



Short communication

# A concept of dual optical detection using three light emitting diodes

Marta Pokrzywnicka, Robert Koncki, Łukasz Tymecki\*

University of Warsaw, Department of Chemistry, Pasteura 1, 02-093 Warsaw, Poland

## ARTICLE INFO

## Article history:

Received 18 November 2009

Received in revised form 6 April 2010

Accepted 16 April 2010

Available online 22 April 2010

## Keywords:

Light emitting diodes

Fluorometry

Photometry

Quinine

## ABSTRACT

In this paper a concept of very simple measurement system dedicated for simultaneous photometric and fluorometric detection is presented. Only three ordinary unmodified light emitting diodes (LEDs) can be applied in this analytical device: one of them is used in the conventional way as a source of nearly monochromatic light inducing fluorescence, whereas two others are applied as photometric and fluorometric detectors of light. In this study quinine is chosen as a model analyte. The reported device enables simultaneous detection of this analyte in the micromolar range of concentration. The practical utility of prototype dual detector for complex sample analysis is illustrated by its application for determination of quinine in tonic water samples.

© 2010 Elsevier B.V. All rights reserved.

## 1. Introduction

Although optical detection dominates in analytical chemistry, for many practical applications highly advanced and expensive spectrophotometers as well as spectrofluorometers are not necessary. For many dedicated analytical uses simple optical detectors based on economic optoelectronic components are sufficient.

Light emitting diodes (LEDs) are used very often in analytical photometric methods as extremely cheap, low-power, high efficiency, robust, long lifetime and very stable sources of nearly monochromatic light [1,2]. They are also suitable as excitation light sources inducing analytically useful fluorescence [2–5]. The role of LEDs as light sources for optical methods of analysis is well established.

On the other hand, it is known, that the internal photoelectric effect (opposite to electroluminescence phenomena) allows the use of LED as light detector [6,7]. Absorbance detectors exploring LEDs as light detectors are reported in the analytical literature [8–17]. As analytically useful signal generated by LED-based light detectors the discharging time [8–12] or electric potential [13–17] can be applied. Pairing of two LEDs, playing role of light emitter and detector, leads to construction of complete photometric devices useful in analytical chemistry. These are very simple photometers dedicated for educational purposes [17] as well as more advanced absorbance detectors for chromatography [8–10] and flow injection analysis [11–16].

In this communication the first-time example of the utility of LED as fluorescence detector is demonstrated. Moreover, the ability

of simultaneous absorbance and fluorescence measurements using extremely simple three-LED-based device without any additional optical and optoelectronic components is presented.

## 2. Experimental

Quinine (as sulphate salt) was obtained from Sigma-Aldrich (Germany). All other reagents were of analytical grade and were obtained from POCh (Poland). Doubly distilled water was used throughout. The set of quinine standards was prepared in 0.05 M sulphuric acid. The oxygen from quinine solutions was removed immediately before measurements by 5 min bubbling with argon. Disposable acrylic cuvettes (1 cm optical pathlength) were obtained from Sarstedt (product no. 67.755, Germany). The cuvettes with solutions were thermostated (usually at 30 °C) using water bath. The anionite resin Amberlite IRA-904 used in the course of real sample (drinks contained quinine) analysis was obtained from Bio Rad Laboratories. Drink samples were diluted with 0.1 M sulphuric acid. For each sample the measurements performed using the prototype device were six times replicated.

The prototype of dual photo/fluorometer is shown in Fig. 1. The holder for 1 cm × 4 cm × 4 cm cuvettes has been constructed using Technic™ LEGO® bricks. Typical holes in Lego bricks are suitable for LED mounting. In the course of measurements the holder was additionally isolated from incident light using ordinary black box. The holder enables mounting of one LED emitter, one LED-absorbance detector and two LED-fluorescence detectors. Optionally, fiber optic coupling the cuvette with conventional fluorometer can be mounted. Similar constructions have been shown in our previous papers [13,17].

Declared nominal powers of 365, 370 and 375 nm UV LEDs applied in this investigation were 3, 6 and 11 mW, respec-

\* Corresponding author. Fax: +48 22 8225 996.

E-mail address: [luktym@chem.uw.edu.pl](mailto:luktym@chem.uw.edu.pl) (Ł. Tymecki).

