Novel Integrated Paired Emitter-Detector Diode (PEDD) as a Miniaturized Photometric Detector in HPLC

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Abstract

A novel low power, low cost, highly sensitive, miniaturized light emitting diode (LED) based flow detector has been used as optical detector for the detection of sample components in high performance liquid chromatography (HPLC). This colorimetric detector employs two LEDs, one operating in normal mode as a light source and the other is reverse biased to work as a light detector. Instead of measuring the photocurrent directly, a simple timer circuit is used to measure the time taken for the photocurrent generated by the emitter LED (λ_{max} 500 nm) to discharge the detector LED (λ_{max} 621 nm) from 5 V (logic 1) to 1.7 V (logic 0) to give digital output directly without using an A/D converter.

Employing a post-column reagent method, a Nucleosil 100-7 (functionalised with iminodiacetic acid (IDA) groups) column was used to separate a mixture of transition metal complexes, manganese (II) and cobalt (II) in PAR. All optical measurements were taken by using both the in built HPLC variable wavelength detector and the proposed paired-emitter-

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detector-diode (PEDD) optical detector configured in-line for data comparison. The concentration range investigated using the PEDD was found to give a linear response to the Mn (II) and Co (II) PAR complexes. The effects of flow rate and emitter LED light source intensity were investigated. Under optimised conditions the PEDD detector offered a linear range of 0.9-100 μM and LOD of 0.09 μM for Mn-PAR complex. A linear range of 0.2-100 μM and LOD of 0.09 μM for Co-PAR complex was achieved.

Keywords: Light emitting diodes, optical sensing, miniaturized HPLC detector, post column detection, Colorimetric analysis.

1. Introduction

Sensor research is driven by the need to generate a selective response to a particular analyte [1]. Separation prior to detection essentially eliminates the need for highly selective detection for many applications [2]. HPLC as a separation technique offers excellent selectivity through well established combinations of stationary phase and mobile phase and this is often the method of choice for routine Lab-based assays [3]. During the last decade there has been a general trend towards the miniaturization of separation techniques. There are significant advantages that include increased chromatographic resolution, reduced sample volume and reduced solvent consumption [4,5]. These characteristics are also essential criteria for making chromatography instrumentation field-deployable. Coupled with wireless communication, miniaturized field deployable LC systems will have an important role in the future realisation of widely distributed environmental monitoring networks [6].

However, there are considerable challenges associated with the miniaturization of all components including pumps, columns and detectors into fully integrated systems. Significant

advances have been made in recent years in the development of innovative fluid handling based on electro-osmotic [7] and micro pumps [5,8,9] and of short columns [10]. Comparatively fewer advances have been made in optical detection and UV-vis variable wavelength detectors are still the most commonly used detectors in HPLC. They are expensive (> $3,500), relatively large (32 x 12 cm), have high power consumption, and are therefore not suitable for field deployment.

Basic components in a chromatographic separation system include the sample introduction port, the separation column, connecting tubing, fluid control systems (pump, valves), and detector. For miniaturized systems, components should ideally be integrated, small, low reagent consumption, low power and low cost. The work presented here focuses on developing a miniaturized optical detector for HPLC. LEDs provide convenient source of light illumination in almost the entire visible spectral range [11] and have long been used as light sources [12,13] as they offer the advantages of being inexpensive, small in size (available as surface mount), cover a broad spectral range from UV to near-IR, robust and exhibit long lifetimes. In sensor research LEDs to date have been primarily used as light sources in optical sensors. Hauser [14-17] has long advocated the advantages of using LEDs and was the first to report the use of a blue LED as a spectrophotometric source coupled with a photodiode as a detector. Photodiodes are one of the most commonly used detectors in optical sensors [15,18-21]. Schmidt et. al. produced a flow cell detector based on an LED-photodiode configuration for post column detection of transition metals. This device successfully detected cations such as copper, manganese and cobalt [21].

In most micro-analytical systems the light source and the photodetector are normally separate units integrated with the microfluidic manifold [22]. We have previously demonstrated the use of the integrated PEDD device for colour and pH measurements in static solution [23,24] and

subsequently fabricated a single unit containing light source, fluid channel and detector for colorimetric flow analysis detection [25]. The PEDD was successfully employed for pH monitoring by using bromocresol green and subsequently the determination of its pK_a [25]. The PEDD integrated flow cell detector is highly sensitive, low power and extremely low cost (< $1).

In this paper we present for the first time the use of an integrated PEDD flow cell as a simple, small, highly sensitive, low cost photometric detector for HPLC. Using a post column colorimetric detection method commonly used for transition metal ions [26,27], Mn (II) and Co (II) PAR complexes are separated on a miniature Nucleosil 100-7 IDA functionalized column [28]. The coloured metal complexes formed by reacting the transition metal ions with a post column reagent (PCR) mixture containing 4-(2-pyridylazo) resorcinol (PAR) and ammonia were detected inline by the HPLC variable wavelength detector and the PEDD device. Spectrophotometric detection of metal complexes with PAR is a rapid, sensitive and convenient technique used for quantitative metal analyses [29].

