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Novel fused-LEDs devices as optical sensors for colorimetric analysis

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Abstract

The development of a novel, low power optical sensing platform based on light emitting diodes (LEDs) is described. The sensor is constructed from a pair of LEDs fused together at an angle where one LED functions as the light source and the other LED is reverse biased to function as a light detector. Sensor function is based on the level of light received by the detector diode, which varies with the reflectance of the interface between the device and its environment, or the chemochromic membrane that covers the device. A simple microprocessor circuit is used to measure the time taken for the photon-induced current to discharge the detector LED from an initial 5 V (logic 1) to 1.7 V (logic zero). This sensing device has been successfully used for colour and colour-based pH measurements and offers extremely high sensitivity, enabling detection down to the sub micro molar level of dyes.

Keywords: Fused-LEDs; Optical sensor; pH sensing; Reflectance measurements; Colorimetric analysis; Acidochromic dyes; Evanescent wave

1. Introduction

The increasing range of inexpensive optical components such as light emitting diodes (LEDs), optical fibres and photodiodes coupled with the recent advances in electronic, material sciences, computer technologies, and the wide range of synthetic reagents for colorimetric analysis have made optical and chemochromic systems a very attractive and important area of sensor research.

In most optical sensor configurations the use of LEDs as the light source has become very common as a result of the wide range of devices available, covering the ultraviolet to infrared range (ca. 380–900 nm). They also offer the advantages of low power requirements, long working lifetime, stable emission, availability in various sizes and shapes, and low cost. In general an optical sensor consists of a light source (LED), a light detector (e.g. a photodiode), a power source (mains or batteries) and electronic circuitry to run the system that includes a means of data transfer (to a PC) or a data display unit. The heart of the assembly is the LED and the photodiode, which are either arranged on the same side of the sensor platform for reflectance measurements, or are arranged such that the colorimetric chemistry occurs between the light source and the detector for transmittance/absorbance measurements. These design constraints have been resolved somewhat by using fibre-optics where light can be directed to wherever the configuration requires.

Numerous optical sensing systems reported in the literature have used these basic designs [1–7]. Matias et al. [8] developed a very simple and low-cost reflectometer for diffuse reflectance measurements. This device uses an LED as a light source and a light dependent resistor (LDR) as a detector for reflectance measurements.

Photodiodes are more commonly used as a detector along with an LED as a light source. Worsfold et al. [9] designed a double beam photometric detector that has an LED and a photodiode all enclosed in a 20 cm^3 box. Optical fibres have been adapted for chemical sensing [10–13] and as wave-guides. Hauser et al. [14,15] have used seven light-emitting diodes of different colours guided, one at a time, into a measuring cell by means of a fibre-optic coupler. Detection is carried out with photodiodes. These

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sensing devices have proved to be successful for specific applications.

In this paper, we propose a novel, simple optical sensor device based on a pair of LEDs. One of the LEDs is used as the light source and the other LED is reverse biased to act as the light detector. The LEDs are fused together to form a unique sensor platform where the light source and the detector are a single unit. This paper discusses the use of this device to measure colour, and to monitor colorimetric chemical reactions (pH induced colour change).

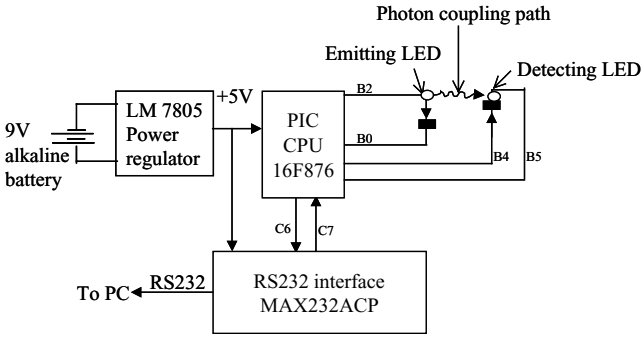

Fig. 2. Block diagram of circuitry used for the optical probe.

