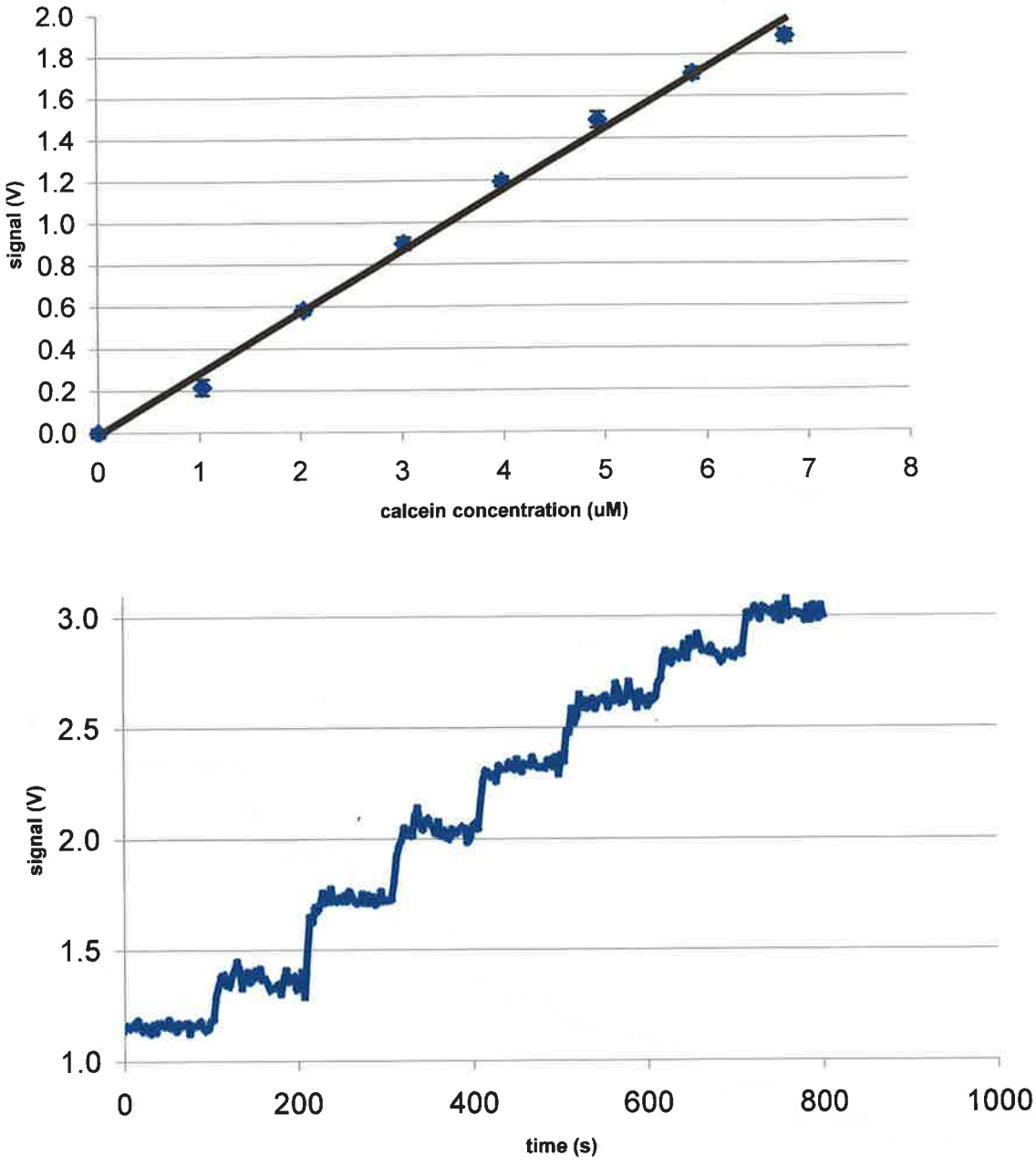
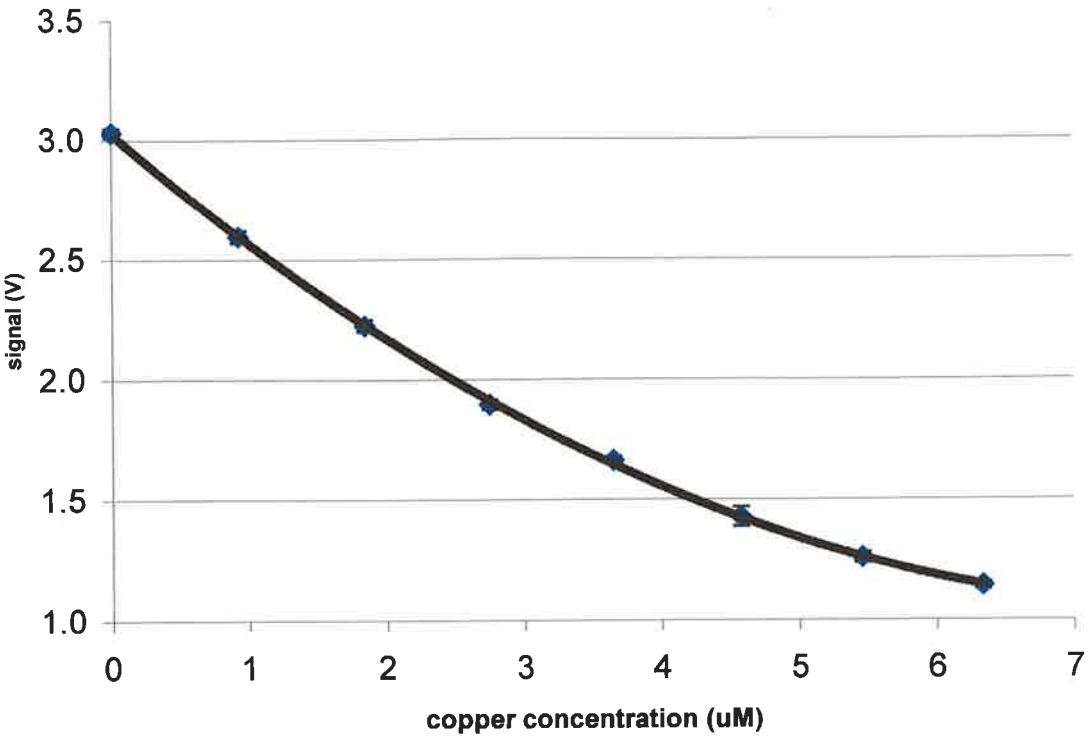


