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Appendix 3

SUMMARY OF SCIENTIFIC ACHIEVEMENTS

(AS ATTACHMENT FOR A HABILITATION PROCEDURE PROPOSAL)

Warsaw, November 2015r

TABLE OF CONTENTS

1 habilitation candidate's personal data

Łukasz Tymecki, age**:** 38

2 information about diplomas held and obtained academic degrees

2001 (Faculty of Chemistry, University of Warsaw) **MSc**
"Screen-printed potentiometric sensors for determination of copper ions." accomplished under supervision of Professor Stanisław Głąb.

2005 (Faculty of Chemistry, University of Warsaw) **PhD** in the field of Chemical Sciences, dissertation defended with honors: "*Screen-printed cells for potentiometric measurements."* accomplished under supervision of Professor Robert Koncki.

3 information on previous employment in scientific/artistic units

From **October 1st, 2005** till now as assistance professor in the Laboratory of Fundamental Aspects of Analytical Chemistry, Division of Inorganic and Analytical Chemistry, **Faculty of Chemistry, University of Warsaw**

4	*indication of the achievements resulting from Article 16, paragraph 2 of the Act of 14 March 2003 on Academic Degrees and Title in Art (Journal of Laws No. 65, item. 595, as amended.)*
A	*type of scientific achievement:*

monothematic series of publications entitled:
„Paired Emitter Detector Diodes and their Analytical Use."

B	*publications included in the scientific achievement*

[H1]. **Tymecki Ł.** ✉, Pokrzywnicka M., Koncki R., (2008), *Paired Emitter-Detector Diodes (PEDD)-based photometry - an alternative approach.,* **Analyst**, 133, 1501-1504

[H2]. **Tymecki Ł.** ✉, Koncki R., (2009) *Simplified Paired Light Emitting Diodes – based photometry with improved sensitivity.,* **Analytica Chimica Acta**, 639, 73-77

[H3]. Pokrzywnicka M., **Tymecki Ł.** ✉, Koncki R., (2009), *A very simple photometer for educational purposes based on paired emitter-detector diodes.,* **Chemia Analityczna**, 54, 427-435

[H4]. **Tymecki Ł.** ✉, Brodacka L., Rozum B., Koncki R., (2009), *UV-PEDD photometry dedicated for bioanalytical uses.,* **Analyst**, 134, 1333-1337

[H5]. Mieczkowska E., Koncki R., **Tymecki Ł.** ✉**,** (2011), *Hemoglobin determination with paired emitter detector diode.,* **Analytical and Bioanalytical Chemistry**, 399, 3293-3297.

[H6]. Pokrzywnicka M., Koncki R., **Tymecki Ł.** ✉, (2010), *A concept of dual optical detection using three light emitting diodes.,* **Talanta**, 82, 422-425.

[H7]. **Tymecki Ł.** ✉, Pokrzywnicka M., Koncki R., (2011), *Fluorometric paired emitter detector diode (FPEDD).,* **Analyst**, 136, 73-76.

[H8]. Fiedoruk M., Mieczkowska E., Koncki R., **Tymecki Ł.** ✉ , (2014), *A bimodal optoelectronic flow-through detector for phosphate determination.,* **Talanta**, 128, 211- 214

[H9]. Pokrzywnicka M., **Tymecki Ł.**, Koncki R. ✉, (2012), *Low-cost optical detectors and flow systems for protein determination.,* **Talanta**, 96, 121-126.

[H10]. **Tymecki Ł.** ✉, Korszun J., Strzelak K., Koncki R., (2013), *Multicommutated flow analysis system for determination of creatinine in physiological fluids by Jaffe method.,* **Analytica Chimica Acta**, 787, 118-125

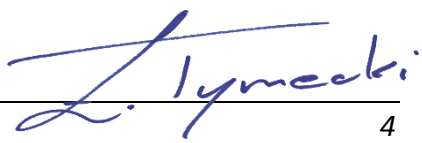
[H11]. Strzelak K. ✉, Misztal J., **Tymecki Ł.**, Koncki R., (2015), *Bianalyte multicommutated flow analysis system for microproteinuria diagnostics.,* **Talanta,** (in press) http://dx.doi.org/10.1016/j.talanta.2015.04.021

[H12]. Strzelak K., Koncki R., **Tymecki Ł.** ✉**,** (2012), *Serum alkaline phosphatase assay with paired emitter detector diode.,* **Talanta**, 96, 127-131.

[H13]. **Tymecki Ł.** ✉, Strzelak K., Koncki R., (2013), *Biparametric multicommutated flow analysis system for determination of human serum phosphoesterase activity.,* **Analytica Chimica Acta**, 797, 57-63

[H14]. **Tymecki Ł.** ✉, Rejnis M., Pokrzywnicka M., Strzelak K., Koncki R., (2012), *Fluorimetric detector and sensor for flow analysis made of light emitting diodes.* **Analytica Chimica Acta**, 721, 92-96.

[H15]. Pokrzywnicka M., Koncki R., **Tymecki Ł.** ✉ **,** (2015), *Towards optoelectronic urea biosensors.,* **Analytical and Bioanalytical Chemistry**, 407, 1807-1812



The total impact factors of publications included in the monothematic series, giving an opportunity for the proposal for habilitation procedures initiation are:

$$H\sum IF_{2014}=55.490$$
$$H\sum IF_{\text{according to year of publication}}=54.183$$

(InCites™ Journal Citations Reports®)

$$H\sum cit=152$$
$$H\sum cit_{\text{withouth self-citations}}=66$$

(Web of Science: 06.09.2015)

C | *discussion of the above-mentioned scientific work and the obtained results, including evaluation of their potential use.*

I | *scientific goal*

Developing the concept of analytical optical detection measurement methodology using the paired light emitting diodes - the characterization, optimization and adaptation to the flow measurement systems as well as the demonstration of usefulness potential of proposed detection systems in a variety of real analytical scenarios.

II | *introduction*

Analytical Chemistry is a part of Science which is utilitarian to the needs of society. Although analysts are perceived sometimes as craftsmen, the result of their research is reflected immediately in practice, in the form of commercially available products for analytics. Developing societies need analytical methods as a diagnostic and monitoring tools to effective treating, taking better care of the environment as well as for control of more and more sophisticated technological processes. Therefore, innovative chemical detection schemes are developed, but also apparatus for analytical measurement are being improved constantly. With the use of other

science disciplines achievements analytical chemists miniaturizing instruments, minimizing the size of the samples needed to perform the analyzes, reducing the influence of analysis on the environment through the development of more sensitive methods, that do not require the consumption of large amounts of reagents, but still are characterized by high accuracy and precision.

The constant progress allows not only mechanization of analytical procedures, but also automation in chemical analysis. Modern analytical equipment, to be called "cosmic" several years ago, today is available for many research laboratories, as well as for routine analytics. As a rule, modern analytical instrumentation are large, very complex devices, with lots of dedicated technical solutions. Such equipment is also extremely expensive, both in terms of price of the device itself and its operation. Their unquestionable advantage is that they are maintenance free and there is no need for an analytical background of their operator. Unfortunately, this advantage stay in opposite to the need of service interventions, which are expensive and should to be frequent. What is worth noticing, the endless race for pole position between companies producing equipment for analytical methods could be observed. Surprisingly, the result of this race is not lowering prices of products, but the appearance of more expensive equipment, only sometimes with objectively better parameters. Due to the obvious need for profit-taking by corporations producing analytical equipment, routine analyst (clinical, environmental and industrial) benefits from the achievements of the scientific community with a significant delay.

This trend may be stopped, and truly utilitarian character of analytical chemistry restored, through the development of devices which still do not require the knowledge to handle them, but as simplest, cheapest, automatic and reliable as possible. In a large part of the practical applications, there is no need for using universal device with sophisticated operating parameters. That is especially important for less developed countries where there is no access to modern methods of chemical analysis. Low-cost analytical systems are gaining in popularity and more and more research teams provides solutions induced by this concept. The team, where I realize my research passions is involved to developing of relatively simple integrated devices and miniaturized flow measurement systems for the determination of specific substances. Part of the research carried out by me, focused on developing and optimizing optoelectronic flow-through detectors, designing and validating the flow systems with developed detectors in real analytical cases. The results of these studies, collected in monothematic series of publications **[H1-H15]** are the base of proposal of performing habilitation procedure.

III light emitting diodes as light sources and photodetectors in the analytical devices

Light-emitting diodes are used in everyday life for decades, and almost as long they are used in the construction of analytical instrumentation. LED is a semiconductor electronic component, which main task is the emission of electromagnetic radiation. The energy of the electromagnetic wave, emitted by the LED, depends on the chip diode layers composition. LED chip is built in the form of a *p*/*n*-type semiconductor connector, wherein the *n*-type is characterized by the presence of donor atoms and the *p*-type by the presence of acceptor atoms (which causes the appearance of electron gaps in the crystal lattice structure). Such gaps are colloquially called „holes". Thermodynamic equilibrium of such system is reflected in the continuous recombination of diffusing electrons from *n*-type semiconductor with holes from *p*-type semiconductor. In the nearby of the semiconductors contact depletion layer exist. Diffusion potential, which arises as a result of this process determines the size of energy barrier, require to overcome the electrons and holes for recombination. Applying a voltage to the semiconductor in the conducting way, providing new carriers and reduces the depletion layer as well as the energy barrier. Further voltage increase and gradual reduction of barrier's energy resulting in the increase of the amount of carriers recombination, and as a consequence, an increase in conductivity and current flowing through the diode. Polarization in the opposite direction - biased - reverses the direction of carriers migration. The consequence of this is increasing the depletion layer [1].

Recombination of carriers in the junction region can cause the radiative recombination - photons emission. The higher the voltage applied to the diode in the forward direction, the processes of radiative recombination is more likely to occur and light emission has higher intensity. Significant for this work is to emphasize that the proper manipulation of the composition of the semiconductor connector allows obtaining LEDs with almost any color of the emitted radiation. This is important in an unusual use of light-emitting diodes - in the role of light detectors.