2. Experimental

2.1. Fabrication of fused-LEDs sensor and optical probe

2.1.1. Fused-LEDs sensor

The optical system was fabricated using 2 identical LEDs (broad band emission with λ_{max} at 621 nm) (LiteOn Corp. LTL-2F3VAKNT, USA). The optical system was fabricated by grinding the epoxy housing of each of the LEDs from the centre of the tip at a 45° angle (to the base of the LED) so that the two LEDs join together to form a 90° angle (Fig. 1A). The ground surface is then polished with increasingly fine grades of silicon-carbide abrasive of up to #400 to smooth the surface prior to bonding. The LEDs are then fused together using UV curable epoxy glue (Edmund Scientific: Orland 81 extra fast curing, USA), and placed under UV light (280 nm) for 30 min. The finished fused-LEDs detector is shown in Fig. 1A.

2.1.2. Optical probe

An optical probe was prepared as follows; The fused-LEDs detector prefitted with connection leads was inserted into an opaque poly(propylene) tubing (L (10 cm) × D (1 cm)) (Atlantic Homecare, Dublin, Ireland) so that the tip of the detector was ∼5 mm from the opening and the leads were exposed at the other end. The tube was then filled with fast set epoxy glue (Lennihans, Dublin, Ireland) so that only the tip of the optical device (ca.2 mm) was exposed. This configuration allows the sensor to operate in solution and restrict the amount of ambient light from reaching the detector LED.

2.2. Circuitry and operation of fused-LEDs sensor

2.2.1. Circuitry

Fig. 2 shows the simple circuitry that makes use of the internal parasitic capacitance of the LED itself to indirectly measure the incoming light intensity in terms of decay time. The main components are a microprocessor (PIC 16F876), a voltage regulator, and a MAXIM MAX232 RS232-TTL level shifter for communication with a PC. The emitter LED is driven directly from microprocessor control pins. The detector LED is also connected to the microprocessor I/O pins, so that it can be charged to +5 V very quickly, and then the control pins switched into the high-impedance (approximately 10^{15} ohm resistance) digital input state. This decreases the leakage current through the control pin down to on the order of 2×10^{-14} A (0.002 pA). Compared to

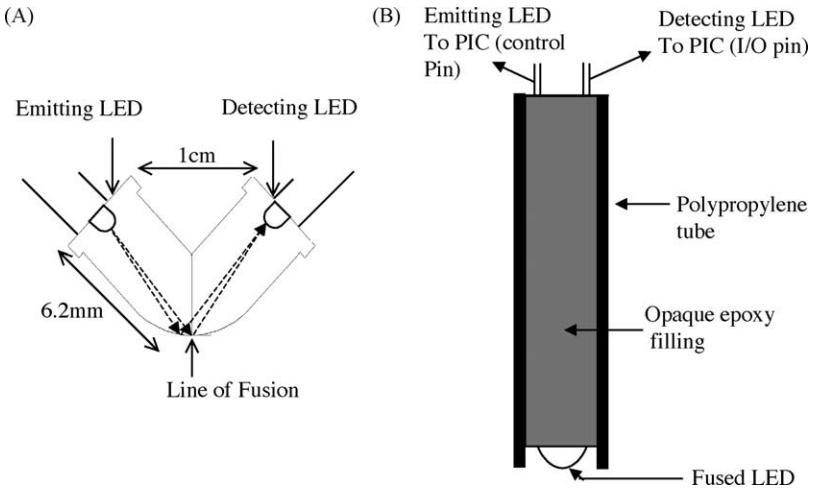

Fig. 1. Schematic of (A) a fused-LEDs and (B) cross-section of the optical probe.

a typical photocurrent of 50 pA through the diode itself, this 0.002 pA pin leakage current is insignificant. Once this control-pin changeover to digital input mode is done, the PIC 16F876 microcontroller measures the time needed for the 5 V charge on the LED to decay down to 1.7 V (logic zero) and communicates this information to the controlling PC.

2.2.2. Measurement rationale

The photon emitted from the light emitting LED hits the detector LED (reverse biased LED). This results in a small photocurrent being produced. The photocurrent produced is in the range of 10^{-12} A (1 pA) in total darkness to about 10^{-6} A (1 μA) under strong illumination. With normal room illumination the current produced is in the order of ca. 50 pA. This is a very small current and measuring it directly would require expensive instrumentation. Instead, the internal parasitic capacitance of the LED itself is used to reproducibly measure the current.