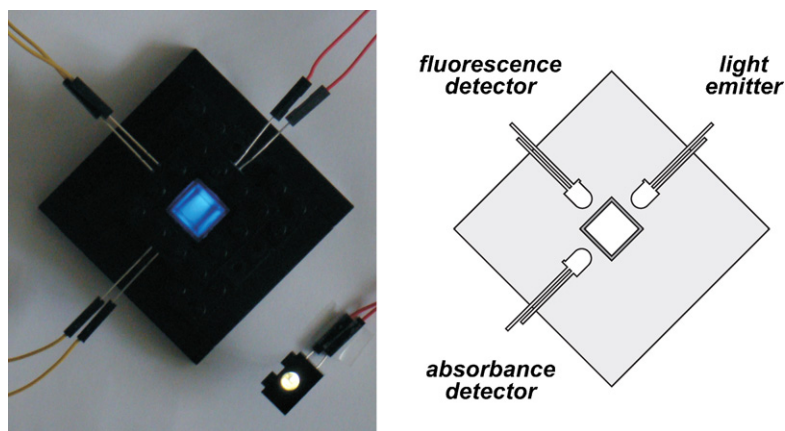


Fig. 1. The prototype of three-LED-based device for cuvette measurements.

tively. These 5 mm LEDs were supplied by Amecam (product numbers: LED365003-40AWOM06, LED3700006-50AWOM06 and LED375011-50AWOM06, respectively). 5 mm blue (470 nm) and red (630 nm) LEDs were obtained from Optosupply Honkong (product numbers OSUB5131A-PQ and OSHR53E1A-LM, respectively).

For supplying the LED emitter a home-made circuit was constructed using a solderless board and typical electronic components obtained from TME (Poland). As instrument measuring analytical signal (voltage generated by LED-based detectors) a digital pH-meter from Radelkis (model OP208/1, Hungary) was applied. The scheme of complete device for dual optical measurements is depicted in Fig. 1.

The reference measurements of fluorescence were performed using fiber optic fluorometer (model USB2000FLG from Ocean Optic Inc. (OOI), USA). The reference photometric measurements were performed using a UV–vis Shimadzu spectrophotometer (model 2401/PC, Japan).

### 3. Results and discussion

#### 3.1. Selection of LED emitter

In this study quinine, often applied for educational demonstrations of fluorescence phenomenon [18–21], has been chosen as a model analyte. Quinine is easily detectable by both, absorbance and fluorometric methods. In sulphuric acid solutions, quinine has two analytically useful absorption/excitation wavelengths, 250 and 350 nm. Regardless of which excitation wavelength is used, the wavelength of maximum fluorescence is 450 nm. Thus, as a light source, UV LEDs were applied.

In primary experiments the LED induced fluorescence was measured using OOI fluorometer. For these experiments the silica fiber optic connecting the instrument with the cuvette holder was mounted in place of LED-fluorescence detector. The highest fluorescence intensity of quinine (0–50  $\mu\text{M}$ ) was induced by 375 nm LED with the slope of  $19.6 \pm 0.2$  [counts per  $\mu\text{M}$ ] ( $R^2 = 0.9996$ ). For comparison, the sensitivities for 370 and 365 nm LEDs were  $8.8 \pm 0.1$  ( $R^2 = 0.9985$ ) and  $4.3 \pm 0.1$  ( $R^2 = 0.9998$ ), respectively. The results suggest that the intensity of LED emitter is more significant parameter than the compatibility of light source spectrum with absorption spectrum of quinine. In the other words, due to straightforward energy transfer mechanism, quinine absorbs reasonable strongly up to 375 nm and as long as it happens and the LED intensity is greater, then there will be more energy transferred and the emission will be enhanced. As expected, longer-wavelength red and blue LEDs did not induced fluorescence of quinine. Obviously, the emission spectrum of fluorescence-induced-LED is compatible

with absorption spectrum of quinine. This way the selected LED can also play the role of light source in the course of absorbance mode measurements.

#### 3.2. Selection of LED-fluorescence detector

LEDs could play a role as non-selective detectors for light of higher energy than the light emitted by them. Additionally, halfwidths of the emission spectra of the LEDs used in the course of investigations are  $\pm 10$  nm. Thus, potentially all tested LEDs can detect light generated by 375 nm LED emitter, whereas only blue and red LED detectors could measure quinine fluorescence. As expected UV LEDs are insensitive for fluorescence. Useful calibration graphs for fluorometric quinine detection are shown in Fig. 2. Analytically useful calibration graphs for photometric quinine detection are given in Fig. 3. According to the operation theory of paired LED-absorbance detectors based on compilation of Shockley equation and Lambert–Beer law [13], for absorbance mode of measurements the voltage signal is directly proportional to quinine concentration. As the intensity of fluorescence is directly proportional to the analyte concentration, the fluorometric calibration curves are expected to be linear in the logarithmic scale of concentration.

