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A remote-controlled immunochemical system for nephelometric detection of human serum transferrin

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ABSTRACT

The fully-mechanized Multicommutated Flow Analysis (MCFA) system for determination of transferrin in human serum has been developed. The analytical methodology concerns immunoprecipitation measurements with the use of LED-based nephelometric flow-through detectors with polymeric light guides. To improve the mechanization degree of the MCFA system, the Do-It-Yourself electronic module for mobile phone control has been developed. The design and structure of an Android software (build using open source application) controlling the operation of presented flow system has been presented. To bypass the problem of nonlinearity of calibration curve caused by the nature of antigen-antibody interactions, the on-line dilution module has been integrated into the presented manifold. Under optimized conditions, the developed flow analysis system is characterized by relatively low limit of detection and quantitation (2.0 and 4.9 mg L^{-1}, respectively), good precision (RSD < 4%), low reagent and sample consumption per one measurement (16 μL of undiluted reagent and 20 μL of undiluted sample without further on-line dilution, respectively) and relatively high throughput (approximately 35 signal recordings per hour). The developed MCFA system enabled to analyze 16 serum samples with the transferrin concentration from 90 to almost 350 mg dL^{-1} with statistical agreement to the reference method.

1. Introduction

Flow analysis techniques have been recognized as a very useful tool for contemporary analytical chemistry (Horstkotte et al., 2018; Rocha et al., 2002; Trojanowicz and Kołacinska, 2016). Its main attributes, in the form of various on-line operations of sample processing, large sampling-rate, improved precision as well as reduced consumption of reagents and sample and possibility of kinetic/stopped flow measurements, enable to apply flow methodologies in very demanding areas of chemistry, such as clinical and biomedical analysis (Culbertson et al., 2014; Hartwell and Grudpan, 2011; Mahato et al., 2017). The recent significant feature, which has come with the development of cheap, easy-to-obtain electronic components and everyday life IT equipment (e.g. mobile phones or webcams), concerns using Do-It-Yourself (DIY) devices to improve the mechanization (or even automation) process of flow analysis systems. Over the past few years, many propositions of DIY constructions applied in flow analysis manifolds appeared in the literature (Urban, 2015). The range of given options covers hardware for controlling flow devices (da Costa et al., 2014; González et al., 2016), software designing and programming for managing the flow system operation (Barbesi et al., 2017), using everyday life electronic devices as detection systems (Grudpan et al., 2015) or even preparation of flow-through cells made by low-budget 3D printer (Michalec and Tymecki, 2018).

In this publication, the dedicated flow analysis system with nephelometric detection for immunoassay combined with DIY mobile phone control is presented. The construction of the manifold is based on Multicommutation Flow Analysis (MCFA) methodology, which is the one of flow techniques with the greatest potential for medical diagnostics. It is partially thanks to the possibility of arranging flow devices (mostly solenoid pumps and valves) into analytical modules responsible for different operations of sampling processing (e.g. dilution, incubation or separation) (Llorent-Martinez et al., 2010; Rocha et al., 2002; Trojanowicz and Kołacinska, 2016). Such systems have already been used for determination of many, clinically important analytes, such as: electrolytes (Fiedoruk-Pogrebniak and Koncki, 2015; Lopes et al., 2006; Rocha and Rocha, 2013), metabolytes and uremic toxins (Michalec et al., 2016; Rocha and Rocha, 2010; Strzelak et al., 2016), enzymes (Bzura et al., 2018) and proteins (Pokrzywnicka et al., 2012; Strzelak et al., 2017). A separate issue is the control of entire system with the use of Android mobile phone to provide easier and more intuitive setting of flow system parameters. In this case, the connection between MCFA system, Arduino microcontroller (Lake et al., 2017; McClain, 2014; Milanovic et al., 2018) and Bluetooth module is shown. All presented

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investigations are enriched with briefly discussion about design and operation of Android software created with the use of open-source web application.

This contribution presents the application of MCFA system for determination of human transferrin - a transportation glycoprotein involved in iron distribution and homeostasis (Gkouvatsos et al., 2012). Only in the last few years, a wide variety of analytical methods for transferrin determination have been published. Many of such methodologies include either immunochemical recognition (Matysiak-Brynda et al., 2017) or additional preparation step for transferrin separation in the form of gel electrophoresis (Feng et al., 2015; Ye et al., 2013) and liquid chromatography (Yu et al., 2012). Some of them propose imprinted nanoparticles (Zhang et al., 2018) or even boronic acid monolayer (Mayang et al., 2017) as a specific units for selective binding of target protein. Above-mentioned publications describe whole spectrum of detection methods including fluorometry as well as mass spectrometry that are mostly characterized by very low limits of detection and quantitation, especially in comparison to the transferrin range of concentrations in human serum, which is 2.0–3.6 $g\,L^{-1}$ (Bishop et al., 2010).