Due to the configuration of the light paths in the fused-LEDs assemblies, a significant amount of light produced by one LED can strike the other LED. Typically, with a photocurrent of 50 pA in normal ambient laboratory lighting (emitting LED off), and a capacitance (LED) of 20 pF, the voltage decay rate is approximately 2.5 V/s. Energising the exciter LED increases the photocurrent typically by an order of magnitude. By means of a small program executing in the PIC 16F876 microcontroller we can accurately measure the time required for this discharge, and hence measure the diode photocurrent, thus indirectly measuring the incoming illumination on the LED itself. This obviates the need to directly measure these extremely small photocurrents using expensive instrumentations. By alternately measuring the decay times with the exciter LED on and off, we can make a differential measurement and compensate for the effects of ambient lighting. As implemented, the base sample rate is 14 Hz., alternating between measurements taken with the emitter LED on and off for differential measurement. Hence, the signal is measured in time units, typically microseconds (see Figs. 4–7).

2.3. Chemicals and reagents

All the reagents used were of analytical grade. The pH indicator dyes used were nitrazine yellow (NY), bromocresol purple (BCP), and rhodamine B (RB) (all from Sigma–Aldrich, Dublin, Ireland). All samples were made from ultra pure water, and a 0.1 M pH 9 buffer solution was made from phosphate buffer tablets (Lennox, Dublin, Ireland) according to the instructed method. Stock solutions of 4 mM NY and RB were prepared by dissolving 0.21 and 0.19 g, respectively, in 100 ml of pH 9 buffer solution. BCP stock solution (0.92 mM) was prepared by dissolving 50 mg solid dye in a 100 ml pH 9 buffer solution, from which a 1.85 μM BCP solution was made up in the same buffer solution.

2.4. Data capture

Data was acquired via the PC serial port by a custom software interface that was developed in-house using Visual Basic 6 (Microsoft). The microcontroller board was programmed such that the sampling rate was 7 data points/s (differential readings). Raw data collected was exported into Excel (Microsoft) for subsequent analysis and visualisation.

2.5. Calibration procedure

The following was the calibration procedure; the optical probe was dipped into a 150 ml beaker containing 50 ml of pH buffer or dye solution and secured with a clamp, with the end of the device immersed approximately 1 cm into a 2.5 cm deep liquid. To vary dye concentrations small aliquots (4–400 μl) of stock dye solutions (NY, RB and BCP) were added into a pH 9 buffer solution to give calibration curves shown in Figs. 4 and 5. For pH titration experiments, A 1.85 μM BCP solution was made up in ultra pure water and adjusted to pH3 with a small amount of concentrated HCl (ca. 1–2 μl). Aliquots of 2–4 μl 4 M NaOH were added to 50 ml of this BCP solution to change the pH. A Metrohm pH meter (model 691) and glass electrode (Metrohm, Switzerland) were used to continuously monitor the actual pH of the solution during the experiment. In all experiments the reagents were added and stirred for ca. 2–3 min to ensure thorough mixing and to allow the pH meter reading to stablise. Then the stirrer was turned off to minimise turbulence and the light dependence discharge time of the detector LED was measured continuously for 10 s (sampling rate: 7 data points/s).

3. Results and discussion

3.1. Optical probe configuration and colour detection

The configuration of the fused-LEDs optical probe prevents one LED from directly illuminating the other LED. Instead, light must either exit the transparent plastic case, reflect from an external material, and re-enter the case, or the light must reflect directly from the inner surface of the case (internal reflection). It is supposed that if the fused-LEDs assembly is immersed in a liquid, the liquid in close proximity to the surface of the assembly can have a large effect on the amount of light propagated from one LED (emitter) to the other LED (detector) due to a change of refractive index of the medium. It follows that if the surface of the fused-LEDs assembly is modified with a coloured material, then the absorption characteristics of the material may have a large effect on the amount of light reaching the detecting LED. These hypotheses form the basis of our present and future investigations. The first optical probe was constructed from a pair of identical broad band orange LEDs (emission band ca. 400–800 nm, Fig. 3) which is convenient