Figure 11. Calibration curve (upper) and signal transient (lower) for calcein (measured using a xenon lamp and grating monochromator).



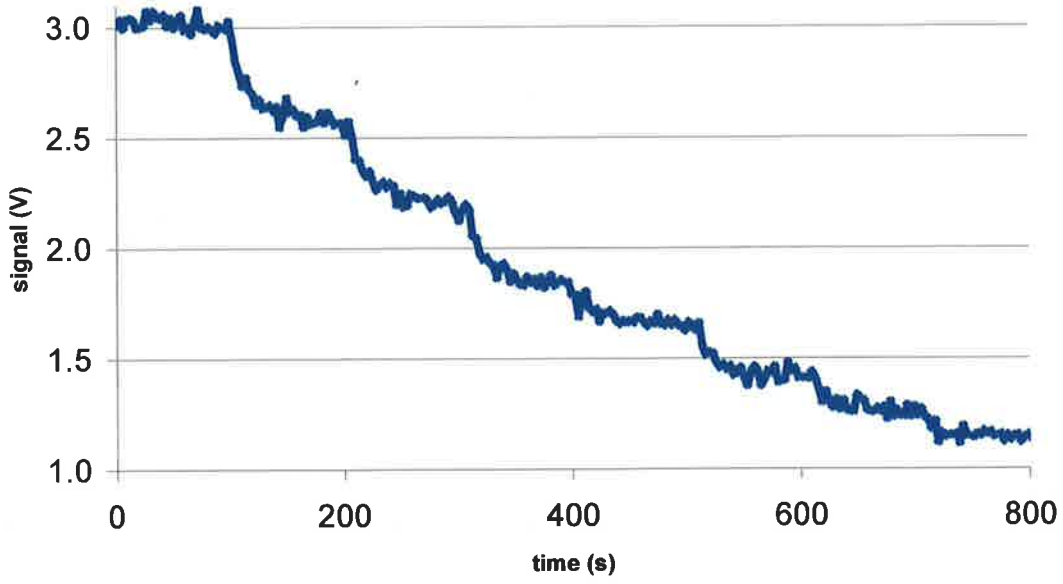


Figure 12. Calibration curve (upper) and signal transient (lower) for calcein/copper (II) complex (measured using a xenon lamp and grating monochromator).