The construction of the diode is simple - the semiconductor chip is located in the funnel-shaped metallic reflector and connected to the electrodes. All of it is embedded in a transparent cover. Cover is made of a polymer with high refractive index so that radiation can escape from the space where it is generated and directed to the forehead of the diode. LEDs are efficient light sources, characterized by low power consumption, and in comparison to conventional light emitters are incomparably smaller, more durable, cheaper and more reliable. For many years

the relative monochromatic emission of the LEDs is an advantage which makes them useful in miniature measuring systems, including analytical systems [2-3].

In order to create a complete set of measurements, for different uses, light emitting diodes, as radiation sources, are usually combined with photodiodes as radiation detectors (which are compatible with size and stability of LEDs). The photodiode is constructed similarly to the LED, but works in the opposite way to the diode (thanks to the internal photoelectric effect). In order to improve the efficiency a composition of photodiode's semiconductor junction is different than in the LED - the area of light acquisition in the photodiode is larger than the LED's emission area. Diodes are used in electronic circuits, connected in the reverse direction. This results in a greater difference in potential, rapid separation of the carriers and, consequently, greater sensitivity of a device.

Because of similarity in construction between photodiodes and LEDs it was considered for many years whether there is the possibility of using the LED as a detector of electromagnetic radiation, including analytical applications. Already in 1976 a work [4] describes a simple photometer with an LED as the source of radiation and a second diode connected to an amplifier operating and acting as detector was published. Such way the use of light-emitting diode has been popularized in non-analytical applications in the 90s of the twentieth century by F.M. Mims III. He constructed a device for studying the intensity of sunlight which is designed to assess the state of global warming [5]. The device was simple enough that reproduction not caused problems. This mean that the measurements with the use of this device could be carried out by enthusiasts all over the world, and as a target this was to allow the creation of global climate change database. Although the use of LEDs as a detector looks exotic, the literature provides examples of devices in which light-emitting diode with the operational amplifier and a digital multimeter form a detection system. Among them you can find examples of simple photometers and their usefulness has been confirmed by studies in meteorology, medicine, telecommunications, astronomy and physics [6-12]. You can also find publications describing reports on this topic, closely related to analytical chemistry [13]. In the literature you can also find examples of measurement systems, using current-voltage converters with emitting diode (reversed biased) as the detector [11,12]. An interesting finding, having implications for the results presented in this work is also the fact that the light emitting diode has a different spectral sensitivity as compared to the photodiode. Unlike the photodiode (where current values do not vary over a wide range of wavelengths) - current signal LED response change only for light with

a wavelength shorter than itself emits [11,12]. This fact is an important concern in the context of achievements described in this work.

Light emitting diode connected in the electronic circuit in reverse mode and illuminated with light of an appropriate wavelength generates a photocurrent having such a small value (pA-nA) that measuring it requires sophisticated instrumentation. In the '90s only use LED as the detector have a demonstrative effect - the quality of the characteristics was not satisfactory. Except of mentioned publication [13] analytical applications of this concept didn't exist and in the nineties, the research group led by prof. D. Diamond (Dublin City University - DCU) developed a methodology of practical using LEDs as light detectors and an acronym "PEDD" (*Paired Emitter Diode Detector*) was introduced [14].

In a series of publications [14-21] it has been shown an electronic system that allows measuring the discharge time of the illuminated LED. Reverse biased diode (like a photodiode) behaves like a capacitor - the flow of the photocurrent generated by light, forces decrease the voltage applied to the electrodes of the diode. The time at which this fall takes place, is a function of photocurrent, and this is implication of the light intensity. This discharge time (in microseconds) was the analytical signal of paired detector diode - an equipment consisted of two light-emitting diodes only. Although groundbreaking and innovative, proposed in the DCU system was not without drawbacks. The construction of a complex system with PIC microcontroller, power source components and power regulators as well as the communication interface was so complicated that the simplest modification requires knowledge and experience in the field of practical electronics. From this reason it was also not available to the average analytical chemist. Moreover, the authors mention that the system is characterized by a high sensitivity (more than one hundred times larger than the photodiode used in such a circuit), but it is characterized by an unfavorable signal to noise ratio and provide less accurate results (even in comparison to photoresistor) [16]. In addition - the proper operation of the microelectronics requires that LED should generate light pulses of millisecond duration.

table 1. *Selected applications of the photometric paired emitter detector diode concept with the measuring of LEDs discharge time as analytical signal. (from the group of prof. D. Diamond - DCU Ireland; data covers the period up to 2008).*

analyte / reagent	***PEDD (application)***	***REF.***
Nitrazine Yellow, Bromocresol Purple, Rhodamine B	refractometric: LED*em** – 621nm / LED*det*** – 621nm (dyes's concentration determination and determination of their pK_a value)	[14]
Methyl Red, Bromocresol Purple, Aniline Blue	refractometric: LED*em* – 400; 465; 565; 590; 630; 660; 880nm / LED*det* – 940nm (determination of dyes concentration)	[15]
Bromocresol Green, complexes of Fe (II) with Phenanthroline	absorbtion: LED*em* – 610nm / LED*det* – 610nm (demonstration of diode photometer under stationary and flow conditions)	[16,17]
complexes of Pb (II), Cd (II) with Malachite Green	absorbtion: LED*em* – 610nm / LED*det* – 610nm (stationary measurements: determinations of metal ions in samples)	[18]
Phosphate ions	absorbtion: LED*em* – 636nm / LED*det* – 660nm (demonstration of diode photometer under flow conditions)	[19]
complexes Mn(II) Co(II) with PAR	absorbtion: LED*em* – 500nm / LED*det* – 621nm (demonstration of diode photometer and flow conditions as HPLC detector)	[20]
complexes Mg(II), Ba(II), Ca(II), Sr(II) with o-crezoloftalein	absorbtion: *LEDem* – 500nm / LED*det* – 621nm (demonstration of diode photometer and flow conditions as ion chromatography detector)	[21]

*LED*em –emitter diode;*

**LED*det – diode as a detector*

Despite of imperfections of the system mentioned above, team of prof. D. Diamond has consistently promoted such solutions in their publications. Probably, the concept of measuring the discharge time of illuminated LEDs is so complicated that it discourages other research groups for the use of such instruments in their research (only one group, beside the DCU -prof. Captain-Vallvey, Spain [22]).

However, in the literature there are a lot of publications from DCU research team, demonstrating the analytical usefulness of PEDDs. These demonstrations were made using model

substances and examples of this achievements are presented in Table 1. The information in this chapter show the state of research and related literature reports to the moment when I have started a research project to which this dissertation is devoted.

IV	*alternative methodology of analytical measurement using a paired LEDs*

IVa	*photometric measurements*

Publications of the team led by prof. D. Diamond cited in the previous chapter were the inspiration for my research. I have assumed that the idea of using LEDs as the detector, presented by this group is attractive, because two LEDs are compatible with each other and fabrication of flow-through cell with an integrated light source and detector will be easy. In 2008 a paper **[H1]** was published, which began the cycle, which is the subject of the habilitation proposal. In this work I have presented alternative way of analytical signal acquisition in comparison to those described previously in the literature [14-21]. The illuminated LED generates a small photocurrent, but also easy to measure electromotive force, whose magnitude is dependent on the intensity of light reaching the semiconductor chip diode. The electromotive force is stable over time and can be easily measured under current-less conditions with a standard laboratory pH meter (high-impedance millivoltmeter). Surprisingly, it turned out that the potential values obtained are significant, what with high-resolution equipment, conventionally used in potentiometry, has created the possibility of obtaining no-cost device with high precision and accuracy of its readings.

The LED of a particular color, was placed in front of second diode, which was connected to a pH-meter. This second LED was intended to be used as a radiation detector. Between LEDs a standard cuvette for optical measurements, filled with color solution was placed. This solution absorbed light from emitter diode. In case when stabilized current-voltage parameters was used for the emitter, the detector was solely dependent on the absorption properties of the solution between LEDs. This way the first prototype of a dedicated photometer has been created. In the same work **[H1]** I provided a mathematical description of photometric detector PEDD, used in the proposed measurement configuration. Mathematical transformation of Shockley's law (**equations (1-7)/[H1]**) for the ideal diode shows that in such measurement conditions the potential difference generated at the electrodes of the illuminated LED may be treated as a unit of absorbance while maintaining linear characteristics over a wide range of concentrations.

In other words, the ideal measuring system composed of two light-emitting diodes satisfies the equation of Lambert-Beer law. The stabilization speed of measured potential was fast, and this is due to the same reasons as almost immediately reaching full value of the light emission with the conventional use of LED [1-3].

In the first work in the series I showed results of simple photometric determinations of a model dye - Bromothymol Blue, as well as iron and cobalt ions, determined by the use of thiocyanate method. During the study the fact of spectral selectivity of light-emitting diodes was confirmed: appropriate combinations of LED (Emitter: blue – Detector: red, E: red – D: red, E: blue – D: blue) showed signal changes and other (E: red – D: blue) did not. This is confirmation that the LED is sensitive only when illuminated by electromagnetic wave having an energy greater (or equal) than is itself is able to emit (**Fig.3/[H1]**). I have shown the analytical utility of this phenomenon by demonstrating the results of determination of a $Fe(SCN)_6^{3-}$ (in the range of 1-5 ppm Fe^{3+}) in the presence of the $Co(SCN)_6^{3-}$ by a pair of diodes (blue and red). Similarly, by a pair of diodes: red-red, it was possible to perform a calibration for the cobalt complex (in the range of 5-25ppm Co^{2+}) in the presence of iron complex (**Fig.5/[H1]**). The residual sensitivity to iron was present due to two facts – the first: thiocyanate iron complex absorption spectrum is wide, and thus it was possible absorption of the radiation emitted by the red LED, and the second: range of spectral sensitivity of LEDs is difficult to calculate, but is guaranteed to be on the side of the shorter waves.