2. Experimental

2.1. Equipment

A series 1050 Hewlett Packard HPLC system (Agilent Technologies., Dublin) was used to deliver the eluent at a flow rate of 0.7 mL/min. Samples were injected using an automated injector with a sample loop of 100 µL. A Nucleosil 100-7 column covalently functionalised with IDA groups of 4 x 14 mm in size was used for the ion separation. A Gilson (Miniplus 3)

peristaltic pump, (Anachem, UK) at a flow rate setting of 0.38 mL/min was used for the introduction of the post-column reagent (PCR), which was mixed at room temperature with the eluent using 0.5 m of PEEK reaction coil (0.25 mm i.d., VICI® AG International, Switzerland). A single channel PEDD flow cell (Emitter λ_{max} 500 nm, Detector λ_{max} 621 nm, Fig. 1) was used inline with the Hewlett Packard (series 1050) UV-vis variable wavelength detector (Agilent Technologies, Dublin) for the detection of the metal complexes.

2.2. Chemicals and Reagents

The PCR reagent used for the detection of the transition metal ions consisted of a mixture of 0.4 mM 4-(2-pyridylazo) resorcinol monosodium salt hydrate (PAR) (Sigma Aldrich, Dublin, Ireland) and 0.5 M ammonia (35%, BDH, Poole, England), which was adjusted to pH 10.5. Manganese chloride tetrahydrate and cobalt (II)-nitrate hexahydrate (Sigma Aldrich, Dublin, Ireland) standards were prepared daily from 1 mM stock solutions. The mobile phase, stock solutions and standard solutions were prepared using water from a Millipore Milli-Q water purification system. All samples were filtered through a 0.45 μm filter and degassed by sonication. The mobile phase used was 3 mM Nitric acid (70%, East Anglia Chemicals, Suffolk, England).

2.3. Fabrication and operation of integrated PEDD flow cell detector

The integrated detector cell was fabricated as previously described [25] using two 5 mm LEDs (Kingbright, Ireland) as shown in Fig. 2. The detector used was a red LED with a λ_{max} at 621 nm which can detect any wavelength below this point. A green LED with a λ_{max} at 500 nm was used as the emitter.

A 9 V battery was used as the power source to drive the circuitry from which a voltage regulator was used to control the voltage supply to the LEDs. The light detector LED in input mode was charged up to 5 V for 100 µs and then switched to output mode. Then the photon emitted from the green LED hit the red detector LED to generate a small photocurrent to discharge the detector LED. The time taken for the discharge process to go from an initial value of 5 V (logic 1) to a preset value of 1.7 V (logic 0) was measured with a simple timer circuit [23,25].

2.4. Measurement Procedure

Various concentrations of Mn (II) and Co (II) were made up in Milli-Q water and 100 µl aliquots of sample were injected onto the nucleosil 100-7 column at a flow rate of 0.7 mL/min. The analytes were first detected using the UV-vis detector at a wavelength of 500 nm followed directly by the PEDD flow cell. All experiments were carried out in triplicate (n = 3). The chromatograms obtained from the UV-vis variable wavelength detector were analysed using Agilent Chemstation for LC and LC/MS systems software. The data acquired from the PEDD was transported to a PC via RS232 port and captured with HyperTerminal software (Microsoft Inc., USA), saved as a text file and then analysed using Excel™ (Microsoft Inc., USA).

3. Results and discussion

3.1. Optimisation of HPLC and PEDD flow cell conditions

Fig. 1 shows that the absorption spectra of manganese (II) and cobalt (II) PCR complexes overlap with the emission spectrum of the PEDD device. The emission spectrum of the green emitter LED was obtained by using an ocean optic spectrometer (OOIBase 32™, Ocean Optics. Inc., Dunedin, USA). The absorbance spectra of manganese (II) and cobalt (II) PAR complexes were acquired using the μQuant™ platewell reader (Bio – Tek Instruments, Inc., USA). The overlap between the absorbing species and the light source provide high sensitivity for the detection of Mn-PAR and Co-PAR.

The data obtained by the PEDD is based on the theoretical model derived by Lau et al. [23,24]

$$\text{Log}\,(t) = \varepsilon Cl + \log\,(t_0) \qquad (1)$$

where l is the optical pathlength through the solution (cm), ε the molar extinction coefficient (mol l^{-1} cm^{-1}) at a particular wavelength, C the concentration of the absorbing species (mol l^{-1}), t_0 a constant that represents discharge time in the absence of the coloured species in solution (μs), t is the discharge time of the detector (μs).