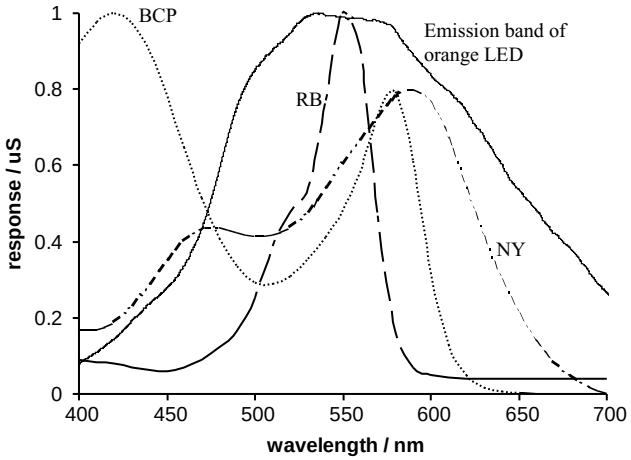

Fig. 3. Emission spectrum of LED used in optical probe and the absorption spectra of nitrazine yellow (NY), rhodamine B (RB) and bromocresol purple (BCP).

for demonstration purposes because this broad region covers wide range of dyes that are used for colorimetric analysis. For more specific applications the emitter wavelength should be chosen to be more compatible with the dye absorption spectrum for optimal sensitivity whereas the choice of detector is less critical because an LED is sensitive to all wavelengths of light equal to or shorter than the emission wavelength.

The initial study involved a simple calibration of a change in colour intensity (concentration) of a pH indicator dye, nitrazine yellow in a pH 9 buffer, in which only the deprotonated form (Fig. 3, absorption $\lambda_{max} = 590$ nm) dominates and efficiently absorbs the emission light of the source LED. Fig. 4 is a plot obtained from three repeats that shows a detection range between 0 and ca.120 μM with a linear range of approximately 0.4–100 μM nitrazine yellow. Lower detection ranges were observed with rhodamine B (ca. 0–10 μM, linear range 0 to <5 μM, Fig. 5) and BCP (ca. 0–4 μM, linear range 0 to <3 μM, data not shown). The limit of detection was not fully investigated but was in the order of hundreds of nanomolar in each case. It can be seen from Figs. 4 and 5 that the standard deviation of the measurements ($n = 3$, shown as error bars) are relatively high (ca. 10%). It was due to the electrical noise picked up from the long connecting leads between the fused-LEDs and the PIC. This has since been improved by reducing the length of connection leads used and by attaching the fused-LEDs directly onto a smaller circuit board.

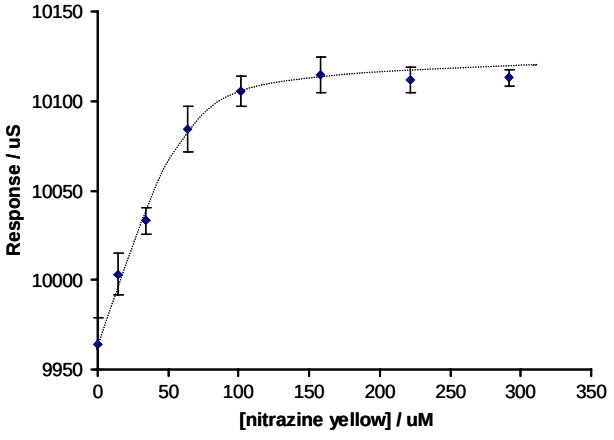

Fig. 4. Response of the fused-LEDs optical probe to a change in nitrazine yellow concentration. Each data point represents an average of three repeats and the error bars represent the standard deviation. The dotted line acts only as a visual aid.

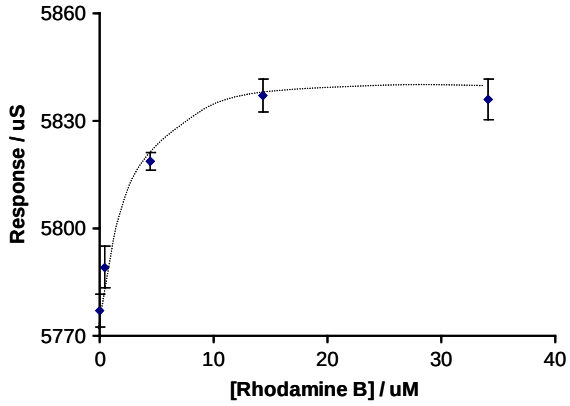

Fig. 5. Response of the fused-LEDs optical probe to a change in rhodamine B concentration. Each data point represents an average of three repeats and the error bars represent the standard deviation. The dotted line acts as a visual aid.

The stability of the fused-LED sensor is good. The data reported is from an optical probe that has been in continual use for at least 10 months. This same optical probe is currently in use and continues to function. The response time of the sensor to a change in colour is fast and is in the order of milliseconds.