In summary - monothematic series of publications documenting achievement of the scientific goal, has been opened by work **[H1]**, where it was proved that a simple variant of the photometric measurement using conventional LEDs is possible by using instruments available in each analytical laboratory and the analytical signal (electromotive force) - is proportional to the concentration of the color substance. Next researches was designed for continuation of simplifying the detection system using light emitting diodes. Received and published **[H2]** results confirmed that the high resistance pH-meter can be replaced in a variety of applications, with a low-cost digital multimeter, plays role of millivoltmeter. However, this conversion does not take place without changing the nature of the received analytical signal (**Fig.2/[H2]**). Measurement with pH meter, due to high input impedance of the device and currentless measurement conditions, keep linear relationship between the obtained electromotive force (analytical signal) and the concentration of the color substance. What is more, pH-meter is equipped with electronical amplification of the signal and this resulting in signal acquisition in almost ideal conditions. Simple, low-resistance multimeters allow such measurement also, however, in the

absence of lighting, multimeter "discharge" LED detector. In the situation when electromagnetic radiation reaches the diodes, connected to the low resistance meter, analytical signal is the result of steady state between the two processes - the aforementioned "discharge" and constantly LED "charging" by illumination. For this reason (as difference to measurement using a pH-meter), calibration curves, obtained using the photometer are not linear but sigmoidal with the linear fragments in the narrower range of concentrations. On the one hand range of responses will be limited by diode saturation and on the other end of curve by complete discharge diode detector (**Fig.2/[H2]**). However, this solution has one undisputed advantage. In relation to the pH-meter measurement linear characteristic over a wide concentration range is lost, but sensitivity of measurement will arise several dozen times (**Fig.3/[H2]**). Fragment with the highest slope of the curve changes when intensity of light achieving the diode detector is changing. As a result, changing the value of the supply current for the LED emitter, we can adjust the range of maximum sensitivity, needed for a particular application. In the course of the studies, a series of calibration curves of color model substances (Bromothymol Blue - dye often used to validate the photometric systems) confirming this thesis. In this way I have demonstrated that complete photometer of PEDD type can be a simple, inexpensive, easy to use, and thanks to the size of universal multimeter, small device.

In the next publication **[H3]** I have stated that, in some applications, it is not necessary to use stabilized electronic circuit for supplying light-emitting diode. Some of the multimeters are in fact equipped with a slot through the transistors are tested. Using this current source (with additional current-limiting resistor) allows to obtain a compact unit consisting of only two diodes and the meter (**Fig.1/[H3]**). Analytical signals received in such system are stable and repeatable. Battery powered multimeter allow 72-hour operation without changing the device parameters. In my opinion, such photometer is a very attractive tool for teaching and exactly this was an intention for developing this device. Using a simple and cheap "instrument", each student in the group classrooms can perform individual experiments, which illustrate the fundamental absorption laws. Carrying out a bit more sophisticated measurements is also possible with the presented device. Those that lead to: pKa determination of the color pH indicators (**Fig.2/[H3]**), the construction of an optical system to detect the titration end point, or to investigate the activity of enzyme samples (**Fig.3/[H3]**). Examples for such student's exercises with a DIY photometer (Do-It-Yourself) was collected in **[H3]**.

Another manuscript **[H4]** describes LED detection system for the determination of *p*-nitrophenol. In this publication research aimed at selecting the appropriate components – LEDs,

as well as suitable potential meter for the best operating parameters – is presented (**Fig.1/[H4]**). A solution of *p*-nitrophenol exhibits absorption with a maximum at approximately 400 nm. The maximum absorption of the analyte indicates the scope of the search for an optimum LED emitter in the developed PEDD - in this case the choice was made on the 405nm LED. Having basic information that the light of such short wavelength is possible to detect by the LED of any color (emitting an electromagnetic wave of lower energy) number of current-voltage characteristics for different pairs of LEDs has been made. PEDD was constructed after the selection of available light-emitting diodes and after verification of dozen from the most promising LEDs combination. LED acting as a detector in this set was blue one (470nm). After the tests, both with the use of precise pH-meter and simple millivoltmeter, it turned out that in this application, multimeter allows the acquisition of the signal above the value 2V, which for most pH-meters is not possible, and it was necessary to perform research with a blue LED as a detector (**Fig.2/[H4]**). Results, presented in **[H4]** indicated that the PEDD diode photometer can be successfully used to determine the activity of alkaline phosphatase which catalyzes the hydrolysis reaction of *p*-nitrophenyl phosphate.

Just during in the initial optimization process I have shown that with the proposed analytical system, it is possible to measure in stationary mode for distinguish between the pathological and physiological activity of alkaline phosphatase (ALP) in the blood serum - a parameter which is significant from the clinical point of view. Such use of the detector in the form of paired light-emitting diodes for bioanalytical applications presented in **[H4]** was the best of my knowledge, the first case reported in the literature. Previous publications related to PEDD applications concerned the determination of dyes and indirect determination of metal ions. My further research was mainly associated with clinical analytics.

As an example, in **[H5]** I showed that properly selected two light-emitting diodes enable the construction of a simple hemoglobinometer, useful in the clinical analysis. The system of twin diodes (570nm) was used for the determination of total hemoglobin in the blood. The choice of LEDs was carried out according to the analysis of current-voltage and calibration curves for 6 different LEDs (36 combinations). The study has been concluded that all three methods used for the determination of hemoglobin (modification of all forms of hemoglobin present in the blood sample to a stable: cyanmethemoglobin, methemoglobin complex with sodium lauryl sulphate, or "reduced" Hb using sodium dithionite), may be implemented using a small and uncomplicated photometer. Presented data confirmed that for the clinical samples analysis of major significance; multifunctional, versatile and expensive spectrophotometer is not needed.

The evidence was given, because for these methods of determination good agreement between the results for the LED photometer constructed and conventional clinical analyzer (**Fig.3/[H5]**) have been obtained. Miniature set-up for stationary tests allows hemoglobin determination in two hundred times diluted sample of blood (20µL blood) in the range up to 1000 mg/L of hemoglobin.

Summarizing, in the course of preliminary tests, an alternative method of photometric paired light emitting diodes has been developed. I explained why the observed phenomena essence of electromotive force generation during measurements using a pH meter **[H1]** and a simple multimeter **[H2]** could be used in the field of analytical chemistry. I showed that further simplifying of the measurement set-up with paired light-emitting diodes can be an interesting illustration of the instrumental analysis teaching practice **[H3]**. In subsequent research **[H4,H5]** I have presented a methodology for the selection of the LEDs dedicated to the determination of the desired analyte. I have also indicated the potential application of PEDD in clinical analytics giving example of enzymatic activity determination of ALP in the blood. Development of uncomplicated hemoglobinometer, has completed the first part of research and was the starting point for further work, devoted to mechanical flow systems with dedicated diode detectors for the determination of various substances essential in clinical diagnosis. It will be discussed in next chapters.

IVb | *fluorimetric measurements*

The surprising for me was the lack of literature reports about the possibility of using light emitting diodes as detectors of radiation generated by fluorescence. With a very low amount of publications on this issue in the context of the photometry the answer of this situation explanation was pretty obvious. In common photometric applications signal acquisition capabilities are severely limited (because of nA current). On the other hand analytically useful fluorescence is characterized by the emission of light of a far lower intensity than that in the photometric techniques. Preliminary studies with measuring electromotive force as analytical signal instead of the current or diode discharge time showed that proper selection of LEDs allows construction a fluorometer as simple as photometric set-ups described above **[H1-H5]**.

In the course of research on the Fluorometric Paired Emitter Diode Detector (FPEDD) – I demonstrated the possibility of fluorimetric determination of quinine in drinks samples using a dedicated fluorometer, working on the basis of the proposed measuring methodology **[H6]**.

When properly selected set of LEDs, appropriately oriented relative to each other in space (so that the fluorescence inductors were not directly lit by LEDs acting as the detector) was used, simultaneous and mutually non-disturbing photometric and fluorimetric measurements could be performed. Relatively large and well-shaped analytical signals were obtained in this simple system thanks to good match of emitter spectral properties with the optimum excitation of the fluorescence at one of the absorption maxima of quinine. With the use of such simple analytical system recording the typical phenomena was possible: fluorescence quenching by concentration or quenching by specific ions (in the case of quinine typical quenchers are chloride ions (**Fig.3/[H6]**). The results of samples analysis after interferents removal (sorption on chloride ions on exchange microcolumn), both in a PEDD stationary system (photometric measurement) as well as with the FPEDD system (fluorimetric measurement) were in good agreement with the results obtained by fiber-optic spectrofluorimeter (**Tab.1/[H6]**).

Using similar concept, I created a prototype optoelectronic systems, consisting of "blue" fluorescence inductor and the "red" detector oriented to each other at an angle of 90deg. These detectors allow determination of the fluorescein as accurately as dedicated to such tasks optical CCD detector (USB2000-FLG Ocean Optics) (**Fig.1/[H7]**). The utility of the analytical fluorometric detector, constructed entirely of light emitting diodes, was confirmed by the results of the analysis of mineral waters for the content of calcium ions, obtained with the fluorimetric method with calcein (**Fig.5/[H7],Tab.1/[H7]**). It turned out that LEDs, as a highly efficient light source, induces fluorescence effectively and analytical signal acquisition method gives reliable results. The results for the developed FPEDD and fiber optic CCD detector were almost identical.

It is worth mentioning that the possibility of constructing photometric detectors as well as fluorimetric in such simple way, opens the possibility for similar research with the use of turbidimetric (equivalent of photometric measurement) and nephelometric methods (fluorimetric). Such work were conducted recently [23,24], also in the group in which I am realize my research [25,26].