Optimisation of HPLC and PEDD parameters such as flow rate and light intensity were carried out. A concentration of 2 μM Mn (II) solution was selected to carry out the study as it provided reproducible responses and good peak size and shape. Optimal separation conditions were determined to be: eluent flow rate of 0.7 mL/min and peristaltic pump rate setting of 0.38 mL/min. Previous studies have shown that on decreasing the light intensity of the emitter LED, the change in discharge time (i.e. the resolution) can be improved by up to a factor of 4 [25]. A (0-10 kΩ) variable resistor was used to determine the optimum light intensity of the

green emitter LED to achieve optimum resolution i.e. by varying the electrical current applied to the emitter LED. The detector response increases linearly with an increase in electrical resistance at the LED emitter. However, the increase in response was accompanied with an increase in noise and a decrease in baseline stability as shown in Fig. 3. A resistance of 1.5 kΩ was found to provide the optimum light source intensity giving high sensitivity, while maintaining a smooth baseline without drift. These effects are clearly demonstrated in Fig. 3.

For example, at a resistance of 0.005 kΩ, the difference in discharge time (Δt) i.e. peak height was 18.33 ± 0.58 μs with an R.S.D. (n = 3) of 3.15%. At an additional applied resistance of 1.5 kΩ the peak height obtained was 142.67 ± 1.15 μs with 0.81% R.S.D. (n = 3). At a resistance of 4 kΩ the peak height achieved was 457.33 ± 13.20 μs with 2.89% R.S.D. (n = 3). Increasing the resistance to 1.5 kΩ improved the change in discharge time by a factor of 8. Further increase in resistance increased the peak height even more, but caused more baseline drift and higher R.S.D. values as shown in Fig. 3.

The optimal PCR delivery rate was determined experimentally. The optimal flow rate was 0.38 mL/min.

3.2. Separation and Detection of Manganese (II) and Cobalt (II) PAR complexes

To investigate whether the PEDD flow cell caused additional peak broadening a 5 μM mixture of manganese (II) and cobalt (II) was prepared and injected (n = 3). The chromatogram obtained from the UV-vis variable wavelength detector shown in Fig. 4A shows two well-resolved peaks (Table 1) with retention times of 1.3 and 2.1 minutes for manganese (II) and cobalt (II) PAR complexes respectively. The average peak area calculated for Mn-PAR and Co-PAR had relative standard deviations of 0.29% and 2.71% respectively.

The green light intensity transmitted in the PEDD flow cell efficiently overlaps the absorbance spectrum of PAR complexes with manganese (II) and cobalt (II) as shown in Fig. 1. The PAR complex detection data (discharge time *t*/μs) was plotted against retention time (minutes) and presented in Fig. 4B. As shown in Fig. 4B the plot obtained from the PEDD shows identical retention times to that acquired from the UV-vis variable wavelength detector. The mean peak height (discharge time/μs) calculated for Mn (II) and Co (II) both had a relative standard deviation of 0.04%. The peak efficiencies calculated for both the UV-vis variable wavelength detector and the PEDD flow device are presented in Table 1. The results obtained show similar peak efficiencies and indicate that the PEDD flow device does not cause additional peak broadening to the sample peaks.

3.3. Calibration using the PEDD flow cell and the UV-vis spectrophotometer

Mixtures containing various concentrations of manganese (II) and cobalt (II) were passed through the HPLC system using the optimal conditions (carrier flow rate of 0.7 mL/min, PCR flow setting of 0.38 mL/min and a 1.5 kΩ current limiting resistor on the emitter LED). Each injection was carried out in triplicate (n = 3). Results were acquired simultaneously using both a UV-vis variable wavelength detector in-line with the PEDD flow cell.

Fig. 5 shows results obtained from the PEDD flow cell. The peak heights (log *t*/μs) of each transition metal complex detected were plotted against the input ion concentration (C) in accordance with the model (Eq. 1). A large detection range from 0.09 μM to 500 μM (Inset A, Fig. 5) with linear ranges of 0.9 – 100 μM for Mn-PAR (R^2 = 0.997) and 0.2 – 100 μM Co-PAR (R^2 = 0.9997) were obtained. An LOD of approximately 0.09 μM was achieved for both

Mn-PAR and Co-PAR as shown in Fig. 6. It can be seen from Fig. 5 that the relative standard deviation of the measurements (n = 3, shown as error bars) were very low (<0.08%).

For the data obtained from the UV-vis detector, the peak area was plotted against concentration (C) and the results were presented in Fig. 7 for both manganese and cobalt complexes. A similar detection range from 0.09 μM to 500 μM (Inset A, Fig. 7) was achieved for Mn-PAR. A detection range of 0.5 μM to 500 μM was obtained for Co-PAR (Fig. 7, inset A). The linear ranges obtained were 0.9 – 100 μM for Mn-PAR (R^2 = 0.9981) and 0.7 – 100 μM for Co-PAR (R^2 = 0.9997). An LOD of 0.09 μM was obtained for Mn-PAR but a much higher LOD value of 0.7 μM was achieved for Co-PAR (Fig. 6). It can be seen from the inset of Fig. 7 that the relative standard deviations of the measurements (n = 3, shown as error bars) are in the region of ca. 10%.