In this work, to estimate the amount of transferrin molecules, the direct creation of turbidity in the course of antigen/antibody interactions is monitored. Unfortunately, this process depends on the ratio of antigen and antibody concentrations resulting in the specific bell-shaped calibration curve (so-called Heidelberger curve (Bishop et al., 2010)), which can be divided into three parts: antibody excess, equivalent zone and antigen excess. Therefore, at least one dilution step is needed in analytical procedure. As it has been proven recently (Strzelak and Koncki, 2015), a suitable combination of solenoid devices can be used as a multipoint dilution unit to perform such immunoprecipitation measurements. Moreover, to improve the light scattering detection, a novel kind of nephelometric detector based on LEDs with specific light guides is presented and compared to the construction used earlier (Strzelak and Koncki, 2013).

2. Experimental

For preliminary results (chapters 3.1 and 3.2), the bovine serum albumin (A7906, ≥ 98%) and 5-sulfosalicylic acid (S2130, ≥ 99%) were bought from Sigma-Aldrich (USA). The immunoprecipitation measurements (chapters 3.3 and 3.4) were performed using Human Transferrin (ca. 25% of a proteins saturation with Fe(III)) (T3705), apo-Transferrin (T1147) and holo-Transferrin (T0665) as antigens and whole antiserum anti-Transferrin (T2027) with the antibody concentration of 4.5 $mg\,mL^{-1}$. These reagents were obtained from Sigma Aldrich. For all experiments doubly distilled water was used throughout. Other reagents of analytical grade, were obtained from Avantor Performance Materials (Poland). For the dilution of antiserum, 10 $mmol\,L^{-1}$ phosphate buffer (pH 7.4) was used. The human serum samples with known transferrin levels were obtained from the Central Clinical Laboratory of Medical University of Warsaw. The reference serum analyses were performed with clinical analyzer Cobas Integra 6000 (Roche Diagnostics, Switzerland) using the immunoturbidimetric assay for Roche/Hitachi systems designated as Tina-quant Transferrin ver. 2.

The nephelometric detection of precipitated albumin and antigen-antibody complex was based on LEDs-based flow-through detector (Strzelak and Koncki, 2013). Previously selected LEDs (Strzelak and Koncki, 2015) used as emitters (630 nm, OSHR5161A-NO) and detector (650 nm, L-53SRC-F) were purchased from TME (Poland). The flow-through cells were made by material processing of polyether ether ketone (Plastics Group, Poland) using lathe and milling machine. For fabrication of light guided detector (called 4LEDs detector), the PMMA light guides with 3 mm diameter (1216.1003, Mentor, Germany) and drilled LEGO® bricks as emitter-diodes holders were used. Emitter-diodes were powered with stable current using Arduino Mega 2560 microcontroller board based on the ATmega2560 (Arduino, Italy). The universal board and electrical wires were obtained from TME (Poland). The analytical signal in a form of electromotive force, generated by detector-diode was measured and recorded using UT70B multimeter (UNI-T, China) connected with data storage computer via RS232 interface.

The presented Multicommutation flow analysis system consists of solenoid micropumps (indicated stroke volume of 20 µL, product no. 120SP12120-5TV) and three-way solenoid microvalves (product no. 100T3MP12-62-5) which were purchased from Bio-Chem Fluidics (USA). All flow system connections were arranged using PTFE Microbore tubing (ID 0,8 mm) from Cole-Palmer (USA). All flow devices were powered and controlled by above-mentioned Arduino Mega 2560 through linear integrated circuit ULN 2803 (TME). Moreover, the wireless control of MCFA system was possible thanks to Bluetooth module (HC-05, TME) connected to microcontroller via Universal Asynchronous Receiver/Transmitter (UART) communication. The software for system control was prepared by MIT App Inventor (http://appinventor.mit.edu) which works on LG Nexus 5 mobile phone with Android operating system. Both, the electronic system for wireless control and the mobile phone software are presented in details in the Supplementary material.

3. Results and discussion

3.1. Flow system design - construction, operation and mobile control

In this section both aspects concerning the developed MCFA system are discussed: the architecture of flow system manifold and the electronic circuits enabling its operation with wireless control. Firstly, the construction and operation of flow analysis system will be discussed. In Fig. 1A the fully-mechanized MCFA system for immunoprecipitation assay is presented as the arrangement of four solenoid micropumps and four solenoid microvalves. Such a setup is similar to the system shown elsewhere (Strzelak and Koncki, 2015) except that the current manifold has a minimal amount of devices (there are only one microvalve V1 for antibody solution introduction and one microvalve V2 with one micropump P4 for antigen transport) through which segments of antibody and antigen solutions are transported before reaching the detector flow cell. It reduces the dispersion of liquid zones by removing the unnecessary components characterized by its internal geometry and additional dead volume.