V | *PEDD flow-through detectors*

From the very beginning of a researches on the development of optical measurement methodology using simple optoelectronic sensors, consisting only of light emitting diodes I have tried to confirm the usefulness of developed analytical devices for measurements in flow conditions. This was important, because as a practitioner I see a huge mechanization and

automation potential of chemical procedures by flow methods. Moreover, thanks to the great simplicity of the flow analysis idea, trends of modern analytical chemistry as miniaturization, computerization and attention to meet the environmental requirements are reflected here.

Analytical measurements in the flowing media require special flow-through detectors. Innovative needs desire dedicated solutions. All detectors used to achieve the objectives of individual studies, were designed and fabricated by me. In order to clarify this discussion - the description of the flow detectors design, with an indication of their intrinsic characteristics - is placed in a separate part of thesis (Chapter VII).

Va | *photometric flow analytical systems*

The dynamics of the light generation by the LED, and the resulting fast response of LED-detector to light, causes the paired light emitting diodes detectors much faster detectors than a data acquisition devices in the flow analysis should be. In the course of preliminary studies I have presented the different PEDD calibrations performed in equilibrium and disequilibrium regime (**Fig.4/[H1]**). During such experiments fast achieving of signals, lack of noise and high repeatability were observed. Determination of iron (III) and cobalt (II) as a colored thiocyanate complex was carried out in a system consisted of peristaltic pump, a manual injection valve and two, dedicated to the detection of the corresponding complexes PEDD detectors (**Fig.5/[H1]**) and the resulting peaks were well-shaped and repetitive. In another work, illustrating the results of preliminary research on the design and optimization of the flow detector for the determination of p-nitrophenol with the use of simple instrumentation for flow analysis the results were also satisfactory (**Fig.3/[H4], Fig.5/[H4]**). In all these publications the results of research shows that the integrated diode detector is suitable for use under nonequilibrium conditions. Another example uses PEDD flow-through detector in a classical flow system for photometric determination of phosphate ions with molybdate, leading to the creation of the heteropolyacid which has been on-line reduced to blue form subjected to detection [H8]. Validation of the system with a dedicated detector was performed using samples of beverages and postdialysate produced by an artificial kidney in dialysis process (**Fig.S3/[H8]**).

In my research I have used miniature flow equipment belongs to a group of devices, commonly known as flow control microsolenoids elements that can be used for construction of modular flow systems of any architecture and complexity. These systems belong to the category

MPFA (Multi Pumping Flow Analysis - where were used only to the microsolenoid pumps) or MCFA (Multicommutated Flow Analysis - where microsolenoid valves also were used).

I have constructed a miniature flow-through detector for the determination of total protein after reaction with the Coomasine Blue **[H9]**. The microsolenoid flow control elements open the possibility of the analytical procedure mechanization, and their small size allows miniaturization of the entire analyzer, which has a size compatible with the designed detector. Meticulous LEDs selection, construction of the detector and finally construction of flow system, were finished with validation, using certified protein standards from human serum. Obtained results were sufficiently well-correlated with the results from analysis made by recommended routine methods. This indicated the applicability of the system in real clinical analysis also in the analysis of urine, which in terms of the protein content is more important diagnostic parameter and the urine is a much less complicated sample than the blood serum, because of matrix (**Tab.1/[H9]**).

The analyte of the same high-impact diagnostic significance, and which determination is carried out almost every time as a part of routine package of biochemical blood analysis is creatinine. For this compound there are also available recognized and used for over a century, colorimetric detection method (Jaffe method with alkaline picrate). From that reasons it is possible to construct a PEDD detector dedicated for such determinations. A pair of LEDs for the construction of PEDD was chosen after determining the current-voltage characteristics, so as to ensure maximum sensitivity of the detector. It was found that the most effective diodes pair for the determination of creatinine after reaction with picric acid will the couple of diodes: 505 nm (LED emitter), 525 nm (LED detector) **[H10]**. Miniature device wherein the selected pair of diodes was located opposite one to another in the flow-through detector (Chapter VII) was a part of a non-complicated flow system, which uses electromagnetic pump and valves for dilution creatinine solution (according to previously developed concept of mechanical process of calibration in MCFA systems [27]). The electromagnetic flow control elements provide alkalized picrate solution to the sample and enable to transfer the reaction products to the detection zone.

Despite of high simplicity of the proposed flow system (**Fig.4/[H10]**) it was possible to use advanced analytical signal processing methods. Measurements were held with stopping of the reaction zone inside the detector. Stopping the flow was followed immediately after mixing of the sample with the reagents, so that progress of the reaction could be observed from initiation in real time. Such kinetic measurement variant resulted in decreasing of diode detector

electromotive force (**Fig.2/[H10]**). The shape of the curves and the value of analytical signal were the result of the creatinine concentration in the sample. Due to the fact that the determination of creatinine by Jaffe method has a very large group of interfering substances, in this case it is necessary to perform differential measurements (**Fig.5/[H10]**), and a suitable system architecture enabled to use the discrimination time phenomenon (interfering compounds react with other kinetics than creatinine). It was particularly important in the analysis of serum samples due to their complex matrix. In addition, in the course of research we have developed a method of processing measurement results, mainly - choosing a certain period of time for "observing" the reaction, and choosing the right delay time (from the start of the reaction). The results are consistent with similar data obtained for the samples analyzed in the hospital laboratory, both the serum and the urine (**Fig.7/[H10]**).

The same kind of detector dedicated for the determination of creatinine, as shown in **[H10]**, together with a detector for turbidimetric detection of total protein [25,26] was used in the biparameter flow system **[H11]**. The described microsolenoid system was dedicated to the microproteinuria diagnostics. The concentration of protein in the urine above the value of physiological limit (150mg/L) is considered to be a pathological condition called proteinuria. It was noted that in many kidney diseases, in urine are present specific protein content of less than 150 mg/L, where routinely performed colorimetric test strip indicates a negative result. This situation (subclinical proteinuria - microproteinuria) can occur not only in the case of progressive renal failure or diabetic nephropathy but also in cases of many other diseases. The composition of urine varies throughout the day and for this reason in analytics most often used method is daily urine collection to obtain a representative sample. Such a method of obtaining the sample is time consuming and inconvenient for the patient. It is estimated that more than 25% of urine samples [28] obtained in daily collections are collected in a wrong manner. From this reason, an alternative for urine collection, two-parameter analysis is proposed for proteins determination. In addition to the protein assay, determination of creatinine is performed, which is an internal secretory marker. In this situation protein concentration is expressed in the relation of the concentration of creatinine, and this reduces fluctuations in the volume of the urine sample and the degree of dilution/concentration. This methodology is reflected in the analytical practice in the microproteinuria diagnosis and has been implemented in our analytical system with two PEDD detectors. In this case, the result of analysis for individual samples (of several µL volume) is in agreement with the results obtained from the reference analysis **[H11]**. An important aspect of the analysis in this case was to prove

that designed analyzer, despite of small size and low cost allowed correct diagnosis for those patients with renal impairment, as well as for the exclusion of such failure in patients for who the concentration of total protein in daily urine (so as is done routinely) was measured only (**Fig.4/[H11]**).

Development of a detector designed to determine the concentration of *p*-nitrophenol presented in **[H4]** was the starting point for publications documenting the successive stages of the development of modern flow system for simultaneous detection of acid and alkaline phosphatases activity in real samples.

At least three potential reasons for interest phosphatases (alkaline ALP, EC 3.1.3.1., acid ACP EC 3.1.3.2.) could be identified in the context of analytical chemistry. First of all – these are enzymes which activity is most often determined in the routine clinical analysis because the ALP activity in the serum provides information on potential cancer of liver or skeletal system. On the other hand ACP activity levels helps to determine the presence of prostate cancer. Secondly - ALP is used as a marker in multiple immune- or gene-assays detection schemes. A third aspect of the analytical applications is the ability of indirect determination of inhibitors/activators of this enzyme.

Routinely, activity of phosphatases is determined photometrically using *p*-nitrophenyl phosphate as a substrate, by monitoring the appearance of the product, *p*-nitrophenol - having an absorption maximum at 405 nm. The dephosphorylation product (NPP) is acid-base dye. Intensely colored is its alkalized form. For this reason, in order to increase the sensitivity of the assay, the reaction mixture should be alkalized, which also stops the enzymatic reaction. Unfortunately, serum components and the substrate absorb radiation in the range close to the *p*-nitrophenol, but separating the serum matrix before the analysis is economically unjustified. For this reason, two-point differential measurement is generally performed, which means that the first signal is recorded for background (coming from sample components), then the signal from the sum of the background and the products of the enzymatic reaction is recorded. Developed flow-through PEDD detector, in conjunction with a flow cell, was used in the classic two-channel flow system to detect *p*-nitrophenol and parameters of the system have been optimized. Properly selected electrical parameters and further optimization of the flow system operating conditions enabled the receiving resolvable signals for pathological and physiological levels of activity **[H4]**. The experience gained during these studies have been used to carry out further work.

In the next publication **[H12]** on this subject, different design of flow-through detector for monophosphoesterases activity determination was presented (Chapter VII). Flow-through vessel with an integrated light source and detector worked in a flow system consisting of a solenoid valves. The flow of solutions was forced by a peristaltic pump. The flow control elements were programmed (**rys.1B/[H12]**) for the achieving possibility of stopping the segment of the sample in the detector when mixed with substrate solution, buffered at the optimum pH for the action of ALP (9.0) immediately after the start of the reaction. After a specified time, the flow was resumed, and then signal after the enzymatic reaction was recorded. Whole procedure resulted in the two points differential measurement (**Fig.2b/[H12]**). Before the detector a solenoid valve was located, providing alkalizing solution. Despite the fact that the analyzed sample (serum) is a solution of a relatively high viscosity, and a peristaltic pump was placed at the end instead of at the beginning of the system (to avoid dilution of the sample zone and therefore dispersion) determining the activity of ALP with a high degree of correlation to the reference method is possible. Replacing alkaline solution to the acid solution should be such system making suitable for the determination of the acid phosphatase (ACP) activity (optimum pH for the operation of the ACP is less than 5.0). Unfortunately, the activity of serum ACP - 1-2 orders smaller than the ALP activity - in this system were difficult to determine.