Fluorometric measurements with red LED detector allow wider linear range of determination, whereas blue LED detector offers higher sensitivity but worse detection limit and reproducibility. Moreover, the graphs shown in Fig. 2 illustrate effect from the intensity of light inducing fluorescence. In both cases for lower intensities worse detection limits are observed caused by limited sensitivity of LEDs as light detectors.

#### 3.3. Selection of LED-absorbance detector

For absorbance measurements only data for red and 365 nm LEDs detectors are shown (Fig. 3) because analytical signals generated by other LEDs were out of scale of the used pH-meter (the electromotive force generated by illuminated LEDs was higher than 1999.9 mV). As it was shown in our previous paper [14] only high impedance pH-meters offer ideal currentless conditions for measurements of voltage signal generated by LED detectors. Simpler electronic circuits give no ability to demonstrate real characteristics and phenomena of linear LED response, what is intention of this paper. On the other hand, pH-meters are common laboratory equipment. Thus, the suggested way of experiments is acceptable for analysts without experience in electronics.

For red LED detector surprising inversion of calibration graph was obtained (Fig. 3). Apparently, with an increase of quinine

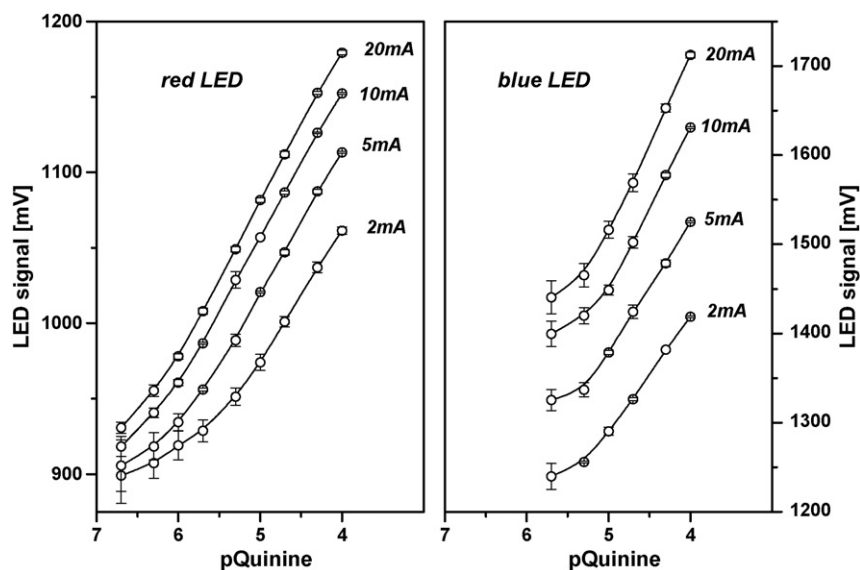


Fig. 2. Calibration graphs for fluorometric measurements with red (left) and blue (right) LED used as detector. Currents supplying fluorescence inducing 375 nm LED emitter are given at curves.

concentration an decrease of absorbance was measured. Regular calibration graph was obtained in the presence of 1 M excess of chloride ions. This effect can be explained by nonselectivity of red detector, which is sensitive for both absorbance and fluorescence wavelengths. Chloride ions are quenching fluorescence of quinine and therefore expected calibration graph is obtained. On the other hand, these observations indicate limitations of LEDs as absorbance detectors. In case of 365 nm LED detector such effect is not observed because this LED is insensitive for quinine fluorescence. Thus, as shown in Fig. 3, no effect from chloride ions is observed. UV LED offers significantly higher sensitivity than red LED. Finally, for further experiments red LED-based fluorescence detector and 365 nm LED-based absorbance detector have been applied.