The analytical procedure for precipitate analysis with the use of developed MCFA system has to meet three main assumptions in the form of:

- Introduction into the manifold analyte (antigen) and precipitant (antibody) solution zones with specific volume (100 µL and 80 µL, respectively).
- Simultaneous transport of aforementioned zones into the detector cell and flow stop for the fixed period of time. Commonly, measurements were performed with 60 s stop flow.
- Possibility of online dilution of a sample due to the nature of antigen-antibody complex formation (as it was mentioned in the Introduction section).

All above-mentioned tasks can be performed by appropriate programming of solenoid microdevices. The basic measurement procedure is based on the following scheme. According to Fig. 1A, the loading of the antibody solution is performed by the actuation of pump P2 (4 cycles) and switching V1 valve to NC position. Whereas, in the case of undiluted antigen solution, the operation of P4 (5 cycles) and V2 and V4 in NC position is required. Afterwards, both segment are transported in the direction of the nephelometer by the actuation of P1 and P3 with V2 and V4 switched to NO position and V3 enabling the flow through the detection cell (NC state). After 5 cycles the flow is stopped and the

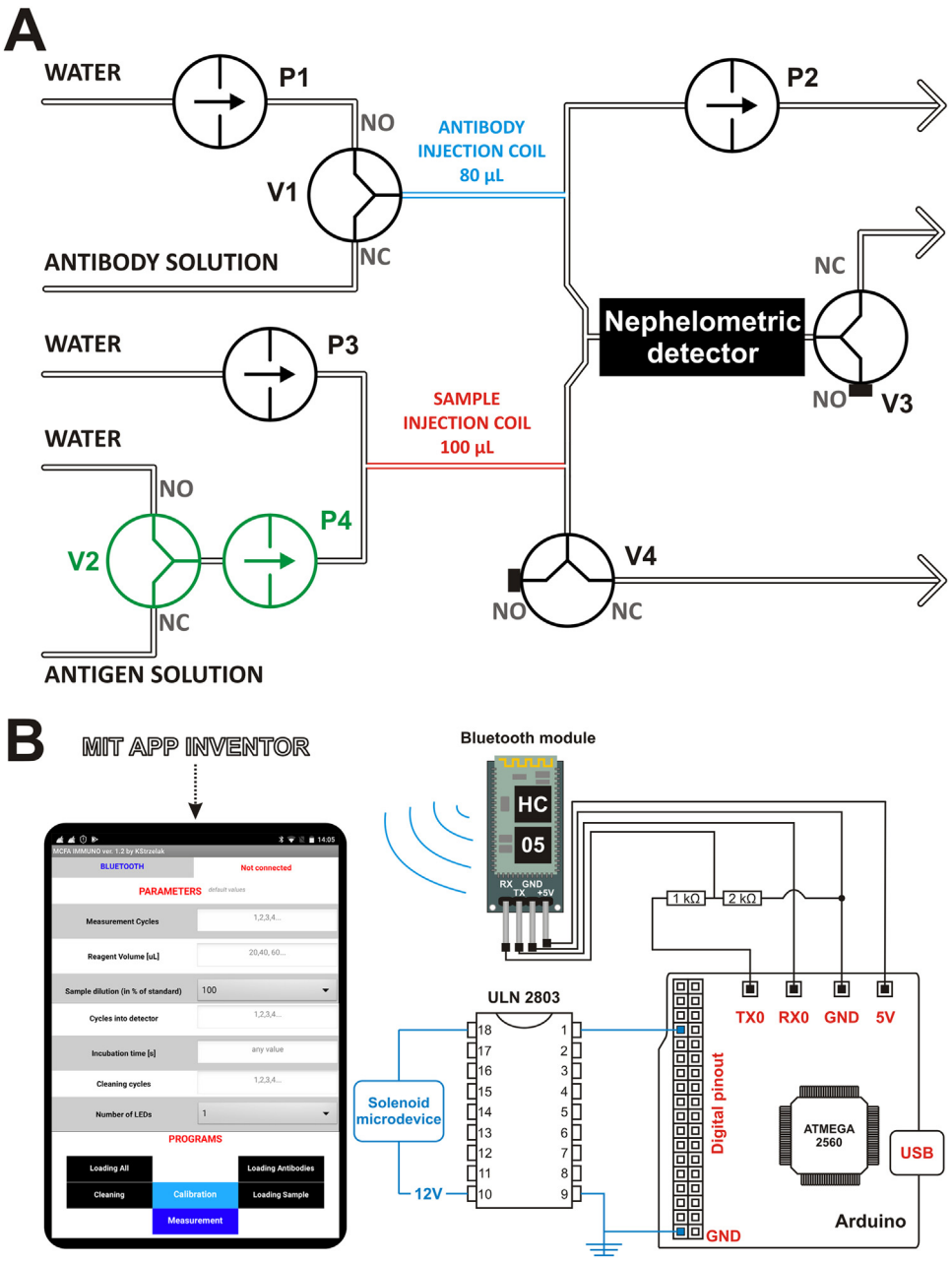

Fig. 1. A: The scheme of MCFA manifold with the selections of antibody solution injection coil (blue), sample injection coil (red) and standard dilution unit (green). Abbreviations used in the figure: V – solenoid microvalve, P – solenoid micropump, NC – normally closed, NO – normally open. B: The outline of connection between Arduino microcontroller, solenoid microdevice, Bluetooth module and mobile phone to configure and control the fully-mechanized flow system. Details are given in the text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

PEDD signal is monitored as kinetic recordings (for more details please see chapter 3.3). After 60 s, the procedure is continued as a cleaning step (30 cycles) and preparation of the system for next measurement. In this case, the whole measurement cycle takes ca. 100 s, giving the average throughput of about 35 signal recordings per hour. The graphical presentation of operation of the MCFA system and the source code of Arduino program are posted in the Supplementary material file.

A separate issue for immunoprecipitation methodology is an online dilution procedure carried out by V2 and P4 devices (green components in Fig. 1A). Such an online dilution unit has been already described and discussed in details elsewhere (Strzelak and Koncki, 2015; Tymecki et al., 2009). In brief, the solenoid micropump has its own dead volume estimated to 100 microliters. With its stroke volume of 20 µL and proper operation of the valve (changing the position between sample and water as a diluent) this online micro solenoid unit enables even 5-fold dilution. This is the reason why the injection volume of a sample was adjusted for 100 µL. To illustrate the appropriateness of its use, the comparison between manually prepared standards of