Therefore, modification of the microsolenoid system, presented in **[H12]**, additionally supported by optimization of the detector architecture (Chapter VII), with the use of different measurement methodology, which takes into account the difference in the types of activities between monophosphoesterases in blood serum was made. Geometry optimization of the flow detector was carried out by comparing the results for devices with different optical path length and aperture, which also is an universal way of dealing with selection of optimal working conditions with PEDD detectors. A much more advanced MCFA-PEDD system (**Fig.2/[H13]**), based solely on microsolenoid devices (valves and pumps) allowed simultaneous determination of ALP and ACP, taking into account the need for differential measurements. Consequently, this meant that the receiving of four analytical signals from one serum sample (**Fig.4/[H13]**) was needed. In the designed system, the flow system architecture can be divided into four functional modules: Module (I) take the reactants (substrate and buffer), the module (II) is a sample delivery unit, the module (III) is a reaction (including two holding coils) unit, and a module (IV) is a detector unit (containing PEDD). A first module, similarly to the previous system, is used for transferring a segments of the substrate dissolved in a buffer (acid or alkaline). The second module enable delivering exactly the same volume of serum samples, regardless of its viscosity.

The third module contains two reaction loops for incubation the sample with the substrate in an acidic (determination ACP) and alkaline (ALP assay) buffer. The fourth module, followed by alkalization which inhibits enzyme as well as improves the accuracy and sensitivity of photometric determination. Despite the non-linear characteristics of the PEDD detector, obtained results of the analysis proves system reliability in the simultaneous activity determination of ACP as well as ALP in serum samples (**Fig.6/[H13]**).

Vb | *fluorimetric flow analytical systems*

Publication already cited here **[H7]**, contains the results of studies on adaptation of the concept of paired diode detectors to the format of the flow analytical equipment. The paper presents an innovative construction of the measuring cell and identifies its analytical usefulness for determining model fluorophores (fluorescein) as well as the device for the determination of calcium ions in mineral waters (reaction with calcein) using the classical instrumentation for flow analysis. In addition to the model fluorophores, in the studies compounds that are products of chemical reactions and are important from the clinical point of view were used.

An alternative for the photometric determination of total protein by reaction with the Coomasine Blue is a fluorimetric method with the use of fluoresceamine. This compound reacts with a protein to form a derivative, absorbing at 390nm and expressing an emission with a maximum at 480nm. Appropriate selection of diodes (emitters 405nm, 630nm detector) allowed the construction of the PEDD detector **[H9]**. The use of two fluorescence inductors increased sensitivity. Detector with low dead volume was used for fluorimetric determination of total protein in serum and urine in a non-complicated multicomutated flow system consisting of four microsolenoid flow control components. During collecting results for this work, interesting phenomenon was observed, which is further evidence of the attractiveness of the described PEDD concept, due to the spectral selectivity of the diodes. One channel of the flow system was supplying acetone (as a solvent for fluoresceamine). Mixing acetone with aqueous streams results in the refractive index gradient formation in the detection zone. This resulted in partial reflection of excitation radiation directly towards the detector, which was the source of the noise of an apparatus. This phenomenon has been recorded in the case of using a conventional fluorimeter fiber detector (CCD – Ocean Optics USB2000FLG) and signal monitoring at 405 nm (maximum emission of emitters used) (**Fig.4/[H9]**). It has been found that the observed Shllieren effect can be eliminated by the selection of the detector diode in such a way that it is not

sensitive to light emitters. In this case, the LEDs as miniature optical components for chemical analysis are an interesting alternative to any other non-selective detectors (photodiodes, photoresistors, etc.), because due to the lack of need for additional filters. Selectivity of the LEDs can be equivalent to the use of a monochromator in classical spectrofluorimeter **[H9]**.

The fact that the spectral selectivity of the diodes has been exploited also for the construction of a miniature detector for determination of riboflavin **[H14]**. The relation between the emission spectrum of the emitter diode, spectral sensitivity spectrum of the LED, acting in the role of the detector and the compatibility of these spectra with excitation and fluorescence spectra for riboflavin (**Fig.2/[H14]**) illustrate the importance of the LEDs choice for the construction of the FPEDD detector. In this detector three diodes were used - two fluorescence inductors, whereby is shown that with the use of LEDs substances having a low fluorescence intensity can be the determined. In addition, this paper presents the multimedia material illustrating a method of producing an universal detector (**SM,FPEDD.wmf/[H14]**). The results of determinations in these circumstances made it possible to achieve low detection limits (ppm levels), which is sufficient for most applications of analytical determination of riboflavin in natural samples – i.e. samples of food supplements and medicines.

The concept of carrying out fluorescent assays using the three LEDs in the flow system is continued in other works. So far, practical applications of such detectors were presented for determinations of the bioavailable phosphates in soil samples [29], and applications in the field of clinical analysis: determination of calcium in blood serum [30] as well as for the simultaneous measurement of calcium and phosphate ions in the serum [31].

Looking for alternative approaches regarding the concept of optical analytical measurement using light-emitting diodes resulted also in example of the bimodal device - configured so that measuring in both photometric and fluorimetric modes is allowed **[H8]**. The flow-through type of this detector is based on a system of paired diodes for phosphate fluorimetric assay [29]. Except diodes necessary for fluorimetric measurement (two inductors/one receiver), in the detector construction additional diode was used as a photometric detector. finally the device was composed of four LEDs (**Fig.1/[H8]**) and this allows demonstration both modes of operation. For example, the determination of phosphate: the aforementioned photometric (Chapter Va/, page 17/18) and fluorimetric, based on rhodamine fluorescence quenching by phosphomolybdate, proven analytical utility of such detector. The differences in the ranges of determination (from ppb to ppm) in both modes of operation may be used to analyze samples with different contents of phosphate **[H8]**.

VI PEDDs with integrated sensitive layers and/or bioreactors

The small size of paired diode detectors predisposes these devices to integrate them with biosensing layers and bioreactors. When I started research on integrated detectors, existed publications were reported only on sensor applications related to gaseous samples only. Among them there can be find a sensor with the dye (*p*-nitrophenylonitrozoamine), immobilized in a gel matrix for the determination of ammonia gas [32] or chemical nose (matrix of sensors) to test the gas acidity [33]. My goal was to present opportunities for integration of sensor layers in a small format for the analysis of liquid samples.

I am a co-author of works [34,35] devoted to sensors of paired light-emitting diodes composed with a thin layer of Prussian Blue placed inside the flow-through cell. A sensor was initially characterized for determination of ascorbic acid which reversibly reduced the film of Prussian Blue, and was used for determination of vitamin C in pharmaceutical formulations [34]. Similarly, using the spectral properties of the oxidized/reduced Prussian Blue this type of sensor was characterized as a tool for the determination of hydrogen peroxide which is the product of many enzymatic reactions. Finally, this detection scheme was used to determine the concentration of glucose [35]. This was possible thanks to the layer of glucose, immobilized on the sensor surface.

The enzymatic layers, placed directly at the detector allow the observation in situ of kinetic biochemical processes [36, **H15**]. PEDD-bioreactor configurations were demonstrated [36] as an example of device sensitive to *p*-nitrophenol and another one for determination of urea hydrolysis products **[H15]**. PEDD-sensors overview was shown in the publications describing enzymatic determination of glucose [35] and urea **[H15]**.

Using light-emitting diodes it is also possible to construct miniature fluorimetric quasi-sensor device. This is shown as FPEDD-sensor for riboflavin determination. In this case, sorbent (C18) providing the riboflavin retention was placed directly in the space between inducers and fluorescence detectors **[H14]**. It was shown that such modification of the detector allows to obtain the detection limit in the range of ppb (a fluorimetric detector, described above, allowed the designation of riboflavin in the ppm range).

VII | *construction of PEDD detectors, sensors and biosensors*

Analytical optical measurements require proper orientation of each detection system component. The detector in photometric and turbidimetric techniques should be located in the opposite to the light source. In fluorometric and nephelometric techniques locations of emitters and detector should be different than 180 degrees, and the assumed value of 90 degrees is optimal. In my research focused on the development of the PEDD concept it was necessary to create such measurement methodology that it is easy to make, cheap and modifiable.

In the first phase of research on PEDDs, when it was necessary to perform a large number of measurements with a variety of different light-emitting diodes I had designed a measurement module made of LEGO bricks. A Technics kind of this toy has elements with holes, where, without modification, stably and reversibly fixing the light emitting diodes of standard size (5mm) is possible. Proper selection of LEGO blocks formed into a measuring module (Fig.1) with space for a standard (12x12x39mm) cuvette.

