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A Very Simple Photometer Based on Paired-Emitter-Detector Diodes

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According to the concept of paired-emitter-detector diode (PEDD)-based photometry, a light emitting diode (LED) used as a light source together with a second LED serving as a light detector comprises a novel kind of a photometric device. It has been found that coupling of PEDD with an ordinary multimeter used simultaneously as a power supply of LED-emitter and as an instrument measuring the signal generated by LED-detector allows form a complete, extremely simple, and low-cost photometer. Owing to these advantages such photometer is especially useful for student teaching, although it could be used also for more advanced analytical applications. This paper reports on the set of experiments with a use of the developed photometer demonstrating its practical utility, including determination of selected dyes in the ppm concentration range, pH detection with the use of acid-base-indicators, enzymatic detection of urea, and finally bioassays for evaluation of urease and alkaline phosphatase activities.

W pracy opisano detektor fotometryczny zbudowany z dwóch diod elektroluminescencyjnych, pełniących rolę źródła i detektora promieniowania. Takie sparowane diody połączone ze zwykłym miernikiem uniwersalnym zasilającym diodę-emiter i jednocześnie rejestrującym potencjałowy sygnał analityczny generowany przez diodę-detektor mogą pełnić funkcję prostego i bardzo taniego a zarazem kompletnego fotometru, który może być stosowany w wybranych pomiarach analitycznych. W pracy przedstawiono przykłady kilku prostych oznaczeń analitycznych z użyciem tego urządzenia, w tym pomiary stężeń wybranych barwników, pomiary pH, enzymatyczne oznaczanie mocznika oraz oznaczanie aktywności dwóch enzymów: ureazy oraz fosfatazy alkalicznej. Ze względu na prostotę wykonania oraz znikome koszty fotometru, a także przykładowych procedur analitycznych, mogą być one zaadaptowane w procesie kształcenia w zakresie analizy instrumentalnej.

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Light emitting diodes (LEDs) are the simplest and cheapest optoelectronic components mass-produced in semiconductor technology. These extremely low-cost, low-power, robust, and intensive light emitters with narrow emission spectra covering broad spectral range from UV to NIR are widely applied in many commercial photometers and spectrophotometers. Several analytical applications of LEDs as effective light sources for photometric systems have been reviewed recently [1, 2].

LED consists of a p-n junction, which emits a characteristic narrow band of light frequencies when forward biased. Surprisingly, LED operates reversibly: if light is incident upon the p-n junction, a small current, proportional to the light intensity, is generated. Thus, LEDs applied in the reverse mode can be used as light detectors, alternatively to phototransistors, photodiodes, light dependent resistors, and photomultiplier tubes commonly applied in photometers [2]. LEDs are significantly cheaper than the above mentioned detectors, but less sensitive. Unfortunately, they produce picoamperic currents difficult for direct measurement without special signal amplification. To bypass this problem, a special electronic circuit has been developed recently [2–4]. Its operation is based on the computer-controlled measurement within the microsecond time range required for discharging of the illuminating LED.

Recently we have announced an alternative approach based on the measurement of electromotive force generated by LED-detector using a precise potentiometric pH-meter [5]. The theory of operation for such photometric-transduction system, based on the compilation of Shockley equation for non-ideal diode with Lambert-Beer law for analytical photometry, leads to the conclusion that the voltage signal generated by paired emitter detector diodes is directly proportional to the absorbance and thus to the analyte concentration. In this work we have demonstrated the possibility of further simplification of the transducing system, which results in the construction of extremely cheap instrument consisting of only two LEDs and an ordinary electronic multimeter. Such complete photometric system costs less than 10 US dollars and can be easily customized and modified even with no experience in electronics. These features are especially important from educational point of view. Some examples of simple analytical and bioanalytical detections illustrating practical utility of the designed photometer have been presented.