The optical probe developed is based on measuring the light reflected back from the LED/solution interface that discharges the reverse biased detector LED, it is therefore possible to relate the measured discharge time to reflectance. One can take the capacitance C to be a constant as a result of a constant applied voltage of 5 V. Based on the equation

$$C = \frac{Q}{V} \tag{3}$$

where C is the capacitance, Q the charge accumulated and V is the applied voltage.The voltage change when the capacitor (LED) is discharged from an initial voltage V to a predefined voltage V' is $\delta V = V - V' =$ constant.

The charge Q being dissipated by the photocurrent i is $Q = it$, Eq. (3) becomes $\delta C = Q/\delta V$ or $t = C/\delta Vi$ and, as $\delta C/\delta V$ is a constant, then

$$t = \frac{k_1}{i} \tag{4}$$

The photocurrent i is proportional to the amount of light I reaching the detector, i.e.

$$i = k_2 I, \quad \text{where } k_2 \text{ is a constant} \tag{5}$$

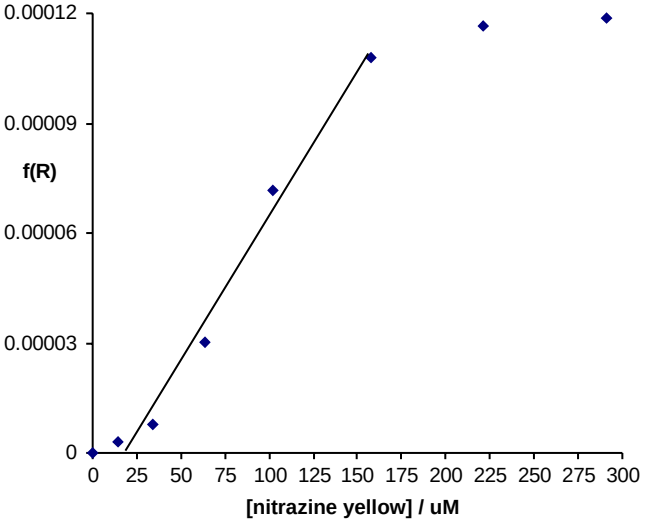

Fig. 6. The Kubelka–Munk function $f(R)$ vs. C plot obtained with the fused-LED sensor probe and nitrazine yellow.

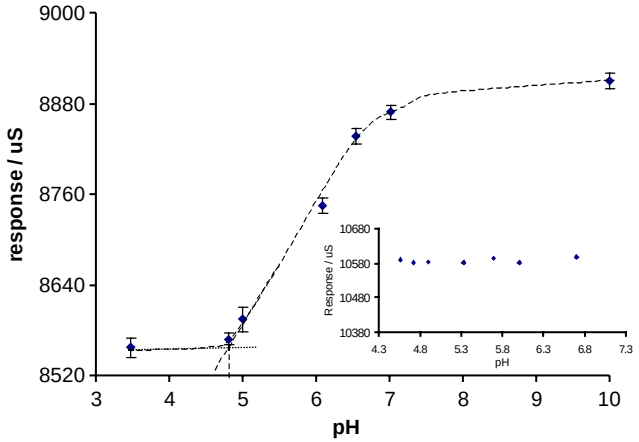

Fig. 7. Response of the fused-LEDs optical probe to a change in pH of 1.85 μM BCP solution. Each data point represents an average of three repeats and the error bars represent the standard deviation. Inset: Response of fused-LEDs optical probe to a change in pH of water acidified with HCl to pH 3.

Therefore, combining Eqs. (4) and (5), we get

$$t = \frac{k_3}{I} \tag{6}$$

where $k_3 = k_1/k_2$

Because I is related to the concentration of coloured species in the solution; it follows that the measured time t required for the photocurrent i to discharge from V to a preset level V' (the logic zero) is related to the concentration of the analyte in solution.

For absorbance, the optical density A for a light that passes through a solution of concentration C and a thickness l is given by Beer–Lambert law:

$A = \log(I_0/I) = \varepsilon cl$ where ε is the molar absortivity of the analyte measured at a given wavelength.

In reflectance analysis the optical density A_R of the reflected measurement is given by $A_R = -\log R$ and $R = I/I_0$. The value of R therefore varies from 0 to 1 and is equal to 1 when an ideal white surface (e.g. compressed $BaSO_4$) is used to reflect all incident light.