The simple PEDD flow cell was successful as a post column detector for the HPLC. The data obtained from this inexpensive, small, power efficient optical detector were comparable to those obtained from the HPLC variable wavelength detector, and sensitivity is significantly higher. For detection of the Mn-PAR complex the PEDD response matched that of a variable wavelength and was 6 times more sensitive for the detection of Co-PAR complex. In comparison to the performance of the typical LED-photodiode device employed by Schmidt et. al. the PEDD achieved similar sensitivity with a path length 4 times shorter than that of the reported device. This is partly due to the availability of an improved LED light source which more efficiently overlaps with the absorbance of the metal complexes. The improved performance is largely due to the high sensitivity of the PEDD device. A simple PEDD detector cannot replace the existing variable wavelength detector because of the limited bandwidth a single LED can cover, however a more sophisticated PEDD device with multiple LED sources covering a wider range of wavelengths could make this possible. For specific

applications where an appropriate operation wavelength can be selected for the analytes a single PEDD flow detector is ideal and preferable to a more complex multi-source detector. This small flow detector when coupled with a miniature low pressure separation column, such as the one used in this work, and a pressure/pumping unit, such as a small gas cylinder, could form a complete miniature HPLC system suitable for rapid small sample analysis. Apart from working as a small bench top analytical device, such a system may also be developed as a field deployable autonomous monitoring device.

4. Conclusions

The results obtained using the integrated PEDD flow analysis cell have demonstrated that this simple low cost, low power device can be used as a photometric detector in HPLC. Under optimised conditions the PEDD detector offered a linear range of 0.9-100 μM and LOD of 0.09 μM for Mn-PAR complex. A linear range of 0.2-100 μM and LOD of 0.09 μM for Co-PAR complex was achieved. The PEDD flow cell can detect lower concentration levels of Co-PAR than that of an expensive, commercially available bench top instrument. This optical sensor has the potential for very broad analytical applications, given that it offers high sensitivity and precision with excellent signal-to-noise characteristics. The PEDD flow cell is therefore ideal for detection of multiple analytes when coupled with a separation technique.

Acknowledgements

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Figure 1

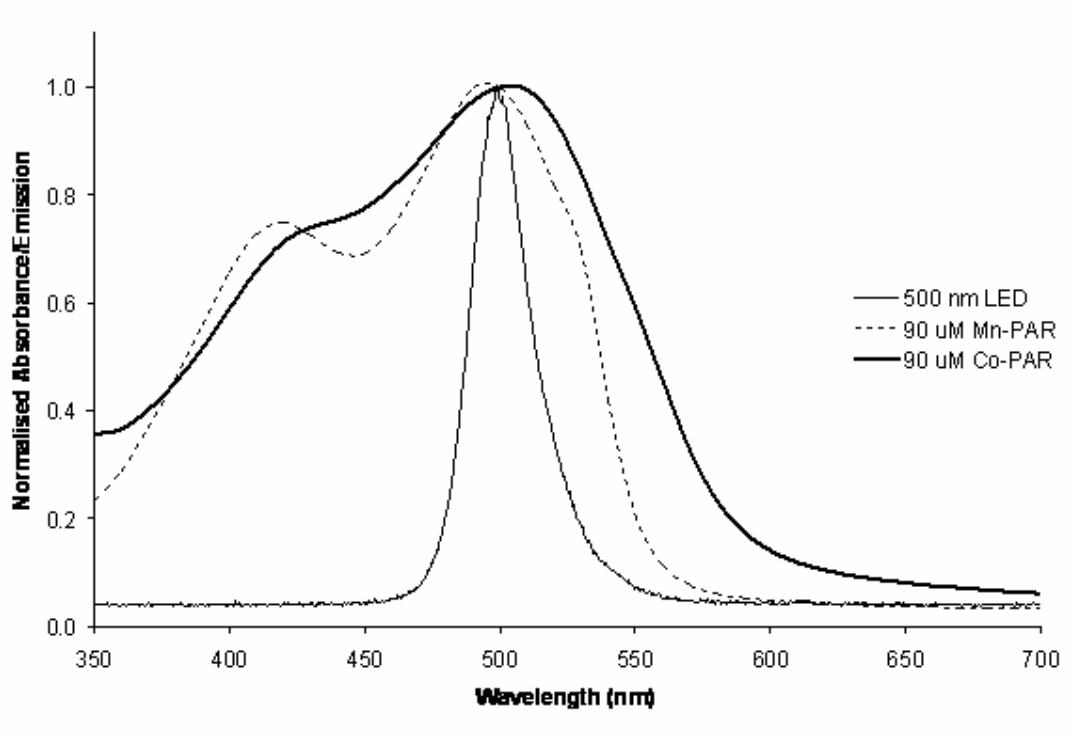
Normalised Absorbance/Emission
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
600
650
700
Wavelength (nm)
500 nm LED
90 uM Mn-PAR
90 uM Co-PAR