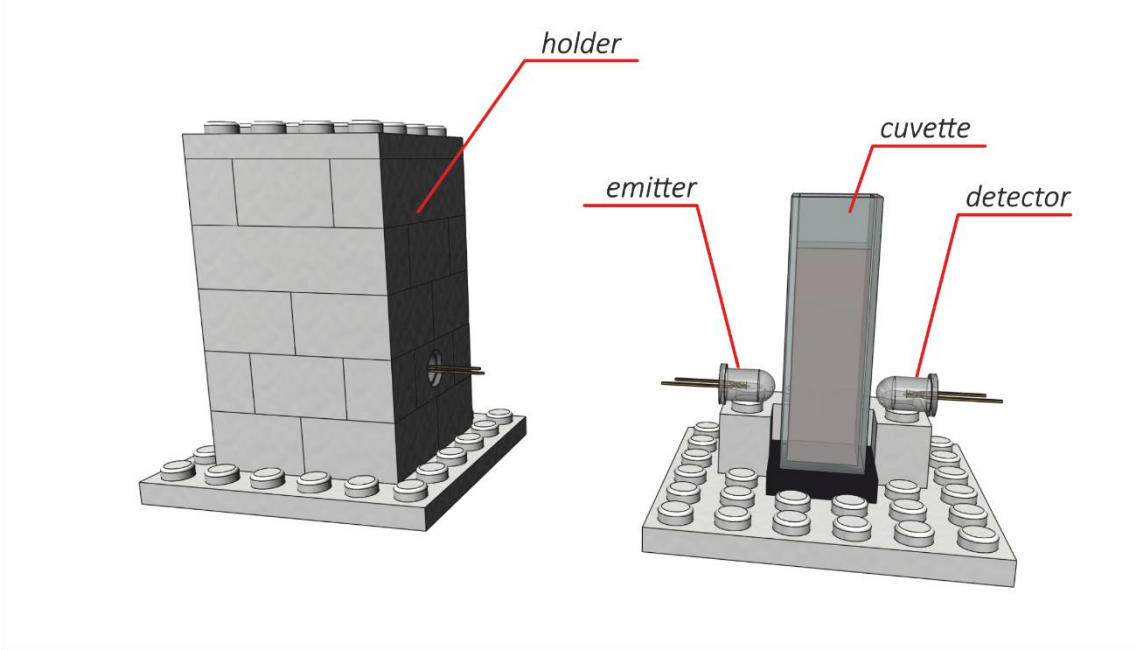


Figure 1. *Stand made of LEGO bricks that allows permanent fixing of the light-emitting diodes in photometric measurements.*

Opaque elements provided interior insulation from variable lighting conditions in a laboratory. By using such cuvette holder, it was possible to perform measurements for the two LEDs, acting as a light emitter and detector as well as in cases where the universal fiber optic

detector was used. This kind of measurements in the course of preliminary tests should had confirm the feasibility of measurement or have been used for reference measurements.

Similarly to the photometric / turbidimetric measurement method using a holder made of LEGO bricks, fluorimetric / nephelometric measurements could be performed (Fig.2). Despite the aperture, equal to the diameter of diodes (5 mm) and close distance between diodes in the measuring unit, the light reaching the diode detector, was rudimentary, which has been proven in an experiment where in the role of fluorescence detector universal fiber optic device was used **[H7]**. In addition, the previously mentioned spectral selectivity of LEDs reduced the effect of nonspecific signals from the light emitted by a light emitting diode.

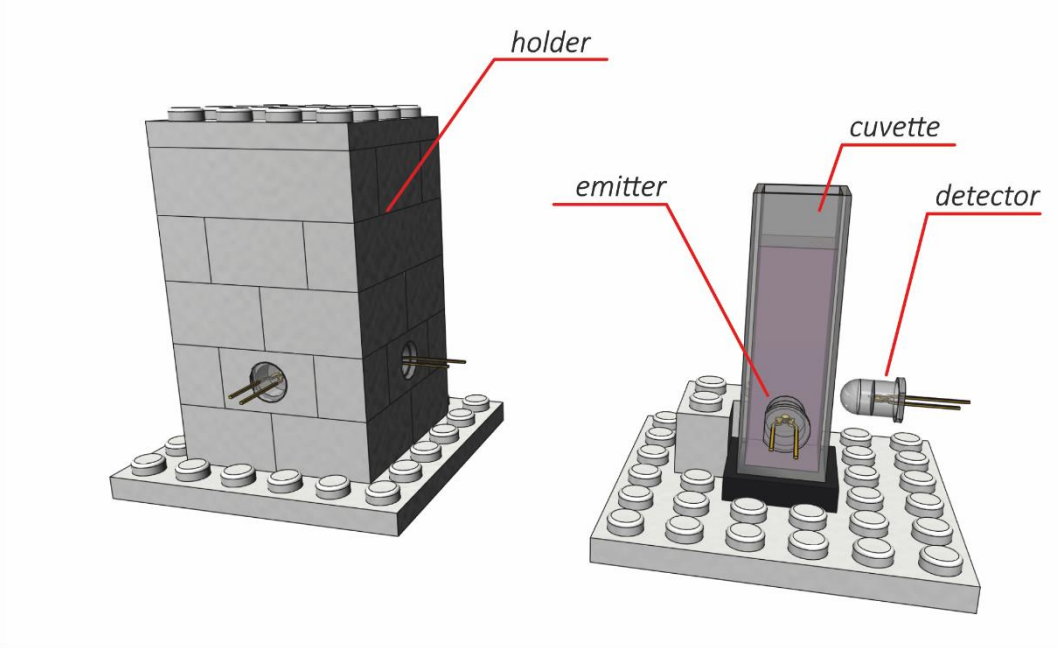


Figure 2. *Stand made of LEGO bricks that allows permanent fixing of the light-emitting diodes in fluorimetric measurements.*

Almost all of integrated diode detectors were used in flow analysis systems. Measurements methodology under the flow conditions requires a flow-through element to detect analytical signal with the appropriate measurement technique. Exploring PEDD concept in such conditions has been performed with the use of laboratory-made polycarbonate flow-through cell, placed inside the holder descrived above (Fig.3). The size of aperture, the position of the central channel and the outer dimensions of the flow cell were chosen to fit into the holder and the axis of the LED coincides with the axis of the center channel. The walls of the flow cell were blacked so that the light passed only through the center channel rather than by the body of the polycarbonate cuvette.

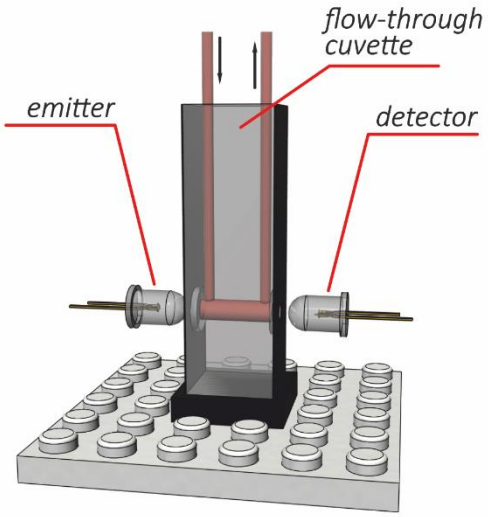


Figure 3. A flow-through cell used in a preliminary photometric studies with PEDD.

The same concept has been implemented in the course of flow measurements with fluorimetric detection with LEDs. The polymeric cuvette for such measurements, placed in a LEGO holder consisted of opaque elements - made of polyimide and forming the edges of the fluorometric cuvette and transparent one s- made of polymethyl methacrylate and forming a space for light transmission (Fig.4). On the cross-section of the cuvette could be seen clear symmetrical cross on the plan of opaque square and the flow channel running along the height of the cuvette.

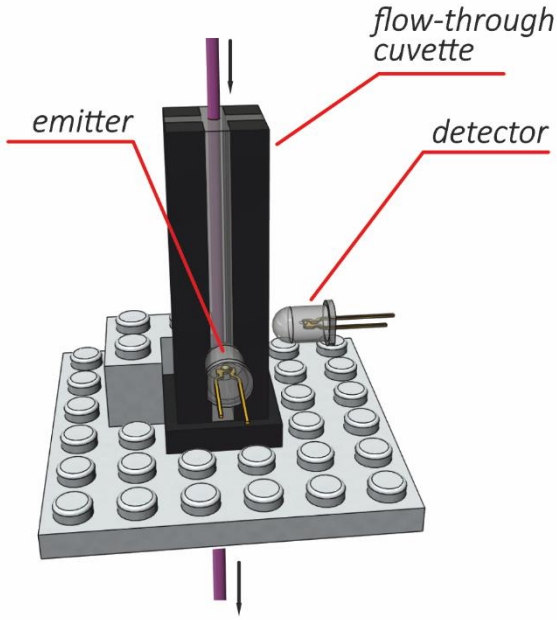


Figure 4. *A flow-through cell used in a preliminary fluorimetric studies with PEDD.*

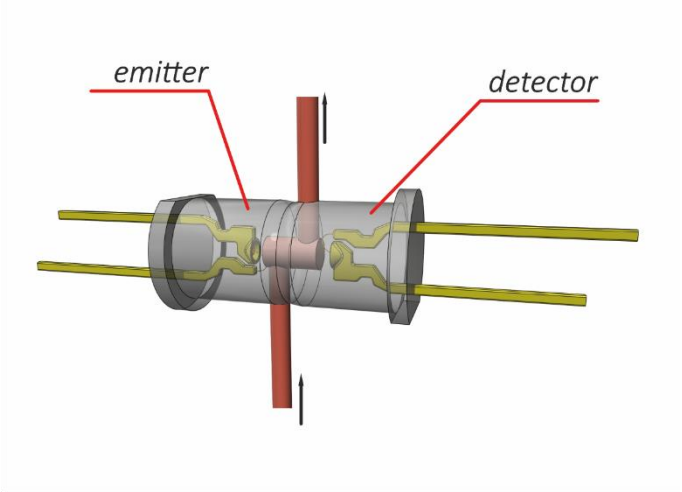
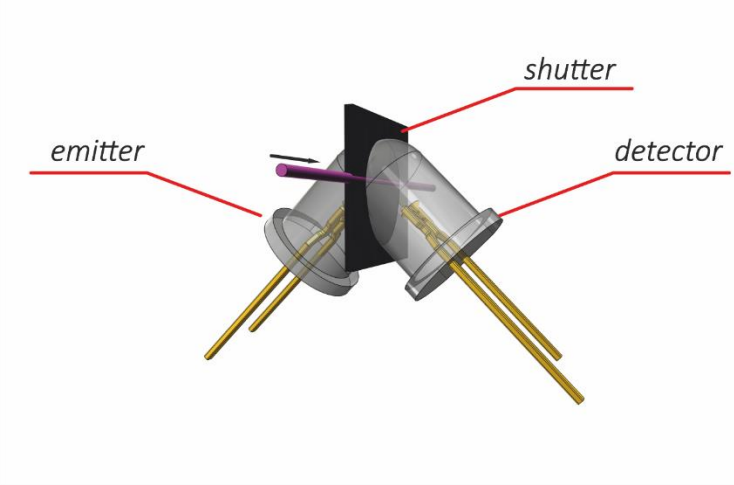