bovine serum albumin (used as a model protein) in the range of 10–200 mg L^{-1} and online dilution using two standards of 100 and 200 mg L^{-1} was prepared. After mixing protein solution with 5-sulfosalicylic acid as precipitin agent the colloid is formed and turbidity of a sample appears (so-called Exton method). The 4LEDs-based nephelometric flow-through device, which in details is discussed in chapter 3.2, was used as light scattering detector. The results presented in Fig. 2 confirm the lack of differences between analytical signals for manual preparation of standards and its online diluted equivalents. All points corresponding to the standards prepared using mechanized unit lay within the range of

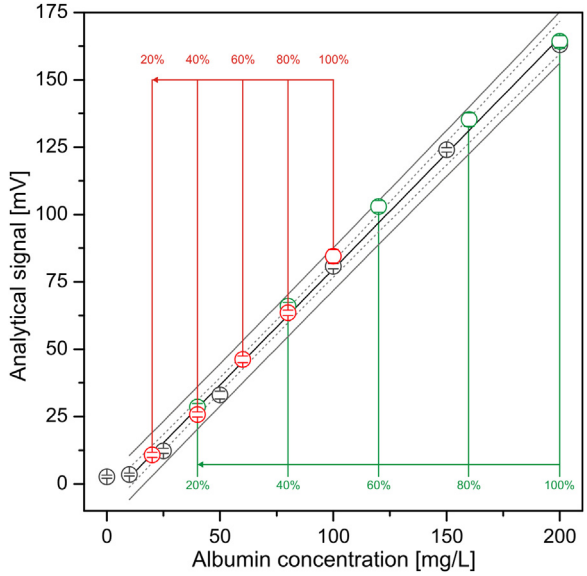

Fig. 2. The comparison between calibration curve with the use of manually prepared standards (black points with black linear fit and 95% confidence and prediction bands) and points which come from the dilution of 100 and 200 mg L^{-1} standards of albumin. The Exton method with 5-sulfosalycilic acid was used for precipitation assay.

prediction bands with confidence level of 95% (solid grey lines) appointed by linear fit of manually prepared standards.

The idea of measurements under flow analysis conditions using MCFA system has been already presented. Nonetheless, for operation of solenoid microdevices also electronic systems are necessary. For proper and effective operation of the MCFA system, an external 12 V power supply and microcontroller are required. The combination of Arduino and linear integrated circuit ULN 2803 (powered by 1 A AC/DC adapter with 12 V output), as it is depicted in Fig. 1B, is sufficient for presented application. Moreover, the Arduino board is connected to Bluetooth module via UART communication ports - the Bluetooth ports: RX, TX, Ground (GND) and 5 V are connected to Arduino TX0, RX0, GND and 5 V, respectively. Although the Bluetooth is powered by 5 V power supply voltage, its communication RX port works with 3.3 V, whereas the Arduino TX port generates 5 V. So the crucial issue is to apply voltage divider to lower the voltage on RX port (1 kΩ and 2 kΩ resistors were used). The Bluetooth module connected to Arduino enables wireless control of presented MCFA system via Bluetooth communication with mobile phone. For this purpose, dedicated software was created with the use of open-source web application – MIT App Inventor. The simple block editor allows to prepare Android software (called MCFA IMMUNO) for establishing a connection between microcontroller and mobile phone, setting up parameters of flow measurement (such as: number of measurement cycles, reagent volume, incubation time in flow cell, dilution of a standard or number of LEDs emitting light in detector) and choosing one of prepared flow programs (loading all reagents, loading only antibody solution or only sample, cleaning, full measurement of a sample and calibration using one standard). The interface of software is presented in Fig. 1B. Programs for Arduino microcontroller and MCFA IMMUNO software are presented in details in the Supplementary material.

