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Short communication

A bimodal optoelectronic flow-through detector for phosphate determination

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ABSTRACT

A miniature flow-through detector useful for bimodal, photometric and fluorimetric, determination of phosphates has been developed. This optoelectronic device made of four light emitting diodes (LEDs) integrated in the form of 85 μL optical cell is easily applied in flow analysis manifolds. These LEDs play the roles of light source for photometric measurements, fluorescence inductors and detector of absorbance and fluorescence. For photometric mode of determinations a phosphomolybdenum blue method has been applied. The fluorimetric method of phosphate determination is based on quenching of rhodamine fluorescence by the heteropolyacid. The developed detector used in a simple three-channel flow injection analysis (FIA) system allows photometric or fluorimetric determination of phosphate in the wide range of concentration. The detection limits found for photometric and fluorimetric modes of FIA measurements are 5.5 mg L^{-1} and 10.4 μg L^{-1}, respectively. The potential utility of the flow-through detector for the needs of food and clinical analysis has been demonstrated.

1. Introduction

A great number of flow analysis systems are coupled with optical detectors. It is obvious that for such systems heavy, highly advanced and expensive optical instruments are not necessary. Therefore, one of current trends in modern flow analysis is the development of integrated miniature detectors of selected analytes based on economic optoelectronic devices [1]. Light emitting diodes (LEDs) are widely applied in modern analytical systems as nearly monochromatic, very stable and extremely cheap sources of light [2]. However, since 1976 it is known that LEDs can also be used as light detectors [3], thus pairing of respective LEDs can result in complete photometric device consisting of light source and detector. Paired emitter detector diodes (PEDDs) for the needs of analytical chemistry have been developed by Diamond group [4,5]. The comparison of photometric LED–LED detectors with LED-photodiode devices has been demonstrated recently [6]. Our group has shown that the analytically useful voltaic signal generated by PEDD offers high sensitivity of measurements [7-9]. A predominant number of analytical PEDDs are developed for photometric measurements [4–20]. However, recently it has been demonstrated that the PEDD concept can be effectively adapted for fluorescence detection [20–25], as well as for turbidimetric [26–28] and nephelometric measurements [27]. PEDD-based detectors and sensors have been applied for detection of selected cations [4,7,10,25] and gases [13,24]. They have also found applications in pharmaceutical analysis (determinations of calcium [25], vitamin B [23] and vitamin C [12]), environmental analysis (measurements of water pH and turbidity [26], inorganic nitrogen content [17,18], dissolved organics content [19]) as well as in clinical analysis (determination of glucose [15], creatinine [16], hemoglobin [11], proteins [20,27] and enzymes activity [9-14]).

Till now only two PEDDs for phosphate determination have been reported in the analytical literature: O'Toole et al. [29] demonstrated photometric PEDD for phosphorus determination based on a phosphomolybdate-malachite green method, whereas Saetear et al. [28] presented sequential injection analysis system with PEDD applied for turbidimetric detection of phosphate (as calcium salt suspension). On the other hand, recently the potential possibility of dual optical detection using LEDs only has been announced [30]. In this work we present a novel LED-based optoelectronic flow-through detector allowing bimodal (photometric and fluorimetric) determination of phosphate—one of the most requested targets of modern flow analysis [31,32].

2. Experimental

Ammonium heptamolybdate tetrahydrate was purchased from MERCK (Product no. 31109320/11). Fluorescent dyes, rhodamine B (Product no. 2576380) and rhodamine 6G (Product no. 8260) were

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obtained from BDH. A poly(vinyl alcohol) PVA (Product no. 341584) was obtained from Sigma-Aldrich. All other reagents of analytical grade were obtained from POCh (Poland). For all experiments doubly distilled water was used throughout.