Figure 2

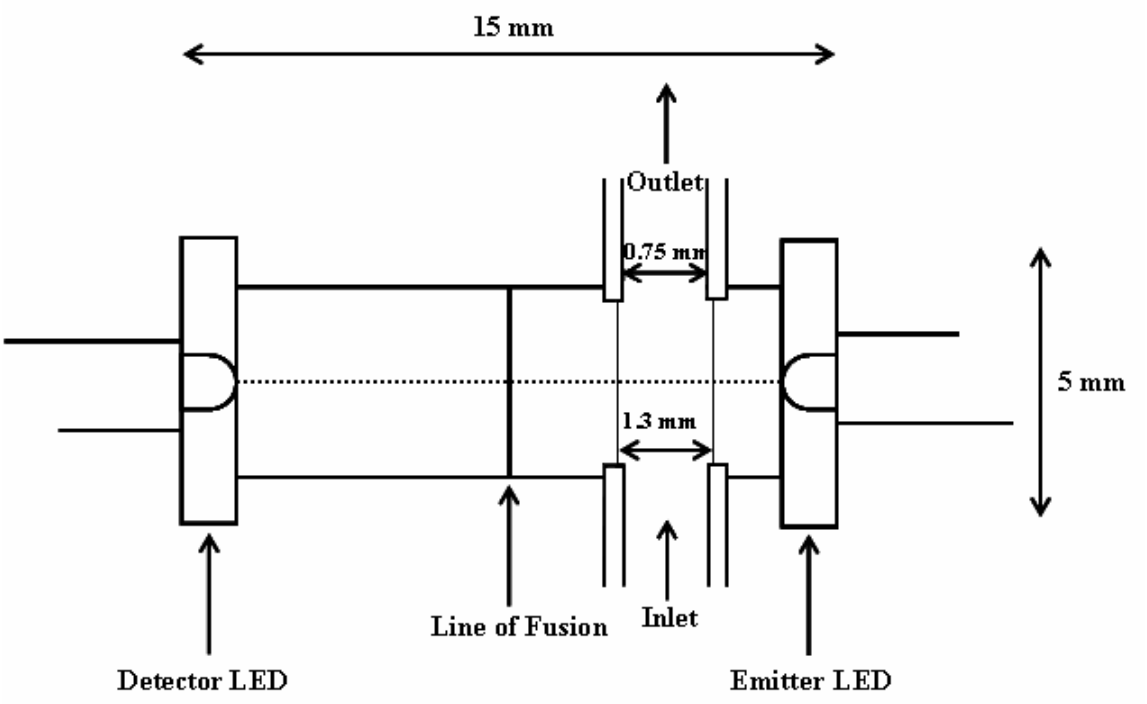
15 mm
Outlet
0.75 mm
5 mm
1.3 mm
Line of Fusion
Inlet
Detector LED
Emitter LED

Figure 3

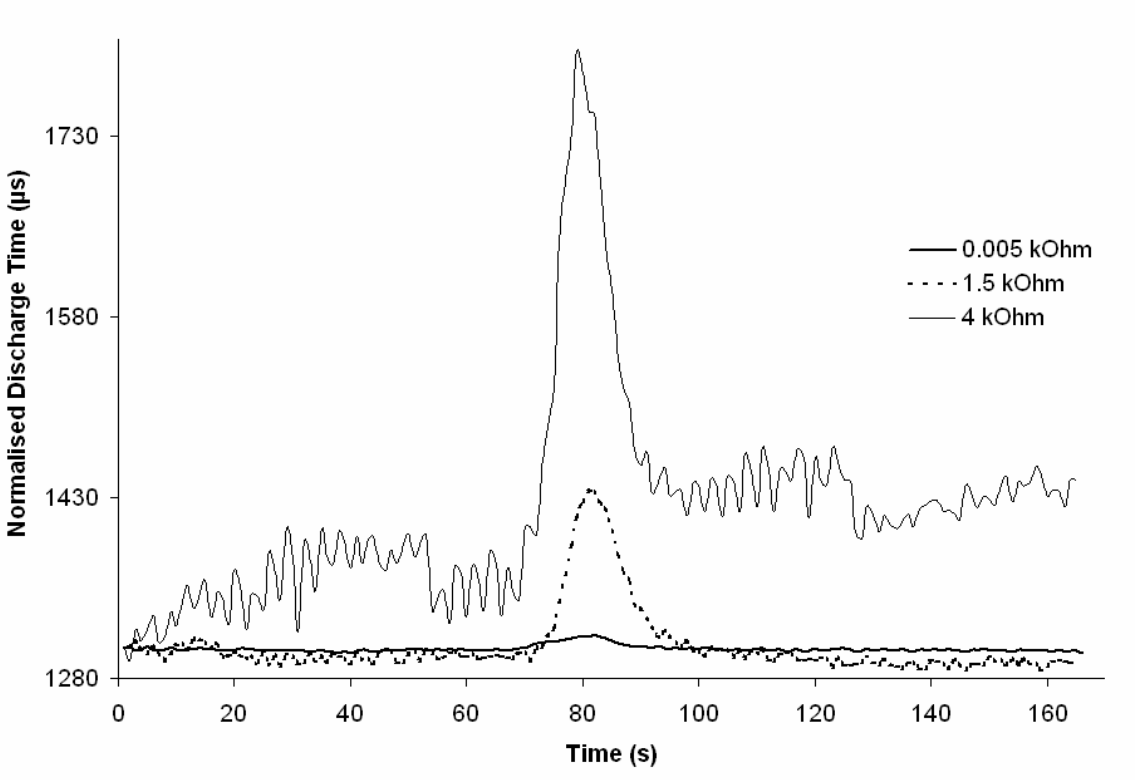
Normalised Discharge Time (µs)
1730
1580
1430
1280
0
20
40
60
80
100
120
140
160
Time (s)
0.005 kOhm
1.5 kOhm
4 kOhm