Figure 5. *Flow-through cel made of two LEDs permanently conected to each other.*

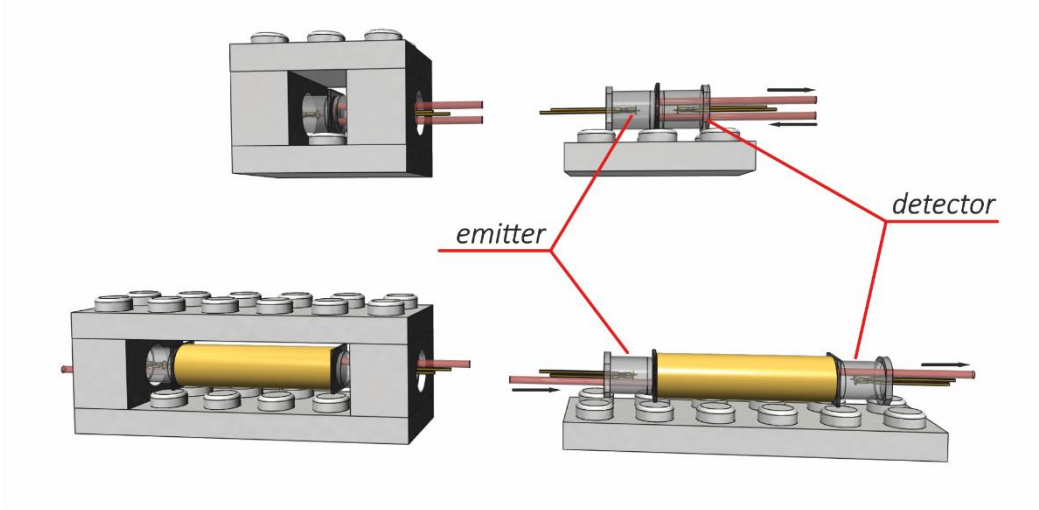
The ability for direct connection of the LEDs to form a flow-through cell has been tested by drilling small (1mm) axial channels in the diodes - (without prejudice to the space of the semiconductor chip diode) and next to this - perpendicular channels. After gluing one LED to another the vessel has been created with integrated light source and detector as well as with the inlet and the outlet of the solution (Fig.5). This modification is very attractive as a concept and due to is applicable potential, but in many cases, such solution was not optimal. The space of the diodes body is limited. On the other hand designing flow-through cell for the particular application and the particular chemical detection scheme, sometimes it is necessary to use larger than the standard (10mm) optical path in order to obtain the analytical signal with better quality. In the laboratory conditions, it is difficult to repeatedly obtain such sophisticated structure. The fabrication of such two LEDs element with channels in the structure of LEDs lenses make risk of irreversible damage to the LED chip.

A similar LEDs integration for the fluorimetric measurements was not possible to carry out in way other than modifying the diode covers (milling at angle of 45deg to the axis of the diodes), and adhering them by non-transparent part of the shutter interposed in between (Fig.6). In the shutter and partially on both sides - in the structure of LEDs glued together, the channel was fabricated for introduction the transparent tubing from the flow system. This approach was characterized by the sensitivity almost twice larger than the flow cell described above (Fig.4).



Rysunek 6. *Flow-through cell made of two LEDs connected permanently for the fluorimetric measurements*

Proper LED positioning and fixing them using manual procedures it is not a simple procedure and percentage of successful attempts was not satisfactory. The use of elements from a wide range of LEGO bricks can solve this problem. After a slight modification of blocks used (milling holes in original LEGO Technics bricks), construction of a flow-through photometric vessel of discrete changing the optical path (of distance resulting from the width of a single piece of LEGO) is possible. This configuration of LEDs has been proven in sensor variant of PEDD [34,35] (Fig.7), but it is possible to use such a flow-through cell in photometric research.



Rysunek 7. *Photometric flow-through cells with light-emitting diodes, made without gluing. (Left side: - assembled, Right side: disassembled)*

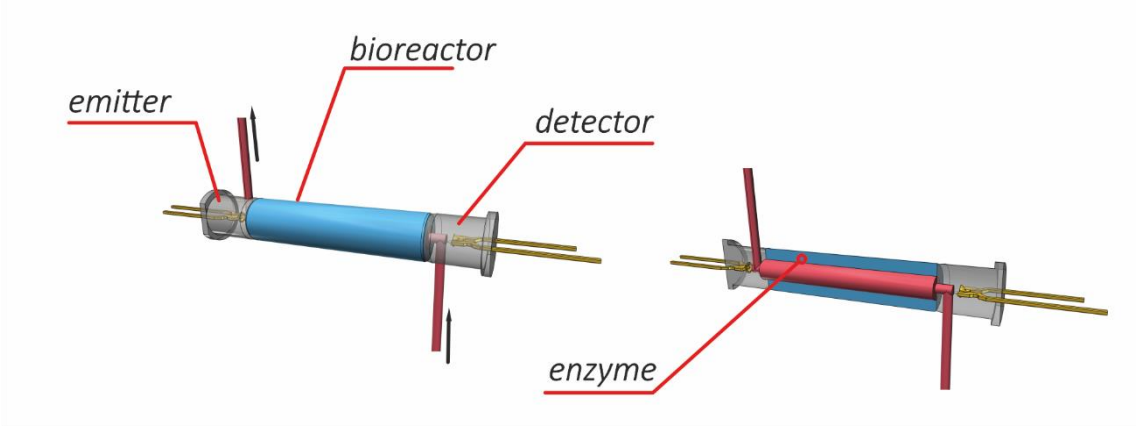


Figure 8. *Flow-through cell with integrated enzymatic bioreactor. Left: complete cell , Right: cross-section of the cell.*

In a similar manner it is possible to fabricate a photometric flow-through vessel with integrated enzymatic bioreactor. Also in this case, channels are formed in the diodes covers and the functionalized bioreactor was adhered to the faces of the modified diodes.

During further studies other construction of the detector was designed, requiring no mechanical interference with the structure of the light emitting diode. The most commonly used, in the course of measurements and described in the publications, is a construction of the flow detector was made of chemically inert polymer (polyetheretherketone, PEEK), subjected to conventional machining to form a space for the flow of the solution and allows reversible and precise positioning of light emitting diodes on both sides and inside of the polymeric block .

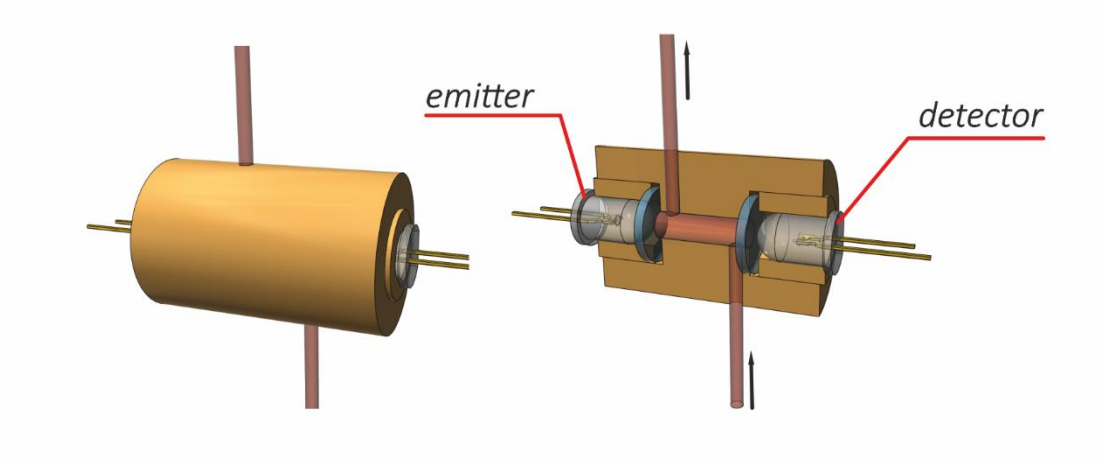


Figure 9. *Flow-through photometric cell with unmodified interchangeable LEDs.*

Part of the polymer bar with a main channel fabricated along what defining the aperture and the optical path length. Also, it had formed perpendicular channels, located at the beginning and the end of the optical path. In addition, the components included: circular transparent "windows" and bushes having an external diameter which allows stable immobilization of "windows" and an internal diameter, allowing stable fixation of the LED (Fig.10). The advantage of this solution was to exchange the diodes so that the flow cell was versatile and can be used in studies of various types. Furthermore, with the use of conventional methods of micromachining, it was possible to produce cell of any internal size (channel diameter and length) so that the detector geometry optimization processes for a specific detection scheme could be conducted effectively. Flow-through vessel of this type is also allowed for further modifications. Typical changes to the original solution was, among others, placing in the space between the light emitting diodes the tube which inner walls was functionalized by enzyme layers to produce integrated optical bioreactor. Another example of modification was to place instead of the usual transparent PMMA sheets of plastic as “windows”, the slides coated with Prussian Blue (with or without an enzyme) to produce a flow-through optical (bio)sensor with integrated light source and detector (Fig.9).