All LEDs tested in this work and applied for the detectors fabrication were obtained from Optosupply (Hong Kong). They have common shape, 5 mm diameter and transparent lens. Full width at half-maximum of LEDs declared by manufacturer is 20 nm. LEDs operating as light emitters were powered by stable over time currents using homemade low-voltage circuit based on L272 chip, which contains two operational amplifiers and can independently supply two separate emitters. All electronic components used for the fabrication of supply unit were obtained from TME (Poland). Electromotive force generated by illuminated LED (and treated as an analytical signal of LED-detector [8]) was measured and recorded with a Voltcraft multimeter (model VC820, Germany) operating as an ordinary voltmeter. The multimeter was connected with data storage computer using RS232 interface.

The 4-LED-based detector presented in this communication is schematically depicted in Fig. 1 (top). The fabrication of the detector in the form of flow-through cell integrated with LEDs was reported in detail elsewhere [23,25] (including technical tutorial as a supplementary video material [23]). The internal volume of flow-through cell is ca. 85 μL. For photometric measurements two LEDs located facing each other are used. The distance between them (optical pathlength) is 5.0 mm. Two next LEDs intended to fluorescence excitation are located in the perpendicular position to LEDs forming photometric PEDD. In both modes of measurements the same LED plays the role of light detector.

The investigations of developed flow-through detector were performed in a three-channel FIA system consisting of peristaltic pump (model Minipuls 3 from Gilson, France), manual rotary injection valve (model 5020 from Rheodyne, USA) and 0.8 mm ID Teflon tubing (Cole-Palmer, USA). The scheme of the FIA manifold is shown in Fig. 1 (bottom). The common flow rate and injection volume applied in the course of all measurements were 2.0 mL min^{-1} and 0.25 mL, respectively.

Reference analyses of natural samples were performed using a common manual photometric phosphomolybdenum blue method [33], disposable polystyrene cuvettes from Saerstedt (Germany) and Shimadzu PC-2401 spectrophotometer (Japan). If necessary before analysis samples were diluted with water.

3. Results and discussion

The method for phosphorus determination commonly used in routine analytics is based on the reaction of analyte with