From Eq. (6), R can be rewritten as

$$R = \frac{t_0}{t} \quad \text{and} \quad A_R = -\log\left(\frac{t_0}{t}\right) \tag{7}$$

Kubelka–Munk theory is widely used to relate the reflectance R to the concentration C of the analyte.

$$f(R) = \left[\frac{(1-R)^2}{2R}\right] = \frac{\varepsilon C}{S} \tag{8}$$

where ε is the molar absorptivity of the analyte and S is the scattering coefficient. Plotting $f(R)$ versus C yields a straight line.

Fig. 6 is a Kubelka–Munk plot for the LED based optical device using the nitrazine yellow data shown in Fig. 4. The plot shows a linear region between ca. 15–150 μM natrazine yellow with an R^2 value of 0.985. Deviations from linearity below 15 μM and above 150 μM were observed indicating the lower limit of detection of the device for this particular dye and when the system is driven into saturation. This result shows that the fused-LED device is suitable to use as an optical device for measuring reflectance when Kubelka–Munk equation is obeyed.

3.2. Optical probe to monitor colour changes

The optical probe has been used to monitor the pH-dependent colour change of BCP. The calibration curve obtained for BCP using similar procedure to nitrazine yellow indicates that above a concentration of ca. 4 μM the responses were independent of concentration (i.e. response saturation); a dye concentration of 1.85 μM was therefore chosen to avoid saturation. Fig. 7 shows the results of three replicates of pH titration with an inset plot to show the lack of response to pH changes of water acidified with HCl to pH 3 in the absence of the dye. The calibration resulted in a sigmoidal shaped curve with a linear range between pH 4.8 and 7.2. The pK_a was determined to be 6.1 which is comparable to the reported pK_a value of 6.3 for BCP [16].

This experiment has demonstrated not only that the optical probe is able to follow the pH-dependent colour change of a species; with the known initial dye concentration and the pK_a determined, it is easy to estimate the lowest concentration of the deprotonated BCP dye detected by the optical probe using the following equation [17]:

$$\%\,\text{ionization} = \frac{100}{1 + 10^{-(\text{pH}-\text{p}K_a)}} \tag{1}$$

for acid dissociation reactions of the type

$$\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^- \tag{1'}$$

where, in this case, HA is the ionisation of the BCP dye in acid form (HA, yellow colour). The addition of NaOH gives

the blue deprotonated form (A^-); and

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad (2)$$

By using the intercept between the base line and the linear part of the response curve of Fig. 7, we can estimate that the initial response of the titration started from ca. pH 4.8. Therefore equation 1 estimates that the optical probe detects as low as ca. 90 nM deprotonated BCP in this particular configuration.

3.3. Detection mechanism

These results have shown that the change in colour intensity (therefore the change in dye concentration) and a change of colour (i.e. a shift of wavelength) of the environment that the probe is in, both have a significant impact on the amount of light reaching the detector LED. This would allow the optical probe to be used as an analytical tool for colour measurements. An unexpected, but interesting observation is that the probe operates at a very low concentration (nanomolar to micromolar) range. The exact mechanism is not entirely clear at this stage and is still under investigation. However, simple experiments have been carried out to elucidate the possible detection mechanisms. The pH calibration experiment was carried out with a small amount (10 μl) of ethylene glycol added into the solution, resulting in a significant reduction of signal (Fig. 8, curves A and B). This result helps to rule out the bulk effect because if the emitted light from the source LED has to travel out into the bulk solution, and reflect/scatter back to the detector LED, there should not be any suppression of response as the bulk dye concentration is virtually unchanged. It can also be ruled out that the response is due to simple total internal reflection because this phenomenon is independent of external colour changes.

