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# Photometric detection in flow analysis systems using integrated PEDDs

Martina O' Toole, King Tong Lau, Dermot Diamond *

*Adaptive Information Clusters, National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Dublin 9, Ireland*

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**Abstract**

A novel inexpensive optical-sensing technique has been developed for colorimetric flow analysis. This sensing system employs two LEDs whereby one is used as the light source and the other as a light detector. The LED used as light detector is reverse biased with a 5-V supply so that the photocurrent generated by the incident light discharges the capacitance. Direct digital output is provided by a simple timer circuit that measures the time taken for this discharge process from 5 V (logic 1) to 1.7 V (logic 0).

This sensing concept has been applied in flow analysis by constructing an optical flow cell with a pair of LEDs. Calibration of the integrated optical flow cell using a dye resulted in a linear response that obeys the Beer–Lambert law. The flow rate, dynamic range, sensitivity and limits of detection were investigated. The system was also used for pH determination in the range of pH 2.5–6.8 using bromocresol green (BCG). The p$K_a$ of BCG was successfully determined by this technique.


*Keywords:* LEDs; Optical sensing; pH sensing; Transmission measurements; Colorimetric analysis

## 1. Introduction

At present optical sensing is one of the most active areas of research in analytical chemistry. This is largely due to the availability of inexpensive, low power-consumption components such as LEDs, photodiodes and data acquisition technologies. Desirable qualities like these are vital requirements in the development of (micro) total analysis systems, particularly, with respect to field deployable micro-instruments [1].

Light-emitting diodes (LEDs) are the most energy-efficient means of producing light emission, and are ideal for miniature analytical devices [2]. LEDs are increasingly popular for the fabrication of optical sensors as they offer advantages of being inexpensive, small in size, available over a broad spectral range from ultraviolet to near-infrared (ca. 380–900 nm), have a long lifetime, robust and easy to fabricate into various configurations. When used as light detectors for chemical sensing by measuring the intensity-dependent discharge of capacitance, they have been found to be highly sensitive and could detect sub-micro-molar of dye [3].

Optical sensor configurations generally combine LED light source with photodiodes, which measure the light intensity after passage through the sample [2,4–8]. The use of LEDs in photometric detectors has been reported as early as 1978 when Betteridge et al. [9] used a gallium phosphide LED and a silicon phototransistor for the detection of metal ions. Since then LEDs have improved, for example, in increased range and intensity, and a variety of LED-based optical sensors has been developed [10–12]. Hauser et al. [13] employed seven light-emitting diodes ranging from blue to infrared as a light source with photodiodes as detection. The light from each LED was guided into a single measuring cell using a fibre-optic coupler. Using this configuration, it was possible to determine copper using the cuprizone method and ammonia using the indophenol method with 90% of the sensitivity of a spectrometer. LEDs have also been used in fluorescence intensity-based sensors [14] and phase fluorimetry [15].

* Corresponding author. Tel.: +353 1 7005404; fax: +353 1 7008002.
*E-mail address:* dermot.diamond@dcu.ie (D. Diamond).

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A novel configuration for optical sensing is the use of an LED as a light detector. In this approach, two LEDs are employed, whereby one is used as a light source and the second as the light detector. Mims and Forrest [16,17] first investigated using a LED as a crude light detector to measure photocurrent generated by direct sunlight. Previously, we demonstrated the use of a paired emitter detector diode (PEDD) device for colour and pH measurements in static solution [3]. A PEDD consists of two LEDs arranged in various configurations in which one LED is used to provide illumination and the other is used as a light detector for the transmitted or reflected light.

In this paper we propose a novel, simple optical-sensing device for flow analysis using a pair of LEDs. An optical flow cell was constructed using two LEDs which allowed sample to flow inside the cojoined LEDs along the light path. The use of this integrated colorimetric flow analyser to measure colour and pH is reported.

## 2. Experimental

### 2.1. Chemicals and reagents

The pH indicator dye used was bromocresol green (BCG) (from Sigma Aldrich, Dublin, Ireland). All samples were made up in a pH 7 buffer stock solution made from buffer tablets (Lennox, Dublin, Ireland) and ultrapure water according to the instructed method. All the reagents used were of analytical grade. Stock solution of 25 mM BCG was prepared by dissolving 0.70 g dye in 250 ml of pH 7 buffer solution from which dilutions were prepared.