3.2. Light scattering detector

As it was highlighted in the introduction section, for determination of proteins in the course of precipitation assays, the nephelometric LEDs-based detectors have been successfully used (Strzelak and Koncki, 2015, 2013). So far, the construction of such detectors was based on polymeric cylinder with the hole of 4.9 mm diameter drilled through, perpendicular to the main axis of a symmetry - two LEDs-emitters are pressed tightly to these holes (2LEDs detector). Then, the 5 mm orifice is drilled parallel to aforementioned axis. Furthermore, places for acrylic windows and sleeves, playing a role of diode holders (in the shape of cylinders with the 5 mm orifice for LED-detector) are required. It forms the flow cell of a volume of ca. 85 μL. In this case, the sleeve of a length of 25 mm was needed to reduce the signal which comes from the illumination of a LED-detector by the operation of LED-emitters (Strzelak and Koncki, 2013). It has to be emphasized that in this construction only two LED-emitters could be applied - the attempt to add more diodes ended with the rupture of the detector. Similar micromechanical fabrication process has been described and visualized as a video presentation elsewhere (Tymecki et al., 2012).

In this paper, a novel construction of optoelectronic light scattering detector is presented. In this case, the first step of fabrication is to drilled through PEEK block two 2.9 mm holes perpendicular to each other, instead of one with 4.9 mm diameter, to place four poly(methyl methacrylate) light guides with the diameter of 3 mm. On the top of these light guides, four LED-emitters are placed inside the drilled LEGO® bricks playing a role of holders – this kind of detector is called 4LEDs detector. Then the drilling process is similar to the one described in the paragraph above. This fabrication changes affect two structural issues: there are four emitting diodes, which are significantly moved away from the interior of a flow cell and inner volume of a flow cell can be reduced – the diameter of a main hole drilled parallel to the main axis of symmetry remains the same (5 mm) but the width of a flow cell changes from ca. 4.4–3.1 mm, which gives volume of ca. 60 μL. No additional distance between emitters and detectors was created (the sleeves have the length of ca. 8 mm). Its 3D model is depicted in the Supplementary material.

Both presented flow-cell constructions are able to perform light-scattering measurements under flow analysis conditions but their analytical usefulness is different. The introduction of light guides into detector construction has significant effects on analytical parameters presented by developed flow system. In Fig. 3, the comparison between analytical responses of 2LEDs and 4LEDs-based detectors for albumin levels in the range of 25–200 mg L^{-1} is shown under different measurement conditions (different current values for emitter-diodes). Moreover, to confront obtained results, the same specimen of LED-detector was taken. It can be seen that analytical signals for 4LEDs-based detector is much higher than for this one based on two LEDs, with the optimal current of 6 mA. In addition, the dependence between the ratio of analytical signals to the background signals is similar except the 10 mA current (too high intensity of a light illuminating LED-based detector causes reaching of upper determination limit of a detector and decrease of device sensitivity (Strzelak and Koncki, 2013)). On the basis of these results a few conclusions can be drawn. First of all, analytical signals for 4LEDs-based detector are higher because of the implementation of light guides which enables to introduce more emitters without damaging the structure of polymeric cylinder. It results in the reduction the flow cell dimensions and thus also the dispersion of a sample. But it would not have been possible without the special light guides feature. In the case of 2LEDs-based detector, the increase of a current supplying LEDs-emitters also contributes to significant increase of a background signal due to the fact that LED-detector is illuminated directly by emitters (in the case of light scattering measurements, detector unit is sensitive for the light emitted by diode-emitters). In this situation no notable improvement of a sensitivity is observed. By applying light guides into the construction, diode detector is not illuminated so much by LEDs-emitters which are located at the top of light guides. Moreover, the cone of light emitted by LEDs becomes trimmed by a light guides and collimated. It gives large increase of scattered light intensity with a much smaller increase of nonspecific signal of a baseline. For all these reasons, for further investigations 4LEDs-based detector has been applied.