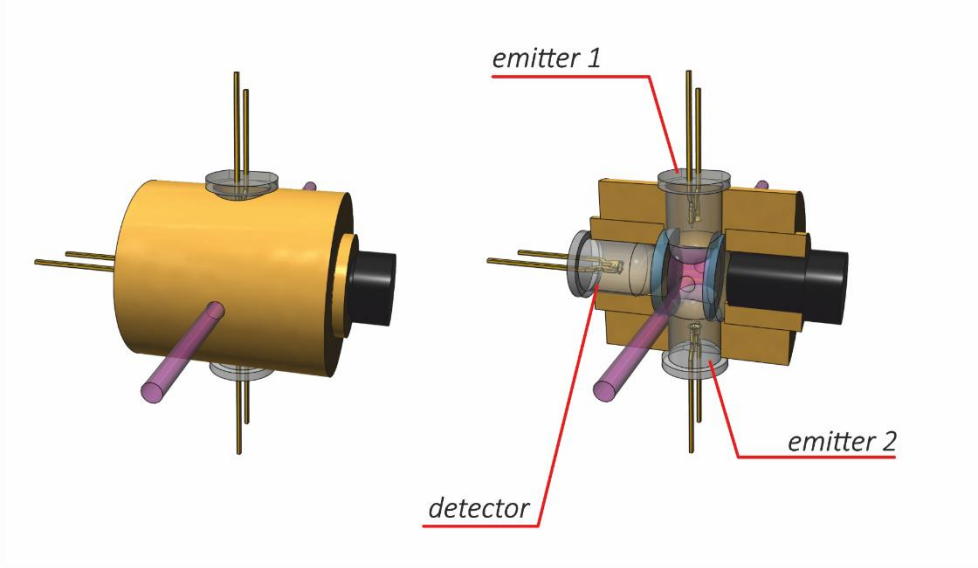


Figure 10. *Photometric flow-through cell (cross-section) with unmodified interchangeable LEDs, which allows placing bioreactor and/or chemosensitive layer/layers inside.*

The most versatile, efficient, and easiest-to-manufacturing flow detector for fluorimetric measurements made of LEDs was based on the above mentioned idea. Chemically inert polymer (PEEK) have been micromachined so that beside the channel fabricated along an axis (forming aperture) and perpendicular channels for moving solution, holes for LEDs having induce fluorescence of the analyte were prepared in the cell body. Emitting diodes, tightly placed inside were subsequently milled on the inner side in the way that did not damage structural integrity of the LEDs chips maintaining as much aperture as possible. After closing the detector with the similar to the above-described "windows" flow-through cell was complete and allowing fluorescence measurement by any exchangeable light-emitting diode, positioned by the “window”. The inner volume of such vessel is less than 60µL. Location of the sockets: an inlet and an outlet, facing each other effectively eliminated the problem of air bubbles in the detector (Fig. 11).

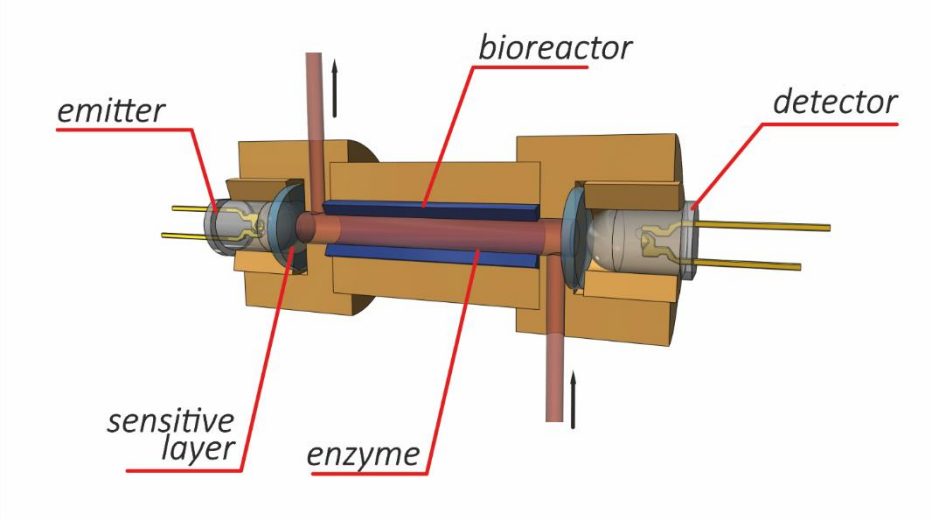


Figure 11. *FPEDD with interchangable LEDs. (complete vessel on the left , cross-section on the right)*

The proposed construction was characterized by simplicity, high degree of the project feasibility and also allowed the modification aimed at using fluorimetric quasi-sensoric materials. This was done by placing inside the detector, between the "windows", powder of solid sorbent C18 which filling completely interior space of the detector. The wetted sorbent was so clear that it was possible to measure the fluorescence of the substance adsorbed on its surface.

Similar flow-through vessel has been designed and optimized due to optical properties and served as a detector for the photometric or fluorometric determination (Fig.9), depending on the type of analyzed sample.

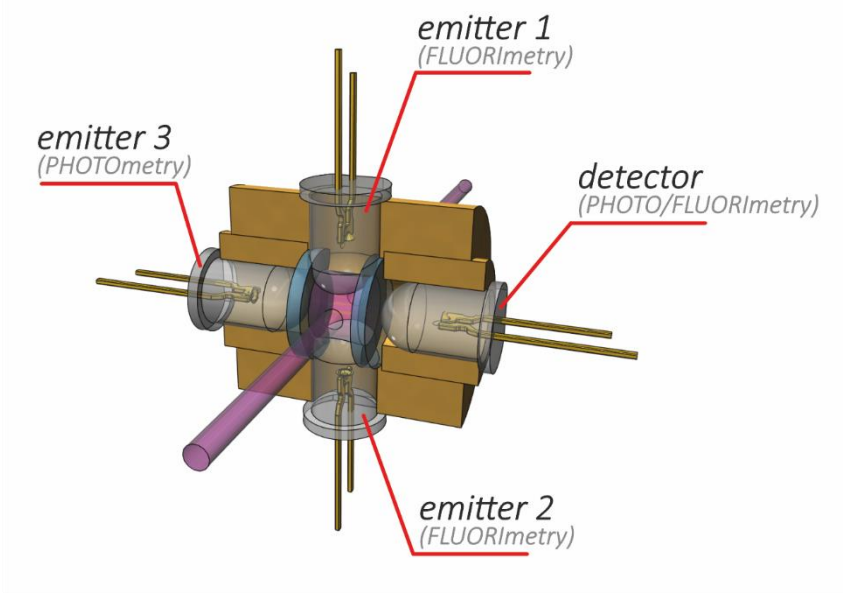


Figure 12. *Flow-through cell allowing dual photometric/fluorimetric detection.*

VIII summary

Publications series, discussed in this dissertation provides the results of my research on the achieving the scientific goal, stated an the very beginning of this work.

In my opinion, the most important achievements are:

1. Development of innovative methods for the measurement with the photometric PEDD detectors, based on the electromotive force acquisition as an analytical signal
2. Adapting the PEDD concept for fluorescence measurements.
3. Indication of PEDD sensors and biosensors microfabrication methods.
4. Design, fabrication and analytical characterization of flow-through PEDD detectors.
5. Giving the evidence for the analytical usefulness of designed detectors and analytical systems in the real chemical analysis, especially in clinical analysis (Table 2)

I have proposed an alternative, simpler and cost effective, than presented in the scientific literature, method of acquisition the analytical signal generated by the light emitting diode and also explained the reasons for the observed phenomena. I proved that the fact of LEDs spectral selectivity may be analytically useful. From this reason LEDs can be used in such applications where conventional optoelectronic components for light detection requires additional equipment (optical filters). I have constructed and characterized a variety of analytical flow systems with several different configurations of light emitting diode detectors. All of this form a research platform, ready to use, since neither the design nor the detector operation is not required expertise knowledge. The only limitation is the need to physical fabrication of the detector, but in a number of publications there are included detailed descriptions and diagrams as well as multimedia materials **[H14]** illustrating the process of detectors manufacturing.

Table 2. *Applications of diode detectors with electromotive force as analytical signal used for the determination of analytes important from a clinical point of view.*

analyte	***reagent (method)***	***PEDD … -metric***	***system***	***sample***	***application***	***REF.***
Hb*	potassium cyanide sodium lauryl sulfate sodium dithionite	photo-	ST**	whole blood	hemoglobinometry	[H5]
PO_4^{3-}	molybdic acid rhodamine	photo fluori	MCFA	blood serum	hiperphosphatemia diagnostics	[H8]
total protein	Coomasine Blue fluoresceamine	photo- fluori-	MCFA	blood serum, urine	general diagnostics	[H9]
creatinine	picric acid (Jaffe)	photo-	MCFA	urine, blood serum	general diagnostic	[H10]
(PCR) creatinine/ protein	picric acid (Jaffe) sulfosalicilic acid (Exton)	photo- turbidi-	MCFA	urine	nephrologic diagnostics	[H11]
ALP	*p*-nitrophenyl phosphate	photo-	MCFA	blood serum	okcologic diagnostics	[H12]
ALP/ACP	*p*-nitrophenyl phosphate	photo-	MCFA	blood serum	okcologic diagnostics	[H13]

**Hb – total hemoglobin*
***ST –stationary measurtements.*

Ideas and technological solutions presented in this work exist in various forms in foreign publications. Concepts and solutions proposed in this dissertation have been used in other studies of which I am a co-author [28,29,34-36] and in the work of other authors [25,26,30,31,37-41].

Up to date, the literature contains reports of PEDD detectors measured electromotive force as an analytical signal in photometric determinations of: ammonium ions in urine [37], inorganic ions in water [38-40]; fluorometric determinations of: phosphate soil extracts [29],

calcium ions in the blood serum [30], simultaneous measurements for calcium ions and phosphate ions in the plasma [31], or chlorophyll in water [41] and nephelo- and turbidimetric determinations of total urinary protein [25] as well as protein in the cerebrospinal fluid [26].

Despite the initial controversy that featured topic had raised, a recent review articles [42-44] suggest that the PEDD concept, in the version proposed by me, may permanently be entered in the package of available modern analytical tools. In my opinion the most promising directions for future research in this area are multi-detector/sensor systems, monitors and portable Point of Care Testing systems. All of these have great potential of applicability and commercial implementation.

Dissertation consists of fifteen publications - in seven of them, I am the first author whereas I am the corresponding author of fourteen of them. The total impact factor for these works is **55.490**

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