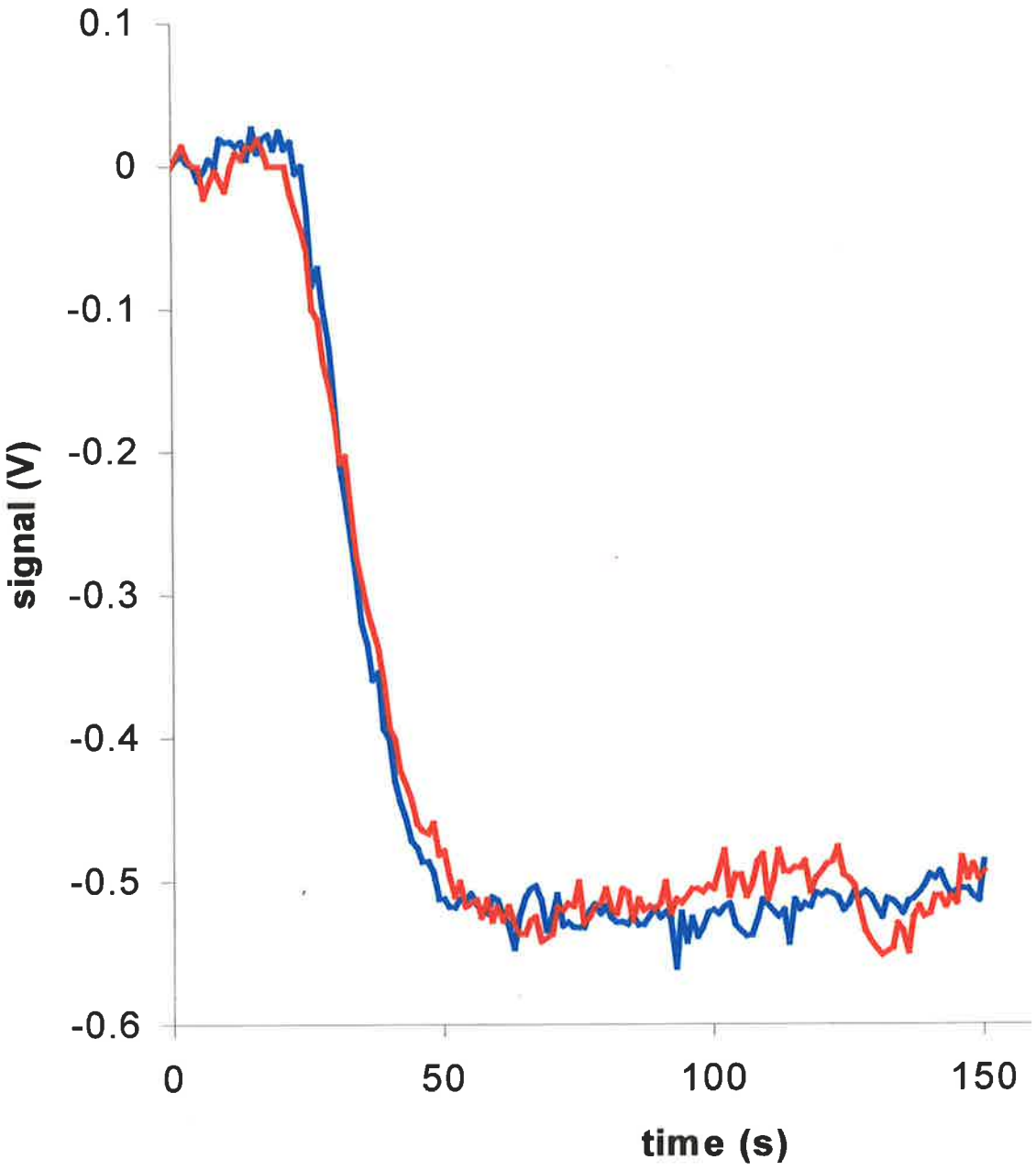


Figure 13. Signal transients for fluorescence spectroelectrochemical determination of 200nM copper (measurements made using a tungsten-halogen lamp and linear variable filter).