EXPERIMENTAL

Dyes: Congo red (CR), bromphenol blue (BPB), bromcresole green (BCG), bromthymol blue (BTB), thymol blue (TB) and thymolphtalein (TP) were obtained from POCh (Poland). Urease isolated from Jackbean (EC 3.5.1.5, powder 83 U mg^{-1}), alkaline phosphatase isolated from bovine intestinal mucosa (ALP, EC 3.1.3.1, powder 10 U mg^{-1}) and sodium monofluorophosphate (MFP) were obtained from Sigma–Aldrich (USA). Urea and other reagents of analytical grade used for buffers preparation were obtained from POCh (Poland). Doubly distilled water was used throughout.

Red LEDs (5 mm diameter; transparent lens, flat front; 140° view angle, 1560 mcd average luminous intensity at 20 mA current supply; λ_{max} = 628nm, product No OSR5MA57E1A) were obtained from Optosupply (Hong Kong). A cuvette holder was made of black Lego® bricks with mounted pairs of LEDs coupled with a UNI Trend multimeter (model UT30C, Hong Kong). Disposable acrylic plastic cuvettes (1 cm optical path length) used for measurements were obtained from Sarstedt (product No 67.755, Germany). Reference optical measurements were performed using a Shimadzu spectrophotometer (model PC2401, Japan).

RESULTS AND DISCUSSION

Photometer operation

A scheme of the complete photometer is shown in Figure 1. Two red LEDs are mounted into black Lego® bricks serving as a holder for common 1 × 1 × 4 cm cuvettes. The multimeter plays simultaneously two roles. LED used as a light detector is coupled with a multimeter working in the voltmetric mode. LED used as an emitter is powered by the multimeter operated in the transistor tester function (emitter (E) is positive and collector (C) is negative in p-n-p-type transistors). An additional resistor (70 Ω) enables long-time, stable, and reproducible measurements. The multimeter can be used for at least three days for continuous supplying of LED-emitter and recording of a voltage signal generated by LED-detector without a need of battery exchange. Moreover, without this resistor, supplying of LED-emitter slightly influences simultaneous readings of LED-detector voltage.

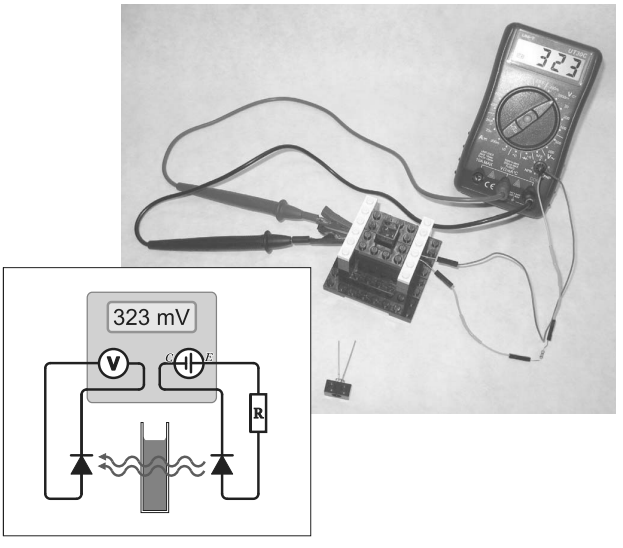

Figure 1. A photo and a scheme of a complete photometer

Contrary to pH-meters, common multimeters, like the one used in this study for voltage measurements, offer negligible input resistance. In consequence, they are discharged by the measurement of electromotive force (EMF) generated by LED-detector. Such EMF measurement causes total discharge of LED when the detector is not illuminated, so the signal for darkness is zero volts. When LED-detector is illuminated, the measured voltage signal is the resultant of the illumination-related charging and measurement-related discharging. On the other hand, this non-ideal way of EMF measurement causes a significant expansion of the available signal range. Although the responses are linear in narrower ranges than in case of the measurements performed with a high-impedance input pH-meter [5], they are still useful from the analytical point of view.