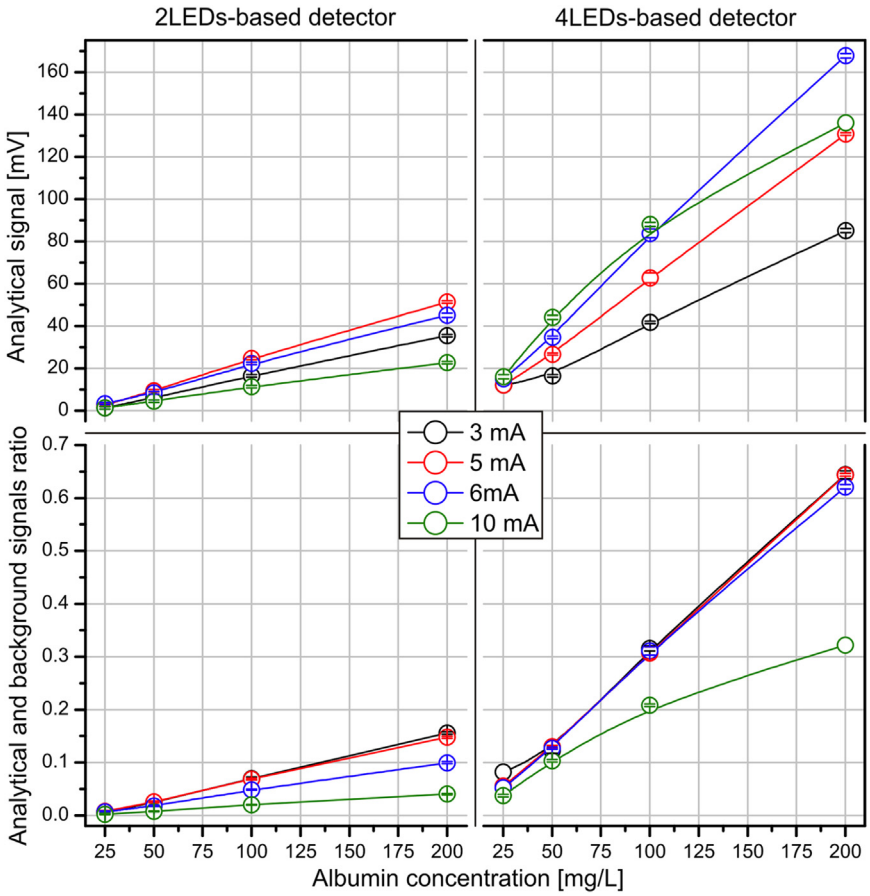

Fig. 3. The analytical responses of 2LEDs and 4LEDs-based detectors for different currents supplying LEDs-emitters. For the precipitation analysis (Exton method) of bovine serum albumin, both analytical signals and the ratio of analytical signals to background signals are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

3.3. Transferrin determination and serum samples analysis

The developed MCFA system combined with 4LEDs-based nephelometric detector has been applied to the immunoprecipitation assay for transferrin determination in human serum samples. To check the analytical characteristics of this method, three dilution factors of antibody solutions were used (for final concentrations of 0.5, 0.7 and 0.9 mg mL^{-1}). The calibration curves (with the typical shapes for Heidelberger curve) were obtained using the online dilution unit to achieve 60, 120, 180, 240, 300 mg L^{-1} and 0, 10, 20, 30, 40 and 50 mg L^{-1} of proteins by dilution of 300 mg L^{-1} and 50 mg L^{-1} standards, respectively. In Fig. 4A, the calibration bell curves for each antibody concentration with corresponding recordings are shown as the dependence between analytical signal and the ratio of transferrin and antibody concentrations. Two observations can be made. First of all, the analytical signal is proportional to the absolute concentration of antibody. Secondly, the top of precipitation curves, which corresponds to the equivalence zone does not depend on the amount of antibodies but the character of antigen antibody interaction (the kind of antibody molecules). For further experiments, 0.9 mg mL^{-1} of antibody was chosen, which gives 10 mL of antibody solution. Such a volume was sufficient to perform over 100 measurements.

The transferrin is iron-dependent protein and can occur in blood in two biological forms - with or without iron ions bound to the protein molecules (Strzelak et al., 2017). Thus, the effect of transferrin speciation on obtained analytical signal was checked. For this purpose, apo-transferrin, holo-transferrin and transferrin with physiological Fe (III) saturation were used. In Fig. 4B, signals for each form in the range of 10–50 mg L^{-1} of transferrin is depicted as column graph. It can be seen that there is no statistical difference in signal values between presented izoforms of transferrin, which can be used interchangeably. It also means that presented immunochemical assay enables to estimate concentration of total transferrin. For standard preparation in further investigations, physiological transferrin form was taken.