A series of dye solutions each containing 40 μM BCG at various pHs (2.5–6.8) was also prepared.

### 2.2. Fabrication of PEDD optical flow cell

The optical device was fabricated using two identical (5 mm) LEDs ($\lambda_{max}$ at 621 nm) (Knightbright, France), with emission shown in Fig. 2. The original length of each LED was 10 mm. The PEDD sensor was prepared by first cutting 0.25 mm from the tips of each LED to give a flat top, rather than the usual curved surface. The total length of the PEDD was now 15 mm. The surface of the LEDs was then sanded down using general purpose, fine-grade paper (Homebase, Dublin) to make them smooth and flat before bonding. Using a drill bit of size 1.3 mm, a channel was machined into each LED to a depth of 1.8 mm, resulting in a pathlength of 3.6 mm as shown in Fig. 1. An inlet and outlet were also machined using the same method. Prior to bonding, the two LEDs were accurately aligned to ensure the line of fusion was perfectly round. The LEDs were then fused together using UV curable epoxy glue (Edmund Scientific: Orland 81 extra fast curing, USA), and placed under UV light (380 nm) for 30 min. Green peek tubing (i.d. 0.75 mm) was placed at either end of the channel and sealed into place using araldite epoxy glue (Homebase, Dublin). The PEDD cell was left to dry at room temperature for a further 30 min. The optical cell was then painted black to reduce stray light effects.

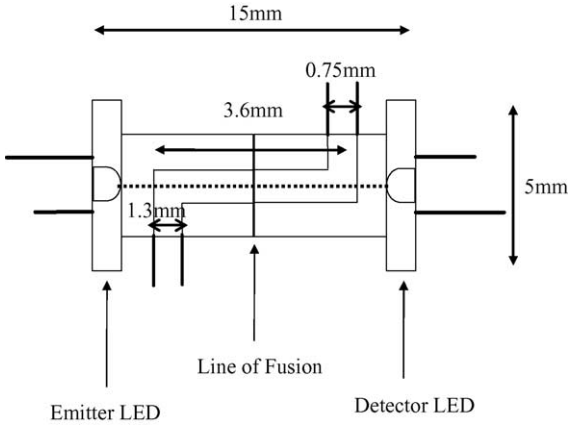


Fig. 1. A schematic of the integrated PEDD flow analysis device used for colorimetric detection.

### 2.3. Light measurement

A 9-V battery was used 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. The photon flux from the emitter LED strikes the detector LED, generating a small photocurrent which gradually discharges the capacitor voltage. 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.

### 2.4. Measurement procedure

Various concentrations of BCG were made up in pH 7 buffer solution and passed through the PEDD flow cell at a flow rate of 0.6 ml/min for ca. 4 min while continuously monitoring the absorbance. All experiments were carried out in triplicate with the exception of BCG calibration, which was repeated 8 times. The data are transported to a PC via RS232 port and captured with the HyperTerminal software, and then saved as a text file for further analysis using Excel™ (Microsoft, Inc., USA).

## 3. Results and discussion

### 3.1. Colour measurement using PEDD flow cell

In this study, the measurement is based on the following theoretical model, which has been derived by Lau et al. [18]:

$$\log(t) = \varepsilon Cl + \log(t_0) \quad (1)$$

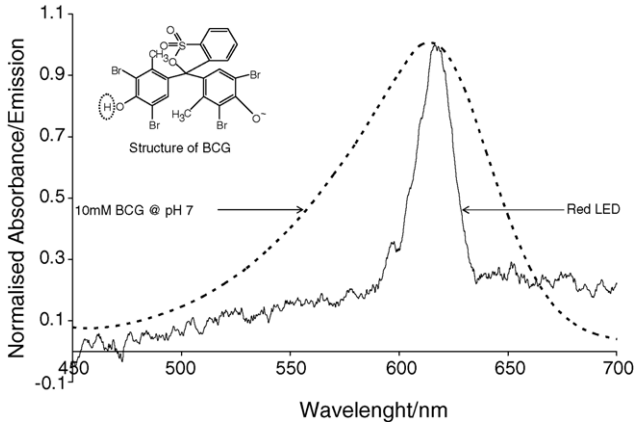


Fig. 2. Emission spectrum ($\lambda_{max}$ 620 nm) of the LED used in the integrated PEDD flow analysis device and the absorption spectrum ($\lambda_{max}$ 616 nm) of 10 mM BCG at pH 7 ($\varepsilon = 1.2 \times 10^4$ l mol$^{-1}$ cm$^{-1}$). The inset is the structure of the deprotonated BCG dye used. Emission and absorbance normalised to range 0–1 by dividing values by $\lambda_{max}$.

where $l$ is the optical pathlength through the solution (cm), $\varepsilon$ 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).

Initial studies carried out involved the calibration of a change in colour intensity or concentration of a pH indicator dye, Bromocresol green in a pH 7 buffer. BCG was selected due to its large molar extinction coefficient of $1.2 \times 10^4$. The light intensity transmitted in the flow cell was measured with an LED ($\lambda_{max}$ 621 nm), which efficiently overlaps the absorbance spectrum of BCG ($\lambda_{max}$ 620 nm) as shown in Fig. 2. The emission spectrum of the LED was obtained by using ocean optic spectrometer (OOIBase 32$^{TM}$, Ocean Optics, Inc., Dunedin, USA). Various concentrations of BCG were made up in pH 7 buffer solution and passed through the PEDD flow cell for ca. 4 min per sample, at a flow rate of 0.6 ml/min. The log of the discharge times (log $t$, μs) was plotted against dye concentration ($C$) in accordance with the model (Eq. (1)) and the result is presented in Fig. 3. Inset (a) in Fig. 3 shows a large detection range from ca. 0 to 20.5 mM BCG from which a linear range of approximately 0.9–250 μM BCG ($R^2$ value 0.998) was observed as shown in the main feature plot. It can be seen from that the relative standard deviation of the measurements ($n = 8$, shown as error bars) are very low (ca. 0.4%) and LOD of 0.9 μM.

As a comparison study, the absorbance of the same BCG concentrations was acquired using the μQuant$^{TM}$ platewell reader (Bio-Tek Instruments, Inc., USA). As shown in Fig. 4, the absorbance at $\lambda_{max}$ plotted against the dye concentration ($C$) resulted in a linear detection range of 0.5–250 μM ($R^2$ value 0.999), with an R.S.D. ($n = 3$) of 4% and an LOD of 0.9 μM. It can be seen that the performance of the simple LED-based device matched that of a conventional bench top instrument.

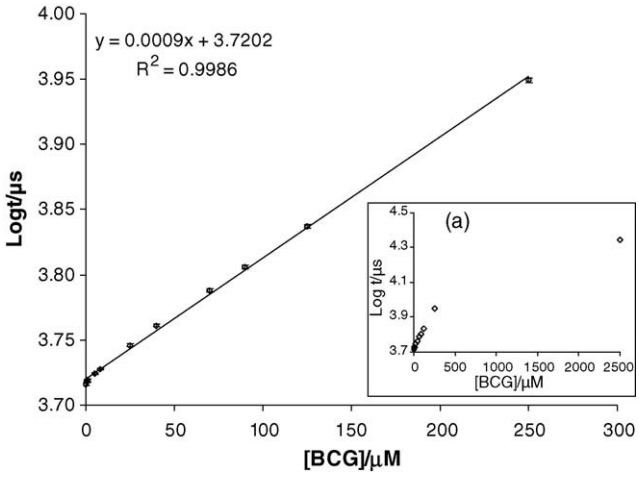


Fig. 3. Linear calibration plot of log of the discharge times log($t$) vs. BCG dye concentration. The error bars represent the standard deviations for $n = 8$. The inset (a) shows the full range of responses obtained from the calibration.

### 3.2. Detector responses to varying light source intensity

A study has been carried out to investigate the effect of varying illumination intensity on the sensitivity of the detector. The emitter intensity was controlled by using a variable resistor connected directly to the emitter LED as the amount of electrical current passing to the emitter LED is directly proportional to its emission intensity. Therefore an increase in the resistance results in a decrease in the light intensity of the emitter LED, which in turn results in an increase in the time taken to discharge the detector LED.