LEDs can be used for detection of light of a higher energy than the light emitted by themselves [5]. Thus, red LED operates as a non-selective detector in the whole range of visible radiation. Selectivity of photometric measurements with such detector is defined only by a narrow emission spectrum of LED applied as a light source. Obviously, red LED-emitter determines the application of a photometer to optical detection of blue compounds having absorption spectra compatible with the emission spectrum of LED itself.

Analytical measurements

The absorption spectra of several investigated blue dyes as well as the emission spectrum of red LED are shown in the left part of Figure 2. The absorption coefficients ε (λ_{max} [nm]) of these dyes are: 48 (585), 117 (589), 12 (616), 48 (616), 61 (598) and 79 (594) L cm^{-1} g^{-1} for CR, BPB, BCG, BTB, TB, and TP, respectively. Although the spectra of some dyes (especially BPB, but also TB and TP) are not fully compatible with red LED spectrum (λ_{max} = 628 nm), the photometer can be still used for their detection.

Figure 2 also presents calibration graphs of all tested dyes obtained using the designed photometer. The plots are linear in a relatively wide range of concentrations. Even if the spectra of some dyes are not fully compatible with LED spectrum (BPB case) and their absorption coefficients are different (the lowest for BCG), determination of dyes in the ppm concentration range is still possible.

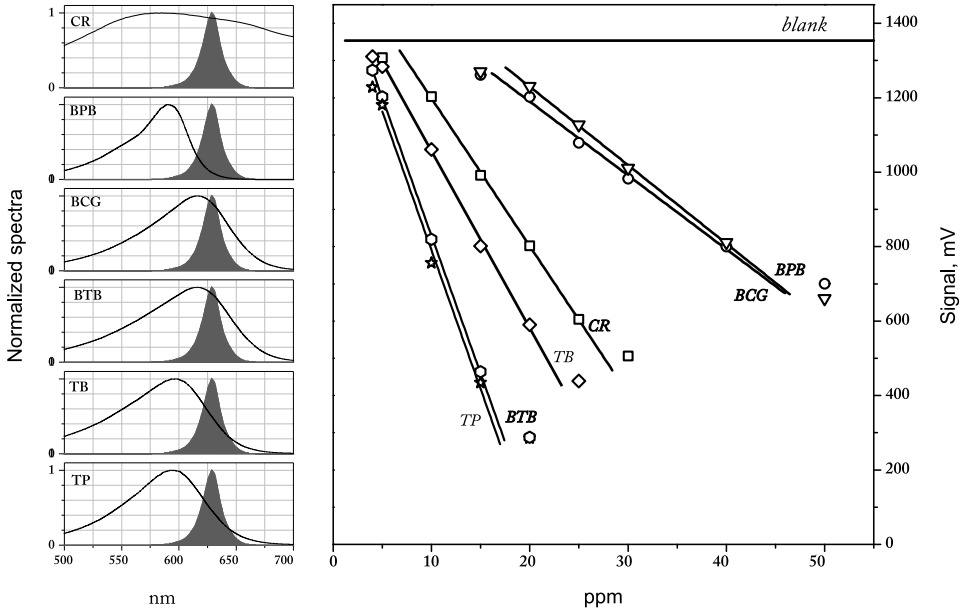

Figure 2. Absorption spectra of the analyzed dyes and emission spectrum of red LED applied as an emitter (left) and photometric calibration plots for dyes in quasi-linear concentration ranges (right). Measurements were performed in 0.01 mol L^{-1} HCl or 0.01 mol L^{-1} NaOH solutions, in which blue protolytic forms of the investigated dyes were predominant