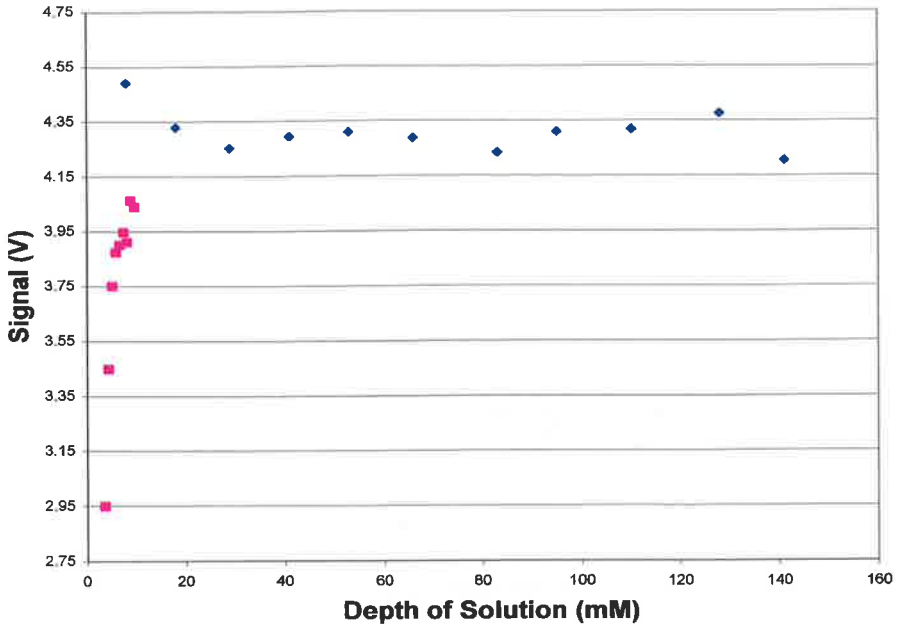


Figure 14. Signal Intensity as a Function of Solution Depth

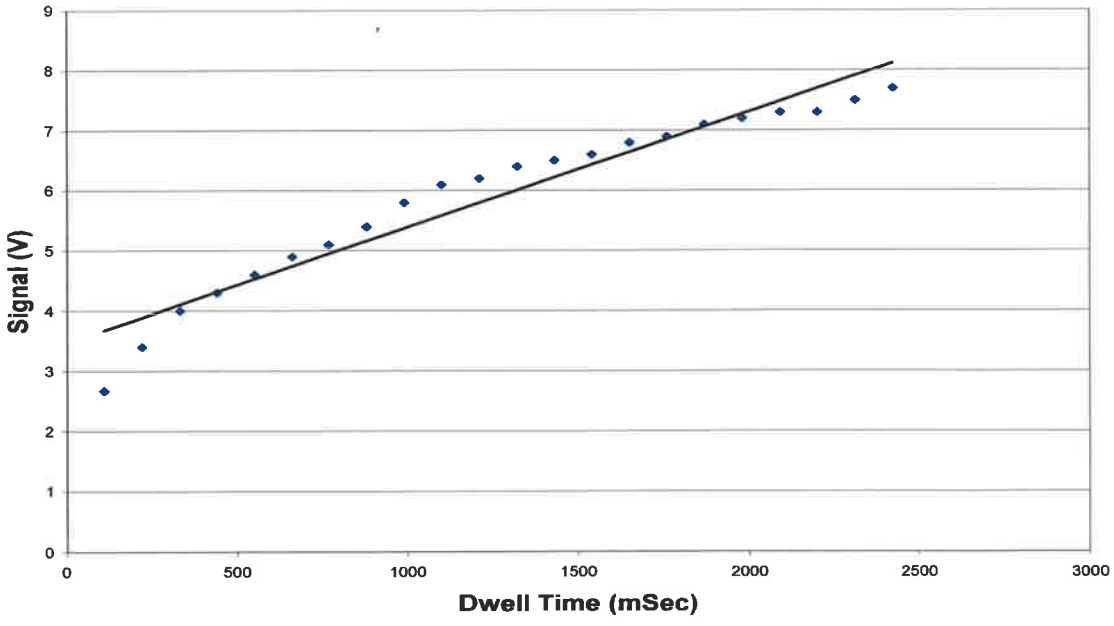


Figure 15. Signal Intensity as a Function of Dwell Time