The analytical and clinical usefulness of presented MCFA system was proven by human serum samples analysis. The analytical procedure consisted of two measurements concerning different dilution factors for a sample – 1.0 for undiluted sample and 1.(6) for 60% of primary sample. The two-step analysis is a consequence of nonlinear calibration curve mentioned before and it was sufficient for successful transferrin determination (Strzelak and Koncki, 2015). The final results were calculated as a mean and standard deviations of two values for undiluted and diluted samples. The optimized MCFA system was characterized by low reagent and sample consumption per one measurement (16 μL of undiluted reagent and 20 μL of undiluted sample), relatively low limits of detection (LOD = 2.0 mg L^{-1}) and quantitation (LOQ = 4.9 mg L^{-1}), satisfactory precision (RSD below 4%) and efficient throughput of 35 signals recordings per hour. In Table 1, recently published papers devoted to analytical methods for transferrin determination are compared with presented MCFA methodology. It can be noticed that there is no methodology offering significant improvement in all investigated analytical parameters. For example, the amazingly low LOD and LOQ have been obtained at the expense of time analysis (Matysiak-Brynda et al., 2017) or precision (Mayang et al., 2017). On the other hand, the methodologies offering higher LOD have better precision even if the measurement step is preceded by separation step (Yu et al., 2012; Zhang et al., 2018). From this point of view, presented method using developed MCFA system seems to be attractive - the analytical parameters of such a method are sufficient for transferrin determination with fast measurement procedure, with no need to perform any pretreatment steps.

The developed bioanalytical system was validated with 16 human serum samples. All samples, with the concentrations of transferrin covering both physiological and pathological ranges (from about 90 to

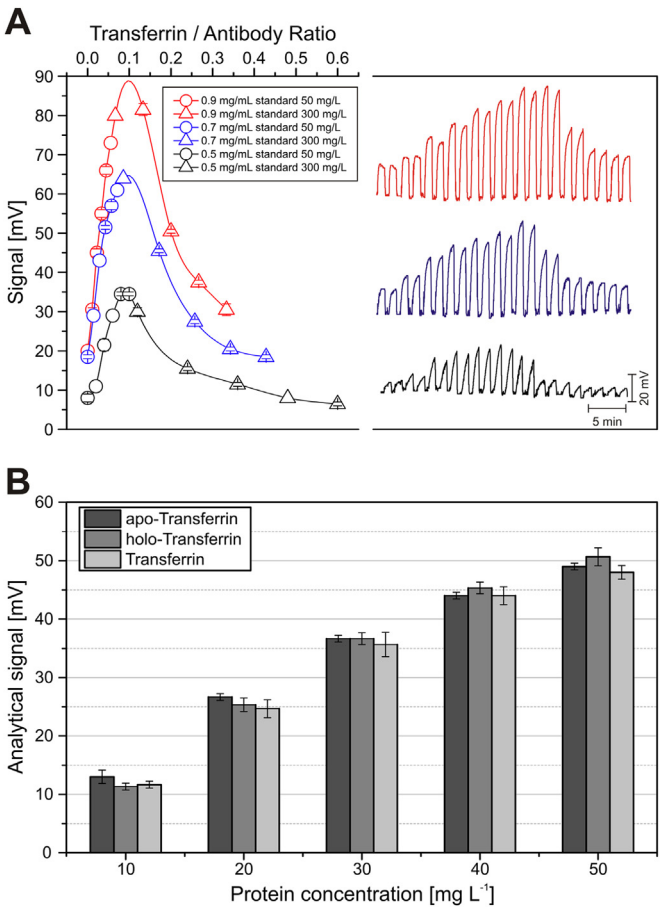

Fig. 4. A: The calibration curves for different antibody concentrations (left) and corresponding recordings (right). B: The effect of different transferrin forms (apo-transferrin, holo-transferrin and saturated transferrin in ca. 25%) on analytical signals.

almost 350 mg dL^{-1}), were pretreated by manual 10-fold dilution. For each dilution of sample (100% and 60% of a sample), three signals were obtained. The analytical signal was counted as the difference between the signal generated on the detector after 1 min of incubation and the signal value just after the flow stops. In Fig. 5, the correlation graph between values obtained by reference method and investigated system is shown. The regression line was $y = (0.98 \pm 0.06)\,x + (8.1 \pm 13.4)$ with the coefficient of determination on the level of 0.95. For the comparison of accuracy of MCFA measurements with the reference methodology the two-tail paired Student's t-test with 15 degrees of freedom at a 95% confidence level was used. The calculated t-value was 1.141 and does not exceed the tabulated one (2.131). Moreover, the