All dyes investigated in this work are pH-indicators. In each case one of their protolytic forms is blue and thus optically detectable using the developed photometer. These indicators exhibit different pKa values, so they can be used for optical detection of pH in a wide range (Fig. 3). Moreover, the results shown in Figure 3 can be applied for experimental estimation of pKa values. pKa values were obtained in the series of universal buffers and equal (literature data [6] are given in parentheses): 4.2 (4.0), 4.2 (4.1), 4.8 (4.9), 7.0 (7.2), 9.1 (8.9) and 10.0 (9.7) for CR, BPB, BCG, BTB, TB, and TP, respectively. Taking into account quasi-linearity of detector response in wider concentration ranges of dyes (Fig. 2), caused by incompatibility of dye light source spectra, the correlation between the literature and experimental data is satisfactory and absolutely sufficient for the needs of student exercise. To improve quality of measurements with some dyes, other red LEDs exhibiting maximum absorbance at slightly shorter wavelength could be applied. Such an approach is in agreement with the general concept of PEDDs (based on careful selection of LED-emitter), in which the devices are dedicated for a particular kind of an analyte.

Bioanalytical measurements

Protolytic properties of products generated in the course of many enzymatic reactions offer a possibility of bioanalytical measurements based on pH changes. Such biosensing schemes can be used for detection of either a substrate or enzyme activity, or both. A common example of such an approach is biosensing of urea using urease. The products of enzymatic hydrolysis are slightly alkaline (pH of the enzyme reaction environment increases up to 9.3 [7]), whereas maximal activity of urease occurs at pH 6÷8. Thus, the expected range of pH changes caused by the biocatalytic process is from 6 to 9 and fully covers the characteristic pH range for BTB (Fig. 3).

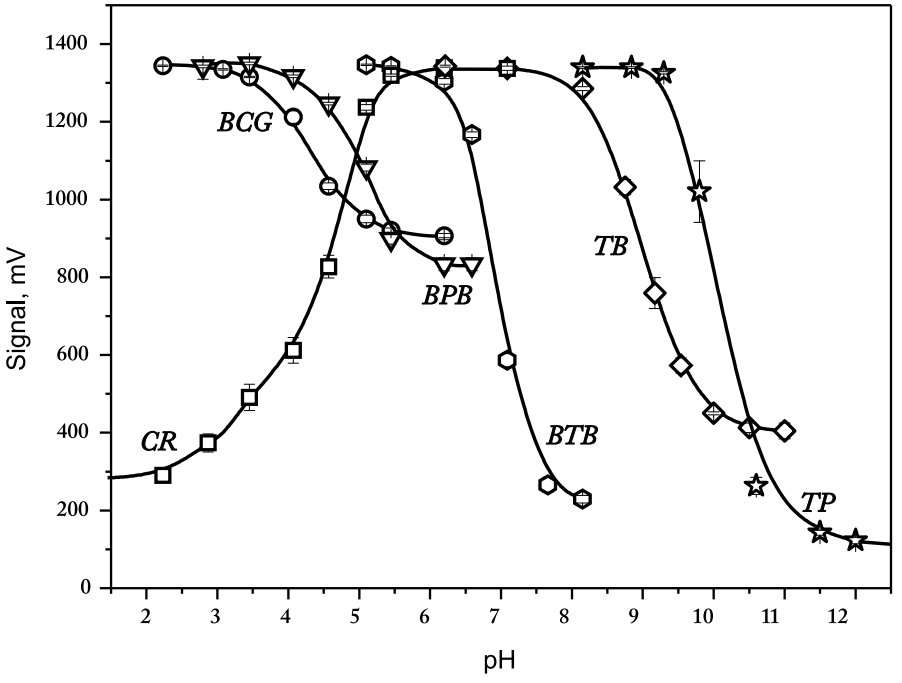

Figure 3. Photometric pH measurements using selected dyes. Measurements were performed in a series of universal buffers with selected acid-base-indicators

Figure 4A shows an example of photometric detection of urea in the millimolar concentration range, performed in 10 mmol L^{-1} phosphate buffer (pH = 6.2) doped with BTB (50 mg L^{-1}) and urease (5 U mL^{-1}). The same kinetic scheme of measurements can be applied in the reverse mode for optical detection of enzyme's activity. The results of urease assay performed in the same buffer doped with BTB and urea (100 mmol L^{-1}) are shown in Figure 4B.