#### 3.4. Possible modifications of the detection system

It is worth to notice that the reported device was constructed using only three ordinary unmodified LEDs without any additional monochromators, filters, lenses, collimators, changes of LED geometry etc. Sensitivity of absorbance measurements could be improved by changing of signal transduction method and careful adjustment of LED emitter powering [14]. Sensitivity of fluorometric determination could be improved by modifications of LED-emitter geometry [3–5], by using brighter LEDs or their arrays for example, the replacing of absorbance LED detector with the second identical LED emitter in the cuvette holder shown in Fig. 1 resulted in nearly twice increase of sensitivity for detection with fluorescence LED detector. Investigations of more advanced and

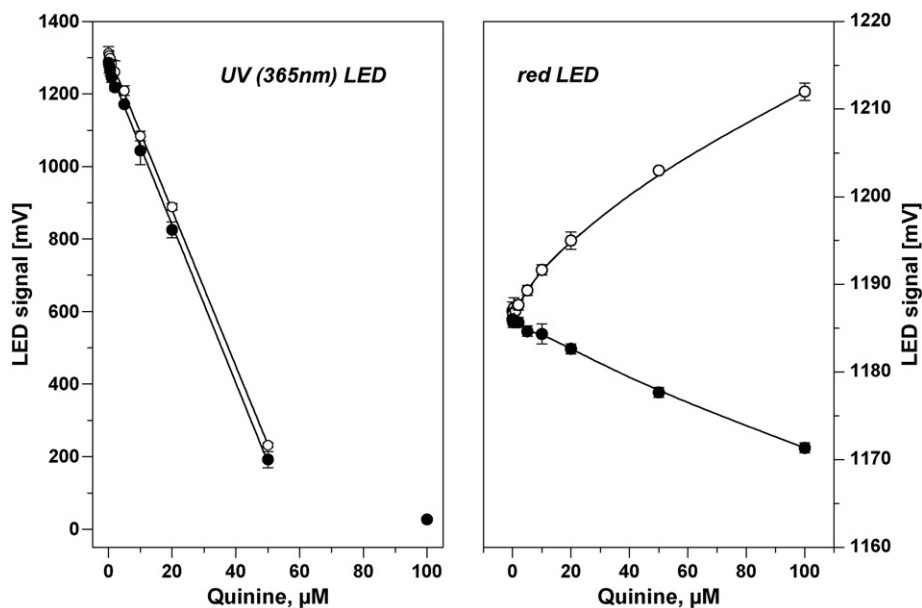


Fig. 3. Calibration graphs for absorbance measurements with 365 nm LED (left) and red LED (right) used as detector. 375 nm LED emitter was supplying with 20 mA current. Data for quinine standards with (filled points) and without (open points) 1 M chloride ions additive.

**Table 1**

Quinine determinations in tonic waters performed with dual optical detector. Fluorescence measurements performed with and without (data in brackets) chloride ions removal. Reference analysis were performed photometrically at 350 nm.

Sample	Quinine content [ $\mu\text{M}$ ]		
	Reference method	LED-based photometry	LED-based fluorometry
Schweepes	110 $\pm$ 1	110 $\pm$ 2	109 $\pm$ 1 (59 $\pm$ 4)
Kinley	99 $\pm$ 2	100 $\pm$ 3	98 $\pm$ 2 (71 $\pm$ 3)
Zbyszko	50 $\pm$ 1	50 $\pm$ 2	50 $\pm$ 2 (34 $\pm$ 2)
Ustronianka	35 $\pm$ 1	36 $\pm$ 1	34 $\pm$ 1 (28 $\pm$ 1)

improved detectors based on the concept announced in this paper are in progress. The most promising direction is the development of compact detectors dedicated for chromatography and flow analysis.

### 3.5. Analytical application of three-LED system

In both cases of measurement mode the resulting determination ranges were similar. Although these ranges are worse than those offered by advanced detectors, they are still useful for some kinds of complex sample analysis, for example for quinine determination in drinks. The utility of the extremely simple device (Fig. 1) in real scenario as a prototype of dual optical detector for cuvette measurements has been confirmed. Results of tonic waters analysis using the reported device are given in Table 1.

Primary experiments shown no effects from several species (alkaline cations, carbonates, sulphates, nitrates, glucose, ascorbic and citric acid at 1 mM concentration) on calibration graphs in ppm range. Only chloride anions at high concentrations influence both kinds of detection as discussed above. The concept of simultaneous detection seems to be attractive because it allows the control of interfering effects (i.e. quenching of quinine fluorescence by chlorides or influencing of absorbance measurements by other absorbing species) using two independent methods. It is well illustrated by the results of fluorometric determinations performed with and without chloride removal using of microcolumn with sulphate anion exchanger (Table 1).