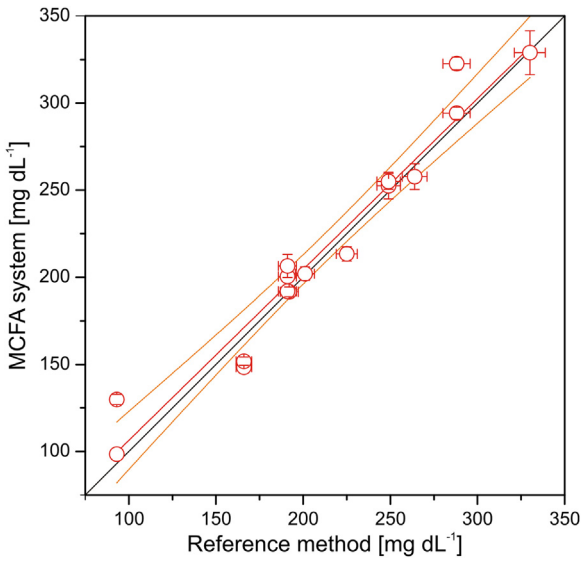

Fig. 5. The results of human serum analysis using presented MCFA system and reference clinical laboratory method. Black line indicates perfect correlation dependence (y = x); red line is a real correlation trend line; orange curves are confidence bands (95% confidence level) appointed by a linear fit of obtained correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

ideal dependence line y = x (black line in Fig. 5) lays within the range of confidence bands (95% confidence level, solid orange lines) appointed by linear fit of obtained correlation. All above-mentioned statistical results clearly confirm that presented bioanalytical system can be used for determination of human serum transferrin.

4. Conclusions

To the best of authors knowledge, this is the first literature report on the remote-controlled multicommutated immunochemical system dedicated for human transferrin determination. To achieve satisfactory analytical parameters of immunoprecipitation assay, the novel construction of nephelometric flow-through detector made of 4 LEDs-emitters integrated with light guides is proposed and its homemade construction process is also described. The combination of such a detector with flow analysis system led to form fully-mechanized and efficient bioanalytical MCFA system. In this contribution, the analytical usefulness was presented by the immunochemical determination of total transferrin in human serum samples. The developed system presented satisfactory LOD and LOQ parameters (2.0 and 4.9 mg L^{-1}, respectively), good precision (less than 4%), low consumption of antibody and antigen solutions (16 µL and 20 µL without further dilution, respectively) and fast determination procedure (less than 2 min for single

Table 1
The comparison of different methods for transferrin determination.

Analytical method	LOD [mg L^{-1}]	LOQ [mg L^{-1}]	Precision (RSD)	Comments	Ref.
Liquid chromatography with tandem mass spectrometry (LC/MS/MS)	n/a	0.5	≤ 4.9%	Additional sample preparation steps	(Yu et al., 2012)
Fluorometry on western blots with polymer dots	0.01	n/a	n/a	Additional sample preparation steps	(Ye et al., 2013)
ICP-MS with isotope dilution	550	n/a	≤ 3%	Separation step of proteins	(Feng et al., 2015)
Immunosensor with electrochemical quartz crystal microbalance	$1.2{\cdot}10^{-4}$	0.005	≤ 9.5%	30 min of incubation and 15 min of regeneration	(Matysiak-Brynda et al., 2017)
Surface plasmon resonance with boronic acid monolayer	0.34	0.8	≤ 15.3%	60 min of a measurement cycle	(Mayang et al., 2017)
Fluorometry with magnetic molecularly imprinted nanoparticles	7.5	25	≤ 7.7%	Nanoparticles used for detection + extraction procedure	(Zhang et al., 2018)
Immunonephelometric detection integrated in MCFA system	2.0	4.9	< 4%	ca. 100 s of a measurement cycle	This work

assay). The obtained analysis results of 16 serum samples stay in good statistical agreement with the values of reference analysis performed in the clinical laboratory settings.

The presented system is all-embracing tool for immunoprecipitation measurements and it can be successfully applied for other protein determination, provided that the forming antigen-antibody complexes could be detected by proposed optoelectronic nephelometer (it depends on characteristics of antigen/antibody interactions). Moreover, this contribution is also focused on the increase of a mechanization degree which is a significant aspect in modern analytical chemistry and clinical analysis. The idea of mobile phone control via Bluetooth module was based on DIY electronic devices and open source web application for Android software preparation. Such an approach contributes to the increase of convenience of flow system operation by remote-adjusting system parameters (there is no need to change parameters in an Arduino code editor) and somehow the reduction of time needed for system settings. The biggest advantage of the presented solution is the possibility to connect flow analysis devices with electronic circuits and devices without specialist knowledge in the field of electronics. It also proves a huge versatility and adaptability of flow analysis to modern technical approaches. In the near future, such features can lead to the development of point-of-care flow analysis systems and personalized medicine devices, what would be a big step forward for medical diagnostics and consequently public health.

Acknowledgements

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Supplementary material

Electronic supplementary material containing a figure of 3D models of 2LEDs and 4LEDs-based detectors, graphical presentation of MCFA system operation, description of software programming and full source code is given as PDF file.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.12.011.

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