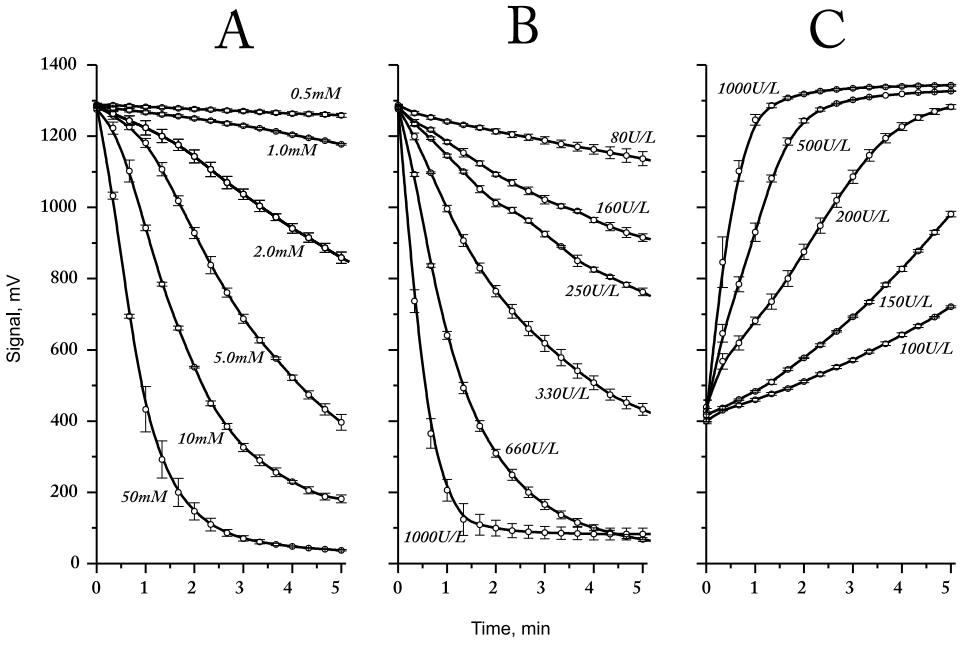

Figure 4. Cuvette assays of urea (A), urease activity (B), and ALP activity (C). Analyte concentrations/ activities are given in the Figure. Conditions of measurements are described in the text. Each measurement was repeated in triplicate

ALP is another example of biomedically important enzyme, easily available in the form of cheap, stable, and highly active enzyme preparations, what is acceptable for educational needs. pH sensing of ALP activity is possible using very cheap MFP as the enzyme substrate [8]. Maximal ALP activity is observed above pH 9, whereas the products of MFP biodegradation acidify the environment down to pH 7. TB is a useful acid-base indicator for the monitoring of pH changes in this range (Fig 3). The results of ALP activity assays with the use of the proposed photometer are shown in Figure 4C. The measurements were performed in saturated magnesium hydroxide solution doped with TB (50 mg L^{-1}) and MFP (10 mmol L^{-1}), reported as optimal for this kind of pH-based bioassay [9]. Although all presented bioassays were performed without any special control of experimental conditions (at the room temperature), their results were well reproducible (Fig. 4).

CONCLUSION

Exciting experiments are crucial for effective teaching of chemistry. In analytical chemistry it means the need of experiments easily understandable by students, easy-to-adapt to their experimental settings, and clearly demonstrating the features of the instrumental analysis. The need of experimental simplicity and reduction of outlays is obvious. In practice it means that the use of expensive reagents and instrumentation should be mostly reduced. Moreover, experiments should not be complicated, tedious, and time-consuming. In this paper we have developed an extremely simple instrument and a set of analytical and bioanalytical experiments fulfilling all these demands.

In conclusion, it should be strongly stressed that the complete photometer shown in this work can be easily customized for more sophisticated and advanced analytical systems, for example as a miniaturized and compact flow-through detector with extremely simple powering and signal transduction system for flow injection analysis [5, 10] or chromatographic systems [11, 12].

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