Figure 4

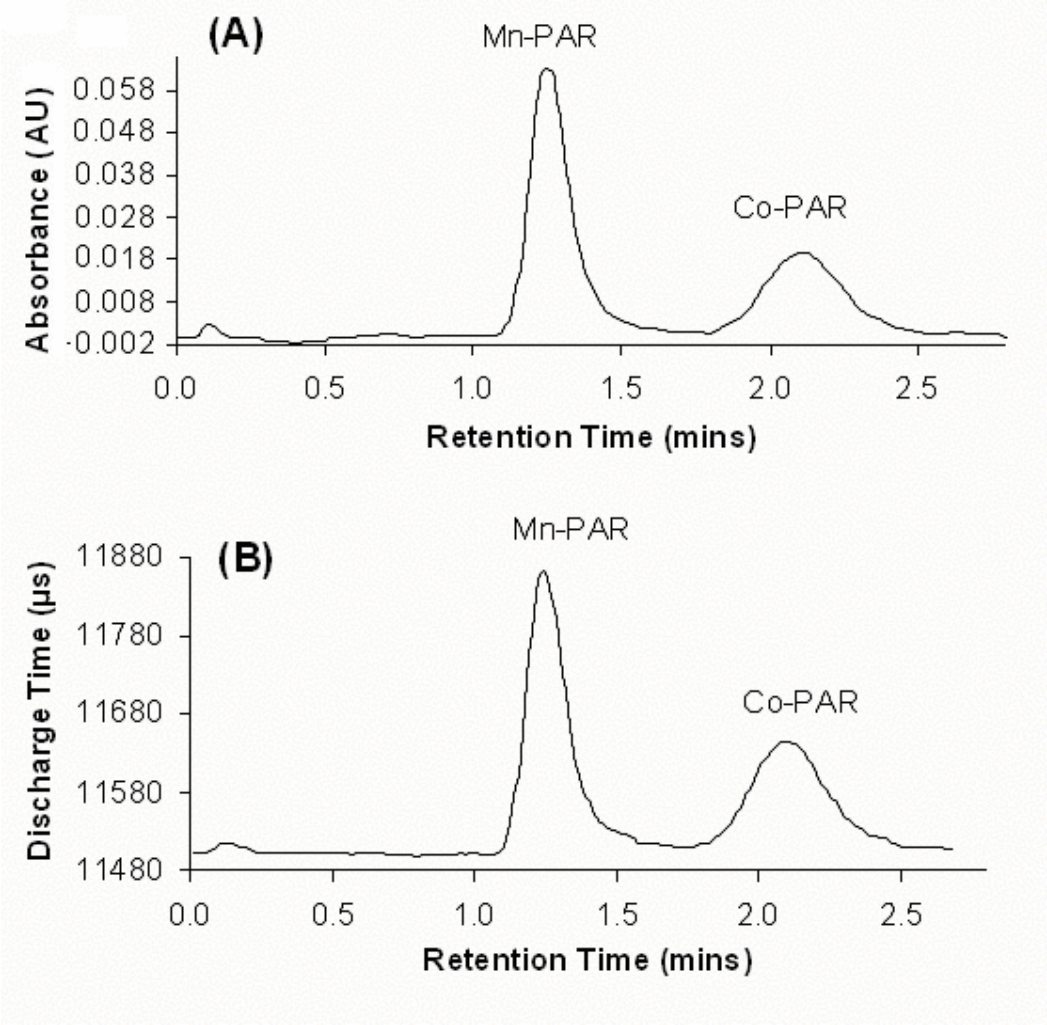
(A)
Mn-PAR
Co-PAR
Absorbance (AU)
0.058
0.048
0.038
0.028
0.018
0.008
-0.002
0.0
0.5
1.0
1.5
2.0
2.5
Retention Time (mins)
(B)
Mn-PAR
Co-PAR
Discharge Time (µs)
11880
11780
11680
11580
11480
0.0
0.5
1.0
1.5
2.0
2.5
Retention Time (mins)