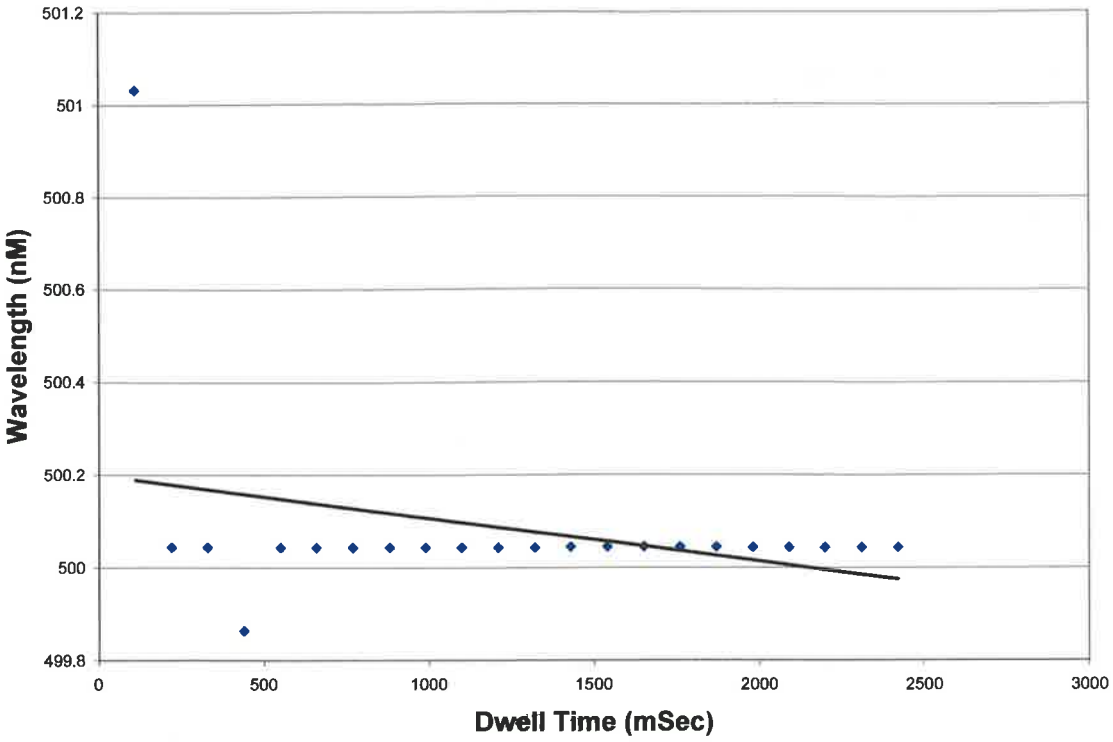


Figure 16. Signal Wavelength as a Function of Dwell Time

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