A pH 7 buffer solution was passed through the flow cell for ca. 4 min, followed by 8 μM BCG at pH 7 at a flow rate of 0.6 ml/min. This sample was selected as it gave a significant change in response (discharge time of ca. 100 μs) compared to the pH 7 background. Various light intensities were examined and the results obtained are shown in Fig. 5. The data shows that increasing resistance to reduce light intensity results in a linear increase in responses. The largest R.S.D. ($n = 3$) shown in Fig. 5 appears to be big at ca. 12%. This was

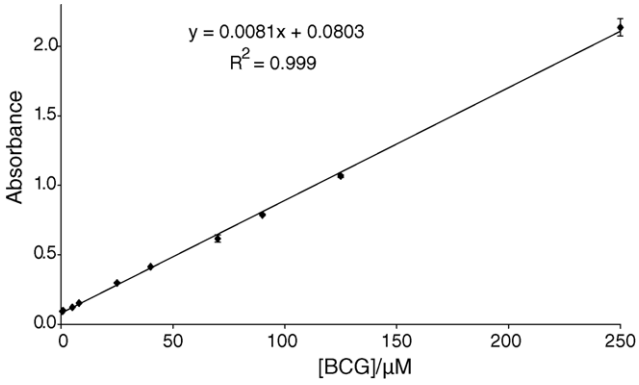


Fig. 4. Calibration plot of absorbance at $\lambda_{max}$ vs. BCG dye concentration obtained using a platewell reader. The error bars represent the standard deviations for $n = 3$.

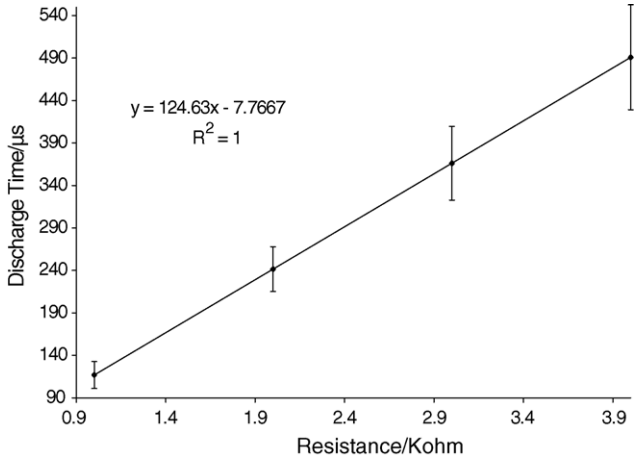


Fig. 5. A plot to illustrate the effect of varying light intensity on the discharge time detected for 8 μM BCG by changing the resistance of the variable resistor connecting to the LED. The error bars represent the standard deviations for $n = 3$.

due to the difficulties associated with using a manual variable resistor to reproduce the exact resistance values after each experiment, which contributed to the big error margin. With fixed resistors the data obtained were much better as shown in Fig. 6, which shows a comparison of the reproducibility of the system with: (1) no additional resistance and (2) at 2 kΩ. Note that the baselines of the two traces were very similar and smooth (R.S.D. 0.03%). The average response to 8 μM BCG obtained from (1) was $104.5 \pm 1.9$ μs ($n = 3$) whereas the average response from (2) was $416.2 \pm 11.6$ μs ($n = 3$). Increasing the resistance by 2 kΩ therefore improved the sensitivity by approximately a factor of 4.

### 3.3. Using PEDD to monitor colour changes

The PEDD flow cell has also been used to monitor the pH-dependent colour change of BCG. A dye concentration of 40 μM was chosen to demonstrate this application as it has good colour density. The plot obtained shown in Fig. 7 is