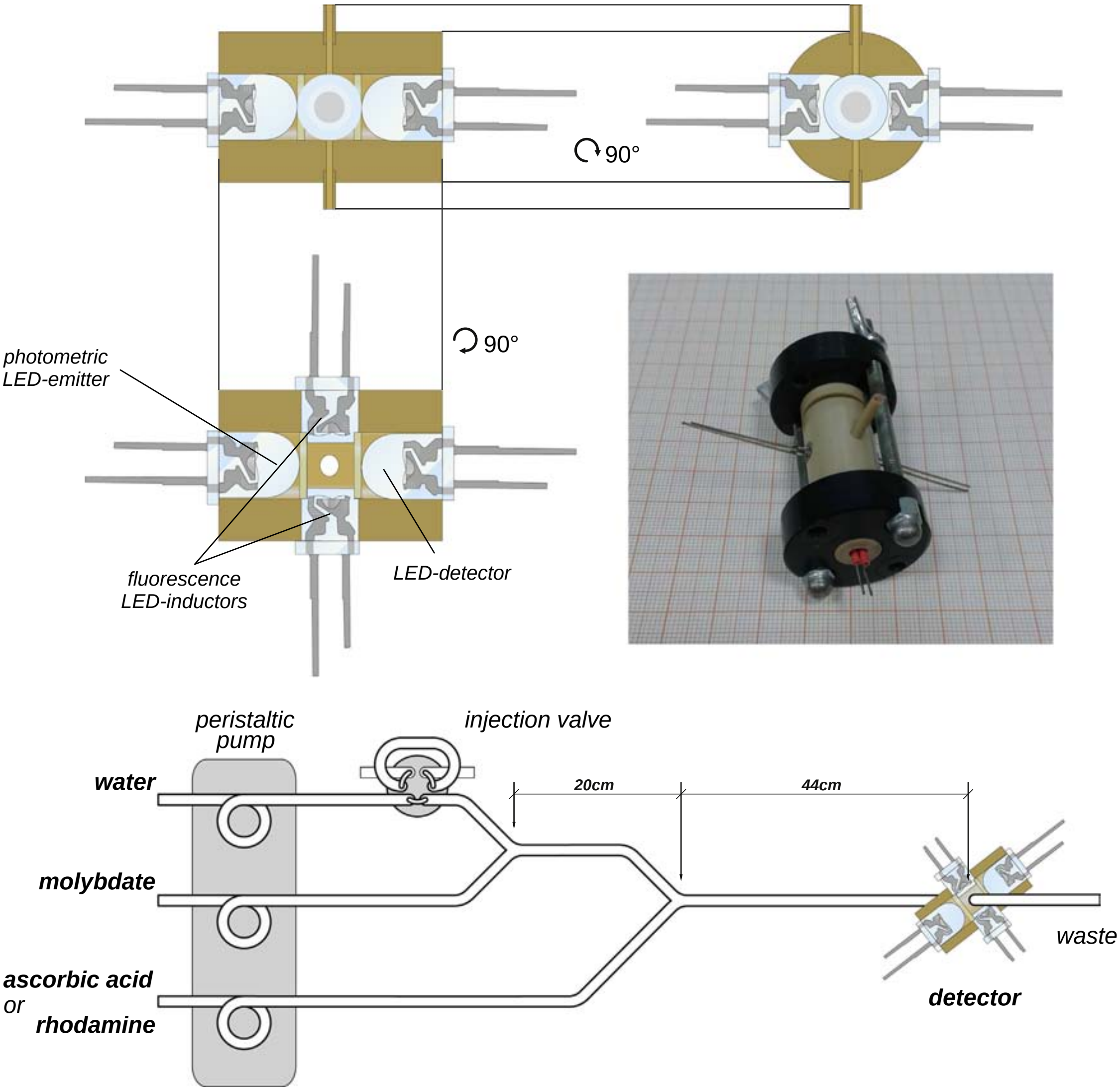

Fig. 1. The scheme and photograph of optical flow-through cell integrated with four light emitting diodes (top) and the scheme of FIA manifold (bottom).

molybdate to form yellow heteropolyacid. Subsequently, to improve the sensitivity of detection the product is reduced to intensively colored blue form. This photometric phosphomolybdenum blue method, in several variants and using different reductants, is widely applied in predominant number of FIA systems dedicated to phosphorus determination [31,32]. In this work as reducing agent ascorbic acid has been used. The phosphomolybdenum blue strongly absorbs the light in the 600–900 nm range of wavelength. In the course of preliminary cuvette tests (using simple experimental setup shown elsewhere [30]) several combinations of LEDs have been tested and most sensitive PEDD for phosphomolybdenum blue detection was obtained by pairing of two 655 nm LEDs. These LEDs were used for fabrication of the flow-through detector and tested under FIA conditions. As reported earlier [8], the current supplying PEDD causes the shift of photometric calibration graph enabling the control of determination range. As shown in Supplementary material (Fig. S1), this effect is also observed in the course of non-stationary flow measurements. The highest sensitivity was found at 2.0 mA and this current was applied for all further photometric measurements. Under given measurement conditions the developed FIA system allows fast and well-reproducible determination of phosphate in wide range of concentrations. The calibration of the system and corresponding calibration graph are shown in Fig. 2.