Figure 5

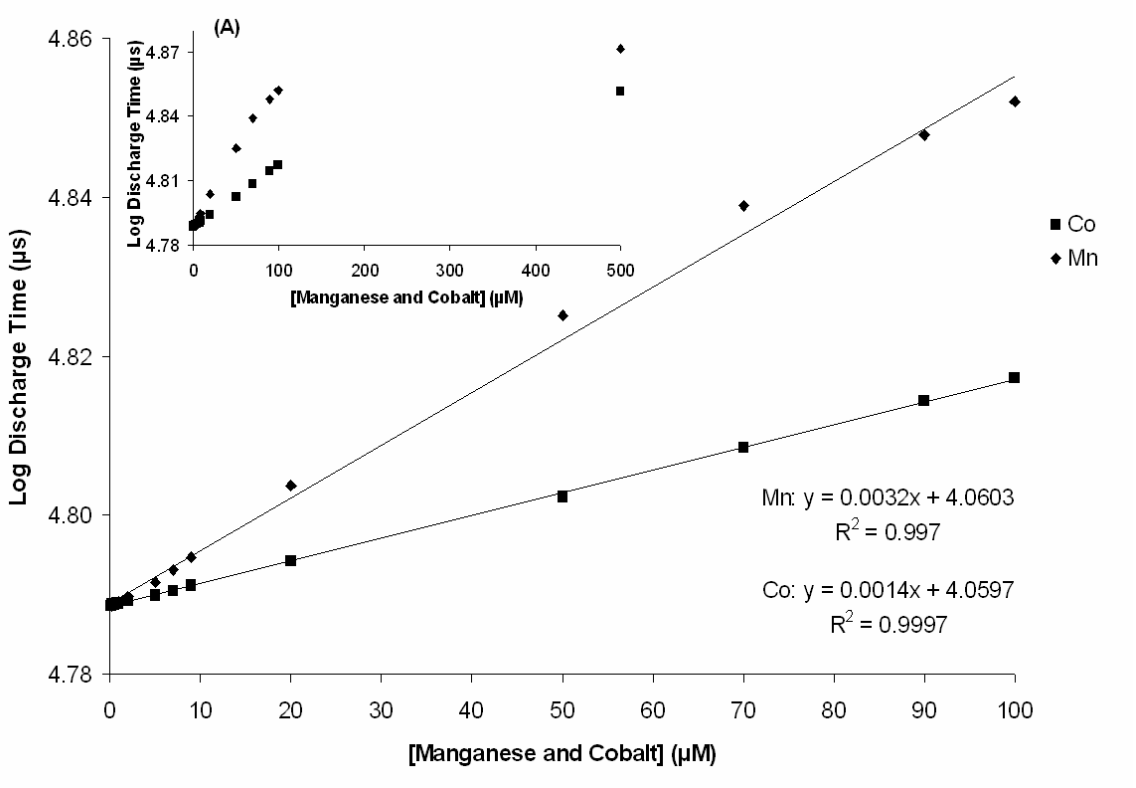
(A)
Log Discharge Time (µs)
4.87
4.84
4.81
4.78
0
100
200
300
400
500
[Manganese and Cobalt] (µM)
Log Discharge Time (µs)
4.86
4.84
4.82
4.80
4.78
0
10
20
30
40
50
60
70
80
90
100
[Manganese and Cobalt] (µM)
Co
Mn
Mn: y = 0.0032x + 4.0603
R2 = 0.997
Co: y = 0.0014x + 4.0597
R2 = 0.9997