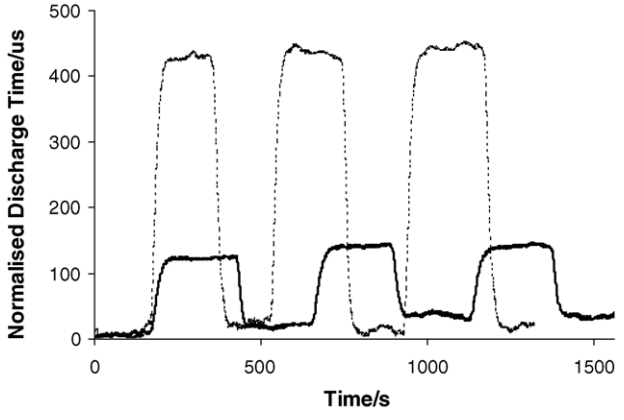


Fig. 6. Real-time traces obtained for 8 μM BCG solution buffered at pH 7 using two resistances (0.006 kΩ, solid line and 2 kΩ, dashed line). The flow rate used was 0.6 ml/min.

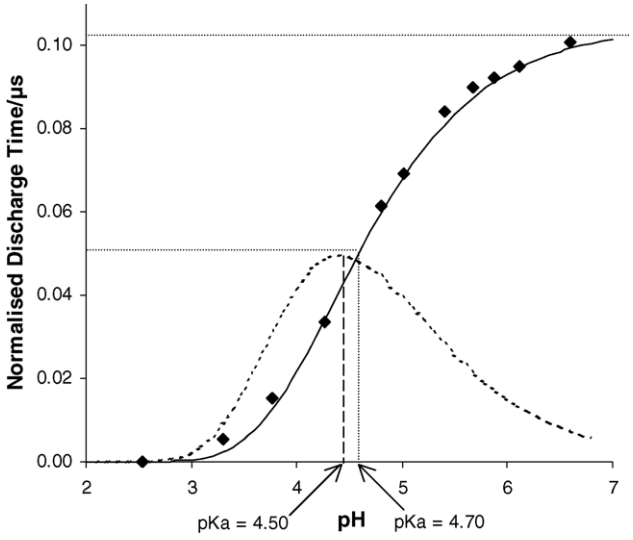


Fig. 7. A plot to illustrate the change in pH of 40 μM BCG solution with the discharge time of the PEDD flow detector (♦). The first derivative (dashed line) of the best-fit line (solid line) for the data gave an estimated p$K_a$ value of 4.50.

sigmoidal in shape with a linear range between pH 3.8 and 5.3. Using Microsoft™ Excel solver and method developed by Diamond and Hanratty, a best-fit line was found for the experimental data [19]. The p$K_a$ was determined to be 4.50 by taking the first derivative of the fitted line, which is slightly lower than the reported p$K_a$ value of 4.74 for BCG at room temperature [20]. This was probably due to the limitation of the data range, which resulted in a non-symmetrical Gaussian plot. An alternative method for p$K_a$ evaluation was used: At pH 2–3, the acid form of the dye was present at about 100%. This corresponds to a discharge time of 0.00 μs. At pH 7, the deprotonated form dominated (close to 100%) and this corresponded to a discharge time of 0.11 μs. At a discharge time of 0.055 μs, there should be equal amounts of these species corresponding to a p$K_a$ of ca. 4.70, which agrees better with the literature data.

### 3.4. Future developments

The results obtained using the integrated PEDD flow analysis cell has demonstrated that this simple low-cost device can be used as a very sensitive optical detector for colorimetric flow optical sensor for colorimetric flow analysis. The high sensitivity, already evident in this study, may be further improved by increasing the pathlength through modification of the channel geometry. The PEDD flow cell may be used in various colour-based chemical analyses, e.g. detection of phosphates [21] or nitrites [22]. To enable chemical analyses using multiple reagents, a multichannel flow cell is required to perform reagent mixing. With different configurations, the PEDD flow cell can also be used for fluorescence detection. This sensing approach therefore has potential for very broad analytical applications, given that it has the advantages of very low cost, low power consumption

and offers high sensitivity with excellent signal-to-noise characteristics.

## 4. Conclusions

We have demonstrated that the integrated PEDD flow analysis system is useful for colorimetric analysis. This device while small, compact and inexpensive, is nevertheless highly sensitive and is clearly capable of providing limits of detection in the nanomolar concentration range. The power consumption required is extremely low and the sensor can be operated from a 9-V battery. The PEDD flow cell is therefore very suitable for scale-up and field deployment in autonomous monitoring instruments.

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