Fluorimetric determination of phosphates applied in this study is based on the reaction of phosphomolybdate with rhodamines [34,35]. The formation of ion pair causes the quenching of dye fluorescence. Such scheme of detection has been successfully applied for development of rather sophisticated flow analysis systems dedicated for ultrasensitive determination of phosphates [36,37]. The simple FIA manifold used in this work (Fig. 1) can be also applied for such measurements. As previously, preliminary experiments performed using ordinary cuvettes [30] were focused on the selection of the best LED pairs for detection of rhodamine B and rhodamine 6G. For both rhodamines 525 nm LED was found as the most effective fluorescence inductor, whereas 590 nm LED and 655 nm LED were found as the most sensitive detectors of fluorescence of excited rhodamine 6G and rhodamine B, respectively. Both the most promising LED combinations have been integrated in the form of flow-through detectors and calibrated on respective rhodamines. The intensity of LED-induced fluorescence is proportional to energy emitted by the light source [21,25] and therefore to obtain maximal sensitivity the measurements were performed at relatively high currents supplying LED emitters (35 mA). As shown in the Supplementary material (Fig. S2), both detectors allow determination of rhodamines at ppm levels. For higher concentrations (over 40 mg L^{-1}) self-quenching of dye fluorescence is observed. Both fluorimetric detectors have been calibrated on phosphates using respective rhodamines in carriers solutions at concentrations for which the highest signals for dyes were found (20 mg L^{-1} for both rhodamines). The increase of phosphate concentration causes quenching of dye fluorescence and consequently the decay of analytical signal. As shown in Fig. 3, both flow-through detectors offer fast and reproducible fluorimetric determination of phosphates in the sub-ppm range of concentrations.

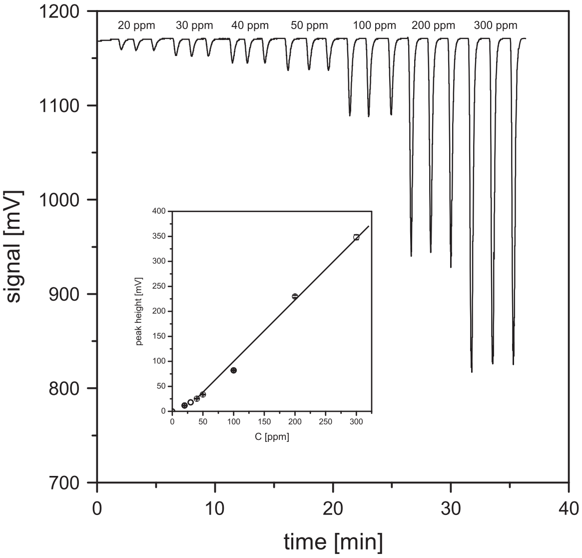

Fig. 2. Calibration of the detector operating in photometric mode of measurements. Corresponding calibration graph is shown in the inset.

Finally, the bimodal flow-through detector shown in Fig. 1 has been fabricated using two 525 nm LEDs applied as fluorescence inductors and two 655 nm LEDs—one used as a light emitter in case of photometric mode and the second operating as light detector in both measurements modes. The flow-through detector was applied in three channel FIA manifold shown in Fig. 1. The phosphate standards as well as samples were injected into water line. The second channel delivered 10 mM ammonium molybdate in 0.8 M sulfuric acid. The last channel delivered reducing agent (120 mM ascorbic acid) in case of photometric measurements or fluorescent dye (0.04 mM rhodamine B) in case of fluorimetric mode of measurements. The common calibrations obtained for both modes of measurements are shown in Fig. 2 and Fig. 3 (left).

In case of photometric measurements the detector offers linear range of determination up to 300 mg L^{-1} with 1.05 mV L mg^{-1} sensitivity. The determination and detection limits are 11.3 mg L^{-1} and 5.5 mg L^{-1} of phosphate, respectively. In case of fluorescence measurements the system allows phosphate determination in 0–1 mg L^{-1} range of linear response with 600 mV L mg^{-1} sensitivity. The determination and detection limits are 33.5 μg L^{-1} and 10.4 μg L^{-1} of phosphate, respectively. These data were obtained in routine way, using standard deviation (SD) of a blank signal (noise of baseline). Detection and determination limits are given as concentration for 3SD and 10SD signal values, respectively. Unfortunately, in case of fluorimetric measurements a slow drift of baseline caused by deposition of dye on the walls of FIA system is observed. To avoid this effect the carrier can be spiked with 0.05% (w/v) poly(vinyl alcohol), however such additive causes some decrease in sensitivity (410 mV mg L^{-1}). Alternatively, it was found that the flow-detector can be effectively cleaned by periodical washing with ethanol. The utility of the developed bimodal PEDD-based FIA system for analysis of selected natural samples is demonstrated in the Supplementary material (Fig. S3).