The ethylene glycol has a tendency to adhere onto the surface of the LED casing, which is an organic epoxy polymer, so it is likely that the observation was due to a surface effect and that an evanescent wave, propagating along the interface surface between the organic epoxy polymer case and the solution may be involved. The emitted light from the source LED hits the surface of the LED casing and because there is a change in refractive index from the LED casing to the solution, an evanescent wave traverses along this interface and is able to penetrate into the solution to interact with the dye molecules. Because the evanescent wave can only penetrate a limited depth, typically a few hundred nanometers, with the light energy decrease exponentially into the solution; when there is a layer of ethylene glycol molecules covering the casing surface the light–dye interaction is greatly reduced and this results in reduced sensitivity. This may also explain why the calibration curves shown in Figs. 4 and 5 saturate at low concentrations. The limited light energy can only detect low concentration of dye molecule nearest to the surface. It must be stressed that this interpretation of the observation is somewhat vague, as there may be interactions between the dye molecules and ethylene glycol that may change the chemical equilibrium (deprotonation process). Further studies of concentration change of a non-pH-sensitive dye in the presence of ethylene glycol will help to clarify this. Pre-treating the probe with a very thin layer of hydrophobic material (e.g. paraffin oil) may also give useful information about the proposed surface effect.

This observation also raises an important issue that the presence of some organic compounds may affect the response obtained by the optical senor if they adhere onto the surface of the LEDs. This effect of organic materials other than ethylene glycol on the response has not yet been investigated and will be an important part of future studies. However, this interferent effect may be minimised if the surface of the LEDs is made more polar, possibly through plasma treatment, to enhance surface-dye interactions.

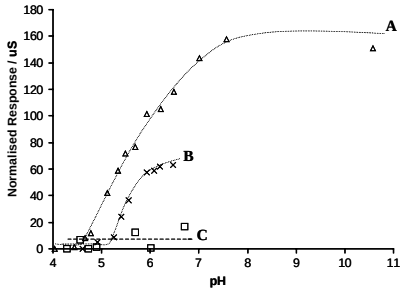

Fig. 8. Effects of ethylene glycol to pH titration of (A) BCP dye solution, (B) mixture of BCP + EG and (C) EG only.

3.4. LED–LED sensor platform versus LED-photodiode optical system

A distinctive advantage of using the fused-LED optical sensor in comparison with widely used LED-photodiode system is that the LED–LED combination is a lot cheaper in both component cost (35 US cents per sensor), and the cost for the signal transduction circuitry. With the LED–LED system, because the output as seen by the microprocessor digital input as a direct pulse-duration-modulated signal, so there is no need for a relatively expensive A/D converter. This proposed device uses much less power (can operate in microwatts range), can detect low absolute light levels (∼0.0001 lx), responds to a broad bandwidth (350 to >900:nm, i.e. non-selective detector) and, with digital filtering, can achieve very low S/N ratio.

This LED–LED sensing system is very versatile. It may be configured as a single fused-light source-detector device as proposed in this paper, or they may be used as two separate components similar to conventional LED-photodiode optical system. More importantly, the LED–LED sensing system may be used directly in contact with the analyte which may minimise the loss of signal through scattering. It is also

probable to use the fused-LED device (or just the detector LED) as a generic optical sensor platform where the surface is modified with specific chemochromic reagents to achieve discrete selectivity. This method of sensor fabrication is already under investigation in our laboratory.

4. Future developments

The current research on this fused-LEDs sensor has shown that the device can be used as an optical tool for colorimetric analysis in solution, and also when the device is modified with a chemochromic reagent for gas/liquid phase detection [18]. This device detects extremely low concentrations of dye molecules, opening up a new and exciting area of research for low detection limit optical devices based on simple and very inexpensive components. These optical devices may form a generic platform for a number of different colour-based detections, especially in the biosensor field where low detection limit is an important prerequisite. Since the LED sensor can be easily coated with a polymeric film containing chemochromic reagents, it makes it an extremely versatile sensing platform for constructing solid-state sensors for various colorimetric analysis. Furthermore, the detection principle is not confined to the fused-LEDs mode. A pair of LED may be arranged in conventional configurations which normal LED-photodiode systems adopt for reflectance and transmittance measurements, although these arrangements may not offer as low a detection limit. Given that LEDs come in different sizes and shapes (the smallest surface mount LEDs are just a few millimetres square in surface area), they should be extremely useful for the development of microfluidic/lab-on-a-chip sensing devices, which, at present, commonly rely on relatively large light sources and detectors.

5. Conclusions

We have demonstrated that these fused-LEDs devices coupled with measuring the light-induced voltage decay time of the detector LED are useful for colorimetric analysis. This optical sensing system uses very inexpensive optical and electronic components and is simple to construct. Its power consumption is low and can be operated from a common 9 V battery for months. The high sensitivity observed with these optical probes makes them interesting tools for optical detection at low concentrations.

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