Figure 6

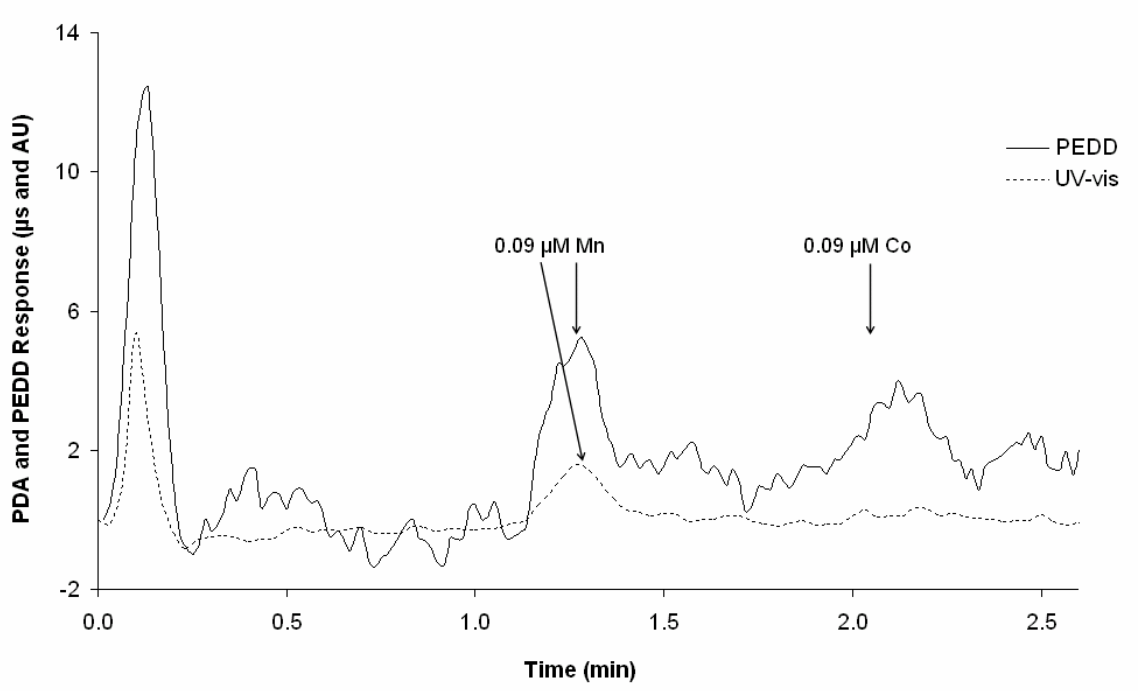
14
10
6
2
-2
0.0
0.5
1.0
1.5
2.0
2.5
PDA and PEDD Response (µs and AU)
Time (min)
PEDD
UV-vis
0.09 µM Mn
0.09 µM Co

Figure 7

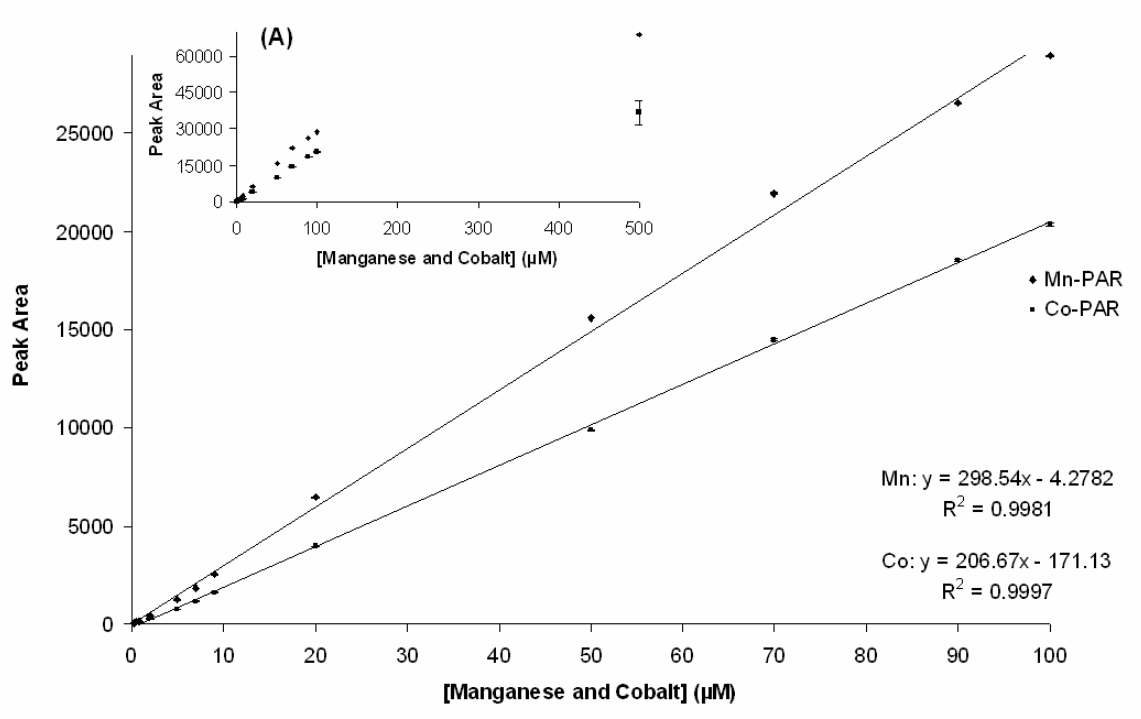
(A)
Peak Area
60000
45000
30000
15000
0
0
100
200
300
400
500
[Manganese and Cobalt] (µM)
Peak Area
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
70
80
90
100
[Manganese and Cobalt] (µM)
Mn-PAR
Co-PAR
Mn: y = 298.54x - 4.2782
R2 = 0.9981
Co: y = 206.67x - 171.13
R2 = 0.9997

Table 1

	PEDD	*VWD*
Resolution	2.1	2.0
Mn-PAR efficiency, *N*	365.7	365.7
co-PAR efficiency, *N*	311.6	224.4