4. Conclusions

This short communication reports on bimodal optoelectronic detector made of four light emitting diodes integrated in the form of optical flow-through cell useful for phosphate determination under flow analysis regime. This miniaturized device as well as its supply and signal recorder (ordinary voltmeter) is extremely economic. To the best of our knowledge, this is the first example of bimodal photometric/fluorimetric optoelectronic detector designed for flow measurements reported in the analytical literature.

Without any special efforts nor system optimization the detector allows dual photometric determination of phosphate in the mg L^{-1} range of concentrations as well as fluorimetric determination below this range. For the switching of detection mode only one easily available reagent (ascorbic acid or rhodamine) has to be changed. It is expected that the detector can be easily applied in

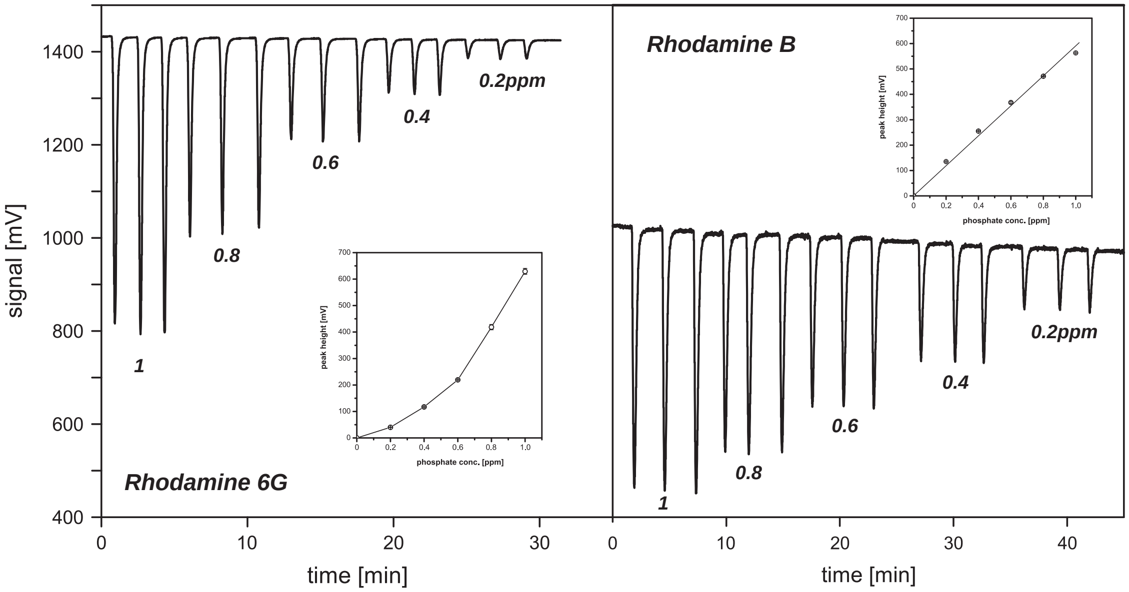

Fig. 3. Calibration of the detector operating in fluorimetric mode of measurements. Corresponding calibration graph for phosphates is shown in the inset. Results for systems with rhodamine 6G (left) and rhodamine B (right).

more sophisticated flow systems reported in the literature [31,32,35–37] offering further improvement of sensitivity and utility for trace and ultratrace analysis. Alternatively, further improvement of parameters of fluorimetric detection can be done by the use of additional lenses and filters [38]. However, as shown in this communication (Supplementary material), for some kinds of natural samples such modifications are not necessary. At present, the detector reported in this work is tested in advanced flow systems dedicated for the needs of environmental and clinical analysis and the results of these investigations will be presented in the future [39].

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.04.086.

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