## 4. Conclusions

In our opinion, the experiments performed with very simple measurement setup and presented in this communication give rise to important conclusions. To the best of our knowledge, this is the first evidence that LED can be applied as analytically useful fluorescence detector. Moreover, this is the first report on the three-LED-based system allowing simultaneous dual optical detection. The concept reported in this communication is illustrated by quinine detection, however it seems to be applicable for many other species, only if appropriate LEDs playing the roles of light source

and light detectors compatible with analyte spectra will be selected. Finally, the concept presented in this paper can be adapted for development of more advanced devices useful in analytical practice, like fluorometric as well as dual photometric/fluorometric flow-through detectors, similar to those working in absorbance mode of measurements, mentioned in the Introduction to this note.

Finally it should be stressed that the use of LEDs as light detectors is very attractive from economic point of view, because these are the cheapest optoelectronic components. LEDs are usually in 1–2 orders cheaper than conventional photodiodes (for example see Table 1 in paper [5]). Obviously, LEDs prices are beyond comparison with costs of the simplest photomultiplier tubes (PMTs), predominantly applied as fluorescence detectors. On the other hand, LEDs are significantly easier for integration and miniaturization endeavors than PMTs, so the concept of application of LEDs as fluorescence detectors could be stimulating for development of compact optical devices dedicated for selected analytes.

## Acknowledgements

These investigations were supported by research grant from the Polish Ministry of Science and Higher Education (project no. N N204 029636). MP kindly acknowledges PhD scholarship co-funded by European Social Fund and National Budget within Integrate Operating Program of Regional Development for Mazovian province (Operations 2.6 “Regional Innovative Strategy and Transfer of Knowledge”). ŁT acknowledges fellowship from Foundation for Polish Science (START, 2009).

## References

- [1] M. O'Toole, D. Diamond, *Sensors* 8 (2008) 2453.
- [2] P.K. Dasgupta, I.Y. Eom, K.J. Morris, J. Li, *Anal. Chim. Acta* 500 (2003) 337.
- [3] B. Yang, Y. Guan, *Talanta* 59 (2003) 509.
- [4] B. Yang, H. Tian, J. Xu, Y. Guan, *Talanta* 69 (2006) 996.
- [5] F.B. Yang, J.Z. Pan, T. Zhang, Q. Fang, *Talanta* 78 (2009) 1155.
- [6] R.H. Lindsay, *Am. J. Phys.* 44 (1976) 188.
- [7] F.M. Mims III, *Appl. Opt.* 31 (1992) 6965.
- [8] L. Barron, P.N. Nesterenko, D. Diamond, M. O'Toole, K.T. Lau, B. Paull, *Anal. Chim. Acta* 577 (2006) 32.
- [9] L. Barron, M. O'Toole, D. Diamond, P.N. Nesterenko, B. Paull, *J. Chromatogr. A* 1213 (2008) 31.
- [10] M. O'Toole, L. Barron, R. Shepherd, B. Paull, P. Nesterenko, D. Diamond, *Analyst* 134 (2009) 124.
- [11] M. O'Toole, K.T. Lau, D. Diamond, *Talanta* 66 (2005) 1340.
- [12] M. O'Toole, K.T. Lau, R. Shepherd, C. Slater, D. Diamond, *Anal. Chim. Acta* 597 (2007) 290.
- [13] Ł. Tymecki, M. Pokrzywnicka, R. Koncki, *Analyst* 133 (2008) 1501.
- [14] Ł. Tymecki, R. Koncki, *Anal. Chim. Acta* 639 (2009) 73.
- [15] Ł. Tymecki, L. Brodacka, B. Rozum, R. Koncki, *Analyst* 134 (2009) 1333.
- [16] Ł. Tymecki, K. Strzelak, R. Koncki, *Talanta* 79 (2009) 205.
- [17] M. Pokrzywnicka, R. Koncki, Ł. Tymecki, *Chem. Anal.* 54 (2009) 427.
- [18] J.E. O'Reilly, *J. Chem. Educ.* 52 (1975) 610.
- [19] J.E. O'Reilly, *J. Chem. Educ.* 53 (1976) 171.
- [20] S. Pandey, T.L. Borders, C.E. Hernández, L.E. Roy, G.D. Reddy, G.L. Martinez, A. Jackson, G. Brown, W.E. Acree, *J. Chem. Educ.* 76 (1999) 85.
- [21] J.H. Gutow, *J. Chem. Educ.* 82 (2005) 302.